LOW FREQUENCY FATIGUE OF QUADRICEPS MUSCLE AFTER PERFORMING MAXIMAL ISOMETRIC CONTRACTIONS AT DIFFERENT MUSCLE LENGTH

by

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Immediately after performing isometric exercise (males, age 22.9±1.8 years, n=12) with maximal intensity (60 s) a greater muscle fatigue and, espacially, low-frequency fatigue (LFF) after exercise performed at longer muscle length (knee joint angles of 90 degrees) than after the exercise performed at shorter muscle length (knee joint angles of 135 degrees) is observed and it does not depend on muscle length (shorter or longer) at which the muscle fatigue has been registered. There are two periods in recovery of muscle fatigue: rapidly recover (to 3 min) and slow recover. There is an increase in low frequency muscle fatigue during slow muscle recovery period. It is more pronounced after performing exercise at longer muscle length but when LFF registered at shorter length.

Key words: electrical stimulation, low frequency fatigue, recovery, isometric exercise, maximal intensity

Introduction

In humans, low frequency fatigue (LFF) occurs in concentric, isometric and eccentric exercise (Newham et al. 1987, Ratkevicius et al. 1995; Skurvydas et al. 1999). Although the mechanism for production of LFF is unknown, both metabolite build-up and elevation in intracellular Ca^{2+} concentration as well as muscle mechanical damage have been suggested to play a role in the development of LFF (Newham et al. 1987, Westerblad et al. 1993, Chin et al. 1997).

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In the series of experiments designed to investigate the effects of length, isometric exercise were performed at both short and long muscle length and the muscles were subsequently tested at an intermediate length pain (Jones et al. 1989). The contractions at long length resulted in greater low frequency fatigue and pain, despite the fact that they generated less force than those at the short length. Furthermore, there is a length-dependent component in the generation of low frequency fatigue and muscle pain (Jones et al. 1989). It has been shown, that isometric contractions at long lengths will lead to overextension of some sarcomeres according to the non-uniform sarcomere hypothesis by Morgan (1990). To test this theory of muscle damage it would be necessary to verify whether there had been a change in force-length relationship. Saxton and Donnelly (1994) have shown that the force loss in human biceps, damaged by lengthening contractions, was the greatest when the muscle was tested at short lengths and the least when the muscle was extended, indicating a shift to the right of the length-tension relationship.

We hypothesis that muscle low frequency fatigue is dependent on the coexistence of four components: a) fast developing and rapidly recovering**,** muscle potentiation and metabolite build-up dependent component; b) fast developing, slow recovering, metabolite dependent component; c) slow developing, slow recovering $[Ca^{2+}]_i$ -time integral dependent component); d) fast developing but slow recovering, muscle mechanical damage dependent component. Verifying this hypothesis was our main objective. To verify this hypothesis a research protocol was chosen during which isometric exercise inducing muscle fatigue caused both by metabolic and non-metabolic changes as well as muscle damage was performed. In addition, during such loads muscle post-tetanic potentiation is also markedly expressed. We think that isometric exercise of maximal intensity with the duration of 60 s performed at short and long muscle length suited this purpose most.

Methods

Subjects

Healthy untrained men (age 22.9±1.8 years, n=12) (weight 77.5±5.2 kg) gave their informed consent to take part in all experiments within the study.

The untrained subjects were physically active but did not take part in any formal physical exercise or sport program.

General protocol

Two experiments with an interval of 8 weeks were performed. Experiments 1 and 2 were designed to examine time-course of recovery of muscle contraction and relaxation properties after isometric MVC- 60 s performed at knee angles of 90^{o} (full extension 180^{o}, long muscle length, LL) and 135^{o} (short muscle length, SL). The subjects performed experiments in a randomised way.

Force measurements

The equipment and technique for measuring force was the same as has been used in a previous study (Ratkevicius et al. 1995, Skurvydas et al. 2000). Subjects were placed in an experimental chair. They sat upright in the experimental chair with a vertical back support. A strap secured the hips and thighs to minimise uncontrolled movements. The right leg was clamped in the force-measuring device with the knee at an angle of 90^{o}. A 6 cm wide plastic cuff, placed around the right leg just proximal to the malleoli, was tightly attached to a linear variable differential transducer. The output of the transducer, proportional to isometric knee extension force, was amplified and digitised at a sampling rate of 1 kHz by a 12-bit analogue-to-digital converter installed in an IBM-compatible personal computer. The digitised signal was stored on the hard disk for subsequent analysis. The output from the force transducer was also displayed on a voltmeter in front of the subject.

Electrical stimulation

Equipment and procedure for electrical stimulation were essentially the same as has been described previously (Ratkevicius et al. 1995, Skurvydas et al. 2000). A high voltage stimulator (MG 440, Medicor, Budapest, Hungary) was used. Electrical stimuli to the quadriceps muscle were delivered through surface electrodes (9x18 cm) padded with cotton cloth and soaked in saline solution. One stimulation electrode was placed just above the patella, while the other one covered a large portion of the muscle belly in the proximal third of the thigh. The electrical stimulation was always delivered in trains of square wave pulses

of 1 ms duration (voltage 150 V, which induces 65-85 per cent of maximal voluntary contraction force; MVC). With an aim of recruiting the greatest number of fibres the highest stimulation voltage possible was chosen. The subjects were introduced to electrical stimulation before the experiments began.

The following data were measured: the force of the quadriceps muscle, aroused by electrical stimulation at 20 Hz (P20) and 50 Hz (P50) frequencies (the duration of each electrical stimulation series was 1 s) and MVC (top of the MVC was reached and maintained some 2 seconds before relaxation). Rest interval between muscle electrostimulations was 3 s and between MVC was 1 min. The ratio of P20/P50 was calculated for the evaluation of LFF (Skurvydas et al. 2000). The contractile force was measured at knee joint angles of 135 degrees (SL) and 90 degrees (LL) in a randomised way.

Experimental protocol

The subject was seated in the experimental chair and after 5 min, muscle contractile properties were recorded in the following sequence: P20, P50 and MVC (MVC was reached 2 times). During the period of recovery MVC was evaluated but once. Immediately after the exercise (A0) and 3 (A3), 7 (A7) and 15 (A15) min following the exercise the contractile properties of skeletal muscle were tested.

Data and statistical analysis

The two-way analysis of variance (two way ANOVA) for repeated measures was used to determine differences between the groups. When the ANOVA was significant, a paired Student's t test was used to determine differences between the groups. One-way ANOVA for repeated measures was used to test the statistical differences within each group (pre- vs. post-fatigue). When the ANOVA was significant, a paired Student's t test was used to determine differences between separate measurements. Statistical significance was set at $P<0.05$.

Results

The results of our study have shown that both the force evoked by electrostimulation and relaxation time are muscle length-dependent: force-

frequency curve registered at 135 degrees of knee angle (SL) is steeper than the one at 90 degrees (LL) since P20/P50 is significantly smaller ($P<0.05$) (Table 1).

Table 1. Control values of indices of men's electrostimulation-induced contractions of quadriceps muscle and MVC (mean ±SD)

Angle	P20, N	P50, N	MVC, N	P20/P50
90	348.4 51.4	442.7 68.4	719.8 79.2	0.77 0.06
135	422.7 60.1	634.8 79.2	747.1 89.5	0.66 0.06
Diffeerence between 90 and 135 angles	<0.05	<0.05	n.s.	<0.05

P20 and P50 – muscle contraction force evoked by stimulating quadriceps muscle at 20 Hz and 50 Hz frequencies. MVC - maximal voluntary contraction force.

Is time-course of recovery of muscle contractile properties length-dependent after performing exercise at 90 degrees of knee angle (LL)?

The results of our study have shown that immediately after the exercise (A0) there was a significant ($P<0.05$) decrease in muscle force induced by low (20 Hz) and high (50 Hz) stimulation frequencies and MVC (it is not muscle length-dependent) and it did not recover to its initial (pre-exercise) level 15 min after the end of exercise (Table 2). During recovery period there was a significant ($P<0.05$) decrease in P20/P50 that is indicative of origin of low frequency fatigue (Fig. 1). From 3 min to 15 min during recovery LFF increased significantly ($P<0.05$) and it is muscle length-dependent, because LFF is more pronounced ($P<0.05$) in SL than LL (Fig. 1).

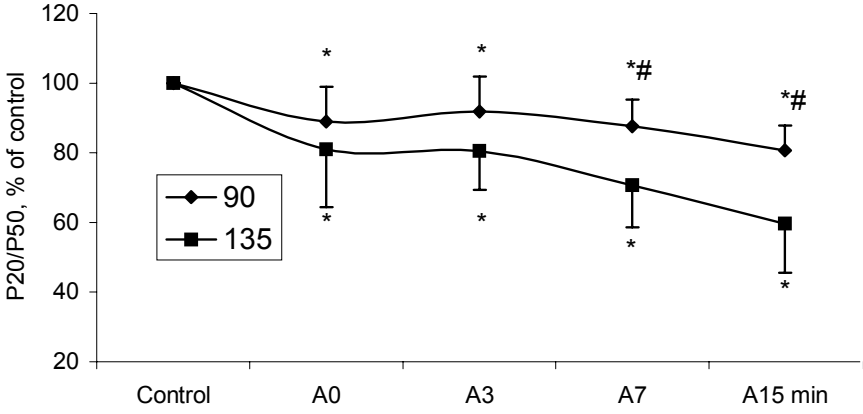

Fig. 1. The changes in P20/P50 after performing izometric exercise with maximal intensity lasting 60 s (knee-joint angle of 90°). A0, A3, A7 and A15 - immediately, 3 min, 7 min and 15 min after exercise. P20 and P50 – muscle contraction force evoked by stimulating quadriceps muscle at 20 Hz and 50 Hz frequencies. * - significant (P<0.05) to initial mean level. # - P<0.05 between 90 and 135 knee degre angles mean level.

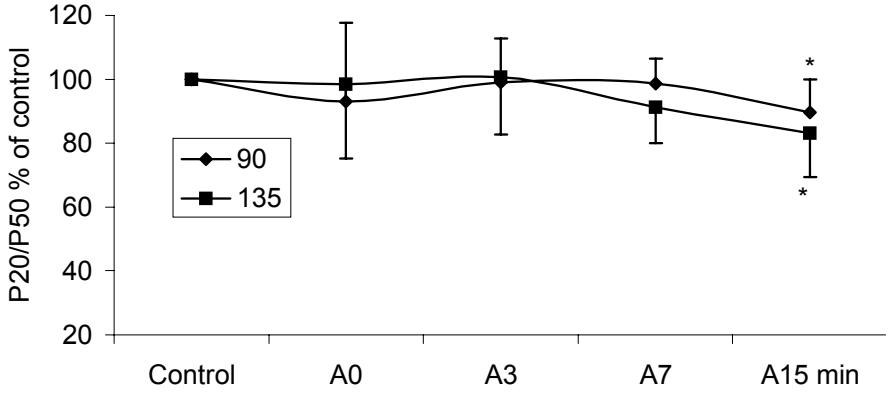

Fig. 2. The changes in P20/P50 after performing izometric exercise with maximal intensity lasting 60 s (knee-joint angles of 135°). A0, A3, A7 and A15 - immediately, 3 min, 7 min and 15 min after exercise. P20 and P50 – muscle contraction force evoked by stimulating quadriceps muscle at 20 Hz and 50 Hz frequencies. * - significant (P<0.05) to initial mean level.

Is time-course of recovery of muscle contractile properties length-dependent after performing exercise at 135 degrees of knee angle (SL)?

Table 2. Mean values (mean±SD) in per cent compared to pre-exercise values of electrostimulation-induced muscle contractions and MVC immediately (0 min), 3 min, 7 min and 15 min after isometric exercise of maximal intensity performed 60 s (knee-joint angle of 90°).

Properties	Angle	0 min	3 min	7 min	15 min
P20	90	41.9* 10.3	75.8* 13.2	74.2* 11.8	65.6*# 10.8
	135	40.2* 14.1	67.1* 11.2	57.5* 11.3	46.6*# 11.2
	Difference between 90 and 135 angles	ns.	ns.	<0.05	<0.05
P50	90	47.2* 9.9	85.4* 6.7	84.2* 8.1	81.2* 8.2
	135	50.9* 12.4	83.1* 7.4	81.5* 9.2	78.6* 9.9
	Difference between 90 and 135 angles	ns.	ns.	ns.	ns.
MVC	90	38.8* 6.7	87.7* 6.8	89.7* 8.1	89.4* 7.1
	135	41.7* 7.3	86.8* 7.9	88.1* 9.1	87.4* 7.3
	Difference between 90 and 135 angles	ns.	ns.	ns.	ns.

P20 and P50 – muscle contraction force evoked by stimulating quadriceps muscle at 20 Hz and 50 Hz frequencies. MVC – maximum voluntary contraction force. *-significant (P<0.05) to initial mean level. # - P<0.05 from 3 min after the end of exercise level.

The results of our study have shown that after performing exercise at SL there was a significant (P<0.05) decrease in muscle force induced by low (20 Hz) and high (50 Hz) stimulation frequencies and MVC and it recovered to its initial (pre-exercise) level 3 min after the end of the exercise (it is not muscle length dependent) (Table 3); 2) there was a non-significant (P>0.05) decrease in P20/P50 (it is not muscle length dependent) (Fig. 2); from 3 min to 15 min

during recovery there was a secondary decrease (P<0.05) in force evoked at low frequencies (20 Hz) stimulation and at 15 min following the end of exercise manifested LFF but it is not muscle length-dependent (Fig. 2).

Table 3. Mean values (mean±SD) in per cent compared to pre-exercise values of electrostimulation-induced muscle contractions and MVC immediately (0 min), 3 min, 7 min and 15 min after isometric exercise of maximal intensity performed 60 s (knee-joint angle of 135^{o})

Properties	Angle	0 min	3 min	7 min	15 min
P20	90	59.1* 20.2	98.6 17.6	96.9 14.9	85.8* 11.9
	135	58.5* 24.1	97.1 15.4	90.6 11.8	80.4*# 15.1
	Difference between 90 and 135 angles	ns.	ns.	ns.	ns.
P50	90	64.2* 15.2	101.7 26.4	99.8 20.9	97.7 19.9
	135	57.9* 15.6	97.5 13.6	100.4 11.2	96.8 10.9
	Difference between 90 and 135 angles	ns.	ns.	ns.	ns.
MVC	90	50.1* 14.2	92.9 13.2	97.4 11.4	98.3 14.2
	135	52.7* 15.5	91.2 12.6	95.8 124.4	96.2 12.7
	Difference between 90 and 135 angles	ns.	ns.	ns.	ns.

P20 and P50 – muscle contraction force evoked by stimulating quadriceps muscle at 20 Hz and 50 Hz frequencies. MVC – maximum voluntary contraction force. *-significant (P<0.05) to initial mean level. # - P<0.05 from 3 min after the end of exercise level.

Differences in muscle fatigue after performing exercise at SL and LL are as follows: 1) after isometric exercise performed at longer muscle length (LL) greater low frequencies fatigue than that after performing exercise of the same intensity at shorter muscle length (SL), is observed; 2) it is during the period of

recovery and at a short muscle length (SL) that LFF is most markedly expressed (Table 2, 3; Fig. 1, 2).

Discussion

Our main findings are as follows: 1) there is an increase in low frequency muscle fatigue during muscle slow recovery period; 2) it is more pronounced after performing exercise at long muscle length than at short length and especially when low frequency fatigue registered at short muscle length.

These results of our study support the theory that muscle low frequency fatigue during performing isometric exercise at maximal intensity are dependent on the coexistence of four components: a) fast developing and rapidly recovering muscle potentiation and metabolite build-up dependent component; b) fast developing and slow recovering metabolite dependent component; c) slow developing and slow recovering $[Ca^{2+}]_i$ -time integral dependent component); d) fast developing but slow recovering muscle mechanical damage dependent component.

It has been shown that quadriceps muscle endurance is greater, when muscle contraction at shorter muscle length (knee angles of 40-45 degrees) than at longer (knee angles of 90 degrees) (Ng et al. 1994). This is in accordance with the results of our study, namely, that muscle fatigue and, especially, low-frequency fatigue is smaller when performing MVC-60 s at shorter muscle length. Fitch and McComas (1985) hypothesised that the greater fatigue rates at longer muscle length than those at shorter length were due to greater cross-bridge interaction at longer length resulting in greater ATP turnover. However, more recent work, using ^{31}P-NMR spectroscopy, has shown that the metabolic cost of contraction at shorter muscle length is similar to contraction at optimal muscle length (Sacco et al. 1994). Thus, greater fatigue resistance at shorter muscle length does not appear to be due to a smaller metabolic demand at shorter length relative to optimal length. Failure of the contractile machinery may be due in part to factors independent of metabolic rate. These factors, however, have not been exactly defined yet.

The results of our study, however, do not indicate that post-tetanic potentiation could have influence on muscle contraction force induced by

stimulating the muscle at 20 Hz since changes taking place in force were independent of the muscle length. Therefore alteration in P20/P50 according to which LFF arising after physical exercise was estimated in our case could not be dependent on post-tetanic potentiation.

The isometric exercise chosen by us was analogous to the one performed by Houston and Grange (1990), i.e. maintaining MVC for 60 s. They have shown that after such exercise there was a significant decrease in ATP and phosphocreatine (PCr) concentration whereas lactate increased markedly. There are not doubts, therefore, that muscle fatigue arising after 60-s MVC is dependent on metabolic factors. It has been shown that 10 min after the 60-s MVC, twitch tension was similar to control values and phosphorylation of myosin regulatory light chain was similar to the one observed at rest, ATP and PCr concentration returned to control level while lactate concentration remained elevated (Houston and Grange 1990). Therefore rapid recovery of muscle contraction force within 3 min after the exercise in our case could also be dependent on metabolite factors (Table 2, 3).

It has been established that one of the mechanisms of LFF depends on the decrease in sarcoplasmic reticulum Ca^{2+} release (Westerblad et al. 1993). Kabbara and Allen (1999) in their recent study provide strong evidence that the reduction in releasable Ca^{2+} is in some way related to the metabolic changes associated with fatigue and it is consistent with the hypothesis that Ca^{2+} precipitates with phosphate in the sarcoplasmic reticulum. It has been shown that there is a delay of several minutes between the rise of inorganic phosphate and the development of Ca^{2+} and inorganic phosphate precipitate (Fryer et al. 1997). This mechanism, therefore, could also manifest itself in our case (during recovery) since there was an unquestionable increase in inorganic phosphate following MVC-60 s.

Smith et al. (1999) have shown that after concentric exercise (forty maximal voluntary contractions of he biceps muscle) all subjects showed a progressive decrease in twitch response with a minimum after 10-30 min and a subsequent recovery complete within 1-2 h. The development of LFF thus occurs during the period of metabolic recovery and at a rate apparently dependent on the metabolic work done. These results are consistent with the hypothesis of a metabolic trapping of calcium compounded by damage to SR.

(Smith et al. 1999). In our case changes in the muscle contraction force after MVC-60 s exercise also indicate the presence of a secondary increase in LFF during recovery period.

It has been shown that recovery of force and $[Ca^{2+}]_i$ after fatigue follows a complex time course. One component of force and $[Ca^{2+}]_i$ recovers within 15-30 min and then there is a slow-recovering component which requires more than 60 min (Westerblad et al. 1993). The elevated $[Ca^{2+}]_i$-time integral induces prolonged reduction in Ca^{2+} release in the absence of any metabolic alterations associated with fatigue, since it is believed to activate some process that results in the disruption of proteins involved in excitation-contraction coupling (Lamb et al. 1995).

Within 7-15 min after the exercise the metabolic component may have fully recovered (Baker et al. 1993), but the $[Ca^{2+}]_i$-time-integral dependent component may be active enough to counteract recovery of the metabolic-dependent component. It may be that this Ca^{2+} dependent long lasting component of LFF has a longer onset time than the metabolic component. This would explain the rapid initial decrease in LFF at 3 min of recovery and a subsequent increase in LFF at 15 min of recovery. Previous researches have typically examined LFF at 10, 30 and/or 60 min of recovery (Westerblad et al. 1993, Chin et al. 1997) and so would not have detected the changes in low-frequency force response between 3 and 15 min seen here although a similar decrease and subsequent increase in LFF have previously been reported (Ratkevicius et al. 1995, Skurvydas et al. 2000).

Slow recovery of muscle contraction force after exercise indicates that fatigue is of non-metabolic origin and that it might be associated with muscle damage which is a fast developing but slow recovering muscle fatigue component. The phenomenon of heterogeneity in sarcomere length plays a key role in the mechanical events that initiate contraction-induced injury (Morgan 1990). During maximal activation of single permeabilized fibers held at optimum muscle length, the stronger sarcomeres get shorter or stay at the same level, whereas the weaker sarcomeres are stretched onto the descending limb of their length-force relationship. The reduction in the sarcomere length of the active sarcomeres at a fixed muscle length has a number of consequences. It is known that the activation curve of muscle shifts to higher Ca^{2+} at shorter

sarcomere lengths (Endo 1973), possibly explaining the apparent reduced Ca^{2+} sensitivity after eccentric exercise (Balnave and Allen 1995). Therefore with an increase in partial sorcomere damage due to exercise there should be a more marked manifestation of LFF in the muscle at a smaller muscle length. Consequently, when performing isometric physical exercise at longer muscle LFF is greater because of greater muscle damage. If fatigue were associated with metabolic factors it should disappear altogether.

In conclusion, immediately after performing isometric exercise with maximal intensity a greater muscle low frequency fatigue is observed after exercise performed at longer muscle length and it does not depend on the length (shorter or longer) at which LFF was registered. There are two periods in recovery of muscle fatigue: rapid recovering (to 3 min) and slow recovering. There is an increase in low frequency muscle fatigue during muscle slow recovery period. It is more pronounced after performing exercise at longer muscle length if compared to low frequency fatigue registered at shorter length. These results support the theory that muscle low frequency fatigue during performing isometric exercise at maximal intensity is dependent on the coexistence of four components: a) fast developing and rapid recovering muscle potentiation and metabolite build-up dependent component; b) fast developing and slow recovering, metabolite dependent component; c) slow developing and slow recovering $[Ca^{2+}]_i$-time integral dependent component); d) fast developing but slow recovering muscle mechanical damage dependent component.

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