Response of compressed skinned skeletal muscle fibers to conditions that simulate fatigue

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Myburgh, Kathryn H., and Roger Cooke. Response of compressed skinned skeletal muscle fibers to conditions that simulate fatigue. J. Appl. Physiol. 82(4):1297–1304, 1997.—During fatigue, muscles become weaker, slower, and more economical at producing tension. Studies of skinned muscle fibers can explain some but not all of these effects, and, in particular, they are less economical in conditions that simulate fatigue. We investigated three factors that may contribute to the different behavior of skinned fibers. 1) Skinned fibers have increased myofilament lattice spacing, which is reversible by osmotic compression. 2) A myosin subunit becomes phosphorylated during fatigue. 3) Inosine 5′-monophosphate (IMP) accumulates during fatigue. We tested the response of phosphorylated and unphosphorylated single skinned fibers (isometric tension, contraction velocity, and adenosinetriphosphatase activity) to changes in lattice spacing (0–5% dextran) and IMP (0–5 mM) in the presence of altered concentrations of P, (3–25 mM), H (pH 7–6.2), and ADP (0–5 mM). The response of maximally activated skinned fibers to the direct metabolites of ATP hydrolysis is not altered by osmotic compression, phosphorylating myosin subunits, or increasing IMP concentration. These factors, therefore, do not explain the discrepancy between intact and skinned fibers during fatigue.

pH; phosphate; force; velocity; mechanics; inosine 5′-monophosphate; phosphorylation; lattice compression

PROLONGED PHYSICAL ACTIVITY results in an accumulation of metabolites in muscle and in a number of changes in the mechanics and energetics of contraction (for review, see Ref. 17). For example, during a lengthy tetanic contraction, tension, velocity and energy expenditure all are inhibited, although not all to the same extent. Energy expenditure is decreased more than tension so that the economy, or energy cost, of tension production improves (4, 11, 12, 16, 18). The tension economy of living fibers has been defined as the tension-time integral divided by the ATP used, and here we define the tension economy of skinned fibers as the ratio of isometric tension (P) divided by the adenosinetriphosphatase (ATPase) activity of the fiber. Despite considerable effort, the exact mechanisms by which these changes are produced remain controversial. Previous work in skinned muscle fibers has shown that increases in the concentrations of some metabolites, e.g., phosphate (P) and H , can apparently account for some of the decrease in force observed during fatigue and an increase in H  concentration inhibits velocity (for review, see Ref. 26). However, these studies do not explain a number of other observations.

For example, there is controversy regarding the role of pH during fatigue, particularly in vivo (1, 2, 6, 27), and a mechanism for improved economy during fatigue has yet to be found despite several observations of this effect in intact fast skeletal muscle during isometric contractions (4, 11, 12, 16) and isometric tetani (13). This effect has also been shown in rat diaphragm, which has both fatigue-sensitive and fatigue-resistant fibers (8) and in human quadriceps (15). The magnitude of increase in economy is between two- and fourfold (11, 15). In contrast, the economy of contraction of skinned fibers is decreased in the presence of either phosphate (0.7-fold) or H (0.7-fold) or both (0.5-fold) (26, 10, 29).

In living fibers the contraction velocity decreases before tension; however, in skinned fibers the conditions that simulate fatigue inhibit tension more than velocity. These discrepancies between the responses of intact fibers to fatigue and of skinned fibers to the increased metabolites of fatigue suggest that some mechanisms that occur in vivo are lost in the skinned fiber preparation.

One important difference between intact and skinned fibers is that the diameter of muscle fibers increases when they are skinned, resulting in increases in the lattice spacing between adjacent thick filaments in relaxed fibers of up to 28% (19). However, the lattice spacing can be returned to physiological spacing by osmotic compression (19), which increases tension production by 4% but decreases ATPase activity by 26%, thus increasing the economy of contraction by ~40% (22, 35). We hypothesize that the economy of compressed fibers may undergo further changes in response to the conditions of fatigue so that their contractile properties become more akin to that seen in vivo. This hypothesis has been investigated, in part, in a study by Martyn and Gordon (25), who showed that osmotic compression of skinned fibers did not alter the effect of changing pH from 7 to 6. However, this has not been done in the presence of other metabolites, such as P , ADP, or inosine 5′-monophosphate (IMP), or a combination of several of these.

Myosin P light chains of intact fibers in animal models (31) and in human muscle (21) become phosphorylated during fatigue, and this occurs at about the same time as economy increases (31). Phosphorylation is associated with twitch potentiation (21, 31) but may not be associated with increased economy of contraction (4). However, myosin phosphorylation appears to have little effect in maximally activated skinned fibers (20, 28). Because phosphorylation occurs during fatigue, it is plausible that its effect may become evident only in the presence of the metabolites of fatigue. Alternatively, its effect may be enhanced by simultaneous compression to physiological lattice spacing. The myosin head is sufficiently long that it more than spans the interfilament distance at in vivo spacing, and there is
METHODS

Simulating fatigue. Among these metabolites, IMP is a likely candidate to play a role in modifying muscle function during fatigue (14, 33) because it is a direct product of nucleotide degradation. Furthermore, in a recent study of intact muscle in which high levels of IMP were maintained by inhibiting its resynthesis to AMP, the muscles had an increased economy of contraction (18).

We hypothesized that either myosin light chain phosphorylation or increased IMP concentration, or both, might alter the response of fully activated skinned fibers to the direct metabolites of ATP hydrolysis, especially under conditions of simultaneous myofilament lattice compression. In particular, we hypothesized that one or more of these additional factors would lessen the decrease in force production previously observed or enhance the decrease in the velocity of contraction or ATPase activity so that the economy of tension generation would improve. In contrast to expectation, we found that none of these factors, either singly or in combination, produced effects that could explain the apparent discrepancies in mechanics and energetics of the actomyosin interaction between fatigued intact fibers and skinned fibers tested under conditions simulating fatigue.

METHODS

Muscle fiber preparation. Thin strips of psoas muscle, 2–3 mm in diameter, were dissected from a rabbit and glycerinated as previously described by Cooke et al. (10). To prepare fibers with phosphorylated myosin light chains, (in mM) 5 potassium fluoride, 5 MgATP, and 20 P; were added to the basic skinnning solution before dilution with glycerol. Fibers were gently shaken in this solution for 24 h at 0°C before being stored in a fresh but similar solution at 20°C until used. The extent of phosphorylation of fibers from each preparation was monitored by isoelectric focusing gel electrophoresis (IEF gel) before mechanical testing. The IEF gel method has been previously described in detail (Refs. 28, 31; see Fig. 1 for a representative gel). Only fiber preparations that were 90–100% phosphorylated were used for mechanical testing. Control fibers were between 0 and 10% phosphorylated.

Measurement of mechanical properties and sarcomere lengths. Single fibers or bundles of 2–3 fibers were connected to a solid-state force transducer on one end and to a rapid servo-controlled motor at the other, as described previously (10). Sarcomere length was monitored by laser diffraction before and during activation. Resting sarcomere lengths were between 2.2 and 2.4 μm. The position of the first-order diffraction pattern was measured by a position-sensitive photodiode (United Technology, Cupertino, CA). The signal from the photodiode was calibrated by translating the photodiode rapidly by 0.5 mm and by applying a series of step changes to the length of the fiber during relaxation. The strength of the first-order diffraction was monitored visually, and fibers for which the strength decreased appreciably during activation were discarded.

After activation, the peak P0 and the isotonic contraction velocity (lengths/s) were measured as described in Cooke et al. (10). To measure the velocity at 10% of P0 (V10), the load was maintained at 10% of P0 for 40 ms by a feedback loop operating at 5 kHz as described in more detail by Cooke et al.; the fiber was then shortened by 10% of its length, and the zero tension of the transducer was determined. Representative traces of displacement vs. time for fibers obtained under four conditions are shown in Fig. 2. Sarcomere length was also monitored by laser diffraction during isotonic releases (Fig. 2). The position of the line was monitored during a force clamp at 2–5 kHz while force was maintained via feedback of the force signal to the motor position. A distinct possibility exists that the different fiber forces produced under different bathing conditions introduced heterogeneities in sarcomere lengths and that motor position was thus not a good monitor of fiber velocity. Both the motor position and the sarcomere length are plotted in Fig. 2 for four conditions (see Fig. 2 legend).

Experimental conditions. The basic rigor buffer contained (in mM) 120 KAc, 5 MgCl2, 3 phosphate, 1 ethylene glycolbis(β-aminoethyl ether)-N,N,N′,N′′-tetraacetic acid, and 20 3-(N-morpholino)propanesulfonic acid (pH 7) or 40 2-(Nmorpholino)ethanesulfonic acid (pH 6.2). To this, 20 mM creatine phosphate, 4 mM ATP, and 1 mg/ml creatine kinase were added to relax the fiber. In all conditions, the temperature of the solutions was maintained at 10°C and fibers were maximally activated with calcium chloride. Initially, several fibers were titrated with increasing amounts of calcium to establish the concentration of calcium required for full activation. Because all fibers were fully activated by addition of 1.1 mM calcium (≈0.1 mM free Ca2+), this quantity of calcium was subsequently routinely added.

Compression of the fiber lattice can be achieved by bathing the fiber in a solution containing a high-molecular-weight polymer, such as dextran. These large molecules do not penetrate the myofibrillar space but increase the osmolarity of the solution bathing the fiber. Water and electrolytes are removed from the myofibrillar space, causing a decrease in cross-sectional area. Normal lattice spacing is achieved by the addition of between 3 and 5% Dextran T-500 (Sigma Chemical, St Louis, MO) (19). There were, therefore, four buffers in which baseline mechanical measurements were made, namely,
pH 7, 3 mM Pi, with and without 5% dextran, and pH 6.2, 3 mM Pi, with and without dextran. To these baseline buffers, the metabolites ADP, Pi, or IMP were added. Ionic strength was maintained at the same level as the basic rigor buffers by varying KAc concentration. The experiments in which ADP concentration was varied were carried out in the presence of 2.5 mM ATP and the absence of creatine phosphate or creatine kinase because the competition between ATP and ADP is difficult to measure in the presence of an ATP regeneration system. For experiments in which ADP was added, the rigor buffer contained 100 µM diadenosine pentaphosphate, which inhibited the activity of adenylyl kinase. The effect of 5% dextran was determined in two ways: first, by using a two-well system we alternated between wells containing solutions with either 0 or 5% dextran, starting in the 0% dextran solution and switching to the 5% dextran solution or starting in the 5% dextran solution and washing out the dextran by rapid mixing in a 0% dextran solution; second, by using a one-well system we determined whether there was any difference in the effect of addition of metabolites of fatigue in the presence or absence of dextran.

The effect of phosphorylation on mechanics of contraction was determined by using two methods: first, by phosphorylating the fibers during glycerination as described above and, second, by measuring P0 and Vmax in activated control fibers and subsequently in the same bundle of 2–4 fibers after incubation in a relaxing solution containing calcium-insensitive myosin light chain kinase (MLCK). Aliquots of calcium-independent MLCK were clipped by incubation for 15 min. MLCK was a gift of Dr. J. Stull.

To determine the effect of phosphorylation on mechanics of contraction, the following two conditions were compared in the presence of dextran: 1) 4 mM ATP and 3 mM Pi, pH 7, and 2) (in mM) 1 ATP, 30 Pi, 1 ADP, and 4 IMP, pH 6.2. No ATP regeneration system was used for these two experiments. For a more complete picture of the effects of fatigue on force and velocity of contraction, data were obtained from force clamps ranging between 5% and 40% of P0 for construction of a force-velocity curve.

Determination of myosin ATPase activity. Bundles containing 3–4 skinned fibers were mounted between two stainless steel pegs by wrapping the ends around the pegs and gluing with acetone-diluted nail polish. The fibers and pegs were immersed in activating and relaxing solutions of different compositions in wells containing 25 µl of solution at 10°C before the second set of mechanical measures were made. The entire fiber or fiber bundle was used in an IEF gel to determine the extent of phosphorylation. Samples were >80% phosphorylated by incubation for 15 min. MLCK was a gift of Dr. James Stull.

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Figure 3 shows the effect of increasing Pi from 3 to 25 mM, at different time points (0–6 min) on Po and ATPase activity. The Pi production of the remaining portion of the fibers in these control experiments was 7 ± 4% (n = 8) of the overall ATPase activity, indicating that the suspended portion of the fibers utilized 20% of the total fiber ATPase activity. The economy of force production for ATPase activity was measured by using two types of experimental protocols. In one, the fibers were activated in a particular set of conditions and switched between two solutions, one of which included dextran. In these control experiments, the fibers were suspended in a solution containing 5% dextran (n = 8). The presence of 5% dextran did not change the mechanical response of skinned psoas fibers to increases in ADP concentrations, with Po increased by 15 ± 2% and V10 decreased by 20 ± 7% (n = 9, respectively; changes not significant; P > 0.1). Although the concentrations of ADP utilized in these experiments were greater than those found to occur in vivo, it is evident that the competition between ATP and ADP for ADP [competition that depends on both the dissociation constant (Km) for ATP and the inhibition constant (Ki) for ADP] was not changed by fiber compression. The values of Km and Ki measured previously show that the buildup of ADP in vivo would be sufficient to alter muscle properties appreciably (26). Although the K, for inhibition of fiber velocity or ATPase activity is 200 µM, indicating tight binding of ADP, the binding of ATP is far stronger, Km 15–150 µM, so that physiological levels of ADP do not compete effectively with ATP, even in severe fatigue (26).

ATPase activity. Fiber ATPase activity was measured in a separate series of experiments in the absence of dextran, the addition of 5 mM ADP increased Po by 15 ± 2% and decreased V10 by 27 ± 5% (n = 3). The presence of 5% dextran did not change the mechanical response of skinned psoas fibers to increases in ADP concentrations, with Po increased by 15 ± 2% and V10 decreased by 20 ± 7% (n = 9, respectively; changes not significant; P > 0.1). Although the concentrations of ADP utilized in these experiments were greater than those found to occur in vivo, it is evident that the competition between ATP and ADP for ADP [competition that depends on both the dissociation constant (Km) for ATP and the inhibition constant (Ki) for ADP] was not changed by fiber compression. The values of Km and Ki measured previously show that the buildup of ADP in vivo would be sufficient to alter muscle properties appreciably (26). Although the K, for inhibition of fiber velocity or ATPase activity is 200 µM, indicating tight binding of ADP, the binding of ATP is far stronger, Km 15–150 µM, so that physiological levels of ADP do not compete effectively with ATP, even in severe fatigue (26).

Fig. 3. Effects of dextran on Po(A) and velocity at 10% of Po(V10,B) at increasing levels of Pi, for each of following 4 conditions: pH 7 (○), pH 7 + 5% dextran (●), pH 6.2 (□), and pH 6.2 + 5% dextran (■) (n = 3–5 fibers at each data point). Only increase in tension at pH 7 with addition of dextran is significant (P < 0.05).

RESULTS

Lattice compression does not alter the effects of the products of ATP hydrolysis. The effects of the direct products of ATP hydrolysis on Po and V10 were measured by using two types of experimental protocols. In one, the fibers were activated in a particular set of conditions and switched between two solutions, one of which included dextran. In the second, the responses in two conditions were measured, keeping dextran constant at either 0 or 5%. The effect of dextran on the width of single fibers mounted and bathed in a relaxing solution was measured. Changing a relaxing bathing solution to a similar solution containing 5% dextran decreased fiber width by 1.4% ± 1.4% (n = 11; P < 0.001).

Figure 3 shows the effect of increasing Pi from 3 to 25 mM, at either pH 7 or 6.2, on fiber tension and velocity. Compression of the fiber increases tension at pH 7. However, compression has much less effect at pH 6.2. In the absence of dextran, the effect of Pi was similar to previous results, and compression did not alter this effect.

To test whether lattice compression alters the competition between ATP and ADP in the fiber, the effect of increasing the ADP concentration was measured. In the absence of dextran, the addition of 5 mM ADP increased Po by 15 ± 2% and decreased V10 by 27 ± 5% (n = 3). The presence of 5% dextran did not change the mechanical response of skinned psoas fibers to increases in ADP concentrations, with Po increased by 15 ± 2% and V10 decreased by 20 ± 7% (n = 9, respectively; changes not significant; P > 0.1). Although the concentrations of ADP utilized in these experiments were greater than those found to occur in vivo, it is evident that the competition between ATP and ADP for ADP [competition that depends on both the dissociation constant (Km) for ATP and the inhibition constant (Ki) for ADP] was not changed by fiber compression. The values of Km and Ki measured previously show that the buildup of ADP in vivo would be sufficient to alter muscle properties appreciably (26). Although the K, for inhibition of fiber velocity or ATPase activity is 200 µM, indicating tight binding of ADP, the binding of ATP is far stronger, Km 15–150 µM, so that physiological levels of ADP do not compete effectively with ATP, even in severe fatigue (26).

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added P at pH 7 or 6.2. Figure 4 shows the effects of 5% dextran on P/ATPase activity and economy (P/ATPase activity). Dextran increased P0 and decreased ATPase activity at both pH 7 and 6.2. The effect of compression on the ATPase activity was more dramatic at pH 7 than at 6.2, leading to less improvement in the economy of force production at the lower pH (Fig. 4).

Phosphorylated fibers. We addressed the question of whether light chain phosphorylation affected fiber properties in conjunction with a change in a metabolite (increasing P, and decreasing pH) or with fiber compression, or both. As shown in Fig. 4, phosphorylation did not significantly alter the tension, ATPase activity, or economy, or the changes in these on addition of dextran. The effect of compression on the ATPase activities of phosphorylated fibers was also determined by phosphorlyating the fibers via incubation in MLCK, as described in METHODS. Phosphorylation did not alter ATPase activity, as determined by measuring P production in an activating solution before and after incubation with MLCK for 15 min. The ATPase activity of control fibers incubated for 15 min in a relaxing solution that did not contain MLCK decreased slightly over that time period (–6.3 ± 11.9%, n = 3). The change in ATPase activity from pre- to postincubation with MLCK was 1.8 ± 5.5% at pH 7, 0% dextran (n = 6), and 6.2 ± 4.5% (n = 7) in the presence of 5% dextran. At pH 6.2, incubation with MLCK decreased ATPase activity by 12.5 ± 4.9%, (n = 2) without dextran. With 5% dextran, ATPase activity increased by 4.4 ± 8.2% (n = 7). The above data show that phosphorylation of skinned fibers by incubation with a kinase does not greatly alter ATPase activity in either the absence or the presence of dextran (P > 0.1).

We also examined the effects of increasing P in phosphorylated fibers. Increasing P concentrations from 3 to 25 mM in phosphorylated fibers resulted in a decrease in the tension of between 29 and 37%. There was essentially no difference in the response to P, at pH 7 or 6.2, with or without dextran, and the mean decrease for these conditions was 32 ± 3%, which is similar to that observed in unphosphorylated fibers. Increased P generated small increases in velocity at pH 7 (9 ± 4 and 21 ± 6%, without and with dextran, respectively) and small decreases at pH 6.2 (–12 ± 3 and –6 ± 6%, without and with dextran, respectively). These changes were not significantly different from those in unphosphorylated fibers (see Fig. 3). We conclude that phosphorylation has little effect on the mechanics of fully activated fibers and that this result is not affected by increased P, decreased pH, or fiber compression.

Effect of IMP on fiber mechanics in control and phosphorylated fibers. The effect of IMP on fiber mechanics was determined in control fibers under four conditions (see Fig. 5). IMP did not alter force production or V10 in control or phosphorylated fibers at either pH 7 and 3 mM P or at pH 6.2 and 25 mM P, with or without dextran. Mean values for the change from 0–5 mM IMP ranged between –4 and +4% and were not significantly different (P > 0.1). The results obtained from fibers phosphorylated in the skinning procedure were not different from those obtained from control fibers, and the results of both types of preparation were averaged to produce the data in Fig. 5.

Combined conditions. Because some of the above conditions may act in concert in modifying the activity of muscle cross bridges, we assayed fibers under conditions in which many of the possible modifiers were combined. Each fiber was activated in a solution simulating the intracellular medium of an unfatigued fiber: rigor buffer plus 4 mM ATP, 3 mM P, and 5% dextran at...
pH 7. The fiber was then switched to a solution simulating a fatigued fiber: rigor buffer plus (in mM) 1 ATP, 30 Pi, 1 ADP, and 4 IMP, as well as 5% dextran at pH 6.2. The velocity of contraction was inhibited by 30 ± 4% under the conditions simulating the fatigued state (Fig. 6). However, P0 was also inhibited in the fatigue conditions by 52 ± 2%.

We also measured the ATPase activity of the fibers (n = 14), again contrasting two conditions: simulating 1) unfatigued fibers and 2) fatigued fibers, with 5% dextran included in both solutions. One difference between these experiments and the mechanical experiments described in the paragraph above was that we kept P0 low in solutions simulating both fresh and fatigued conditions so that we could assess the ATPase activity by using the sensitive Malachite Green assay for phosphate. The two solutions contained either 1) rigor solution + 4 mM ATP, pH 7 (unfatigued condition), or 2) rigor solution + (in mM) 1 ATP, 1 ADP, and 4 IMP, pH 6.2 (fatigued condition). We found that the ATPase activity of the fibers in the presence of dextran was inhibited only slightly on transfer from unfatigued to fatigued solutions (0.69 ± 0.05 to 0.57 ± 0.03 s). However, in a parallel set of measurements made with the same solutions and fibers from the same preparations, we found that the tension was inhibited by 35 ± 4% (n = 10). Thus tension is inhibited to a greater extent than ATPase activity, showing that the fibers with in vivo lattice spacings also display a decrease in tension economy when they are shifted into conditions that simulate fatigue. The inhibition in the ATPase activity was about the same as was observed for the inhibition of velocity under slightly different conditions (from 1.4 to 1.15 lengths/s). Together these results suggest that tension economy of skinned fibers was not increased by conditions that simulate fatigue, that contraction velocity is inhibited less than force, and that a synergistic action of the various conditions did not occur.

**DISCUSSION**

Although the basic contractile machinery is clearly functional in skinned fibers, there remain a number of conditions in which it appears to respond differently than it does in vivo. The most dramatic difference occurs in the tension economy, which appears to increase in vivo during fatigue but to decrease in the skinned fibers under conditions that simulate fatigue.

There appears to be definitive evidence that the tension economy increases at least twofold during fatigue in vivo. The economy of tension production, calculated from changes in either force production or energy consumption, or both, has been shown to improve with fatigue in frogs (13), mice (4, 11, 12), and in human subjects (7, 15). This issue is, however, somewhat complex. For example, increases in economy have been shown predominantly with continuous isometric stimulation protocols (7) and in muscle containing predominantly fast-twitch fibers (11), although it is also present in muscle of mixed fiber type (8, 15). In the skinned muscle fiber model, economy is calculated as the ratio of force produced to the ATPase rate. In vivo, the lower ATPase activity, which produces the improved economy, is correlated temporally with a decrease in the velocity of contraction (3), and both parameters are inhibited at a point where tension remains relatively high. In skinned fibers, however, increases in the concentrations of metabolic products inhibit tension more than either velocity or ATPase activity, leading to a decrease in the tension economy. A major purpose of this study was to attempt to understand these differences between skinned and intact fibers.

One observation made here, and by other investigators, is that compression of skinned fibers to physiological widths increases their force production, decreases their ATPase activity, and thus increases their tension economy under conditions simulating the unfatigued state (22, 35). We hypothesized that a further improvement in tension economy might be obtained under conditions simulating fatigue. If our hypothesis proved correct, it could be concluded that increased myofila-ment lattice spacing in uncompressed skinned fibers was a factor that prevented them from showing an increased economy of tension production under conditions that simulate fatigue. In the absence of compression, skinned fibers in our study responded predictably to increases in the metabolites of fatigue: either phosphate, H+, or both decreased force production and velocity of contraction, and increased ADP concentration increased force production and decreased velocity of contraction. These effects have previously been observed in our and other laboratories (see Ref. 26 for review). However, contrary to the above hypothesis, compression did not alter the mechanical response of
skinned fibers to increased concentrations of either P,
ADP, or H+ . Nor was the economy of tension production
increased by a combination of compression and condi-
tions that simulate fatigue. Although ATPase activity
was not measured at high P, concentration, it is un-
likely that the economy would be different under condi-
tions of high P, concentration because velocity, which
correlates with ATPase activity (3), was similar in
compressed and uncompressed fibers under those condi-
tions.

An additional difference between fatigued intact
fibers and the skinned fiber model is that intact fibers
become phosphorylated with fatigue (31). Although
myosin phosphorylation has been found to have little
effect (<5%) on fully activated skinned fiber mechanics
or economy (20, 28), it could be that the effects of
phosphorylation are enhanced under metabolic condi-
tions simulating fatigue or when the myofilament
lattice spacing is at physiological widths, or both.

However, the responses of phosphorylated fibers in the
present study followed similar patterns to those of
unphosphorylated fibers on changes in either lattice
compressions or metabolite concentrations, or both.

The large changes in IMP concentration from rest to
fatigue (70- to 80-fold) (14, 33) make it a reasonable
effect of muscle mechanics and energetics, as previ-
ously suggested by Westra et al. (33), who found an
excellent correlation between increasing IMP concen-
tration and decreasing P, in rat muscle in situ. However,
we did not observe any changes in skinned fiber mechan-
ics on addition of 5 mM IMP (approximately equal to
that obtained in vivo) to fibers under a variety of
conditions, suggesting that previous results were due
to other factors.

Although the skinned fiber model has been exten-
sively used to investigate the effects of potential effec-
tors on the mechanics of contraction, these effectors
have been studied separately or in pairs. Because there
is a possibility that such effectors may act in concert,
we have investigated several additional combinations.
In all of these investigations, we found little synergistic
effect between various factors.

In conclusion, our data confirm that compression of
skinned fibers to physiological widths increases their
force production, decreases their ATPase activity, and
thus increases their tension economy under conditions
simulating the unfatigued state (22, 35). From the
present study, we also conclude that the different
response to fatigue of fully activated, skinned fibers
compared with intact fibers is due to unphysiologi-
cal, expanded lattice spacing or myosin phosphoryla-
tion or to high IMP concentration. Thus the discrep-
ancies between the mechanics of intact fatigued fibers
and skinned fibers in conditions that simulate fatigue re-
main an enigma.

It is possible that the improved economy of intact
fibers is due to different Ca2+ handling during fatigue,
as suggested by several recent reports (23, 24). These
studies have described, in particular, decreased cal-
cium release, subsequent to decreased capacity for
calcium reuptake by the sarcoplasmic reticulum (for
review, see Ref. 2). However, exactly how and when the
alterations in calcium handling might affect economy
of force production during fatigue are unclear. Alterna-
tively, economy of force production during fatigue
could be due to another factor, such as an additional metabo-
lite or phosphorylation of proteins other than the
myosin light chain, which have not yet been investi-
gated in skinned fibers. The observation that the
mechanical economy in skinned fibers improves as a
result of compression to physiological widths suggests
that all future experiments, and particularly those
relating to fatigue, should be done with fibers with
physiological lattice spacings.

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