Muscle Fatigue: Lactic Acid or Inorganic Phosphate the Major Cause?

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Intracellular acidosis due mainly to lactic acid accumulation has been regarded as the most important cause of skeletal muscle fatigue. Recent studies on mammalian muscle, however, show little direct effect of acidosis on muscle function at physiological temperatures. Instead, inorganic phosphate, which increases during fatigue due to breakdown of creatine phosphate, appears to be a major cause of muscle fatigue.

The energy consumption of skeletal muscle cells may increase up to 100-fold when going from rest to high-intensity exercise. This high energy demand exceeds the aerobic capacity of the muscle cells, and a large fraction of the ATP required will come from anaerobic metabolism. High-intensity exercise also leads to a rapid decline in contractile function known as skeletal muscle fatigue. It therefore seems logical that there is a causal relationship between anaerobic metabolism and muscle fatigue; that is, some consequence(s) of anaerobic metabolism causes the decline in contractile function.

Anaerobic breakdown of glycogen leads to an intracellular accumulation of inorganic acids, of which lactic acid is quantitatively the most important. Since lactic acid is a strong acid, it dissociates into lactate and H^+. Lactate ions would have little effect on muscle contraction (16); however, the increase in H^+ (i.e., reduced pH or acidosis) is the classic cause of skeletal muscle fatigue. However, the role of reduced pH as an important cause of fatigue is now being challenged, and several recent studies (5, 14, 19, 20) show that reduced pH may have little effect on contraction in mammalian muscle at physiological temperatures.

Besides acidosis, anaerobic metabolism in skeletal muscle also involves hydrolysis of creatine phosphate (CrP) to creatine and inorganic phosphate (P_i). Creatine has little effect on contractile function, whereas there are several mechanisms by which increased P_i may depress contractile function. Thus, on the basis of recent findings (6–8, 10–12), increased P_i rather than acidosis appears to be the most important cause of fatigue during high-intensity exercise. This brief review will outline the results that form the basis for the switch from acidosis to increased P_i as the major fatigue factor in mammalian muscle. We will focus on studies in which fatigue develops on the time scale of minutes, in which the consequences of anaerobic metabolism would be of greatest importance. With even more intense activation (e.g., a continuous maximal contraction), other factors, like failure of action potential propagation, may become increasingly important. Conversely, with more long-lasting types of exercise (e.g., marathon running), factors such as depletion of carbohydrate stores and dehydration become increasingly important.

To study the mechanisms underlying fatigue, we frequently use isolated muscle cells (fibers), which are fatigued by repeated tetani of short duration. The present review will focus on results obtained in such studies as well as studies on skinned muscle fibers (i.e., muscle cells where the surface membrane has been chemically or physically removed). This is because studies on single muscle fibers provide the most direct way to address cellular mechanisms of fatigue. It may be argued that conclusions drawn from studies on single fibers are not relevant to the fatigue experienced by humans during various types of exercise. However, available data indicate that the mechanisms of fatigue are qualitatively similar in diverse experimental models, ranging from exercising humans to single fibers (2). The differences that inevitably must exist appear to be mainly of a quantitative nature.

The rise and fall of lactic acid as a direct cause of skeletal muscle dysfunction in fatigue

During intense muscle activity, the intracellular pH may fall by ~0.5 pH units. There are two major lines of evidence that have been used to link this decline in pH to the contractile dysfunction in fatigue. First, studies on human muscle fatigue have often shown a good temporal correlation between the decline of muscle pH and the reduction of force or power production. Second, studies on skinned skeletal muscle fibers have shown that acidification may reduce both the isometric force and the shortening velocity.

However, in humans the temporal correlation between impaired contractile function during fatigue and reduced pH is not always present. For instance, force sometimes recovers more rapidly than pH after the end of fatiguing contractions (18). This means that if reduced pH has a direct force-depressing effect in human muscles, this effect must have been counteracted by some other factor that increases force to the same extent. Such a force-potentiating factor has not been identified, and hence the obvious conclusion is that there is no causal relationship between acidosis and reduced force production.

Important evidence in favor of acidosis causing reduced force production comes from studies on skinned muscle fibers that were performed at ≤15°C (14). Recent studies have focused on the temperature dependence of the pH effects on force, and the results of these studies further challenge the role of H^+ in mammalian muscle fatigue. Some early studies con-

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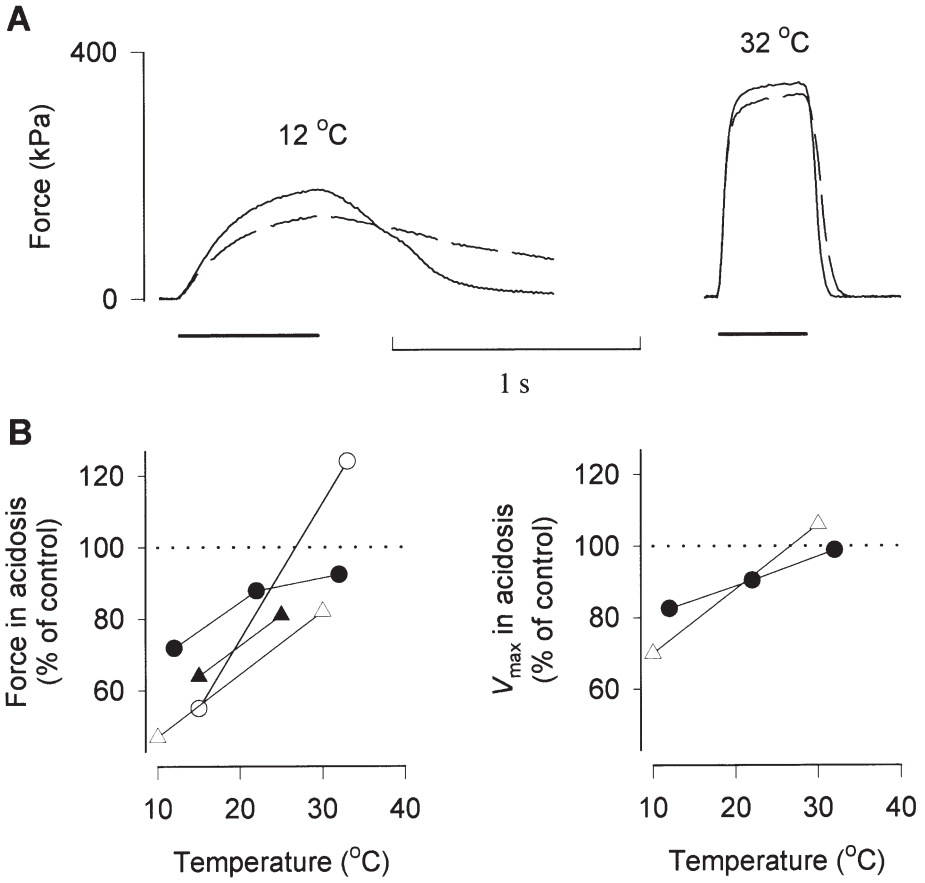

FIGURE 1. *A*: original force records from tetanic contractions produced in an intact, single muscle fiber from a mouse toe muscle. Records were obtained under control conditions (solid line) and when the fiber was acidified by ~0.5 pH units by increasing the bath CO_2 concentration (dashed line). Note that the effect of acidification is markedly larger at 12°C than at 32°C. Period of stimulation is indicated below force records. *B*: depressive effect of acidosis on force production (*left*) and maximum shortening velocity (V_{max}; *right*) diminishes as the temperature is raised. Data were obtained from intact mouse fibers (●; Ref. 19), skinned rabbit fibers (△; Ref. 14)), intact rat muscle (○; Ref. 17), and intact mouse muscle (▲; Ref. 20). Dotted line indicates no difference between acidosis and control. *A* is Adapted from Ref. 19.

ducted more than 10 years ago showed that acidification, if anything, resulted in an increased tetanic force at physiological temperatures (17). More recently, Pate and colleagues (14) studied skinned rabbit psoas fibers and observed the expected large depressive effect of lowered pH at 10°C, but the effect of acidification on force production was small at 30°C. Similar results have subsequently been obtained in isolated single mouse muscle fibers (19) and whole mouse muscles (20) (Fig. 1*A*).

Acidification has been considered to be an important factor behind the reduced shortening speed in fatigue. However, using skinned rabbit muscle fibers, Pate and colleagues (14) showed that acidification has little effect on the shortening speed at 30°C. Similarly, in intact mouse muscle fibers, the maximum shortening velocity was reduced by ~20% at 12°C, whereas there was no significant reduction at 32°C (19). Thus in mammalian muscle studied at physiological temperatures, cross-bridge function (i.e., the cyclic attachment and detachment of myosin heads to actin that results in muscle contraction) is little affected by acidification (Fig. 1*B*).

Another mechanism by which intracellular acidosis may induce fatigue is by inhibition of energy metabolism. Key enzymes in glycogenolysis and glycolysis are phosphorylase and phosphofructokinase, respectively. Both of these enzymes are inhibited at low pH in vitro, and hence the rate of ATP supply to energy-requiring processes [e.g., cross-bridge cycling and sarcoplasmic reticulum (SR) Ca^{2+} pumping] might be diminished in muscles that become acidic during fatigue. However, a recent human study failed to detect a reduction of the rate of glycogenolysis/glycolysis in acidified muscle (4). Furthermore, a 0.4-pH-unit acidification did not affect the endurance of isolated mouse muscle fibers fatigued by repeated brief tetani at 28°C (5). Thus the inhibition of phosphorylase and phosphofructokinase induced by acidosis in vitro appears to be counteracted by other factors in vivo, and fatigue development does not seem to be accelerated by acidosis at near-physiological temperatures. Acidosis has also been suggested to decrease muscle performance during fatigue by inhibiting Ca^{2+} release from the SR. Such inhibition will decrease the degree of activation of the contractile machinery and hence lead to decreased force production. Although it has been repeatedly shown that there is a decrease in free myoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) during contractions in fatigue (see Fig. 2*A*), it is doubtful if this is related to acidosis. One finding that might support a role of decreased pH in this aspect is that acidosis reduces the open probability of isolated

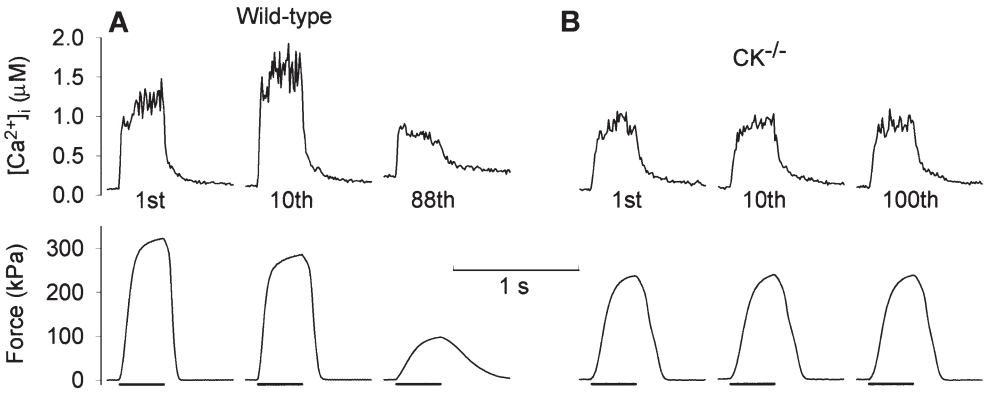

FIGURE 2. Typical force and free myoplasmic Ca^{2+} concentration ($[Ca^{2+}]_i$) records obtained during fatigue induced by repeated, brief tetani in a wild-type fiber (*A*) and a fiber completely deficient of creatine kinase ($CK^{-/-}$; *B*). The wild-type fiber shows typical changes, with an early increase of tetanic $[Ca^{2+}]_i$ accompanied by a slight reduction of force. Then follows a decline of both tetanic $[Ca^{2+}]_i$ and force until fatiguing stimulation is stopped after 88 tetani. Conversely, neither tetanic $[Ca^{2+}]_i$ nor force is altered during 100 fatiguing tetani in the $CK^{-/-}$ fiber, which fatigues without inorganic phosphate (P_i) accumulation. Note also the lower force in the unfatigued state in the $CK^{-/-}$ fiber, which has a higher P_i concentration at rest. Periods of stimulation are indicated below force records. Figure adapted from Ref. 7.

SR Ca^{2+} release channels (i.e., the ryanodine receptors). However, acidification has no obvious depressive effect on depolarization-induced SR Ca^{2+} release in skinned fibers with an intact transverse-tubular SR system (13).

To sum up, acidosis has little direct effect on isometric force production, maximum shortening velocity, or the rate of glycogen breakdown in mammalian muscles studied at physiological temperatures. Therefore, if acidosis is involved in skeletal muscle fatigue, the effect may be indirect. For instance, extracellular acidosis may well activate group III–IV nerve afferents in muscle and hence be involved in the sensation of discomfort in fatigue. This would make good sense from an athlete's point of view. Training regimes for top athletes in endurance-type sports often emphasize "lactic acid training," i.e., training protocols that induce high plasma lactic acid levels. An effect of this type of training may then be to learn to cope with the acidosis-induced discomfort without loosing pace and technique and in this way get the maximum effect out of muscles, which in themselves are not directly inhibited by acidosis. An alternative mechanism by which lactic acid formation may impose a limit on performance is during long-lasting types of exercise in which glycogen depletion is a key factor. With extensive lactic acid production, the total amount of ATP produced from the stored glycogen is lower than with complete aerobic breakdown, because each glycosyl unit gives 3 ATP when lactic acid is produced and 39 ATP when it is completely metabolized in the mitochondria to CO_2 and H_2O. Thus the glycogen store is more rapidly depleted when large amounts of lactic acid are produced and muscle performance is severely depressed at low glycogen levels. Finally, the frequently observed temporal correlation between declining pH and decreased muscle function may be coincidental rather than causal. That is, a marked acidification implies that the energy demand exceeds the capacity of aerobic metabolism and that anaerobic pathways are used to generate ATP. It could then be that rather than acidification, some other consequence of anaerobic metabolism is the actual cause of impaired muscle function, and increased P_i is a strong candidate in this respect.

The rise and rise of P_i accumulation as a major cause of skeletal muscle fatigue

The concentration of P_i increases during intense skeletal muscle activity mainly due to breakdown of CrP. Most models of cross-bridge action propose that P_i is released in the transition from low-force, weakly attached states to high-force, strongly attached states. This implies that the transition to the high-force states is hindered by increased P_i. Therefore, fewer cross-bridges would be in high-force states and the force production would decrease as P_i increases during fatigue development. In line with this, experiments on skinned fibers consistently show a reduced maximum Ca^{2+}-activated force in the presence of elevated P_i.

The hypothesis that increased P_i reduces maximum cross-bridge force has been difficult to test in intact muscle cells, since it has proven difficult to increase myoplasmic P_i without imposing other metabolic changes as well. We recently showed (6, 7) that genetically modified mice completely lacking creatine kinase (CK) in their skeletal muscles ($CK^{-/-}$ mice) provide a reasonable model to study the effects of increased P_i. CK catalyzes the transfer of high-energy phosphate groups between CrP and ATP. During periods of high energy demand, the net result of the CK reaction is that CrP breaks down to Cr and P_i but the ATP concentration remains almost constant. Fast-twitch skeletal muscle fibers of $CK^{-/-}$ mice display an increased myoplasmic P_i concentration at rest; furthermore, during fatigue there is no significant P_i accumulation. The maximum Ca^{2+}-activated force of unfatigued $CK^{-/-}$ fast-twitch fibers is markedly lower than that of wild-type fibers, which supports a force-depressing role of increased P_i (6). Furthermore, during fatigue induced by repeated brief tetani, fast-twitch fibers with intact CK display a 10–20% reduction of maximum Ca^{2+}-activated force quickly, after ~10 tetani. This force decline, which

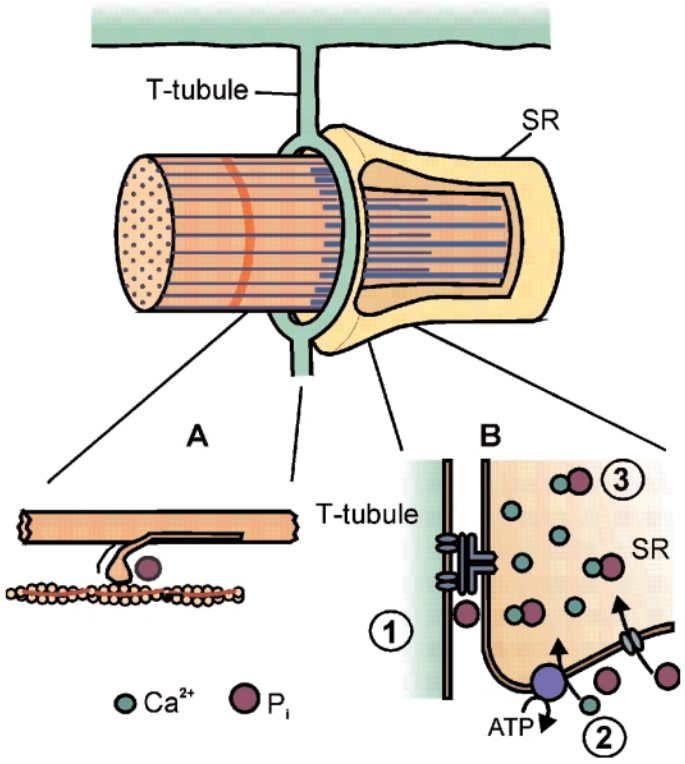

FIGURE 3. Schematic figure illustrating sites where increased P_i may affect muscle function during fatigue. Increased P_i may act directly on the myofibrils and decrease cross-bridge force production and myofibrillar Ca^{2+} sensitivity (*A*). By acting on sarcoplasmic reticulum (SR) Ca^{2+} handling (*B*), increased P_i may also increase tetanic $[Ca^{2+}]_i$ in early fatigue by stimulating the SR Ca^{2+} release channels (*1*); inhibit the ATP-driven SR Ca^{2+} uptake (*2*); and reduce tetanic $[Ca^{2+}]_i$ in late fatigue by entering the SR, precipitating with Ca^{2+}, and thereby decreasing the Ca^{2+} available for release (*3*).

has been ascribed to increased P_i, does not occur in $CK^{-/-}$ fibers (7). Even after 100 fatiguing tetani, force was not significantly affected in $CK^{-/-}$ fibers, whereas by this time force was reduced to <30% of the original in wild-type fibers (Fig. 2). Additional support for a coupling between myoplasmic P_i concentration and force production in intact muscle cells comes from experiments in which reduced myoplasmic P_i is associated with increased force production (15). Thus increased myoplasmic P_i may decrease force production during fatigue by direct action on cross-bridge function. Altered cross-bridge function may also affect the force-$[Ca^{2+}]_i$ relationship via the complex interaction between cross-bridge attachment and thin (actin) filament activation. In this way, increased P_i may also reduce force production by causing a reduced myofibrillar Ca^{2+} sensitivity, which is a frequently observed characteristic in skeletal muscle fatigue.

In recent years, it has become increasingly clear that increased P_i also affects fatigue development by acting on SR Ca^{2+} handling. In this respect, there are several mechanisms by which increased P_i may exert its effect, and the result may be both increased and reduced tetanic $[Ca^{2+}]_i$. Important mechanisms include the following:

Direct action. P_i may act directly on the SR Ca^{2+} release channels, increase their open probability, and facilitate Ca^{2+}-induced Ca^{2+} release (3). This action of P_i would lead to increased tetanic $[Ca^{2+}]_i$ and may be involved in the increase of tetanic $[Ca^{2+}]_i$ normally observed in early fatigue. In support of this notion, $CK^{-/-}$ fibers do not display this early increase of tetanic $[Ca^{2+}]_i$ (7).

Inhibition of Ca^{2+} uptake. Increased P_i may inhibit the ATP-driven SR Ca^{2+} uptake (9). In the short term, inhibition of the SR Ca^{2+} uptake will result in an increased tetanic $[Ca^{2+}]_i$ (assuming that the amount of Ca^{2+} released stays constant). In the long term, on the other hand, Ca^{2+} might accumulate in other organelles (e.g., mitochondria) or possibly leave the cell. In this way, the Ca^{2+} available for release may substantially decline, resulting in reduced tetanic $[Ca^{2+}]_i$. Although it is theoretically possible that loss of Ca^{2+} from the cell contributes to the decline of tetanic $[Ca^{2+}]_i$ in fatigue, we are not aware of any experimental findings that support this.

Ca^{2+}-P_i precipitation. P_i may enter the SR, which may result in Ca^{2+}-P_i precipitation and hence decrease the Ca^{2+} available for release. This mechanism has recently gained support from studies using many different experimental approaches. In initial experiments on skinned fibers with intact transverse-tubular SR systems, Fryer and colleagues (10) showed that increased P_i might depress SR Ca^{2+} release. These authors also provided indirect evidence that P_i may reach a concentration in the SR high enough to exceed the threshold for Ca^{2+}-P_i precipitation in this high-Ca^{2+} environment. Since this pioneering work, it has been shown that the Ca^{2+} available for release is actually reduced in fatigued single fibers from cane toad muscles (11). Measurements of the SR Ca^{2+} concentration also show a decrease in fatigued cane toad fibers (12). Furthermore, the decline of tetanic $[Ca^{2+}]_i$ during fatigue is delayed when the P_i accumulation is prevented by inhibition of the CK reaction, either pharmacologically (8) or by gene deletion ($CK^{-/-}$) (7).

One weakness of the hypothesis that raised P_i causes Ca^{2+}-P_i precipitation in the SR is that P_i increases rather early during fatiguing stimulation but the decline of tetanic $[Ca^{2+}]_i$ generally occurs quite late. Moreover, in mouse fast-twitch fibers the decline of tetanic $[Ca^{2+}]_i$ temporally correlates with an increase in Mg^{2+}, which presumably stems from a net breakdown of ATP (2), and the coupling between Ca^{2+}-P_i precipitation in the SR and increased Mg^{2+}/reduced ATP is not obvious. However, a recent study provides a reasonable explanation for these apparent difficulties: P_i probably enters the SR via an anion channel, which increases its open probability as ATP declines (1). This can explain both why P_i enters the SR with a delay and why there is a temporal correlation between increasing Mg^{2+} and declining tetanic $[Ca^{2+}]_i$. Interestingly, in fibers where the CK reaction is pharmacologically inhibited and fatigue occurs without major P_i accumulation, an increase in Mg^{2+} is not accompanied by reduced tetanic $[Ca^{2+}]_i$ (8). Together, results obtained with a variety of experimental approaches indicate that Ca^{2+}-P_i precipitation in the SR is the major cause of reduced tetanic $[Ca^{2+}]_i$ in fatigue induced by repeated, brief tetani.

Figure 3 illustrates the various mechanisms by which P_i may affect muscle function during fatigue. It shows that increased P_i may depress force production by acting directly on the myofibrils or on sites in the excitation-contraction pathway within

muscle cells. The depressive effect of increased P_i may, like the effect of acidification described above, diminish as the temperature is increased to that prevailing in mammalian muscles in situ. Little information is available regarding the temperature dependence of P_i effects on muscle contraction, and most skinned fiber studies looking at P_i effects have been performed at low temperatures. Studies performed on intact mouse fibers in our laboratory show marked depressive effects on force production that can be ascribed to elevated P_i. These studies have generally been performed at ~25°C, which is close to the in situ temperature at rest (31°C) of the superficially situated toe muscles used (5). Nevertheless, studies on mammalian muscle performed at normal body temperature (~37°C) are required to make sure that the effects of increased P_i remain as the temperature is increased.

Conclusion

The data presented above provide substantial support for increased P_i having a key role in skeletal muscle fatigue. For acidosis, on the other hand, most recent data indicate that its depressive effect on muscle contraction is limited.

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References

1. Ahern GP and Laver DR. ATP inhibition and rectification of a Ca^{2+}-activated anion channel in sarcoplasmic reticulum of skeletal muscle. *Biophys J* 74: 2335–2351, 1998.
2. Allen DG, Lännergren J, and Westerblad H. Muscle cell function during prolonged activity: cellular mechanisms of fatigue. *Exp Physiol* 80: 497–527, 1995.
3. Balog EM, Fruen BR, Kane PK, and Louis CF. Mechanisms of P_i regulation of the skeletal muscle SR Ca^{2+} release channel. *Am J Physiol Cell Physiol* 278: C601–C611, 2000.
4. Bangsbo J, Madsen K, Kiens B, and Richter EA. Effect of muscle acidity on muscle metabolism and fatigue during intense exercise in man. *J Physiol (Lond)* 495: 587–596, 1996.
5. Bruton JD, Lännergren J, and Westerblad H. Effects of CO_2-induced acidification on the fatigue resistance of single mouse muscle fibers at 28°C. *J Appl Physiol* 85: 478–483, 1998.
6. Dahlstedt AJ, Katz A, and Westerblad H. Role of myoplasmic phosphate in contractile function of skeletal muscle: studies on creatine kinase-deficient mice. *J Physiol (Lond)* 533: 379–388, 2001.
7. Dahlstedt AJ, Katz A, Wieringa B, and Westerblad H. Is creatine kinase responsible for fatigue? Studies of skeletal muscle deficient of creatine kinase. *FASEB J* 14: 982–990, 2000.
8. Dahlstedt AJ and Westerblad H. Inhibition of creatine kinase reduces the rate of fatigue-induced decrease in tetanic $[Ca^{2+}]_i$ in mouse skeletal muscle. *J Physiol (Lond)* 533: 639–649, 2001.
9. Duke AM and Steele DS. Characteristics of phosphate-induced Ca^{2+} efflux from the SR in mechanically skinned rat skeletal muscle fibers. *Am J Physiol Cell Physiol* 278: C126–C135, 2000.
10. Fryer MW, Owen VJ, Lamb GD, and Stephenson DG. Effects of creatine phosphate and P_i on Ca^{2+} movements and tension development in rat skinned skeletal muscle fibres. *J Physiol (Lond)* 482: 123–140, 1995.
11. Kabbara AA and Allen DG. The role of calcium stores in fatigue of isolated single muscle fibres from the cane toad. *J Physiol (Lond)* 519: 169–176, 1999.
12. Kabbara AA and Allen DG. The use of fluo-5N to measure sarcoplasmic reticulum calcium in single muscle fibres of the cane toad. *J Physiol (Lond)* 534: 87–97, 2001.
13. Lamb GD, Recupero E, and Stephenson DG. Effect of myoplasmic pH on excitation-contraction coupling in skeletal muscle fibres of the toad. *J Physiol (Lond)* 448: 211–224, 1992.
14. Pate E, Bhimani M, Franks-Skiba K, and Cooke R. Reduced effect of pH on skinned rabbit psoas muscle mechanics at high temperatures: implications for fatigue. *J Physiol (Lond)* 486: 689–694, 1995.
15. Phillips SK, Wiseman RW, Woledge RC, and Kushmerick MJ. The effect of metabolic fuel on force production and resting inorganic phosphate levels in mouse skeletal muscle. *J Physiol (Lond)* 462: 135–146, 1993.
16. Posterino GS, Dutka TL, and Lamb GD. L(+)-lactate does not affect twitch and tetanic responses in mechanically skinned mammalian muscle fibres. *Pflügers Arch* 442: 197–203, 2001.
17. Ranatunga KW. Effects of acidosis on tension development in mammalian skeletal muscle. *Muscle Nerve* 10: 439–445, 1987.
18. Sahlin K and Ren JM. Relationship of contraction capacity to metabolic changes during recovery from a fatiguing contraction. *J Appl Physiol* 67: 648–654, 1989.
19. Westerblad H, Bruton JD, and Lännergren J. The effect of intracellular pH on contractile function of intact, single fibres of mouse muscle declines with increasing temperature. *J Physiol (Lond)* 500: 193–204, 1997.
20. Wiseman RW, Beck TW, and Chase PB. Effect of intracellular pH on force development depends on temperature in intact skeletal muscle from mouse. *Am J Physiol Cell Physiol* 271: C878–C886, 1996.