Muscle Fatigue: The Role of Metabolism

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Abstract/Résumé

This paper examined the role of metabolites in causing muscle fatigue. Previous studies have shown that Pi ($H_2PO_4^-$, $HPO_4^{2-}$) and $H^+$ may be important factors in causing fatigue. A key question is the potential interaction between metabolic end-products and calcium related excitation-contraction coupling fatigue (ECC). An in vivo rat muscle model was used to measure tension development and metabolic end-products in response to electrical stimulation. Two stimulation protocols were used, high intensity stimulation followed by a medium intensity stimulation (High Group), and low intensity stimulation followed by a medium intensity stimulation (Low Group). Metabolic fatigue was based on concentrations of $H_2PO_4^-$ measured with phosphorus magnetic resonance spectroscopy. ECC fatigue was measured as the fatigue in excess of metabolic fatigue, and as the relative decline of force at low compared to high stimulation frequencies. During the initial stimulation period, the High Group had greater metabolic fatigue (p < 0.001) and greater ECC fatigue (p = 0.007). During the second stimulation period and recovery, the High Group had no difference in metabolic fatigue (p = 0.07) and greater ECC fatigue (p = 0.015). These results present a method for determining the relative amounts of metabolic and ECC fatigue, and suggest that metabolites can increase the amount of ECC fatigue.

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Cet article analyse le rôle des produits du métabolisme dans l’installation de la fatigue musculaire. Des études antérieures ont montré que l’ion Pi (\(H_2PO_4^-\), HPO4-2) et l’ion H+ pouvaient être des facteurs importants dans l’installation de la fatigue. À ce sujet, il y a lieu d’analyser l’interaction potentielle des produits du métabolisme avec le calcium impliqué dans la défaillance du couplage électromécanique (fatigue ECC). Les auteurs étudient la tension et les produits du métabolisme consécutifs à la stimulation électrique d’un muscle de rat in vivo. Deux groupes expérimentaux sont formés: l’un (High group) recevant une stimulation forte suivie d’une stimulation de moyenne intensité et l’autre (Low group), recevant une stimulation faible suivie d’une stimulation de moyenne intensité. La fatigue métabolique est établie d’après la concentration de \(H_2PO_4^-\) obtenue par spectroscopie RMN du phosphore. La fatigue ECC est définie par la fatigue au-delà de la fatigue métabolique et par le déclin relatif de la tension sous basse stimulation comparativement à celle sous forte stimulation. Au cours de la phase initiale de stimulation, le groupe à forte stimulation se fatigue plus rapidement aux niveaux métabolique \((p < 0.001)\) et électromécanique \((p = 0.007)\). Au cours de la phase subséquente de stimulation et durant la récupération, la fatigue métabolique du groupe à forte stimulation est semblable \((p = 0.07)\), mais sa fatigue électromécanique est plus forte \((p = 0.015)\). Cette étude illustre une méthode pour établir la fatigue relative attribuable au métabolisme et au couplage électromécanique et indique que les produits du métabolisme peuvent accroître la fatigue ECC.

**Introduction**

Muscle fatigue is a well studied phenomenon, with clear implications as to how skeletal muscle functions. However, a number of questions remain to be resolved. For the purposes of this article, we will define fatigue as the development of less than the expected amount of force as a consequence of muscle activation. This definition attempts to take into account that force can be altered by changing stimulation patterns, that potentiation (increase in force with stimulation) can occur independently of fatigue, and to exclude losses in force development due to injury or factors not directly related to activation. In one of the better thought out models of muscle fatigue, Edwards proposed that inhibition of force development (fatigue) can occur at almost all steps in the activation, stimulation, contraction, and relaxation process (Edwards, 1982). A key aspect of this model of fatigue is that skeletal muscle commonly operates at metabolic intensities that are far from equilibrium, that is, far from sustainable levels. Thus, the fatigue that results from skeletal muscle activity serves as a mechanism to inhibit muscle activation and maintain intracellular homeostasis (Edwards, 1982).

Evidence that fatigue can occur at the various steps along the muscle activation/relaxation pathway have been presented (Baker et al., 1993; Kent-Braun et al., 1993; Miller et al., 1987; Wilson et al., 1988), generally supporting the model presented by Edwards (1982). One of the key remaining questions is: at what step or steps does fatigue occur in vivo? Is there one step that can be considered a “critical” step, with the other steps being relatively unimportant? Or do we need to develop multiple -component models to determine the relative importance of multiple steps? An example of a multiple component model is the one used by Chance et al. (1986) to study the control of oxidative metabolism. A fair amount of current research on muscle fatigue has
focused on calcium kinetics and excitation contraction coupling as a primary location for muscle fatigue (ECC fatigue). Recently, the interaction of metabolic byproducts (phosphate) and calcium have been suggested as a mechanism for fatigue (Kabbara and Allen, 1999). The purpose of this article is to examine the role of metabolic end-products in inhibiting muscle contraction (metabolic fatigue). This article will review previous studies and present new evidence on the role of metabolic fatigue.

Evidence for Metabolic Fatigue

Metabolic fatigue is thought to be due to an inhibition of myosin crossbridge formation secondary to a change in either the free energy state of the muscle or level of intracellular metabolites (Nosek et al., 1987). In vitro approaches use skinned or intact fiber preparations and measure the force and velocity of activated muscle in the presence of known concentrations of inorganic phosphate, hydrogen ion, and ADP. However, ADP does not appear to be a potential fatigue causing metabolic in physiological concentrations (Cooke and Pate, 1985; Kentish, 1986). A number of different in vitro studies have shown that increasing both inorganic phosphate and H+ decreased force development of skinned fibers (Cooke and Pate, 1985). For example, force development was decreased in the presence of 20 mM Pi to 30% of maximal force (Kentish, 1986). Inorganic phosphate is thought to inhibit the transition from the pre power-stroke actin*myosin*ADP*Pi state to the force producing actin*myosin*ADP state (Chinn et al., 2000). The effects of H+ are controversial, with some evidence that increasing H+ depresses force, reduces velocity, and improves economy (Myburgh and Cooke, 1997). Interestingly, there was some suggestion in early studies of an interactive effect of inorganic phosphate and H+ (Figure 1). However, recent studies have questioned the role of pH in metabolic fatigue. Most of the previous studies which have shown strong evidence of the effect of pH on fatigue have been conducted at relatively low temperatures < 15 °C. However, warmer temperatures that approach physiological temperatures (30 °C) show much less of an inhibitory effect of pH (Dibold and Fitts, 2000; Pate et al., 1995). For example, the influence of low pH on force development went from 50% at 10 °C to 18% at 30 °C (Pate et al., 1995).

Evidence that metabolic products are associated with fatigue has also been found in vivo. In vivo preparations use intact human or animal skeletal muscles and correlate the amount of fatigue to changes metabolite levels. The advent of magnetic resonance spectroscopy has allowed for measurements of Pi and H+ during activation in intact muscle (Dawson et al., 1978). Studies on humans have found that muscle fatigue correlates best with changes in protonated inorganic phosphate, suggesting an interaction effect of Pi and pH (Dawson et al., 1978; Miller et al., 1987; Wilson et al., 1988). In support of metabolic fatigue, the kinetics of muscle fatigue and changes in protonated inorganic phosphate are often similar. Wilson et al (1988) found correlation coefficients ~0.9 for pH and \( H_2PO_4^- \) and force. As shown in Figure 2, high intensity exercise is associated with rapid increases in metabolites, with relatively rapid recovery once the exercise stops. Fatigue during exercise and recovery of force afterwards seems to occur in discrete phases, with the most rapid phase being associated with first
changes in Pi and second phase with changes in pH (Miller et al., 1987). This is in contrast to some of the more recent in vitro studies which have suggested that pH might not be a critical factor in muscle fatigue (Dibold and Fitts, 2000; Pate et al., 1995). Further studies are needed to resolve the differences between the interpretation of in vitro and in vivo results, particularly on the role of H⁺. Not all in vivo studies report good relationships between fatigue and Pi and H⁺ (Adams et al., 1991; LeRumer et al., 1990). In particular, highly trained muscle seemed to be associated with reduced sensitivity to fatigue from Pi and H⁺ (McCully et al., 1991).

While the current study focuses on metabolic fatigue as the accumulation of metabolic products that directly inhibit muscle activation, there is some evidence that metabolic fatigue can occur via different mechanisms. One is that the depletion of metabolic substrates might produce muscle fatigue. Depletion of muscle glycogen (Welton et al., 1998) and tricarboxylic acid cycle intermediates (Lee and Davis, 1979; Spencer et al., 1991) have also been suggested as mechanisms to cause muscle fatigue by reducing oxidative muscle metabolism. This potential mechanism is speculative and and there is some evidence that reduced tricarboxylic acid cycle intermediates are not reduced during exercise after glycogen depletion, a condition where metabolic depletion would be expected to be important (Gibala et al., 1999). The depletion of substrates should have very different fatigue characteristics compared to accumulation of metabolic products, such as a much slower onset and recovery.
Figure 2. An example of changes in muscle metabolites (HPO\textsuperscript{-} and ADP) in human calf muscles during and after 64 seconds of voluntary rapid plantar flexion. Note that changes in metabolites can occur rapidly, supporting the rapid development of metabolic fatigue.

Another mechanism for metabolic fatigue is that metabolic products might reduce calcium release from the sarcoplasmic reticulum (Kabbara and Allen, 1999). For example, high levels of inorganic phosphate might precipitate with calcium and reduce free calcium levels (Fryer et al., 1995). Metabolites might also inhibit calcium uptake by the sarcoplasmic reticulum. One of the methods used to identify ECC fatigue is to make use of the frequency-force relationship (Edwards et al., 1977). Reduced calcium release with each action potential should have a greater relative effect on force at low stimulation frequencies than at higher stimulation frequencies. High stimulation frequencies can produce saturating levels of calcium even if the amount calcium released per action potential is reduced. The relatively lower force at lower stimulation frequencies has been termed “low-frequency” fatigue (Edwards et al., 1977). While the onset of fatigue might be fairly rapid, once calcium has been sequestered, it may take hours for this calcium to return to the pool of free calcium (Edwards et al., 1977).

The aim of the present experiments was to use an in vivo model to quantify the effects of metabolic fatigue and ECC fatigue on force development of skeletal muscle. A stimulation paradigm was developed based on the assumption that metabolic fatigue recovers quickly and ECC fatigue recovers slowly. If ECC fatigue is
altered by metabolic fatigue, a high intensity protocol should result in greater ECC fatigue than a low intensity protocol during a subsequent stimulation bout.

Methods

Male Sprague-Dawley rats (six animals per group) weighing 300–400 gms were used according to guidelines established by the University of Pennsylvania regulations (where the experiments were performed). Anesthesia was induced by pentobarbital injection (40 mg/kg, i.p.). The left Achilles tendon was isolated with part of the heelbone, and linked to a non-magnetic isometric transducer (West Coast Research Corp) (Roth et al., 1989). The leg was fixed at the ankle and the knee. Platinum electrodes were placed in contact with the sciatic nerve through an incision just proximal to the knee. The stimulation voltage was adjusted to 150% of that needed to give a maximal twitch (4–6 V) with 0.5 ms duration. The muscle length was adjusted to produce maximum twitch tension.

Muscle activation was performed using isometric contractions produced by electrical stimulation (computer controlled Grass S8800 stimulator). Three stimulation levels were used, low, medium, and high. Low consisted of 1 x 500 ms @ 50 Hz train of every 6 s, medium consisted of 2 x 500 ms @ 50 Hz trains every 6 s, and high consisted of 4 x 500 ms @ 50 Hz trains every 6 s. The stimulation period divided into two 8 min periods, and the animals given one of two different stimulation patterns. The High Group received the high stimulation during the initial stimulation period followed by the medium stimulation in the last period. The Low Group received the low stimulation during the initial stimulation period followed by the medium stimulation in the last period. Force-frequency curves were obtained at the beginning and end of each stimulation period, and during recovery, by sending 1 x 500 ms train each at 20 Hz, 35 Hz, 50 Hz, 80 Hz and 120 Hz with 1 second between trains. The tension-time integral (TTI) was calculated from the recorded force.

To make the metabolic measurements, the rat leg was placed into a 4-tum solenoid single-tuned coil with a phosphorus frequency of 47 MHz. The animal was then placed in a 30 cm free-bore 2.7 T supraconductive magnet system (Otsuka Electronics, USA). Two-min spectra with a 6 s repetition time were acquired, signal acquisition was gated to occur between muscle contractions. The areas of the peaks were corrected for saturation differences using a T1 of 5.4s for PCr and 3.9 s for Pi (Authier et al., 1988). Pi/PCr ratios and intracellular pH determined and H₃PO₄ levels were calculated assuming PCr + Pi to be constant and equal to 37 mmol/L, with a pK of 6.75. The value of PCr + Pi concentration was calculated assuming a 67% intracellular water content and a PCr + Pi content of 24 umol/gm wet weight obtained in a previous experiment by perchloric acid extracts of rat muscle (Authier et al., 1988). The animals were sacrificed immediately after the end of the experiment by a pentobarbital overdose. The gastrocnemius and plantaris muscle group was dissected and weighed.

Statistics: the results were compared by two-way analysis of variance, ad hoc tests were done using Fishers least significance difference. All results are expressed as mean ± SEM. A significance threshold of p < 0.05 was adopted. The relationship between H₃PO₄ and force was examined with linear regression analysis.
Results

Prior to the stimulation protocol, there was no difference in resting mechanical and biochemical variables between the rats selected to perform the two different protocols (Low Group and High Group). The average body mass was 340 ± 20 gm in the Low Group and 347 ± 20 gm in the High Group. The gastrocnemius/plantaris mass was 2.27 ± 0.2 gm vs 2.47 ± 0.2 gm and the maximum exerted force (500 ms tetanus at 120 Hz) was 4.39 ± 0.4 N vs 4.64 ± 0.2 N. At rest, Pi/PCr was 0.12 ± 0.03 vs 0.13 ± 0.02, pH 7.15 ± 0.02 vs 7.12 ± 0.02 and H$_2$PO$_4^-$ was 1.23 ± 0.4 vs 1.32 ± 0.3 mmol/L. None of these differences were statistically significant.

The two protocols resulted in significantly different mechanical and metabolic responses (Figure 3). The High Group developed significantly more tension (TTI) during the initial stimulation period that the Low Group, and was associated with higher Pi/PCr ratios, lower pH values, and higher calculated H$_2$PO$_4^-$ concentrations. In the final stimulation period (medium level), the TTI decreased for the High Group and increased for the Low Group. The TTI was decreased in the High Group compared to the Low Group, indicating greater fatigue in the High Group. There were no significant differences in the Pi/PCr ratios, pH, or H$_2$PO$_4^-$ concentrations between the High and Low groups during the medium stimulation level.

The frequency versus force curves (Figure 4) showed a greater fatigue at lower frequencies than at higher frequencies in both the High and Low groups. This was particularly true 10 min after the stimulation protocol. This time point

![Figure 3](image-url)

**Figure 3.** Force and metabolite levels during and after the stimulation protocols. Time zero is the resting measurements prior to the two stimulation periods. Arrows indicate the different stimulation periods. A) tension-time integral expressed as a fraction of the resting 50 Hz force, B) Pi/PCr ratios, C) intracellular pH, D) calculated H$_2$PO$_4^-$ concentrations. * significant difference ($p < 0.05$) between High Group and the Low Group.
Figure 4. Force-frequency curves for the two stimulation protocols. The force for each stimulation frequency is plotted as a percentage of the resting force at that frequency. A) The Low group. B) The High group. The recovery measurements were made 10 min after the stimulation protocol stopped.

was chosen because by 10 min metabolic recovery is essentially complete, and any potentiation effects from the stimulation are gone. The high stimulation (first stimulation period) in the High Group caused significantly more fatigue at all frequencies. The force values reached during the second stimulation period differed significantly between the High Group and the Low Group only at 35 to 50 Hz. During recovery, force increased significantly at both 80 and 120 Hz, but the High Group was more fatigued than the Low Group at all frequencies. Thus, force remained depressed relative to the initial resting force even after \(\text{H}_2\text{PO}_4^-\) levels had returned to normal suggesting the presence of low-frequency fatigue.

To examine the relationship between \(\text{H}_2\text{PO}_4^-\) levels and force, regression lines were calculated for all the time points in the two groups for the different stimulation frequencies (Table 1). For the initial stimulation period, there were significant correlations for all the stimulation frequencies except 20 Hz. For the second stimulation period and during recovery, no significant correlations were seen. To examine the relative contribution to fatigue by metabolites, a regression line was developed based on the resting \(\text{H}_2\text{PO}_4^-\) levels and the average X-intercept in Table 1. For any given \(\text{H}_2\text{PO}_4^-\) concentration, it was assumed that the amount of fatigue due to \(\text{H}_2\text{PO}_4^-\) would be on this regression line. Any additional decline in force (fatigue) was assumed to be due to other mechanisms (ECC fatigue). We assumed a linear relationship rather than a hyperbolic or exponential one (Kentish, 1986). This is a simplification, but the difference between a linear relationship and a curvilinear one in the range of \(\text{H}_2\text{PO}_4^-\) concentrations in this study (see Figure 2) should be small. The lower stimulation frequencies showed much more divergence from the extrapolated regression line, suggesting more fatigue due to ECC (Figure 5). The data in Figure 5 are also summarized in Figure 6. This figure demonstrates the relative contribution to the observed fatigue attributed to metabolic and ECC mechanisms. During the first stimulation period, the High Group showed greater metabolic fatigue \((p < 0.001)\) and ECC fatigue \((p = 0.007)\) than the Low Group. In addition, the relative contribution of ECC to fatigue was higher (although still small). During the second stimulation period and recovery, the High
Table 1. Linear Regression Analysis of the Relationship Between Force and H$_2$PO$_4^-$

<table>
<thead>
<tr>
<th>Frequency</th>
<th>$r$</th>
<th>Slope (Y intercept)</th>
<th>X intercept (mM H$_2$PO$_4^-$)</th>
<th>Stimulation 2 medium</th>
<th>Recovery 10 min post</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>-.32</td>
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<td>65</td>
<td>16.6</td>
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<td>-.59*</td>
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<td>17.5</td>
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<tr>
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<td>-.73*</td>
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<td>90</td>
<td>17.7</td>
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<tr>
<td>80</td>
<td>-.78*</td>
<td>-5.30</td>
<td>94</td>
<td>17.7</td>
<td>-0.22</td>
</tr>
<tr>
<td>120</td>
<td>-.75*</td>
<td>-6.22</td>
<td>109</td>
<td>17.4</td>
<td>-0.35</td>
</tr>
<tr>
<td>Extrapolated line</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.33</td>
</tr>
</tbody>
</table>

The values are from all rats. The extrapolated line was determined from the mean resting H$_2$PO$_4^-$ values (100% force) and the averaged X intercept. The extrapolated line was used to determine the amount of fatigue assigned to metabolic and ECC mechanisms. *Indicates significance at a level of $p < 0.05$.

Figure 5. A) Force recorded at 35 Hz before, during and after the stimulation as a function of calculated H$_2$PO$_4^-$. B) Force recorded at 120 Hz before, during and after the stimulation as a function of calculated H$_2$PO$_4^-$. In both graphs, the dotted line is the extrapolated relationship between H$_2$PO$_4^-$ and fatigue based on resting H$_2$PO$_4^-$ values and the average intercept of H$_2$PO$_4^-$ versus force relationships during the first stimulation period.
Figure 6. The relative contribution of metabolic and ECC to the observed fatigue during the stimulation protocol. Note that during the initial period, more fatigue was observed with the high protocol. * ECC fatigue significantly greater for high-medium group (High) than for low-medium group (Low). # Metabolic fatigue significantly greater for high-medium group (High) than for low-medium group (Low).

Group had greater ECC fatigue ($p = 0.015$) with metabolic fatigue that was not significantly greater than the Low Group ($p = 0.073$).

Discussion

The experiments presented here represent an attempt to quantify multiple mechanisms of muscle fatigue. Some attempts have been made to quantify different mechanisms of fatigue (Baker et al., 1993), but this still represents an under-utilized approach to studying muscle fatigue. We found that metabolic fatigue associated with elevated $\text{H}_2\text{PO}_4^-$ levels varied with stimulation intensity and did not vary with the frequency of stimulation (similar with 35 or 120 Hz). ECC fatigue was higher during the second stimulation period compared to the first, independent of stimulation intensity, and was higher after the high stimulation bout than after the low stimulation bout. These results are similar to previous studies that have shown that metabolic fatigue is more rapid in onset and recovery than ECC fatigue (Baker et al., 1993; Edwards et al., 1977). It is also consistent with previous \textit{in vitro} studies that have suggested an interaction between metabolic and ECC fatigue (Kabbara and Allen, 1999).

One of the experimental questions addressed in the present study is the role of metabolism in influencing ECC fatigue. In other words, is there an interactive effect between muscle metabolism and Calcium kinetics? This study found a significant interaction between metabolic intensity and ECC fatigue, consistent with previous \textit{in vitro} studies (Kabbara and Allen, 1999). The alternative hypothesis is that ECC fatigue is related to the number of action potentials rather than metabolism. The metabolic effect on ECC in this study was of long duration, lasting at least 10 min into recovery (18 min after the transition from either high or low stimulation intensity to medium stimulation intensity). This is consistent with
metabolic products such as Pi sequestering Calcium, with a long duration needed to free Calcium. It is not consistent with a rapid and reversible effect on sarcoplasmic reticulum function (Ca uptake).

In this paper we have referred to metabolic fatigue as metabolic end products directly inhibiting actin and myosin force development. This was not measured directly in this paper, and thus must be considered an assumption. Previous in vitro studies have suggested such a mechanism. There are some inconsistencies between the in vitro and in vivo measurements, a primary one being the role of pH. According to recent in vitro studies, pH does not appear to be important to inhibiting actin-myosin function at physiological temperatures. But how this fits with the in vivo results is not clear. Some of the in vitro studies have used the lack of correlation of fatigue with metabolic products such as Pi and pH to discount this mechanism. However, this argument depends on the assumption that there is only one primary mechanism of muscle fatigue. If there is more than one, then it would be possible to disprove all single mechanisms, by simply designing an experiment that enhanced the importance of other fatiguing mechanisms. Clearly using force development during the recovery process after muscle activation favors fatigue mechanisms of long duration such as ECC. As shown in this paper, fatigue protocols that use short duration activity favor metabolic mechanisms.

In summary, this study demonstrates an approach which provides quantitative separation of metabolic fatigue from low frequency fatigue using phosphorous NMR spectra and force frequency response curves obtained simultaneously in the intact contracting skeletal muscle. The initial force levels and H_2PO_4^- levels were used in the estimate metabolic fatigue. During subsequent measurements, the increased amount of fatigue in excess of the metabolic fatigue was used to indicate ECC fatigue. This approach was supported by the time course of appearance of low frequency fatigue. This study found a statistically significant impact of muscle metabolism on ECC fatigue. Future studies should make use of a multi-component model to study muscle fatigue, which will allow testing of the relative importance of the various components of mechanisms to muscle fatigue.

References


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