

# Low-frequency fatigue at maximal and submaximal muscle contractions

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Skeletal muscle force production following repetitive contractions is preferentially reduced when muscle is evaluated with low-frequency stimulation. This selective impairment in force generation is called low-frequency fatigue (LFF) and could be dependent on the contraction type. The purpose of this study was to compare LFF after concentric and eccentric maximal and submaximal contractions of knee extensor muscles. Ten healthy male subjects (age:  $23.6 \pm 4.2$  years; weight:  $73.8 \pm 7.7$  kg; height:  $1.79 \pm 0.05$  m) executed maximal voluntary contractions that were measured before a fatigue test (pre-exercise), immediately after (after-exercise) and after 1 h of recovery (after-recovery). The fatigue test consisted of 60 maximal (100%) or submaximal (40%) dynamic concentric or eccentric knee extensions at an angular velocity of  $60^\circ/\text{s}$ . The isometric torque produced by low- (20 Hz) and high- (100 Hz) frequency stimulation was also measured at these times and the 20:100 Hz ratio was calculated to assess LFF. One-way ANOVA for repeated measures followed by the Newman-Keuls *post hoc* test was used to determine significant ( $P < 0.05$ ) differences. LFF was evident after-recovery in all trials except following submaximal eccentric contractions. LFF was not evident after-exercise, regardless of exercise intensity or contraction type. Our results suggest that low-frequency fatigue was evident after submaximal concentric but not submaximal eccentric contractions and was more pronounced after 1-h of recovery.

Key words: Eccentric contractions; Concentric contractions; Knee extensor torque; Electrical stimulation

Research supported by CAPES (Master's scholarship), CNPq (Research scholarship).

Received April 6, 2008. Accepted January 19, 2009

## Introduction

Mammalian skeletal muscle is capable of producing impressive force and power levels when activated. However, repeated muscle activations of moderate to high magnitude are accompanied by progressive failure, which is clearly observed as a rapid reduction of performance that has been called fatigue (1). Although fatigue is a biological phenomenon that is present in several types of physical activity, the precise mechanisms of fatigue are not fully understood. In addition, fatigue is a complex phenomenon due to the various sites where it may occur,

and this has led to different definitions and/or different types of fatigue.

One of these fatigue types was described in the 1970's and was called low-frequency fatigue (LFF), since it affected force production in a more severe way when the muscle was activated with low frequency stimulation (2). Jones (3) suggested that a reduction of calcium release by the sarcoplasmic reticulum was one of the likely mechanisms of LFF. This has been confirmed with direct measurement of  $\text{Ca}^{2+}$  in the presence of LFF (4).

LFF is also called long-lasting fatigue due to the slow recovery (3,5). This slow recovery implies that structural

damage, requiring protein synthesis for return to normal function may be an integral part of LFF. Structural damage and LFF have also been associated with eccentric contractions. The explanation for LFF in this circumstance has been that there appears to be a rightward shift in the force-length relationship (6) due to increased compliance. If this was the case, then sarcomere length would be shorter at any given whole muscle length and the selective depression of force at low frequencies could be explained by the known length dependence of submaximal force (7,8). However, it has been shown that the apparent rightward shift in the force-length relationship is similar after isometric contractions and after eccentric contractions (9) and it has been suggested that this shift may be an artifact of the manner in which active force is calculated (10). Furthermore, it has been demonstrated that fascicle length is not affected by a series of fatiguing isometric contractions (11).

Considering the different mechanisms proposed, it would be of interest to make a direct comparison of the consequences of concentric and eccentric contractions with respect to LFF.

It would be expected that maximal eccentric contractions (where muscle damage is more evident) (12) would produce greater LFF. Also, assuming that submaximal contractions cause lower levels of fatigue, it would be expected that LFF would be greater after maximal compared to submaximal fatigue protocols. Since daily life activities generally require submaximal levels of force production and are associated with recruitment of motor units at low frequencies (3), studying LFF after submaximal contractions may allow for a better understanding of the mechanism that may limit human performance during daily activities. Therefore, the purpose of this study was to compare LFF levels between concentric and eccentric maximal and submaximal contractions of knee extensor muscles.

## Material and Methods

Ten healthy male subjects (age:  $23.6 \pm 4.2$  years, mass:  $73.8 \pm 7.7$  kg, height:  $1.79 \pm 0.05$  m), without history of neuromuscular disease, gave informed written consent to participate in the study. The Ethics Committee of the Federal University of Rio Grande do Sul approved the study (Protocol #200140).

### Torque measurements

The measurement of knee extensor torque was obtained using a Cybex NORM isokinetic dynamometer (Lumex & Co., USA). Subjects sat on the dynamometer

chair and were secured with Velcro straps. All subjects held onto the handles at the side of the chair for better body stabilization while performing the muscular efforts.

To measure the maximal isometric voluntary contraction (MIVC) the highest torque value from three initial MIVCs was obtained. Each MIVC was performed at a nominal knee angle of  $60^\circ$  of knee flexion from total knee extension ( $0^\circ$ ). All subjects were instructed to reach maximal torque in at least 1 s, and to maintain it for 3 s (13). In order to avoid fatigue, a 2-min rest was permitted between contractions.

To induce fatigue, 60 maximal dynamic voluntary contractions (MVCs) were performed in the maximal protocol, whereas for the submaximal protocol 60 dynamic contractions were performed at a level of 40% MIVC. The subjects performed these tests concentrically or eccentrically. During these tests, contractions of both knee extensor and flexor muscle groups were performed, in such a way that no concentric contractions were performed in the eccentric tests and no eccentric contractions were performed in the concentric tests. All dynamic contractions were executed at an angular velocity of  $60^\circ/\text{s}$ . Visual feedback of the contractile performance was given through an oscilloscope that was positioned in front of the subjects.

### Electrical stimulation

A Grass (S88, USA) stimulator, with an isolation unit approved for use with human subjects (SIU8T), was used for transcutaneous electrical stimulation of the femoral nerve. Two surface electrodes ( $4.5 \times 10$  cm) were placed over the skin with a conductive gel after skin preparation using standard procedures (14). The electrodes were positioned a) proximally in the anterior-medial surface of the thigh, over the femoral nerve, and b) distally over the distal portion of the quadriceps muscles. This procedure was similar to that used in previous studies (15-17). Electrical stimulation consisted of pulses of 1-ms duration (18,19) at frequencies of 20 and 100 Hz (20,21), and a train duration of 2 s. The criterium to determine the individual voltage of stimulation was that the torque generated at the frequency of 100 Hz should reach a level equal to or greater than 40% MIVC.

### Exercise protocols

Warm-up and familiarization with the equipment were performed prior to each experimental protocol. Four exercise protocols were performed by each subject, with an interval of at least 1 week (22).

During protocol 1 (maximal concentric test), three MIVCs were performed, for 5 s each, with 2 min between contractions. The highest MIVC was assumed to represent the

pre-exercise state. Ten minutes later, contractions were elicited by electrical stimulation to determine the appropriate stimulation voltage. This interval allowed for dissipation of post-tetanic potentiation (18). After the stimulation voltage was determined, electrical stimulation was applied at the nominal frequencies of 20 and 100 Hz (21), respectively. The maximal concentric test was initiated 10 s after the electrically elicited contractions, and consisted of three series of 20 repetitions of concentric contractions, performed at maximal effort (MVCs) with 25 s between consecutive series. Immediately after the exercise protocol, electrical stimulation (20 and 100 Hz) was applied again and the torque was measured. Five seconds after the 100-Hz contraction, an MIVC was performed. Electrical stimulation was applied again at these frequencies 60 min after the exercise protocol. A final MIVC was performed 5 s after the 100-Hz contraction (last train of electrical stimulation). Protocol 2 (maximal eccentric test) was similar to protocol 1, but fatigue was induced by maximal eccentric contractions. Protocols 3 (submaximal concentric test) and 4 (submaximal eccentric test) were similar to protocols 1 and 2, but with fatigue being induced by submaximal (40% of the MIVC) concentric and eccentric contractions, respectively.

#### Data analysis

The ratio between the torque produced with 20-Hz stimulation and that produced with 100 Hz (low frequency: high frequency, LF:HF) was used to assess LFF. The LF:HF ratio was obtained before the fatigue test (pretest), after-exercise and after-recovery. A fatigue index was

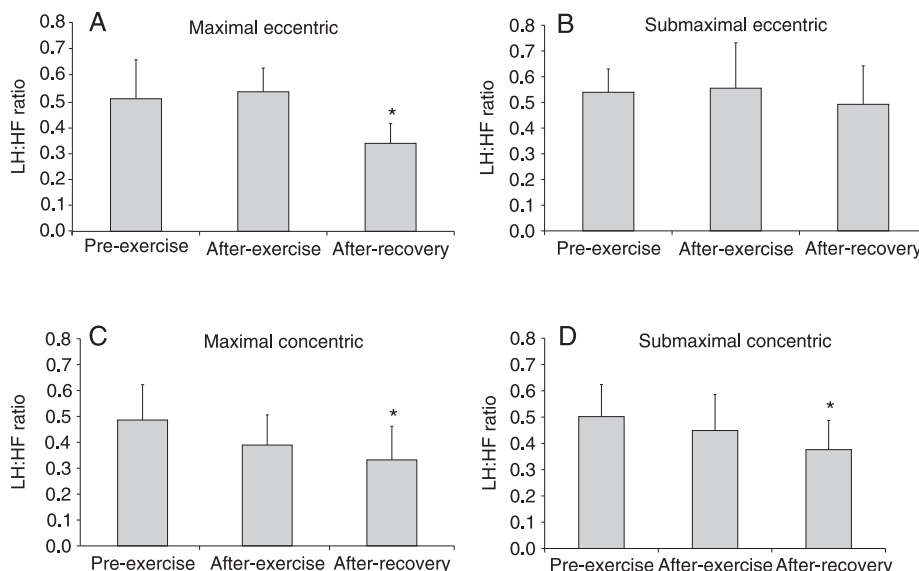
calculated as:  $1 - \text{LF:HF}_e / \text{LF:HF}_i$ . Here, the subscript *e* refers to after-exercise or after-recovery and the subscript *i* refers to initial or pretest. The maximum value for the fatigue index would be 1 if the LF:HF ratio decreased to zero as a consequence of the exercise. Results are reported as means  $\pm$  SD.

#### Statistical analysis

One-way analysis of variance (ANOVA) for repeated measures (with a significance level of  $P < 0.05$ ) was used to determine differences between the values of LF:HF pre-exercise, after-exercise and after-recovery obtained after each of the four different fatigue protocols (maximal concentric, maximal eccentric, submaximal concentric and submaximal eccentric). The Newman Keuls *post hoc* test was used to identify the specific differences when the ANOVA revealed significance. Statistical analysis was done with the Statistica software (Satsoft, USA).

#### Results

The ratio between the torque produced with 20-Hz stimulation and that produced with 100-Hz stimulation was used to assess LFF. Figure 1 shows the results for eccentric and concentric contractions. Maximal eccentric contractions resulted in no change in the ratio immediately after the exercise, but there was a substantial and significant ( $P < 0.001$ ) decrease evident after-recovery (see Figure 1A). This indicates the presence of LFF only after-recovery. The fatigue index was  $-0.12 \pm 0.29$  after-exercise and  $0.30 \pm 0.2$  after-recovery. There were no signifi-



**Figure 1.** LF:HF ratio of eccentric and concentric contractions. Ratio of torque from low-frequency (20 Hz) stimulation to that from high-frequency (100 Hz) stimulation (LF:HF) is shown. Pre-exercise basal values were obtained prior to each test. There was no significant change from the pre-exercise to the after-exercise value. However, after 60 min of recovery, there was low-frequency fatigue evident after maximal (A) but not submaximal (B) eccentric contractions and in both maximal (C) and submaximal (D) concentric contractions. \* $P < 0.05$  compared to pre-exercise (one-way ANOVA with Newman Keuls *post hoc* test).

cant changes in the LF:HF ratio for the submaximal eccentric contractions ( $P = 0.5$ ). Fatigue index was  $0.02 \pm 0.23$  after-exercise and  $0.09 \pm 0.20$  after-recovery (Figure 1B). There was a trend for a change in LF:HF ratio after-exercise for maximal concentric contractions ( $P = 0.06$ ) and a significant change ( $P < 0.02$ ) after-recovery (see Figure 1C). Fatigue index was  $0.16 \pm 0.31$  after-exercise and  $0.29 \pm 0.30$  after-recovery. There was no significant change in the LF:HF ratio after-exercise following the submaximal concentric contractions. However, the after-recovery ratio was significantly different from that of the pretest (see Figure 1D). The fatigue index was  $0.10 \pm 0.22$  after-exercise and  $0.22 \pm 0.24$  after-recovery.

## Discussion

The main findings of this study were: 1) there was greater LFF after maximal compared to submaximal eccentric fatigue tests, 2) there was greater LFF after-recovery compared to the after-exercise period in both concentric tests, and 3) submaximal eccentric contractions did not result in LFF. These results provide evidence that maximal muscle contractions are more effective in generating LFF, and that LFF is more pronounced 1 h after-recovery, and not immediately after-exercise, when fatigue would be expected to be greater. This latter result is consistent with the long-lasting property of LFF (2). It is not clear from these measurements whether the masking of LFF immediately after-exercise is due to a counterbalancing of post-activation potentiation, which dissipates over several minutes, or the LFF is caused by a change in the muscles associated with post-exercise protein degradation or loss of  $Ca^{2+}$  from binding proteins in the cytoplasm.

The fact that LFF was not evident after-exercise when contractions were eccentric was surprising. In contrast, concentric contractions at least tended to have a decreased LF:HF ratio at this time. It would appear that there is at least one component of LFF that is very different when eccentric and concentric contractions are compared.

Daily life activities occur predominantly with motor units being recruited at low frequencies, whereas in maximal efforts high motor unit firing rates are observed (23). Also, different exercise intensities are related to the recruitment of different muscle fiber types (24). The submaximal exercise in this study could have been accomplished with predominantly slow-twitch motor unit activation. Rijkelijhuizen et al. (25) studied regions of the rat's gastrocnemius muscle with different fiber type distribution and found greater levels of LFF in the fast glycolytic compared to the fast oxidative muscle fibers. Some investigators attribute such results to the greater susceptibility of

fast contraction fibers to fatigue and injury (26,27) compared to the slow fibers. This would be consistent with the differences we observed between maximal and submaximal contractions.

One important reason to study the mechanisms of LFF is related to the fact that fatigue is probably one of the major limiting factors of muscle function in patients with central nervous system dysfunctions. Functional electrical stimulation is aimed at helping these patients to produce simple patterns of motion. However, long-lasting artificially elicited contractions are subject to LFF. Studies comparing low-electrical stimulation frequencies (9.1 and 14.3 Hz) to high frequencies (33.3 and 100 Hz) found greater levels of LFF at the low stimulation frequencies early in the recovery period, but comparable levels across stimulation frequencies later in the recovery. These low frequencies are consistent with the physiological range of stimulation frequencies used by humans. Also, Binder-Macleod and Russ (28) reported greater LFF at 13 min of recovery compared to 2 min of recovery, consistent with the delayed development of LFF in our experiments. Furthermore, force produced at low stimulation frequencies during recovery does not seem to be influenced by the frequency or pattern of stimulation, which induced fatigue.

It has been proposed that LFF is the result of impairment in the excitation-contraction coupling process, leading to a reduction of  $Ca^{2+}$  release by the sarcoplasmic reticulum. As eccentric exercise is known to be more destructive to the muscle fibers than other types of contraction (29,30), damage to the sarcoplasmic reticulum leading to diminished  $Ca^{2+}$  release, as well as sarcolemmal damage that could interfere with  $Ca^{2+}$  homeostasis (22,25), might have been greater in the maximal eccentric fatigue test. However, as we did not examine  $Ca^{2+}$  concentrations, we cannot say which effects each one of our four protocols had on these variables.

Several studies have shown that repeated eccentric contractions may modify the optimal length of muscle force production, and some evidence suggests that with 30-Hz stimulation there is a greater fatigue if the muscle is in a shortened position. In addition, *in vivo* studies show that even isometric contractions may generate changes in muscular architecture leading to alterations in tendon rigidity, as well as changing the geometry of the in-series structures and, therefore, affecting force production capacity (31). Such changes in muscle architecture might therefore affect fatigue, limiting force production *in vivo* (32). Thus, it is possible that uncontrolled changes in the force-length relationship might have influenced torque production and the LFF responses in this study. Furthermore, low stimulation frequencies correspond to the steep part of

the force- $pCa^{2+}$  curve, whereas high frequencies relate to the horizontal part (25). Therefore, according to the force-frequency relationship, little variations in intra-muscular  $Ca^{2+}$  concentrations result in greater changes in the force produced with low stimulation frequencies compared to that produced with high stimulation frequencies (3,33).

LFF seems to be a phenomenon capable of compromising daily life activities, which are likely produced by low stimulation frequencies and therefore low levels of voluntary effort or of muscle contraction. However, LFF is probably also a consequence of high-intensity and long-lasting exercises that are typical of sports activities. Studies addressing the presence of LFF in sports should be developed in order to better determine the potential impairment of muscle performance. Studies looking at the force-fre-

quency relationship and the length dependence of fatigue in an *in vivo* model might give some insight to this problem.

Maximal concentric and eccentric contractions and submaximal concentric contractions are capable of generating significant LFF. This phenomenon is not obvious immediately after a fatiguing exercise, but appears later, and is quite evident after 1 h of recovery. LFF was similar between contraction types, and was greater after maximal compared to submaximal eccentric fatiguing contractions.

## Acknowledgments

The authors would like to thank Cristian Kohmann and João Breno, from the Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil, for technical help.

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