Eur J Appl Physiol (1999) 80:276–284

ORIGINAL ARTICLE

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Surface mechanomyogram reflects the changes in the mechanical properties of muscle at fatigue

Accepted: 9 April 1999

Abstract The contractile properties of muscle are usually investigated by analysing the force signal recorded during electrically elicited contractions. The electrically stimulated muscle shows surface oscillations that can be detected by an accelerometer; the acceleration signal is termed the surface mechanomyogram (MMG). In the study described here we compared, in the human tibialis anterior muscle, changes in the MMG and force signal characteristics before, and immediately after fatigue, as well as during 6 min of recovery, when changes in the contractile properties of muscle occur. Fatigue was induced by sustained electrical stimulation. The final aim was to evaluate the reliability of the MMG as a tool to follow the changes in the mechanical properties of muscle caused by fatigue. Because of fatigue, the parameters of the force peak, the peak rate of force production and the peak of the acceleration of force production (d^2F/dt^2) decreased, while the contraction time and the half-relaxation time (½-RT) increased. The MMG peak-to-peak (p-p) also decreased. The attenuation rate of the force oscillation amplitude and MMG p-p at increasing stimulation frequency was greater after fatigue. With the exception of ½-RT, all of the force and MMG parameters were restored within 2 min of recovery. A high correlation was found between MMG and d^2F/dt^2 in un-fatigued muscle and during recovery. In conclusion, the MMG reflects specific aspects of muscle mechanics and can be used to follow the changes in the contractile properties of muscle caused by localised muscle fatigue.

Key words Muscle contraction physiology · Muscle fatigue · Mechanomyogram · Tibialis anterior

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Introduction

The mechanical properties of muscle are investigated mainly using electrical stimulation. The analysis of the force signal recorded provides information about the mechanical features of muscle. The contractile properties of muscle are studied by analysing the peak force, the contraction time (CT), the half-relaxation time (½-RT) and other parameters calculated from the force signal recorded during a single twitch (Takamori et al. 1971; McComas et al. 1986; Winter 1990). It has been demonstrated that twitch characteristics depend upon the relative proportion of fast or slow motor units in the muscle under investigation (Bellemare et al. 1983), or on the presence of fatigue (Thompson et al. 1992a).

The force versus stimulation rate relationship is another tool that can be used for characterising the contractile properties of muscle. The faster the twitch of the muscle, the higher the frequency at which fusion of the evoked mechanical events takes place (Cooper and Eccles 1930; Burke 1981). As a corollary, the force fluctuation at each frequency is larger in muscles that have fast twitches, and the force fluctuation amplitude versus stimulation frequency relationship reflects the contractile properties of the muscle (Cooper and Eccles, 1930).

When supramaximally stimulated, muscle behaves as "a large artificial motor unit" (Partridge 1965). Its contraction is accompanied by dimensional changes of the transverse diameter of the muscle, as detected by Marey, at the end of last century, in isolated and in vivo muscles (see Luciani 1923). These dimensional changes generate "in vivo" muscle surface oscillations that can

be recorded by means of an accelerometer (Barry 1992; Barry et al. 1992). A similar surface mechanical oscillation due to motor unit activity can even be recorded during voluntary contractions (Gordon and Holbourn 1948; Jorgensen and Lammert 1976; Petitjean and Maton 1995). When several motor units are active, the summation of the surface oscillations generates a signal that has been labelled a surface mechanomyogram (MMG; Orizio et al. 1996). This term has recently been suggested (CIBA Foundation Discussion Meeting: "Investigating muscle sounds by mechanomyography", London, December 12, 1995) as being preferable to the previously used ones (e.g. soundmyogram, phonomyogram, acousticmyogram) because it emphasizes the mechanical nature of the phenomenon independently of the transducer used to detect it.

The relationships between the parameters of the MMG and force signal have never been investigated while simultaneously spanning from single twitches to high stimulation rates. Even very recent papers on MMG (Celichowski et al. 1998; Esposito et al. 1998; Shinohara et al. 1998) have not examined MMG together with force during stimulated muscle contraction.

The aims of this study were: (1) to study, by comparison of the force signal and the MMG recorded during in vivo human muscle electrical stimulation, the relationship between MMG and the force parameters, and (2) to verify the reliability of the MMG as a tool to follow the changes in the contractile properties of muscle at fatigue.

Methods

Seven male subjects (age 20–40 years) gave their informed consent to participate after being given a full explanation of the experimental procedure. The local Ethical Research Committee approved the proposed experimental design.

Experimental set-up and signal detection

The experimental set-up is shown in Fig. 1. The cathode of the stimulator was positioned at the most proximal motor point of the tibialis anterior muscle (TA). The accelerometer and the probe with surface electrodes for MMG and EMG detection, respectively, were placed about 2 cm distal of the motor point. The force exerted by the ankle flexors was transduced by a load cell.

Force

The ergometer shown in Fig. 1 allowed us to record the force produced by the ankle flexors during isometric contractions at a neutral joint angle. The load cell (Interface, model SM-100 N) operated linearly between 0 and 100 N. The whole detection apparatus had a resonant frequency of >200 Hz. After conditioning (bandwith: DC 128 Hz) the electrical signal was stored on a personal computer (sampling rate 4096 Hz).

Mechanomyogram

The muscle surface oscillation was detected with the aid of an accelerometer (Entran EGASY- 25D, dimensions: $0.5 \times 0.5 \times 1.0$ cm; mass: 1 g; sensitivity: 4 mV/g; resonant frequency >1000 Hz). The detector was placed on the TA 2 cm distal of the most proximal motor point and 0.5 cm from the tibial crest. In this position an outward movement of the muscle surface is always present during TA stimulation (Orizio et al. 1994). The accelerometer was secured to the skin with double-sided sticky tape (Barry 1992). The long axis of the accelerometer was transverse to that of the TA. The accelerometer was placed in such a way that its sensitive axis was in the same direction of the muscle displacement during single twitches, and so that an initial positive deflection of the MMG was produced during muscle bulging (Barry 1992). The resulting MMG was stored on a personal computer (sampling rate 4096 Hz). The monoaxial accelerometer we used is able to pick up muscle surface movement in only one plane. As a consequence, given that in the region of the muscle surface we used to detect the MMG the sensitive axis of the accelerometer was in the same plane of the larger surface displacement, lesser movements of the skin in the other two planes were not revealed by this detection technique.

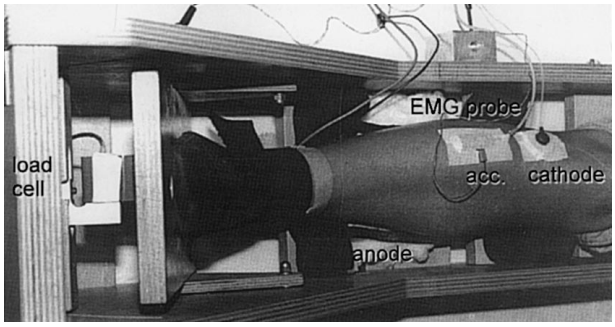

Fig. 1 The experimental set-up. The load cell for force measurement, the accelerometer (*acc*) for surface mechanomyogram (MMG) detection, the electromyogram (*EMG*) electrodes, the cathode of the stimulator (the *black rounded button* close to the knee), the anode of the stimulator (a *large rectangular sponge* below the gastrocnemious) and the reference electrode (the *strip* at the ankle) are indicated

Electromyogram

The EMG was detected by two silver bars (0.5×1.0 cm) spaced 1 cm apart. The signal was amplified and filtered (bandwith: 10–480 Hz). The stimulation artefact was eliminated from the EMG signal using the technique and apparatus described in Knaflitz and Merletti (1988). The EMG signal was also sampled at 4096 Hz and stored on a personal computer. The EMG probe was fixed to the skin by a special tape (Fixomull stretch, BDF, Germany) that allowed the muscle surface to move in order to avoid interference with the MMG recordings.

Electrical stimulation

A monopolar supramaximal stimulation was delivered to the TA. A large positive electrode (10×14 cm) was placed on the gastrocnemius muscle, while a rounded negative electrode (diameter 2 cm) was placed at the most proximal motor point of the TA. The duration of the stimuli was 100 μs. The pattern of stimulation, reported in Fig. 3, was as follows:

1. Test of the muscle contractile properties in un-fatigued muscle. Six single twitches (with 1-s interval followed by a 5-s period of repetitive stimulation in which the frequency was increased by 1 Hz between one stimulus and the next, in the 1–50 Hz range. Henceforth, this repetitive stimulation with increasing rate will be referred to as a "frequency sweep".

2. Fatiguing stimulation at 35 Hz for 40 s (not shown in Fig. 3).
3. Test of the muscle contractile properties in fatigued muscle. The same protocol reported in 1. above was repeated at 1 s (for sake of clarity the results of this trial will be reported at the 0-min time mark during recovery), 0.5 min, 1 min, 2 min, 3 min, 4 min, and 6 min after the fatiguing phase.

Experimental protocol All of the recordings were made in a room at constant temperature (22°C). After the identification of the most proximal motor point, a stimulation level that was 15% greater than that which induced a maximal compound motor action potential (cMAP) was chosen. The previously described stimulation pattern was delivered to the TA of the subject under investigation. Each subject took part in just one session.

Analysis of the data Single twitches: the force, the MMG and the EMG recorded during the six single twitches before fatigue at each time mark during recovery (from 0 to 6 min) were all averaged. The following parameters were calculated from the average force signal: the peak twitch force (P_t), the CT, which is defined as the interval between the force onset (when the force signal value was larger than three standard deviations of the baseline noise for at least three samples) to the peak force, the ½-RT which is defined as the time taken for the force to decline to half of the peak force value during the relaxation phase, the peak rate of force development (dF/dt), and the acceleration peak of the force development (d^2F/dt^2). The latter two parameters were calculated from the first and second derivative of the "on" phase of the force twitch. The peak-to-peak (p-p) value was measured from the average MMG signal. This parameter was compared to the force acceleration (see Fig. 5).

1–50 Hz frequency sweep: During the 5-s period, the MMG p-p and the force oscillation (FO) values for each time interval between two stimuli, from 1 s (1 Hz) to 20 ms (50 Hz), were measured. The relative MMG amplitude and FO at each stimulation frequency of the sweep was calculated by scaling their actual value to that measured at 1 Hz. The FO was calculated as the "delta" force added by each stimulus before the next one was delivered. The area (hatched in Fig. 2) beneath the relative MMG amplitude or FO versus stimulation rate (1–50 Hz) relationship was calculated. The ratio between these areas and the rectangle with height 1 (signal reference value) and the 1–50 Hz range as width were all calculated for MMG and FO. A ratio equal to 1 would indicate that no signal amplitude reduction took place when increasing the stimulation rate. On the contrary, the lower the ratio, the greater the degree of the fusion of the mechanical events evoked by the pulses administered at an increasing rate. At the end of the 1–50 Hz sweep, P_0 (the force at 50 Hz, i.e. at tetanic rate), was measured in un-fatigued muscle and during recovery. The p-p value and the average rectified value of the evoked cMAP, the mean of the six single twitche responses, before fatigue and at each time mark throughout recovery (see Fig. 3), were calculated.

Statistics

Since our data-sets consist of repeated measures, the significance of any difference in means was tested by a linear-mixed-effects model for repeated measures. The linear-mixed-effects model extends the generalized linear model in one important way: it allows a better evaluation of the correlation of intra-subject outcomes. With this model, we have analysed the following parameters: P_t, P_0, dF/dt, d^2F/dt^2, CT, ½-RT, MMG p-p, FO and MMG area ratio with respect to time. We have also studied the relationship between force d^2F/dt^2 and MMG p-p with a linear model.

A probability level of $P \leq 0.05$ was considered to be statistically significant. The statistical analysis was performed using the "proc Mixed" program from the SAS statistical package (Carry, N.C., USA).

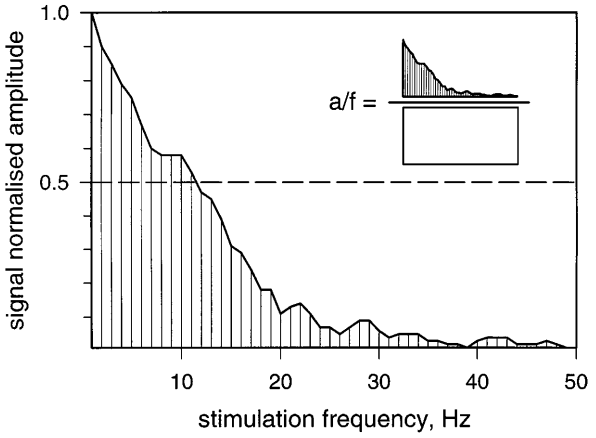

Fig. 2 Representation of the areas considered for the area ratio calculation. The *hatched area* is delimited by the relationship between the normalised signal amplitude (force oscillation or MMG peak-to-peak amplitude at a given frequency scaled to the value at 1 Hz) and the stimulation rate. The lower the ratio between this *hatched* area and the *white rectangle*, the larger is the fusion of the mechanical events that occur with the increasing stimulation rate

Results

Force and MMG

The force and MMG during single twitches and the frequency sweep before, immediately after and at 360 s of recovery are shown in Fig. 3. For clarity the intermediate recovery stimulation (at 0.5. 1, 2 and 4 min) are not reported.

Single twitches

The average (SD) (through the subjects, $n = 7$) of the force parameters and MMG amplitude before the fatiguing stimulation and at the different time marks of recovery are shown in Fig. 4. On average, P_t, dF/dt and d^2F/dt^2 were reduced by about 50% compared to the pre-fatigue value, and most of their recovery took place within 2 min: statistical analysis revealed that the difference with respect to the reference value was not significant at 2 min for P_t and dF/dt or at 1 min for d^2F/dt^2. The following 4 min of recovery showed no further change in the values of the three parameters, with no significant differences found at 2, 4 and 6 min.

As expected, the CT and ½-RT increased because of fatigue, being 130% and 150% of the reference value, respectively. At the 0.5-min time mark, CT and ½-RT values were closer to the pre-fatigue values than after 1 and 2 min of recovery. After the fatiguing stimulation, the average values were only significantly different from the reference values at the 30-s time mark for CT. For ½-RT, however, average values were significantly different from the reference value for the whole recovery period.

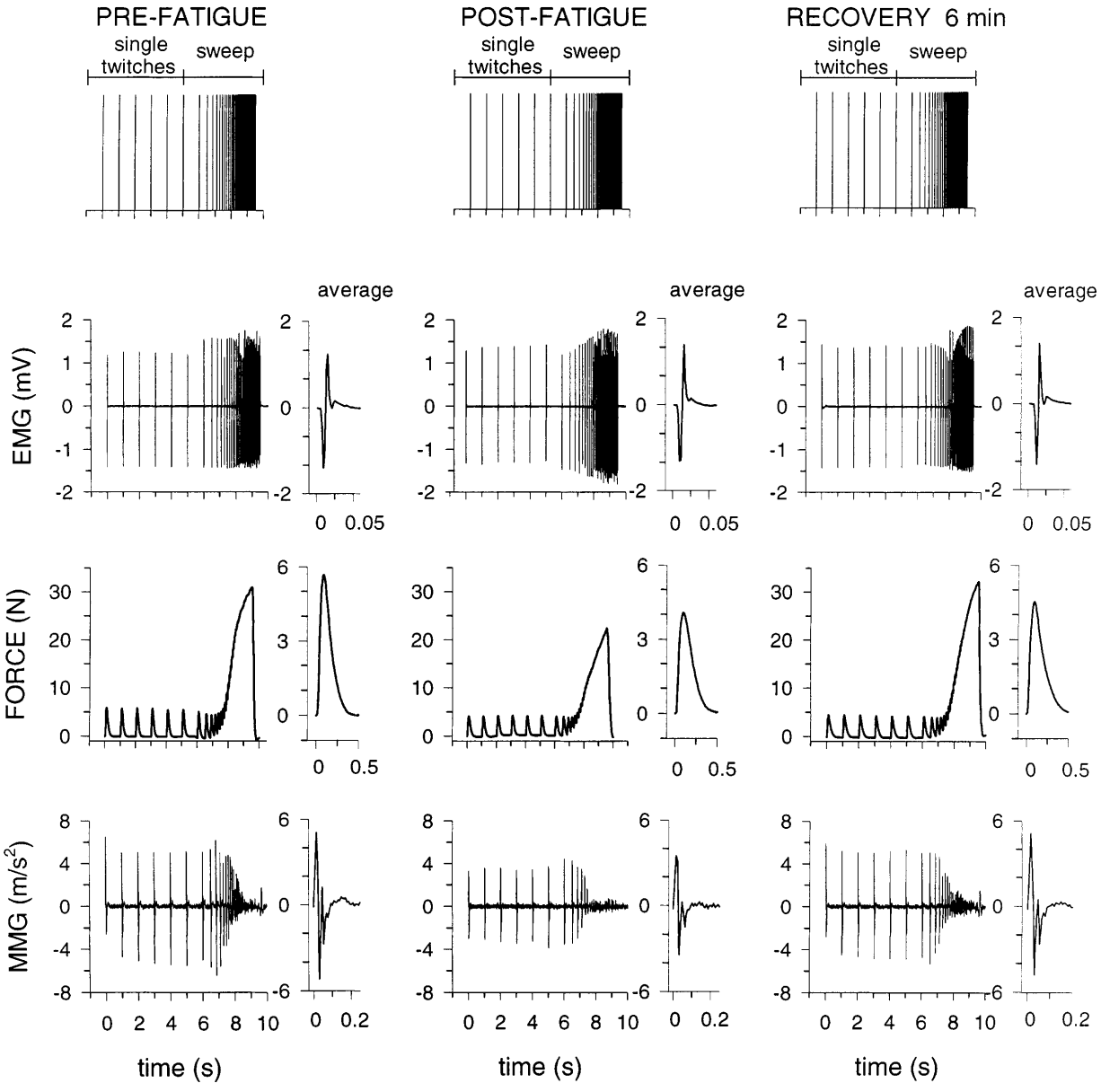

Fig. 3 In the four rows from top to bottom are the stimulation pattern (six single twitches and the 1–50 Hz sweep), the EMG, the force, and the MMG. For clarity only the responses before (PRE-FATIGUE), immediately after (POST-FATIGUE), and 6 min after the fatiguing stimulation (RECOVERY 6 min) are reported. The average of the electrical and mechanical responses during the six single twitches have been added to the right of the three signals. For details see text

The behaviour of the MMG amplitude was similar to the d^2F/dt^2 trend (see Fig. 4). As for d^2F/dt^2, the MMG p-p had recovered and was not significantly different from the pre-fatigue value after 1 min of recovery. The relationship between the pooled data of force twitch acceleration (49 individual data points) and the corresponding MMG (49 individual data points) is reported in Fig. 5. The regression line equation is: MMG p-p (m/s^2) = 1.3 + 1.23 d^2F/dt^2 (N/s^2) (R^2 = 0.76; adjusted R = 0.75; $P < 0.0001$).

1–50 Hz frequency sweep

Because of the fatiguing stimulation, the force at 50 Hz (P_0) was reduced, on average, by about 30% with respect to its pre-fatigue value (see Fig. 6). A consistent recovery had already occurred in the first 0.5 min of recovery, and no significant differences with respect to the pre-fatigue value were detectable in the remaining 1- to 6-min period. The behaviour of the area ratios of FO and MMG were similar. The degree of change of MMG area ratio at fatigue (−40% on average) was larger compared to that of the force (−22% on average). The FO area ratios were significantly different from the reference value only immediately after the fatiguing stimulation, while the MMG area ratio did not present a significant improvement even at 2 min of recovery. Throughout recovery, the area ratios had a reversed trend compared to the force twitch time variables (CT and ½-RT).

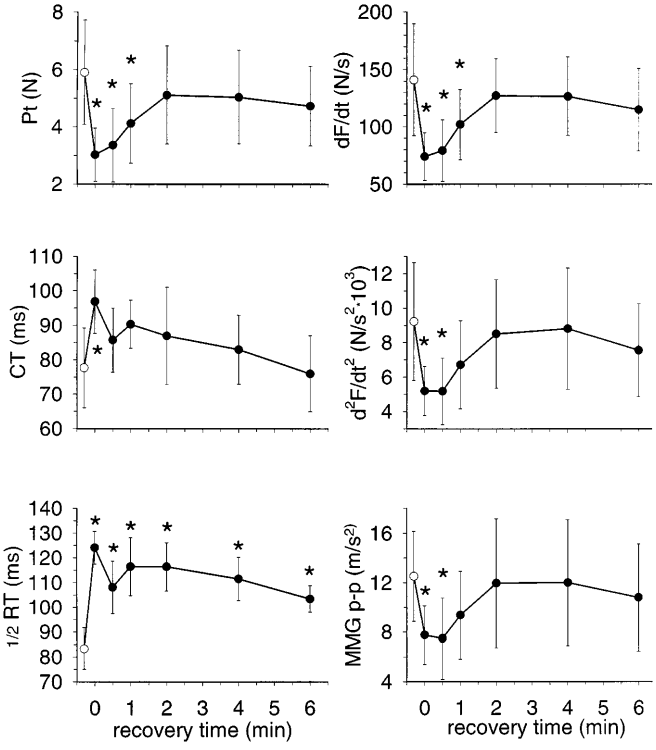

Fig. 4 Single twitches. Force and MMG parameters in un-fatigued muscle (*open circle*) and throughout recovery (*filled circles*). The data points are average ± SD across the seven subjects at each time mark. The *asterisks* indicate a significant difference from the reference value before fatigue. For details see text. (*Pt* Peak twitch force, *CT* contraction time, *½RT* half-relaxation time, *dF/dt* peak rate of force development, d^2F/d^2t acceleration peak of the force development, *p-p* peak-to-peak)

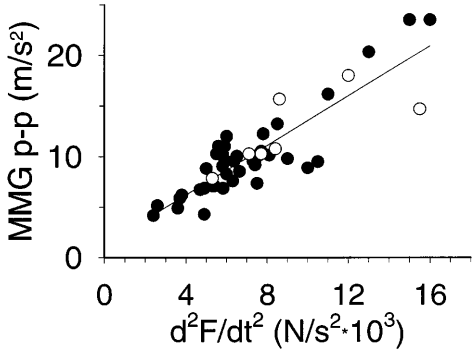

Fig. 5 MMG p-p versus d^2F/dt^2 relationship [49 points, 7 points for each subject: pre-fatigue data (*open circles*) plus the 6 data points throughout recovery]. The relationship demonstrates that the changes in the force acceleration are mirrored by the changes in the MMG p-p

EMG

In un-fatigued muscle, during single twitches, the values of the cMAP amplitude and of the average rectified value of the EMG signal, averaged across the subjects, were 2.32 (1.19) mV and 14 (5) mV, respectively. After the fatiguing stimulation these parameters showed no significant reduction.

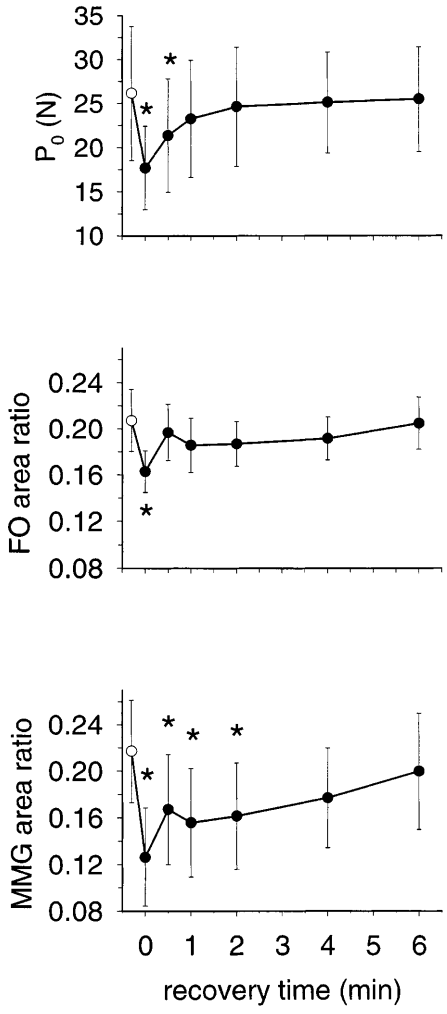

Fig. 6 Frequency sweep. Force at 50 Hz (P_0), force oscillation (*FO*) and MMG area ratios in un-fatigued muscle (*open circles*) and throughout recovery (*filled circles*). The data points are average ± SD across the seven subjects at each time mark. The *asterisks* indicate a significant difference with the reference to the value obtained before fatigue. For details see text

Discussion

Within the limits of the experimental design we used, our results showed that the surface MMG properties were related to the rate at which the muscle mechanical response was produced. On this basis, if these results are proven to be the same in different muscles and under other experimental conditions, it can be suggested that MMG can be used to describe the fatigue effect on some of the properties of muscle mechanics. An important future preliminary step should involve the analysis of the timing between the electrical activity of the muscle, the force signal at the tendon and the MMG. Indeed, as recently emphasized by Orizio et al. (1999), the understanding of the full significance of the MMG needs investigations aimed at designing an electromechanical model that is able to explain the existence of different

simultaneous EMG versus force, EMG versus MMG, and MMG versus force relationships.

Preliminary considerations

The goal of the fatiguing stimulation was to alter the contractile properties of the muscle in order to check whether the MMG and force reflected, to the same extent, some of the mechanical parameters of contraction in un-fatigued muscle, and to study the changes in these parameters as a result of fatigue. A good compromise between providing mechanical fatigue, stimulation time and the lowest possible discomfort for the subject (which is mainly related to the stimulation frequency) was found by using the 35 Hz for 40 s protocol. This was similar to the 35 Hz for 30 s protocol used by Rossi et al. (1993) and the 36 Hz for 45 s protocol used by Thomas (1997). The analysis of the EMG and force signals allowed us to define a hypothesis about the type of fatigue induced by the adopted stimulation. The EMG parameters, cMAP amplitude and integrated cMAP value presented only minor changes after the 40-s stimulation period at 35 Hz, as previously shown by Thomas (1997). This suggests that our fatiguing protocol mainly affected the mechanical response of the muscle to single twitches and the frequency sweep.

A significant change in the electrical response is a peculiar aspect of high-frequency fatigue of "in situ" stimulated human muscle (Bigland-Ritchie et al. 1979; Jones 1981). Therefore, this type of fatigue may be excluded from our study. On the contrary, low-frequency fatigue (LFF) may be the result of our sustained 35 Hz stimulation. In fact, LFF, due to a dysfunction in electromechanical coupling, presents a force reduction during single twitches and low stimulation rates such as 20 Hz (Bigland-Ritchie et al. 1986; Binder-Macleod et al. 1995). The larger relative reduction in P_t compared to the force at 50 Hz (P_0) at each time mark during recovery, and the faster normalisation of P_0 (see Figs. 4 and 6) suggest that some aspects of LFF are present after stimulation of the TA for 35 s at 40 Hz.

The variables considered for the force signal characterisation during single twitches and the 1–50 Hz sweep are well known (Takamori et al. 1971; Fitts 1994). In particular, the d^2F/dt^2 observed during the contraction phase of the single twitch has been compared to the MMG p-p value (Takamori et al. 1971). The rationale behind this comparison arises from the fact that during isometric muscle activity the force-generation process is paralleled by changes in muscle thickness (Partridge and Benton 1981). This can be explained by the fact that during isometric contractions, muscle fibres shorten (Griffiths 1991; Narici et al. 1996). In this situation the muscle behaves as a near-constant-volume system (Huijing 1995), with a shortening of the long axis (Griffiths 1991; Narici et al. 1996) and an obvious change in the transverse diameter. The result is that the tension generated at the tendon is accompanied by muscle surface displacement. The second derivative of this displacement with respect to time is represented by its acceleration. This is the variable of the displacement that is transduced by an accelerometer applied to the muscle surface during evoked contractions. As a consequence, the most convenient comparison can be made only with the second derivative of the other simultaneous mechanical event (i.e. the force produced at the tendon).

Un-fatigued and fatigued muscle contractile properties estimated by the force signal

In this study the P_t value was lower than expected from the torque moment values reported during motor nerve stimulation (McComas et al. 1986; Moglia et al. 1995). This may be due to the fact that we did not stimulate the whole TA, but only those motor units close to the most proximal motor point. However, we preferred to use motor-point surface stimulation to obtain an easier selective activation of the TA. All other parameters of the force twitch studied (CT, ½-RT, dF/dt, d^2F/dt^2) were similar to the data reported in current literature in un-fatigued muscle. To the authors' knowledge, studies dealing with alterations of the parameters of the human muscle force signal, measured during single twitches and repetitive stimulation, and evaluated immediately after and throughout recovery from electrically induced muscular fatigue are scarce in the literature. As a consequence, in order to discuss the influence of fatigue on muscle contractile properties and the characteristics of the time course of the force parameters during recovery, we will refer mostly to data obtained from non-human muscles.

The reduction in P_t, P_0 and dF/dt that we observed with fatigue (about 55%, 70% and 57% of the un-fatigued value, respectively) and their recovery time course are quite similar to these reported by Metzger and Fitts (1987) in diaphragm muscle when stimulated at 5 Hz for 1.5 min. CT and ½-RT increased with fatigue (+25% and +50%, respectively). However, while CT reached its reference value after 1 min of recovery, ½-RT was still significantly longer 6 min after the end of fatiguing stimulation. Our data confirm the larger and longer deviation from the reference value of ½-RT than CT immediately after fatiguing stimulation and along the recovery phase reported for rat diaphragm (Metzger and Fitts 1987) and for frog semitendinosus (Thompson et al. 1992a, b). The fact that CT and ½-RT are prolonged in fatigued TA may explain the better fusion of the mechanical events evoked during the frequency sweep, and the lower reduction and faster recovery of P_0 with respect to P_t (see Fig. 4).

The changes in pH, inorganic phosphate concentration ([Pi]), and Ca^{++} release and re-uptake that take place at fatigue may alter the efficiency of the cross-bridge cycle during both contraction and relax-

ation, and may influence the value of the force parameters we used to characterise the contractile properties of the muscle (Metzger et al. 1989; Fitts, 1994; Allen et al. 1995). In fatigued muscle the tension recovery process takes place in two phases; the first is shorter (about 2 min) than the second (up to tens of minutes; (Fitts 1994). The fast phase of contractile restoration may involve an increase in Ca^{++} release from the sarcoplasmic reticulum (SR) (Metzger and Fitts 1987) which was previously impaired by the fatiguing activity. Enoka and Stuart (1992) stated that the fast phase of contractile recovery may be related to the re-establishment of the pre-fatigue values of the concentrations of some metabolites, such as [Pi] (its normalisation within 2 min may play an important role; Miller et al. 1987). In our study P_t, P_0, dF/dt and d^2F/dt^2 were not significantly different from their unfatigued values after only 2 min of recovery from the fatiguing stimulation, suggesting that our fatiguing protocol did not produce the dramatic metabolic changes that require tens of minutes to recover but only alterations in the electromechanical coupling that recover quickly.

At fatigue, the increases in CT and ½-RT are due to the decrease and elongation of the Ca^{++} transient (Thompson et al. 1992a). As shown in Fig. 3, the CT and ½-RT values at the 30-s time mark were shorter than at 1 min. It may be hypothesised, therefore, that in the 1st min of recovery, factors that tend to normalise the characteristics of the Ca^{++} transient may coexist with factors that still deviate from normality and influence negatively the Ca^{++} release or re-uptake processes. Metzger and Fitts (1987) reported that pH "continued to fall in the 1 min of recovery". This may dominate in the second half of the 1st min of recovery. In our study, the ½-RT was the sole force parameter that even after 6 min of recovery was still significantly different from the reference value. The same result was obtained by Thompson et al. (1992a) for frog semitendinosus muscle after 30 min of recovery from intermittent fatiguing stimulation. This suggests that the our fatiguing protocol produces a "long-lasting element of fatigue" (Metzger and Fitts 1987), related to the SR Ca^{++} re-uptake capacity, that in turn determines the persisting alteration in ½-RT.

Similarly to CT, the FO area ratio was completely recovered by 30 s after the end of fatiguing stimulation. This suggests that the time taken by the muscle to reach P_t is crucial in determining the degree of FO at a particular frequency.

Analysis of MMG and comparison with the force signal

The use of an accelerometer to monitor the surface mechanical oscillation during muscle electrical stimulation is a relatively new technique (Barry et al. 1992; Orizio et al. 1996). Before and after an isometric voluntary contraction of the first dorsal interosseus muscle, the electrically evoked MMG was shown to change from about 17 m/s^2 to about 5 or 10 m/s^2 (Barry et al. 1992). The degree of MMG reduction was related to the intensity of the fatiguing isometric voluntary effort. The larger acceleration of the muscle surface displacement reported in the study of Barry et al. (1992) compared to our results in un-fatigued muscle may be due to the larger proportion of fast-twitch fibres in the first dorsal interossues muscle in comparison with the TA (Johnson et al. 1973). Moreover, in the study of Barry et al. (1992), the whole muscle was activated by supramaximal stimulation of the ulnar nerve. The parallel decrease of both the muscle lateral displacement acceleration and force twitch with fatigue, led the investigators to conclude that changes in the properties of the surface oscillation may be a tool to monitor the mechanical aspects of fatigue. In an attempt to provide more insight into this topic we compared the MMG and the d^2F/dt^2 during single twitches. A comparison of the time course of d^2F/dt^2 and MMG p-p during single twitches (Fig. 4) showed that the behaviour of the force acceleration and that of the muscle surface acceleration were well correlated. The d^2F/dt^2 probably reflects the intensity of the active state related to the amount of Ca^{++} released from the SR (Takamori et al. 1971). Therefore, it may be that d^2F/dt^2 and MMG p-p during single twitches mirror the changes in the properties of the Ca^{++} transient. The linear correlation between the pooled data of the d^2F/dt^2 and MMG p-p (Fig. 5) indicates that the surface MMG is linked to specific aspects of the force-generation process. This confirms that the production of tension at the tendon is always coupled with muscle dimensional changes, as reported previously (Partridge and Benton 1981; Winter 1990; Orizio et al. 1999).

Our data show that in the un-fatigued TA, the FO and MMG versus stimulation rate relationships are similar to those reported by Stokes and Cooper (1992) for the human adductor pollicis muscle. These authors obtained their relationships by stimulating the muscle at 10, 20, 30, 50, 70 and 100 Hz. As a consequence, their data are not directly comparable with ours. Nonetheless, our results and those reported by Stokes and Cooper (1992) confirm that the MMG amplitude reflects, as does the FO, the degree of fusion of the mechanical events evoked by stimulation of the muscle at a given frequency.

Because of fatigue, contrary to the FO area ratio, the MMG area ratio took more than 2 min to normalise (Fig. 6). This means that during repetitive stimulation at an increasing rate, the amplitude versus frequency relationship of the acceleration of the muscle displacement (i.e. the MMG) required a longer time to recover than that of FO. Since the MMG amplitude during the single twitch normalised within 1 min, we have to conclude that the recovery of the acceleration of the muscle surface oscillation is faster during a single twitch than during repetitive increasing-rate stimulation. To explain this dual behaviour of the MMG we have to make some hypotheses:

1. The relaxation process is altered for a long time after fatiguing stimulation (see the ½-RT time course during recovery). This may determine a slower reduction of the muscle cell transverse diameter between one motor command and the next at increasing frequencies. As a consequence, a reduction of the surface displacement related with each stimulus, and hence of its second derivative transduced by the accelerometer, may occur. This phenomenon may be not evident at 1 Hz because in this case enough time is provided to the fibre for the recovery of the initial transverse diameter. Future studies aimed at measuring the absolute muscle displacement will contribute to verifying this hypothesis.
2. Takamori et al. (1971) related the maximum acceleration of the force during the "on" phase of a single twitch to the amount of Ca^{++} released by the SR. Given the relationship between force acceleration and MMG that we verified (see Fig. 5), it may be suggested that, contrary to the single twitch, at increasing stimulation rates the mechanism of Ca^{++} release reveals that recovery is still not complete 2 min after the end of fatiguing stimulation. Future studies designed to compare the force acceleration and MMG at increasing stimulation rates will verify the reliability of this hypothesis.
3. During isometric contraction, the shortening of the muscle fibres corresponds to a lengthening of the connective tissue (Taylor et al. 1997). The connective tissue is the visco-elastic component of the muscle mechanical model; alteration in the properties of the viscous or elastic elements with fatigue may be revealed during repetitive stimulation and may persist for a long time.

In the un-fatigued muscle a direct relationship exists between the number of active cross-bridges and the force output as well as the muscle active stiffness (Metzger and Moss 1990). The observation that in un-fatigued muscle the evoked MMG exhibited a larger amplitude and frequency content at increasing evoked output tensions led Barry (1992) to suggest that the MMG could be an indirect measure of muscle stiffness. In fatigued muscle the reduction in force is much larger than the reduction in stiffness: this indicates that the number of active cross-bridges is poorly influenced by fatigue, which is likely to reduce the force per cross-bridge (Fitts 1994). Our results indicate that the relative changes in MMG and force as a result of fatigue are quite similar. On this basis, it may be hypothesised that at fatigue the changes of the MMG amplitude may correlate with variations in the rate of force production, while it does not reflect muscle active stiffness.

Conclusions

The comparison between the force twitch parameters, especially the d^2F/dt^2, with the surface MMG, particularly the MMG p-p and the analysis of the MMG area ratio time course during recovery after fatigue, suggests that the surface MMG reflects specific aspects of muscle contraction. Moreover, when the mechanical properties of muscle change because of fatigue, the MMG may be used to point out the phenomenon. This is of particular interest when force measurements are difficult to obtain from a specific muscle group.

Acknowledgements We thank Dr. Ornella Gabriotti for her skilful contribution to the iconographical section. This work was supported partly by the Italian MURST grant 960547 and by "Centro Studi di Fisiologia del Lavoro Muscolare e dello Sport" dell'Università di Brescia.

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