Combined in situ analysis of metabolic and myoelectrical changes associated with electrically induced fatigue

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Darques, J. L., D. Bendahan, M. Roussel, B. Giannesini, F. Tagliarini, Y. Le Fur, P. J. Cozzone, and Y. Jammes. Combined in situ analysis of metabolic and myoelectrical changes associated with electrically induced fatigue. J Appl Physiol 95: 1476–1484, 2003.—Electrical muscle stimulation (Mstim) at a low or high frequency is associated with failure of force production, but the exact mechanisms leading to fatigue in this model are still poorly understood. Using 31P-magnetic resonance spectroscopy (31P-MRS), we investigated the metabolic changes in rabbit tibialis anterior muscle associated with the force decline during Mstim at low (10 Hz) and high (100 Hz) frequency. We also simultaneously recorded the compound muscle mass action potential (M-wave) evoked by direct muscle stimulation, and we analyzed its post-Mstim variations. The 100-Hz Mstim elicited marked M-wave alterations and induced mild metabolic changes at the onset of stimulation followed by a paradoxical recovery of phosphocreatine (PCr) and pH during the stimulation period. On the contrary, the 10-Hz Mstim produced significant PCr consumption and intracellular acidosis with no paradoxical recovery phenomenon and no significant changes in M-wave characteristics. In addition, the force depression was linearly linked to the stimulation-induced acidosis and PCr breakdown. These results led us to conclude that force failure during 100-Hz Mstim only results from an impaired propagation of muscle action potentials with no metabolic involvement. On the contrary, fatigue induced by 10-Hz Mstim is closely associated with metabolic changes with no alteration of the membrane excitability, thereby underlining the central role of muscle energetics in force depression when muscle is stimulated at low frequency. Finally, our results further indicate a reduction of energy cost of contraction when stimulation frequency is increased from 10 to 100 Hz.

THE FAILURE OF FORCE PRODUCTION during voluntary efforts may result from different mechanisms, including an alteration of muscle membrane excitability, an impaired excitation-contraction coupling, a reduction of intramuscular concentration of high-energy phosphates, an accumulation of by-products of metabolism, e.g., lactate, inorganic phosphate (P,) (20, 24), and also the development of central fatigue involving spinal as well as supraspinal factors (25).

The direct repetitive electrical muscle stimulation (Mstim) allows production of muscle fatigue without any central fatigue. This fatigue protocol has been widely used in animals (1, 8, 16, 55) and also in humans (18, 25, 35, 41). However, the physiological events associated with the loss of force-generating capacity during Mstim vary according to the pattern of electrical stimulation (19, 20), indicating that fatigue is a complex, multifactorial phenomenon that should be investigated with the use of combined approaches. It is well documented that at high stimulation frequency (≥80 Hz) force is maintained for a very short period of time (a few seconds) and then dramatically decreases on continuation of muscle stimulation, whereas when a muscle is repeatedly stimulated at 20 Hz or less (low frequency) the force is kept constant for a longer period of time and its rate of decline may be very slow (1, 15, 16, 35, 41, 62). These results are highly dependent on duty cycle, pattern of stimulation, and fiber types. In addition, after high-frequency Mstim the muscle is able to reproduce the control maximal force and Twitches within a few minutes, whereas recovery is not complete 20 min after low-frequency Mstim trial (16, 24). Numerous studies have already shown that the high-frequency Mstim elicited a marked slowing of the membrane propagation of myopotentials, illustrated by recordings of the evoked compound muscle mass action potential (M-wave) and suggesting the existence of an altered membrane excitability (1, 3, 7, 16, 35, 41, 42, 48, 62). On the other hand, this altered muscle electrical activity is absent or modest after low-frequency Mstim (1, 16).

From a metabolic point of view, Mstim-induced fatigue elicited in in vitro muscle preparation is associ-
Rabbits were housed in an environment-controlled facility and the French Law on Animal Handling and Protection. Static contractions in humans, our laboratory has pre-

were not investigated. During and after voluntary and intramuscular acidosis in rabbit muscle stimu-

changes such as phosphocreatine (PCr) consumption have combined myoelectrical and metabolic recordings. In vivo studies are of more physiological interests given that muscles in situ are functioning at their normal temperatures, rather than the lower temperatures often used for in vitro work, and their blood supply is intact, which should diminish any diffusion issues. Ryschon et al. (58) have reported typical metabolic changes such as phosphocreatine (PCr) consumption and intramuscular acidosis in rabbit muscle stimulated at low frequency, but the myoelectrical aspects were not investigated. During and after voluntary static contractions in humans, our laboratory has previously reported (4, 5), using 31P-MRS, that changes in muscle energetics prevail, whereas other studies have shown that altered muscle membrane excitability is absent (18). Overall, myoelectrical and metabolic as-

pects of fatigue in relation to stimulation frequency have been investigated separately in the past. We aimed in the present study at examining in situ these aspects together during two fatigue schemes.

**METHODS**

**General animal care and muscle preparation.** Fourteen adult rabbits, weighing 2.5–3.0 kg, were used in these experiments according to the guidelines of the National Research Council Guide for the Care and Use of Laboratory Animals, and the French Law on Animal Handling and Protection. Rabbits were housed in an environment-controlled facility (12:12-h light-dark cycle; 22°C) and received water and standard rabbit food ad libitum until the time of experiment. At the end of experiments, animals were immediately eutha-
nized by an intracardiac injection of pentobarbital sodium.

Anesthesia was induced by an injection of a solution of ethyl-carbamate (1 g/kg urethane, Sigma Chemical, St. Louis, MO) in the marginal ear vein and was maintained by regular injections. Animals were tracheotomized, paralyzed by injections of pancuronium bromide (0.4 mg/kg iv, Pavulon, Organon Technica, Fresne, France), and ventilated at con-

stant volume (10 ml/kg) and frequency (40 breaths/min) with a volumetric pump. Both tibialis anterior muscles were dis-

sected and freed from surrounding tissues. The knee and ankle were firmly held on a dedicated cradle to avoid limb motions during electrical muscle stimulation.

**Electrically induced fatigue protocols and force measure-

ments.** The tibialis anterior muscle was directly stimulated via two silver electrodes as previously reported (38). They were positioned in the belly of the muscle (3 cm apart) for direct muscle stimulation. A conventional neurostimulator (S8800 Grass Instruments, Quincy, MA) delivered rectangu-

lar pulses of constant amplitude and frequency through an isolated unit (SIU5 Grass Instruments). Contractions were produced by trains of stimulation lasting 500 ms and re-

peated every 1.5 s (40 trains/min). Within each train, shocks were repeated at low (10-Hz; pulse duration = 1 ms, inten-

sity = 80 V) or high frequency (100-Hz; pulse duration = 0.1 ms, intensity = 120 V). These protocols were referred as 10-Hz Mstim and 100-Hz Mstim, respectively. The total stimulation period was 5 min at low frequency and 3 min at high frequency to elicit the same fall in force. According to previous observations (15, 16), we decided not to prolong the period of 100-Hz Mstim beyond 3 min to avoid deleterious effects, namely the disappearance of M-waves. It was techni-

cally impossible to have similar pulse duration for both fatigue schemes. Indeed, pulse duration larger than 0.1 ms repeated at 100 Hz tended to produce stimulator overheating, whereas 0.1 ms duration pulse repeated at 10 Hz did not produce a significant reduction of force. To measure the force output, the distal tendon was attached to a home-built iso-

metric strain gauge (range of gain: 500–1,000). Force was continuously recorded on a personal computer and analyzed by use of a dedicated software (ATS, Sysma, Aix-en-Pro-

vence, France), and it was expressed as the tension-time integral (g × s). In each animal, the two tibialis anterior muscles were studied, but only one stimulation protocol (10-Hz or 100-Hz) was used in each muscle. The rate of force during the activation muscle stimulation scheme was determined by dividing the maximal force decline (in g) by the total duration of the trial (3 min for 100 Hz and 5 min for 10 Hz). Because of the presence of potentiation during the 10-Hz protocol, the rate of force decline was then calculated with or without potentiation.

**M-wave recordings.** Two other nonmagnetic electrodes were positioned on the belly of the muscle (5 mm interelec-
trode distance), and the M-waves were elicited by direct muscle stimulation with single pulses (1 Hz, pulse dura-

tion = 0.05 ms, supramaximal intensity level). M-waves were recorded before each fatigue trial, immediately after the end of each stimulation scheme, and also at 2, 5, and 10 min during the recovery period of Mstim. The electrophysiological signal was amplified (Tektronix differential amplifier AM 502, Beaverton, OR, gain 500–1,000), filtered (30 Hz to 1 KHz), displayed on a storage oscilloscope (DSO 400 Gould, Illford, UK), and fed to a chart recorder (Gould 6120, Clev-

land, OH). Two parameters of the M-wave were analyzed: peak amplitude and duration.

**31P-MRS measurements.** Once the cradle was inserted into the 4.7-T superconducting magnet of the magnetic resonance (MR) spectrometer (Biopspec-Bruker), the dedicated coil was tuned to both 31P and 1H frequencies. The shimming proce-

dure was performed on the proton signal of muscle water. Acquisition of 31P MR spectra was gated to electrical muscle stimulations. The gating sequence was initiated by a pulse from the spectrometer, which triggered the stimulus output from the stimulator. During 31P MR spectroscopy, the follow-

ing parameters were used: sweep width 4 kHz, block size 2.048 points, interpulse delay 1.5 s, and a pulse width of 120 µs, which was selected to optimize the signal-to-noise ratio. The combination of these parameters and 16 scans/spectrum resulted in a 24-s temporal resolution. Metabolic parameters (PCr, P1, and intracellular pH) were continuously recorded before, during, and after each run of electrically induced Mstim. Free induction decays were processed with the use of NMR1 software (Syracuse, NY). After Fourier analysis, line broadening (exponential multiplication of 20 Hz), and baseline correction, the peak areas of P1 and PCr were derived by Lorentzian fitting. Intracellular pH was calculated by using the chemical shift of P1, as previously described (5).

**Statistical analysis.** Statistical analysis was performed with a commercially available software program (Sigmastat, Jandel Scientific). One-way repeated-measures ANOVA was used to depict Mstim-induced changes in force and M-wave

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characteristics, i.e., amplitude and duration. Regarding the biphasic profiles of PCr concentration ([PCr]) and pH during the high-frequency fatigue scheme, paired t-tests were used to compare three values during each fatigue scheme, i.e., the resting values, the minimum values, and the end-of-stimulation values. We used linear regressions, with 95% confidence interval, to compare the decline in force and the [PCr] and pH values reported for the same muscle (58). No significant difference was measured for any of the ratios or values reported for the same muscle (58). No significant difference was measured for any of the ratios or intracellular pH between both fatigue schemes (Table 1 and are in agreement with summaries in Table 1). On the contrary, end-of-stimulation values and minimum values were not recorded at the same time during the 100-Hz Mstim, thereby illustrating a biphasic profile (Fig. 3, B and C, and Table 1). Both PCr and pH transiently and significantly decreased whereas a paradoxical recovery occurred as the time-dependent pattern of force was not modified. When the failure of force production throughout the 10-Hz Mstim protocol was considered, a significant reduction of force output was recorded after a stimulation period of 120 s, while almost at the same time [PCr] and pH were both significantly reduced. The discrepancies between the responses to 10-Hz Mstim and 100-Hz Mstim were highlighted when the variations of metabolic variables ([PCr] and pH) were compared with the corresponding fall in force (Fig. 4). With 10-Hz Mstim, the force decrease was significantly correlated with the corresponding PCr consumption (Fig. 2A, $r = 0.975$, $P < 0.001$) and the intracellular acidosis (Fig. 2B, $r = 0.897$, $P < 0.001$). These relationships were not found under conditions of 100-Hz Mstim. In addition, ATP changes were not recorded during either 10- or 100-Hz Mstim, with end-of-stimulation values being 90 ± 3 and 96 ± 6% of the resting values for both conditions, respectively.

As shown in Fig. 5, M-wave duration did not change during the 10-Hz Mstim scheme; however, a small but not significant amplitude decrease was recorded. On the contrary, 100-Hz Mstim elicited significant changes in both variables with a 53.8 ± 9% decrease in amplitude and a 10.7 ± 1.5% increase in duration. As a matter of consequence, we found no correlation between the changes in M-wave characteristics (only present in the 100-Hz Mstim protocol) and the changes in muscle energetics (only measured in the 10-Hz Mstim protocol).

DISCUSSION

The present study is the first combining 31P-MRS measurements of muscle energetics and M-wave recordings in an attempt to investigate the different mechanisms of force failure during electrically induced fatigue at high and low frequency.

We have mainly shown that fatigue induced by low-frequency Mstim was associated with changes affecting the high-energy phosphate compounds and the intracellular pH, with no alteration of the M-wave characteristics. On the contrary, when the muscle was stimulated at high frequency, the marked force reduction was accompanied by M-wave variations, whereas changes in PCr level and intracellular pH were moderate and transient. A paradoxical recovery phenome-
non occurred for both variables during the stimulation scheme so that PCr level and pH had recovered their resting values by the end of the stimulation procedure. These very limited metabolic changes recorded during the high-frequency stimulation scheme may appear surprising given that mechanical work was produced at the same time. It also pointed out that the apparent energy cost of high-frequency contractions is lower than that of low-frequency contractions, as previously reported (56, 57). First of all, because of the inherent low sensitivity of MRS, metabolic changes are not recorded instantaneously but are time averaged. Because of this signal-accumulation procedure, an absence of changes may reflect either a real effect or a tight coupling between ATP production and consumption, resulting in a balance between both mechanisms that would ultimately result in no apparent changes. Such a balance resulting in no recordable changes in muscle energetics despite significant force production has been reported previously in chronically stimulated animal muscles (14, 58) during similar high-frequency stimulation procedures (68, 69) and is comparable to the reduction of metabolic changes mediated by endurance training (40). As reported previously, such a reduction of ATP cost of contraction and the resulting very limited metabolic changes is intimately linked to the frequency of stimulation (12, 32, 51, 66). During repeated contractions, ATP is used for both contractile and noncontractile processes, the latter process being related to ion transport associated with activation-

Fig. 2. Raw data for tension (A and B) and M-wave (C–F) traces. A and B: muscle tension time-dependent changes recorded during low- and high-frequency stimulation, respectively. C and D: M-wave traces recorded before and immediately after the low-frequency stimulation protocol. E and F: M-wave traces recorded during the high-frequency stimulation protocol.
relaxation cycle of muscle contraction. Therefore, any reduction of one of these processes throughout muscle activity would account for the overall reduction in metabolic changes. This assumption is supported by the fact that, for a given duration of contraction, ATP cost of contraction is lower for a single sustained isometric contraction when compared with repeated isometric contractions (12, 32, 56, 66) and is in agreement with previous results showing a reduction of ATP cost throughout a fatigue scheme in rat skeletal muscle (28). Actually, during a single sustained isometric contraction, ATP is mainly hydrolyzed to maintain tension whereas additional ATP consumption is required for ion transport between contraction during repeated isometric contractions, and it has been previously reported that 20–50% of the ATP utilized during conduction would be used for $Ca^{2+}$ pumping across the sarcoplasmic reticulum membrane (6, 32, 39).

Such a reduction of ATP cost of contraction when stimulation frequency is increased has also been reported by Russ and colleagues (56, 57), who provided a different explanation as to the reduction of ATP cost. They concluded from their investigations that the ATP cost reduction was due to the fact that the metabolic cost of attaining tension is greater than that of maintaining force during electrically induced isometric contraction in human skeletal muscle (56, 57). Overall, it is clear from this study and others that the ATP cost of contraction can be reduced when muscle stimulation frequency is increased, thereby reducing energy consumption associated with ion transport during relaxation phases and energy needed to attain force. Such a reduction together with the M-wave changes recorded during the 100-Hz Mstim most likely accounts for the very limited metabolic changes reported in the present study.

On the other hand, the paradoxical recovery phenomenon recorded during the high-frequency stimulation scheme would be in keeping with fibers becoming inactivated, this mechanism being due to membrane inexcitability as previously suggested (43) and illustrated by M-wave changes in the present study. This paradoxical recovery phase during the stimulation period has been reported previously in animal and human muscles (27, 34, 38). M-wave changes reported in the present study are in agreement with previous results (1, 7, 10, 15, 16, 18), which have been related to the inability to stimulate muscle fibers as a result of impaired action potential propagation. In addition, an increased blood flow to these fibers as a result of the lack of force production would permit the regeneration of PCr and proton export. Overall, M-wave changes could both explain the limited metabolic changes and the paradoxical recovery phenomenon during the high-frequency stimulation but the impairment of action potential propagation.

Table 1. Metabolic changes recorded throughout both HF and LF fatigue schemes.

<table>
<thead>
<tr>
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<th>LF Fatigue Scheme</th>
<th>HF Fatigue Scheme</th>
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<tbody>
<tr>
<td>Resting values</td>
<td></td>
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<tr>
<td>PCr/ATP</td>
<td>3.9 ± 0.76</td>
<td>3.5 ± 0.63</td>
</tr>
<tr>
<td>PCr/Pi</td>
<td>10.5 ± 2.51</td>
<td>8.9 ± 1.44</td>
</tr>
<tr>
<td>pH</td>
<td>7.05 ± 0.05</td>
<td>6.99 ± 0.06</td>
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<tr>
<td>Minimum values during stimulation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ΔPCr, %</td>
<td>-30.2 ± 4.6</td>
<td>-9.2 ± 0.4†</td>
</tr>
<tr>
<td>ΔpH</td>
<td>-0.46 ± 0.06</td>
<td>-0.23 ± 0.04‡</td>
</tr>
<tr>
<td>Time, s</td>
<td>214 ± 13.8</td>
<td>118 ± 7.6*</td>
</tr>
<tr>
<td>End-of-stimulation values</td>
<td></td>
<td></td>
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<tr>
<td>ΔPCr, %</td>
<td>-27.8 ± 5</td>
<td>0.45 ± 1.09*</td>
</tr>
<tr>
<td>ΔpH</td>
<td>-0.36 ± 0.04</td>
<td>-0.12 ± 0.03*</td>
</tr>
<tr>
<td>ATP, %</td>
<td>90 ± 3</td>
<td>96 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SE. LF, low frequency; HF, high frequency; ΔPCr and ΔpH, difference between PCr or pH value at this time (minimum value or end-of-stimulation value) and value at rest. *Significant difference between values recorded during the HF and LF protocols; †significant difference between minimum value and value recorded at end of stimulation period.
potential propagation is not related to any energetics failure.

The M-wave changes reported in the present study after 100-Hz Mstim are similar to what our laboratory has previously recorded in vitro (1) and are in keeping with the reduced action potential amplitude and the slowing and prolongation of action potential waveform reported during high-frequency Mstim in humans (7, 18), rats (10), and rabbits (1, 15, 16). They have been clearly related to the pattern of stimulation, regardless of the muscle composition (1, 7), given that they have been recorded in both fast-twitch and slow-twitch muscles (1) and considering that they could be reversed when changing the stimulation frequency (7). From the present results, a failure of energy production as a causative factor of 100-Hz Mstim-induced muscle fatigue may be discarded. Extensive K⁺/H⁺ shifts have been reported with exercise resulting in a dramatic K⁺/H⁺ accumulation in the extracellular space (64). It has been suggested that these shifts may cause fatigue through the effect on muscle excitability and action potentials, eventually leading to inexcitable fibers (10, 54, 63). Again, these data could both explain the paradoxical recovery occurring during the high-frequency muscle stimulation and the M-wave changes recorded immediately after the fatigue scheme, although the role of potassium in muscle fatigue still remains debated (2, 64) and calcium-mediated mechanisms would also play a significant role in high-frequency fatigue (1, 23, 30). On the other hand, Nielsen et al. (52) reported in isolated rat gastrocnemius muscle that K⁺ alone was related to a depression of force whereas force reduction and M-wave changes were absent when K⁺ (4 mM) and lactic acid (20 mM) were added together, thereby illustrating a potential protective effect of lactic acidosis on force production. Unfortunately, this hypothesis could not be accurately verified with our results.

The absence of an altered M-wave after 10-Hz Mstim is in agreement with in vivo (16) as well as in vitro (1) animal observations related to M-wave changes after low-frequency Mstim. This clearly shows that fatigue induced by low-frequency Mstim cannot be explained by an altered muscle electrical activity failure. Moreover, the present study shows that 10-Hz Mstim is closely linked to metabolic changes such as intracellular acidosis and PCr degradation. Several metabolic candidates as factors accounting for muscle fatigue have been put forth, particularly intracellular acidosis (9, 33, 37, 45, 46, 60, 61), Pi accumulation or more
exactly its diprotonated form (17, 31, 53, 71, 72), and ATP depletion (21, 59, 70). The present results clearly excluded ATP depletion as a cause of low-frequency fatigue given that ATP homeostasis was not altered. However, both PCr depletion (a mirror of Pi accumulation) and intracellular acidosis were linearly coupled to force depression. Accumulation of diprotonated Pi would directly inhibit the contractile process (31, 53, 71, 72). Such a mechanism has been demonstrated in NaCN-poisoned frog muscle undergoing repeated isometric contractions (17), in skinned rabbit muscle fibers (53), and in humans sustaining maximum voluntary contraction (72). Recent analyses of correlated evolution of phosphoric acid concentration ([H2PO4\(^-\)]) and force lead to the hypothesis that force production could be uncoupled by increased [H2PO4\(^-\)] (13) and that the inhibitory effect of Pi accumulation on force production could be actually mediated by calcium (26). Regarding intracellular acidosis as a factor involved in fatigue, its role might have been underestimated (37). Indeed, during a 4-min maximal isometric exercise, Kent-Braun reported that intracellular acidosis was significantly linked to both the fall in force production and the integrated EMG, thereby suggesting that such relationships might be consistent with the presence of a feedback loop between intramuscular metabolism and central motor drive during fatigue. Such a direct relationship between fatigue and H\(^+\) has been already reported in different muscles in humans (9, 33, 46, 61), in both cat biceps and soleus muscles (45), and under conditions of hypercapnic acidosis (60). Interestingly, NH\(_4\)Cl-induced metabolic acidosis results in both a larger decrease of pH and a larger depression of force, further indicating that the decrease of force is well correlated with the intracellular acidosis (60). In addition, the force vs. pH relationships were reported in stimulated cat muscles but not when similar pH changes were induced by hypercapnia, indicating that pH does not act directly on muscle force production (i.e., on cross-bridge cycling) but rather indirectly at least under conditions of low-frequency stimulation (45). Several potential indirect mechanisms mediated by intracellular acidosis have been reported, such as changes in the release, binding, and uptake of calcium by the sarcoplasmic reticulum (22); the calcium affinity for troponin (33); the inhibitory effect of low pH on phosphofructokinase activity; and the transformation of glycogen phosphorylase b to a in skeletal muscle (11). A similar causative relationship between pH changes and loss of force has not been reported during the recovery period after exercise (46, 47, 71). However, given that PCr resynthesis is coupled to H\(^+\) generation during this period, it is not surprising that the relationship between H\(^+\) and force is no more linear during the recovery period, and it seems likely that fatigue and recovery from fatigue may involve distinct processes. Recent experiments in contracting rat gastrocnemius muscle failed to report a correlation between pH and force reduction (13), but the discrepancy with our results could be due to the use of fluorinated anesthetics, which could interfere with the motor end-plate transmission.

Muscle fatigue is a multifactorial process involving many levels of organization. Our study has combined noninvasive techniques to unravel the complexities of fatigue process in the rabbit tibialis anterior muscle submitted to low- and high-frequency stimulation. We mainly illustrated that low-frequency \(M_{\text{stim}}\) is closely linked with metabolic changes, i.e., intracellular acidosis and/or Pi accumulation associated with PCr utilization without any altered muscle electrical activity. On the contrary, when muscle is stimulated at higher frequencies, both metabolic changes and ATP cost of contraction are reduced, and this reduction is accompanied by marked alterations of action potential propagation.

REFERENCES


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