Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Physiology

Bioenergetic basis of skeletal muscle fatigue

Christopher W Sundberg[1,2] and Robert H Fitts[1]

Energetic demand from high-intensity exercise can easily exceed ATP synthesis rates of mitochondria leading to a reliance on anaerobic metabolism. The reliance on anaerobic metabolism results in the accumulation of intracellular metabolites, namely inorganic phosphate (P_i) and hydrogen (H^+), that are closely associated with exercise-induced reductions in power. Cellular and molecular studies have revealed several steps where these metabolites impair contractile function demonstrating a causal role in fatigue. Elevated P_i or H^+ directly inhibits force and power of the cross-bridge and decreases myofibrillar Ca^{2+} sensitivity, whereas P_i also inhibits Ca^{2+} release from the sarcoplasmic reticulum (SR). When both metabolites are elevated, they act synergistically to cause marked reductions in power, indicating that fatigue during high-intensity exercise has a bioenergetic basis.

Addresses
[1] Department of Biological Sciences, Marquette University, Milwaukee, WI, United States
[2] Department of Physical Therapy, Marquette University, Milwaukee, WI, United States

Corresponding author: Sundberg, Christopher W (christopher.sundberg@marquette.edu)

Current Opinion in Physiology 2019, **10**:118–127

This review comes from a themed issue on **Exercise physiology**

Edited by **Harry B Rossiter** and **Brian Glancy**

For a complete overview see the Issue and the Editorial

Available online 10th May 2019

https://doi.org/10.1016/j.cophys.2019.05.004

Introduction

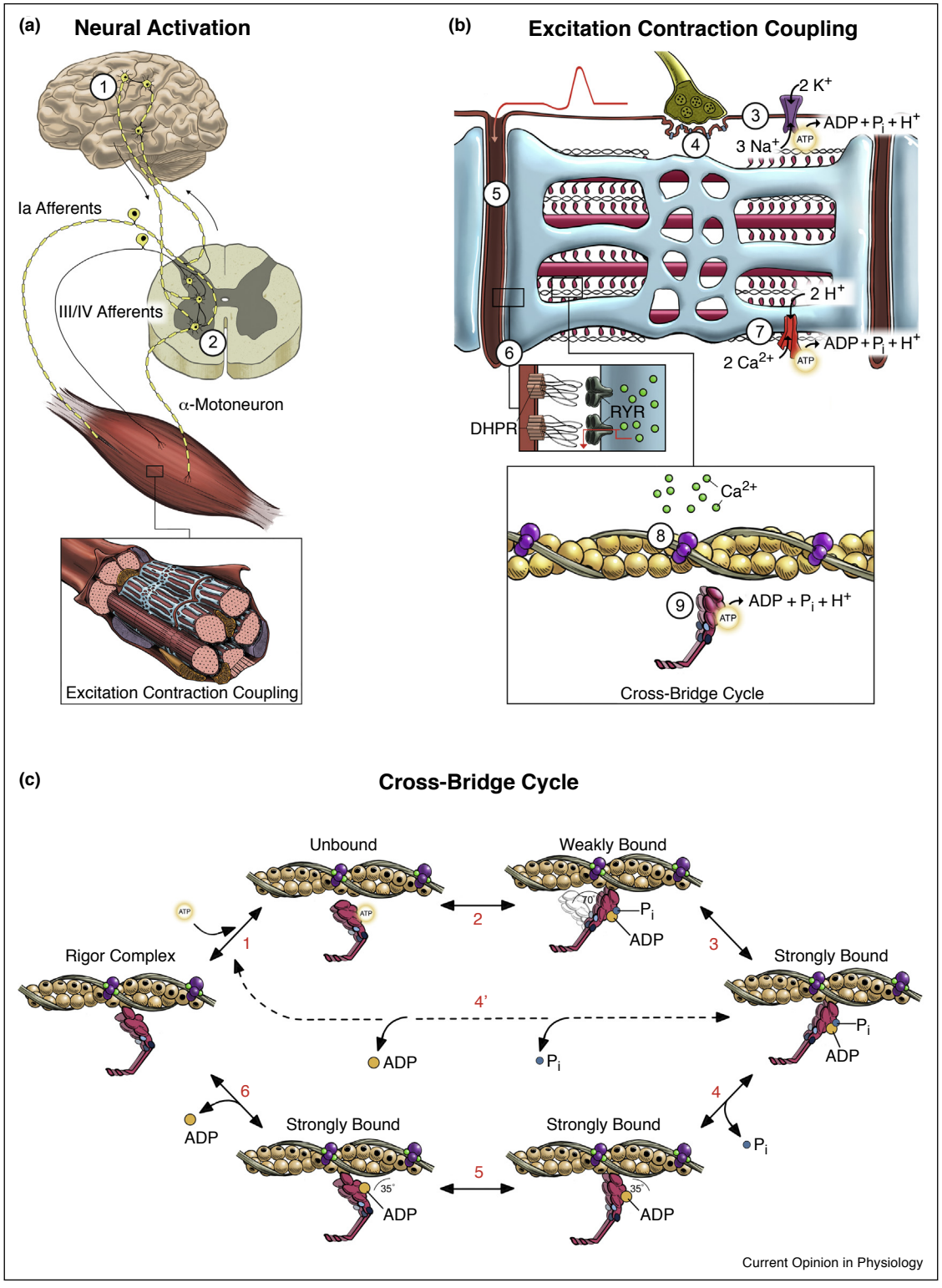
Skeletal muscle produces movement by converting chemical energy into mechanical energy through the hydrolysis of ATP. The total energetic demand of this process is the sum of the ATP hydrolyzed for ion transport (SR-Ca^{2+} and Na^+/K^+ ATPases) and the chemo-mechanical transduction of the myosin–actin interaction (myofibrillar ATPase). When the energetic demand of a motor task is low enough that ATP synthesis can be met primarily by oxidative phosphorylation, the mechanical power and force outputs necessary to perform the exercise can be sustained for hours. In contrast, as soon as the exercise intensity exceeds the ATP synthesis rates of oxidative phosphorylation requiring an increased reliance on anaerobic metabolism, force and power become impaired, that is, fatigue develops, and failure to sustain the exercise occurs within seconds to minutes of the onset of contractile activity [1,2•]. Any alterations to the mechanics of the contraction, such as contractile frequency, shortening velocity, or duty cycle, will inevitably change 1) the energetic demand and extent the exercise relies on anaerobic metabolism and 2) the rate at which fatigue develops [3]. The observation that the rate of fatigue development has a bioenergetic basis determined by the extent the exercise relies on anaerobic metabolism has been consistently documented in isometric and dynamic contractions [3–5], whole-body and isolated-limb exercises [1,3,6], and in men and women [2•].

Although it is generally accepted that fatigue during high-intensity exercise has a bioenergetic basis, identifying the mechanisms by which a reliance on anaerobic metabolism causes fatigue has proven considerably more challenging. This is in large part because fatigue can originate at multiple locations along the motor pathway (Figure 1), and the central nervous system is tightly tuned to the metabolic state of the muscle through sensory feedback from group III/IV afferents [7–9]. A preponderance of evidence, however, indicates that most of the fatigue during high-intensity exercise is due to impaired contractile function within the muscle [4,6], and that this observation is true for healthy young and old men and women [10••,11]. Identifying how a reliance on anaerobic metabolism impairs muscle force and power in such a predictable manner is particularly important because in whole-body exercises, such as cycling, ~60–70% of the power outputs generated by the neuromuscular system are non-sustainable and elicit failure within seconds to minutes [1,2•]. In this brief review, we integrate findings from *in vivo* to isolated cellular and molecular studies to identify how a reliance on anaerobic metabolism causes fatigue by disrupting contractile function in the muscle.

Anaerobic metabolism impairs contractile function through the accumulation of intracellular metabolites

Intracellular concentrations of ATP ([ATP]) in quiescent skeletal muscle are low (~5–6 mmol/kg wet weight or ~8.2 mM) and could be depleted in less than two seconds during high-intensity exercise. The depletion of ATP would cause the contractile proteins to enter a state of rigor which does not occur *in vivo*; rather, intracellular [ATP] is maintained relatively stable via the synchronized activation of the creatine kinase and adenylate kinase reactions, glycolysis and oxidative phosphorylation. Under the most severe fatigue conditions,

Figure 1



Schematic of potential sites of fatigue along the motor pathway.
Potential sites of fatigue are labeled 1–9 starting with the ability of the nervous system to activate the muscle **(a)** and progressing to excitation contraction coupling **(b)** and the cross-bridge cycle **(c)**. During volitional contractions, skeletal muscle is activated via signals originating in the motor cortex (site 1) that are transmitted to the α-motoneurons in the spinal cord (site 2). The output of the polydendritic α-motoneuron is determined by the ensemble synaptic input from thousands of sensory and descending neural pathways, with the group III/IV afferents

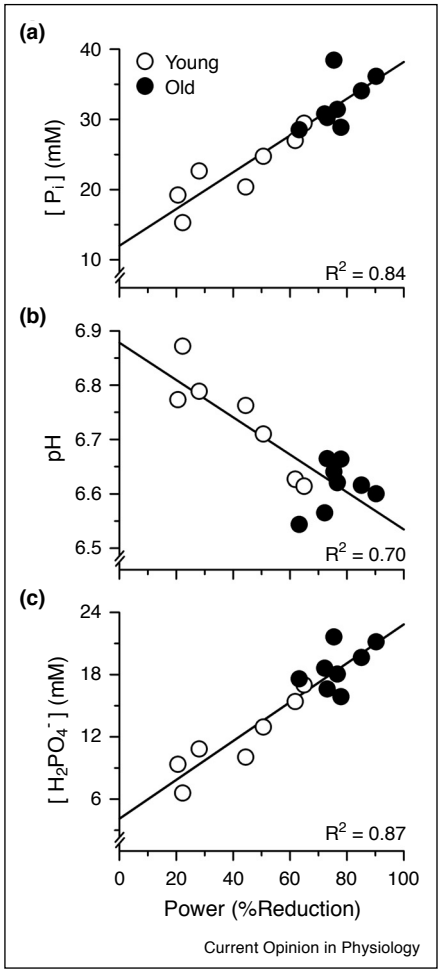
intracellular [ATP] rarely falls below 60–70% of resting values in the whole-muscle [12–14], with the most severely depleted muscle fibers reaching ~20% of resting [ATP], or ~1.6–1.8 mM [15]. Even in these severe conditions, the [ATP] has not reached the level necessary to observe impairments in contractile function [12,13]. Thus, barring the unlikely event of a pronounced intracellular compartmentalization of ATP, there is little to no evidence that high-intensity exercise is limited by the rate at which ATP can be synthesized and supplied to the myofibrillar and ion transport ATPases. However, buffering the fall in [ATP] with glycolysis and the creatine kinase and adenylate kinase reactions results in marked disruptions in intracellular homeostasis through the accumulation of ATP hydrolysis metabolites, ADP, inorganic phosphate (P_i), and hydrogen (H^+), the latter causing pH to decrease ($pH = -\log_{10}[H^+]$).

Direct measures of intramuscular bioenergetics via phosphorus nuclear magnetic resonance spectroscopy (^{31}P-MRS) have provided unprecedented detail of the time course and extent of intracellular metabolite accumulation that occurs during high-intensity exercise *in vivo*. In quiescent human skeletal muscle, intramuscular pH is ~7.0, [P_i] ~3–5 mM, and [ADP] only a few μM [16]. At the onset of high-intensity exercise, ATP is synthesized primarily via the creatine kinase reaction resulting in an exponential decline in the concentration of phosphocreatine ([PCr]) with concomitant increases in [P_i] that can reach >30 mM in human skeletal muscle during volitional exercise [17•]. Similarly, other than the first few seconds of exercise where the intracellular pH becomes more alkaline by ~0.1 pH units due to PCr hydrolysis ($PCr + ADP + H^+ \leftrightarrow ATP + Cr$) [18], pH declines precipitously and can reach levels between 6.5 to 6.2 during high-intensity exercise [17•,19,20], with more severe acidic states possible within regions of the muscle. In contrast, due to the creatine kinase and adenylate kinase reactions, [ADP] remains relatively stable until [PCr] is nearly depleted, after which [ADP] increases by a few hundred μM, which are levels that do not appear to significantly disrupt contractile function [12]. It is important to note that other compounds accumulate during high-intensity exercise, such as, AMP, IMP, creatine, lactate, extracellular K^+, Mg^{2+}, and reactive oxygen and nitrogen species, that have been implicated in fatigue; however, with the possible exception of K^+ at high-firing frequencies [21••], either 1) do not impair contractile function or do not reach the concentrations during volitional exercise where impaired contractile function occurs, 2) are not correlated with the reductions in force or power, or 3) cause structural damage to proteins that requires days to recover from and arguably can no longer be considered a mechanism of fatigue [13,21••,22–24]. Below we present evidence to suggest that a majority of the fatigue during high-intensity volitional exercise can be explained by the multifaceted and synergistic effects of elevated H^+ and P_i.

Although *in vivo* studies are unable to determine whether the accumulation of H^+ and/or P_i causes fatigue, two primary observations suggest that these metabolites play a major role in limiting force and power production during high-intensity exercise. The first is the further the exercise intensity exceeds the level that can be supported by oxidative phosphorylation, the more rapidly H^+ and P_i accumulate in the muscle [5,25] and the decrements in contractile function occur [4]. Accordingly, reducing O_2 availability through inspired hypoxic gas [26] or blood flow occlusion [27] results in a more rapid accumulation of intracellular H^+ and P_i and more rapid impairments in contractile function [28,29]. The second, and perhaps more important observation, is that the extent of intracellular H^+ and P_i accumulation is closely associated with the decrements in 1) force production during volitional [20,30] and electrically evoked isometric contractions [17•] and 2) power production during volitional dynamic exercise (Figure 2) [31••]. Although these observations do not indicate a causal role for H^+ and P_i in fatigue, they do provide the premise for mechanistic studies employing isolated

(Figure 1 Legend Continued) transmitting signals specific to the metabolic state of the muscle. The propagation of the action potential across the neuromuscular junction (site 4) and into the t-tubule (site 5) is detected by the dihydropyrodine receptor (DHPR) that initiates the release of Ca^{2+} (Site 6) through the ryanodine receptors (RYR) on the SR membrane. This process requires proper ion gradients and maintained excitability determined, at least in part, by the Na^+/K^+ pump (site 3) and SR-Ca^{2+} pump activity (site 7). The binding of Ca^{2+} to the troponin-tropomyosin complex induces a series of conformational changes in the regulatory proteins (site 8) that allows the interaction of myosin and actin to initiate the power stroke (site 9). Contemporary cross-bridge theory depicted in (c) suggests that the chemo-mechanical transduction of the myosin–actin interaction is partitioned into ~6-strain-dependent structural transitions (steps 1–6) that make up the power stroke [32,53,72,73]. Briefly, starting in the rigor complex, ATP binds to the catalytic site on myosin and dissociates myosin from actin (step 1). The hydrolysis of ATP reprimes and cocks the myosin head which attaches to actin in a weakly bound state with the hydrolysis metabolites still bound to the catalytic site (step 2). In the conventional power stroke, the weakly bound cross-bridge transitions to the strongly bound state through unknown mechanisms (step 3). P_i is released from the catalytic site initiating the power stroke where myosin pivots at the light chain domain (step 4). The power stroke continues with an isomerization step where ADP is still bound to the catalytic site (step 5), followed by the release of ADP (step 6). *Sites where elevated H+ and Pi disrupt muscle contraction*: A majority of the fatigue during high-intensity volitional exercise can be explained by the multifaceted and synergistic effects of increased levels of H^+ and P_i. Elevated H^+ disrupts contractile function by 1) reducing the sensitivity of the myofilaments to Ca^{2+} (site 8), 2) decreasing force of the cross-bridge by inhibiting the low- to high-force transition (step 3), and 3) slowing shortening velocity by inhibiting the ADP isomerization step (step 5) and/or the release of ADP (step 6). Elevated P_i disrupts contractile function by 1) decreasing the free [Ca^{2+}] available for release from the SR (site 6), 2) reducing the sensitivity of the myofilaments to Ca^{2+} (site 8), and 3) decreasing force by inducing an unconventional power stroke where myosin dissociates from actin early in the high-force state before the release of P_i and ADP (step 4').

Figure 2



Associations between the accumulation of metabolites and the reductions in mechanical power during a fatiguing knee extension exercise.

Young (22.7 ± 1.2 yrs) and old participants (76.4 ± 6.0 yrs) performed a high-intensity fatiguing knee extension exercise consisting of 120 maximal velocity contractions (1 contraction per 2-s) lifting a load equivalent to 20% of the individual-specific maximal voluntary isometric contraction. The reduction in power during the 4-min dynamic fatiguing exercise was closely associated with the $[P_i]$ **(a)**, pH **(b)**, and concentration of diprotonated phosphate $[H_2PO_4^-]$ **(c)** measured over the final 64-s of the exercise. The greater fatigue in the old compared with young adults was accompanied by an ~30% greater increase in the $[H^+]$ (pH 6.61 versus 6.73) and an ~42% greater increase in the $[P_i]$ (32 versus 23 mM P_i). The combination of the greater decrease in pH and increase in $[P_i]$ resulted in an ~59% greater increase in the $[H_2PO_4^-]$ in the old compared with the young adults. The figure is reprinted with permission from Ref. [31••].

cellular and molecular preparations. In the remainder of the review, we focus primarily on recent discoveries of the effects of H^+ and P_i on contractile function from experiments using the chemically skinned fiber preparation and the *in vitro* motility and laser trap assays [10••,32,33••,34]. The advantage of these approaches is they permit precise control over the milieu surrounding the contractile proteins to systematically study both the individual and combined effects of elevated metabolite levels on contractile function.

Effects of acidosis, H^+, on contractile function

Although the role of H^+ in fatigue remains a topic of intense debate [35,36], the argument against acidosis as a putative mechanism of fatigue is based primarily on the modest effect H^+ has on isometric force under saturating Ca^{2+} conditions. Indeed, in saturating Ca^{2+} (pCa = 4.5 where $pCa = -\log_{10}[Ca^{2+}]$) a pH of 6.2 elicits a relatively small, albeit still significant, reduction in peak isometric force of 4–18% in skinned rat and rabbit fibers at 30°C [37,38•]. These findings are consistent with both the 10% decline in peak isometric force elicited by a pH of ~6.67 in living mouse muscle fibers at 32°C [39] and the 20% decline in force elicited by a pH of 6.5 in a mini-ensemble of myosin studied in a laser trap assay at 30°C [34]. The observation that elevated H^+ inhibits force production in both the laser trap assay with an unregulated thin filament [34] and in the skinned fiber preparation in saturating Ca^{2+} [37,38•] suggests that acidosis elicits decrements in force production, at least in part, by directly inhibiting the cross-bridge. While the mechanism remains unresolved, the acidosis induced decrements in isometric force may involve a reduction in the number of bound cross-bridges and/or the force generated per cross-bridge. The finding that the rate of force redevelopment (k_{tr}) following a slack re-extension maneuver of maximally Ca^{2+}-activated human fibers was slowed by elevated H^+ and P_i (pH 6.2 + 30 mM P_i) compared with a control condition (pH 7.0 + 4 mM P_i) suggests that acidosis may reduce the force per cross-bridge by inhibiting the low-force to high-force state of the cross-bridge cycle [10••]. This hypothesis is supported by the decreased high-force generating events and prolonged cross-bridge attachment times elicited by a pH of 6.5 in a mini-ensemble of myosin studied in a laser trap assay [34,40]. Thus, acidosis appears to inhibit peak isometric force in saturating Ca^{2+} primarily by reducing the force per cross-bridge via slowing the low- to high-force transition (step 3 Figure 1c) rather than reducing the number of bound cross-bridges.

In addition to the effect H^+ has on peak isometric force in saturating Ca^{2+}, intracellular acidosis contributes to fatigue by decreasing the sensitivity of the myofilament to Ca^{2+}. Because the relationship between the $[Ca^{2+}]$ and isometric force is sigmoidal, the acidosis-induced decrease in myofibrillar Ca^{2+} sensitivity manifests as a rightward shift in the force–pCa relationship and much greater reductions in isometric force when rat fibers were activated in pH 6.2 and submaximal compared with saturating Ca^{2+} [41]. The mechanisms for the decreased Ca^{2+} sensitivity are not fully elucidated; however, there is compelling evidence from skinned rabbit fibers that elevated H^+ reduces the affinity of the binding sites on troponin C (TnC) to Ca^{2+} [42,43] (site 8 Figure 1b). A subsequent study using the *in vitro* motility assay and a specific mutation in TnC that slows the release of Ca^{2+} from the binding sites revealed

that acidosis slows the rate that Ca^{2+} binds to TnC rather than accelerating the rate at which it's released [44]. In addition to the decreased Ca^{2+} affinity, there is also evidence that a H^+-binding residue on troponin I (TnI) may contribute to the acidosis-induced decrease in Ca^{2+} sensitivity by altering the binding affinity of TnI to TnC [45]. Irrespective of the mechanism, the evidence that the free [Ca^{2+}] decreases in the myoplasm during high-intensity contractions to subsaturating levels [46••] suggests that the H^+-induced decrements in isometric force studied in saturating Ca^{2+} likely underestimate the depressive effects of acidosis on force production during fatigue *in vivo*.

Perhaps more important than the effect acidosis has on isometric force is the effect it has on shortening velocity and the ability to generate mechanical power (Figure 3). In saturating Ca^{2+}, a pH of 6.2 inhibits the maximal shortening velocity of skinned rat and rabbit fibers by 11–30% at 30°C, regardless of whether the velocity was measured with the slack test or extrapolation of the force–velocity curve [38•,47••]. These findings were corroborated by the observation that the actin filament velocity slowed markedly under acidic conditions in the *in vitro* motility assay at 20–30°C, which could be explained quantitatively by the acidosis-induced increase in the cross-bridge attachment times measured in the single molecule laser trap assay [33••,48]. The increased attachment times and slowed shortening velocity is thought to be due primarily to an inhibition of the ADP isomerization step of the cross-bridge cycle and/or the rate of ADP release [32,44] (steps 5 and 6 Figure 1c). The combination of the acidosis-induced decrease in both force and velocity resulted in an 18–34% reduction in peak power of rat fibers activated in pH 6.2 at 30°C [38•]. Thus, acidosis has a detrimental effect on contractile function by directly inhibiting force, velocity, and power of the cross-bridge and decreasing the sensitivity of the myofilament to Ca^{2+}.

Effects of inorganic phosphate, P_i, on contractile function

Similar to elevated H^+, activating rat and rabbit fibers in saturating Ca^{2+} and 25–30 mM P_i reduced peak isometric force by 5–19% at 30°C [49,50]. However, the observation that the rate of force redevelopment following a slack re-extension maneuver of a maximally activated fiber (k_{tr}) is accelerated in the presence of elevated P_i [51,52] but slowed when H^+ and P_i are elevated together [10••] suggests that the mechanisms for the reduction in force differ for P_i compared with H^+. While the mechanism is not fully elucidated, elevated P_i is thought to inhibit force, at least in part, by inducing an unconventional power stroke where myosin dissociates from actin early in the high-force state of the cross-bridge cycle before the release of P_i and ADP [32,53,54•] (step 4' Figure 1c). This mechanism is consistent with the increased ATP cost of contraction observed in skinned fibers that occurs from the P_i-induced decrease in isometric force but a maintained myofibrillar ATP hydrolysis rate [54•,55]. Further support for this mechanism comes from the observation that 30 mM P_i elicited a 65% decrease in the cross-bridge attachment times of a mini-ensemble of myosin studied in a laser trap assay at 30°C [56•]. Thus, elevated P_i appears to inhibit peak isometric force in saturating Ca^{2+} primarily by accelerating the detachment of myosin from actin, which reduces the number of bound cross-bridges.

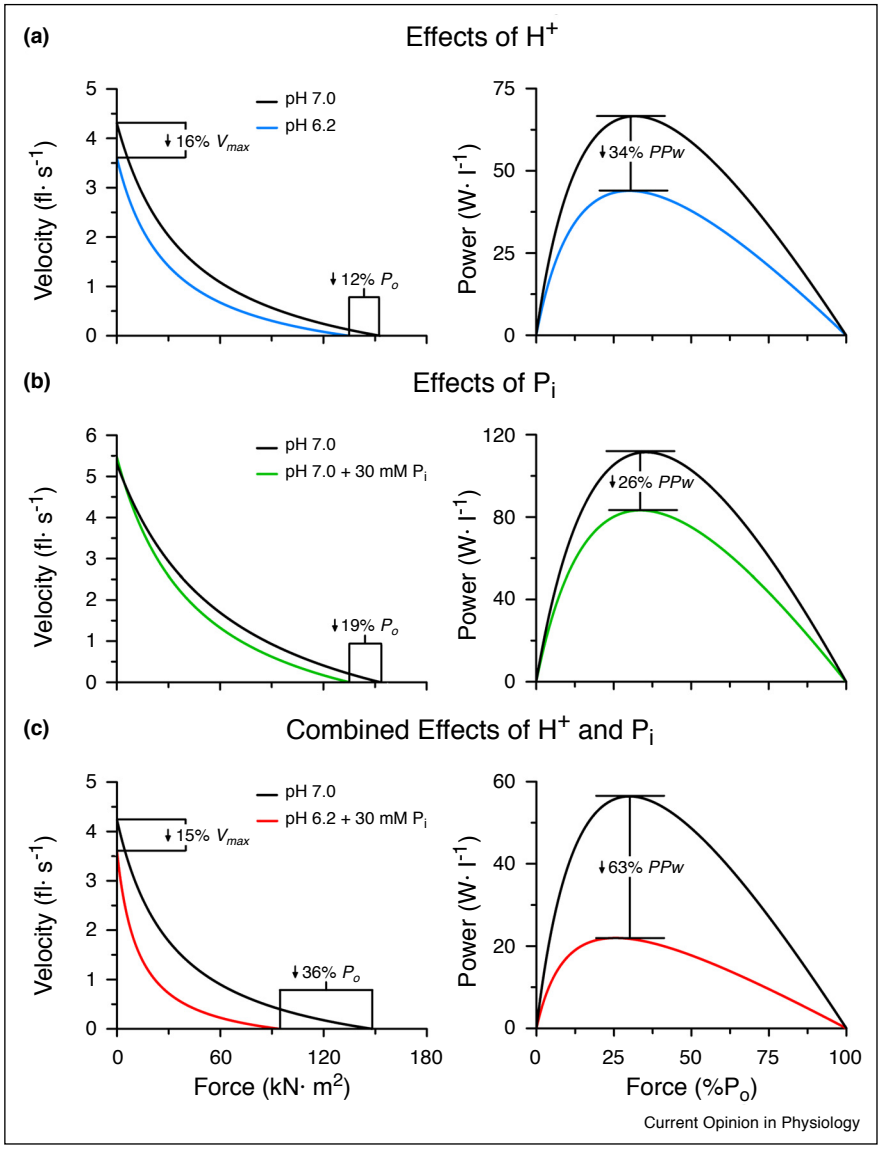
In addition to the effect P_i has on isometric force in saturating Ca^{2+}, elevated P_i contributes to fatigue by decreasing the sensitivity of the myofilament to Ca^{2+} [57] and inhibiting Ca^{2+} release from the sarcoplasmic reticulum (SR) [13,58,59]. The mechanism for the P_i-induced decrease in myofibrillar Ca^{2+} sensitivity is unknown and needs further investigation; however, it is clear that, unlike H^+, elevated P_i does not alter the affinity of the binding sites on TnC to Ca^{2+} [42]. In contrast to the lack of understanding of how elevated P_i decreases myofibrillar Ca^{2+} sensitivity, there is more evidence describing the mechanism by which elevated P_i inhibits Ca^{2+} release from the SR [13,58,60]. Briefly, P_i is thought to reduce the amount of free [Ca^{2+}] available for release from the SR by diffusing into the SR through a P_i-permeable channel [61] and binding to Ca^{2+} to form a precipitate [58–60,62] (site 6 Figure 1b). Similar to H^+, the reduced free [Ca^{2+}] available in the myoplasm to subsaturating levels coupled with the decreased myofibrillar Ca^{2+} sensitivity suggests that the P_i-induced decrements in isometric force studied in saturating Ca^{2+} likely underestimate the depressive effects of P_i on force production during fatigue *in vivo*.

Unlike elevated H^+, P_i does not alter the shortening velocity of rat, rabbit or human fibers activated at 30°C [10••,47••,50]. However, athletic prowess and the ability to perform daily activities are determined more by the muscle's ability to generate mechanical power, and 30 mM P_i elicited an 18–26% reduction in peak power in rat fibers activated at 30°C [50] (Figure 3). Thus, elevated P_i has a detrimental effect on contractile function by directly inhibiting force and power of the cross-bridge and by decreasing both the free [Ca^{2+}] available for release from the SR and the sensitivity of the myofilament to Ca^{2+}.

Synergistic effects of H^+ and P_i on contractile function

Although studies on the individual effects of H^+ and P_i are important, studying their effects when elevated together is more pertinent to understanding how these metabolites contribute to the decrements in contractile function that occur during high-intensity fatiguing exercise *in vivo* [17•,19,20,31••]. Given the evidence that elevated H^+ and P_i contribute to fatigue by different mechanisms, it

Figure 3



Individual and combined effects of elevated H^+ and P_i on force, velocity, and power of rat slow MHC I fibers at 30°C. Force–velocity and force–power curves obtained from rat MHC I fibers in the control pH 7.0 condition compared with pH 6.2 **(a)**, 30 mM P_i **(b)**, and combined pH 6.2 and 30 mM P_i **(c)** at 30°C. Shortening velocity (fiber lengths per second) and power (W l^{-1}) are plotted as a function of the force expressed relative to the fiber cross-sectional area (kN m^2) and as a percentage of the peak isometric force (%P_o), respectively. Error bars around the mean curves have been omitted for clarity. Findings from fast fibers are qualitatively similar to those presented here. The figure is reprinted with permission from Ref. [32] and the data obtained from Refs. [38•,50,63•].

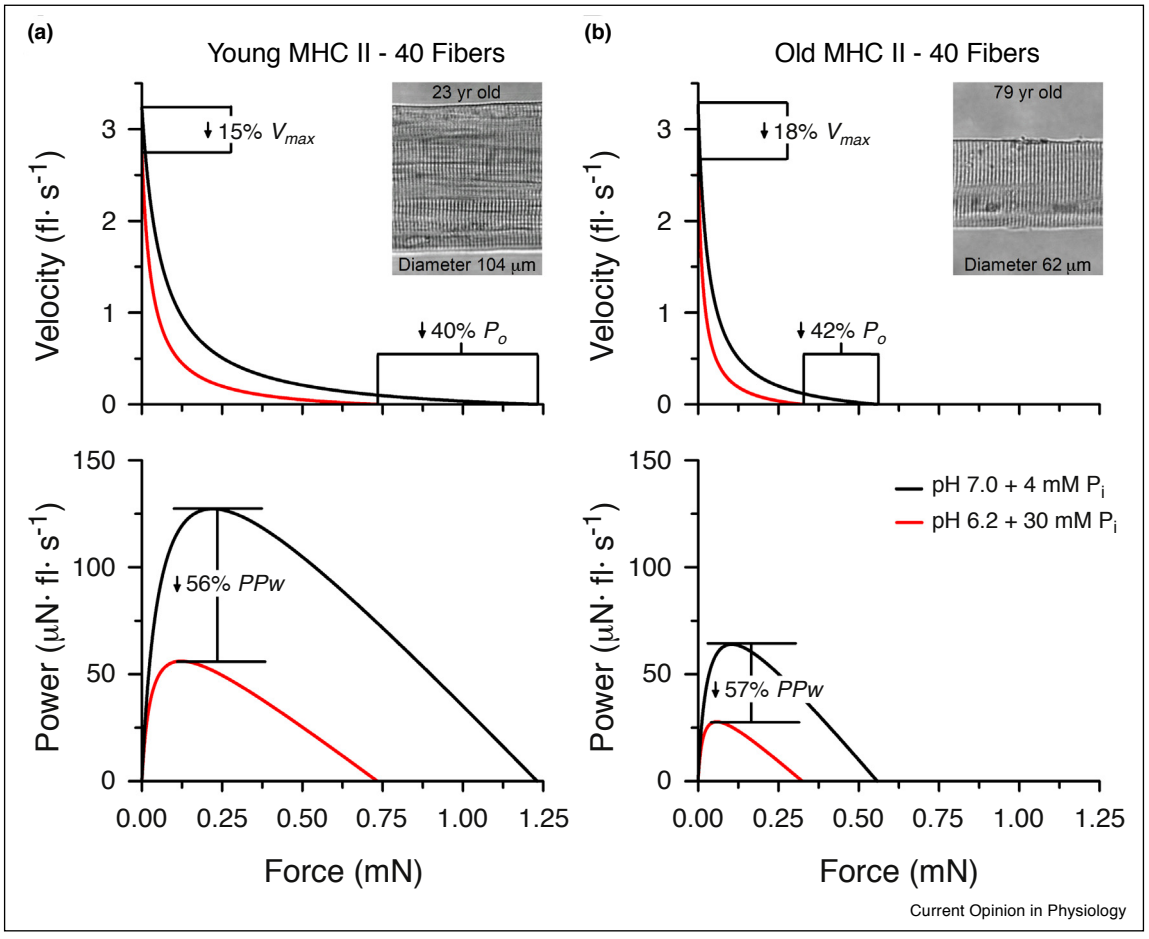
is perhaps not surprising that when studied in combination their depressive effects on contractile function are additive. For example, a combined pH 6.2 and 30 mM P_i condition decreased peak isometric force of rat fibers by 36–46% in saturating Ca^{2+} at 30°C [63•] and caused a considerably greater decrease in the sensitivity of the myofilament to Ca^{2+} than either metabolite alone [41,57]. Interestingly, the combined condition had a synergistic effect where the decrements were greater than would be predicted from the sum of the individual metabolite effects, particularly for peak power of rat and rabbit fast fibers [38•,47••,50,63•]. The combined pH 6.2 and 30 mM P_i condition decreased peak power by 55–63% in rabbit and rat fibers at 30°C (Figure 3), which was exacerbated to a 70% decline in power in rabbit fast fibers when the myosin regulatory light chain was phosphorylated [47••,63•]. Extrapolating these findings to the whole-muscle suggests that ~55–70% of the reduction in the ability to generate power can be attributed to the synergistic effects of elevated H^+ and P_i directly inhibiting the

cross-bridge, which is likely exacerbated by the effects these metabolites also have on myofibrillar Ca^{2+} sensitivity and the release of Ca^{2+} from the SR. The mechanisms to explain the synergistic effects are unknown [34,40], but may include alterations in the binding affinities of the myosin–actin interaction at different steps in the cross-bridge cycle [64].

Translating the bioenergetics perspective to understanding fatigue in old adults

The ability of old adults to generate power is severely compromised by the combination of the atrophy of muscle fibers expressing the fast myosin heavy chain isoforms [10••,65,66] and the increased fatigue that occurs when old adults perform moderate- to high-velocity contractions [11,67–70]. The mechanisms for the age-related increase in fatigue are unresolved; however, studies employing non-invasive stimulation procedures to the intact neuromuscular system have localized the primary site to within the muscle rather than the nervous system [10••,11,68,69,71]. Translating the understanding of the bioenergetic basis of fatigue to this problem, we tested whether age-related changes of the muscle resulted in either 1) an increased sensitivity of the cross-bridge to a given concentration of metabolite accumulation or 2) an increased production of metabolites due to a greater reliance on anaerobic metabolism. To test these hypotheses, we exposed fibers from the vastus lateralis of young and old men to a condition mimicking quiescent skeletal muscle (pH 7.0 + 4 mM P_i) and the

Figure 4



Force–velocity and force–power curves of fast MHC II fibers from young and old men at 15°C.
Absolute peak power (*PPw*) and peak isometric force (P_o) of the fast MHC II fibers from young adults **(a)** were ~twofold greater than in fibers from the old adults **(b)**. The age differences in absolute *PPw* and P_o were explained entirely by differences in the size of the fibers between young and old adults. The insets are digital images taken of young and old adult fast fibers at 800× magnification that are representative of the average size of the fast MHC II fibers studied in the young and old adults. The combined pH 6.2 and 30 mM P_i condition caused significant decreases in V_{max}, P_o, and *PPw* compared to the condition mimicking quiescent human skeletal muscle (pH 7.0 + 4 mM P_i) in fibers from young and old adults; however, the relative reductions did not differ with age. The variances around the mean curves were omitted for clarity. The figure was modified with permission from Ref. [10••].

combined pH 6.2 and 30 mM P_i condition (Figure 4). While the data confirmed that these metabolites act synergistically to impair cross-bridge function, the decrements in force, velocity, and power from the combined pH 6.2 and 30 mM P_i condition did not differ in the fibers isolated from young compared with old men [10••]. In contrast, when we had young and old adults perform a dynamic fatiguing knee extension exercise while simultaneously measuring the intracellular metabolite accumulation with ^{31}P-MRS, the greater fatigue in the old compared with young adults was accompanied by an ~30% greater increase in the [H^+] (pH 6.61 versus 6.73) and an ~42% greater increase in the [P_i] (32 versus 23 mM P_i) [31••]. Importantly, the reductions in power during the fatiguing exercise were closely associated with the intracellular metabolite accumulation (Figure 2) suggesting that the increased fatigue in old adults during dynamic exercise has a bioenergetic basis explained by an increased accumulation of metabolites within the muscle [31••].

Concluding remarks

Integrating findings from *in vivo* and isolated cellular andmolecular studies has provided considerable advancements in our understanding of how a reliance on anaerobic metabolism disrupts contractile function during high-intensity exercise. While many of the mechanisms are not fully resolved, the data suggest that fatigue during high-intensity exercise is determined, in large part, by the rate and extent of intracellular metabolite accumulation. We conclude that a majority of the fatigue in healthy young and old adults performing high-intensity volitional exercise can be explained by the multifaceted and synergistic effects of elevated H^+ and P_i acting to impair contractile function within the muscle. Translating this understanding of the bioenergetic basis of fatigue to clinical populations may help guide studies aimed at identifying the mechanisms of fatigue and designing targeted therapies to offset the detrimental effects of fatigue in these alternative populations. Future studies are needed to identify how elevated H^+ and P_i act synergistically to impair cross-bridge function and decrease myofibrillar Ca^{2+} sensitivity so that we may ultimately develop treatments to attenuate the effects of these metabolites on contractile function.

Conflict of interest statement

Nothing declared.

Author contributions

C.W.S. prepared the figures and drafted the manuscript; C.W.S. and R.H.F. edited and revised the manuscript. Both authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

Funding

This work was supported by an American Heart Association postdoctoral fellowship (19POST34380411) to Christopher Sundberg and a National Institute of Aging R01 (AG048262) to Robert Fitts.

Acknowledgements

Because of journal restrictions on manuscript length and references, the literature on neuromuscular fatigue and skeletal muscle bioenergetics could not be incorporated and acknowledged to the extent warranted by the existing scholarship. We are sincerely grateful to Ethan Claunch for the illustration in Figure 1.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Bundle MW, Weyand PG: **Sprint exercise performance: does metabolic power matter?** *Exerc Sport Sci Rev* 2012, **40**:174-182.

2. • Sundberg CW, Hunter SK, Bundle MW: **Rates of performance loss and neuromuscular activity in men and women during cycling: evidence for a common metabolic basis of muscle fatigue**. *J Appl Physiol (1985)* 2017, **122**:130-141.
This study compared the neuromuscular activity and rates of power loss between men and women during multiple exercise bouts that spanned the full range of power outputs that could be generated by the neuromuscular system. The data revealed that while the absolute cycling power outputs differed considerably between the sexes, the rate-limiting mechanisms were similar between men and women and determined by the extent the exercise relied on anaerobic metabolism.

3. Sundberg CW, Bundle MW: **Influence of duty cycle on the time course of muscle fatigue and the onset of neuromuscular compensation during exhaustive dynamic isolated limb exercise**. *Am J Physiol Regul Integr Comp Physiol* 2015, **309**:R51-R61.

4. Burnley M, Vanhatalo A, Jones AM: **Distinct profiles of neuromuscular fatigue during muscle contractions below and above the critical torque in humans**. *J Appl Physiol (1985)* 2012, **113**:215-223.

5. Jones AM, Wilkerson DP, DiMenna F, Fulford J, Poole DC: **Muscle metabolic responses to exercise above and below the "critical power" assessed using ^{31}P-MRS**. *Am J Physiol Regul Integr Comp Physiol* 2008, **294**:R585-593.

6. Burnley M, Jones AM: **Power-duration relationship: physiology, fatigue, and the limits of human performance**. *Eur J Sport Sci* 2018, **18**:1-12.

7. Bigland-Ritchie BR, Dawson NJ, Johansson RS, Lippold OCJ: **Reflex origin for the slowing of motoneuron firing rates in fatigue of human voluntary contractions**. *J Physiol* 1986, **379**:451-459.

8. Amann M, Blain GM, Proctor LT, Sebranek JJ, Pegelow DF, Dempsey JA: **Implications of group III and IV muscle afferents for high-intensity endurance exercise performance in humans**. *J Physiol* 2011, **589**:5299-5309.

9. Gandevia SC: **Spinal and supraspinal factors in human muscle fatigue**. *Physiol Rev* 2001, **81**:1725-1789.

10. •• Sundberg CW, Hunter SK, Trappe SW, Smith CS, Fitts RH: **Effects of elevated H^+ and P_i on the contractile mechanics of skeletal muscle fibres from young and old men: implications for muscle fatigue in humans**. *J Physiol* 2018, **596**:3993-4015.
This is the first study to examine the combined effects of elevated H^+ and P_i on contractile function of fibers isolated from human skeletal muscle. The data confirmed previous non-human studies that the metabolites act synergistically to impair cross-bridge function, but that the effects were similar in fibers isolated from young and old men. The findings highlight the importance of the metabolites in human muscle fatigue, while also suggesting that the age-related increase in fatigue during dynamic

exercise cannot be explained by an increased sensitivity of the cross-bridge to H^+ and P_i.

11. Sundberg CW, Kuplic A, Hassanlouei H, Hunter SK: **Mechanisms for the age-related increase in fatigability of the knee extensors in old and very old adults**. *J Appl Physiol (1985)* 2018, **125**:146-158.

12. Cooke R: **Modulation of the actomyosin interaction during fatigue of skeletal muscle**. *Muscle Nerve* 2007, **36**:756-777.

13. Allen DG, Lamb GD, Westerblad H: **Skeletal muscle fatigue: cellular mechanisms**. *Physiol Rev* 2008, **88**:287-332.

14. Fitts RH: **Cellular mechanisms of muscle fatigue**. *Physiol Rev* 1994, **74**:49-94.

15. Karatzaferi C, de Haan A, Ferguson RA, van Mechelen W, Sargeant AJ: **Phosphocreatine and ATP content in human single muscle fibres before and after maximum dynamic exercise**. *Pflugers Arch* 2001, **442**:467-474.

16. Kemp GJ, Meyerspeer M, Moser E: **Absolute quantification of phosphorus metabolite concentrations in human muscle in vivo by ^{31}P MRS: a quantitative review**. *NMR Biomed* 2007, **20**:555-565.

17. • Broxterman RM, Layec G, Hureau TJ, Amann M, Richardson RS: **Skeletal muscle bioenergetics during all-out exercise: mechanistic insight into the oxygen uptake slow component and neuromuscular fatigue**. *J Appl Physiol (1985)* 2017, **122**:1208-1217.
This study shows the association between the increase in intracellular metabolite accumulation and the reduction in the electrically evoked twitch following an all-out, isometric knee extension exercise. The associations provide compelling evidence that elevated H^+ and P_i have an important role in limiting force production *in vivo* by disrupting contractile function.

18. Adams GR, Foley JM, Meyer RA: **Muscle buffer capacity estimated from pH changes during rest-to-work transitions**. *J Appl Physiol (1985)* 1990, **69**:968-972.

19. Cady EB, Jones DA, Lynn J, Newham DJ: **Changes in force and intracellular metabolites during fatigue of human skeletal muscle**. *J Physiol* 1989, **418**:311-325.

20. 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 (1985)* 1988, **64**:2333-2339.

21. •• Pedersen KK, Nielsen OB, Overgaard K: **Contractile benefits of doublet-initiated low-frequency stimulation in rat EDL muscle exposed to high extracellular [K^+] or fatiguing contractions**. *Am J Physiol Cell Physiol* 2019.
This study shows that elevated extracellular K^+ (11 mM) does not alter the contractile response of the rat EDL muscle to high-frequency doublet stimulations (300 Hz) when elicited among a train of near *in vivo* firing frequencies (50 Hz) but inhibits power when elicited among a train of high-firing frequencies (150 Hz). The data suggest that elevated extracellular K^+ may play a role in fatigue at high-firing frequencies but does not likely have a major role at the firing frequencies typically observed during volitional exercise *in vivo*.

22. Cheng AJ, Yamada T, Rassier DE, Andersson DC, Westerblad H, Lanner JT: **Reactive oxygen/nitrogen species and contractile function in skeletal muscle during fatigue and recovery**. *J Physiol* 2016, **594**:5149-5160.

23. McKenna MJ, Bangsbo J, Renaud JM: **Muscle K^+, Na^+, and Cl^- disturbances and Na^+-K^+ pump inactivation: implications for fatigue**. *J Appl Physiol (1985)* 2008, **104**:288-295.

24. Brooks GA: **The science and translation of lactate shuttle theory**. *Cell Metab* 2018, **27**:757-785.

25. Vanhatalo A, Fulford J, DiMenna FJ, Jones AM: **Influence of hyperoxia on muscle metabolic responses and the power-duration relationship during severe-intensity exercise in humans: a ^{31}P magnetic resonance spectroscopy study**. *Exp Physiol* 2010, **95**:528-540.

26. Hogan MC, Richardson RS, Haseler LJ: **Human muscle performance and PCr hydrolysis with varied inspired oxygen fractions: a ^{31}P-MRS study**. *J Appl Physiol (1985)* 1999, **86**:1367-1373.

27. Lanza IR, Larsen RG, Kent-Braun JA: **Effects of old age on human skeletal muscle energetics during fatiguing contractions with and without blood flow**. *J Physiol* 2007, **583**:1093-1105.

28. Romer LM, Haverkamp HC, Lovering AT, Pegelow DF, Dempsey JA: **Effect of exercise-induced arterial hypoxemia on quadriceps muscle fatigue in healthy humans**. *Am J Physiol Regul Integr Comp Physiol* 2006, **290**:R365-R375.

29. Romer LM, Haverkamp HC, Amann M, Lovering AT, Pegelow DF, Dempsey JA: **Effect of acute severe hypoxia on peripheral fatigue and endurance capacity in healthy humans**. *Am J Physiol Regul Integr Comp Physiol* 2007, **292**:R598-R606.

30. Lanza IR, Wigmore DM, Befroy DE, Kent-Braun JA: **In vivo ATP production during free-flow and ischaemic muscle contractions in humans**. *J Physiol* 2006, **577**:353-367.

31. •• Sundberg CW, Prost RW, Fitts RH, Hunter SK: **Bioenergetic basis for the increased fatigability with ageing**. *J Physiol* 2019.
This study shows the association between the increase in intracellular metabolite accumulation and the reduction in power during an all-out, volitional knee extension exercise. The data also reveal that the age-related increase in fatigue during dynamic exercise is accompanied by an ~30% greater increase in the [H^+] (pH 6.61 versus 6.73) and an ~42% greater increase in the [P_i] (32 versus 23 mM P_i), suggesting that the increased fatigue in old adults has a bioenergetic basis explained by an increased accumulation of H^+ and P_i.

32. Debold EP, Fitts RH, Sundberg CW, Nosek TM: **Muscle fatigue from the perspective of a single crossbridge**. *Med Sci Sports Exerc* 2016, **48**:2270-2280.

33. •• Debold EP, Longyear TJ, Turner MA: **The effects of phosphate and acidosis on regulated thin-filament velocity in an in vitro motility assay**. *J Appl Physiol (1985)* 2012, **113**:1413-1422.
This study shows that pH 6.5 causes marked reductions in the actin filament velocity in the *in vitro* motility assay, which confirms findings from skinned fibers that acidosis inhibits velocity. The study also presents data suggesting that the mechanism by which acidosis slows shortening velocity is by inhibiting the ADP isomerization step of the cross-bridge cycle (step 5 Figure 1c).

34. Woodward M, Debold EP: **Acidosis and phosphate directly reduce myosin's force-generating capacity through distinct molecular mechanisms**. *Front Physiol* 2018, **9**:862.

35. Fitts RH: **The role of acidosis in fatigue: pro perspective**. *Med Sci Sports Exerc* 2016, **48**:2335-2338.

36. Westerblad H: **Acidosis is not a significant cause of skeletal muscle fatigue**. *Med Sci Sports Exerc* 2016, **48**:2339-2342.

37. 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* 1995, **486**:689-694.

38. • 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* 2006, **575**:887-899.
This study tested the effects of elevated H^+ on force, velocity, and power of rat slow and fast fibers at near *in vivo* temperatures of 30°C. The data revealed that pH 6.2 elicited a 4–12% decrease in isometric force, a 25–32% decrease in unloaded shortening velocity, and an 18–34% decrease in peak power, indicating that acidosis has an important role in fatigue by directly disrupting cross-bridge function.

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

40. Jarvis K, Woodward M, Debold EP, Walcott S: **Acidosis affects muscle contraction by slowing the rates myosin attaches to and detaches from actin**. *J Muscle Res Cell Motil* 2018, **39**:135-147.

41. Nelson CR, Fitts RH: **Effects of low cell pH and elevated inorganic phosphate on the pCa-force relationship in single muscle fibers at near-physiological temperatures**. *Am J Physiol Cell Physiol* 2014, **306**:C670-678.

42. 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* 1994, **480**:45-60.

43. Parsons B, Szczesna D, Zhao J, Van Slooten G, Kerrick WG, Putkey JA, Potter JD: **The effect of pH on the Ca^{2+} affinity of the Ca^{2+} regulatory sites of skeletal and cardiac troponin C in skinned muscle fibres**. *J Muscle Res Cell Motil* 1997, **18**:599-609.

44. Longyear TJ, Turner MA, Davis JP, Lopez J, Biesiadecki B, Debold EP: **Ca^{++}-sensitizing mutations in troponin, P_i, and 2-deoxyATP alter the depressive effect of acidosis on regulated thin-filament velocity**. *J Appl Physiol (1985)* 2014, **116**:1165-1174.

45. Robertson IM, Holmes PC, Li MX, Pineda-Sanabria SE, Baryshnikova OK, Sykes BD: **Elucidation of isoform-dependent pH sensitivity of troponin i by NMR spectroscopy**. *J Biol Chem* 2012, **287**:4996-5007.

46. •• Allen DG, Clugston E, Petersen Y, Roder IV, Chapman B, Rudolf R: **Interactions between intracellular calcium and phosphate in intact mouse muscle during fatigue**. *J Appl Physiol (1985)* 2011, **111**:358-366.

This study is the first to show that both the myoplasmic free Ca^{2+} and SR Ca^{2+} decline from the onset of contractile activity and continue to decrease in conjunction with the decrease in force during a fatiguing protocol in an *in situ* mouse muscle preparation. The decrease in isometric force production was associated with the increase in the $[P_i]$ suggesting that P_i may be responsible for the changes in free $[Ca^{2+}]$. Importantly, the fatiguing protocol in this preparation elicited an exponential decline in force production, which is similar to what is observed in humans during all-out, volitional exercise.

47. •• Karatzaferi C, Franks-Skiba K, Cooke R: **Inhibition of shortening velocity of skinned skeletal muscle fibers in conditions that mimic fatigue**. *Am J Physiol Regul Integr Comp Physiol* 2008, **294**: R948-R955.

This is the first study to demonstrate that elevated H^+ and P_i act synergistically to inhibit peak power production in rabbit fast fibers at near *in vivo* temperatures of 30°C. Specifically, the combined pH 6.2 and 30 mM P_i condition elicited a 55% decrease in peak power that was exacerbated to a 70% reduction when the myosin regulatory light chain was phosphorylated.

48. Debold EP, Beck SE, Warshaw DM: **Effect of low pH on single skeletal muscle myosin mechanics and kinetics**. *Am J Physiol Cell Physiol* 2008, **295**:C173-C179.

49. Coupland ME, Puchert E, Ranatunga KW: **Temperature dependence of active tension in mammalian (rabbit psoas) muscle fibres: effect of inorganic phosphate**. *J Physiol* 2001, **536**:879-891.

50. Debold EP, Dave H, Fitts RH: **Fiber type and temperature dependence of inorganic phosphate: implications for fatigue**. *Am J Physiol Cell Physiol* 2004, **287**:C673-C681.

51. Wahr PA, Cantor HC, Metzger JM: **Nucleotide-dependent contractile properties of Ca^{2+}-activated fast and slow skeletal muscle fibers**. *Biophys J* 1997, **72**:822-834.

52. Tesi C, Colomo F, Piroddi N, Poggesi C: **Characterization of the cross-bridge force-generating step using inorganic phosphate and BDM in myofibrils from rabbit skeletal muscles**. *J Physiol* 2002, **541**:187-199.

53. Caremani M, Melli L, Dolfi M, Lombardi V, Linari M: **The working stroke of the myosin II motor in muscle is not tightly coupled to release of orthophosphate from its active site**. *J Physiol* 2013, **591**:5187-5205.

54. • Linari M, Caremani M, Lombardi V: **A kinetic model that explains the effect of inorganic phosphate on the mechanics and energetics of isometric contraction of fast skeletal muscle**. *Proc Biol Sci* 2010, **277**:19-27.

This study developed a model that accurately describes the increased ATP cost of contraction with the increase in the $[P_i]$ in skinned fibers. The model predicts that P_i inhibits isometric force production by inducing an unconventional power stroke where myosin detaches from actin early in the strongly bound state before the release of P_i and ADP (Step 4' Figure 1c).

55. Kerrick WG, Xu Y: **Inorganic phosphate affects the pCa-force relationship more than the pCa-ATPase by increasing the rate of dissociation of force generating cross-bridges in skinned fibers from both EDL and soleus muscles of the rat**. *J Muscle Res Cell Motil* 2004, **25**:107-117.

56. • Debold EP, Walcott S, Woodward M, Turner MA: **Direct observation of phosphate inhibiting the force-generating capacity of a miniensemble of myosin molecules**. *Biophys J* 2013, **105**:2374-2384.

This study shows that elevated P_i inhibits force in a miniensemble of myosin studied in the laser trap assay and decreases the cross-bridge attachment times by 65%. Using mechanical modeling of the cross-bridge cycle, the study was able to accurately predict the P_i-induced effects by incorporating an unconventional power stroke where myosin detaches from actin early in the strongly bound state before the release of P_i and ADP (Step 4' Figure 1c).

57. Debold EP, Romatowski J, Fitts RH: **The depressive effect of P_i on the force-pCa relationship in skinned single muscle fibers is temperature dependent**. *Am J Physiol Cell Physiol* 2006, **290**: C1041-C1050.

58. Allen DG, Trajanovska S: **The multiple roles of phosphate in muscle fatigue**. *Front Physiol* 2012, **3**:463.

59. Fryer MW, Owen VJ, Lamb 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* 1995, **482**:123-140.

60. Allen DG, Westerblad H: **Role of phosphate and calcium stores in muscle fatigue**. *J Physiol Lond* 2001, **536**:657-665.

61. Laver DR, Lenz GK, Dulhunty AF: **Phosphate ion channels in sarcoplasmic reticulum of rabbit skeletal muscle**. *J Physiol* 2001, **535**:715-728.

62. Dutka TL, Cole L, Lamb GD: **Calcium phosphate precipitation in the sarcoplasmic reticulum reduces action potential-mediated Ca^{2+} release in mammalian skeletal muscle**. *Am J Physiol Cell Physiol* 2005, **289**:C1502-C1512.

63. • Nelson CR, Debold EP, Fitts RH: **Phosphate and acidosis act synergistically to depress peak power in rat muscle fibers**. *Am J Physiol Cell Physiol* 2014, **307**:C939-C950.

This is the first study to demonstrate that elevated H^+ and P_i act synergistically to inhibit peak power production in slow fibers at low (15°C) and near *in vivo* temperatures (30°C), and showed an ~60% decline in peak power in both slow and fast fibers at both temperatures.

64. Karatzaferi C, Adamek N, Geeves MA: **Modulators of actin-myosin dissociation: basis for muscle type functional differences during fatigue**. *Am J Physiol Cell Physiol* 2017, **313**: C644-C654.

65. Lexell J: **Human aging, muscle mass, and fiber type composition**. *J Gerontol A Biol Sci Med Sci* 1995, **50 Spec No**: 11-16.

66. Nilwik R, Snijders T, Leenders M, Groen BB, van Kranenburg J, Verdijk LB, van Loon LJ: **The decline in skeletal muscle mass with aging is mainly attributed to a reduction in type II muscle fiber size**. *Exp Gerontol* 2013, **48**:492-498.

67. McNeil CJ, Rice CL: **Fatigability is increased with age during velocity-dependent contractions of the dorsiflexors**. *J Gerontol A Biol Sci Med Sci* 2007, **62**:624-629.

68. Dalton BH, Power GA, Vandervoort AA, Rice CL: **The age-related slowing of voluntary shortening velocity exacerbates power loss during repeated fast knee extensions**. *Exp Gerontol* 2012, **47**:85-92.

69. Dalton BH, Power GA, Vandervoort AA, Rice CL: **Power loss is greater in old men than young men during fast plantar flexion contractions**. *J Appl Physiol (1985)* 2010, **109**:1441-1447.

70. Callahan DM, Kent-Braun JA: **Effect of old age on human skeletal muscle force-velocity and fatigue properties**. *J Appl Physiol (1985)* 2011, **111**:1345-1352.

71. Baudry S, Klass M, Pasquet B, Duchateau J: **Age-related fatigability of the ankle dorsiflexor muscles during concentric and eccentric contractions**. *Eur J Appl Physiol* 2007, **100**:515-525.

72. Geeves MA, Fedorov R, Manstein DJ: **Molecular mechanism of actomyosin-based motility**. *Cell Mol Life Sci* 2005, **62**:1462-1477.

73. Caremani M, Melli L, Dolfi M, Lombardi V, Linari M: **Force and number of myosin motors during muscle shortening and the coupling with the release of the ATP hydrolysis products**. *J Physiol* 2015, **593**:3313-3332.