TITLE

Neuromuscular fatigue and physiological responses after five dynamic squat exercise protocols

RUNNING HEAD

Responses to five different squat exercise protocols

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Abstract

The present study aimed to analyze neuromuscular, physiological and perceptual responses to a single bout of five different dynamic squat exercise protocols. In a randomized and counterbalanced order, fifteen male resistance-trained athletes (mean±SD; age: 23.1±1.9 years, body mass: 77.4±8.0 kg) completed a traditional multiple sets (MS: 4 x 6, 85% 1RM), drop sets (DS: 1 x 6, 85% 1RM + 3 drop sets), eccentric overload (EO: 4 x 6, 70% 1RM concentric, 100% 1RM eccentric), flywheel YoYo™ Squat (FW: 4 x 6, all-out), and a plyometric jump protocol (PJ: 4 x 15, all-out). Blood lactate (La), ratings of perceived exertion (RPE), counter movement jump height (CMJ), multiple rebound jump performance (MRJ), maximal voluntary isometric contraction force (MVIC), serum creatine kinase (CK) and delayed onset muscle soreness (DOMS) were measured. Immediately post-exercise, La was significantly (p<0.001) higher in FW (mean±95% CL; 12.2±0.9 mmol·L−1) and lower in PJ (3.0±0.8 mmol·L−1) compared to MS (7.7±1.5 mmol·L−1), DS (8.5±0.6 mmol·L−1) and EO (8.2±1.6 mmol·L−1), accompanied by similar RPE responses. Neuromuscular performance (CMJ, MRJ) significantly remained decreased (p<0.001) from 0.5 to 48 h post-exercise in all protocols. There was a significant time x protocol interaction (p<0.05) in MRJ with a significant lower performance in DS, EO and FW compared to PJ (0.5 h post-exercise), and in EO compared to all other protocols (24 h post-exercise). A significant main time effect with peak values 24 h post-exercise was observed in CK serum concentrations (p<0.001), but there was no time x protocol interaction. In conclusion, (1.) metabolic and perceptual demands were higher in FW and EO compared to MS, DS and PJ, (2.) neuromuscular fatigue was consistent up to 48 h post-exercise in all protocols, and (3.) EO induced the greatest neuromuscular fatigue.

Keywords: eccentric overload, flywheel training, strength training methods, muscle damage
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Introduction

In strength and power dominated sports like team or racquet sports, the implementation of high-intensity lower-limb strength training sessions to the regular training routine is of great practical relevance. It has been stated that maximal-dynamic strength and power of the lower limbs are strongly correlated to many sport-specific variables of athletic performance, such as vertical jump height, 10-m shuttle run time, and sprint times over several distances (10 - 30m), respectively (35, 42). However, for well-designed strength training sessions it is required to have a comprehensive knowledge about the short-term fatigue effects of different strength training methods. This can be considered a fundamental prerequisite for strength training prescription in resistance-trained athletes.

An extensive variety of certain strength training methods has been developed as an alternative approach to traditional multiple-set (MS) strength training, in an attempt to elicit higher gains in muscle hypertrophy, strength and power. However, the magnitude of fatigue caused by different strength training methods highly depends on the arrangement and interaction of multiple determinants such as training volume, intensity, rest intervals, muscle action (concentric, eccentric, isometric), range of motion (ROM), time under tension (TUT), or volitional muscular failure, respectively, making the strength training design a complex process (6, 40). For example, gradually reducing the training load after volitional muscular fatigue with very short inter-set rest intervals within a strength training session, such as the use of Drop Sets (DS), has been shown to produce greater fatigue of motor units due to an increased TUT causing higher levels of metabolic stress and ischemia within skeletal muscle, which in turn may promote a greater hypertrophy response (23, 37). Moreover, eccentric overload (EO) training represents an opportunity to provide the muscle with a higher mechanical load (near or above the maximal concentric load) during the eccentric phase (32-34). Eccentric contractions are capable to produce greater muscle forces compared to pure isometric or concentric muscle actions and are less metabolically demanding (27). Research indicates that EO training, evoked by the use of maximal or supra-maximal loads, eccentric strength training devices (e.g., flywheel YoYo™ squat; FW) or high-intensity plyometric jump (PJ) exercises, is superior.
than traditional strength training in eliciting muscle hypertrophy and muscular strength and power gains due to a higher myofibrillar protein synthesis, a greater accumulation of anabolic hormones, and an enhanced satellite cell activation caused by severe skeletal muscle damage (27, 32-34). Additionally, these changes may provoke greater levels of fatigue followed by a prolonged recovery process.

The participation in a single high-intensity strength training session leads to a transient decline in neural activation, muscular force, and jump performance due to central and/or peripheral fatigue mechanisms (2, 10, 13, 36). Particularly, EO training protocols seem to produce large decrements in jump and strength performance lasting several days (13, 26, 36). Moreover, these protocols may even cause marked increases in serum creatine kinase (CK) activity and sensations of muscle stiffness and/or muscle pain (delayed onset muscle soreness; DOMS) with peak values usually observed within 24 to 48 hours post-exercise (13, 14, 36). However, a great number of studies analyzing short-term fatigue effects to strength exercise do not cover a broad range of strength training methods and mostly used single-joint exercises (knee extensors, elbow flexors) associated with an inappropriately high training volume (9, 10, 21, 26).

From an applied point of view, these studies cannot provide useful information for the coach or scientist regarding strength training prescription. Thus, the purpose of the present study was to analyze the extent of fatigue and recovery time among five different squat exercise protocols of realistic training volume on neuromuscular performance (CMJ, MRJ, MVIC) as well as on the response of physiological (La, CK) and perceptual (RPE, DOMS) markers.
Methods

Experimental Approach to the Problem

A randomized, counterbalanced repeated-measures design was used throughout the 8-week study period (Figure 1). All subjects attended a laboratory familiarization visit to introduce and practice the testing and training procedures, and also to ensure that any learning effect was minimal for the baseline measurements. Baseline values were collected on two discrete occasions separated by one week including counter movement jump height (CMJ), multiple rebound jump performance (MRJ) maximal voluntary isometric contraction force (MVIC) in half-squat and estimated one-repetition maximum strength (1RM_{est}) in parallel squat. In the following five-week training phase, the subjects performed one of a total of five different squat exercise protocols, which were randomized and counterbalanced to eliminate order effects.

For a given subject, each testing and training session were conducted within 1.5 - 2 hours of the same time of day, separated by one week, to minimize diurnal variations. Measurements were taken at Baseline, 30 minutes before (Pre) and immediately after the strength training session (Post 0) as well as 30 minutes (Post 0.5), 24 hours (Post 24) and 48 hours post-exercise (Post 48) including different experimental procedures: (1.) Jump and Strength Tests: CMJ, MRJ, MVIC, (2.) Blood Measures: blood lactate (La), serum creatine kinase concentration (CK), and (3.) Perceptual Measures: ratings of perceived exertion (RPE), delayed onset muscle soreness (DOMS). More precisely, CMJ, MRJ and MVIC were determined at Baseline and Post 0.5, 24 and 48. La was measured at Pre and Post 0, while RPE was taken at Post 0.

CK was assessed at Pre, Post 24 and 48, while DOMS was measured at Post 24 and 48. Within the length of this investigation, the subjects were asked to refrain from strenuous exercise for 24 hours before each strength training session to the time of 48 hours post-exercise, and also to avoid engaging in further types of vigorous sport activities. Furthermore, they were instructed to maintain their normal dietary intake and habitual lifestyle. During training and testing only water was allowed to drink ad libitum.

[Figure 1 near here]
Responses to five different squat exercise protocols

Subjects

Fifteen male resistance-trained athletes (soccer, handball, tennis) volunteered to participate in this study (mean ± SD; age: 23.1 ± 1.9 years, body mass: 77.4 ± 8.0 kg, height: 180.0 ± 10.0 cm). The subjects had to meet the following inclusion criteria: (1.) estimated one-repetition maximum (1RM\textsubscript{est}) performance in parallel squat of at least 100% of the subject’s body mass, (2.) experience in strength training of one year at least with minimum of two strength training sessions per week, and (3.) free from any cardiovascular or orthopedic disease, as judged by an medical history questionnaire. The subjects’ parallel squat performance data were: absolute 1RM\textsubscript{est} = 114 ± 16 [kg] and relative 1RM\textsubscript{est} = 1.5 ± 0.2 [1RM\textsubscript{est} [kg] · body mass [kg]\textsuperscript{-1}], respectively. In accordance with the Declaration of Helsinki, before any participation, the experimental procedures and potential risks were explained fully to the subjects, and the study was approved by the local Ethics Committee. The subjects were free to withdraw from this study without disadvantages at any time, and they all provided written informed consent.

Procedures

Body Composition. Within the first laboratory visit, anthropometrical data was collected including body mass by means of a customary weighing scale (Soehnle Professional, Germany) and body height using a customary height measure system (Seca Leicester, UK).

Counter Movement Jump Test. The CMJ were performed at baseline and at time points post-exercise (Post 0.5, 24, 48) by means of a contact platform (Haynlelektronik, Germany). During the CMJ, the subjects were asked to place the hands on the hips and to drop down to a self-selected level before jumping for maximal height. The flight time was used to calculate the jump height. At any measurement point, the subjects performed two CMJ and the mean jump height was taken for later analysis. The intra-class correlation coefficient (ICC) with 95% confidence limits (CI) and the typical error (TE) were previously calculated and considered highly reliable in CMJ [cm], n = 38: ICC (95% CI) = 0.915 (0.837 – 0.956), TE = 1.86.
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Multiple Rebound Jump Test. The MRJ were performed at baseline and at time points post-exercise (Post 0.5, 24, 48) by means of a contact platform (Haynl Elektronik, Germany). At any measurement point, the subjects performed multiple rebound jumps for maximal jump height with minimal ground contact times for a total of 15 seconds (22). During the MRJ, the subjects were instructed to place the hands on the hips and to keep their legs as straight as possible enabling powerful rebounds with mostly the ankle joint. The ground contact times and the flight times of the five best jumps based on the jump efficiency score (flight time² [s] · ground contact time [s]⁻¹) were recorded. The reactive strength index (RSI = jump height [mm] · ground contact time [ms]⁻¹) was calculated for the predetermined five best jumps (4) and the mean MRJ was taken for later analysis. Previously calculated reliability scores in MRJ [RSI], n = 38, were regarded as highly reliable: ICC (95% CI) = 0.914 (0.834 - 0.955), TE = 0.13.

Maximal Voluntary Isometric Contraction Force Test. The MVIC were performed at baseline and at time points post-exercise (Post 0.5, 24, 48). MVIC was registered by recording maximal isometric force output in Newton [N] by means of a Multitrainer 7812-000 (Kettler Profiline, Germany) and analogous user software (DigiMax Version 7.X). The subjects were directed to position under the shoulder upholstery into a shoulder bride stand with an external foot rotation of five to ten degrees. Perpendicular to the middle of the shoulder upholstery a floor marker functioned as a point of reference to stand parallel and to standardize the adjustment of the axis of the ankle joint. Subsequently, the subjects were set up into a testing position up to a knee-joint angle of 90° using a customary goniometer. Without moving explosively, but with a slow force development, they were asked to produce a maximal voluntary isometric contraction over a 3 second time interval (7). At any measurement point, the subjects performed two MVIC trials interspersed with 2 min rest, and the mean MVIC force of both attempts was taken for later analysis. Reliability scores in MVIC [N], n = 38, were previously calculated and considered highly reliable: ICC (95% CI) = 0.920 (0.845 – 0.958), TE = 124.

One Repetition Maximum Strength Test. 1RMest was performed at baseline, functioning as an individual calculation of the subject’s training loads throughout the study, by means of a Smith machine.
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(The TechnoGym Multipower, Italy). The subjects were instructed to position into a shoulder bride stand with an external foot rotation of five to ten degrees under the barbell of the smith rack machine. The barbell was placed on the trapezius muscle (pars descendens) and posterior deltoid muscle (the high-bar back squat). Perpendicular to the middle of the barbell a colored floor marker functioned as a point of reference to stand parallel and to standardize the adjustment of the axis of the ankle joint. The squat depth was determined to a parallel squat position, i.e. the knees are flexed until the inguinal fold is in a straight horizontal line with the top of the knee musculature (25). A laser imager served as an acoustic stimulus to standardize the range of motion of approximately 110-120°. 1RM<sub>est</sub> comprised the assessment of five to less than ten maximal repetitions until concentric failure following the recommendations described elsewhere (30). The test was stopped when the subjects were unable to raise the barbell with a proper technique or when the help of the supervisors were needed. If the subjects exceeded the limit of ten repetitions, the test was also stopped by the supervisors and the intensity was gradually increased. 1RM strength was estimated (1RM<sub>est</sub>) using the formula proposed by Brzycki (30) This formula has been shown to be valid in accurately predicting 1RM performance, particular for loads ranging between 7-10RM (17), with an underestimation of 1RM squat performance by an average of 5% (28). Previously calculated reliability scores in 1RM<sub>est</sub> [kg], n = 38, were regarded as highly reliable: ICC (95% CI) = 0.959 (0.921 - 0.979), TE = 5.2.

Blood Measures. Capillary whole-blood samples for La analyses were taken at Pre and immediately after cessation of the last repetition of the final set of each protocol (Post 0) from the hyperemized earlobe (Finalgon®) with 20 µl capillary tubes, hemolyzed in 1 ml microtest tubes and analyzed enzymatic amperometrically by the Biosen S_Line, Germany (EKF Diagnostic, Germany). Capillary whole-blood samples for CK analyses were collected at Pre, Post 24 and Post 48 from the hyperemized earlobe, hemolyzed in 200 µl microtest gel tubes (Kabe Labortechnik, Germany), positioned upright to clot at room temperature for 10 minutes, centrifuged and analyzed by the COBAS INTEGRA® 400 plus (Roche Diagnostics, Germany).
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Perceptual Measures. Immediately after cessation of the last repetition of the final set of each protocol (Post 0), the subjects were asked to rate their perceived exertion using the CR-10 RPE scale (8). A rating of 0 meant rest (no effort) and a rating of 10 was quantified as maximal effort. DOMS was measured Post 24 and Post 48 by means of a visual analogue scale (VAS) consisting of a 10 cm line whose endpoints were labeled by “no pain” (left) and “unbearable pain” (right). The subjects were asked to palpate their lower limbs and mark a vertical line at a point on the line that best represented their rating of soreness at the time of measurement. The score was the distance in cm from the left side of the scale to the point marked (29).

Training protocols

The squat exercise was selected as basic movement due to its similar biomechanical and neuromuscular characteristics to many athletic movement activities. Furthermore, the squat exercise can be regarded as an integral part across large areas of strength training and conditioning, and represents an overall measure of lower-body strength (38). However, squat exercise protocols comprising a wide range of strength training methods that may trigger different physical response mechanisms (increased metabolic stress, mechanical tension, or muscle damage) are poorly studied. Moreover, the respective short-term fatigue effects are not specifically clarified and are therefore of great practical relevance. Following this, five different squat exercise protocols were designed with the objective of incorporating a broad range of practically relevant strength training methods based on commonly used training prescriptions (volume, intensity) (6), taken from scientific literature. Thus, the present squat exercise protocols comprised a traditional Multiple-Sets (MS), a Drop Sets (DS), an Eccentric Overload (EO), a Flywheel YoYo™ Squat (FW), and a Plyometric Jump protocol (PJ). All training sessions were preceded by a ten minute standardized dynamic warm-up (jogging, high knee skipping and running, heel ups, lunges with trunk rotation, straight leg skipping, straight-leg deadlift walk, deep squatting, bi-lateral jumps), core stability exercises (plank, side plank and bridging), and five repetitions of the subjects’ 50% 1RMest in parallel squat. A detailed description of the squat exercise protocols is shown in Table 1.
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**Multiple Sets (MS).** A Smith machine with a guided barbell (Techno Gym Multipower, Italy) was used for training. The subjects performed parallel squats (knees are flexed until the inguinal fold is in a straight horizontal line with the top of the knee musculature) using a laser imager as an acoustic stimulus to standardize the range of motion (ROM) of approximately 110-120° (25). The MS protocol contained four sets of six repetitions at an intensity of 85% of subject’s individual 1RM$_{est}$ (18) with a cadence per repetition of two seconds in eccentric mode and an intent to move explosively in concentric mode, approximately 72 seconds of total TUT for all four sets, and three minutes rest intervals between sets.

**Drop Sets (DS).** The subjects performed parallel squats with the same machine and ROM as described for MS. The DS protocol comprised four sets of six repetitions starting at an intensity of 85% of subject’s individual 1RM$_{est}$ and a cadence per repetition of four seconds in eccentric and two seconds in concentric mode, approximately 130 – 150 seconds of total TUT for all four sets, and ten seconds rest intervals between sets (23). Immediately after termination of the first set, the load was gradually (70%, 55% and 40% 1RM$_{est}$) reduced for the next three sets. The subjects were then asked to perform another six repetitions in all of the three drop sets, unless the subjects had to stop prematurely due to volitional concentric muscle failure, which was defined as the point when the muscles involved could no longer produce force enough to sustain the given load (43). Prior to the start of this study, an own executed pilot study revealed that gradual load reductions of 15% from the initial relative load (85% 1RM$_{est}$) are most appropriate to continue with another six repetitions close to volitional concentric muscle failure.

**Eccentric Overload (EO).** The EO protocol combined concentric with eccentric overload muscle actions and the subjects performed parallel squats with the same machine and ROM as the two protocols described before. EO consisted of four sets of six repetitions at a load of 100% in eccentric mode and 70% in concentric mode of subject’s individual 1RM$_{est}$ (43) with three minutes rest intervals between sets. Each repetition was performed in a cadence of two seconds in eccentric mode, one second in isometric mode, and an intent to move explosively in concentric mode resulting in approximately 96 seconds of total TUT for all four sets. Additionally, the subjects were instructed to perform a short isometric hold of maximum
one second in a complete upright body position at the end of the concentric phase. The training weight was changed within the isometric holding phase at the end of each concentric and eccentric phase, respectively, by two experienced spotters.

_Flywheel Yo Yo™ Squat (FW)._ In FW, the subjects also performed parallel squats using a gravity-free training device, the flywheel YoYo™ squat (YoYo™ Inertial Technology, Stockholm, Sweden), equipped with a 2.7 kg flywheel with a moment inertia of 0.07 kg · m². From a starting position of approximately 60-70° of internal knee angle, the flywheel rotation was initiated through a powerful pull of a strap anchored to the flywheel shaft. Within the concentric phase of the squat movement (knee and hip extension), the strap unwinds off the flywheel shaft and force and energy is transferred to the flywheel. At the end of the concentric pulling phase at approximately 165° of internal knee angle, the strap rewinds by release of the kinetic energy of the flywheel. While attempting to resist the force produced by the pull of the rotating flywheel, which recoils the strap, the trainee then executes an eccentric muscle action. (34). The full ROM of around 95-105° was carefully controlled by an experienced supervisor. The FW protocol contained four sets of six repetitions preceded by two repetitions for initial movement acceleration due to inertial loading characteristics, approximately 96 seconds of total TUT for all four sets, and three minutes rest intervals between sets. The subjects were asked to perform each repetition with maximum-effort, accelerating and decelerating (generating quick braking forces) the flywheel with intent to move explosively during the concentric and eccentric phase, respectively (33, 34).

_Plyometric Jumps (PJ)._ The PJ protocol contained four sets of 15 plyometric jumps from a 60 cm jump box with five seconds and three minutes rest intervals between the repetitions and the sets, respectively (16). The subjects were asked to land until the knees are flexed of about 90° followed by a simultaneous explosive knee extension and arm swing for maximum jump height.

_Time under Tension (TUT)._ TUT was defined as the total time spent in which the muscles were generating force, and was measured by means of a digital chronometer (Hanhart, Germany).
Session Duration. The session duration was defined as the total training time including the standardized warm-up routine.

[Table 1 near here]
Statistical Analyses

Values are presented as mean ± SD (95% confidence intervals; 95% CI) or as mean ± 95% confidence limits (95% CL). The assumption of normality was verified by means of Shapiro-Wilks-Test, before calculating any parametric tests. Since RPE responses were measured immediately post-exercise (Post 0), a one-factor analysis of variance (ANOVA) with repeated-measures was calculated to determine differences between protocols (main effect for protocol). If ANOVA main effect was significant, Bonferroni post-hoc tests were conducted to allow multiple comparisons between protocols. A two-factor repeated-measures ANOVA was performed for La, CMJ, MRJ, MVIC, CK and DOMS to determine differences between the measurement points (main effect for time), between the protocols (main effect for protocol) and for the changeover time in response to the different protocols (time x protocol interaction). Violation of sphericity was adjusted by Greenhouse-Geisser. If significance was found, Bonferroni post-hoc analyses were performed enabling multiple comparisons between the measurement points and protocols. Paired samples t-tests were also used to detect significant differences between protocols. To allow a better interpretation of the results, effect sizes (partial eta squared, $\eta^2_{\text{partial}}$) were also calculated. Values of 0.01, 0.06 and >0.14 were considered small, medium, and large, respectively (15). The $p \leq 0.05$ criterion was used to constitute statistical significance. Data analyses were performed with SPSS statistical package (SPSS Inc., version 18, Chicago, IL, USA). We also conducted a post hoc power and sensitivity analysis with G*Power software (G*Power, version 3.1.9.2, Kiel University, Germany) in order to firstly test whether power was acceptable to detect differences, and secondly to calculate minimum effect sizes to which the applied statistical tests were at least adequately sensitive to. In all conditions, statistical power (1-$\beta$) was sufficient with 1-$\beta$ > 0.80 (> 80% probability to detect differences), and minimum effect sizes (partial $\eta^2$) ranged between 0.07 and 0.12.
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1 Results

Metabolic and perceptual demands.

The two-factor repeated-measures ANOVA revealed a significant main time effect (p<0.001, $\eta^2_{\text{partial}}=0.967$) and main protocol effect (p<0.001, $\eta^2_{\text{partial}}=0.780$) as well as significant time x protocol interaction in La levels (p<0.001, $\eta^2_{\text{partial}}=0.802$). Immediately post-exercise (Post 0), La concentrations were significantly higher (p<0.001) in FW (mean ± 95% CL; 12.2 ± 0.9 mmol·L$^{-1}$) compared to all other protocols (mean ± 95% CL; MS = 7.7 ± 1.5 mmol·L$^{-1}$, DS = 8.5 ± 0.6 mmol·L$^{-1}$, EO = 8.2 ± 1.6 mmol·L$^{-1}$, and PJ = 3.0 ± 0.8 mmol·L$^{-1}$), and significantly lower (p<0.001) in PJ relative to all other protocols (MS, DS, EO, FW). No significant differences in La values (p>0.05) were found between all protocols at Pre as well as between MS, DS and EO at Post 0 (Figure 2a). A significant main protocol effect was found in RPE responses (p <0.001, $\eta^2_{\text{partial}}=0.827$). Significantly lower (p<0.001) RPE values were observed in PJ (mean ± 95% CL; 5.1 ± 0.8) compared to all other protocols (mean ± 95% CL; MS = 8.7 ± 0.6, DS = 9.1 ± 0.5, EO = 9.3 ± 0.5, and FW = 9.9 ± 0.1), while there were found significantly higher (p<0.01) RPE levels in FW relative to MS and DS (Figure 2b).

[Insert Figure 2 near here]

Neuromuscular fatigue.

There were significant main time effects in CMJ (p<0.001, $\eta^2_{\text{partial}}=0.668$), MRJ (p<0.001, $\eta^2_{\text{partial}}=0.580$) and MVIC (p<0.001, $\eta^2_{\text{partial}}=0.462$), but no main protocol effects (p>0.05) were found in all neuromuscular performance variables (CMJ, MRJ, MVIC) analyzed (Table 2). Neuromuscular performance in CMJ and MRJ remained significantly decreased in all protocols from 0.5 to 48 h post-exercise (p<0.01), whereas there were no significant differences between baseline and 24h post-exercise as well as between baseline and 48 h post-exercise in all protocols regarding MVIC (p>0.05). There was a significant time x protocol interaction (p<0.05) in MRJ with a significant lower performance in DS, EO and FW compared to PJ (0.5 h post-exercise), and in EO compared to all other protocols (24 h post-
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Responses to five different squat exercise protocols, as shown in Table 2. The percentage changes (mean ± 95% CI) in performance relative to baseline were also calculated for CMJ, MRJ and MVIC (Figure 3a-c). All protocols caused percentage performance decreases from 0.5 to 48 h post-exercise of -10.2 ± 4.5 to -5.9 ± 4.1 % in CMJ, -13.6 ± 6.3 to -9.0 ± 5.9 % in MRJ and -11.0 ± 7.3 to -3.0 ± 7.3 % in MVIC, respectively. The highest percentage performance decrements induced EO at any time point in CMJ (-12.5 ± 4.8 to -7.5 ± 4.3%) and MRJ (-17.0 ± 8.1 to -11.5 ± 6.3%) as well as 24 and 48 h post-exercise in MVIC (-7.1 ± 7.7%).

[Insert Table 2 and Figure 3 near here]

Muscle damage and muscle soreness.

A significant main time effect with peak values 24 h post-exercise was observed in CK levels (p<0.001, \( \eta^2_{\text{partial}}=0.624 \)), but not in DOMS (p>0.05). There were no significant differences between the protocols as well as no significant time x protocol interactions in either CK levels or in DOMS (p>0.05) following 24 and 48 h after squat exercise training (Figure 4a-b).

[Insert Figure 4 near here]
Discussion

The aim of the present study was to analyze the extent of fatigue and recovery time among five different squat exercise protocols reflected by neuromuscular performance variables (CMJ, MRJ, MVIC) as well as by physiological (La, CK) and perceptual (RPE, DOMS) markers. The major findings were as follows: (1.) the protocols FW and EO induced higher metabolic and perceptual demands compared to MS, DS and PJ, (2.) neuromuscular fatigue in CMJ and MRJ was consistent up to 48 h post-exercise in all protocols, while EO caused the highest percentage performance decrements 24 h and 48 h post-exercise in all neuromuscular performance variables and produced a stronger decrease in MRJ performance until 24 h post-exercise compared to all other protocols, and (3.) CK serum concentrations peaked 24 h post-exercise in all protocols and remained elevated following 48 h after squat exercise training.

Metabolic and perceptual demands.

The present results showed significant differences in La levels between the squat exercise protocols, with FW producing the highest (> 12 mmol · L⁻¹) and PJ the lowest (< 4 mmol · L⁻¹) La concentrations as against the other protocols (~8 mmol · L⁻¹), which corresponded to the perceptual RPE responses (Figure 2a-b). Previous research in this field reported La concentrations ranging from about 3 to 15 mmol · L⁻¹ after performing different strength training methods in combination with distinct strength exercise prescriptions (1, 11, 20). Evidently, the immediate La response to strength training is highly affected by the training method (e.g., single- vs. multi-joint exercises, magnitude of the involved muscle mass, training equipment) as well as by training volume, intensity, and TUT (11, 20).

In case of FW, the crucial factor in eliciting the highest La and RPE responses may be related to TUT (Table 1) and to training method (inertial loading characteristics) resulting in a greater muscle activation (11, 34). More precisely, the inertia of the flywheel enables unrestricted high resistance forces within the total concentric and eccentric range of motion requiring a permanently high energy flow as opposed to gravity-dependent strength exercises, where the level of muscle activation is highly dictated by the efficiency of biomechanical levers and muscle lengths (33, 34).
In contrast, the lowest La and RPE values were induced by PJ and could be attributed to the overall low TUT (Table 1) during landing and take-off which is likely to result in the primary use of high-energy phosphates (ATP + PC system). A study by Cadore and colleagues analyzing different plyometric exercise volumes reported similar low La levels (10). Moreover, it is supposed that the storage of elastic energy during the eccentric phase (landing) and the subsequent energy release during the concentric muscle action (take-off) accompanied by enhanced reflex activity potentiates the muscle contractile response and consequently contributes to less metabolic demands (41).

Surprisingly, DS caused similar La concentrations and RPE responses as against MS and EO, respectively, despite an overall high TUT and the presence of very short inter-set rest periods (Table 1) assuming a higher involvement of anaerobic glycolytic energy pathways. It can be speculated that the gradual reduction of the mechanical load (from 85 to 40% 1RMest) as well as the relatively long-lasting eccentric phase (4s), requiring less metabolic demands (19), may provide an explanation for the similar La response in DS.

Neuromuscular fatigue.

The present data indicate that all squat exercise protocols similarly inhibit neuromuscular function from 0.5 to 48 h post-exercise reflected by an overall decline in vertical jump (CMJ, MRJ) and isometric strength (MVIC) performance (Table 2). It has been generally accepted that high-intensity strength and plyometric training protocols evoke short-term detrimental effects on jump and strength performance due to central and/or peripheral fatigue mechanisms (1, 2, 9, 10, 13, 24).

Regarding jump performance, neuromuscular fatigue was significantly consistent up to 48 h post-exercise in all protocols with mean considerable decreases of approximately -14 to -6% within 0.5 to 48 h of recovery (Table 2, Figure 3a-b), which is in agreement with previous studies (9, 10, 13). Based on the current data, it is concluded that in case of high-intensity squat exercise protocols and regardless of strength training method, jump performance is impaired for at least 48 h post-exercise, which needs to be taken into account when planning and prescribing strength training programs prior to competitive events.
Concerning isometric strength, significant performance decreases were found 0.5 h post-exercise in all protocols, but after 24 h of recovery there were no significant differences from respective baseline values (Table 2, Figure 3c), indicating different temporal patterns of recovery between jump (CMJ, MRJ) and isometric strength (MVIC) performance. Evidently, MVIC showed a more rapid recovery, whereas the loss in jump performance was still preserved until 48 h post-exercise (Table 2). It can be assumed that the decline in neuromuscular performance and the resulting fatigue is primarily provoked by the selective damage of the fast-twitch muscle fibers (2, 9) which in turn are markedly involved in explosive movement activities like jumps, throws and sprints. However, the time available to develop force considerably differs between the jump and strength tests. While the subjects need to generate force in minimal time during the jump tests, MVIC was recorded over a three second time interval indicating that the fast-twitch muscle fibers are not solely (higher involvement of the slow-twitch muscle fibers) the performance determining aspect in the present MVIC measurement.

EO caused the highest percentage performance decrements 24 h and 48 h post-exercise in all neuromuscular performance variables (Figure 3a-c) and produced a significantly stronger decrease in MRJ performance until 24 h post-exercise compared to all other protocols (Table 2). These data indicate that EO induced the greatest drop in overall neuromuscular performance and that MRJ performance is more strongly impaired until 24 h post-exercise. This relevant aspect could be attributed to the eccentric overload used in EO causing a higher mechanical tension on the involved muscle fibers during eccentric muscle actions resulting in greater myofibrillar disruptions (27), which in turn may induce a prolonged neuromuscular fatigue relative to all other protocols. This is of important practical relevance for coaches and scientists and need to be considered regarding strength training prescription. Besides this, MRJ performance was significantly higher in PJ compared to DS, EO and FW 0.5 h post-exercise (Table 2). The lesser detrimental effect on MRJ performance in PJ could be explained by reduced metabolic demands reflected by an overall low TUT and the primary use of elastic energy associated with enhanced reflex activity (41), which is likely to result in reduced neuromuscular fatigue.
Muscle damage and muscle soreness.

The serum concentrations of CK showed a significant main time effect with peak values observed 24 h post-exercise (Figure 4a), which is in line with previous research reporting on elevated CK levels within 12 h to 48 h post-exercise after high-intensity strength or plyometric training (5, 13). CK is generally accepted as a marker for muscle damage and highly affected by volume and intensity of the preceding exercise stimulus, particularly eccentric or unaccustomed movement activities (5). It has been proposed, that the primary cause of muscle damage is a high mechanical load rather than metabolic stress (3). As expected, MS and EO tended to cause the highest CK concentrations compared to all other protocols. This can be attributed to the constant high mechanical loads used in MS and EO resulting in greater skeletal muscle traumas which in turn cause a higher CK release from muscle tissue to the blood (19). Moreover, it has been shown that high mechanical loads in fast velocity eccentric muscle actions lead to greater levels of muscle damage than comparable slow velocity eccentric muscle actions due to the higher peak torque output in the eccentric-concentric transition phase, which results in greater mechanical stress to the active muscle fibers (12). Interestingly, DOMS tended to be higher rated in EO than MS (Figure 4b) indicating that traditional load configurations are accompanied by less sensations of muscle soreness and perceptually better tolerated rather than EO muscle actions (43).

Surprisingly, CK and DOMS responses tended to be less in FW compared to all other protocols despite its inertial loading characteristics. It is supposed that the high metabolic rate in FW may impair a proper movement execution (at least in the final reps) resulting in premature fatigue and an inability to induce an eccentric overload for short episodes in skeletal muscle (34), which in turn is associated with stronger myofibrillar disruption (greater CK release from muscle tissue to blood) and higher ratings of DOMS (27).

In conclusion, the present results indicate that acute metabolic and perceptual responses as well as neuromuscular fatigue and muscle damage are specifically depending on strength training method and the characteristics of strength training prescription. Neuromuscular fatigue reflected by an overall decline in jump performance (CMJ, MRJ) is consistent up to 48 h post-exercise. This is of great practical relevance.
for coaches and scientists when planning and prescribing lower-limb strength training programs and requires the proper management of certain program variables such as training volume, intensity, muscle action, TUT, exercise modality, and their respective impact on fatigue and recovery time.
**Methodological Limitations**

This study aimed to analyze the extent of fatigue and recovery time reflected by neuromuscular performance variables as well as by physiological and perceptual markers among five different squat exercise protocols covering a broad range of practically relevant strength training methods. The latter were derived from scientific literature and were designed as likely to cause different physical response mechanisms (metabolic stress, mechanical tension, or muscle damage) based on strength training prescriptions (e.g., training volume, intensity) in general use (6). According to this, the present study has a major limitation, as the analyzed squat exercise protocols were not volume equated characterized by different volume load configurations (repetitions [no.] x external load [kg]) and TUT. Although it has been proposed different methods to quantify and monitor volume in strength exercise, none of them appeared to be appropriate to calculate volume in the present squat exercise protocols analyzed (31). To the best of our knowledge, it seems to be impossible to quantify volume by means of an overall integrative measure when including eccentric overload and plyometric jump training protocols.

In addition, the training loads in this study were calculated from an estimated 1RM ($1\text{RM}_{\text{est}}$) rather than a true 1RM. This might affect the accuracy of load estimations in the MS, DS and EO protocols which in turn could mask the true impact on the analyzed dependent variables. Furthermore, a Smith machine was used to determine $1\text{RM}_{\text{est}}$ and also to perform the training sessions comprising the MS, DS and EO protocols. According to this, the observed effects in MS, DS and EO are solely related to machine squats and therefore, these protocol effects cannot simply be transferred to free-weight barbell squats due to varying absolute loads, various levels of muscle activation (39), and different metabolic and perceptual responses likely to be expected. This may have an impact on the extent of neuromuscular fatigue and subsequent recovery time and need to be considered by practitioners and scientists.

Moreover, the total volume in the DS protocol was partially affected because there were a few subjects that were not capable to perform the required six repetitions in the three drop sets due to premature
volitional concentric muscle failure. Therefore, total TUT was not consistent and ranged between 130 and 150 seconds.
Responses to five different squat exercise protocols 22

1 Practical Applications

The present results indicate that a single bout of squat exercise training using different strength training methods (drop sets, eccentric overload, plyometric jumps) diversely affect the acute metabolic and perceptual responses. Particularly, our data suggest that when exercise intensity was focused on training for strength, neuromuscular function is impaired from 0.5 up to 48 h post-exercise reflected by an overall decline in jump (CMJ, MRJ) and strength performance (MVIC). More precisely, MVIC is restored more rapidly than jump performance which remained reduced for 48 h post-exercise. Caution is however needed when using eccentric overload muscle actions as they are very likely to cause greater amounts of neuromuscular fatigue and muscle damage reflected by higher preserved decrements in jump performance and greater CK serum concentrations, respectively. These results are of practical relevance and need to be considered when prescribing high-intensity, lower-limb strength training programs for resistance-trained athletes. However, it should also be taken into account that when multiple lower-limb exercises per session (e.g., squats, lunges, deadlifts) are included in the training routine, which is more frequent in the practical field, the extent of neuromuscular fatigue and muscle damage might be amplified. Based on the present data, during the off- or pre-season, it is recommended to perform a maximum of two sessions per week of lower-limb EO training separated by at least two days of rest to enable adequate recovery time. During the season or competitive phase lower-limb EO training should be reduced to one session at the beginning of the week.
Responses to five different squat exercise protocols 23

Figure and Table Legends

Figure 1. Schematic representation of the study design. Legends. CMJ=counter movement jump, MRJ=multiple rebound jumps, MVIC=maximal voluntary isometric contraction, 1RM_est=estimated one-repetition maximum, La=blood lactate, CK=serum creatine kinase, RPE=ratings of perceived exertion, DOMS=delayed onset muscle soreness.

Figure 2. Effects of different squat exercise protocols on La (a) and RPE at Post 0 (b). Data are mean ± 95% CL. *significantly different to all other protocols #significantly different to MS and DS

Figure 3. Percentage changes in CMJ (a), MRJ (b) and MVIC (c) performance relative to baseline. Data are mean ± 95% CL.

Figure 4 Effects of different squat exercise protocols on CK (a) and DOMS (b). *All protocols significantly different to Pre.

Table 1. Schematic representation of the different squat exercise protocols.

Table 2. Effects of different squat exercise protocols on neuromuscular performance variables.
1 References


Responses to five different squat exercise protocols


Responses to five different squat exercise protocols


Table 1. Schematic representation of the different squat exercise protocols.

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Volume:</th>
<th>Intensity:</th>
<th>Cadence [s]:</th>
<th>Intra-set/Inter-set</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sets x Reps</td>
<td>% 1RM&lt;sub&gt;est&lt;/sub&gt;</td>
<td>ECC – ISO - CON</td>
<td>Total TUT [s]</td>
<td>Rest Intervals[s]</td>
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<tr>
<td>MS</td>
<td>4 x 6</td>
<td>85</td>
<td>2 – 0 - x</td>
<td>~72</td>
<td>-1/180</td>
</tr>
<tr>
<td>DS</td>
<td>4 x 6 (incl. 3 drop sets)</td>
<td>85 (70-55-40)</td>
<td>4 - 0 - 2</td>
<td>~130-150</td>
<td>-10</td>
</tr>
<tr>
<td>EO</td>
<td>4 x 6</td>
<td>100 ECC - 70 CON</td>
<td>2 - 1 - x</td>
<td>~96</td>
<td>-1/180</td>
</tr>
<tr>
<td>FW</td>
<td>4 x 6 (+2 Acc Reps)</td>
<td>max-effort</td>
<td>x – 0 - x</td>
<td>~96</td>
<td>-1/180</td>
</tr>
<tr>
<td>PJ</td>
<td>4 x 15</td>
<td>max-effort</td>
<td>max-explosive</td>
<td>~30</td>
<td>5/180</td>
</tr>
</tbody>
</table>

Legends. Reps=repetitions, 1RM<sub>est</sub>=estimated one-repetition maximum, TUT=time under tension, ECC=eccentric, ISO=isometric, CON=concentric, Acc=acceleration, max=maximum, x=with an intent to move explosively. MS=multiple sets, DS=drop sets, EO=eccentric overload, FW=flywheel YoYo<sup>TM</sup> squat, PJ=plyometric jumps.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Protocol</th>
<th>Post 0.5</th>
<th>Post 24</th>
<th>Post 48</th>
<th>Time</th>
<th>Time x Protocol</th>
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</thead>
<tbody>
<tr>
<td>MS</td>
<td>37.0 ± 5.2*</td>
<td>(34.2-39.9)</td>
<td>40.0 ± 4.0*</td>
<td>(37.8-42.2)</td>
<td>40.4 ± 3.7*</td>
<td>(38.3-42.4)</td>
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<tr>
<td>DS</td>
<td>39.0 ± 3.8*</td>
<td>(36.9-41.1)</td>
<td>39.7 ± 3.6*</td>
<td>(37.7-41.7)</td>
<td>40.0 ± 3.3*</td>
<td>(38.2-41.8)</td>
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<tr>
<td>CMJ [cm]</td>
<td>42.7 ± 3.9*</td>
<td>(40.5-44.9)</td>
<td>37.2 ± 3.9*</td>
<td>(35.1-39.4)</td>
<td>38.3 ± 3.1*</td>
<td>(36.6-40.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>EO</td>
<td>37.2 ± 3.9*</td>
<td>(35.1-39.4)</td>
<td>38.3 ± 3.1*</td>
<td>(36.6-40.0)</td>
<td>39.4 ± 3.7*</td>
<td>(37.3-41.4)</td>
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</tr>
<tr>
<td>FW</td>
<td>38.2 ± 2.8*</td>
<td>(36.7-39.4)</td>
<td>39.5 ± 3.7*</td>
<td>(36.6-40.0)</td>
<td>40.1 ± 2.9*</td>
<td>(37.3-41.4)</td>
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<tr>
<td>PJ</td>
<td>39.8 ± 3.6*</td>
<td>(37.8-41.9)</td>
<td>40.0 ± 3.7*</td>
<td>(37.9-42.0)</td>
<td>40.6 ± 4.1*</td>
<td>(38.7-42.9)</td>
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<tr>
<td>MRJ [RSI]</td>
<td>1.92 ± 0.26</td>
<td>(1.77-2.06)</td>
<td>1.60 ± 0.43*</td>
<td>(1.37-1.84)</td>
<td>1.60 ± 0.37* †</td>
<td>(1.40-1.81)</td>
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<tr>
<td>EO</td>
<td>1.60 ± 0.43*</td>
<td>(1.37-1.84)</td>
<td>1.60 ± 0.37*</td>
<td>(1.40-1.81)</td>
<td>1.71 ± 0.40*</td>
<td>(1.49-1.93)</td>
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<tr>
<td>FW</td>
<td>1.65 ± 0.33*</td>
<td>(1.47-1.83)</td>
<td>1.73 ± 0.37*</td>
<td>(1.53-1.94)</td>
<td>1.77 ± 0.32*</td>
<td>(1.60-1.95)</td>
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<tr>
<td>PJ</td>
<td>1.76 ± 0.36*†</td>
<td>(1.55-1.96)</td>
<td>1.76 ± 0.38*</td>
<td>(1.57-1.99)</td>
<td>1.73 ± 0.35*</td>
<td>(1.53-1.93)</td>
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<tr>
<td>MS</td>
<td>1768 ± 313*</td>
<td>(1584-1952)</td>
<td>1927 ± 317*</td>
<td>(1743-2110)</td>
<td>1950 ± 311*</td>
<td>(1771-2130)</td>
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<tr>
<td>DS</td>
<td>1683 ± 332*</td>
<td>(1492-1775)</td>
<td>1790 ± 335*</td>
<td>(1546-1983)</td>
<td>1818 ± 400*</td>
<td>(1587-2048)</td>
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<tr>
<td>MVIC [N]</td>
<td>1936 ± 313*</td>
<td>(1755-2116)</td>
<td>1697 ± 333*†</td>
<td>(1504-1884)</td>
<td>1763 ± 331*</td>
<td>(1572-1954)</td>
<td>0.001</td>
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<tr>
<td>EO</td>
<td>1697 ± 333*†</td>
<td>(1504-1884)</td>
<td>1763 ± 331*</td>
<td>(1572-1954)</td>
<td>1783 ± 315*</td>
<td>(1601-1964)</td>
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<tr>
<td>FW</td>
<td>1669 ± 313*†</td>
<td>(1488-1849)</td>
<td>1839 ± 321*</td>
<td>(1653-2024)</td>
<td>1893 ± 326*</td>
<td>(1705-2082)</td>
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<tr>
<td>PJ</td>
<td>1805 ± 374*</td>
<td>(1589-2021)</td>
<td>1894 ± 367*</td>
<td>(1682-2106)</td>
<td>1882 ± 398*</td>
<td>(1652-2112)</td>
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Data are mean ± SD (95% confidence intervals; 95% CI).
CMJ=counter movement jump, MRJ=multiple rebound jumps, RSI=reactive strength index, MVIC=maximal voluntary isometric contraction force.
*significantly different from baseline.
†significantly different to DS, EO, and FW.
‡significantly different to all other protocols.
**Timeline**

**Week 1**

**Body Composition:**
- Body Mass, Body Height;

**Squat Exercise Familiarization:**
- Barbell Parallel Squat, Flywheel YoYo Squat, Plyometric Jumps;

**Test Familiarization:**
- CMJ, MRJ, MVIC, 1RM_est;

<table>
<thead>
<tr>
<th>Protocols</th>
<th>Post 0</th>
<th>Post 0.5</th>
<th>Post 24</th>
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<tr>
<td>MS</td>
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<td>CMJ</td>
<td>CK</td>
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<tr>
<td>EO</td>
<td>MRJ</td>
<td>MVIC</td>
<td>MRJ</td>
<td>MVIC</td>
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<tr>
<td>FW</td>
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<td>CMJ</td>
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**Week 2**

<table>
<thead>
<tr>
<th>Baseline Test I:</th>
<th>Baseline Test II:</th>
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<tr>
<td>CMJ, MRJ, MVIC, 1RM_est</td>
<td>CMJ, MRJ, MVIC, 1RM_est</td>
</tr>
</tbody>
</table>

**Week 3**

**Squat Exercise Protocols**

**Multiple Sets (MS)**
- 4 x 6 Reps
- 85% 1RM_est
- 2s Ecc – x Con
- 3 min Rest Intervals

**Drop Sets (DS)**
- 1 x 6 Reps (+3 Drop Sets)
- 85% (70 - 40%) 1RM_est
- 4s Ecc – 2s Con
- 10 sec Rest Intervals

**Eccentric Overload (EO)**
- 4 x 6 Reps
- 100% Ecc–70% Con 1RM_est
- 2s Ecc – 1s Iso - x Con
- 3 min Rest Intervals

**Flywheel YoYo™ (FW)**
- 4 x 6 Reps
- max-effort
- x Ecc - 0 – x Con
- 3 min Rest Intervals

**Flyometric Jumps (PJ)**
- 4 x 15 Jumps (60cm Box)
- max-effort
- max-explosive
- 3 min Rest Intervals

**Weeks 4-8**

Conducted in a randomized but counterbalanced order

**Figure 1.** Schematic representation of the study design.

**Legends.** CMJ=counter movement jump, MRJ=multiple rebound jumps, MVIC=maximal voluntary isometric contraction, 1RM_est=estimated one-repetition maximum, La=blood lactate, CK=serum creatine kinase, RPE=ratings of perceived exertion, DOMS=delayed onset muscle soreness.

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Figure 2. Effects of different squat exercise protocols on La (a) and RPE at Post 0 (b).

Data are mean ± 95% CI.

*significantly different to all other protocols
#significantly different to MS and DS
Figure 3. Percentage changes in CMJ (a), MRJ (b) and MVIC (c) performance relative to baseline. Data are mean ± 95% CL.
Figure 4. Effects of different squat exercise protocols on CK (a) and DOMS (b).
Data are mean ± 95% CL.
*All protocols significantly different from Pre.