

Simultaneous potentiation and fatigue in quadriceps after a 60-second maximal voluntary isometric contraction

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GRANGE, ROBERT W., AND MICHAEL E. HOUSTON. *Simultaneous potentiation and fatigue in quadriceps after a 60-second maximal voluntary isometric contraction*. *J. Appl. Physiol.* 70(2): 726–731, 1991.—Potential mechanisms of fatigue (metabolic factors) and potentiation (phosphate incorporation by myosin phosphorylatable light chains) were investigated during recovery from a 60-s maximal voluntary isometric contraction (MVC) in the quadriceps muscle of 12 subjects. On separate days before and for 2 h after the 60-s MVC, either a 1-s MVC or electrically stimulated contractions were used as indexes to test muscle performance. Torque at the end of the 60-s MVC was 57% of the initial level, whereas torques from a 1-s MVC and 50-Hz stimulation were most depressed in the immediate recovery period. At this time, muscle biopsy analyses revealed significant decreases in ATP and phosphocreatine and a 19-fold increase in muscle lactate. Conversely, isometric twitch torque and torque from a 10-Hz stimulus were the least depressed of six contractile indexes and demonstrated potentiation of 25 and 34%, respectively, by 4 min of recovery ($P < 0.05$). At this time, muscle lactate concentration was still 16 times greater than at rest. An increased phosphate content of the myosin phosphorylatable light chains ($P < 0.05$) was also evident both immediately and 4 min after the 60-s MVC. We conclude that the 60-s MVC produced marked force decreases likely due to metabolic displacement, while the limited decline in the twitch and 10-Hz torques and their significant potentiation suggested that myosin phosphorylation may provide a mechanism to enhance contractile force under conditions of submaximal activation during fatigue.

human muscle; muscle metabolism; myosin phosphorylation; electrical stimulation

Ref. 20). During the period after fatiguing muscle activity, various measurement protocols are employed to characterize the recovery profile of contractile function. For example, Edwards et al. (9) employed a ratio of the tension responses at 20 and 50 Hz to demonstrate that recovery of force at low frequencies of stimulation lags considerably behind recovery at higher frequencies, a phenomenon they described as low-frequency fatigue. In contrast, Miller et al. (24) observed that the force of an MVC reduced to 10% of its control value recovered within 20 min after a fatigue protocol. Confounding interpretation of fatigue mechanisms, however, would be the presence of an opposing tension modulator active during recovery.

Recent studies in human (1, 10, 11) and rat (31) models have shown that the isometric twitch force not only recovers but is potentiated in the period after fatiguing contractions. Studies have shown a correlation between isometric twitch potentiation after a tetanus or during continuous low-frequency contractions and the extent of phosphate incorporation into the phosphorylatable or P-light chains of myosin in fast-twitch and mixed fast-twitch muscles of small animals (17, 21, 25, 26, 29). On the basis of these studies, it seems possible that phosphorylation of myosin P-light chains could provide a mechanism that enhances tension in fatigued muscle.

In this study, we have used a 60-s MVC of the quadriceps muscle group to investigate the relationship between metabolic factors that could contribute to diminished muscle force output and myosin phosphorylation that could lead to force enhancement during recovery. Because skinned fiber studies reveal that myosin phosphorylation potentiates force output at moderate but not saturating calcium levels (22, 30), we have tested muscle force recovery after fatigue with maximal voluntary and electrically stimulated twitch and tetanic contractions at a variety of frequencies.

METHODS

Subjects. Twelve subjects (6 males and 6 females) aged 21 ± 2 (SD) yr volunteered to participate in this study to investigate indexes of voluntary and electrically induced torque output, metabolic response, and myosin P-light chain phosphorylation in the quadriceps after a 60-s MVC. The nature and risks of the experimental procedures were explained to the subjects, and their voluntary written consent was obtained under the guidelines established by the University of Waterloo Committee for Human Research.

IT HAS BEEN PROPOSED by Krarup (18) that the net force output of a muscle after a tetanic contraction or during repetitive low-frequency stimulation is the result of two opposing processes; one of these enhances force while the other diminishes force. On the basis of recent skinned fiber models, it seems likely that increases in the concentrations of protons and inorganic phosphate (P_i) (6, 7, 23) could account for isometric force reduction, whereas phosphorylation of myosin (22, 30, 37) could account for force potentiation in fatigued muscle. Our purpose was to study the relative effect of fatigue and potentiation in human quadriceps by testing a range of contractile activation levels during recovery from a 60-s maximal voluntary isometric contraction (MVC).

During a sustained maximal voluntary contraction, muscle force declines, but the exact cause of the force decrease is controversial and may involve a functional decrement at a number of physiological sites (reviewed in

General protocol. Over the course of this study, each subject performed seven MVCs for 60 s, one for each of six performance indexes and one for a session when muscle biopsies were performed. Before the study began, the subjects underwent a number of practice trials in which they performed prolonged MVC for up to 60 s. During the practice sessions, electrically stimulated trials were undertaken to determine the voltage necessary to elicit maximum twitch tension as well as the voltage needed to produce 50% of the force of an MVC at 50 Hz.

The actual experiment consisted of seven laboratory sessions, conducted on separate days with at least 2 days of rest between experimental trials. Each day consisted of a single 60-s MVC of the right quadriceps muscle group at a knee angle of 90°. Before and at various times after the 60-s MVC, only one of six indexes of muscle performance was tested. The six indexes investigated were a 1-s MVC; a twitch; stimulation frequencies of 10, 20, or 50 Hz; and a final index in which a subject performed a 1-s MVC followed 5 s later by 10-Hz stimulation. The performance indexes were presented in random order. On the last testing day, four muscle biopsy samples were obtained from the right vastus lateralis muscle with the subject in a seated position. Muscle samples were obtained using the needle technique (2) at the following times: at rest before the 60-s MVC, immediately after, and at 4 and 10 min after the 60-s MVC.

Experimental procedures. During the experiment, each subject was seated in a straight-backed padded chair with a restraining belt around the waist, and the tested quadriceps was further stabilized by a Velcro strap. Isometric force (newtons) was measured with a calibrated linear variable differential transformer (LVDT; Schaevitz model FTA-1U-500) affixed to a cuff placed around the subject's lower right leg just above the malleolus. The LVDT contains a mechanical elastic element that produces a small linear deflection when loaded, generating a voltage proportional to the axial load. The signal from the LVDT was amplified by a Daytronic 3230 carrier amplifier and was displayed on a Hewlett-Packard 7402A two-channel pen recorder. Each subject's moment arm length (meters) from the knee's axis of rotation to the level of the tension transducer was kept constant throughout all trials.

MVCs were performed on verbal instruction. Electrical stimulation of the right knee extensor muscles with 50- μ s square pulses was provided by a Grass S-48 stimulator via leads connected to two cloth-covered brass electrodes (7.5 \times 11.5 cm) strapped proximally and distally on the anterolateral surface of the thigh. The cloth was soaked in physiological saline and coated with electrode paste. For each subject, a supramaximal voltage (130–150 V) elicited maximal twitch tension, and a voltage that yielded 50% MVC at a frequency of 50 Hz evoked all frequency responses (33–50 V). Supramaximal voltage was equal to the voltage that elicited maximum tension plus an additional 10 V. Stimulation duration for each frequency index was 1 s, except for 20 Hz (0.2 s).

For a typical testing day, each subject was seated in the testing chair, and the stimulation electrodes were attached, if needed. After 10 min of rest, a control measure for the performance index of that day was made, followed

by another 2 min of rest. Subjects then performed a 60-s MVC, after which the performance index of the fatigued quadriceps was measured during recovery at the following times: 10, 20, 30, 40, and 60 s and 2, 3, 6, 8, 10, 15, 20, 30, 40, 50, 90, and 120 min. During the 60-s MVC, initial tension and the tensions at 30 and 60 s were recorded. A force record in newtons was thus obtained for each subject for each index and its associated MVC. Torque was determined by multiplying a subject's moment arm length in meters by the index tension value in newtons. In addition, relative torque output for a given index was determined by dividing the torque values recorded subsequent to the 60-s MVC by the control torque.

Muscle biopsy samples were obtained at times previously indicated through incisions made in the right vastus lateralis muscle. All biopsy samples were rapidly plunged into liquid nitrogen and then stored at -80°C for subsequent analysis of tissue metabolites and myosin P-light chain phosphate content. The time required to excise the biopsy needle and then freeze it averaged 3 s.

Muscle tissue analysis. For analysis of selected tissue metabolites, a portion of the frozen tissue (5–10 mg) was first lyophilized, then extracted with 0.5 M perchloric acid, neutralized with potassium carbonate (1.1 M), and centrifuged. Concentrations of lactate, ATP, and phosphocreatine (PCr) in the supernatant were determined by the procedures of Harris et al. (13) and the techniques for fluorometric analysis of Lowry and Passonneau (19).

The remaining frozen tissue was used for the determination of myosin P-light chain phosphate content by the procedures of Silver and Stull (33) and Moore and Stull (26) as modified by Stuart et al. (34). Briefly, 5- to 10-mg portions of the frozen muscle were homogenized and diluted and then subjected to pyrophosphate polyacrylamide gel electrophoresis (26) to isolate the myosin. By the use of a Hoefer SE 600 vertical slab unit, resultant myosin gel slices were then subjected to isoelectric focusing in a gel with pH range of 4.5–6.0. Gels were fixed and silver stained (28) and then scanned with an LKB 2202 Ultrosan laser densitometer with attached LKB 2220 recording integrator to establish the relative proportions of the phosphorylated and unphosphorylated P-light chains. Details of these procedures and results from isoelectric focusing of human myosin, including the positions for the unphosphorylated fast (LC2f) and slow (LC2s and LC2s') light chains and their phosphorylated forms (LC2f-P, LC2s-P, and LC2s'-P, respectively) have been previously reported (15, 34).

Statistics. Data analysis was conducted by the use of an analysis of variance (ANOVA) with a repeated-measures design. The Student-Newman-Keuls test was used to determine differences between means for significant main effects. $P < 0.05$ was used to test significance.

RESULTS

Mean values for initial torque and torque at 30 and 60 s of the 60-s MVC were determined for the 12 subjects for each testing condition. Because the mean values did not differ by $>10\%$ for any condition at the three recorded times ($P > 0.05$), the data were pooled. The initial torque, which averaged 233 ± 54 (SD) $\text{N} \cdot \text{m}$ ($n = 84$), declined to

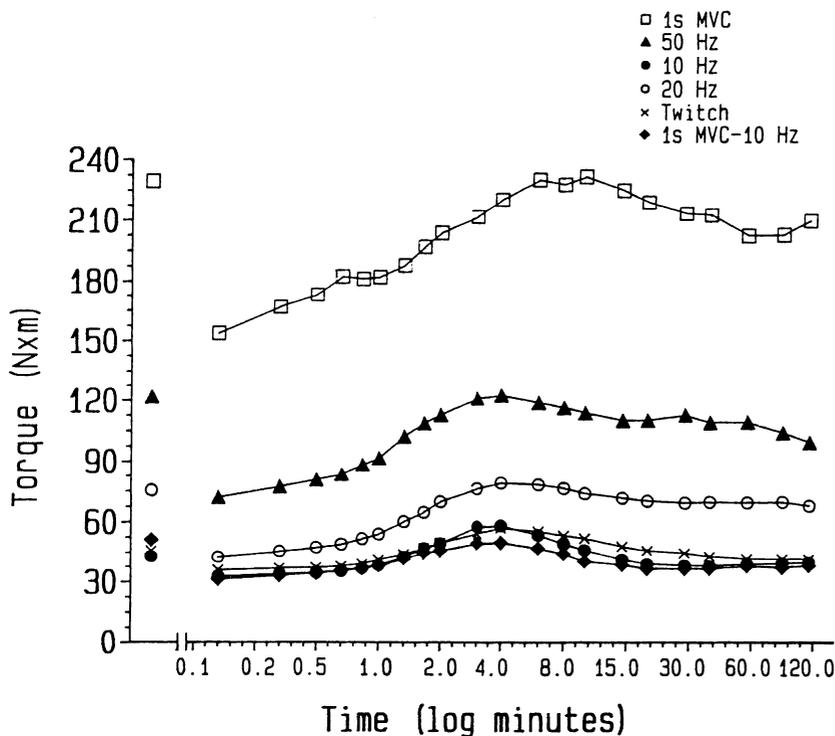


FIG. 1. Voluntary and electrically stimulated muscle torques measured on separate days before and after a 60-s maximal voluntary isometric contraction (MVC). 1s MVC-10 Hz, torque produced by a 10-Hz stimulus after a 1-s MVC.

174 \pm 41 N \cdot m at 30 s and decreased to 101 \pm 24 N \cdot m at the end of the 60-s MVC. The mean percent decrease in torque over the 60-s MVC was 57%.

Absolute torque values for the six performance indexes before and for up to 2 h after the 60-s MVC are shown in Fig. 1. There were significant differences in absolute torque among the six indexes, with the 1-s MVC and 50-Hz torques being consistently greater than the others at all measurement times ($P < 0.05$). Torque at 20 Hz was significantly greater than twitch torque, torque at 10 Hz, or torque at 10 Hz after the 1-s MVC except for the first 100 s of recovery.

When torque values for the six indexes after the 60-s MVC were expressed relative to their resting levels, significant depressions were noted for all indexes for the first 60 s of recovery (Fig. 2). Over the next 5 min of recovery, there were two general responses. Twitch torque and the torque generated by a 10-Hz stimulus train increased more rapidly and demonstrated significant potentiation between 3 and 6 min of recovery, reaching peak values of 125 and 134% of rest values, respectively. None of the other indexes demonstrated potentiation during the recovery period, although these all recovered to pre-MVC levels by 3–4 min. During the later stages of recovery, torque values for the twitch, the 1-s MVC, 10-Hz, and the 10-Hz stimulus that followed a 1-s MVC were significantly depressed. Indeed, the 10-Hz torque that followed the 1-s MVC declined to a value of 75% of the initial level ($P < 0.05$).

Concentrations of ATP, PCr, and lactate in muscle biopsy samples obtained before and after the 60-s MVC are shown in Table 1. In biopsy samples obtained immediately after the MVC, ATP and PCr were decreased by 10 and 81%, respectively, whereas lactate concentration was elevated 19-fold ($P < 0.05$). Four minutes later, ATP concentration was similar to that at rest, whereas PCr

was still 22% less than at rest ($P < 0.05$) and lactate was 16-fold higher than at rest. After 10 min of recovery, the data demonstrate that lactate concentration was still elevated, but ATP and PCr values were similar to those at rest.¹

The phosphate content of the fast and two slow myosin P-light chains before and at three time points after the 60-s MVC are reported in Table 2. It should be noted that the fast P-light chain phosphate level at rest is three times greater in human muscle than in muscle of small mammals (25, 26). Additionally, in humans, both the fast and slow P-light chains incorporate phosphate equally, in contrast to the predominant phosphate incorporation by small mammalian fast P-light chains. This difference has been attributed to the similar myosin light chain kinase (MLCK) and myosin light chain phosphatase (MLCP) enzyme activities of human mixed muscle (15, 34), whereas, in small mammals, the activity of MLCK is greater and MLCP lower in fast compared with slow muscle fibers, respectively (26). Despite these differences, all three light chains demonstrated a significantly greater phosphate content immediately after and 4 min after the 60-s MVC compared with the rest values. The values reported at 10 min of recovery suggest that the phosphate content of the three light chains was decreasing.¹

DISCUSSION

Metabolic factors have been strongly implicated in the depression of muscle force in sustained isometric contractions (reviewed in Ref. 20). The large increase in lactate and decrease in PCr concentrations we observed should be indicative of a low muscle pH and large increase in P_i

¹ Because of missing data at the final muscle sampling time, ANOVA was not performed on these data.

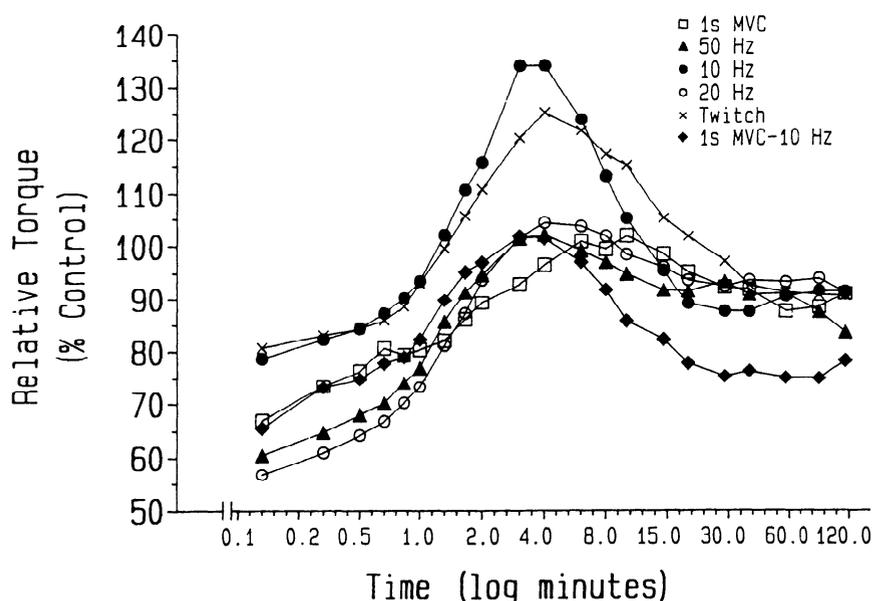


FIG. 2. Relative voluntary and electrically stimulated muscle torques after a 60-s MVC expressed relative to respective torque before 60-s MVC.

concentration on the basis of data from human muscle at fatigue, obtained by the use of ^{31}P nuclear magnetic resonance (40) or chemical analysis of biopsy samples (32). Skinned fiber studies reveal that increases in both hydrogen ions and P_i can lead to marked reductions in isometric force output (6, 7, 23). Therefore the observed metabolic changes could effectively contribute to the marked decline in torque over the 60-s MVC and the reduced torque of the isometric performance indexes early in recovery.

The cellular environment after each 60-s MVC should have been comparable, yet we observed a range of responses for the performance indexes in recovery. Simple reduction of high- or low-frequency fatigue would allow torque output to return to the control index values, but this does not account for the paradoxical recovery of contractile force at low frequencies (i.e., twitch and 10 and 20 Hz). However, simultaneous activation of both force depression and enhancement mechanisms (10, 18, 24, 31) could account for both the minimal depression at 10 s and the potentiation at 3–4 min in the twitch and 10-Hz indexes, respectively. Moreover, the inconsistent responses at low frequency are suggestive of a threshold activation above which force enhancement could not be readily observed. Effective but nonobservable potentia-

tion would obviously confound interpretation of pure fatigue effects.

A definitive mechanism to explain human twitch potentiation after single short- (10-s) (34, 38) and long-duration (60-s) (present study) MVCs remains elusive. One hypothesis is based on evidence that temporally relates the extent of myosin P-light chain phosphorylation and potentiation of the isometric twitch during repetitive low-frequency stimulation (17) and after a tetanus (21, 25, 26) in fast-twitch muscles of small mammals. In addition, skinned fiber models demonstrate that steady-state isometric tension is increased at suboptimal but not optimal calcium concentrations when myosin P-light chains have a high level of covalently bound phosphate (22, 30, 36). In humans, however, the relationship is less clear (14, 34).

An alternative hypothesis is that potentiation results from enhanced sarcoplasmic reticulum Ca^{2+} release or a high residual Ca^{2+} concentration after a fatiguing contraction. The former mechanism has been demonstrated by the use of a 10-s conditioning stimulus before a low-frequency index during recovery from fatigue (12), but this effect is dependent on the duration of the conditioning stimulus. Although a 1-s MVC will induce potentiation in nonfatigued muscle (38), in the present experi-

TABLE 1. Concentrations of ATP, PCr, and lactate in biopsy samples from vastus lateralis muscle obtained before and after a 60-s MVC

	Sampling Time			
	Pre (n = 10)	Post (n = 10)	4 min Post (n = 10)	10 min Post (n = 7)
ATP	27.3±1.6	24.5±1.8*	26.7±2.9	28.6±3.9†
PCr	74.2±14.4	14.4±13.2*	58.0±18.6*	68.3±16.8†
Lactate	4.1±1.6	77.5±20.5*	66.9±18.6*	55.7±35.6†

Values are means ± SD in mmol/kg dry wt; n, no. of observations. * Significantly different from Pre ($P < 0.05$). † Data not included in ANOVA because of missing observations.

TABLE 2. Phosphate content of myosin P-light chains before and after a 60-s MVC

	Sampling Time			
	Pre (n = 9)	Post (n = 9)	4 min Post (n = 9)	10 min Post (n = 6)
LC2f	0.31±0.1	0.63±0.19*	0.44±0.06*	0.37±0.09†
LC2s	0.36±0.13	0.68±0.18*	0.52±0.13*	0.44±0.06†
LC2s'	0.36±0.10	0.69±0.22*	0.54±0.17*	0.41±0.08†

Values are means ± SD in mol phosphate/mol P-light chain; n, no. of observations. Human myosin contains 1 fast (LC2f) and 2 slow (LC2s and LC2s') P-light chains. * Significantly greater than Pre ($P < 0.05$). † Data not included in ANOVA because of missing observations.

ment this effect was lost when the 1-s MVC was employed after the 60-s MVC (1-s MVC-10-Hz index). Nonetheless, the enhanced responses of the twitch and 10-Hz indexes illustrate clearly that a conditioning stimulus is not requisite for potentiation of low-frequency torque after a fatiguing contraction.

An elevated residual cytosolic Ca^{2+} concentration is similarly inadequate to explain the low-frequency potentiation. Blinks et al. (3) observed decreased aequorin luminescence after a tetanus in frog skeletal muscle fibers, which suggests that the amplitude of the calcium transient is depressed at a time when the isometric twitch is potentiated. Surprisingly, this depression may in fact be obligatory to permit potentiation. Augmented tension in the face of a reduced Ca^{2+} transient could result because P-light chain phosphorylation makes activation of the contractile elements more Ca^{2+} sensitive (29). Employing sodium dantrolene to attenuate sarcoplasmic reticulum Ca^{2+} release, Palmer and Moore (29) demonstrated that a greater relative twitch potentiation resulted with high than with low P-light chain phosphorylation levels. Furthermore, they demonstrated that sodium dantrolene-mediated reductions in tetanic tension could still be partially overcome by this mechanism, despite a concomitant reduction in P-light chain phosphorylation, which is also a Ca^{2+} -dependent event (35). These data, therefore, suggest an inverse relationship between the effect of P-light chain phosphorylation and the extent of Ca^{2+} activation of the contractile apparatus (29).

Given the potential for mechanisms of both tension enhancement and depression, observable potentiation in fatigued muscle is likely to be dependent on a number of factors: first, the intensity, duration, and repetitions of the fatiguing protocol; second, the degree of tension loss; third, the contractile index employed; and fourth, the use and duration of a preindex conditioning stimulus. These characteristics suggest complex interactions between the mechanism(s) of potentiation and the sites of both high- (neuromuscular transmission) and low- (excitation-contraction coupling) frequency fatigue and/or the contractile proteins (18).

Distinct differences characterize both fatigue (5) and potentiation responses (35) of muscle fiber types. Coexistence of twitch potentiation and fatigue could be dependent on the viability of the muscle's motor units to nervous excitement (31). Lack of potentiation at higher activation frequencies at 3-4 min of recovery, despite significant P-light chain phosphorylation (Table 2), could occur if motor unit activation was attenuated. Reduced muscle excitability might favor low- rather than a high-frequency stimulation to account for potentiation in the twitch and 10-Hz indexes. Furthermore, Ca^{2+} concentrations at these activation frequencies would be more moderate compared with the saturating levels of higher-frequency activation, and even more so if sarcoplasmic reticulum Ca^{2+} release were impaired (i.e., low-frequency fatigue), thereby favoring potentiation by the P-light chain phosphorylation mechanism.

Coexistence of fatigue and potentiation could also depend on the outcome of their opposing influences at the level of the contractile proteins. P_i and ADP are reported to modulate the strong and weak cross-bridge binding

states, respectively, between actin and myosin (4). Increased H^+ , total P_i , and/or diprotonated P_i concentrations (40) could influence a number of processes that inhibit activation, cross-bridge interaction, or ATP regeneration. Myosin conformational changes associated with P-light chain phosphorylation appear to favor both actin-myosin interaction (8, 22) and regulation of the domains in the myosin head that are thought to be involved in force generation (39). Metzger et al. (22) have recently proposed that phosphorylation could increase the rate constant for myosin cross-bridge attachment to actin. Thus both fatigue and potentiation processes could influence the contractile apparatus directly.

In summary, a 60-s MVC resulted in simultaneous activation of force enhancement and depression processes. Severe metabolic displacement likely contributed substantially to the overall torque decrement. Torque enhancement was likely due to elevated P-light chain phosphorylation, an effect that was observable only in the twitch and 10-Hz indexes at 3-4 min. Nonobservable potentiation at higher frequencies suggests that this process may be limited by an activation threshold, possibly due either to reduced nervous excitability in fast-twitch motoneurons or, conversely, to saturating levels of Ca^{2+} . Both metabolic factors and P-light chain phosphorylation in opposition could directly influence tension generation at the contractile proteins. Finally, simultaneous activation of both fatigue and potentiation processes indicates that both the fatigue paradigm and the stimulus index must be carefully considered in the evaluation of peripheral fatigue.

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