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HIGHLIGHTED TOPIC | *Fatigue Mechanisms Determining Exercise Performance*

The cross-bridge cycle and skeletal muscle fatigue

Robert H. Fitts

Department of Biological Sciences, Marquette University, Milwaukee, Wisconsin

Fitts RH. The cross-bridge cycle and skeletal muscle fatigue. *J Appl Physiol* 104: 551–558, 2008. First published December 27, 2007; doi:10.1152/ japplphysiol.01200.2007.—The functional correlates of fatigue observed in both animals and humans during exercise include a decline in peak force (P_0), maximal velocity, and peak power. Establishing the extent to which these deleterious functional changes result from direct effects on the myofilaments is facilitated through understanding the molecular mechanisms of the cross-bridge cycle. With actin-myosin binding, the cross-bridge transitions from a weakly bound low-force state to a strongly bound high-force state. Low pH reduces the number of high-force cross bridges in fast fibers, and the force per cross bridge in both fast and slow fibers. The former is thought to involve a direct inhibition of the forward rate constant for transition to the strong cross-bridge state. In contrast, inorganic phosphate (P_i) is thought to reduce P_0 by accelerating the reversal of this step. Both H^+ and P_i decrease myofibrillar Ca^{2+} sensitivity. This effect is particularly important as the amplitude of the Ca^{2+} transient falls with fatigue. The inhibitory effects of low pH and high P_i on P_0 are reduced as temperature increases from 10 to 30°C. However, the H^+-induced depression of peak power in the slow fiber type, and P_i inhibition of myofibrillar Ca^{2+} sensitivity in slow and fast fibers, are greater at high compared with low temperature. Thus the depressive effects of H^+ and P_i at in vivo temperatures cannot easily be predicted from data collected below 25° C. In vitro, reactive oxygen species reduce myofibrillar Ca^{2+} sensitivity; however, the importance of this mechanism during in vivo exercise is unknown.

low pH; high inorganic phosphate; contractile properties

THE CAUSES OF MUSCLE FATIGUE are complex, and not totally understood (1, 21). Depending on the circumstance, fatigue may result from disturbances in the central nervous system (26) and/or peripheral factors within the skeletal muscles (1–3, 21–23, 28, 29, 58). By definition, muscle fatigue is characterized by a loss of muscle power that results from a decline in both force and velocity (16, 21). To distinguish fatigue from muscle weakness or damage, it is important to note that the loss of power with fatigue is reversible by rest (21). The etiology of muscle fatigue is an important question, as it can lead to serious limitations in muscle and whole body performance (21, 23), and in clinical situations to respiratory failure and death (34). While the exact causes of muscle fatigue and the relative importance of particular factors remains controversial (1, 21), it is clear that an individual's state of fitness, dietary status, fiber type composition, and intensity and duration of the exercise all affect the process. For example, the factors causing fatigue during high-intensity exercise are distinctively different from those precipitating fatigue during prolonged activity (21). The problem is further complicated as muscle fatigue not only results from multiple factors acting at various sites but in many cases from synergistic actions of two or more agents. The purpose of this minireview is to describe the functional changes that characterize fatigue and then discuss the role of altered cross-bridge events in the fatigue process. Other factors such as central fatigue, and events effecting excitation-contraction coupling or energy supply, will not be considered.

THE CROSS-BRIDGE CYCLE

To understand fatigue mediated by alterations in cross-bridge events, it is important to review the steps involved in cross-bridge cycling and force generation. Although the molecular details of the cross-bridge cycle are not yet fully understood, Fig. 1 reproduced from Geeves et al. (27) shows a minimal mechanochemical scheme for the actomyosin cross-bridge cycle. The myosin and actomyosin ATPase steps are shown in the top and bottom line of Fig. 1*A*, respectively, with the predominant pathway for the actomyosin ATPase shaded. The scheme shown in Fig. 1*B* begins with the rigor complex (A·M, *state a*). As Geeves et al. (27) point out, each step shown has a series of substeps that involve protein conformational changes that alter the affinity of the myosin binding to actin, P_i, or ADP (50). When myosin initially binds to actin, the actomyosin is in a weakly bound low-force state (*state c*). With the subsequent release of P_i (*step d*), the cross bridge transforms into strongly bound high-force state and goes through the power stroke (*state e*). ADP is then released (*state f*), returning the cross bridge to the rigor complex. The strongly bound, high-force states (*states d, e, f,* and *a*) are thought to be the dominant form during a maximal isometric contraction,

Address for reprint requests and other correspondence: R. H. Fitts, Dept. of Biological Sciences, Wehr Life Sciences Bldg., Marquette Univ., P. O. Box 1881, Milwaukee, WI 53201-1881 (e-mail: robert.fitts@mu.edu).
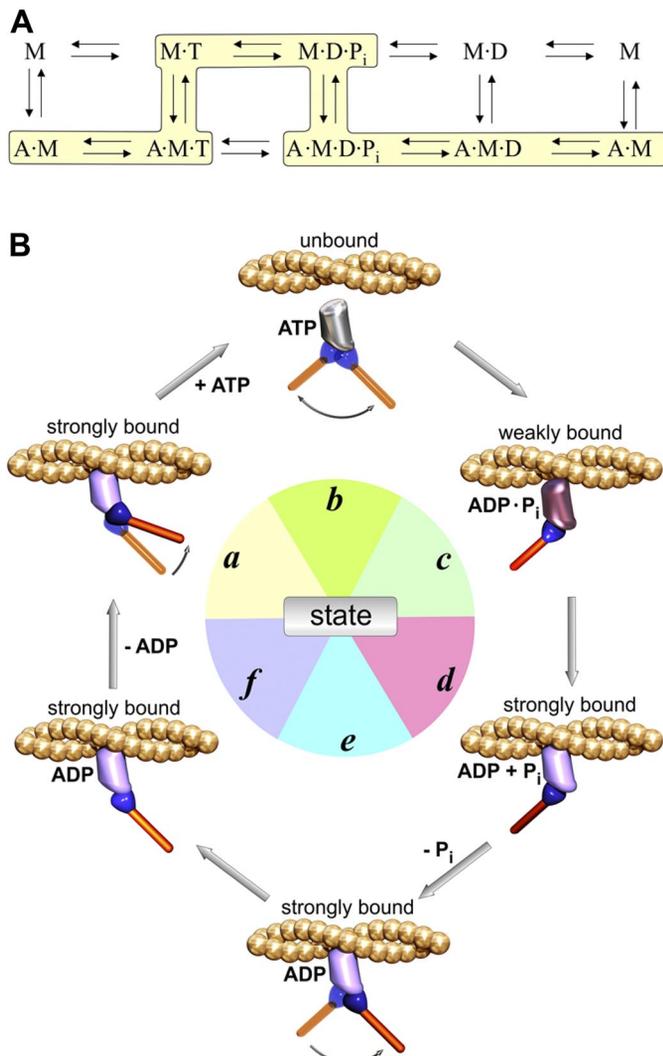
Fig. 1. Actomyosin ATPase cycle. *A*: minimal description of the myosin (M) and actomyosin (A·M) ATPase as defined in solution. Top line represents the myosin ATPase with the following events: ATP (T) binding, and ATP hydrolysis, followed by P_i release and then ADP (D) release. The equivalent steps for actomyosin are shown in the bottom line. Vertical arrows indicate the actin (A) association and dissociation from each myosin complex. In every case, the events shown can be broken down into a series of substeps involving one or more identifiable protein conformational changes. States outlined and with a shaded background represent the predominant pathway for the actomyosin ATPase. *B*: a minimal mechanochemical scheme for the actomyosin cross-bridge cycle. Starting from the rigor complex, A·M (*state a*), ATP binds to rapidly dissociate the complex, and the lever arm is reprimed to the pre-power stroke position (*state b*). This is followed by hydrolysis. The preceding 3 states have been well defined by crystallography, electron microscopy, and solution kinetics. The exact sequence of biochemical, structural, and mechanical events is more speculative. The M·D·P_i complex rebinds to actin, initially weakly (*state c*) and then strongly (*state d*). Binding to actin induces the dissociation of P_i and the power stroke (*state e*). The completion of the tail swing (*state f*) is followed by ADP release to return to the rigorlike complex (*state a*); in some myosins (e.g., smooth muscle myosin II, myo 1b, or myosin-V), ADP dissociation is associated with a further displacement of the lever arm. Actin monomers are shown as golden spheres. The motor domain is colored metallic gray for the free form, purple for the weakly bound form, and violet for the strongly bound form. The converter is shown in blue and the lever arm in orange. [Reproduced from Geeves et al. (27) with copyright permission of Birkhäuser Verlag, Basel, Switzerland.]

whereas during isotonic shortening skeletal muscle myosin spends only 5% of the cycle time in strongly bound states (50). The rate of transition from the weakly bound low-force state to the strongly bound high-force state (difference between the forward and backward rate constants of the *state c*-to-*state d* transition in Fig. 1) is thought to limit the peak rate of force development ($+dP/dt$). In single cells, this parameter is determined by shortening and reextending a fully activated fiber and measuring the rate constant of tension redevelopment (k_{tr}). The k_{tr} is sevenfold higher in fast compared with slow fibers and shows Ca^{2+} sensitivity with lower values observed in suboptimal Ca^{2+} conditions (40). In contrast, the maximal unloaded shortening velocity (V_0) is highly correlated with the actomyosin ATP hydrolysis rate, which in turn appears limited by the ADP dissociation step (*state f*-to-*state a* transition in Fig. 1). This kinetic scheme has proven useful in assessing the cellular mechanisms of muscle fatigue for those factors acting at the cross bridge (21, 23).

ALTERATIONS IN MUSCLE MECHANICS WITH FATIGUE

The functional correlates of fatigue observed in both animals and humans during prolonged endurance and high-intensity, short-duration exercise include a decline in twitch and tetanic force, maximal shortening velocity, and peak power (7, 17, 21, 23, 31). While muscles do not contract in vivo with single twitches, the evaluation of isometric twitches before and after fatigue has proven useful in identifying the cellular sites of fatigue (21). Features of a postfatigued twitch are reduced twitch force (P_t), prolonged contraction and half-relaxation times (CT and ½RT), and reductions in the peak rate of tension development ($+dP/dt$) and decline ($-dP/dt$). The low P_t and prolonged CT and ½RT are known to reflect a reduction in the amplitude and an increase in the duration of the intracellular Ca^{2+} transient, respectively, and thus do not necessarily reflect any direct affect on the cross bridge (1, 2).

The reduced peak force (P_0) with fatigue can be explained by a decline in the force per cross bridge and/or the number of cross bridges in the high-force states (*states d, e, f,* and *a* in Fig. 1). The mechanism can involve a decline in the amplitude of the Ca^{2+} transient and agents that directly inhibit the cross bridges. Allen et al. (1) summarized the mechanisms involved in the reduction of isometric force with fatigue and suggested that the initial decline in force occurred as a direct result of an inhibition of the force-generating steps of the cross-bridge cycle. Simultaneously, there was a right shift of the force-Ca^{2+} relationship, but this had no consequence until later in fatigue when the amplitude of the intracellular Ca^{2+} transient decreased.

With contraction, the forward rate constant for the weakly bound, low-force *state c* (Fig. 1) to the strongly bound, high-force *state d* of the cross bridge (Fig. 1) is accelerated by both cytoplasmic Ca^{2+} and the number of strongly bound cross bridges, both of which decrease with fatigue. In high-intensity exercise, the decline in $+dP/dt$ with fatigue could also be mediated by an increase in H^+ and P_i. The observation that $+dP/dt$ divided by P_0 [($+dP/dt)/P_0$] was not altered by fatigue suggests that the reduced $+dP/dt$ resulted primarily from the decline in P_0, which in turn was caused by the fatigue-induced reduction in the number and force of the strongly bound, high-force cross bridges (21, 36). The latter is dependent on the

concentration of cytoplasmic Ca^{2+}, pH, and P_i. In contrast, $-dP/dt$ declines considerably more than force, and more in fast than slow fibers. This suggests a slower dissociation of actin from myosin in fatigued muscle cells, which could reflect a direct effect on the cross-bridge detachment rate and/or a reduced rate of Ca^{2+} reuptake by the SR pumps (56, 57). The rate of Ca^{2+} dissociation from troponin is thought to be too fast to be rate limiting (1). Relaxation from a tetanus generally occurs in three phases, where force initially shows no change following the final action potential (*phase 1*); then exhibits a slow, linear drop (*phase 2*); and finally a rapid exponential decline (*phase 3*). With fatigue, each of these phases is slowed, and the distinction between them is less clear (1, 60). During *phase 2*, the sarcomere length remains constant, and the force decline is thought to reflect the rate of cross-bridge detachment. The time course of the decline in force is delayed compared with the predicted force determined from the force-pCa relationship. It has been hypothesized that the increased difference between the measured and predicted force in fatigued muscle fibers is due to a slowed relaxation caused in part by a reduced cross-bridge dissociation rate (1, 56, 57).

While peak force has been shown to decline before and to a greater extent than velocity (15), both are known to contribute to the loss of power with fatigue. Peak power shows a greater decline with fatigue in fast muscles because of a greater reduction in both force and velocity (23). The decline in velocity at peak power in fast muscles is in part due to a fatigue-induced increase in the curvature (i.e., lower a/P_0 ratio) of the force-velocity relationship (23, 31). This is particularly important as peak power is elicited at approximately 20–30% of peak force and maximal velocity, where the force-velocity relationship has the greatest curvature (23, 31).

While force always declines first, the reduction in maximal velocity determined from the force-velocity relationship (V_{max}) may ultimately drop by an amount equal to that observed for force (1). However, muscles contracting in vivo never experience zero loads; thus it is important to assess whether the curvature as reflected by the a/P_0 ratio changes with fatigue. In fast muscles, the a/P_0 ratio is three- to fourfold higher compared with slow muscle. Consequently, individuals with a high percentage of fast-twitch fibers can generate higher velocity and power at a given relative load than those with predominantly slow-twitch fibers. There are little data assessing the affect of fatigue on the a/P_0 ratio (23). Jones et al. (31) produced fatigue in the fast-twitch human adductor pollicis muscle and observed the a/P_0 ratio to decline with a similar time course to the loss of peak power. The authors concluded that the loss of force and the reduced a/P_0 ratio contributed equally to the decline in peak power. To my knowledge, there is no comparable information on predominately slow-twitch muscles. Since the extremes of the force-velocity relationship (V_{max} and P_0) in response to a given contractile paradigm are more resistant to change in slow compared with fast muscles, one might expect a similar difference in the stability of the a/P_0 ratio (1, 21, 23).

MUSCLE FATIGUE AND THE CROSS-BRIDGE CYCLE

Inhibition of the high-force states by H^+ and P_i. Intense exercise induces high rates of ATP hydrolysis and glycolysis, and corresponding increases in cell H^+, P_i, and ADP. The first

two are known to directly reduce the force output of the high-force states, while the former by itself would increase force but slow velocity (8, 9, 12, 43). Intracellular pH can reach levels as low as 6.2 (from a resting value of 7.0), while P_i may increase to 30 mM (7, 10, 61). The extent of the change in these ions is directly related to the intensity of the work and fiber type, with the fast glycolytic fibers (type IIx and IIb) showing the lowest pH and highest P_i values (21). Metzger and Moss (37) found fast-twitch fibers from the predominantly type IIb and IIx superficial region of the vastus lateralis of the rat to be more sensitive than slow soleus fibers to the depressive effects of low pH. This difference was not observed when slow type I and fast type IIa fibers were compared (30). The observation that the depressive effects of low pH on peak force are still apparent in the presence of saturating levels of free Ca^{2+} indicates that the mechanism is not simply the result of H^+ inhibition of Ca^{2+} binding to troponin (37). The fact that rigor tension (*state a* in Fig. 1) was reduced at pH 6.2 compared with 7.0 suggests a direct effect of H^+ on the actomyosin cross bridge (19). The effect could involve a decline in the number and/or the force per bridge of the high-force strongly bound cross bridges (Fig. 1). At 15°C, a drop in fiber pH has been shown to reduce the force per cross bridge in both fast- and slow-twitch fibers, while the number of strongly bound (high force) cross bridges was reduced only in fast fibers (38, 39). The latter was attributed to a H^+-mediated inhibition of the forward rate constant for the transition between the weakly and strongly bound cross-bridge states. The observation that stiffness (a marker of the number of strongly bound cross bridges) did not decline in slow fibers, and declined less than force in fast fibers, suggests that the primary means by which protons inhibit force is a reduction in the force per bridge.

Similar to H^+, an increased intracellular P_i is correlated with fatigue in contracting muscles (7, 10, 61) and is known to depress force in maximally activated skinned fibers (12–14, 25, 33, 43, 46). Also, like H^+, it is thought to act by inhibiting the transition from the weakly bound (*state c;* Fig. 1) to the strongly bound (*state d*; Fig. 1) cross bridge, and/or a reduction in the force of the strongly bound states (13, 44). It has been suggested that high P_i reduces force and increases k_{tr} by accelerating the reverse rate constant of the low- to high-force state transition (44). The observation that fiber stiffness is less sensitive to P_i than force suggests that either a part of the force depression is due to less force per strongly bound bridge or that an additional intermediate low-force, strongly bound state exists. This state of the actomyosin would contain both P_i and ADP and would increase in the presence of high P_i (13, 50).

Effect of temperature on the inhibition of force. Up until the mid-1990s, experiments assessing the effect of H^+ and P_i on fiber force were performed at temperatures below 25° C (32, 33, 37–40, 43, 46). Recently, a number of labs, including mine (9, 12, 13, 45, 59), have shown the deleterious effects of high H^+ and P_i on the P_0 of maximally activated fibers to be reduced at 30°C compared with lower temperatures. The reduced effect of these ions is thought to result from the forward rate constant of the force-generating step being more temperature sensitive than the reverse rate constant (62). Zhao and Kawai (62) used sinusoidal analysis to demonstrate that the forward rate constant was greatly accelerated as temperature increased. The observation that P_0 increased with temperature is consistent

with the acceleration of the forward rate constant, and the production of more bridges in the high-force state. In our experiments, for both slow and fast fibers, the inhibiting effect of pH 6.2 on fiber P_0 was reduced from ~30 to 11% with a temperature increase from 15 to 30°C (30). These results were consistent with the data of Pate et al. (45) and Westerblad et al. (59), who found low pH to decrease P_0 by 18% and 10%, respectively, at temperatures between 30 and 32°C. The effect of P_i on P_0 was also tempered by increasing temperature but, unlike H^+, showed a fiber type dependency. At 15°C, 30 mM P_i depressed fiber P_0 by ~50% in fast and slow fibers, while at 30°C the decline was only 5% in fast fibers compared with 19% in slow fibers (12). The mechanism of this difference is not understood but could be due to fiber type-dependent effects of temperature on the forward rate constant of the low- to high-force transition (62). The lack of a fiber type difference in response to low pH (at least between fast type IIa and slow type I fibers) suggests that the mechanism by which P_0 is depressed is fundamentally different for high P_i and low pH. If these ions act at different sites in the cross-bridge cycle, their effects should be additive. Using the skinned fiber, Nosek et al. (43) showed this to be the case as 30 mM P_i and pH 6.2 depressed force considerably more than pH 6.2 and zero added P_i.

Due to problems with fiber stability, studies using skinned fibers are limited to temperatures at or below 30°C. Intramuscular temperature is known to vary with environmental temperature (48) and exercise (20) and thus may vary between environmental and core body temperature. It is difficult to predict the effect on high H^+ and P_i at the high limit of muscle temperatures (37°-39° C). However, the observation that P_0 is unaffected and V_0 only moderately affected by temperature increases from 30°C to 35° C (47) suggests that the inhibiting effects of H^+ and P_i at high muscle temperatures might be relatively the same as those measured at 30°C.

Inhibition of fiber velocity. An increase in myoplasmic H^+ concentration has been shown to depress the maximal shortening velocity as determined by the slack test (V_0) in both living and skinned fibers (15, 37, 52). This effect has been reported to be greater in fast- compared with slow-twitch fibers (37). Recently, we observed this fiber type difference to be reduced at high compared with low temperature. At 15°C, we observed pH 6.2 to inhibit V_0 by 9 and 27% in slow type I and fast type IIa fibers, respectively, while the inhibition was 25 and 32%, respectively, at 30°C (30). Importantly, the H^+ inhibition of the slow fiber V_0 was increased at the higher (near in vivo) temperature. Since V_0 is thought to be limited by the rate of ADP dissociation from the myosin head (*state f*-to-*state a* transition, Fig. 1), it is apparent that low pH inhibits this transition as well as the weakly to strongly bound cross-bridge transition (*state c* to *state d*, Fig. 1). When the pH effects on maximal velocity were determined from the force-velocity relationship (V_{max}), the results were qualitatively the same, but the degree of inhibition was less than observed for V_0 (30). The mechanism by which low pH inhibits velocity is unknown. It may directly inhibit the rate-limiting step (transition from *state f* to *state a*, Fig. 1) and/or cause a decrease in the filament lattice spacing and corresponding increase in internal drag (30, 53).

Controversy exists as to the relative importance of low pH in inhibiting fiber velocity (30, 59). Westerblad et al. (59) studying living fast fibers at 32°C found no significant effect of pH 6.7 on V_0, and Bruton et al. (6) concluded that acidosis to pH 6.6 (produced by 30% CO_2 exposure) did not accelerate the development of fatigue during tetanic stimulation of fast fibers. In contrast, Edman and Mattiazzi (15) observed a significant decline in V_0 and P_0 following the acidification of frog fibers by ~1 pH unit. Collectively, these results suggest that the inhibiting effects of protons on V_0 and P_0 do not occur until pH drops below 6.7. Intense exercise has been shown to reduce intracellular muscle pH to as low as 6.2 (61); however, in many studies the extent of acidosis is less (see Table 1 of Ref. 21). Clearly, the contribution of H^+ to fatigue will depend on the extent of acidosis, muscle temperature, and the amplitude of the intracellular Ca^{2+} transient. The latter is important as H^+ is known to depress myofibrillar Ca^{2+} sensitivity.

High P_i (up to 30 mM) has no effect on slow type I or fast type II fiber V_{max} at cold or near in vivo temperatures (8, 12). Recently, Franks-Skiba et al. (24) reported that at 30°C and in the presence of 0.5 mM of the phosphate analog vanadate, myosin light chain phosphorylation inhibited fast fiber V_{max}. No effect of vanadate was observed in dephosphorylated myosin light chain conditions. The authors suggest that since the vanadate and P_i occupy the same cross-bridge states, a P_i build-up coupled to an increased myosin light chain phosphorylation could contribute to the decline in fiber velocity in fatigued muscles. The mechanism is not clear but might be mediated by a slower release of ADP or to an increased drag caused by weakly bound cross bridges (24).

Force-velocity relationship and peak power. The extent of muscle fatigue is perhaps best related to changes in peak power, as body movement is dependent on the capacity to generate power. Since power is a function of both force and velocity, it is considerably higher in fast fibers and greatly affected by temperature (12, 30). Peak power is elicited at contraction velocities and loads between 20 and 40% of V_{max} and P_0. Given their effects on force and velocity, it is not surprising that both low pH and high P_i depress peak power (Fig. 2). For P_i the inhibition of power is reduced but still significant at 30°C compared with 15°C (12). At the higher temperature, peak power was reduced by 26 and 18% of the control condition (zero added P_i) for slow and fast fibers, respectively (Fig. 2, *B* and *D*). High temperature not only increases peak force, V_0, and peak power, but the a/P_0 ratio allowing fibers to generate more force at a given velocity. At 30°C, high P_i reduces peak power by depressing force and reducing the a/P_0 ratio such that less force is generated at a given velocity (12, 31). The latter is quite substantial. At 30°C, high P_i depressed the a/P_0 ratio by 38 and 30% in fast and slow fibers, respectively (12). The observation that P_i had no effect on the a/P_0 ratio at 15°C suggests that the mechanism cannot by due to an increased internal drag caused by a P_i-induced increase in the number of weakly bound low-force cross bridges (*state c*, Fig. 1) as that effect is greater at the lower temperature. The most likely explanation is that P_i inhibits the cross-bridge detachment rate. In support of this hypothesis, Westerblad and Allen (56, 57) observed a marked slowing of the peak relaxation rate ($-dP/dt$) with fatigue of the mouse flexor brevis muscle, which they attributed to an altered cross-bridge kinetics. The observation that high P_i depresses the a/P_0 ratio but not V_{max} suggests that the step limiting velocity in the cross-bridge cycle (Fig. 1) may be different in loaded vs. unloaded contractions.
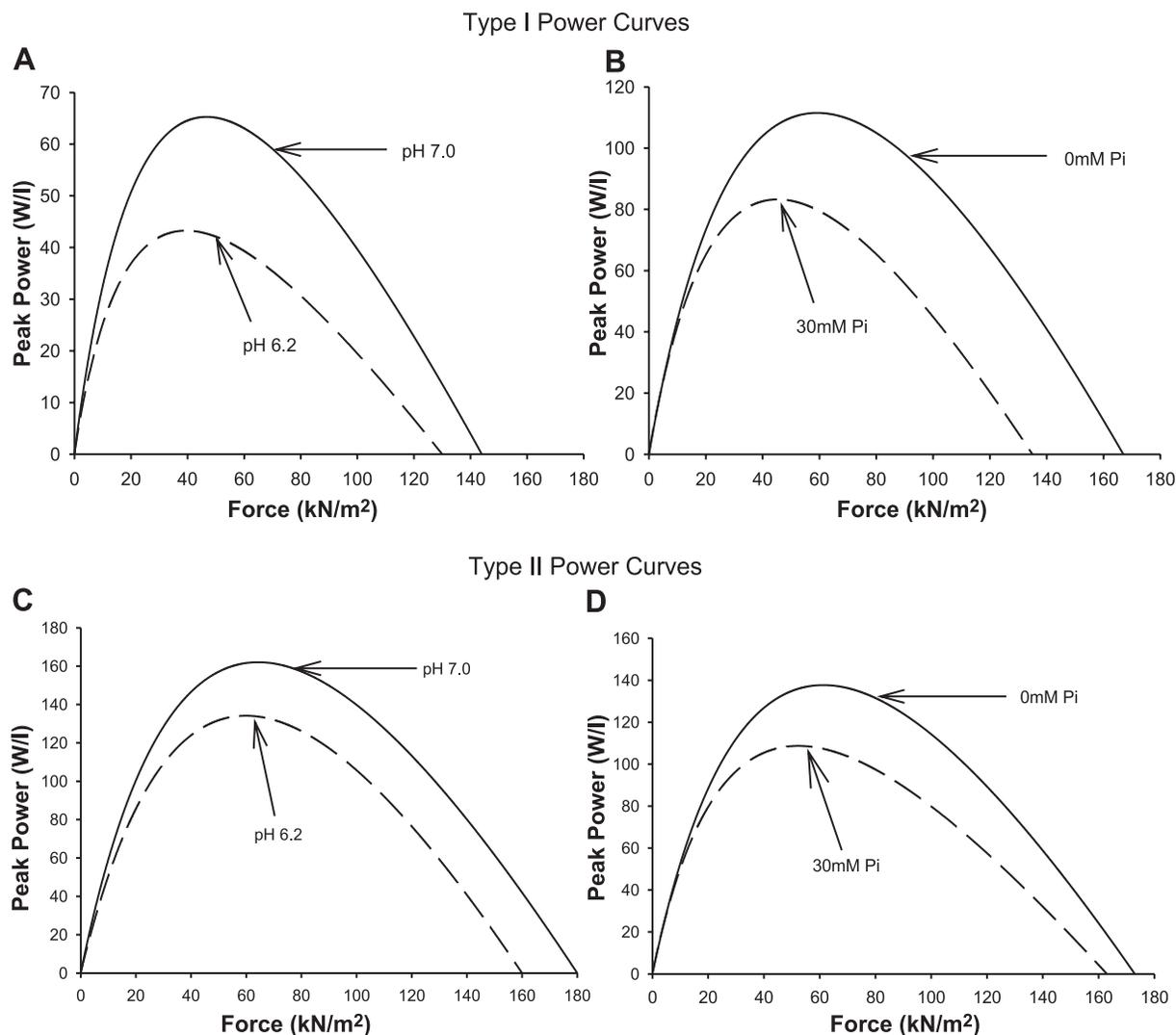
Type I Power Curves

A

B

Type II Power Curves

C

D

Fig. 2. Composite force-power curves for soleus type I fibers determined at pH 7.0 and 6.2 and zero added P_i (*A*), and at zero and 30 mM added P_i and pH 7.0 (*B*). Composite force-power curves for gastrocnemius type II fibers determined at pH 7.0 and 6.2 and zero added P_i (*C*), and at zero and 30 mM added P_i and pH 7.0 (*D*). W/l = watts/liter. Adapted from data plots published in Debold et al. (12) and Knuth et al. (30); all data were obtained at 30°C.

In contrast to P_i, low pH inhibits the peak power of the slow fiber to a greater extent at 30°C then at colder temperatures (30). This effect is attributed to a greater depression of velocity at the higher temperature. Unlike colder temperatures where fast fibers are more sensitive to low pH, at near in vivo temperatures, slow fibers show a greater pH-dependent depression of peak power (compare Fig. 2, *A* and *C*). These data on fiber type susceptibility should be interpreted with caution as the skinned fiber preparation may not reflect the conditions of contracting living fibers. If as suggested by Franks-Skiba et al. (24), myosin light chain phosphorylation exacerbates the inhibitory actions of H^+ and/or P_i, the effect might be greater in fast fibers known to have higher myosin light chain kinase activities.

Low pH depressed the a/P_0 ratio in slow but not fast fibers. Thus the fatigue-induced increase in the curvature of the force-velocity relationship (reduced a/P_0 ratio) observed in fast muscles studied in situ or in vivo might be induced by an increased P_i but does not appear to be caused by low pH (23, 30, 31).

MUSCLE FATIGUE AND THE FORCE-pCA RELATIONSHIP

Both H^+ and P_i not only directly inhibit cross-bridge force but also shift the force-pCa relationship to the right such that higher free Ca^{2+} is required to reach a given tension. As long as the amplitude of the Ca^{2+} transient remains high (pCa > 5.5), peak force will be unaltered by the reduced Ca^{2+} sensitivity (Fig. 3). However, late in fatigue, Allen et al. (2) have shown a precipitous drop in the amplitude of the Ca^{2+} transient. Under these conditions (pCa < 6.0), the reduced Ca^{2+} sensitivity induced by P_i and H^+ would be expected to make a significant contribution to the decline in force (Fig. 3). For low pH, this effect is mediated in part by competitive inhibition of Ca^{2+} binding to troponin-C (55). In addition, the decline in the number of strongly bound, high-force cross bridges (caused by both H^+ and P_i) reduces the thick filament-mediated cooperativity between regulatory thin filament sites, and this is thought to contribute to the right shift in the force relationship. Additionally, at less than maximal Ca^{2+} concentrations, the pH and P_i-induced right shift in the force-pCa relationship would
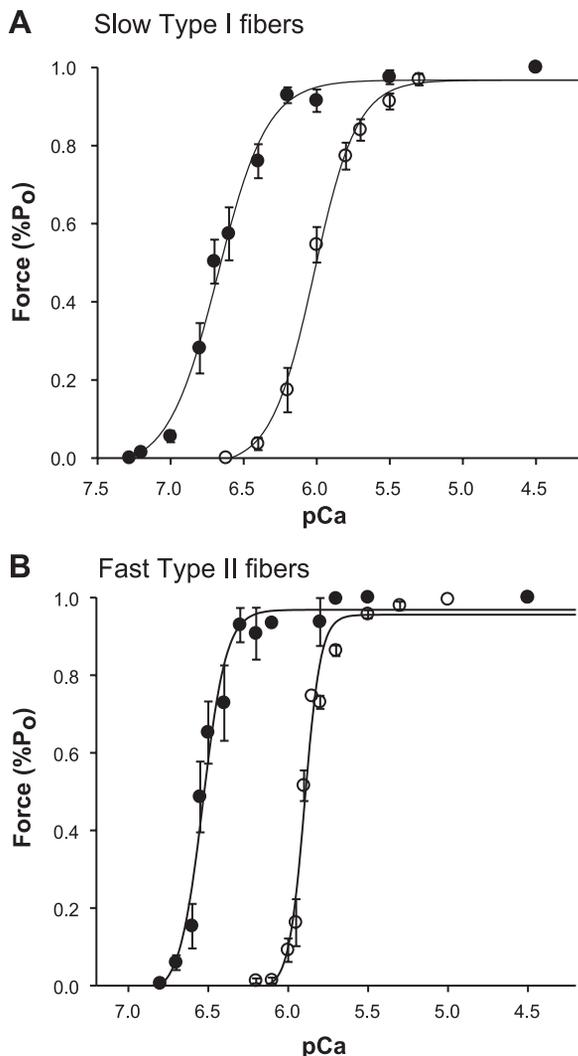
A   Slow Type I fibers

B   Fast Type II fibers

Fig. 3. Mean normalized force-pCa curves for slow type I (*A*) and fast type II (*B*) fibers. P_0, normalized to the level obtained in pCa 4.5 and 30°C at both concentrations of P_i, is plotted against pCa. Each data point represents the mean (±SD) force at each pCa level. Each curve represents data from a separate group of fibers (●, no added P_i; ○, 30 mM P_i added). P_0 = peak force. [Redrawn from data collected at 30°C and presented in Debold et al. (13).]

high-intensity muscle contraction), the deleterious effects of high P_i and H^+ on muscle force are likely to be more important than previously thought. The steeper force-pCa relationship in fast fibers contributes to the greater susceptibility of the fast fiber type to fatigue. For example, in the Debold et al. (13) study, 30 mM P_i resulted in a twofold greater drop in force in fast compared with slow fibers when pCa went from 5.0 to 6.0 (Fig. 3).

REACTIVE OXYGEN SPECIES AND CROSS-BRIDGE FUNCTION

The production of reactive oxygen species (ROS) is known to increase with exercise, and a number of studies have suggested that increases in ROS contribute to the development of fatigue (4, 5, 11 41, 42, 54). Moopanar and Allen (41) found the contribution of ROS to fatigue to be considerably greater at 37° compared with 22° C. Edwards et al. (18) recently confirmed the temperature dependence of ROS production. They found the rate of superoxide ($O_2^{\bullet-}$) production to show little increase between 22°C and 32°C, while it increased fivefold when temperature increased from 22°C to 37°C. Moopanar and Allen (41) observed fatigue elicited by repeated isometric tetani of the flexor digitorum brevis in vitro to be accelerated at body temperature compared with room temperature. Since the effect was blocked by the ROS scavenger Tiron (5 mM), the authors concluded that the fatigue was caused by ROS. The increased rate of fatigue at the higher temperature was not associated with any change in the amplitude of the Ca^{2+} transient or differences in the extent of decline in maximal Ca^{2+}-activated force. Rather, the ROS-mediated fatigue was attributed to a depression in myofibrillar Ca^{2+} sensitivity. ROS production at 37°C without fatigue had no effect on Ca^{2+} sensitivity (18, 42). The exact ROS responsible for the reduced Ca^{2+} sensitivity is unknown. The observation that the superoxide dismutase mimetic Tempol prevented the ROS-induced fatigue eliminates hydrogen peroxide as a causative agent as it is a product of the Tempol reaction with $O_2^{\bullet-}$ (18). The site of oxidative damage of the myofibrillar proteins has not been elucidated. Logical candidates are troponin-C and -I as alterations in either could yield a reduced myofibrillar response to Ca^{2+} (5, 55). Other possible targets of oxidative damage are tropomyosin, actin, and myosin (42). An important unanswered question is whether oxidative damage of myofibrillar proteins, shown to occur with stimulation of in vitro preparations, actually occurs with fatigue during in vivo exercise (42).

SUMMARY AND FUTURE RESEARCH DIRECTIONS

Force, velocity, and power are ultimately determined by the molecular factors controlling the number and force of the strongly bound cross bridges, and the rate of cross-bridge cycling (Fig. 1). With high-intensity muscle contraction, the force per strongly bound, high-force bridge is reduced by both P_i and H^+. In the case of fast fibers, the force reduction is further exacerbated by a decline in the number of strongly bound bridges (Fig. 1, *states d, e, f,* and *a*). Elevated H^+ (pH < 6.7) has the added effect of inhibiting velocity, presumably by slowing the rate of ADP release (Fig. 1 *state f*-to-*state a* transition). With fatigue, the decline in peak power is due to inhibition of both force and velocity, and at least in fast fibers to a depressed a/P_0 ratio. The latter is thought to be caused by P_i inhibition of cross-bridge detachment. Equally important in

also contribute to the decline in k_{tr} (and presumably $+dP/dt$) as the forward rate constant of *state c*-to-*state d* transition (Fig. 1) would be slowed by the reduction in the number of strong-binding, high-force cross bridges (39).

The Ca^{2+} sensitivity of force development is fiber type dependent with fast fibers activating at a higher free Ca^{2+} concentration but with a greater degree of cooperative binding. A rise in muscle temperature has been shown to increase Ca^{2+} sensitivity (shift the force-pCa relationship to the left) in both slow and fast fibers (13, 49, 51). An important finding from the perspective of muscle fatigue is the recent observation by Debold et al. (13) that high P_i depresses Ca^{2+} sensitivity more at 30°C compared with 15°C. Figure 3 shows the extent of the right shift in the force-pCa relationship caused by 30 mM P_i at 30°C for both slow type I and fast type II fibers (13). Although not yet studied, it is likely that the depressive effect of H^+ on Ca^{2+} sensitivity is also temperature dependent. These results suggest that when SR Ca^{2+} release is compromised (as it is in

the fatigue process is that both P_i and H^+ reduce myofibrillar Ca^{2+} sensitivity, which becomes important late in fatigue when the amplitude of the Ca^{2+} transient is depressed. The reduction in myofibrillar Ca^{2+} sensitivity is greater at 30°C compared with 22°C, which suggests that the deleterious effects of high P_i and H^+ may be greater than originally thought from experiments conducted at cold temperatures (10–22°C).

Many of the studies reviewed were conducted using the chemically skinned fiber preparation. A major advantage of this technique is the ability to determine how known quantities of a particular compound (P_i, H^+, ADP, etc.) either individually or collectively alter fiber force, velocity, and power. Importantly, the effects of a particular fatigue agent can be assessed independently of changes in intracellular Ca^{2+}. However, the chemically skinned fiber has limitations in that reliable studies cannot be performed at temperatures >30°C, soluble proteins such as parvalbumin may diffuse out of the fiber, and the phosphorylation state of proteins (such as myosin light-chain 2) may not reflect that existing in living fibers. For these reasons, future studies need to be performed on both skinned (chemically and mechanically peeled) and living fibers.

While it is clear that alterations in myofibrillar events contribute to fatigue (particularly fatigue resulting from high-intensity exercise), the relative importance of increases in P_i and H^+, and the combined effects of high P_i, H^+, and ADP, on cross-bridge properties and the fatigue of slow and fast fiber types is yet to be determined. Future studies are needed to establish the mechanisms by which these compounds inhibit force and power, and how P_i depresses the a/P_0 ratio. Since the effects of fatigue agents acting at the level of the myofibrillar proteins are clearly temperature dependent, it will be important to conduct studies at near in vivo temperatures (≥30° C). Finally, while ROS have been shown to depress myofibrillar Ca^{2+} sensitivity in muscles contracting in vitro, future studies are needed to determine whether this effect occurs during in situ or in vivo muscle contraction; and if it does, the mechanism and site of action of the ROS needs to be established.

REFERENCES

1. **Allen DG, Lännergren J, Westerblad H.** Muscle cell function during prolonged activity: cellular mechanisms of fatigue. *Exp Physiol* 80: 497–527, 1995.
2. **Allen DG, Lee JA, Westerblad H.** Intracellular calcium and tension during fatigue in isolated single muscle fibres from *Xenopus laevis*. *J Physiol* 415: 433–458, 1989.
3. **Allen DG, Westerblad H.** Role of phosphate and calcium stores in muscle fatigue. *J Physiol* 536: 657–665, 2001.
4. **Andrade FH, Reid MB, Allen DG, Westerblad H.** Effect of hydrogen peroxide and dithiothreitol on contractile function of single skeletal muscle fibres from the mouse. *J Physiol* 509: 565–575, 1998.
5. **Brotto MA, van Leyen SA, Nosek CM, Brotto LS, Nosek TM.** Hypoxia and fatigue-induced modification of function and proteins in intact and skinned murine diaphragm muscle. *Pflügers Arch* 440: 727–734, 2000.
6. **Bruton JD, Lännergren J, 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.
7. **Cady EB, Jones DA, Moll A.** Changes in force and intracellular metabolites during fatigue of human skeletal muscle. *J Physiol* 418: 327–337, 1989.
8. **Cooke R, Franks K, Luciani G, Pate E.** The inhibition of rabbit skeletal muscle contraction by hydrogen ions and phosphate. *J Physiol* 395: 77–97, 1988.
9. **Coupland ME, Puchert E, Ranatunga KW.** Temperature dependence of active tension in mammalian (rabbit psoas) muscle fibres: effect of inorganic phosphate. *J Physiol* 536: 879–891, 2001.
10. **Dawson MJ, Gadian DG, Wilkie DR.** Muscular fatigue investigated by phosphorus nuclear magnetic resonance. *Nature* 274: 861–866, 1978.
11. **de Paula Brotto M, van Leyen SA, Brotto LS, Jin JP, Nosek CM, Nosek TM.** Hypoxia/fatigue-induced degradation of troponin I and troponin C: new insights into physiologic muscle fatigue. *Pflügers Arch* 442: 738–744, 2001.
12. **Debold EP, Dave H, Fitts RH.** Fiber type and temperature dependence of inorganic phosphate: implications for fatigue. *Am J Physiol Cell Physiol* 287: C673–C681, 2004.
13. **Debold EP, Romatowski J, Fitts RH.** The depressive effect of P_i on the force calcium relationship in skinned single muscle fibers is temperature dependent. *Am J Physiol Cell Physiol* 290: C1041–C1050, 2006.
14. **Duke AM, Steele DS.** Interdependent effects of inorganic phosphate and creatine phosphate on sarcoplasmic reticulum Ca^{2+} regulation in mechanically skinned rat skeletal muscle. *J Physiol* 531: 729–742, 2001.
15. **Edman KAP, Mattiazzi AR.** Effects of fatigue and altered pH on isometric force and velocity of shortening at zero load in frog muscle fibers. *J Muscle Res Cell Motil* 2: 321–334, 1981.
16. **Edwards RHT.** Biochemical basis of fatigue in exercise performance: catastrophe theory of muscular fatigue. In: *Biochemistry of Exercise,* edited by Knuttgen HG. Champaigne, IL: Human Kinetics, p. 3–28, 1983.
17. **Edwards RHT, Hill DK, Jones DA, Merton PA.** Fatigue of long duration in human skeletal muscle after exercise. *J Physiol* 272: 769–778, 1977.
18. **Edwards JN, Macdonald WA, van der Poel C, Stephenson DG.** $O_2^{•−}$ production at 37°C plays a critical role in depressing tetanic force of isolated rat and mouse skeletal muscle. *Am J Physiol Cell Physiol* 293: C650–C660, 2007.
19. **Fabiato A, Fabiato F.** Effect of pH on the myofilaments and the sarcoplasmic reticulum of skinned cells from cardiac and skeletal muscles. *J Physiol* 276: 233–255, 1978.
20. **Ferguson RA, Krustrup P, Kjaer M, Mohr M, Ball D, Bangsbo J.** Effect of temperature on skeletal muscle energy turnover during dynamic knee-extensor exercise in humans. *J Appl Physiol* 101: 47–52, 2006.
21. **Fitts RH.** Cellular mechanisms of muscle fatigue. *Physiol Rev* 74: 49–94, 1994.
22. **Fitts RH.** Mechanisms of muscular fatigue. In: *Principles of Exercise Biochemistry,* edited by Poortmans JR. Basel: Karger, 2004, vol. 46, p. 279–300 (Med Sport Sci Ser).
23. **Fitts RH.** The muscular system: fatigue processes. In: *Advanced Exercise Physiology*, edited by Tipton C. Philadelphia, PA: Lippincott Williams and Wilkins, 2006, chapt. 8, p. 178–196.
24. **Franks-Skiba K, Lardelli R, Germaine G, Cooke R.** Myosin light chain phosphorylation inhibits muscle fiber shortening velocity in the presence of vanadate. *Am J Physiol Regul Integr Comp Physiol* 292: R1603–R1612, 2007.
25. **Fryer MW, Owen VJ, Lamb GD, GD, Stephenson DG.** Effects of creatine phosphate and P_i on Ca^{2+} movements and tension development in rat skinned skeletal muscle fibres. *J Physiol* 482: 123–140, 1995.
26. **Gandevia SC.** Spinal and supraspinal factors in human muscle fatigue. *Physiol Rev* 81: 1725–1789, 2001.
27. **Geeves MA, Fedorov R, Manstein DJ.** Molecular mechanism of acto-myosin-based motility. *Cell Mol Life Sci* 62: 1462–1477, 2005.
28. **Hermansen L, Osnes J.** Blood and muscle pH after maximal exercise in man. *J Appl Physiol* 32: 302–308, 1972.
29. **Karlsson J, Saltin B.** Lactate, ATP, and CP in working muscles during exhaustive exercise in man. *J Appl Physiol* 29: 598–602, 1970.
30. **Knuth ST, Dave H, Peters JR, Fitts RH.** Low cell pH depresses peak power in rat skeletal muscle fibres at both 30°C and 15°C: implications for muscle fatigue. *J Physiol* 575: 887–899, 2006.

31. **Jones DA, de Ruiter CJ, de Haan A.** Change in contractile properties of human muscle in relationship to the loss of power and slowing of relaxation seen with fatigue. *J Physiol* 576: 913–922, 2006.

32. **Lännergren J, Westerblad H.** Force decline due to fatigue and intracellular acidification in isolated fibers of mouse skeletal muscle. *J Physiol* 434: 307–322, 1991.

33. **Martyn DA, Gordon AM.** Force and stiffness in glycerinated rabbit psoas fibers. Effects of calcium and elevated phosphate. *J Gen Physiol* 99: 795–816, 1992.

34. **McClain J, Hardy C, Enders B, Smith M, Sinoway L.** Limb congestion and sympathoexcitation during exercise. Implications for congestive heart failure. *J Clin Invest* 92: 2353–2359, 1993.

35. **Metzger JM, Fitts RH.** Role of intracellular pH in muscle fatigue. *J Appl Physiol* 62: 1392–1397, 1987.

36. **Metzger JM, Fitts RH.** Fatigue from high- and low-frequency muscle stimulation: contractile and biochemical alterations. *J Appl Physiol* 62: 2075–2082, 1987.

37. **Metzger JM, Moss RL.** Greater hydrogen ion-induced depression of tension and velocity in skinned single fibres of rat fast than slow muscles. *J Physiol* 393: 727–742, 1987.

38. **Metzger JM, Moss RL.** Effects on tension and stiffness due to reduced pH in mammalian fast- and slow-twitch skinned skeletal muscle fibres. *J Physiol* 428: 737–750, 1990.

39. **Metzger JM, Moss RL.** pH modulation of the kinetics of a Ca^{2+}-sensitive cross-bridge state transition in mammalian single skeletal muscle fibres. *J Physiol* 428: 751–764, 1990.

40. **Metzger JM, Moss RL.** Calcium-sensitive cross-bridge transitions in mammalian fast and slow skeletal muscle fibers. *Science* 247: 1088–1090, 1990.

41. **Moopanar TR, Allen DG.** Reactive oxygen species reduce myofibrillar Ca^{2+} sensitivity in fatiguing mouse skeletal muscle at 37°C. *J Physiol* 564: 189–199, 2005.

42. **Moopanar TR, Allen DG.** The activity-induced reduction of myofibrillar Ca^{2+} sensitivity in mouse skeletal muscle is reversed by dithiothreitol. *J Physiol* 571: 191–200, 2006.

43. **Nosek TM, Fender KY, Godt RE.** It is diprotonated inorganic phosphate that depresses force in skinned skeletal muscle fibers. *Science* 236: 191–193, 1987.

44. **Palmer S, Kentish JC.** The role of troponin C in modulating the Ca^{2+} sensitivity of mammalian skinned cardiac and skeletal muscle fibres. *J Physiol* 480: 45–60, 1994.

45. **Pate E, Bhimani M, Franks-Skiba K, Cooke R.** Reduced effect of pH on skinned rabbit psoas muscle mechanics at high temperatures: implications for fatigue. *J Physiol* 486: 689–694, 1995.

46. **Potma EJ, van Graas IA, Stienen GJ.** Influences of inorganic phosphate and pH on ATP utilization in fast and slow skeletal muscle fibers. *Biophys J* 69: 2580–2589, 1995.

47. **Ranatunga KW.** Temperature-dependence of shortening velocity and rate of isometric tension development in rat skeletal muscle. *J Physiol* 329: 465–483, 1982.

48. **Ranatunga KW, Sharpe B, Turnbull B.** Contractions of a human skeletal muscle at different temperatures. *J Physiol* 390: 383–395, 1987.

49. **Stephenson DG, Williams DA.** Calcium-activated force responses in fast- and slow-twitch skinned muscle fibres of the rat at different temperatures. *J Physiol* 317: 281–302, 1981.

50. **Sweeney HL, Houdusse A.** The motor mechanism of myosin V: insights for muscle contraction. *Phil Trans R Soc B* 359: 1829–1841, 2004.

51. **Sweitzer NK, Moss RL.** The effect of altered temperature on Ca^{2+}-sensitive force in permeabilized myocardium and skeletal muscle. Evidence for force dependence of thin filament activation. *J Gen Physiol* 96: 1221–1245, 1990.

52. **Thompson LV, Balog EM, Riley DA, Fitts RH.** Muscle fatigue in frog semitendinosus: alterations in contractile function. *Am J Physiol Cell Physiol* 262: C1500–C1506, 1992.

53. **Umazume Y, Onodera S, Higuchi H.** Width and lattice spacing in radially compressed frog skinned muscle fibres at various pH values, magnesium ion concentrations, and ionic strengths. *J Muscle Res Cell Motil* 7: 251–258, 1986.

54. **van der Poel C, Edwards JN, Macdonald WA, Stephenson DG.** Mitochondrial superoxide production in skeletal muscle fibers of the rat and decreased fiber excitability. *Am J Physiol Cell Physiol* 292: C1353–C1360, 2007.

55. **Wattanapermpool J, Reiser PJ, Solaro RJ.** Troponin I isoforms and differential effects of acidic pH on soleus and cardiac myofilaments. *Am J Physiol Cell Physiol* 268: C323–C330, 1995.

56. **Westerblad H, Allen DG.** The contribution of $[Ca^{2+}]_i$ to the slowing of relaxation in fatigued single fibres from mouse skeletal muscle. *J Physiol* 468: 729–740, 1993.

57. **Westerblad H, Allen DG.** Relaxation, $[Ca^{2+}]_i$ and $[Mg^{2+}]_i$ during prolonged tetanic stimulation of intact, single fibres from mouse skeletal muscle. *J Physiol* 480: 31–43, 1994.

58. **Westerblad H, Allen DG, Bruton JD, Andrade FH, Lännergren J.** Mechanisms underlying the reduction of isometric force in skeletal muscle fatigue. *Acta Physiol Scand* 162: 253–260, 1998.

59. **Westerblad H, Bruton J, Lännergren J.** The effect of intracellular pH on contractile function of intact, single fibres of mouse muscle declines with increasing temperature. *J Physiol* 500: 193–204, 1997.

60. **Westerblad H, Lännergren J.** Slowing of relaxation during fatigue in single mouse muscle fibres. *J Physiol* 434: 323–336, 1991.

61. **Wilson JR, McCully KK, Mancini DM, Boden B, Chance B.** Relationship of muscular fatigue to pH and diprotonated P_i in humans: a ^{31}P-NMR study. *J Appl Physiol* 64: 2333–2339, 1988.

62. **Zhao Y, Kawai M.** Kinetic and thermodynamic studies of the cross-bridge cycle in rabbit psoas muscle fibers. *Biophys J* 67: 1655–1668, 1994.