NEUROMUSCULAR FATIGUE AND PHYSIOLOGICAL RESPONSES AFTER FIVE DYNAMIC SQUAT EXERCISE PROTOCOLS

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ABSTRACT

Raeder, C, Wiewelhove, T, Westphal-Martinez, MP, Fernandez-Fernandez, J, de Paula Simola, RÁ, Kellmann, M, Meyer, T, Pfeiffer, M, and Ferrauti, A. Neuromuscular fatigue and physiological responses after five dynamic squat exercise protocols. *J Strength Cond Res* 30(4): 953–965, 2016—This aimed to analyze neuromuscular, physiological and perceptual responses to a single bout of 5 different dynamic squat exercise protocols. In a randomized and counterbalanced order, 15 male resistance-trained athletes (mean ± *SD*; age: 23.1 ± 1.9 years, body mass: 77.4 ± 8.0 kg) completed traditional multiple sets (MS: 4 × 6, 85% 1 repetition maximum [RM]), drop sets (DS: 1 × 6, 85% 1RM + 3 drop sets), eccentric overload (EO: 4 × 6, 70% 1RM concentric, 100% 1RM eccentric), flywheel YoYo squat (FW: 4 × 6, all-out), and a plyometric jump protocol (PJ: 4 × 15, all-out). Blood lactate (La), ratings of perceived exertion (RPE), counter movement jump height (CMJ), multiple rebound jump (MRJ) performance, maximal voluntary isometric contraction force, serum creatine kinase (CK) and delayed onset muscle soreness were measured. Immediately post exercise, La was significantly ($p < 0.001$) higher in FW (mean ± 95% confidence limit; 12.2 ± 0.9 mmol·L^{-1}) and lower in PJ (3.0 ± 0.8 mmol·L^{-1}) compared with 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 hours post exercise in all protocols. There was a significant time × protocol interaction ($p \leq 0.05$) in MRJ with a significant lower performance in DS, EO, and FW compared with PJ (0.5 hours post exercise), and in EO compared with all other protocols (24 hours post exercise). A significant main time effect with peak values 24 hours post exercise was observed in CK serum concentrations ($p < 0.001$), but there was no time × protocol interaction. In conclusion, (a) metabolic and perceptual demands were higher in FW and EO compared with MS, DS and PJ, (b) neuromuscular fatigue was consistent up to 48 hours post exercise in all protocols, and (c) EO induced the greatest neuromuscular fatigue.

KEY WORDS: 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 (10–30 m), 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, and isometric), range of motion (ROM), time under tension (TUT), or volitional muscular failure, 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 because of 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 with pure isometric or concentric muscle actions and are less metabolically demanding (27). Research indicates that EO training, evoked by the use of maximal or supramaximal 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 because of higher myofibrillar protein synthesis, greater accumulation of anabolic hormones, and 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 because of central and peripheral fatigue mechanisms, respectively (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 or muscle pain (delayed onset muscle soreness; DOMS) with peak values usually observed within 24–48 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 this study was to analyze the extent of fatigue and recovery time among 5 different squat exercise protocols of realistic training volume on neuromuscular performance (counter movement jump height [CMJ], multiple rebound jump performance [MRJ], maximal voluntary isometric contraction force [MVIC]) and on the response of physiological (blood lactate [La], CK) and perceptual (ratings of perceived exertion [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 2 discrete occasions separated by 1 week including CMJ, MRJ, and MVIC in half-squat, and estimated one-repetition maximum strength ($1RM_{est}$) in parallel squat. In the following 5-week training phase, the subjects performed 1 of a total of 5 different squat exercise protocols, which were randomized and counterbalanced to eliminate order effects. For a given subject, each testing and training session was conducted within 1.5–2 hours of the same time of day, separated by 1 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: (a) *Jump and Strength Tests* CMJ, MRJ, and MVIC, (b) *Blood Measures* La, serum CK concentration, and (c) *Perceptual Measures* RPE, DOMS. More precisely, CMJ, MRJ, and MVIC were determined at baseline and post 0.5, 24, and 48 hours. Blood lactate was measured at pre and post 0, while RPE was taken at post 0. Creatine kinase was assessed at pre, post 24 and 48 hours, while DOMS was measured at post 24 and 48 hours. 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 other 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.

Subjects

Fifteen male resistance-trained athletes (soccer, handball, and tennis) volunteered to participate in this study (mean ± *SD*; age: 23.1 ± 1.9 years, age range: 20–27 years; body mass: 77.4 ± 8.0 kg, height: 180.0 ± 10.0 cm). The subjects had to meet the following inclusion criteria: (a) $1RM_{est}$ performance in parallel squat of at least 100% of the subject's body mass, (b) experience in strength training of 1 year at least with minimum of 2 strength training sessions per week, and (c) free from any cardiovascular or orthopedic disease, as judged by a medical history questionnaire. The subjects' parallel squat performance data were absolute $1RM_{est} = 114 \pm 16$ (kg) and relative $1RM_{est} = 1.5 \pm 0.2$ [$1RM_{est}$ (kg)·body mass (kg)], respectively. In accordance with the Declaration

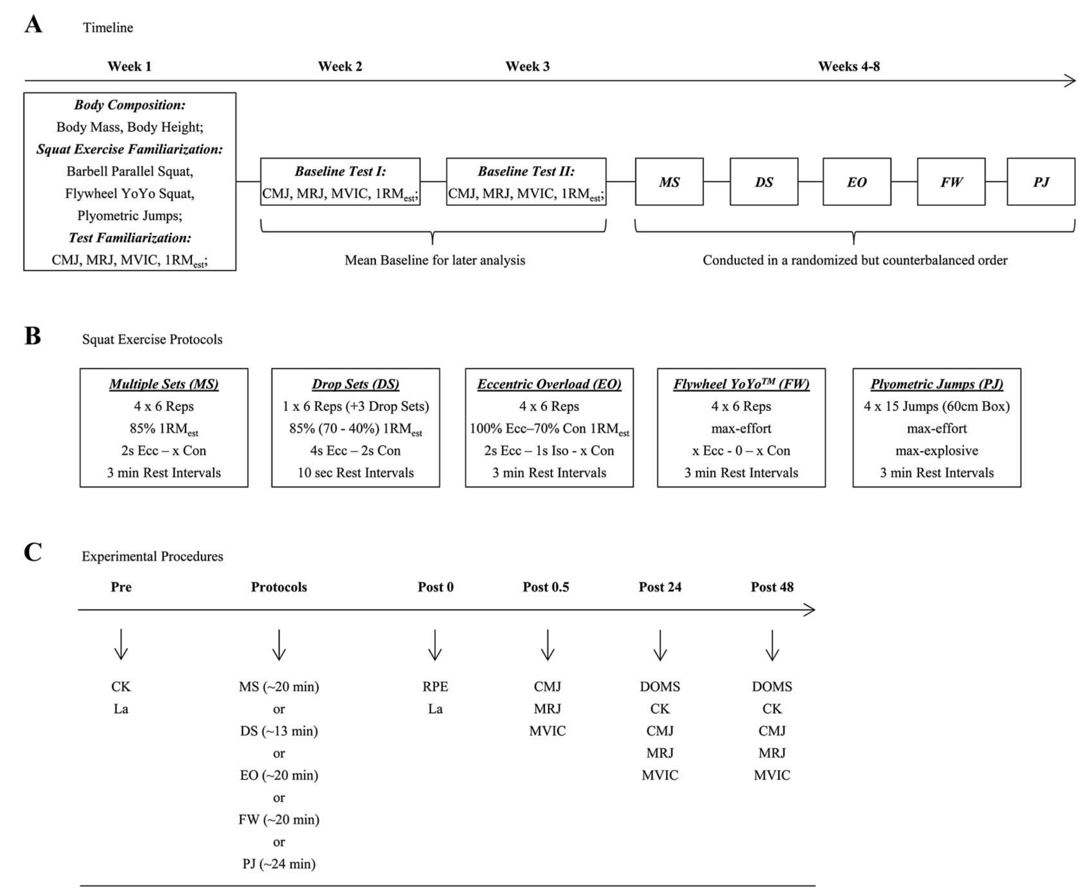

Figure 1. Schematic representation of the study design regarding timeline (A), squat exercise protocols (B), and experimental procedures (C). CMJ = counter movement jump; MRJ = multiple rebound jumps; MVIC = maximal voluntary isometric contraction; $1RM_{est}$ = estimated one-repetition maximum; La = blood lactate; CK = serum creatine kinase; RPE = ratings of perceived exertion; DOMS = delayed onset muscle soreness.

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 were collected including body mass by means of a customary weighing scale (Soehnle Professional, Backnang, Germany) and body height using a customary height measure system (Seca Leicester, Hamburg, Germany).

Counter Movement Jump Test. The CMJ tests were performed at baseline and at time points postexercise (post 0.5, 24, 48) by means of a contact platform (Sport Voss, Sportservice, Doberschütz, 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 2 CMJs and the mean jump height was taken for later analysis. The intraclass correlation coefficient (ICC) with 95% confidence interval (CI) and the typical error (TE) was previously calculated and considered highly reliable in CMJ (cm), $n = 38$: ICC (95% CI) = 0.915 (0.837–0.956), TE = 1.86.

Multiple Rebound Jump Test. The MRJ tests were performed at baseline and at time points postexercise (post 0.5, 24, 48) by means of a contact platform (Haynl Elektronik, Doberschütz, Germany). At any measurement point, the subjects performed MRJs 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 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 5 best jumps based on the jump efficiency score (flight time^2 [seconds]·ground contact time [seconds]) were recorded. The reactive strength index (RSI = jump height [mm] ground contact time [ms]) was calculated for the 5 predetermined 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).

Maximal Voluntary Isometric Contraction Force Test. The MVIC tests were performed at baseline and at time points postexercise (post 0.5, 24, 48). Maximal voluntary isometric contraction force was registered by recording maximal isometric force output in Newton (N) by means of a Multitrainer 7812-000 (Kettler Profiline, Ense-Parsit, 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 5–10°. 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 setup 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 2 MVIC trials interspersed with 2 minutes 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. Estimated one repetition maximum strength test was performed at baseline, functioning as an individual calculation of the subject's training loads throughout the study, by means of a Smith machine (TechnoGym Multipower; Cesena, Italy). The subjects were instructed to position into a shoulder bride stand with an external foot rotation of 5 to 10° 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 ROM of approximately 110–120°. Estimated one repetition maximum strength test comprised the assessment of 5 to less than 10 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. Also, if the subjects exceeded the limit of 10 repetitions, the test was stopped by the supervisors and the intensity was gradually increased. One repetition maximum strength was estimated using the formula proposed by Brzycki (30) This formula has been shown to be valid in accurately predicting 1RM performance, particularly for loads ranging between 7 and 10RM (17), with an underestimation of 1RM squat performance by an average of 5% (28). Previously calculated reliability scores in $1RM_{est}$ (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, Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim am Rhein, Germany) with 20-μl capillary tubes, hemolyzed in l-ml microtest tubes and analyzed enzymatic amperometrically by the Biosen S-Line, Germany (EKF Diagnostics, Barleben, 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, Nürnbrecht, Germany), positioned upright, to clot at room temperature for 10 minutes, centrifuged and analyzed by the COBAS INTEGRA 400 plus (Roche Diagnostics, Rotkreuz, Switzerland).

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. Delayed onset muscle soreness was measured post 24 and post 48 by means of a visual analogue scale 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 because of 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, 5 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 MS, DS, EO, FW, and a PJ protocol. All training sessions were preceded by a 10-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, and bilateral jumps), core stability exercises (plank, side plank, and bridging), and 5 repetitions of the subjects' 50% $1RM_{est}$ in parallel squat. A detailed description of the squat exercise protocols is shown in Table 1.

Multiple Sets. 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 ROM of approximately 110–120° (25).The MS protocol contained 4 sets of 6 repetitions at an intensity of 85% of subject's individual $1RM_{est}$ (18), with a cadence per repetition of 2 seconds in eccentric mode, and an intent to move explosively in concentric mode, approximately 72 seconds of total TUT for all 4 sets, and 3 minutes rest intervals between sets.

Drop Sets. The subjects performed parallel squats with the same machine and ROM as described for MS. The DS protocol comprised 4 sets of 6 repetitions starting at an intensity of 85% of subject's individual $1RM_{est}$ and a cadence per repetition of 4 seconds in eccentric and 2 seconds in concentric mode, approximately 130–150 seconds of total TUT for all 4 sets, and 10 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 3 sets. The subjects were then asked to perform another 6 repetitions in all of the 3 drop sets, unless the subjects had to stop prematurely because of volitional concentric muscle failure that was defined as the point when the muscles involved could no longer produce force, enough to sustain the given load (43). Before 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 6 repetitions close to volitional concentric muscle failure.

Eccentric Overload. The EO protocol combined concentric with eccentric overload muscle actions and the subjects performed parallel squats with the same machine and ROM as the 2 protocols described before. Eccentric overload consisted of 4 sets of 6 repetitions, at a load of 100% in eccentric mode and 70% in concentric mode of the subject's individual $1RM_{est}$ (43) with 3 minutes rest intervals between sets. Each repetition was performed in a cadence of 2 seconds in eccentric mode, 1 second in isometric mode, and an intent to move explosively in concentric mode resulting in

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

Protocol	Volume: sets × reps	Intensity: % $1RM_{est}$	Cadence (s): ecc–iso–con	Total TUT (s)	Intra-set/Inter-set rest Intervals(s)	Session duration (min)	Reference
MS	4 × 6	85	2–0–*x*	~72	–/180	~20	Drinkwater et al. (18)
DS	4 × 6 (incl. 3 drop sets)	85 (70–55–40)	4–0–2	~130–150	–/10	~13	Goto et al. (23)
EO	4 × 6	100 ECC–70 CON	2–1–*x*	~96	–/180	~20	Yarrow et al. (40)
FW	4 × 6 (+2 Acc Reps)	max-effort	*x*–0–*x*	~96	–/180	~20	Norrbrand et al. (31)
PJ	4 × 15	max-effort	max-explosive	~30	5/180	~24	de Villarreal et al. (17)

*Reps = repetitions; $1RM_{est}$ = estimated one-repetition maximum; ECC = eccentric; ISO = isometric; CON = concentric; TUT = time under tension; MS = multiple sets; DS = drop sets; EO = eccentric overload; FW = flywheel YoYo squat; PJ = plyometric jumps; Acc = acceleration; max = maximum; *x* = with an intent to move explosively.

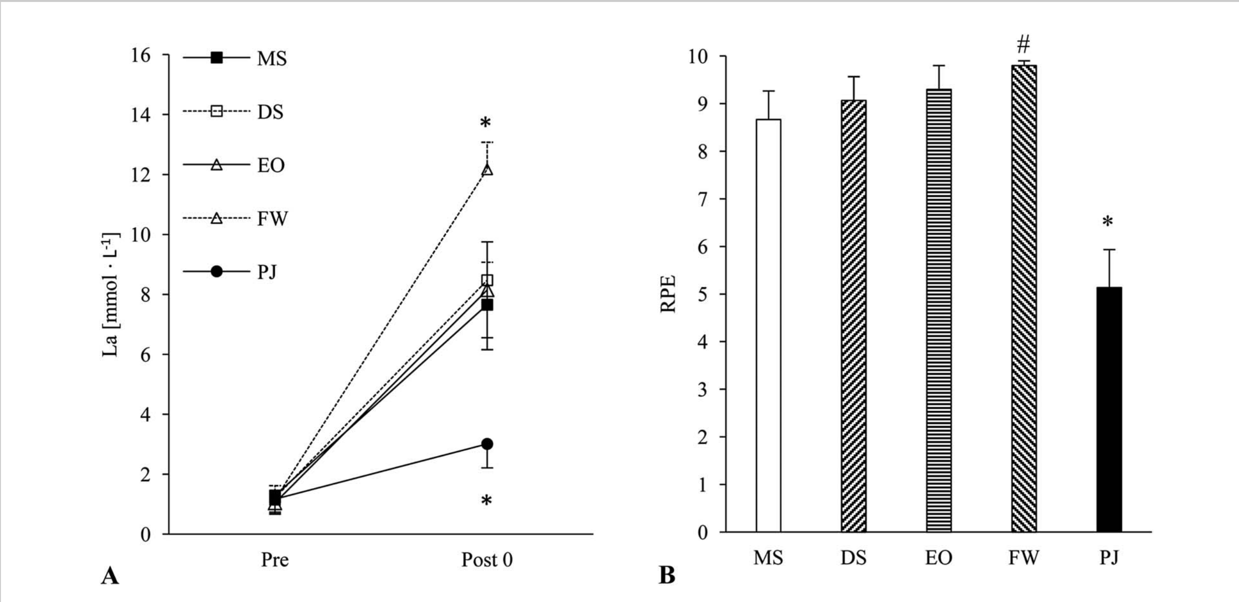

Figure 2. Effects of different squat exercise protocols on blood lactate La (A) and ratings of perceived exertion RPE at post 0 (B). Data are mean ± 95% CL. *significantly different to all other protocols. #significantly different to MS and DS. MS = multiple sets; DS = drop sets; EO = eccentric overload; FW = flywheel YoYo™ squat; PJ = plyometric jumps.

approximately 96 seconds of total TUT for all 4 sets. Additionally, the subjects were instructed to perform a short isometric hold of maximum 1 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 2 experienced spotters.

Flywheel Yo Yo Squat. 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 of inertia of 0.07 $kg \cdot m^{-2}$. 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 4 sets of 6 repetitions preceded by 2 repetitions for initial movement acceleration due to inertial loading characteristics, approximately 96 seconds of total TUT for all 4 sets, and 3 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. The PJ protocol contained 4 sets of 15 plyometric jumps from a 60-cm jump box with 5 seconds and 3 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. Time under tension was defined as the total time spent in which the muscles were generating force, and was measured by means of a digital chronometer (Hanhart, Gütenbach, Germany).

Session Duration. The session duration was defined as the total training time including the standardized warm-up routine.

Statistical Analyses

Values are presented as mean ± *SD* (95% CI) or as mean ± 95% confidence limit (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 postexercise (post 0), a one-factor analysis of variance (ANOVA) with repeated-measures

TABLE 2. Effects of different squat exercise protocols on neuromuscular performance variables.*†

Variable	Baseline		Protocol	Post 0.5		Post 24		Post 48		Time		Time × protocol	
										p	$\eta^2_{partial}$	p	$\eta^2_{partial}$
CMJ (cm)	42.7 ± 3.9	40.5–44.9	MS	37.0 ± 5.2‡	34.2–39.9	40.0 ± 4.0‡	37.8–42.2	40.4 ± 3.7‡	38.3–42.4	0.001	0.668	0.206	0.103
			DS	39.0 ± 3.8‡	36.9–41.1	39.7 ± 3.6‡	37.7–41.7	40.0 ± 3.3‡	38.2–41.8				
			EO	37.2 ± 3.9‡	35.1–39.4	38.3 ± 3.1‡	36.6–40.0	39.4 ± 3.7‡	37.3–41.4				
			FW	38.2 ± 2.8‡	36.7–39.4	39.5 ± 3.7‡	36.6–40.0	40.1 ± 2.9‡	37.3–41.4				
			PJ	39.8 ± 3.6‡	37.8–41.9	40.0 ± 3.7‡	37.9–42.0	40.6 ± 4.1‡	38.7–42.9				
MRJ (RSI)	1.92 ± 0.26	1.77–2.06	MS	1.67 ± 0.40‡	1.45–1.89	1.77 ± 0.38‡	1.56–1.98	1.77 ± 0.41‡	1.53–1.99	0.001	0.580	0.023	0.137
			DS	1.64 ± 0.35‡	1.45–1.84	1.74 ± 0.37‡	1.53–1.94	1.77 ± 0.31‡	1.60–1.94				
			EO	1.60 ± 0.43‡	1.37–1.84	1.60 ± 0.37‡§	1.40–1.81	1.71 ± 0.40‡	1.49–1.93				
			FW	1.65 ± 0.33‡	1.47–1.83	1.73 ± 0.37‡	1.53–1.94	1.77 ± 0.32‡	1.60–1.95				
			PJ	1.76 ± 0.36§‖	1.55–1.96	1.78 ± 0.38‡	1.57–1.99	1.73 ± 0.35‡	1.53–1.93				
MVIC (N)	1,936 ± 313	1,755–2,116	MS	1,768 ± 319‡	1,584–1,952	1,927 ± 317	1,743–2,110	1,950 ± 311	1,771–2,130	0.001	0.462	0.159	0.113
			DS	1,683 ± 332‡	1,492–1,875	1,790 ± 335	1,546–1,983	1,818 ± 400	1,587–2,048				
			EO	1,697 ± 333‡	1,504–1,884	1,763 ± 331	1,572–1,954	1,783 ± 315	1,601–1,964				
			FW	1,669 ± 313‡	1,488–1,849	1,839 ± 321	1,653–2,024	1,893 ± 326	1,705–2,082				
			PJ	1,805 ± 374‡	1,589–2,021	1,894 ± 367	1,682–2,106	1,882 ± 398	1,652–2,112				

*CMJ = counter movement jump; MS = multiple sets; DS = drop sets; EO = eccentric overload; FW = flywheel YoYo squat; PJ = plyometric jumps; MRJ = multiple rebound jumps; RSI = reactive strength index; MVIC = maximal voluntary isometric contraction force.

†Data are mean ± *SD* (95% confidence interval; 95% CI).

‡significantly different from baseline.

§significantly different to all other protocols.

‖significantly different to DS, EO, and FW.

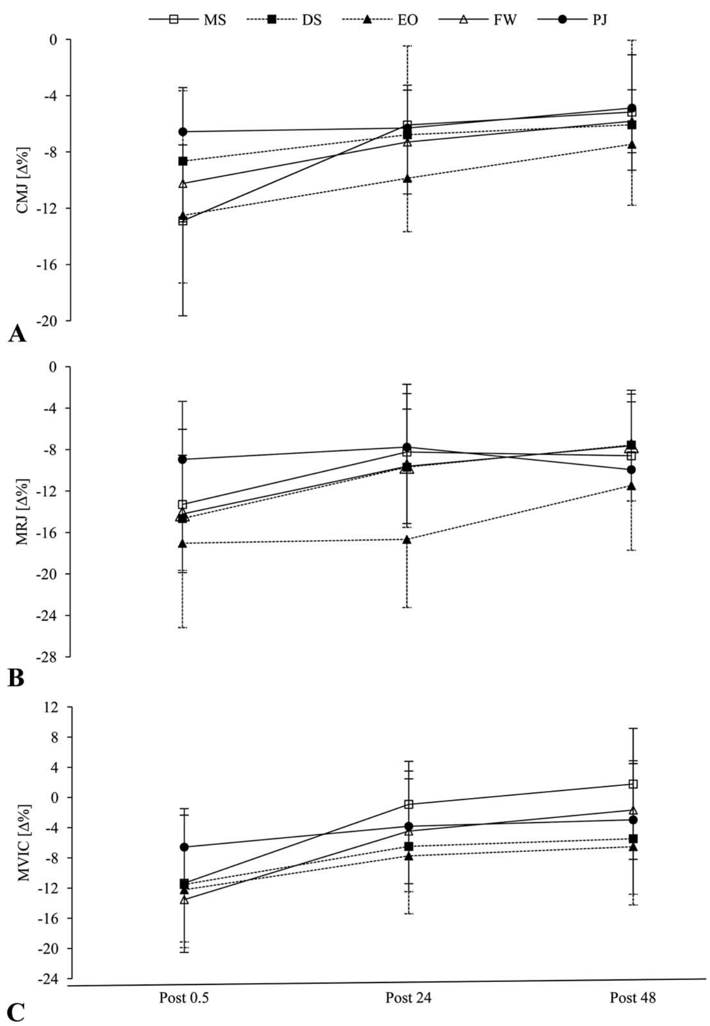

Figure 3. Percentage changes in CMJ (A), MRJ (B), and MVIC (C) performance relative to baseline data are mean ± 95% CL. MS = multiple sets; DS = drop sets; EO = eccentric overload; FW = flywheel YoYo squat; PJ = plyometric jump; CMJ = counter movement jump; MRJ = multiple rebound jumps; MVIC = maximal voluntary isometric contraction.

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 2-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 × 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, $\eta^2_{partial}$) 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) to first test whether power was acceptable to detect differences, and second to calculate minimum effect sizes to which the applied statistical tests were at least adequately sensitive to. In all conditions, statistical power (1-β) was sufficient with 1-β > 0.80 (>80% probability to detect differences), and minimum effect sizes (partial η^2) ranged between 0.07 and 0.12.

Results

Metabolic and Perceptual Demands

The 2-factor repeated-measures ANOVA revealed a significant main time effect ($p < 0.001$, $\eta^2_{partial} = 0.967$) and main protocol effect ($p < 0.001$, $\eta^2_{partial} = 0.780$) as well as significant time x protocol interaction in La levels ($p < 0.001$, $\eta^2_{partial} = 0.802$). 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 with 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, and 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 ($p < 0.001$, $\eta^2_{partial} = 0.827$). Significantly lower ($p < 0.001$) RPE values were

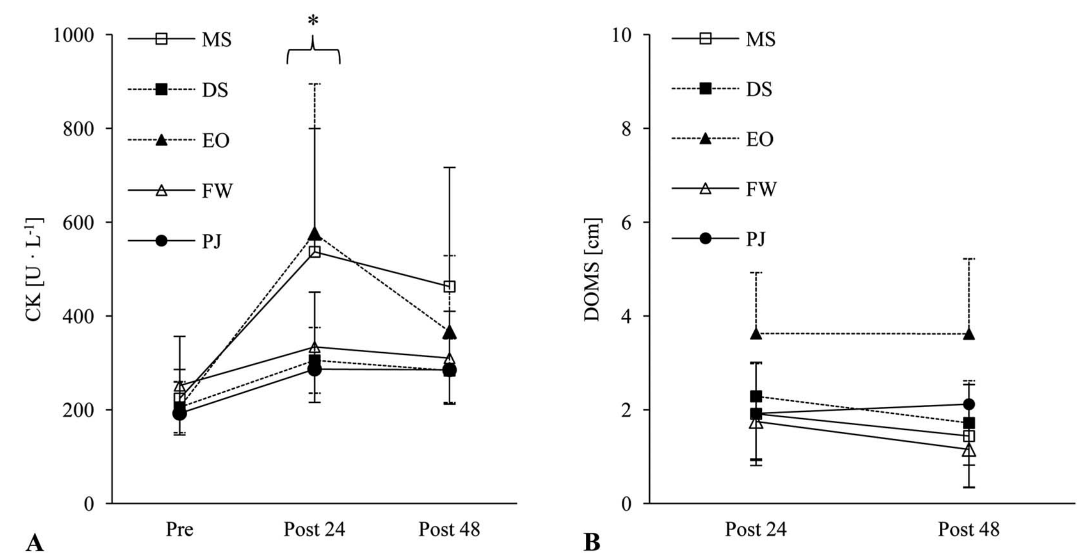

Figure 4. Effects of different squat exercise protocols on CK (A) and DOMS (B). *All protocols significantly different to pre. MS = multiple sets; DS = drop sets; EO = eccentric overload; FW = flywheel YoYo squat; PJ = plyometric jump; CK = creatine kinase; DOMS = delayed onset muscle soreness.

observed in PJ (mean ± 95% CL; 5.1 ± 0.8) compared with 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 significantly higher ($p < 0.01$) RPE levels in FW relative to MS and DS (Figure 2B).

Neuromuscular Fatigue

There were significant main time effects in CMJ ($p < 0.001$, $\eta^2_{partial} = 0.668$), MRJ ($p < 0.001$, $\eta^2_{partial} = 0.580$), and MVIC ($p < 0.001$, $\eta^2_{partial} = 0.462$), but no main protocol effects ($p > 0.05$) were found in all neuromuscular performance variables (CMJ, MRJ, and MVIC) that were analyzed (Table 2). Neuromuscular performance in CMJ and MRJ remained significantly decreased in all protocols from 0.5 to 48 hours post exercise ($p < 0.01$), whereas there were no significant differences between baseline and 24 hours post exercise as well as between baseline and 48 hours post exercise in all protocols regarding MVIC ($p > 0.05$). There was a significant time x protocol interaction ($p \leq 0.05$) in MRJ with a significant lower performance in DS, EO, and FW compared with PJ (0.5 hours post exercise), and in EO compared with all other protocols (24 hours post exercise), as shown in Table 2. The percentage changes (mean ± 95% CL) in performance relative to baseline were also calculated for CMJ, MRJ, and MVIC (Figures 3A–C). All protocols caused percentage performance decreases 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, from 0.5 to 48 hours post exercise. 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 hours post exercise in MVIC (−7.1 ± 7.7%).

Muscle Damage and Muscle Soreness

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

Discussion

The aim of this study was to analyze the extent of fatigue and recovery time among 5 different squat exercise protocols reflected by neuromuscular performance variables (CMJ, MRJ, and MVIC) as well as by physiological (La, CK) and perceptual (RPE, DOMS) markers. The major findings were as follows: (a) the protocols FW and EO induced higher metabolic and perceptual demands compared with MS, DS and PJ, (b) neuromuscular fatigue in CMJ and MRJ was consistent up to 48 hours post exercise in all protocols, while EO caused the highest percentage performance decrements 24 hours and 48 hours post exercise in all neuromuscular performance variables, and produced a stronger decrease in MRJ performance until 24 hours post exercise compared with all other protocols, and (c) CK serum concentrations peaked 24 hours post exercise in all protocols and remained elevated following 48 hours after squat exercise training.

Regarding metabolic and perceptual demands, the present results showed significant differences in La levels between the squat exercise protocols, with FW producing the highest (>12 $mmol \cdot L^{-1}$) and PJ the lowest (<4 $mmol \cdot L^{-1}$) La concentrations as against the other protocols (~8 $mmol \cdot L^{-1}$), which corresponded to the perceptual RPE responses (Figures 2A,B). Previous research in this field reported La concentrations ranging from about 3 to 15 $mmol \cdot L^{-1}$ 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, and training equipment) and 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 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 et al. (10) analyzing different plyometric exercise volumes reported similar low La levels. 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% $1RM_{est}$) and the relatively long-lasting eccentric phase (4s), requiring less metabolic demands (19), may provide an explanation for the similar La response in DS.

According to neuromuscular fatigue, the present data indicate that all squat exercise protocols similarly inhibit neuromuscular function from 0.5 to 48 hours 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 because of central and peripheral fatigue mechanisms (1,2,9,10,13,24).

Regarding jump performance, neuromuscular fatigue was significantly consistent up to 48 hours post exercise in all protocols with mean considerable decreases of approximately −14 to −6% within 0.5–48 hours of recovery (Table 2, Figures 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 hours post exercise, which needs to be taken into account when planning and prescribing strength training programs before competitive events. Concerning isometric strength, significant performance decreases were found 0.5 hours post exercise in all protocols, but after 24 hours 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 hours 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. Whereas the subjects need to generate force in minimal time during the jump tests, MVIC was recorded over a 3-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 hours and 48 hours post exercise in all neuromuscular performance variables (Figures 3A–C) and produced a significantly stronger decrease in MRJ performance until 24 hours post exercise compared with 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 hours 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 needs to be considered regarding strength training prescription. Besides this, MRJ performance was significantly higher in PJ compared with DS, EO, and FW 0.5 hours 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.

The serum concentrations of CK showed a significant main time effect with peak values observed 24 hours post exercise (Figure 4A), which is in line with previous research reporting on elevated CK levels within 12 hours–48 hours post exercise after high-intensity strength or plyometric training (5,13). Creatine kinase 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 with 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 because of 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 with 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 repetitions) 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 hours 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.

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 5 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.] × external load [kg]) and TUT. Although different methods have been proposed 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 ($1RM_{est}$) 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 $1RM_{est}$ 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 because of 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 who were not capable to perform the required 6 repetitions in the 3 drop sets because of premature volitional concentric muscle failure. Therefore, total TUT was not consistent and ranged between 130 and 150 seconds.

Practical Applications

The present results indicate that a single bout of squat exercise training using different strength training methods (drop sets, eccentric overload, and 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 hours 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 hours post exercise. Caution is however needed when using eccentric overload muscle actions because 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, and 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 2 sessions per week of lower-limb EO training separated by at least 2 days of rest to enable adequate recovery time. During the season or competitive phase lower-limb EO training should be reduced to 1 session at the beginning of the week.

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