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Fatigability of motor units estimated by changes in work performed during their repetitive stimulation during the fatigue test

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Abstract. In numerous studies resistance to fatigue is evaluated by measuring the peak tension of motor units in muscle. In the present study, the work performed within successive tetani during the fatigue test of rat medial gastrocnemius motor units was estimated by assessing of the area under the tension record. Resistance to fatigue was evaluated by a modified fatigue index which is expressed as the ratio of the area under a tetanus recorded two minutes after maximal potentiation of tension has been reached to the area under this maximally potentiated tetanus. The values of this modified fatigue index were compared to the standard fatigue index which was taken as the ratio of peak tensions for corresponding tetani. For fast fatigable units, values of the modified fatigue index were significantly lower than those of the standard index. This observation resulted from changes in the shape of unfused tetani accompanying developing fatigue. These changes strongly influenced the area under the tension record whereas the peak tension of these tetani diminished less significantly. For slow and a part of fast resistant to fatigue units (with the standard fatigue index above 0.85) the modified fatigue index was slightly higher than the standard one although the difference was not significant. This phenomenon was due to the prolongation of relaxation which was visible in the last part of the fatigue test. It is being concluded that the modified fatigue index describes more precisely the fatigue-induced changes in tetani during the fatigue test than the standard fatigue index, especially in fast fatigable units.

Short communication

Key words: motor units, resistance to fatigue, work, contraction, tension-time area

Resistance to fatigue is one of the principal properties of motor units. This resistance was used in numerous studies to divide fast motor units into two main categories: fast fatigable (FF) and fast fatigue-resistant (FR) units. Sometimes fast intermediate units (FI) were also distinguished (Burke et al. 1973, Goslow et al. 1977, Jami et al. 1982, 1983, Kernell et al. 1983, Filippi and Troiani 1994, Kwa et al. 1995, Rafuse and Gordon 1996). This division was based on the value of the fatigue index which is expressed as the ratio of tetanic tension of motor units developed after two minutes of repeated tetani within the fatigue test to their maximal initial tension. The fatigue test (stimulation with 330 ms trains of stimuli at 40 Hz, repeated every second during several minutes) was originally introduced for motor units in cat muscles (Burke et al. 1973). This test was later also applied in numerous studies of rat motor units (Gardiner and Olha 1987, Kanda and Hashizume 1992, Bakels and Kernell 1993, 1995, Seburn and Gardiner 1995, 1996, Kadhiresan et al. 1996, De Ruiter et al. 1996). It was observed that the frequency of stimulation of 40 Hz used for these units in this test evoked significantly less fused tetani than in cat muscles. This difference resulted from a different speed of contraction: motor units in rat muscles had shorter contraction time than those in cat muscles (for cat medial gastrocnemius see: Burke et al. 1973, Reinking et al. 1975; for rat medial gastrocnemius see: Grottel and Celichowski 1990, Kanda and Hashizume 1992, De Ruiter et al. 1996). The contractile speed changed during development of fatigue of motor units (Dubose et al. 1987, Celichowski and Grottel 1997). Furthermore, the fusion of successive tetani within this test also changed (Celichowski et al. 1996). In some recent papers we measured the area under the tension record which enabled us to estimate the work performed by a contracting motor unit (Celichowski et al. 1998, Celichowski and Grottel 1998). This method made it possible to describe changes in the work performed during successive tetani within the fatigue test. Therefore, this paper was aimed at describing the fatigue resistance (fatigue index) using not only standard tension measurements but also measurements of the work performed during the fatigue test.

Experiments were performed on 4 adult Wistar rats (weight 260-280 g), under pentobarbital anaesthesia (Vetbutal, 30 mg/kg I.P., supplemented as required during the experiment).

The details of surgery and isolation of single motor units were described earlier (Celichowski 1992a). Briefly, the left medial gastrocnemius muscle was partly isolated from surrounding tissues and its Achilles tendon was connected to a force transducer. The branch of the sciatic nerve and blood vessels supplying the studied muscle were left intact. The remaining muscles of the hindlimb were denervated by cutting their branches of the sciatic nerve. The left hindlimb was immobilised with a steel clamp. This hindlimb and medial gastrocnemius muscle were immersed in a paraffin oil pool. Temperature was kept at a constant level of $37 \pm 1^{o}C$.

Laminectomy was performed over L2-S1 segments and ventral as well as dorsal roots of the spinal cord were cut. The spinal cord was covered with warm paraffin oil ($37 \pm 1^{o}C$) in a pool made of the skin around the laminectomy. L5 ventral root was split into very thin filaments which were stimulated with electrical, rectangular pulses (duration of 0.1 ms and amplitude up to 0.5 V). When the stimulation of these filaments evoked the "all - or - none" type of contraction and action potential, this activity of muscle fibres was accepted as the contraction of a single motor unit.

The action potentials were recorded with a pair of thin-wire silver electrodes inserted into the muscle. The tension was recorded under isometric conditions and during the experiments the muscle was stretched to a passive tension of 100 mN. Under these conditions the maximal twitch tension could be recorded for the majority of motor units in this muscle (Celichowski and Grottel 1992). The tension and action potentials were stored on a computer disc using an analog-digital converter (RTI 800 Utilities). The sampling rate was 10 kHz for tension and 20 kHz for action potential records.

Isolated motor units were subjected to the following stimulations: (1) with 10 repeated stimuli at 1 Hz frequency (an averaged twitch was obtained), (2) with a 500 ms train of stimuli at 40 Hz (an unfused tetanus, the presence of "sag" was controlled), (3) with 200 ms train of stimuli at 150 Hz (a fused tetanus, the tetanic tension was measured), (4) with 330 ms trains of stimuli at 40 Hz, repeated every second for 3 minutes (the fatigue test) (Burke et al. 1973).

The studied motor units were classified as fast when a "sag" was visible in their unfused tetani, whereas units without a "sag" at such stimulation were classified as slow (Grottel and Celichowski 1990). The division of fast units into FF and FR types was based on results of the fatigue test. The *standard fatigue index* was expressed as the ratio of the tetanic tension reached two minutes after the maximal potentiation developed at the

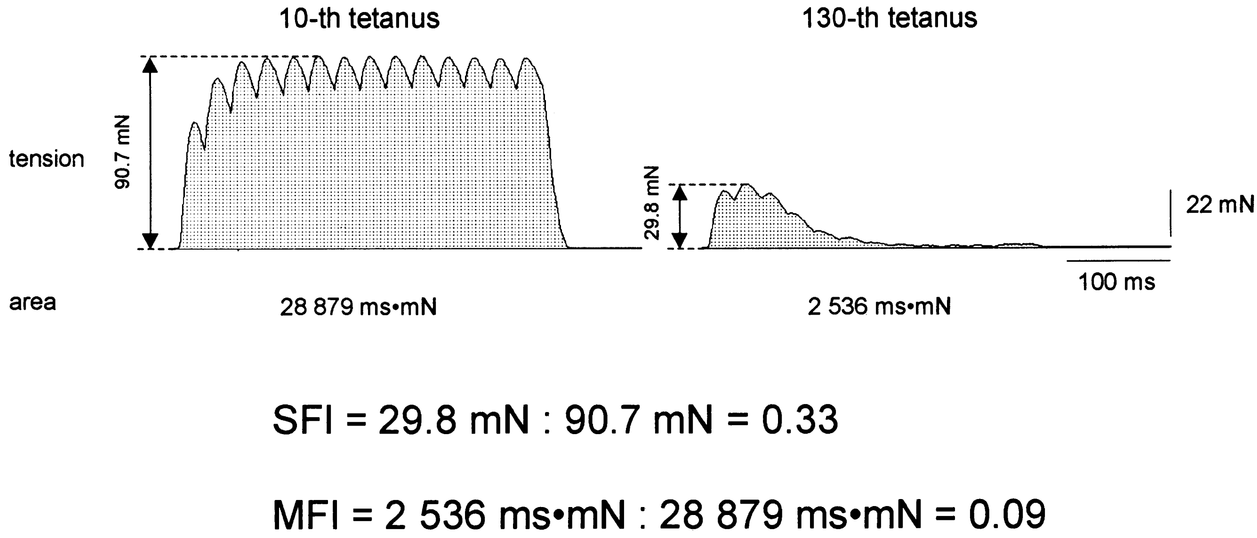

Fig. 1. Main parameters describing the fatigue of motor units. An example of FF type motor unit. Both tetani were recorded during the fatigue test. The 10-th tetanus within the fatigue test is the maximally potentiated one (left, 10th) whereas the next was recorded 120 s later (right). The tension measured from the baseline to the peak of tension record is shown in each diagram. The area between the tension record and the baseline (dotted area) is shown under each record. Both tetani were used to calculate the standard fatigue index (SFI, the ratio of tensions) and the modified fatigue index (MFI, the ratio of areas) as shown in the lower part of the Figure.

onset of the fatigue test to the tension of the most potentiated tetanus (Fig. 1) (Kernell et al. 1975). For tetani recorded within the fatigue test the area under the tension record was calculated using a special computer program. The *modified fatigue index* was additionally calculated. This was expressed as the ratio of areas for tetani used to calculate the standard fatigue index (Fig. 1). Fast units with both fatigue indices lower than 0.5 were classified as FF, whereas those with both indices higher than 0.5 - as FR. Moreover, the tension and area for the first, second and later for each tenth tetanus were calculated and analysed. The tension was measured from the baseline to the peak of the tension record (no matter where this peak occurred within the record). The area was calculated between the baseline and the curve of the record of tension (Fig. 1).

Seventy-two motor units (25 FF, 35 FR and 12 S type units) were investigated in our study. The mean values of principal properties of motor units studied are given in Table I.

Figure 2 shows examples of tetani of FF, FR and S motor units. These tetani were used to calculate both types of fatigue indices. Their values are shown in the Figure.

TABLE I

Mean values and their standard deviations for the principal properties of three types of motor units. CT, contraction time, measured as the time from the beginning of single twitch to its peak; HRT, half-relaxation time, measured from the peak in tension of the single twitch to a decrease of tension to a half of the peak value; TwT, twitch tension, measured for single twitch record; TetT, tension of fused tetanus; SFI, standard fatigue index; MFI, modified fatigue index

	CT (ms)	HRT (ms)	TwT (mN)	TetT (mN)	SFI	MFI
FF	13.5 ± 2.1	13.9 ± 3.8	54.9 ± 27.1	162.5 ± 57.2	0.28 ± 0.14	0.11 ± 0.11
FR	14.5 ± 2.1	16.1 ± 3.1	16.8 ± 12.7	77.3 ± 39.6	0.87 ± 0.09	0.87 ± 0.11
S	25.9 ± 5.2	38.7 ± 9.3	3.6 ± 1.0	28.8 ± 6.7	0.97 ± 0.03	0.99 ± 0.04

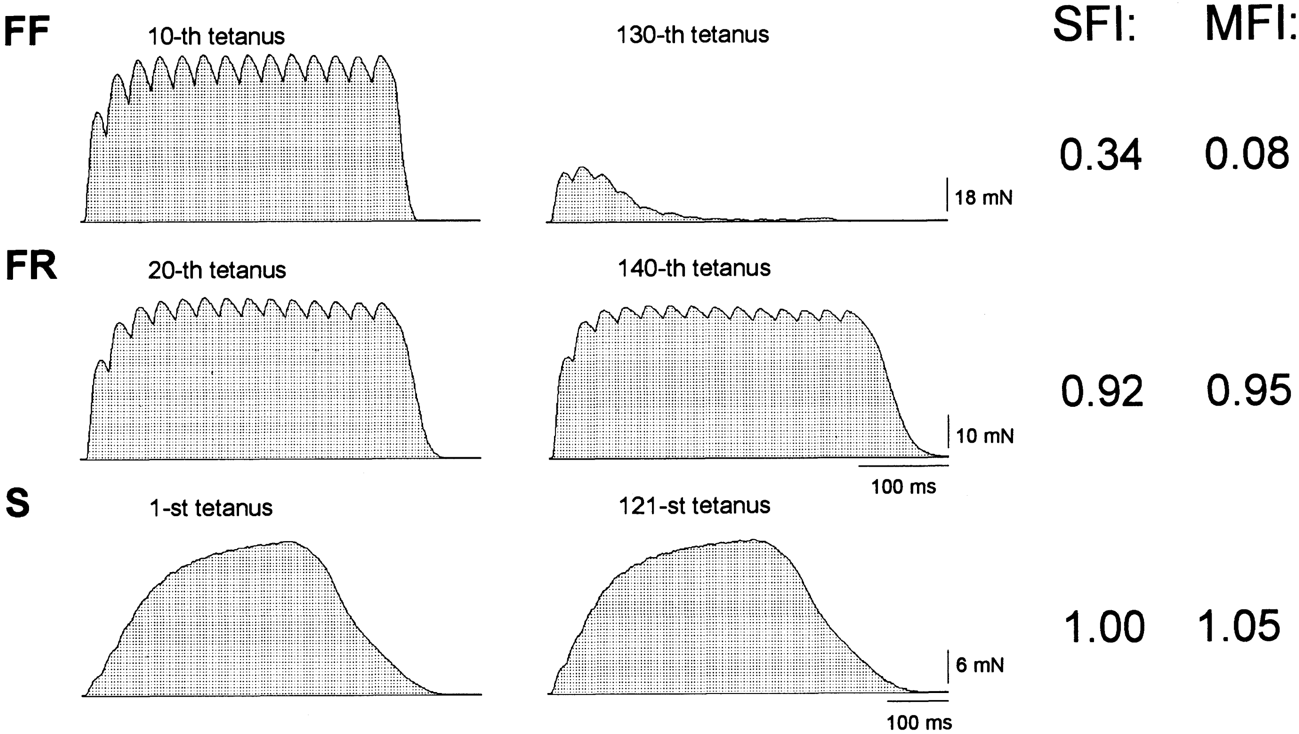

Fig. 2. Examples of tetani recorded within the fatigue test for FF, FR and S motor unit. Tetani presented on the Figure were recorded at 120 s time intervals and were used to calculate both fatigue indices. Tetani of fast units presented on the left side are maximally potentiated tetani within the fatigue test. The values of both fatigue indices are shown on the right. The sequence number of each tetanus within the fatigue test is given over each record.

Figure 3 shows the plot of values of the modified fatigue index as a function of the standard fatigue index. Mean values of both fatigue indices for three types of motor units are given in Table I. For FR and S motor units mean values of both fatigue indices were similar and did not differ significantly (Student *t*-test, $P>0.05$). However, as shown in Fig. 3, for nearly all S and FR motor units with relatively high standard fatigue index (over 0.85) the modified fatigue index was slightly higher than the standard fatigue index (for S units see Table I, for FR units 0.93 ± 0.05 *versus* 0.91 ± 0.04, respectively, although the difference was also not significant). This observation resulted from the slowing of relaxation occurring in these motor units in later parts of the fatigue test (Fig. 2). The half-relaxation time (time of the tension decrease from the peak of the last contraction in tetanus to one-half of this tension) calculated for tetani recorded

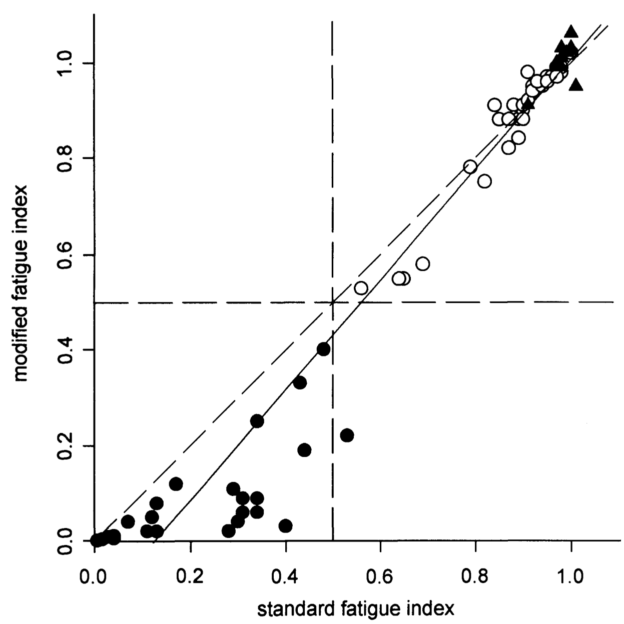

Fig. 3. The modified fatigue index as a function of the standard fatigue index. Interrupted horizontal and vertical lines correspond to the values of 0.5 for both fatigue indices. Filled circles, FF units; open circles, FR units; triangles, S units. Both indices correlated, correlation coefficient $r = 0.979$, $P<0.01$. Diagonal, solid line is the regression line whereas diagonal, broken line denotes equal values of both indices. Equation of regression: $y = -0.1497 + 1.1557x$.

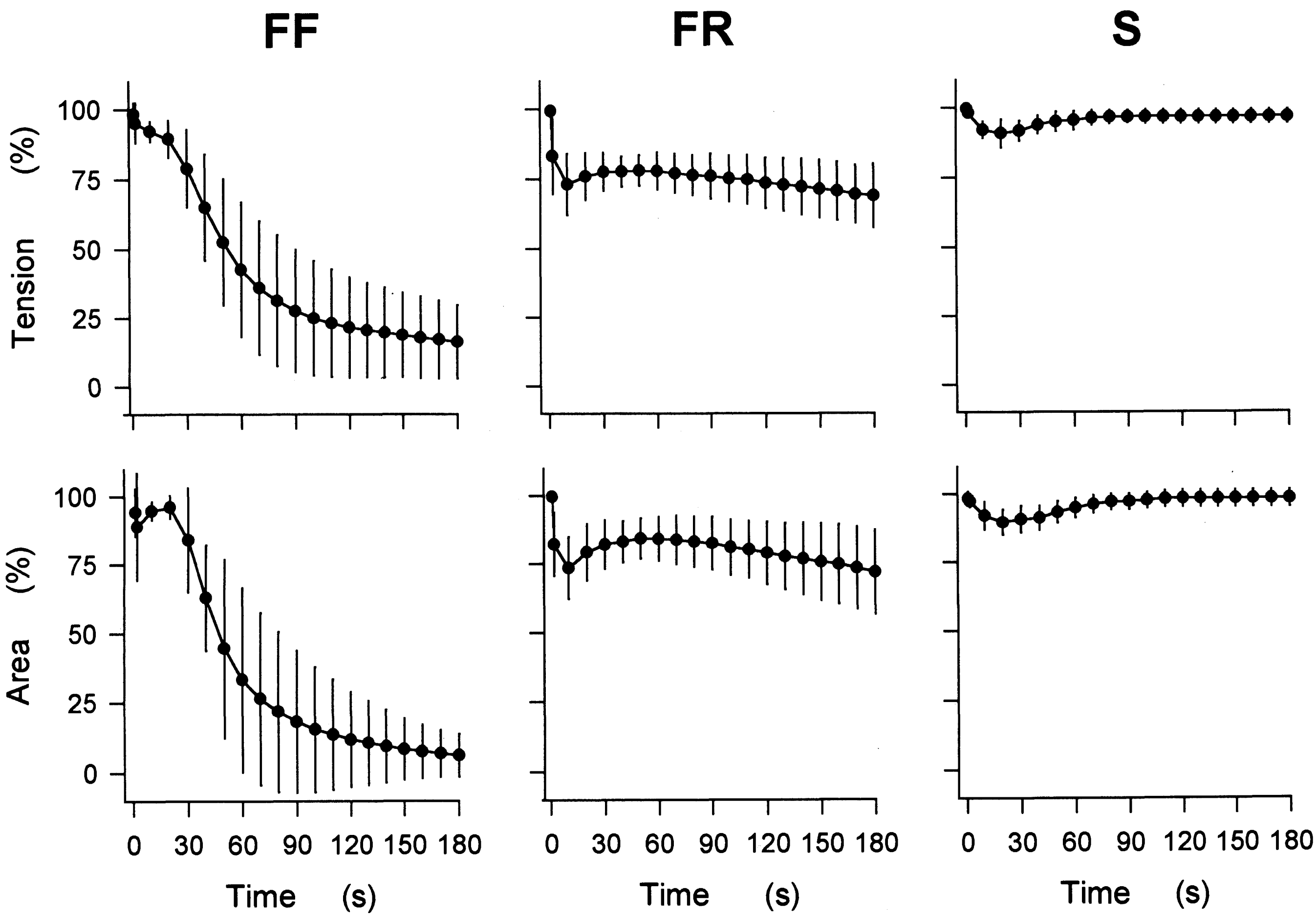

Fig. 4. Plots of changes in the averaged tetanic tension and area during the fatigue test for FF, FR and S motor units. Tetanic tension and tetanic area are given in their relative values; 100% corresponds to the highest average value of presented parameters. Vertical bars are the standard deviations.

after two minutes of fatigue test were prolonged by 7.3 ± 5.6% and 33.2 ± 22.8% for S and FR units, respectively. On the other hand, for all FF units studied, values of the modified fatigue index were lower than values of the standard fatigue index. This result was due to significant changes in the shape of tetani observed in these units after two minutes of the fatigue test. These changes in the shape of tetani were observed also for one motor unit with the standard fatigue index of 0.53. The modified fatigue index amounted in this case to 0.22 (Fig. 3). Therefore, this unit was classified as an FF type. The difference between mean values of both fatigue indices for FF units was significant ($P<0.01$).

Figure 4 presents averaged changes in tension and in area of successive tetani within the fatigue test as a function of time, for motor units of all three types. For FF units a faster decrease in the area than in the tension during the fatigue test was visible. For these units the rate of the decrease in the area measured when it reached 50% of its maximal value was higher than for the tension (2.79 ± 1.42%/s *vs* 1.69 ± 0.95%/s, respectively; difference significant, $P<0.01$). For FR and S units differences between relative values of tetanic tension and area presented on both plots were not significant ($P>0.05$).

In the present paper the method used previously to estimate work performed by a contracting motor unit was used to calculate the modified fatigue index. In our previous paper we have concluded that this method gives better evaluation of work performed during contraction than the measurement of tension alone (Celichowski et al. 1998). Results obtained in the present paper showed that for FF motor units in rat medial gastrocnemius the decrease in this work resulting from the fatigue was stronger than the decrease in the tension measured to the peak. This observation is consistent with our previous observation that the shape of FF motor unit tetani is changing during the fatigue test (Celichowski 1992b, Celichowski et al. 1996). After two minutes of this repeated activity the tension of a tetanus decreased within successive contractions (Fig. 2). It should be stressed that this change in the tetanus profile is a characteristic property of FF units in rat muscle (Gardiner and Olha 1987, Celichowski 1992b, Celichowski et al. 1996). This process results in stronger decrease in the area under the record of this contraction than in the peak tension, which for the first tetanic component remains relatively high. Moreover, our previous studies of the tension-frequency curve in fatigued FF motor units in the rat medial gastrocnemius showed that a steep part of this curve shifted towards higher frequencies in parallel to the shortening of the contraction time (Celichowski and Grottel 1997). Therefore, for fatigued FF units in the studied muscle a stimulation at 40 Hz can be even too low to evoke an unfused tetanus.

The application of measurements of the area under tetanic tension records for rat motor units enabled the estimation of fatigue processes with respect to changes in the shape of successive tetani which were described above. Therefore, the modified fatigue index seems to be more suitable for estimating fatigue resistance than the standard fatigue index. For one out of 25 FF units the modified fatigue index was lower than the standard one which exceeded the value 0.5 (Fig.3). The classification of this unit based on the modified fatigue index was in our opinion more precise because of visible changes in the shape of tetani typical for FF units. Moreover, it is worth stressing that the modified fatigue index gives even better separation of FF and FR units because mean values and their ranges of the modified fatigue index differ more for FF and FR units than corresponding values of the standard fatigue index. This observation results from the higher sensitivity of the tetanic area to processes of fatigue than of the standard parameter - the peak tension. However, this higher sensitivity rather concerns slightly fused tetani. Therefore, for motor units of slower contraction (as in cat gastrocnemius or soleus - Burke et al. 1973, 1974, Reinking et al. 1975) which generate relatively well-fused tetani during the fatigue test, both methods of calculation of fatigue indices should give similar results.

It is worth noting that a modified calculation of the fatigue index was also proposed by Reinking et al. (1975). These authors introduced the so-called cumulative fatigue index. Their fatigue index compared the sum of tetanic tensions recorded within two minutes of the fatigue test to the total sum of these tensions within four minutes of this test. The modified fatigue index, described in the present study, is more similar to the standard fatigue index introduced by Burke et al. (1973).

In conclusion, the modified fatigue index more precisely describes fatigue induced changes in tetani during the fatigue test of rat motor units than the standard fatigue index, especially in fast fatigable units.

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