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Skeletal Muscle Fatigue: Cellular Mechanisms

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Allen DG, Lamb GD, Westerblad H. Skeletal Muscle Fatigue: Cellular Mechanisms. *Physiol Rev* 88: 287–332, 2008; doi:10.1152/physrev.00015.2007.—Repeated, intense use of muscles leads to a decline in performance known as muscle fatigue. Many muscle properties change during fatigue including the action potential, extracellular and intracellular ions, and many intracellular metabolites. A range of mechanisms have been identified that contribute to the decline of performance. The traditional explanation, accumulation of intracellular lactate and hydrogen ions causing impaired function of the contractile proteins, is probably of limited importance in mammals. Alternative explanations that will be considered are the effects of ionic changes on the action potential, failure of SR Ca^{2+} release by various mechanisms, and the effects of reactive oxygen species. Many different activities lead to fatigue,

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and an important challenge is to identify the various mechanisms that contribute under different circumstances. Most of the mechanistic studies of fatigue are on isolated animal tissues, and another major challenge is to use the knowledge generated in these studies to identify the mechanisms of fatigue in intact animals and particularly in human diseases.

I. INTRODUCTION

Muscles that are used intensively show a progressive decline of performance which largely recovers after a period of rest. This reversible phenomenon is denoted muscle fatigue. The phenomenon must have been recognized by perceptive observers since posterity, but studies of the mechanism are relatively recent. Needham (1971) (328) provides a comprehensive account of the history of muscle contraction and metabolism and quotes Berzelius (1807) for the discovery that the muscles of an exhausted stag contained lactic acid. Mosso (1904) (318) introduced the experimental study of the topic with figures illustrating the rapid fatigue in humans when a finger lifts a heavy load. He showed that the rapid fatigue occurs even when the nerve was stimulated electrically, implying that fatigue is in the muscle rather than in the central nervous system. A seminal contribution was made by Hill and Kupalov (1929) (208) who showed that an isolated frog muscle stimulated in N_2 gas fatigued rapidly and accumulated lactic acid. A striking result was that if the muscle was then transferred to a N_2-saturated Ringer solution, the performance recovered as lactic acid diffused out of the muscle, suggesting that lactic acid could cause fatigue. Eberstein and Sandow (1963) (149) were the first to suggest that failure of excitation-contraction (EC) coupling contributed to muscle fatigue by showing that a fatigued muscle could recover much of its force when perfused with caffeine, known to directly facilitate release of Ca^{2+} from the sarcoplasmic reticulum (SR). Burke et al. (1973) (75) stimulated individual motor units in cat muscles to exhaustion and identified the muscle fibers involved by the depletion of glycogen. These influential studies showed that fast fibers fatigued extremely quickly, whereas slow fibers were essentially unfatiguable. The development of the muscle biopsy technique by Bergström et al. (1967) (46) and the application of muscle NMR to fatigue by Dawson et al. (1978) (117) accelerated the understanding of the biochemical changes during fatigue.

One definition of fatigue is any decline in muscle performance associated with muscle activity. This is particularly clear when maximum isometric force is measured in repeated tetani (Fig. 1) and shows that there is a progressive decline that is visible even on the second tetanus of the series. Other aspects of muscle performance also change during fatigue, notably shortening velocity is reduced and the time course of relaxation slows. Most practical activities are dependent on the power output of the muscles involved and, since power output is the product of both force and shortening velocity, the decline in performance can be larger than the decrease in isometric force. Of course, the decline in performance is not immediately apparent if a submaximal activity is performed, and in this situation fatigue manifests itself eventually as a failure to be able to continue the activity at the original intensity (48), often called exhaustion. In such an activity, the progress of fatigue can

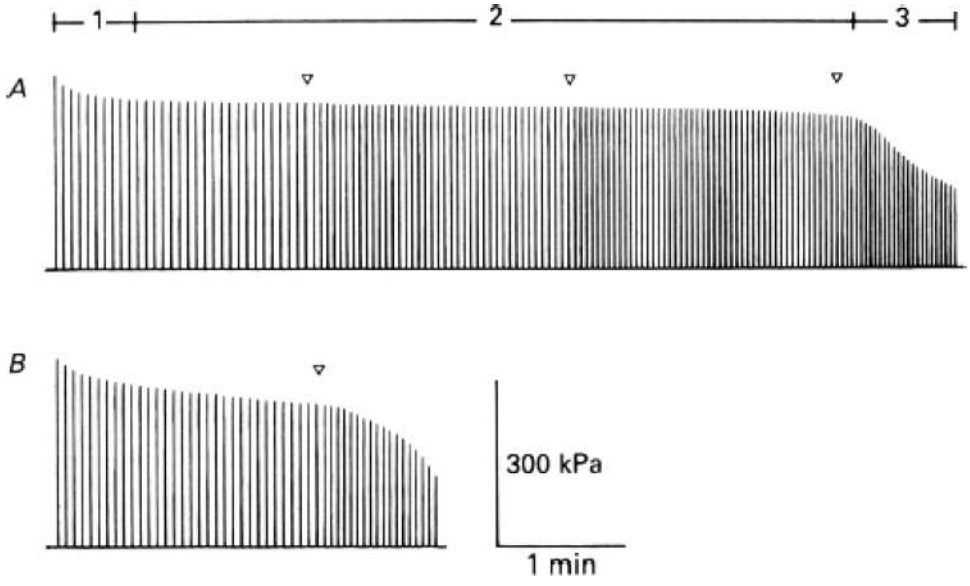

FIG. 1. Force records during fatigue produced by repeated short tetani in an isolated mouse flexor digitorum brevis (FDB) fiber; each tetanus appears as a vertical line. In the *top panel*, the phases of fatigue (see sect. VII) have been indicated. The *bottom panel* shows records from the same fiber fatigued in the presence of cyanide to inhibit mitochondrial oxidative phosphorylation. Stimulation protocol: 350-ms, 70-Hz tetani repeated every 4 s for 2 min, and the interval was decreased by ~20% every 2 min (interval changes indicated by open triangles). Temperature was 25°C. [From Lännergren and Westerblad (268).]

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be estimated by occasionally interpolating a maximal contraction.

In voluntary contractions, muscles are activated by complex pathways starting in the cortex and leading to excitation of lower motor neurons in the spinal cord. The axon of the lower motor neuron carries the action potentials to the neuromuscular junction of the muscle. For simplicity, the processes inside the spinal cord and above are defined as central, whereas the processes in the peripheral nerve, neuromuscular junction, and muscle are defined as peripheral. Clearly fatigue can potentially arise at many points in this pathway and can usefully be divided into central and peripheral fatigue. Early studies by Merton (1954) (308) suggested that in well-motivated individuals, the fatigue in a small muscle of the hand could be entirely peripheral. Later studies suggest that a small degree of central failure of activation often occurs during maximal activation of muscles and that during fatigue there is often a substantial central component (for review, see Gandevia, Ref. 179). Nevertheless, it is universally agreed that much of fatigue arises in the muscles and can therefore be studied in isolated muscle tissues. This review focuses on the components of fatigue that lie within the muscle.

It is usual to make a distinction between muscle fatigue and muscle injury, although undoubtedly the two phenomena overlap. Fatigue is usually defined as the reversible decline of performance during activity, and most recovery occurs within the first hour. However, there is also a slowly reversible component that can take several days to reverse (155). Muscle injury also causes a decline in performance that reverses only very slowly. Muscles that are stretched during contraction (eccentric contractions) are particularly prone to injury or damage. Injury is characterized by structural abnormalities including sarcomeric disorder, membrane damage resulting in the loss of soluble enzymes such as creatine kinase, and inflammatory processes including cytokine release and phagocytic cell infiltration. Recovery from the most serious injuries involves activation of satellite cells and regeneration of damaged fibers. Muscle damage has been reviewed recently (12, 363) and will not be considered here.

A. Continuous Maximal Activity

If a muscle is stimulated continuously at a frequency close to that which gives maximal force, then force production generally shows a rapid decline (Fig. 2) often called high-frequency fatigue (52, 227). A characteristic of this type of fatigue is that the recovery is also very rapid, often having a component of recovery with a time course of only 1–2 s (52, 227). In a human, this type of contraction would be encountered in lifting a very heavy object (e.g., a piano), and it is generally recognized that onset and recovery of fatigue are rapid in this situation. In intact individuals, an important consideration is that when force exceeds ~50% of maximum, then the muscle circulation collapses (33). Consequently, continuous maximal contractions occur in a nonperfused (ischemic) muscle, though perfusion would be present during recovery. Studies of the firing rate of motor neurons in intact individuals show that in such near-maximal contractions, the initial firing rate is high but that this rate falls steadily over a minute or so (50). In experimental studies of high-frequency fatigue, it is common to simply stimulate at a constant high frequency, so it is important to realize that such studies do not accurately reproduce what happens in the intact individual.

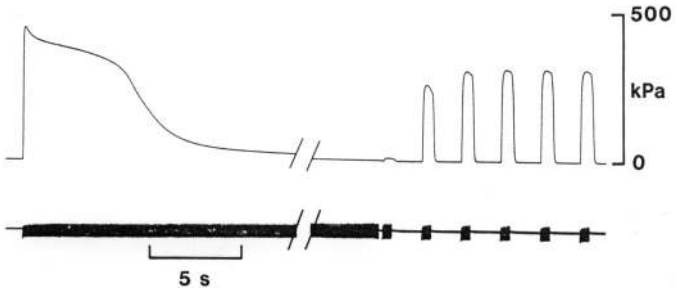

FIG. 2. Force record from a fast-twitch lumbrical muscle fiber of *Xenopus* stimulated at 70 Hz for 30 s. Recovery followed with 500-ms, 70-Hz tetani given at 2-s intervals. Note very rapid recovery after 2 s. [From Lännergren and Westerblad (265).]

B. Repeated Short Tetani

A popular stimulation pattern for the study of fatigue is repeated, short tetani (Fig. 1). Unfortunately, there is little uniformity in the detailed protocols used by different groups. One key variable is the fraction of the time during which the muscle contracts (duty cycle), which is commonly between 0.1 and 0.5. Another key variable is the stimulus frequency during the tetani which determines the degree of activation and will influence the rate of decline of force. This pattern of stimulation, which obviously simulates many natural activities (e.g., walking, running, and breathing), leads to a much slower rate of fatigue than the continuous high-frequency stimulation described above. Commonly the fatigue protocol is stopped after a fixed number of tetani or when the force reaches a certain predetermined level such as 50% of the initial tetanic force. The rate of recovery from this pattern of activity is quite variable. There is usually a phase of recovery that is nearly complete after 5–10 min and sometimes a much slower component (discussed below). Usually recovery of maximum force is virtually complete by 30 min, and it is possible to perform repeated fatigue runs

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with nearly similar time courses after a recovery period of >30 min. This allows the comparison of fatigue runs under different conditions within the same fiber avoiding interfiber variability (90, 203).

C. Delayed Recovery From Fatigue

As noted above, after repeated short tetani there may be a very slow component of recovery. This phenomenon was first described by Edwards et al. (155), who used repeated voluntary contractions in humans under ischemic conditions continued until the force was negligible. Recovery measured in brief tetani at high frequencies (50–100 Hz) was relatively fast ($t_{1/2}$ ~5 min), whereas recovery at low frequencies (10–20 Hz) was very slow ($t_{1/2}$ 1–2 h) with a small component of weakness still persisting after 1 day. They called this phenomenon "low-frequency fatigue," a name we do not recommend for the reasons discussed below. The phenomenon is particularly prominent if the exercise contains a component of muscle stretch (114), which is liable to cause muscle damage, and this is one area in which muscle damage and fatigue appear to overlap. For human subjects, the force is reduced in a frequency range that corresponds to the firing frequencies of human motor units during low- to moderate-force voluntary movements (300), and the phenomenon is probably responsible for the feeling of weakness that can persist for several days after a period of intense exercise. Presumably to achieve the necessary force for a particular activity, the brain must increase the firing rate or activate more motor units for a particular muscle and interprets this information as weakness, even though the muscle may show no decrement in maximal force.

As noted above, high-frequency fatigue is widely used to describe fatigue resulting from continuous maximal contractions. This suggests by analogy that "low-frequency fatigue" should be used for continuous stimulation at low frequencies, but in fact, Edwards et al. (155) used the term to describe a type of recovery that was slower when measured at low frequencies. Thus we recommend using the term *prolonged low-frequency force depression* for this phenomenon and avoid the term *low-frequency fatigue* that is now used for several quite different situations. Furthermore, high-frequency fatigue is sometimes used to describe fatigue induced by repeated short tetani, where the stimulation frequency is high during the tetani. Thus this term may also be misinterpreted, and we recommend that it is also avoided.

II. SEQUENCE OF EXCITATION-CONTRACTION COUPLING IN MUSCLE

The chain of events involved in EC coupling in skeletal muscle is now reasonably well understood (for review, see Refs. 140, 306, 424). Obviously a failure anywhere in this chain of events could contribute to fatigue. The muscle action potential (AP) is initiated at the neuromuscular junction by release of acetylcholine as a consequence of the AP in the motor neuron. The neuromuscular junction appears to provide uniform 1:1 AP transmission under physiological conditions (179), and failure at this point will not be considered in this review. Transmission of the AP along the surface membrane of the muscle fiber depends on local currents activating distant Na^+ channels. Potentially AP transmission is influenced by numerous factors including the membrane potential, the Na^+ and K^+ concentrations extracellularly and intracellularly, the internal and external resistances, and the membrane resistance and capacitance. Many of these factors can vary in fatigue, and possible contributions of the AP transmission to fatigue are considered in section VI. The AP is actively conducted down the transverse tubules (t tubules) into the interior of the muscles (47). Because of the small volume of the t-tubular system, changes in ionic concentrations are particularly prominent in the t tubules. However, because there is no simple way to measure the AP within the t-tubular network, assessing the role of the T system is difficult. The t-tubular membrane expresses high levels of L-type Ca^{2+} channels (or dihydropyridine receptors, DHPRs, or voltage sensors) which change their conformation during an AP, resulting in charge movement (397). The voltage sensors are in close contact with the SR Ca^{2+} release channels (the ryanodine receptors, RyR), which in mammalian skeletal muscle are principally the RyR1 isoforms. Charge movement in the voltage sensors results in opening of the RyR receptor and SR Ca^{2+} release. The mechanism of this interaction has been the subject of intense research and appears to be dependent on movement of the two to three intracellular loop of the DHPR which interacts with the RyR (140, 435). The RyR is a large and multiregulated protein potentially affected by many of the intracellular changes associated with fatigue (see sect. VII). It is known that SR Ca^{2+} release fails in various types of fatigue, and there is an unresolved debate as to whether this occurs through changes in the degree of voltage sensor activation or through the influence of changing myoplasmic metabolites or through depletion of Ca^{2+} inside the SR (see sects. VII, A and C, and IX).

Ca^{2+} released by the SR gives rise to a transient increase in myoplasmic free $[Ca^{2+}]$ ($[Ca^{2+}]_i$), which is relatively easy to measure and can be regarded as the end product of the preceding processes. Ca^{2+} within the myoplasm binds to troponin C, instigates movement of tropomyosin, and allows the cycling of cross bridges, which eventually result in force development (19). The magnitude of the $[Ca^{2+}]_i$ transients depends on the SR Ca^{2+} release and all the Ca^{2+} buffers in the cell, which include troponin C, parvalbumin, the SR Ca^{2+} pump, calmodulin,

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and ATP (36). There are no measurements of Ca^{2+} buffering in fatigue which could assess how the Ca^{2+} buffering is affected by the ionic and metabolic changes of fatigue; consequently, the common assumption that changes in the $[Ca^{2+}]_i$ transient reflect changes in SR Ca^{2+} release may be incorrect.

The sequence of EC coupling ends with cross-bridge activation. Cross-bridge properties can be determined in skinned fiber studies which allow the maximum Ca^{2+}-activated force ($F_{Ca,max}$) and the Ca^{2+} sensitivity to be measured under conditions that simulate any chosen aspect of fatigue. Finally, the muscle relaxes as the elevated Ca^{2+} is pumped back into the SR by the ATP-driven SR Ca^{2+} pumps. The SR pumps are sensitive to many of the metabolic and ionic changes in fatigue, but the contribution of the changing pump properties to the slowing of relaxation in fatigue is still uncertain (8) (see sect. XI).

III. METABOLIC CHANGES IN WORKING MUSCLES

A feature of fast muscle is that it can consume ATP, producing ADP and P_i, much faster than it regenerates it. Because the creatine kinase (PCr + ADP ↔ Cr + ATP) and the adenylate kinase (2ADP ↔ AMP + ATP) reactions are close to equilibrium, the net consumption of ATP leads to relatively stereotyped changes in the concentrations of ATP, ADP, P_i, phosphocreatine (PCr), creatine (Cr), and AMP which can be calculated from the equilibrium constants (10, 85). In essence, during net consumption of ATP, [ATP] is initially unchanged, and the net effect is a fall in [PCr] and rises in [Cr] and [P_i], which have been observed in numerous biopsy and NMR studies of fatiguing muscles (e.g., Ref. 78). Later, when [PCr] reaches low levels (<10 mM), [ATP] starts to fall and [ADP], which under control conditions would be around 10 μM, rises substantially (100–300 μM). When [ADP] reaches such levels, the [AMP] also becomes significant and can be broken down by AMP deaminase to NH_3 and inosine monophosphate (IMP).

Many studies have reported that cytoplasmic [ATP] does not drop below ~60% of the resting level during either imposed stimulation or voluntary exercise (30, 117, 168, 286, 406, 455). However, virtually all of these measurements were made in whole muscle or muscle homogenates and hence reflect the spatially averaged change across all fibers present. Thus these values are difficult to interpret because the rate and extent of ATP usage differ substantially between fibers, depending on their metabolic profile and level of activation. If muscle biopsies are frozen and the individual fibers dissected out and typed, it becomes possible to determine the metabolic changes in identified fiber types. In a study of maximal cycling exercise in humans over 25 s, Karatzaferi et al. (238) showed that when PCr was reduced to 11% (~2.5 mM) in the fastest fibers (type IIX), ATP was reduced to 20% (~1.2 mM) and IMP reached ~5 mM. These changes are much greater than the mean changes across all the fiber types and occurred at a time when the power output had dropped to ~50%. These experiments raise the possibility that changes in [ATP] may be more important than hitherto realized in intense exercise (see sect. VII*C*).

In addition to variations between fiber types, there must also be gradients of metabolites across the cells reflecting the different sites of consumption of ATP compared with its resynthesis. Calculations of the consumption and diffusion across myofibrils (assuming that ATP is consumed in the myofibrils and synthesized at mitochondria surrounding the myofibrils) indicate that the gradients of ATP are generally very small (311). Nevertheless, there may be sites in the cell where diffusion is very restricted, for instance, by multiple binding sites, where high local rates of ATP consumption lead to local depletion (197, 247, 453, 459).

One possible site of localized ATP depletion is the space between the t tubule and the SR (triad junction). ATP consumption in this region is substantial, owing to the presence of calcium pumps on the SR terminal cisternae just outside the junction (173, 392) and Na^+-K^+ pumps and other ATPases in the T-system membrane (206). Approximately 50% of all Na^+-K^+ pumps are in the T system (334), and the T system forms triad junctions with the SR for >90% of its length in mammalian muscle fibers (138). Glycolytic enzymes associated with the triad junction support localized synthesis of ATP, which is used preferentially over cytoplasmic ATP (197). The glycolytic enzymes are well placed to utilize glucose entering the fiber via the T system (276), as well as the glucose-6-phosphate from adjacent glycogen stores. Na^+-K^+ pumps in muscle fibers preferentially use ATP derived from glycolysis (95, 337), including those pumps located in the T system (146). In view of the high density of ATP-consuming and -generating processes in the vicinity of the triad junction, as well as the comparatively small percentage of the cell volume it encompasses, [ATP] in the triad junction quite likely differs considerably from that in the cytoplasm as a whole. Thus this could allow the triad region to play a major role in sensing and responding to changes in cellular energy status, particularly given that the triad junction is the key transduction zone regulating Ca^{2+} release and contraction (see sect. VII*C3*).

When ADP is elevated, it is further hydrolyzed by the adenylate kinase reaction to AMP, which is then rapidly deaminated to IMP. This helps reduce the rise in [ADP] and consequent decrease in free energy for ATP hydrolysis. Most ADP in a resting muscle is bound to F-actin and does not directly influence metabolic reactions (453). During intense exercise, average cytoplasmic free [ADP] rises from ~10 to ~200 μM (168, 455), and reductions in

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[ATP] are nearly matched by equimolar rises in [IMP] (168, 238, 323, 407; see sect. x).

Another important factor is that when [ATP] decreases, free [Mg^{2+}] rises, because ADP, AMP, and IMP all have much lower affinity for Mg^{2+} than does ATP (55, 464). The free [Mg^{2+}] in resting muscle fibers is ~1 mM (246, 464, 467). In single mouse fast-twitch fibers stimulated to fatigue, free [Mg^{2+}] rose rapidly at the time that force declined steeply and reached ~2 mM when force had declined to ~30% (464) (see sect. vii*C3*).

The pathways that resynthesize ATP include anaerobic glycogenolysis and the aerobic breakdown either of glycogen, glucose, or fat. Much current research focuses on the regulation of these pathways in different types of exercise (201, 402, 416) and is beyond the scope of this review. Anaerobic glycolysis is of central importance in muscle fatigue because it is turned on rapidly during activity, and the net reaction is breakdown of glucose units to lactate ions and protons causing the early acidosis associated with rapid-onset muscle fatigue. Typically this can lead to an acidosis of ~0.5 pH units developing over several minutes at the start of intense exercise (for review, see Ref. 168). However, this acidosis is not an invariable accompaniment of muscle activity and presumably depends on the extent to which anaerobic glycolysis is switched on and the rate at which protons leave the muscle on the lactate transporter or the Na^+/H^+ exchanger. Typically after long-lasting activity, particularly at a low duty cycle, there is little or no acidosis (91, 452) (see sect. vii*B*).

Glycogen is the principle store of energy in muscle and is rapidly depleted during intense exercise by anaerobic glycogenolysis and more slowly depleted in aerobic exercise. The twin correlations between muscle glycogen levels and a carbohydrate-rich diet and between glycogen depletion and exhaustion are two of the fundamental findings in exercise science (46); nevertheless, the reason why muscle fatigue is associated with glycogen depletion is still not understood (see sect. vii*D*).

IV. DIFFERENCES BETWEEN FAST AND SLOW FIBER TYPES

There are large differences between skeletal muscle cells regarding their speed of contraction, intracellular Ca^{2+} handling (see sect. ix), glycolytic versus oxidative capacity, fatigue resistance, etc. Several systems have been used to divide muscle cells into different fiber types, and the classification systems have to a large extent been a consequence of the methods available to distinguish between muscle cells (61, 410). At present, the dominating fiber type classification system for mammalian skeletal muscle is based on the expression of myosin heavy chain (MHC) isoforms, and the major fiber types are type I, IIa, IIx, and IIb. While all four types of MHC are expressed in rodent muscles, IIb MHC is not expressed in human muscle (405). There are also muscle fibers that coexpress different MHC isoforms (354). The MHC isoform determines the rate of cross-bridge cycling and hence the maximal shortening velocity of a muscle cell, with type I being the slowest, type IIa intermediate, and IIx/b the fastest (61). However, it should be noted that the maximal shortening velocity differs between cells with the same MHC expression, which shows that it also depends on other factors, including the myosin light chain composition (60). In addition to MHC, there are isoforms of numerous other proteins with expression patterns that differ between muscle cells (61). In many instances, there is a distinguishable pattern of gene coexpression in cells so that the slow MHC type I is coexpressed with "slow" isoforms of other proteins. However, this is not always the situation, and there are multiple mechanisms interacting to control the gene expression of different protein isoforms (410).

A fiber type classification based on MHC isoforms has some relevance in the context of fatigue related to metabolic changes, because a fast isoform will consume ATP at a faster rate than a slow isoform. This can be illustrated by a recent study on isolated mouse fast-twitch fibers, where inhibition of cross-bridge cycling with *N*-benzyl-*p*-toluene sulfonamide (BTS) markedly delayed the fatigue-induced decrease in tetanic [Ca^{2+}]$_i$ (66). Thus fatigue occurs more slowly when cross-bridge ATP consumption is decreased. However, a similar argument could be made for the other major ATP-consuming proteins in skeletal muscles, the SR Ca^{2+} pumps. These pumps exist in two isoforms, SERCA1 in fast type II fibers and SERCA2 in slow type I fibers (293), and the density of pumps is much higher in fast than in slow fibers (159). Furthermore, the major determinant of fatigue resistance is probably the muscle fiber's density of mitochondria and capacity to use oxidative metabolism. Slow type I fibers generally have a higher oxidative capacity than fast type II fibers (158), but in rat muscles, a higher oxidative capacity in type IIa than in type I fibers has been observed (24). In conclusion, "It is now clear that the MHC classification system for fiber types is a MHC, not a fiber type, classification system" (410). In relation to fatigue, slow type I fibers are generally more fatigue resistant than fast type II fibers, but this is mainly due to factors other than their MHC isoform.

V. HOW TO STUDY FATIGUE?

A. Models of Fatigue and Their Limitation

The "gold standard" for fatigue is the intact perfused muscle under central control. For selected muscle

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groups, it is easy to measure the decline of force, and muscles can be studied by electromyography (EMG), NMR, and biopsy. A difficulty with each of these approaches is that muscles are mixtures of fiber types with very different properties, so there are problems either with the representativeness of the sample or the way in which spatial and/or temporal averaging disguises the interpretation. With EMG, it is difficult to distinguish the contributions from slowing of cortical firing rate, dispersion of conduction velocities along the fibers, change in resting potential, and AP amplitude (53). As noted in section I, central fatigue is always a contributor to fatigue in intact animals. A useful variant of this approach is to produce fatigue by stimulating the nerve or muscle directly, thereby eliminating central contribution, and, because the blood flow is intact, eliminating the diffusion problems associated with isolated muscles.

Many studies of fatigue have been performed on isolated whole muscles, stimulated and perfused in a muscle bath. Because of the absence of the circulation, such preparations inevitably develop diffusion gradients of O_2, K^+, and other substances across the muscle, and the apparent mechanisms of fatigue are likely to be biased towards these mechanisms. Barclay (32) has calculated the diffusion gradient of O_2 across isolated mouse or rat muscles and concluded that an anoxic core is likely to develop whenever these preparations are repetitively activated. For instance, a whole mouse soleus can only contract at a duty cycle of 0.5 for ~60 s at 20°C before an anoxic core develops. At 35°C, the anoxic core develops after only 12 s. K^+ is released by active muscle fibers and accumulates in the extracellular space. It will therefore accumulate until a diffusion gradient develops sufficient to allow K^+ to diffuse out of the preparation. Consequently, the concentration of extracellular K^+ will be substantially higher in the center of the muscle than in the perfusate. CO_2 and H^+ and lactate will all tend to accumulate in the extracellular space in the same way. The combination of these issues makes analysis of mechanisms of fatigue in isolated whole muscles problematic. The relative contribution of extracellular accumulation of K^+ or lactic acid or hypoxia was recently studied in isolated mouse muscle fatigued by repeated tetani, and under these experimental conditions, hypoxia was the most important factor (493).

Single fibers dissected from whole muscles eliminate the problems of O_2 supply and the extracellular gradients of K^+ and other products. In addition, there is only one fiber type under investigation, although identifying the fiber type is not trivial. An important additional advantage is the ease of performing fluorescent studies on single fibers. One disadvantage of this preparation is the considerable difficulty of learning the dissection. The absence of changes in O_2 and K^+ can also be viewed as a disadvantage, since in the intact muscle changes in these substances clearly do occur and may contribute to fatigue. Typically single fibers are stimulated with plate electrodes so that the AP is simultaneously generated at multiple points along the fiber, eliminating longitudinal transmission of the AP as a possible mechanism of fatigue. Prolonged high-frequency stimulation applied focally to only one region of a muscle fiber produces greater and faster loss of force than does stimulation applied all along the length of the fiber via parallel electrodes (80, 265), which may indicate that failure of longitudinal AP transmission is an important fatigue mechanism in this situation. However, this force loss may reflect methodological problems with focal electrical stimulation in in vitro experiments, and in our view, this type of failure probably does not normally occur in vivo in healthy humans (see sect. VI) (see Table 1).

Single fibers do not survive well at 37°C or above (267), for reasons which are not entirely clear but seem to involve production of reactive oxygen species (315, 447). Thus a great deal of mammalian fatigue has been studied at unphysiological temperatures, an issue which is assuming increasing importance (see sect. V*B*).

Skinned fibers in which the surface membrane has been removed, either chemically or mechanically, have been widely used to study fatigue. Their main advantage is that the intracellular solution can be completely specified, and it is possible to examine the effects of changing individual metabolites rather than the range of changes that occur in intact fatiguing preparations. Traditionally skinned fibers were used to examine the response of the contractile proteins characterized by the $[Ca^{2+}]$-force relation. However, they can also be used to study SR Ca^{2+} release and uptake using the contractile proteins or an introduced $[Ca^{2+}]$ indicator as a detector. Recently, it has been demonstrated that APs can be generated in the sealed off t tubules of mechanically skinned fibers (361), which means that the voltage sensors and the SR Ca^{2+} release channel and their interaction are amenable to experimental study. Disadvantages of skinned fibers are that it can be unclear whether a protein is present or absent in the preparation and the loss of soluble kinases, for instance, may have important effects on muscle function. In addition, metabolites whose importance has not been recognized by other means are generally not studied.

A great many subcellular components have also been studied as part of attempts to understand muscle function during fatigue. For instance, SR vesicles have been isolated from muscles in an attempt to understand the role of changing SR function in fatigue. Pump function of isolated SR vesicles can be determined, and in principle, such methods can be used to study how changing metabolites affect function or, by using standard conditions, how SR function has been changed by the fatigue process,

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Table 1. *Advantages and disadvantages of various approaches to the study of fatigue*

Muscle in vivo 	Advantages	All physiological mechanisms present Fatigue can be central or peripheral All types of fatigue can be studied Stimulation patterns appropriate for fiber types and stage of fatigue
	Disadvantages	Mixture of fiber types Complex activation patterns Produces correlative data; hard to identify mechanisms Experimental interventions very limited
Isolated muscle 	Advantages	Central fatigue eliminated Dissection simple
	Disadvantages	Mixture of fiber types Inevitable extracellular gradients of O_2, CO_2, K^+, lactic acid Mechanisms of fatigue biased by presence of extracellular gradients Drugs cannot be applied rapidly because of diffusion gradients
Isolated single fiber	Advantages	Only one fiber type present Force and other changes (ionic, metabolic) can be unequivocally correlated Fluorescent measurements of ions, metabolites, membrane potential, etc. possible Easy and rapid application of extracellular drugs, ions, metabolites, etc.
	Disadvantages	Dissection difficult Environment different to in vivo K^+ accumulation and other in vivo changes absent Prone to damage at physiological temperatures Small size makes analysis of metabolites difficult
Skinned fiber	Advantages	Precise solutions can be applied Possible to study myofibrillar properties, SR release and uptake, AP/Ca^{2+} release coupling Metabolic and ionic changes associated with fatigue can be studied in isolation
	Disadvantages	Relevance to fatigue can be questionable May lose important intracellular constituents Relevant metabolites to study must be identified in other systems

assuming that the change is unaffected by isolation. SR release is difficult to study by such methods because the methods of stimulation of release (Ag^+, caffeine) produce a rate of release slower by several orders of magnitude than the physiological mechanism. As in other areas of biology, studies of isolated elements of a cell can be highly effective at defining mechanisms but are generally incapable of demonstrating physiological relevance.

B. Muscle Temperature

Several of the mechanisms that contribute to fatigue (e.g., the effects of intracellular pH and P_i on contractile proteins, reactive oxygen species production) are temperature sensitive (see sects. VII, *A* and *B*, and VIII*A*). Furthermore, fatigue or decline in performance may occur more rapidly at high temperatures compared with low (121, 131, 315). For these reasons, it is important to know the temperature at which muscles normally operate. Muscle temperature is dependent on many factors including activity, blood flow, core temperature, closeness to body surface, and environmental temperature. To eliminate this variability, it is common in studies of peripheral muscles in humans, e.g., adductor pollicis, to preheat the hand in a water bath at 45°C for 20 min, which sets the temperature of a superficial muscle to 37°C (121). The same approach can be used to set peripheral muscle temperature to any chosen temperature between 22 and 37°C (121). If whole body cooling is used to reduce both core and muscle temperature, muscle temperature may start as low as 30°C and increase to 38°C over a 35-min period of activity. This increase arises as a consequence of increased blood flow, increased core temperature, and heat generation by the muscle (58). Maximum muscle activity alone, in the absence of blood flow, causes a temperature rise of ~0.4°C/min (153). Studies in the mouse have shown that the temperature in the subcutaneous space of the foot was ~30°C (71); presumably during activity the muscles will warm up so that, as in humans, the range of temperature over which a muscle operates during a period of activity may be considerable.

In individuals performing intense activity in hot environmental conditions, it is widely appreciated that ex-

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ercise performance declines for a variety of reasons that include muscle temperature, core temperature, and increased competition for blood flow to the muscle (131). Heat exhaustion in humans seems to occur at a relatively constant core temperature of ~40°C (189), and despite a muscle temperature of 40.8°C (189), the failure of muscle performance in hyperthermia appears to be mainly central (439).

VI. EXCITABILITY AND EXTRACELLULAR K^+ ACCUMULATION

A. Basis of Excitability

1. Spread of excitation

As mentioned in section II, normal muscle contraction depends on electrical excitation of the muscle fiber. Provided the muscle fiber is properly polarized, neuromuscular transmission initiates an AP, which propagates very rapidly along the muscle fiber surface (sarcolemma) in both directions (at ~2 to 6 m/s in humans) (57) and then much more slowly (<0.3 m/s) (325) throughout the t-tubular system. The rapid sarcolemmal propagation is necessary to synchronously activate all parts of the muscle fiber to produce a useful contraction, and the conduction in the tubular system can be much slower because of the small distances involved (fiber diameter typically <100 μm). It is necessary to have AP propagation in tubular system because passive spread of surface excitation into the T system is not enough to properly activate the voltage sensors and trigger Ca^{2+} release and contraction (5, 47, 148, 479). Most of the t-tubular system is oriented transversely, but some longitudinally oriented tubules are also present (172, 273). APs can also spread longitudinally inside the muscle fibers via these connections (253, 361), which helps ensure APs normally reach and stimulate every part of the t-tubular system even if conduction in some t tubules is hindered. This may explain why the formation of vacuoles (localized enlargements) in the t-tubular system during exercise does not seem to have any major deleterious effect on force production in most circumstances (262, 271).

2. Types and distribution of ion channels

To understand the changes in excitability that occur with activity, it is necessary to consider the types and locations of the various ion channels involved. In adult muscle, the sodium current is carried by a rapidly activating and inactivating voltage-dependent channel (Nav1.4) (233). The density of these Na^+ channels is higher in fast-twitch than in slow-twitch muscle and is approximately threefold higher near the neuromuscular junction than at the ends of the fiber (384), presumably to provide a safety factor for AP initiation there. Although the total number of Na^+ channels on the sarcolemma is similar to that in the T system (224), the fivefold larger membrane area of the T system (138, 178) means that the Na^+ channel density in the t tubules is only ~20% of the surface density. In broad agreement, modeling of the AP in frog muscle indicated that the limiting Na^+ conductance in the T system is ~5% of that of the sarcolemma (5). The large Na^+ current at the sarcolemma enables fast conduction and increases the likelihood of initiating an AP in each t tubule, where the relatively small tubular Na^+ current is normally adequate for propagation (253).

Most of the resting K^+ conductance is due to the K^+ inward rectifier channels (Kir2.1), which are present at higher density in the T system than at the sarcolemma in mammalian muscle (249). The most common potassium channel on the sarcolemma is the ATP-sensitive K^+ channel (K_{ATP} or Kir6.2) (417); these channels are also found in the T system but at a lower density (331). There are also Ca^{2+}-activated large conductance K^+ channels (referred to as B_K or $K_{Ca}1.1$) at approximately equal density in the sarcolemma and T system (331).

Importantly, normal adult skeletal muscle has a high density of the ClC1 chloride channel (233). In mammalian muscle at rest, including in humans, the Cl^- permeability is approximately four to five times higher than the K^+ permeability (62, 137, 171), and in frog muscle it is approximately two times higher (213). Most of this high Cl^- permeability arises from the T system (100, 137).

3. Importance of membrane potential, Na^+ gradient, and leak conductances

Muscle fibers have to be well polarized to get adequate voltage-sensor activation of Ca^{2+} release. This is because chronic depolarization interferes with two key processes. First, the voltage-dependent Na^+ channels become dysfunctional because of both increased "slow inactivation" (over the course of minutes) (385, 386) and "fast inactivation" (165), and they also require a greater depolarization to be opened (165). Second, the voltage sensors themselves become unresponsive (inactivated) (4, 378) so that even direct depolarization of the T system cannot activate full Ca^{2+} release (139, 378). A large proportion of the Na^+ channels have to be inactivated for there to be complete AP failure, which occurs with prolonged depolarization to potentials less negative than approximately −60 to −55 mV in rat muscle (375) and approximately −50 mV in frog muscle (265). The combined effect of voltage-sensor inactivation and AP failure reduces peak tetanic force in mammalian fibers by ~25% at −60 mV (82), due mostly to voltage-sensor inactivation (93, 352), and fully abolishes responses at approximately −55 mV, due to AP failure.

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AP failure can occur at more polarized potentials if the electrochemical gradient for Na^+ is reduced, either by a decrease in external [Na^+], as might occur in the T system during intense repetitive activation (47, 80, 227, 265), or by a rise in intracellular [Na^+] (335). AP failure can also occur if there is an increase in the permeability of the surface or T-system membranes to K^+ or Cl^-, because an AP can only propagate if the inward Na^+ current exceeds the leak currents carried by K^+ and Cl^- enough to depolarize the adjacent membrane to threshold (423a). For example, repeated stimulation of a muscle fiber (166, 167, 193) can lower intracellular [ATP] sufficiently to activate ATP-sensitive K^+ channels (K_{ATP}) (116, 417) and greatly increase the K^+ leak current, possibly reducing T-system excitability, even though the fiber is well polarized. As discussed later, there are a number of situations where K^+ and Cl^- permeabilities may be modulated, either upwards or downwards, to decrease or enhance membrane excitability, respectively.

B. Alterations in Excitability

1. Changes in electrochemical gradients with activity

There is a large body of literature showing that repeated activation of a muscle causes net K^+ efflux (95, 212), increased extracellular [K^+] ($[K^+]_o$) close to the muscle (232), and decreased intracellular [K^+] ($[K^+]_i$) (403). This is due to the efflux of K^+ occurring with each AP. In rested muscle cells, there is ~160 mM K^+ intracellularly and ~4 mM extracellularly (399). Although only a very small amount of K^+ leaves the muscle cell on each AP (~2 and 10 μM in slow- and fast-twitch mammalian muscle, respectively) (95, 399), repeated activity can increase the extracellular [K^+] near the muscle fibers considerably, with it reaching close to ~9 mM in well-perfused muscle working at high intensity and possibly exceeding 10 mM in localized regions (399). The problem of K^+ accumulation is potentially much greater inside the t tubules (95, 168, 265, 399), because they have ~80% of the total membrane surface area but their volume is only ~1% of the total fiber volume (138, 178).

2. Failure of excitation in in vitro preparations and in vivo with imposed stimulation

In some circumstances, the changes in the electrochemical gradients for K^+ occurring with repeated activation cause substantial membrane depolarization, failure of excitation, and a reduction in force responses. For example, repeated stimulation of whole muscles in vitro for 5–10 min results in a decrease of ~30% or more in $[K^+]_i$ and a depolarization of 10–18 mV in rat fast- and slow-twitch fibers and frog fibers (25, 230). The relatively rapid recovery afterwards of $[K^+]_i$, membrane potential, and some force suggested that part (<30%) of the observed fatigue may have been attributable to inadequate excitation due to the [K^+] changes (25, 438). Similarly, continuous 60-Hz stimulation of rat fast-twitch muscles in vitro caused considerable K^+ efflux from the muscles (and Na^+ influx), with force declining ~80% in 30 s in tandem with a similar decline in the compound surface AP (M-wave) (96). These findings indicate that in certain in vitro situations with imposed stimulation regimes, activity-induced changes in ion concentrations, particularly that of K^+, are not adequately compensated for by activity of the Na^+-K^+ pumps and other means and lead to reductions in muscle excitability and force.

Other experiments have shown that imposing continuous high-frequency stimulation on muscles in vivo in humans caused force to decline rapidly (227) and that this was associated with a reduction in the surface AP (52). Both force and the compound AP rapidly recovered substantially when the rate of stimulation was reduced (52, 227), suggesting that the force reduction was due to failure of excitation arising from the associated [K^+] changes and membrane depolarization.

In isolated single fibers in vitro, continuous high-frequency stimulation has also been shown to cause rapid force decline, fiber depolarization, and reductions in the intracellularly recorded AP (266). It was further observed that as tetanus duration increased, the cytoplasmic [Ca^{2+}] decreased in the middle of the fiber compared with the outer regions, indicating AP failure in the T system (148, 479). As force and cytoplasmic [Ca^{2+}] recovered when the stimulation frequency was reduced, the failure with high frequency of stimulation was likely due to fiber depolarization caused by excessive K^+ accumulation in the T system, and possibly also to an additional effect on AP propagation caused by Na^+ depletion in the T system. Voltage sensor inactivation probably did not contribute substantially to the reduced Ca^{2+} release, because experiments in muscle fiber bundles with comparable stimulation demonstrated that depolarization of the T system with high [K^+] solutions could still elicit a maximal response (81). Other findings in skinned fibers indicate that if a fiber is depolarized sufficiently to affect AP conduction in the T system, it interferes first with AP repriming, such that a single AP is able to propagate into and throughout the T system and elicit force, but subsequent closely spaced APs in a train cannot (147). Such AP failure is also indicated by findings in whole muscles in vitro where following repeated stimulation the response to tetanic stimulation was virtually no bigger than the twitch response to a single AP, and when the muscle was rested briefly, the response to tetanic stimulation recovered rapidly and proportionately much more than did the twitch response (438).

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When the single fibers were subjected to intermittent rather than continuous stimulation, fatigue occurred much more slowly, and excitation-induced Ca^{2+} release was uniform through the fiber (148, 475, 479), indicating AP conduction in the T system. Some studies in frog single fibers, however, found evidence of T-system AP failure even with intermittent stimulation when tested at short muscle lengths (152, 180).

In summary, in certain circumstances AP-induced K^+ movements do appear to reduce muscle excitability and force responses, such as when isolated muscles in vitro are given intense or prolonged stimulation or when all the motor units in a muscle in vivo are stimulated at a relatively high frequency. The occurrence of excitation failure depends greatly on the pattern and frequency of the imposed stimulation and is most marked with continuous high-frequency stimulation.

3. Effects on excitability of raising [K^+] in vitro

It is also well-known that raising the [K^+] in the solution bathing a muscle in vitro can depolarize the fibers and depress excitability and force responses in a graded manner (82, 93, 213, 339, 341, 374), with effects being apparent above ~7 mM K^+ and with complete failure of tetanic force occurring at ~12–13 mM K^+. This depression of excitability and force can be reversed in many circumstances by stimulation of the Na^+-K^+ pump (95, 333, 340). Importantly, these depressive effects of raised [K^+] in the whole muscle experiments take tens of minutes to reach steady state (82). This is not simply due to the delay in K^+ diffusion through the muscle but also reflects the very important role of Cl^- in muscle excitability. As detailed above, the sarcolemma and T-system membranes are more permeable to Cl^- than to K^+, and consequently, even though in the long term Cl^- will passively redistribute across the muscle membrane in accordance with the membrane potential determined largely by K^+ (62), in the short term the depolarizing effect of any increase in extracellular [K^+] is greatly attenuated by the polarizing effects of Cl^- movement (83, 136, 213). Any consideration of the effects of activity-induced ion fluxes in muscle fibers in vivo needs to take in account *1*) the magnitude and locality of the fluxes through the different ion channels and pumps; *2*) the prevailing electrochemical gradients for Na^+, K^+, and also Cl^-; and *3*) the intensity and duration of the exercise or stimulation. The lack of effect of $[K^+]_o$ changes in the short term is likely to be indicative of what happens in vivo early in exercise, and the depressive effect seen in the longer term is possibly more relevant to the effects of very prolonged exercise when $[Cl^-]_i$ may also change and the fibers become substantially depolarized.

4. Does reduced excitability cause muscle fatigue during normal exercise?

The key question then is whether or to what extent muscle excitability decreases in exercising muscles in vivo. A number of studies have examined what happens to the compound surface AP (M-wave) over the course of fatiguing exercising in humans. With continuous maximal voluntary contraction (MVC) of the adductor pollicis muscle for 60 s, force declined by ~40–60%, but the M-wave evoked by a single supramaximal stimulus was increased not decreased (52), indicating that excitation had not failed, at least not at the sarcolemma. It was also shown that direct stimulation of the muscle at various times points produced the same force as voluntary contraction (51). Sustained contraction of the quadriceps at 30% MVC for 3 min, sufficient to reduce twitch response by >50% and raise venous plasma [K^+] from 4 to 6 mM, also caused no change in the M-wave (461). Similarly, with repeated intermittent contractions of the adductor polis muscle at 50% MVC until exhaustion (~5 min), there was no reduction in the M-wave, even though MVC and the evoked force response to 50 Hz direct stimulation were both decreased by ~50% and the twitch response was decreased by ~70% (22, 48, 49). A recent study with cycling exercise also found no change in the M-wave at the point of fatigue (394), though some other studies from the same group have observed some decrement. Thus reduced sarcolemma excitability was not responsible for the muscle fatigue occurring during the exercise in humans in the above cases.

Furthermore, findings of in vitro experiments (see sect. VI*B2*) suggest that excitability problems in the T system would be expected to reduce tetanic force proportionately more than twitch force, but this is not what is observed in exercising humans. In view of this, it seems that the muscle fatigue experienced by the subjects in these exercise studies was not due to problems with excitation of either the sarcolemma or T system but instead caused by "metabolic" changes occurring inside the muscle fibers. As discussed in section VII, the relatively reduced twitch response is likely to be due to reduction in Ca^{2+} release from the SR and changes in Ca^{2+} sensitivity and maximum force production of the contractile apparatus ($F_{Ca,max}$).

C. Factors Helping to Prevent Loss of Excitation During Normal Exercise

The lack of excitability problems during normal exercise may seem surprising. However, there are a large number of factors acting in concert to help reduce or prevent loss of muscle excitation during normal exercise in vivo. These work in large part by reducing the extent of the $[K^+]_o$ increase or its depolarizing effect. Some of

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these mechanisms aid in limiting the number of APs that a fiber undergoes, whereas others involve utilizing the particular ion pathways present in a muscle fiber.

1. Motor unit recruitment

During submaximal force production in vivo, the central nervous system can vary the motor units activated, so as to spread the work load across the pool of motor units and hence reduce the requirements on a given motor unit (157). Less fatiguable motor units can be utilized at lower work loads, and the highest power and most fatiguable units recruited only during very demanding tasks (48). Recruitment of additional motor units explains the increase in EMG occurring when subjects sustain force at a given submaximal level. In fact, the discharge rate of originally active motor units can decrease during prolonged submaximal contractions, despite the fact the overall excitatory drive to the motor neuron pool increases as fatigue develops (181).

2. Activation at optimal firing rate

Fast-twitch and slow-twitch motor units are normally activated in vivo at the firing rate most appropriate to the contractile properties of the motor unit (e.g., at ~30 and 10 Hz, respectively, in humans), such that it is just sufficient to give a fused tetanus of close to maximum force (42, 53). This keeps the number of APs used to excite a muscle fiber to the minimum necessary. Stimulating motor units at higher than their optimal firing rate, as may happen with imposed nerve or muscle stimulation, causes the muscle fibers to fatigue more rapidly than during voluntary contraction (227).

3. Decreasing firing rate

Motor neurons decrease their firing rate during sustained maximal contractions (50). This reduction is normally well-matched to the slowing of relaxation that occurs with muscle activation (see sect. XI), such that the stimulation rate remains just sufficient for eliciting the greatest tetanic force possible at that point in time (28, 51, 53). This phenomenon is often called "muscle wisdom" (157, 179). In addition, impulse trains in vivo typically begin with groups of closely spaced impulses (doublets), which appear to enable force production with less associated fatigue (54), likely because of the force potentiation occurring with the closely spaced impulses (1), which gives more effective force production for a given number of APs.

4. Sarcolemma AP changes

Repeated activation can slow the propagation of the surface AP (231), reduce its size, and increase its duration (28, 265), presumably owing to fiber depolarization. Nevertheless, these changes to the surface AP do not appear to contribute to fatigue (28), as the AP evidently remains sufficient to initiate an AP in the T system because of the large safety factor in the process. The changes in the sarcolemmal properties and AP can also lead to intermittent failure in a train of APs (28, 80), but the accompanying slowing of fusion frequency means that such AP failure does not evidently contribute to decreased force.

5. Na^+-K^+ pumping

The Na^+-K^+ pump plays a vital role in lowering $[K^+]_o$ (95), particularly in the T system. Na^+-K^+ pump activity is stimulated by muscle excitation, raised intracellular $[Na^+]$, β-adrenergic stimulation, and raised temperature (335, 341, 350) and can be increased more than 20-fold above its resting rate. In rat fast-twitch muscle, ~50% of all the α_2-isoform of the pump are in the T system and are estimated to be able to clear K^+ from there at ~4 mM/s (334). In addition, activity of the pump is electrogenic and can hyperpolarize a muscle fiber a further ~8 mV (95).

6. Important role of Cl^- channels

The high resting permeability of the T system to chloride relative to potassium (P_{Cl}/P_K ~4) (100, 137) means that the resting potential (E_M) will be heavily weighted towards the chloride equilibrium potential (E_{Cl}) (83, 213), which will remain relatively constant during activity owing to the high $[Cl^-]$ in the T system (~110 mM) and relatively large amount of Cl^- influx needed to change the $[Cl^-]_i$. For this reason, any inward flux of Cl^- during the repolarizing phase of an AP has much less effect on steady-state conditions than would a matching outward flux of K^+ (62, 137).

Importantly too, the high Cl^- permeability of the T system likely aids in recovering some of the K^+ that does build-up in the T system, because if the $[K^+]$ in the T system increases sufficiently such that E_K across the T-system membrane is less negative than E_M, K^+ will be driven by their electrochemical gradient back into the fiber through the inward rectifier channels. The importance of this effect has been indicated by quantitative modeling (454), though it should be noted that the Wallinga et al. (454) study considerably underestimated the magnitude of the effect as it assumed the T-system chloride conductance was almost 10-fold lower than its true value (62, 100, 137). Thus the high chloride conductance in the T system may not only reduce the rate of accumulation of K^+ there, but also help the recovery of K^+, greatly reducing the problem of K^+ movements on membrane potential and excitability.

The importance of the Cl^- conductance in mammalian muscle is apparent from the problems that occur with muscle function when it is absent (3, 62, 445). Individuals

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with generalized (or Bekker's) myotonia, where there is no muscle Cl^- conductance, are unable to maintain a 60% MVC, and force drops within a few seconds, associated with progressive then complete failure of the sarcolemma AP in most fibers. This effect likely occurs because the high frequency of APs causes a rapid build-up of K^+ in the T system, which depolarizes the fiber as a whole, and causes sarcolemma AP failure (445). Interestingly, the subjects are able to subsequently increase force output over the following 20–60 s to close to 60% MVC again ("warm-up" phenomenon), which appears to be due to the reduced rate of AP stimulation of the muscle occurring at those later times (445).

7. Alterations in leak conductances

If fibers do become depolarized with repeated activity, progressively more of the Na^+ channels will become inactivated. This effect can be beneficial, because the smaller Na^+ influx will mean less K^+ and Cl^- flux is needed for repolarization. However, AP propagation will fail if the Na^+ current is not large enough to overcome the resting leak current, which is carried primarily by Cl^- in mammalian muscle. To help avoid this problem, muscle fibers have mechanisms for reducing the Cl^- leak conductance during activity. Increasing intracellular acidity (e.g., to pH 6.6), as can occur during heavy exercise, reduces the resting Cl^- conductance approximately twofold, enabling APs to continue to generate large tetanic force even when a fiber is depolarized to close to −60 mV (333, 351, 352).

8. Ca^{2+} release feedback

A further factor helping maintain Ca^{2+} release is that the amount of Ca^{2+} released per AP is to some extent kept constant by feedback mechanisms operating on the AP-Ca^{2+} release coupling. The amount of Ca^{2+} release triggered by an AP remains almost constant even if the amount of Ca^{2+} in the SR is increased greatly above its normal endogenous level (360) (see sect. IX*B* and Fig. 5).This occurs because the Ca^{2+} released into the narrow junctional gap *1*) causes Ca^{2+}-dependent inactivation of the SR release channel (35, 346, 398), *2*) speeds the deactivation of the adjacent voltage sensors (345), and *3*) shortens the duration of the AP by activating the Ca^{2+}-activated K^+ channels and possibly Ca^{2+}-activated Cl^- channels in the T system (345). These three factors normally limit Ca^{2+} release (360). Consequently, if the T-system AP becomes smaller and/or the voltage sensors become less activated, resulting in a drop in the rate of SR Ca^{2+} release, the above inhibitory effects will be slower in onset, thus helping keep up the total amount of Ca^{2+} release per AP.

In summary, although many in vitro and in vivo studies show that a loss of muscle excitability can occur with imposed stimulation, this does not usually appear to be the cause of fatigue in exercising humans. This is probably because many compensatory processes act together to prevent changes in $[K^+]$ and fiber depolarization or to reduce their deleterious effects.

VII. METABOLIC CHANGES AND FATIGUE

During locomotion and most other types of muscle activity, muscles are activated with repeated short bursts of APs. In the following section of this review we will therefore focus on models where fatigue has been induced by repeated short contractions. In fast-twitch fibers, this type of fatiguing stimulation results in a characteristic pattern of changes in force and $[Ca^{2+}]_i$. Initially, there is a fast decline of tetanic force by 10–20% that is accompanied by an increase in tetanic $[Ca^{2+}]_i$ (phase 1), then follows a period of relatively constant tetanic force (phase 2); finally, there is rapid decline of both tetanic force and $[Ca^{2+}]_i$ (phase 3) (Figs. 1 and 3*A*). The importance of the decline of tetanic $[Ca^{2+}]_i$ in the phase 3 decline of force has been established by rapid application of caffeine at concentrations that increase the opening of the SR Ca^{2+} channel (149, 268, 462). Caffeine can cause recovery of the tetanic $[Ca^{2+}]_i$ in phase 3 and reverse much of the final phase of force decline. However, this effect has not yet convincingly been demonstrated in intact, perfused muscles (218).

Interestingly, the pattern of force decline is similar in frog, mouse, and rat fast-twitch single fibers (9, 283, 287, 420, 462). A similar pattern of force decline was also described when single fast-twitch motor units were stimulated in anesthetized cats (75). The pattern with an initial increase in tetanic $[Ca^{2+}]_i$ followed by a decline as fatiguing stimulation continues has also been observed in isolated whole mouse extensor digitorum longus (EDL) muscles (203), whereas tetanic $[Ca^{2+}]_i$ decreased monotonically in bullfrog semitendinosus muscles (23).

The duration of phase 2 (i.e., the period with stable force production) differs markedly between cells, which seems to reflect differences in the ability to use oxidative metabolism, because it is markedly shortened by mitochondrial inhibition with cyanide (Fig. 1) (268, 462) or exposure to decreased oxygen pressure (420). Whole muscles generally do not show a clear phase 2, which probably reflects differences in fatigue resistance among the individual fibers within the muscle.

In the experiments described above, the stimulation frequency during the tetani was set close to that required to produce maximal tetanic force. This indicates that the initial force decrease during phase 1, where tetanic $[Ca^{2+}]_i$ actually increases, is caused by a reduction in cross-bridge force-generating capacity. Conversely, the final rapid force decrease during phase 3 would be due to

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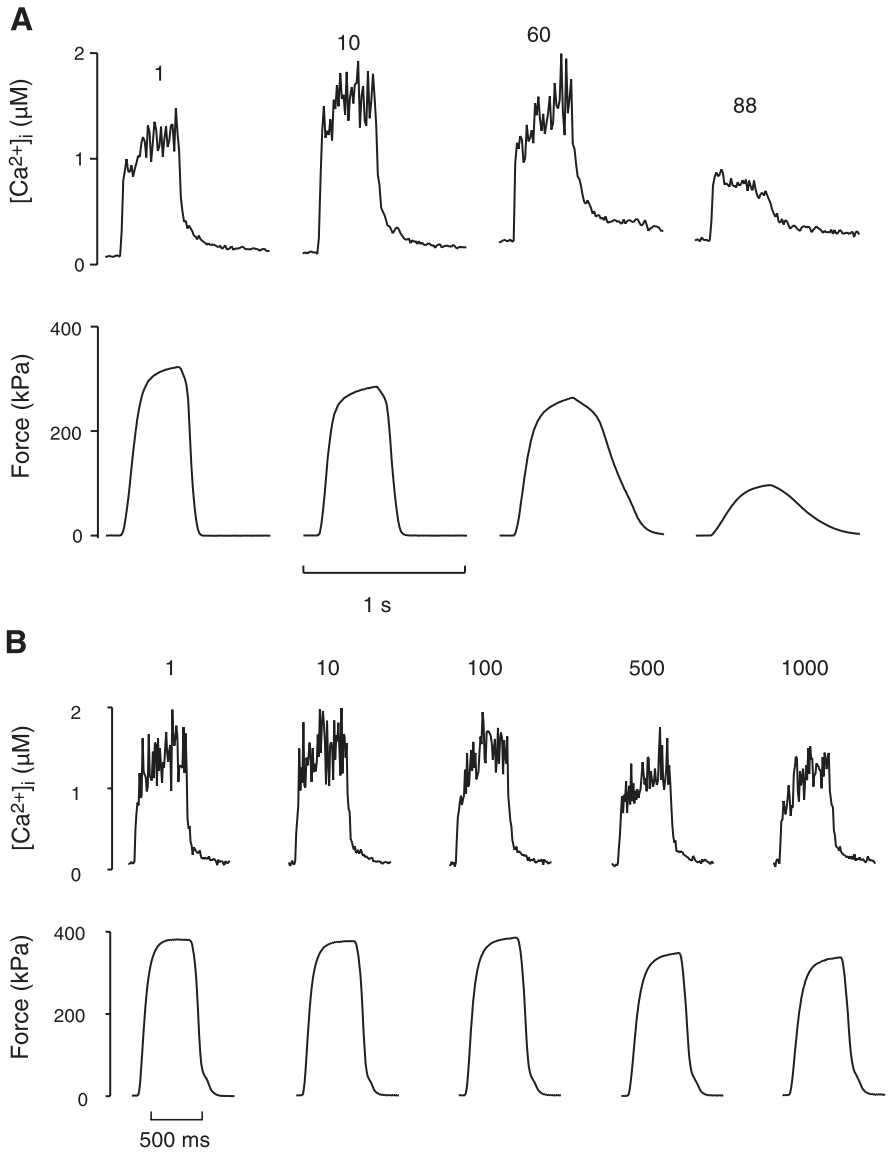

FIG. 3. Tetanic $[Ca^{2+}]_i$ and force records at various phases of fatigue obtained in a fast-twitch (*A*) and a slow-twitch (*B*) mouse fiber. Numbers above $[Ca^{2+}]_i$ records indicate order of tetani. The fast-twitch FDB fiber in *A* shows the normal pattern of easily fatigued fibers: an early increase in tetanic $[Ca^{2+}]_i$ accompanied by ~10% decrease in force (phase 1; tetanus 1–10), followed by a relatively stable period (phase 2; tetanus 10–60), and finally a rapid decrease of tetanic $[Ca^{2+}]_i$ and force (phase 3; tetanus 60–88). Stimulation protocol: 350-ms, 70-Hz tetani given at 2.5-s intervals. The fatigue-resistant soleus fiber in *B* shows little changes in tetanic $[Ca^{2+}]_i$ and force during a markedly more demanding fatiguing stimulation protocol [a total of 1,000 tetani (500 ms, 70 Hz) given at 2-s intervals]. [*A* from Dahlstedt et al. (107); *B* from Bruton et al. (67).]

the combined effect of decreased tetanic $[Ca^{2+}]_i$ (see Fig. 3*A*) and reduced myofibrillar Ca^{2+} sensitivity.

Isolated slow-twitch fibers and motor units are generally highly resistant to fatigue induced by repeated short tetani (67, 75, 188, 250). Changes in tetanic $[Ca^{2+}]_i$ during fatigue of single slow-twitch fibers have been little studied. In mouse soleus fibers, tetanic $[Ca^{2+}]_i$ followed the pattern observed in fast twitch fibers, i.e., an initial increase followed by a decrease (67) (Fig. 3*B*), whereas the early increase in tetanic $[Ca^{2+}]_i$ was not observed in rat soleus fibers (288).

In the following sections we discuss how force is affected by metabolic changes that occur during fatigue. We focus on fast-twitch muscle fibers where metabolic changes generally are larger and the force decrease more marked.

A. Inorganic Phosphate

The exchange of phosphate between ATP and PCr is catalyzed by creatine kinase (CK) according to the following reaction: PCr + ADP + H^+ → Cr (creatine) + ATP. During periods of high energy demand, the ATP concentration initially remains almost constant while CrP breaks down to Cr and P_i. While Cr has little effect on contractile function (321), P_i may cause a marked decrease of myofibrillar force production and Ca^{2+} sensitivity as well as SR Ca^{2+} release. Accordingly, increased P_i is considered to be a major cause of fatigue (470). A causative role of increased P_i has also been implied in other situations with impaired muscle function. For instance, a recent study where human subjects were followed during the rehabilitation after cast immobilization shows a significant in-

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verse relationship between resting P_i and specific force production (349).

1. P_i and cross-bridge force production

According to present models of cross-bridge force production, the myosin head is first bound weakly and then strongly to the actin filament. Thereafter P_i is released, possibly resulting in a further increase in force production (433). This implies that the transition to high-force cross-bridge states is inhibited by increased P_i, and fewer cross bridges would be in high-force states when P_i increases during fatiguing stimulation. In line with this, experiments on skinned fibers consistently show a substantial decrease of $F_{Ca,max}$ in the presence of elevated P_i (314, 348). Mammalian skinned fiber experiments are generally performed at temperatures much lower than those prevailing in vivo, and the P_i-induced inhibition of cross-bridge force production becomes less marked as temperature is increased (101, 112, 123).

The effect of P_i on cross-bridge force production has been difficult to directly test in intact muscle cells, because it has proven difficult to alter myoplasmic P_i without imposing other metabolic changes as well. An experimental model that can be used in this regard is genetically modified mice that completely lack CK in their skeletal muscles ($CK^{-/-}$ mice) (422). Skeletal muscle fibers of $CK^{-/-}$ mice display an increased myoplasmic P_i concentration at rest, and there is no significant P_i accumulation during fatigue (422). The $F_{Ca,max}$ of unfatigued $CK^{-/-}$ fast-twitch fibers is markedly lower than that of wild-type fibers, and this can partly be explained by a P_i-induced depression of cross-bridge force production (106). Furthermore, $CK^{-/-}$ fibers do not display the 10–20% reduction of $F_{Ca,max}$ observed after ~10 fatiguing tetani in fast-twitch fibers (phase 1; see Figs. 1 and 3*A*), which has been ascribed to increased P_i (106, 107). Even after 100 fatiguing tetani, force was not significantly affected in $CK^{-/-}$ fibers, whereas by this time force was reduced to <30% of the original in wild-type fibers. Additional support for a coupling between myoplasmic P_i concentration and force production in intact muscle cells comes from experiments where reduced myoplasmic P_i is associated with increased force production (73, 74, 355).

In conclusion, increased myoplasmic P_i can inhibit force production by direct action on cross-bridge function, and this is a likely mechanism underlying the decrease in tetanic force occurring early during fatigue in fast-twitch fibers. The magnitude of this P_i-induced decrease in cross-bridge force production is probably rather small (~10% of maximum force) in mammalian muscle at physiological temperatures.

2. P_i and myofibrillar Ca^{2+} sensitivity

Altered cross-bridge function may also affect the force-$[Ca^{2+}]_i$ relationship via the complex interaction between myosin cross-bridge attachment and thin (actin) filament activation (192), and reduced myofibrillar Ca^{2+} sensitivity is frequently observed in skeletal muscle fatigue (168, 480). Skinned fiber experiments have shown that increased P_i decreases the myofibrillar Ca^{2+} sensitivity (301, 314). Intriguingly, the inhibitory effect of P_i on myofibrillar Ca^{2+} sensitivity was recently shown to be larger at 30 than at 15°C (124), i.e., opposite to the inhibitory effect of P_i on cross-bridge force production at saturating $[Ca^{2+}]$, which is smaller at high temperature (123). Results from unfatigued $CK^{-/-}$ fibers, which display an increased myoplasmic P_i concentration at rest, also indicate a P_i-induced decrease in the myofibrillar Ca^{2+} sensitivity (106).

In summary, a fatigue-induced increase in P_i can reduce myofibrillar Ca^{2+} sensitivity, which may a have large impact on force production in later stages of fatigue where tetanic $[Ca^{2+}]_i$ decreases.

3. P_i and the increase of tetanic $[Ca^{2+}]_i$ in early fatigue

An increase in tetanic $[Ca^{2+}]_i$ is generally observed early during fatigue induced by repeated tetanic stimulation (phase 1, see above). This increase was not observed in $CK^{-/-}$ muscle fibers (106, 107), but reappeared after CK injection into these fibers (105). A similar result has been observed in *Xenopus* fibers when CK was blocked pharmacologically (243). These results indicate a key role of CK and increased P_i in the early increase in tetanic $[Ca^{2+}]_i$. There are several mechanisms by which changes in CK activity and increased P_i can lead to increased tetanic $[Ca^{2+}]_i$. First, increased P_i might reduce the myoplasmic Ca^{2+} buffering by decreased binding of Ca^{2+} to troponin C due to a reduction in strong cross-bridge attachment (314), but this mechanism appears to be of limited importance in skeletal muscle (192). Second, P_i might act on the RyR and increase SR Ca^{2+} release. Accordingly, elevated P_i has been shown to increase the open probability of isolated RyR incorporated into lipid bilayers as well as the rate of Ca^{2+}-induced Ca^{2+} release in SR vesicles and skinned fibers (27, 174). However, it has since been shown that P_i decreases caffeine- and depolarization-induced SR Ca^{2+} release in mechanically skinned rat skeletal muscle fibers in a Mg^{2+}-dependent manner (135). The reason for these conflicting results is not clear but is likely to involve differences in experimental conditions, e.g., mode of SR activation and the presence or absence of proteins associated with the RyR (135). Third, elevated P_i may inhibit (118, 426) or even reverse (133) SR Ca^{2+} pumping which, at least in the short term, might increase tetanic $[Ca^{2+}]_i$ (468). In addi-

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tion, removal of PCr inhibits SR Ca^{2+} uptake in skinned fibers (132), supposedly caused by decreased ATP buffering via the CK reaction leading to a decreased ATP/ADP·P_i ratio in the vicinity of the SR Ca^{2+} pumps (134, 423). An important role of CK and P_i on SR Ca^{2+} pumping is supported by the finding that the early increase in tetanic $[Ca^{2+}]_i$ during fatigue is accompanied by a marked increase in the amplitude of $[Ca^{2+}]_i$ tails during relaxation in normal fibers but not in $CK^{-/-}$ fibers (106).

In conclusion, an operating CK system and P_i accumulation are required for the early increase in tetanic $[Ca^{2+}]_i$ during fatigue, but the exact mechanism(s) involved remains to be established.

4. P_i and the decrease of tetanic $[Ca^{2+}]_i$ in late fatigue

After the initial increase, tetanic $[Ca^{2+}]_i$ decreases as fatiguing stimulation progresses, and this, together with a decreased myofibrillar Ca^{2+} sensitivity, results in the final rapid decrease of tetanic force (phase 3). CK and elevated P_i appear to play a central role in this decrease because it is markedly delayed in fibers with CK inhibition induced either pharmacologically (108) or genetically ($CK^{-/-}$ muscles) (107). Moreover, CK injection into $CK^{-/-}$ fibers resulted in the normal changes in tetanic $[Ca^{2+}]_i$ during fatigue, i.e., an early increase followed by a decrease (105). We will discuss two mechanisms by which elevated P_i may decrease SR Ca^{2+} release: P_i-induced inhibition of the RyR and Ca^{2+}-P_i precipitation in the SR, causing a reduction of the amount of free Ca^{2+} available for release.

Skinned fiber experiments have shown an inhibitory effect of P_i on caffeine- and depolarization-induced SR Ca^{2+} release (135). This inhibition appears distinct from the P_i effect on SR Ca^{2+} pumping and Ca^{2+}-P_i precipitation in the SR and is attributed to a P_i effect on the SR Ca^{2+} release mechanism (135, 423). Interestingly, this P_i-induced inhibition depends on changes in Mg^{2+} within the physiological range (135): the inhibitory effect was markedly larger at the free $[Mg^{2+}]$ of the myoplasm of fatigued (~1.6 mM) than rested (~0.8 mM) mammalian muscle fibers (464). Thus this Mg^{2+}-dependent P_i-induced inhibition of SR Ca^{2+} release is consistent with an inverse relationship between tetanic $[Ca^{2+}]_i$ and $[Mg^{2+}]_i$ during fatigue under normal conditions, which is completely lost after pharmacological inhibition of CK (108).

Ca^{2+}-P_i precipitation in the SR may be an important mechanism behind the decline in tetanic $[Ca^{2+}]_i$ in late stages of fatigue (11, 142, 423). The underlying theory is as follows: the free $[Ca^{2+}]$ in the SR ($[Ca^{2+}]_{SR}$) is assumed to be ~1 mM, and the Ca^{2+}-P_i solubility product, measured in vitro, is ~6 mM^2 (175). When P_i increases in the myoplasm during fatigue, some P_i ions subsequently enter the SR, and when the Ca^{2+}-P_i solubility product is exceeded, precipitation occurs and the $[Ca^{2+}]_{SR}$ decreases (175, 222). This reduction in the releasable pool of Ca^{2+} in the SR can, depending on the initial SR Ca^{2+} load (360), lead to decreased SR Ca^{2+} release.

The occurrence of SR Ca^{2+}-P_i precipitation has not been directly shown, but indirect evidence of its existence has been presented both in experiments on skinned fibers with intact SR exposed to high P_i solutions (135, 142, 175) and in intact mouse fibers injected with P_i (469). In the latter study, P_i was injected into unfatigued mouse fast-twitch muscle fibers, and this resulted in decreased tetanic $[Ca^{2+}]_i$, faster SR Ca^{2+} reuptake, and decreased resting $[Ca^{2+}]_i$, all of which are consistent with a decreased $[Ca^{2+}]_{SR}$. Several fatigue experiments also support the Ca^{2+}-P_i precipitation mechanism. First, $[Ca^{2+}]_i$ in response to a high dose of caffeine or 4-choro-*m*-cresol is decreased in fatigued mouse (462) and toad muscle fibers (234). These compounds directly stimulate the SR Ca^{2+} release channels, and a decreased response indicates a reduced pool of Ca^{2+} available for release. Second, the $[Ca^{2+}]_{SR}$, measured with the low-affinity Ca^{2+} indicator fluo-5N, declined throughout a period of fatiguing stimulation of toad muscle fibers and recovered afterwards (235). The recovery occurred even in the absence of extracellular Ca^{2+}, which shows that Ca^{2+} did not disappear from the cells during fatigue. On the other hand, recovery was blocked by mitochondrial inhibition with cyanide, showing that it depended on aerobic metabolism.

While there are numerous results supporting an important role of Ca^{2+}-P_i precipitation in fatigue, there are also several results that are difficult to reconcile with this mechanism (423). For instance, the marked increase in P_i develops early during fatiguing stimulation, whereas the decrease in tetanic $[Ca^{2+}]_i$ occurs with a delay. Moreover, in mouse fast-twitch fibers, the decline of tetanic $[Ca^{2+}]_i$ temporally correlates with an increase in cytosolic $[Mg^{2+}]$, which presumably stems from a net breakdown of ATP (464), and it is not obvious why Ca^{2+}-P_i precipitation in the SR should show a temporal correlation with increasing $[Mg^{2+}]$/decreasing [ATP]. However, the SR membrane contains small-conductance chloride channels, which may conduct P_i (6, 279). The open probability of these channels increases at low ATP, which fits the finding that P_i entry into the SR of skinned muscle fibers is inhibited by ATP (359), although others did not observe this ATP dependency (142, 423). Other uncertainties regarding the importance of SR Ca^{2+}-P_i precipitation in fatigue include (423) the concentrations of Ca^{2+} and P_i required to cause precipitation might be higher in the functional SR than in simple salt solutions, Ca^{2+}-P_i may exist in many different forms (456), and unstable complexes dissolve rapidly, which means that more Ca^{2+} might become available as the $[Ca^{2+}]_{SR}$ declines during a tetanic contraction.

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In conclusion, while it is clear that increased P_i can cause a decrease in tetanic $[Ca^{2+}]_i$ in late fatigue, the importance of this in different types of fatigue and the exact processes involved require further attention.

5. Creatine supplementation

There is widespread use of extra Cr intake among athletes, not only in the elite but also among people exercising on a recreational level (for recent reviews, see Refs. 44, 437). Cr supplementation is also used in an attempt to improve muscle function in diseases such as inflammatory and mitochondrial myopathies and muscle dystrophies. Cr enters muscle cells via a Na^+-dependent transporter in the sarcolemma. Inside the cell, Cr is phosphorylated by CK, and the [PCr]/[Cr] ratio basically depends on the energy state of the cell. Extra Cr intake increases the total muscle Cr content by up to ~20%. Cr supplementation has a modest positive effect on muscle performance during bouts of short-term (~10 s) high-power exercise, whereas it does not improve function during more long-lasting types of muscle activity (437). This fits with the fact that PCr breakdown contributes to a relatively large fraction of the ATP supply during the first seconds of high-intensity muscle activity, whereas the PCr contribution is minimal during prolonged exercise.

On the muscle cell level, an increased PCr concentration will provide better ATP buffering during intense activity and delay increases in ADP that might slow cross-bridge cycling (99, 473) and SR Ca^{2+} pumping (132, 222). Thus a high power output could be sustained for a slightly longer time. Moreover, PCr/Cr is osmotically active, and short-term Cr supplementation is generally accompanied by increased body weight due to water accumulation in muscle cells (321, 437). The increased water content in muscle cells might improve function by increasing the myofibrillar Ca^{2+} sensitivity and the $F_{Ca,max}$ (321).

In the context of fatigue at the cellular level, it might be expected that increased PCr loading in muscle cells causes a more marked decrease in contractile function during intense activity due to a larger increase in P_i, which is considered to be a major cause of fatigue inhibiting both myofibrillar and SR function (see above). However, it should be noted that cross-bridge force production and myofibrillar Ca^{2+} sensitivity decline linearly with the logarithm of $[P_i]$ (314, 348); in other words, the depressive effect of an increase in P_i is large at the onset of fatigue where $[P_i]$ is low, but it becomes more and more limited as fatigue progresses and $[P_i]$ reaches high levels. The $[P_i]$ dependency on the fatigue-induced changes in SR Ca^{2+} handling is probably complex, and the present understanding of the processes involved is too limited to predict the effect on these processes of increased PCr loading. It should be noted that the beneficial effect of increased PCr is observed in short-lasting bouts of exercise where inhibitory effects of energy metabolites on SR Ca^{2+} release are limited (see above).

B. Lactate and H^+

The accumulation of lactic acid in muscle has historically been suggested to be the major cause of muscle fatigue (see review in Ref. 168). As outlined in section III, lactate and H^+ are produced in muscles during intense exercise (379), and in humans, the intracellular lactate concentration may reach 30 mM or more (expressed relative to cell water) and the intracellular pH (pH_i) decreases by ~0.5 pH units (389). A close temporal relationship is often observed between decreased muscle force and increased intracellular concentrations of lactate and particularly H^+. However, such correlations break down in many cases, and although increased intracellular levels of H^+ may reduce muscle performance to some degree, it now appears that its deleterious effects have been considerably overestimated and that other, beneficial, effects have been overlooked.

1. Lactate

In humans exercising at different work intensities, lactate levels were found not to correlate well with muscle fatigue (240). Addition of lactate to the outside of muscles in vivo (214) or in vitro (411) resulted in some reduction in tetanic force, but a large part of this effect was likely due not to lactate uptake but instead to the increased extracellular osmolarity causing water to move out of the muscle fibers thereby increasing intracellular ionic strength, which has direct inhibitory effects on force production (259). Experiments with skinned muscle fibers at constant ionic strength have shown that lactate, at concentration even up to 50 mM, has relatively little effect on force production by the contractile apparatus, reducing $F_{Ca,max}$ by <5% and having little or no significant effect on Ca^{2+} sensitivity (16, 86, 143, 358). Furthermore, even though lactate has a mild inhibitory effect on direct activation of SR Ca^{2+} release by caffeine or Ca^{2+} (143, 163), skinned fiber experiments with functional EC coupling have shown that voltage-sensor activation of Ca^{2+} release is little if at all affected, and twitch and tetanic forces are virtually unchanged in the presence of 30 mM cytoplasmic lactate (143, 358). It has also been found recently that increases in intracellular lactate concentration do not cause fiber swelling, owing to compensatory effects of intracellular H^+ changes on the number of osmotically active particles (444). Furthermore, the gradual accumulation of extracellular lactate around muscle fibers during exercise in vivo probably reduces any tendency for intracellular lactate to trigger vacuolation of the

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T system (263). In summary, it appears that the intracellular accumulation of lactate per se is not a major factor in muscle fatigue.

2. pH_i and muscle fatigue

In humans, muscle pH_i at rest is ~7.05 and after exhaustive exercise may drop to as low as ~6.5 (389, 413). In other cases, however, pH_i decreases only to ~6.8 or 6.9 at the point of exhaustion (30, 215), showing that muscle fatigue in humans often occurs without there being any large increase in $[H^+]_i$. Similarly, in rat gastrocnemius muscle stimulated in vivo, tetanic force decreased by 60%, even though the pH_i in the muscle observed by NMR declined only to ~6.9 (21). Very similar results were also found when monitoring pH_i with a fluorescent indicator in isolated single fast-twitch mouse fibers subjected to repeated intermittent stimulation (71, 463). Furthermore, humans with myophosphorylase deficiency are unable to utilize muscle glycogen, and their muscles fatigue faster than normal individuals; this accelerated fatigue development occurs without any change in pH_i (78). Thus, clearly, muscle fatigue can be caused by factors other than raised $[H^+]$.

At the onset of exercise or muscle stimulation, pH_i may initially increase by ~0.1 pH units (215, 463) owing to the H^+ consumed during PCr breakdown, and conversely, after exercise ceases, pH_i can decrease a further ~0.1 pH units as PCr is resynthesized (310, 391). Importantly, in cases where pH_i does drop to low levels in a fatigued muscle, upon ceasing the exercise or stimulation, force typically recovers much faster than pH_i (22, 78, 391, 438), indicating that the low pH per se was not responsible for all of the force reduction. Furthermore, attenuating the decline in pH during a stimulation period did not reduce the extent of fatigue in frog muscle fibers (421).

A poor correlation between decreased pH_i and impaired contractile function is also apparent, particularly in mammalian muscle fibers at near physiological temperature, when pH_i is experimentally decreased, for instance by raising CO_2 concentration. Ranatunga (365) found that twitch and tetanic force in rat EDL bundles at 30–35°C were not decreased at acid pH_i(likely ~6.5), but actually potentiated. In cat muscle in vivo at 37°C, decreasing muscle pH_i to 6.3 by hypercapnia reduced maximum tetanic force by only ~5–10% in soleus and biceps brachii muscles (2). In intact single fibers from mouse at 32°C, decreasing pH_i from 7.17 to 6.67 caused only ~10% reduction in maximum tetanic force and no significant slowing of the maximum velocity of shortening (472). In all these cases, decreasing pH_i to the levels seen in fatigued fibers caused relatively little reduction in tetanic force. Similar conclusions have been reached for frog fibers (20, 373).

3. Effects of low pH on SR Ca^{2+} release and voltage sensor activation

Another way in which low pH previously was thought to reduce force responses was by inhibiting Ca^{2+} release from the SR. Low pH does reduce direct activation of the Ca^{2+} release channel to stimulation by Ca^{2+} and caffeine (277, 294, 482); however, voltage-sensor activation of Ca^{2+} release, the normal physiological mechanism, is not noticeably inhibited even at pH 6.2 (256, 258), nor is the activation of the voltage sensors themselves (26). In accord, when pH_i is lowered in intact fibers, maximum tetanic force is reduced by no more than the amount expected from the reduction in $F_{Ca,max}$ of the contractile apparatus occurring at that temperature (20, 364, 466, 472).

4. Effects of low pH on Ca^{2+} binding to TnC and the SR pump

Low pH also reduces the Ca^{2+} sensitivity of the contractile apparatus (130, 160) probably because the H^+ competes with Ca^{2+} binding to troponin C. This is often presumed to have major deleterious effects on muscle performance. However, low pH also reduces the affinity of Ca^{2+} binding at other sites in the muscle fiber, in particular to the SR Ca^{2+} pump. Thus, even though the affinity of troponin C (TnC) for Ca^{2+} may be reduced, the total amount of Ca^{2+} binding to TnC may not decrease. A single AP normally triggers the release of a comparatively large amount of Ca^{2+} from the SR (~20% of the SR Ca^{2+} content or ~230 μM Ca^{2+} expressed relative to total cell volume) (37, 360), and the rise in free $[Ca^{2+}]$ in the cytoplasm is only a very small fraction of this, because most of the Ca^{2+} binds to TnC, the Ca^{2+} pump, and other sites (37, 307, 360). Lowering pH from 7.0 to 6.3 reduces the affinity of the SR Ca^{2+} pump more than fivefold (0.14–0.72 μM) (488), which is considerably greater than the effect on the contractile apparatus (<2-fold decrease in Ca^{2+} affinity) (20, 160). Consequently, the amount of Ca^{2+} available to bind to TnC is likely higher at acid pH. Furthermore, the greatly reduced Ca^{2+} affinity and rate of uptake by the SR Ca^{2+} pump at acid pH (8, 488) leads to an increase in the resting cytoplasmic free $[Ca^{2+}]$ (466), which would help maintain or even increase the extent of resting occupancy of the various cytoplasmic Ca^{2+} binding sites. As a result of all these effects, the free $[Ca^{2+}]$ in the cytoplasm during contraction actually reaches greater levels at acid pH than at normal pH (20, 466), and the force response to a twitch is actually increased (20, 365) and tetanic force rises faster, at least in frog muscle (364).

In summary, under physiological circumstances, low pH has far less inhibitory effects on the activation of the contractile apparatus and Ca^{2+} release than previously assumed, and its effects on the SR Ca^{2+} pump actually favor force development.

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5. Effect of low pH on membrane conductance

Importantly, as mentioned in section VI*C7*, reduced pH_i can have marked beneficial effects on muscle performance by helping maintain excitability. Nielsen and colleagues (200, 333) demonstrated that lowering pH_i to ~6.6 in both fast-twitch and slow-twitch mammalian muscle helped counteract the reduction in excitability occurring with membrane depolarization. This occurred because lowering pH_i reduced the Cl^- conductance of the T-system membrane by approximately twofold, enabling APs to continue to propagate over both the surface and T-system membranes and hence trigger contraction (351, 352). Juel and colleagues (248) confirmed the finding that lowering pH_i, by addition of extracellular lactic acid, helped restore excitability in depolarized mammalian muscle, but they questioned whether this occurs in active muscle because they found that the rate of force decrease during fatigue was unaffected in muscles stimulated in vitro under such conditions. However, in those experiments, the fibers deeper in the muscles would have become anoxic due to the high stimulation rate and limited O_2 diffusion (32), which would have greatly increased the rate of fatigue (493), thereby masking any beneficial effects of low pH_i. Furthermore, it is quite likely that decreasing pH_i over and above that normally occurring during fatigue has no extra beneficial effects on the excitability. Experiments in which lactate was infused into whole animals while the muscles were stimulated to fatigue by nerve or direct muscle stimulation, found that the presence of extracellular lactate, which likely decreased pH_i, reduced failure of sarcolemma excitability and restored force production (239), consistent with an action on Cl^- conductance.

6. Effects of low pH on the rate of fatigue

It has been shown in isolated muscle fibers that lowering pH_i from 7.18 to 6.77 did not decrease either the initial level of tetanic force or the rate at which the muscle fiber fatigued when given repeated stimulation, even though the final pH_i was much lower at the point where force dropped to 40% (pH_i 6.46 versus 6.91 in control case) (71). In fact, it took 98 ± 18 tetani to cause force to drop to 40% in the acidic conditions compared with 63 ± 10 tetani in the control conditions, suggesting that the rate of fatigue if anything was less at the lower pH_i. This effect might in part have been due to improved excitability in the T system (352) or perhaps was mainly due to a pH-dependent decline in the rate of ATP consumption owing to reduced cross-bridge cycling (71). The results also offered no evidence that low pH_i accelerates muscle fatigue by decreasing glycolysis. Similarly, in humans, glycolysis and glycogenolysis are not inhibited by the pH_i decreases occurring in exhaustive exercise (29, 30, 194), and studies with electrical stimulation in humans have shown that even though glycolysis and glycogenolysis are reduced somewhat when pH_i falls from 6.7 to 6.45, significant activity still remains (415).

Finally, there have been many studies examining the effects of manipulating whole body pH on exercise performance, with some albeit not all showing deleterious effects of acidity and beneficial effects of alkalosis (79). However, altering body pH can have effects on blood oxygen saturation and oxygen unloading, cardiac and local vasculature function, central nervous system drive, and other factors. Experiments with a perfused rat hindlimb preparation, where the effects of some of these variables could be controlled, showed that a decrease in blood pH adversely affected muscle performance, but that this was due to some direct effect of the low extracellular pH itself and not of lowered pH_i (414). The accompanying perfusion pressure data indicated that the effect was quite possibly due entirely to the acidity disrupting the normal local control of blood flow in the muscle vascular beds. Similarly, alkalosis in humans can delay the onset of fatigue (409), but such alkalosis actually causes a decrease in extracellular $[K^+]$, and this may have additional effects in aiding membrane excitability. Significantly, when such changes in $[K^+]$ and other effects were avoided in a perfused hindlimb preparation, alkalosis did not improve muscle performance (412). Finally, increasing muscle carnosine by β-alanine supplementation has been found to delay the onset of fatigue in exercising humans, and this was attributed to increased pH buffering by the carnosine (209), but the beneficial effects of the carnosine are quite likely mainly due to it markedly increasing the Ca^{2+} sensitivity of the contractile apparatus (144, 261).

In conclusion, raised $[H^+]_i$ per se is not the main cause of muscle fatigue, with its direct effects on force production being quite small.

C. ATP and Mg^{2+}

The changes in ATP, ADP, IMP, and $[Mg^{2+}]_i$ that occur when consumption of ATP exceeds resynthesis have been described in section III. To summarize, during intense fatigue, taking account of the greater changes in fast fibers (238), ATP may decline from 7 to 1.2 mM and PCr from 30 to 2.5 mM while ADP may increase from 10 to 200 μM, IMP may increase from undetectable to 5 mM, and $[Mg^{2+}]_i$ may increase from 1 to 2 mM or possibly higher. In slow fibers, on the other hand, there is probably little if any decline in cytoplasmic [ATP] (238, 324). As noted in section III, it is possible that in fast fibers the changes in some restricted regions of the cell are even larger than the measured average cytoplasmic changes. In this section we consider the resulting changes in contractile apparatus and EC coupling that may contribute to fatigue.

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1. Contractile apparatus

Maximum force production in skinned fibers is not reduced unless the [ATP] is <20 μM (98), and is actually increased by ~10% at 0.5–1 mM ATP (145, 185) and also by submillimolar ADP and AMP (185). Ca^{2+} sensitivity is not altered by lowering the [ATP] to 0.5 mM, but is slightly increased by submillimolar ADP (145, 185, 296). One early suggestion was that IMP accumulation may cause fatigue (168); however, 3 mM IMP has no effect on maximum force and causes a slight increase in Ca^{2+} sensitivity (56). Increasing [Mg^{2+}] to 3 mM has no effect on maximum force or the velocity of shortening (145) but causes a substantial decrease in Ca^{2+} sensitivity (1.6-fold increase in [Ca^{2+}] required for 50% maximal force) (56). In fast fibers, the combined effect of all these changes is likely to be a slight increase in maximum force but a substantial decrease in the Ca^{2+} sensitivity of force production. These effects are likely additional to those occurring with raised P_i (see sect. VII*A*) and H^+ (see sect. VII*B*).

2. SR Ca^{2+} pumping and leakage

Studies on skinned fibers show that the rate of relaxation of tetani is reduced 2.5-fold when [ATP] is lowered to 0.5 mM (at constant low [ADP]) (144). This is not due to an effect on the contractile apparatus, but instead is likely due to reduced Ca^{2+} uptake by the SR Ca^{2+} pumps. Although the ATP catalytic site on the SR pumps has a very high affinity (K_m ~1–10 μM), ATP also has a regulatory action on the pump, such that if [ATP] decreases from 5 to 0.25 mM, the Ca^{2+} affinity of the pump is reduced ~10-fold together with a drop in the Ca^{2+} binding cooperativity (327, 432). The latter change may enable the ATPase to continue pumping Ca^{2+} despite a considerable decrease in the free energy of ATP hydrolysis, as only one Ca^{2+} is transported for each ATP molecule hydrolyzed, but this means a decrease in the energy efficiency of the process. If the Ca^{2+} affinity of the SR pump is already greatly reduced by acid pH (e.g., pH ~6.3) (488), reducing [ATP] has little if any additional effect (327). In fast-twitch mammalian fibers, a rise in [ADP] from 10 μM to 0.2 mM causes a small reduction in Ca^{2+} pump rate and induces a twofold increase in leakage of Ca^{2+} from the SR via the pump "slippage" (295). Such changes would slow Ca^{2+} uptake and increase resting [Ca^{2+}] in an intact fiber. The increased SR Ca^{2+} leakage is observed even in the presence of 5–8 mM ATP if CrP is decreased to low levels (132, 134, 295), indicating that the local [ADP] near the Ca^{2+} pumps is poorly controlled if CrP is depleted. Interestingly, in slow-twitch fibers, raised [ADP] has only comparatively small effects on the SR pump rate and leakage (296). Finally, Ca^{2+} uptake in fast-twitch fibers is little if at all affected by raising [Mg^{2+}] from 1 to 3 mM (237) or by the presence of 3 mM IMP (55).

In summary, studies on skinned muscle fibers suggest that the decline in [ATP] and [CrP] can reduce SR Ca^{2+} pumping and increase pump leakage, resulting in elevated resting [Ca^{2+}]$_i$ as typically observed in fatigue. Under some conditions, it may also contribute to the slowing of relaxation in fatigued muscle (see sect. XI).

3. SR Ca^{2+} release

The Ca^{2+} release channel in mammalian skeletal muscle (RyR1) is strongly stimulated by ATP binding at a cytoplasmic regulatory site (K_{app} ~0.5 mM), and ADP and AMP act as weak competitive agonists, but IMP does not (278, 305). The Ca^{2+} release channel is also strongly inhibited by cytoplasmic Mg^{2+} (257, 258, 280, 281, 305) and remains closed at the resting [Mg^{2+}] (~1 mM) unless directly activated by the voltage sensors. Voltage sensor-induced Ca^{2+} release is decreased ~40% by raising [Mg^{2+}] from 1 to 3 mM (145). Voltage sensor-induced Ca^{2+} release is also decreased ~20% by lowering [ATP] to 0.5 mM, and the reduction in Ca^{2+} release is larger when raised [Mg^{2+}] and low [ATP] are combined, and larger still if ATP hydrolysis products, AMP or adenosine, are also present (55, 56, 145, 342). Consequently, it seems likely that this combination of factors could substantially inhibit voltage sensor-induced Ca^{2+} release. The inhibitory effects of increased [Mg^{2+}] and reduced [ATP] on Ca^{2+} release likely underlie the decrease in tetanic [Ca^{2+}]$_i$ observed in $CK^{-/-}$ mice at the onset of high-intensity stimulation (107), but it is apparent that they are not the only metabolic factors involved in reducing Ca^{2+} release in fatigue (108) (see sects. VII*B* and VI*D*).

In conclusion, in intense exercise, the triad junction may play a key role by sensing depletion of cellular [ATP] levels and respond by reducing Ca^{2+} release. This will decrease the rate of ATP usage by reducing both cross-bridge cycling and SR Ca^{2+} uptake, the two main sources of ATP hydrolysis. The cost of this is a reduction in power output, or in other words muscle fatigue, but the benefit is that it ultimately prevents complete exhaustion of all cellular ATP and consequent rigor development and cellular damage.

D. Glycogen

In skeletal muscle, glucose is stored as glycogen, and this store is a major source of energy during most forms of muscle activity. A direct correlation between muscle glycogen concentration and time to fatigue during moderately intense exercise (60–80% of maximal oxygen uptake) was first shown by Hultman and co-workers (46, 204). Their findings have subsequently been confirmed in numerous studies, and the association between glycogen depletion and fatigue during moderately intense exercise is now well established. However, the link between gly-

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cogen depletion and the force decrease during fatigue is not fully understood.

Experiments with simultaneous measurements of force and $[Ca^{2+}]_i$ were performed to shed light on cellular mechanisms linking low glycogen to fatigue. In an initial study, small bundles of fast-twitch flexor digitorum brevis (FDB) muscle fibers were fatigued by repeated tetani, which reduced their glycogen content to ~25% of the control (90). When recovery occurred in the absence of glucose, glycogen did not recover, and fiber bundles fatigued more rapidly in a subsequent fatigue run. Accompanying experiments on single muscle fibers showed that the decrease in tetanic force during fatigue coincided with reduced Ca^{2+} transients both in the initial fatigue run and in the second run, which occurred much faster when recovery took place in the absence of glucose (90). Similar results were subsequently obtained in single cane toad muscle fibers that were repeatedly fatigued by intermittent tetanic stimulation in the absence of glucose (236). Moreover, isolated mouse fast-twitch EDL muscles were fatigued by repeated tetani; allowed to recover for 2 h in zero, normal, or high extracellular glucose; and then fatigued again. Muscles recovering in zero glucose had lower glycogen levels (~50% of the control) at the start of the second fatigue run and fatigued more rapidly than muscles recovering in normal or high glucose. The accelerated fatigue affected both tetanic force and $[Ca^{2+}]_i$ (203). Thus a reduced prefatigue level of glycogen is associated with a faster decrease of tetanic $[Ca^{2+}]_i$ and force during fatigue.

The link between reduced glycogen and decreased $[Ca^{2+}]_i$ transients may be due to glycogen providing acetyl coenzyme A for the tricarboxylic acid (TCA) cycle or maintaining high levels of TCA intermediates (203, 390). Alternatively, the association between low glycogen and impaired SR Ca^{2+} release is not directly linked to glycogen's role in energy metabolism. This alternative originates from a study on skinned toad muscle fibers where the ability to respond to t-tubular depolarizations correlated closely with the muscle glycogen content (nonsoluble component) (425). In these skinned fiber experiments, ATP and PCr were present in the bathing solutions, suggesting that glycogen had a structural rather than a metabolic role. Similar studies were subsequently performed on skinned rat EDL fibers, and these mammalian fibers showed only a small (34) or no (191) ATP- and PCr-independent effect of glycogen on the capacity to respond to depolarizations. Data from fatigue studies on intact muscle cells may be used in support of both a metabolic and structural role of glycogen. Isolated FDB fibers and EDL muscles fatigued more rapidly when glycogen was decreased after recovery in zero glucose, which is consistent with both possibilities (90, 203). However, the glycogen-depleted cells displayed normal changes in other fatigue-induced parameters (i.e., decreased myofibrillar Ca^{2+} sensitivity and maximum force, slowed relaxation, and increased resting $[Ca^{2+}]_i$), which are generally attributed to metabolic changes (90, 203). On the other hand, fatigue in cane toad muscle fibers produced under control conditions was accompanied by a decrease in the rapidly releasable SR Ca^{2+} stores, whereas this store was not decreased after the accelerated fatigue development in glycogen-depleted cells (236). Thus these data on toad fibers indicate different mechanisms behind the fatigue-induced reduction in SR Ca^{2+} release in normal and in glycogen-depleted cells.

In conclusion, depletion of glycogen during prolonged, exhausting exercise may contribute to fatigue by causing decreased SR Ca^{2+} release. The mechanistic link between low glycogen and premature failure of SR Ca^{2+} release remains uncertain.

VIII. REACTIVE OXYGEN SPECIES

There is a large and rapidly growing literature establishing that reactive oxygen species (ROS) are produced in active muscles and have some role in fatigue. The most convincing evidence that ROS contribute to fatigue comes from experiments with exogenously added ROS scavengers which can reduce the rate of fatigue in isolated muscles, in intact animals, and in humans. Despite the overwhelming evidence for some role for ROS in muscle fatigue, many aspects remain uncertain, particularly the source of the ROS, the identification of the particular ROS which are important, and the mechanism(s) by which ROS contribute to fatigue. There are many valuable reviews of ROS in muscle (94, 368, 428), and here we only briefly reiterate the areas covered in those reviews. A comprehensive account of the chemistry and biology of ROS is available (196). Our main focus is current ideas on the mechanisms by which ROS contribute to fatigue.

The most important ROS are superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\bullet}$). In addition, nitric oxide can interact with superoxide to form peroxynitrite ($ONOO^-$), one of a series of reactive nitrogen species, which may also have normal and pathological roles in muscle (369). Superoxide is produced in mitochondria as a by-product of oxidative phosphorylation (443) and also by various enzymes including NADPH oxidase, xanthine oxidase, and lipoxygenases (Fig. 4). Superoxide is only moderately reactive and is rapidly broken down by various superoxide dismutases (SOD) to hydrogen peroxide. Given its negative charge, superoxide does not easily cross membranes but may utilize anion channels in the surface membrane (292) or voltage-dependent anion channels (VDACs) in the mitochondrial membrane for this purpose (443).

Hydrogen peroxide is not highly reactive but diffuses through cell membranes and is thought to have a signaling

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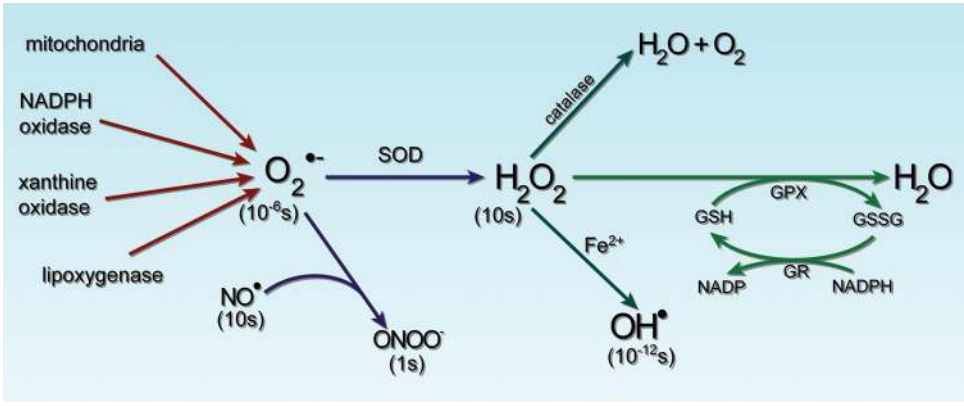

FIG. 4. The major reactive oxygen species in muscle. Numbers in brackets indicate approximate lifetimes of various species (39, 196). GPX, glutathione peroxidase; GR, glutathione reductase. Other abbreviations are as in text.

role in addition to oxidizing some thiol groups on proteins (196). Hydrogen peroxide can be broken down by various pathways; of particular importance is the reaction with free transition metals such as Fe^{2+} (Fenton reaction), which produces the extremely reactive hydroxyl radical. Hydroxyl radicals have exceedingly short lifetimes because they react with virtually any organic molecule and can damage proteins, DNA, and lipids. The Fe^{3+} present in myoglobin, hemoglobin, or cytochrome *c* can be reduced to Fe^{2+} by superoxide radicals (Haber-Weiss reaction) and then allows hydroxyl radical production. Hydrogen peroxide is also broken down harmlessly by catalase ($2H_2O_2 \rightarrow 2\ H_2O + O_2$) or by glutathione peroxidase with the conversion of reduced glutathione (GSH) to oxidized glutathione (GSSG). Given that the important ROS form a cascade from superoxide to hydrogen peroxide to hydroxyl radicals, it is difficult to pinpoint which particular species is exerting an effect.

A. ROS Production Is Accelerated in Active Muscles

It is generally agreed that ROS production is accelerated by muscle activity and by increased temperature which, of course, usually accompanies activity. In early studies, increased ROS production in active muscle was established by electron resonance spectroscopy combined with spin trapping and by nonspecific markers of oxidation, such as lipid peroxidation products and protein oxidation products (115, 127).

Better time resolution and potentially distinction between intracellular and extracellular ROS can be obtained by the use of marker molecules that are located in either the intracellular or extracellular space. Reid et al. (371) used intracellular dichlorofluorescein, which fluoresces in response to various ROS, and found a modest increase in ROS during repetitive tetani. Interestingly, application of SOD and catalase, which would remain extracellular, substantially reduced fluorescence that was interpreted as reducing the extracellular concentrations and thereby preventing back diffusion of superoxide and/or H_2O_2. Extracellular ROS production can be determined by perfusion with cytochrome *c*, which changes its absorption after reduction by superoxide. Increased extracellular superoxide production during activity has been reported (245, 371, 427), which suggests that superoxide is either leaving the cell through anion channels or that it is produced from an extracellular site.

Increases in intracellular and extracellular ROS production as a function of temperature have also been reported by several groups (17, 447, 497). Zuo et al. (498) detected extracellular superoxide and tested whether it was leaving the cell through anion channels by use of the blockers probenecid and DIDS. Neither blocker affected extracellular superoxide, suggesting an extracellular source.

B. Sources of ROS in Muscle

While increased production of ROS during muscle activity is generally accepted, the source of the ROS remains unclear. Mitochondria are generally thought to produce superoxide at a rate of ~1–2% of O_2 consumption, although markedly lower estimates (~0.15%) have also been obtained (418). They contain abundant MnSOD, so much of this superoxide may be broken down to H_2O_2, at least under resting conditions (443). During intense activity, when mitochondrial O_2 consumption increases up to 100-fold (94), it is postulated that the excess superoxide may escape the mitochondria via VDACs or be converted to H_2O_2 that can diffuse across the mitochondrial membrane into the myoplasm (94, 115). Early attempts to test this hypothesis by inhibiting mitochondrial complexes with rotenone failed to reduce muscle ROS production (226, 498). However, a recent study using skinned fibers in which the mitochondria were inhibited with rotenone and succinate (to prevent reverse electron transport across complex I) found that superoxide production was reduced to zero (446).

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Mitochondrial ROS production may also be stimulated indirectly by products of phospholipase A_2 (PLA_2), since inhibition of PLA_2 reduced ROS production (187, 329). Another possible source of ROS is nonphagocytic NAD(P)H oxidase (NOX). There are now several reports of NOX in skeletal muscle that may be associated with the SR (492) or with the t-tubular membrane (207, 226). These studies all showed NAD(P)H-dependent superoxide production, and in two of the studies, inhibitors of NOX (diphenyleneiodonium, DPI) reduced superoxide production by muscle (207, 226). Xanthine oxidase is a further possible source of ROS, and the inhibitor allopurinol has inhibited ROS production in some (427) but not other studies (226). Lipoxygenases (496) are another possible source of superoxide.

Currently there is no consensus about the major sources of ROS production within muscle, but mitochondria and NAD(P)H oxidase seem probable contenders. It is likely that different muscles under various conditions utilize different pathways for ROS production, and it is also likely that the intracellular and extracellular sources of ROS are different.

C. ROS Scavengers Reduce Fatigue

The earliest study of ROS effects on fatigue utilized strips of diaphragm muscle perfused by the circulation and stimulated through the phrenic nerve (400). *N*-acetylcysteine (NAC) injected into the circulation (150 mg/kg body wt) had no effect on the control force but increased the force at the end of a period of intermittent 20-Hz stimulation from 45% control to 66%. Improvement by NAC was present both at low frequencies of stimulation and at high, but NAC did not appear to improve recovery from fatigue. A range of ROS scavengers have subsequently been shown to slow fatigue including SOD, DMSO, catalase (371), Tiron, and Tempol (315). The magnitude of the improvement by ROS scavengers in different studies is quite variable. Two factors that contribute to this are frequency of stimulation (370) and temperature (315). Reid et al. (370) fatigued human tibialis anterior muscles by electrical stimulation using intermittent tetani at either 40 or 10 Hz. During tetani at 40 Hz, the muscles fatigued rapidly, but NAC (150 mg/kg infused intravenously over 1 h) had no effect. In contrast, when tetani were only partially activated by 10-Hz stimulation, fatigue was much slower and NAC produced a moderate improvement. MVCs were also unaffected by NAC. The authors propose that ROS affect either SR Ca^{2+} release or myofibrillar Ca^{2+} sensitivity so that only low-frequency tetani are sensitive.

Muscle temperature appears to be another factor that can explain some of the variability. In a study on mouse single fibers, Moopanar and Allen (315) found that fatigue at 22°C was unaffected by ROS scavengers, while fatigue at 37°C was substantially slowed by ROS scavengers. The increased sensitivity of muscle fatigue to ROS at 37°C may result from the increased rate of ROS production as temperature increases. Since increased activity and temperature both increase ROS production, it is clear that regular exercise will be associated with substantial increases in ROS production. Given the potentially damaging effects of ROS, it is perhaps not surprising that endogenous ROS scavenging pathways are substantially upregulated by regular exercise. For instance, SOD activity and GPX activity are increased in the actively recruited muscles and preferentially in oxidative muscles. There is also evidence that the levels of GSH can increase. These issues have been extensively reviewed elsewhere (362). Furthermore, a recent study showed that PPARγ coactivator 1α, which is a potent stimulator of mitochondrial biogenesis, has a central role in the induction of several ROS defense enzymes in response to oxidative stress (419).

Although improvements in fatigue in response to ROS scavengers have been frequently reported in isolated muscles or intact muscles in animals, improvements in human performance in response to dietary antioxidants (e.g., vitamins C and E) have not usually been observed. An exception is the thiol donor NAC, which seems to have beneficial effects on fatigue performance as already described (370, 400). An important study on trained humans extends the beneficial effects of NAC to a voluntary submaximal cycling in which the time to volitional fatigue was increased by NAC infusion by 26% (304). In a recent extension of this approach, McKenna et al. (303) showed that NAC minimized the reduction in Na^+-K^+ pump activity that usually occurs in exercise (394) and also attenuated the rise in plasma K^+. Given that the Na^+-K^+ pump is redox sensitive (184), the authors propose that ionic changes associated with Na^+-K^+ pump contribute to fatigue and can be ameliorated by the ROS scavenger NAC.

D. Mechanisms by Which ROS Cause Fatigue

As discussed above, there is very strong evidence that ROS contribute to some models of fatigue, but understanding of the mechanisms involved is currently limited. Below we discuss experimental investigation of the mechanisms under three headings, recognizing, of course, that the eventual aim will be to identify specific proteins and specific biochemical changes that underlie these effects.

1. Maximum Ca^{2+}-activated force

Studies of the effects of ROS on $F_{Ca,max}$ have been undertaken both in intact muscles and in skinned fibers.

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In intact preparations, short exposures to H_2O_2 can increase force moderately while longer exposures can decrease force, and at least some of these effects seem to be through changes in $F_{Ca,max}$ (14, 356). In skinned fiber studies, it is clear that very high concentrations of sulfhydryl oxidizing agents, such as 10 mM dithionitrobenzoic acid (485) or 1 mM dithiodipyridine (255), can reduce $F_{Ca,max}$ to zero, but these concentrations are not physiological. Moderate concentrations of ROS have given quite variable results. For instance, in skinned rabbit muscle, Darnley et al. (113) showed that both superoxide and H_2O_2 could produce a 50% enhancement of $F_{Ca,max}$ during an acute exposure, but after the ROS were removed, there was a substantial reduction in force. However, in the same preparation, Callahan et al. (84) found no effects of H_2O_2, while superoxide caused a 15% reduction in $F_{Ca,max}$ and hydroxyl radicals reduced force by 44%. Lamb and Posterino (255), however, found that in rat skinned fibers, with the exception of the high concentration of oxidizing agent mentioned above, none of the oxidants or reductants used affected $F_{Ca,max}$.

There have also been studies that try to dissect the role of ROS during fatigue or high temperature. Moopanar and Allen (315, 316) tested $F_{Ca,max}$ in mouse single fibers fatigued by intermittent tetani. At 22°C, fibers fatigued slowly, and fatigue in this model has been shown to depend on declines in $F_{Ca,max}$, Ca^{2+} sensitivity, and tetanic $[Ca^{2+}]_i$ due to mechanisms discussed elsewhere in this review. When the same fibers were stimulated at 37°C, they fatigued much more quickly, and this increased fatiguability could be reversed by the membrane-permeant antioxidants Tiron or Tempol. However, $F_{Ca,max}$ declined to the same extent at 22 and 37°C, establishing that a decline in $F_{Ca,max}$ was not the cause of the ROS-sensitive component of fatigue at 37°C. This finding is consistent with several reports in which isolated muscles were fatigued and then skinned, but no persisting changes in $F_{Ca,max}$ were noted (111, 483, 484).

During intense exercise, high muscle temperatures can occur, and Van der Poel and Stephenson (447) explored the effects of increasing temperature to 43–47°C for short periods in unstimulated muscles (these temperatures are well above those experienced by muscles during normal exercise). The fibers were then skinned and $F_{Ca,max}$ and Ca^{2+} sensitivity determined as functions of time. The main result was that $F_{Ca,max}$ showed a large fall but then spontaneously recovered. Importantly, if the muscle was treated with Tiron, it prevented both the superoxide production and the reduction in $F_{Ca,max}$. This result, which contrasts with Moopanar and Allen (315) above, suggests that high temperatures in unstimulated muscle cause force decline by a different mechanism to fatigue at 37°C.

2. Ca^{2+} sensitivity

In the intact single fiber study of fatigue at 37°C, Moopanar and Allen (315) found that the rapid decline of force was largely caused by a fall in Ca^{2+} sensitivity. This fall in sensitivity could be prevented by preexposure to Tiron or Tempol and could be reversed by brief application of dithiothreitol (DTT) (316). Studies of skinned fibers have generally not noted pronounced changes in Ca^{2+} sensitivity when ROS were applied to the skinned fiber (84, 113). An exception is the study by Posterino and Lamb (255) who found that prolonged exposure to ROS (H_2O_2 or dithiodipyridine, DTDP) during cycles of activation generally increased Ca^{2+} sensitivity. In addition, exposure to GSH could lead to both increases and decreases in Ca^{2+} sensitivity under different conditions. These results show *1*) that the presence of GSH can modify the effects of oxidants on sensitivity changes, *2*) that probably there are several sites which affect Ca^{2+} sensitivity and are modulated by oxidation state, and *3*) that both the magnitude and direction of sensitivity change can depend on muscle history and concentration and exposure time of oxidants. Because most studies on skinned fibers have not included GSH, this may explain some of the negative results.

Studies of the effects of exogenous H_2O_2 on intact fibers also showed changes in Ca^{2+} sensitivity (14, 15). Initially Ca^{2+} sensitivity increased, but longer exposures to larger concentrations of H_2O_2 caused a large fall in Ca^{2+} sensitivity that was reversed by DTT. Interestingly, DTT caused a small decrease in Ca^{2+} sensitivity when applied alone, suggesting that the endogenous oxidative state of proteins may affect Ca^{2+} sensitivity perhaps by a ROS-sensitive signaling pathway (15).

A series of studies from Nosek's laboratory have explored the effects of hypoxic fatigue on the properties of muscle when subsequently skinned. Fatigue alone had no effect on the function of the subsequently skinned preparation, whereas hypoxic fatigue caused reductions in both $F_{Ca,max}$ and Ca^{2+} sensitivity (63). Subsequently, these changes in the skinned preparations were shown to be associated with degradation of troponins I and C, which the authors speculate may be caused by increased levels of ROS (119).

3. SR Ca^{2+} release

Isolated SR Ca^{2+} channels in a lipid bilayer show an increased open probability in response to Ca^{2+} when H_2O_2 is present at submillimolar concentration (162). In addition, it has long been known that ROS can inhibit the SR Ca^{2+}-ATPase and reduce the rate of Ca^{2+} uptake into the SR (395). Despite these suggestive results on simplified preparations, H_2O_2 had remarkably little effect on the amplitude of the tetanic $[Ca^{2+}]_i$ in single fibers at concentrations and durations which caused substantial changes

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in force due to Ca^{2+} sensitivity changes (14, 15). The only exception was that long exposures to high concentrations of H_2O_2 cause a gradual increase in the magnitude of the Ca^{2+} transient coupled to a slowing of the rate of decline of the Ca^{2+} transient, suggesting that the SR Ca^{2+} pump was being inhibited. Furthermore, in studies of fatigue at 37°C, the rapid decline of force that was ROS sensitive was not accompanied by changes in tetanic $[Ca^{2+}]_i$ (315). Interestingly, when ROS were scavenged and fatigue occurred more slowly at 37°C, the normal pattern of fatigue-induced decline in tetanic $[Ca^{2+}]_i$ was present (see sect. VII).

In skinned rat fibers with intact t-tubule/SR coupling, Brotto and Nosek (65) reported that 5-min exposure to 1 mM H_2O_2 produced a large reduction in the depolarization-induced Ca^{2+} release. These issues were reinvestigated by Posterino et al. (357) using both the same preparation and a skinned preparation in which the sealed t-tubules were electrically stimulated so that the effects of an AP-generated Ca^{2+} release could be examined. H_2O_2 was found to enhance caffeine-induced Ca^{2+} release and Ca^{2+}-induced Ca^{2+} release and to inhibit voltage-induced Ca^{2+} release by an effect on the voltage sensor (since low Mg^{2+} could still trigger Ca^{2+} release). Critically, the AP-induced Ca^{2+} release was unaffected by moderate exposure to H_2O_2 or changes in the GSH/GSSG ratio or DTT. These skinned fiber data suggest that the AP, acting through the voltage sensor, normally completely opens the SR release channels and renders them insensitive to changes in the sensitivity of voltage sensor/RyR coupling. Thus data from intact fibers fatigued at 37°C, intact fibers exposed to H_2O_2, and skinned fibers suggest that changes in SR Ca^{2+} release are not likely to contribute the ROS-sensitive component of fatigue.

In summary, it is clear that ROS are generated in active muscles and contribute to the process of fatigue. How ROS are produced and which ROS are important remain uncertain. Many mechanisms of action are possible given the susceptibility of proteins to oxidative damage, but current evidence points at the contractile proteins and the Na^+-K^+ pump as the sites showing the greatest susceptibility to ROS under physiological conditions.

IX. Ca^{2+} COMPARTMENTS AND MOVEMENTS: ROLE IN FATIGUE

A. SR Ca^{2+} Content and Ca^{2+} Release in Fast- and Slow-Twitch Fibers

The amount of Ca^{2+} contained and released within a muscle fiber is of major importance to its function. In rested muscle, the total Ca^{2+} content of both fast- and slow-twitch rat fibers is ~1.1 mmol/kg wet wt, with most (~1.0 mmol/kg) contained within the SR, equivalent to ~11 and 21 mM when expressed relative to the respective SR volume (176, 183, 343). Total calcium content in human muscle fibers is slightly lower (~0.8 mmol/kg) (338, 393). Most Ca^{2+} in the SR is bound to calsequestrin (38, 45), and the free $[Ca^{2+}]$ is ~0.3–1 mM (175, 274, 275, 382). The SR is normally loaded at only ~25% of maximum capacity in fast-twitch fibers, but at ~70% or greater in slow-twitch fibers (176, 183, 440). The relatively low fractional SR load level in fast-twitch fibers (and consequent low free $[Ca^{2+}]$) likely aids Ca^{2+} uptake in adverse metabolic conditions. The major Ca^{2+}-pump isoform in fast-twitch fibers is SERCA1a and in slow-twitch fibers is SERCA2a, with the faster Ca^{2+} uptake in fast-twitch fibers due primarily to the approximately sixfold greater number of pumps (282, 293, 491).

The rate and amount of SR Ca^{2+} release per AP is approximately three times higher in fast-twitch fibers than in slow-twitch fibers (37). This difference is due primarily to the higher density of both DHPR/voltage sensors and Ca^{2+} release channels in fast-twitch fibers (125, 252, 260, 299). The rapid contraction of fast-twitch fibers requires the presence both of fast MHC (type II) isoforms and fast Ca^{2+} release. Compared with fast-twitch fibers, slow-twitch fibers have fewer Ca^{2+} binding sites on troponin C and the SR pumps, and a lower rate and amount of Ca^{2+} release suffices for contraction, particularly given the much slower contraction rate of the predominant MHC isoform (type I) present (59, 61). Thus SR and contractile properties in a given fiber are generally well matched (440).

B. Effects of SR Content Changes on Ca^{2+} Release

Importantly, if the total amount of Ca^{2+} in the SR of a fast-twitch fiber drops to substantially below its normal level, the amount released by each AP is reduced (360) (see Fig. 5), reducing the force response. Specifically, a 35% decrease in SR Ca^{2+} content (from ~1.1 to 0.71 mmol/kg) reduced peak tetanic force by 46% (142), despite the fact that the amount of Ca^{2+} still available in the SR considerably exceeded the number of Ca^{2+} binding sites on troponin C. Virtually identical reductions in force responses were also seen after exposing a fiber to 30 mM cytoplasmic P_i(142), most likely due to Ca^{2+}-P_i precipitation within the SR reducing the amount of Ca^{2+} available for rapid release (see sect. VII*A*). In contrast, increasing the amount of Ca^{2+} in the SR up to approximately threefold above the endogenous level (to close to maximal capacity) did not alter the amount of Ca^{2+} released by each AP (360) (Fig. 5). This constancy in AP-induced Ca^{2+} release is evidently due to feedback effects of the released Ca^{2+} on further release (see sect. VI*C8*).

In summary, the SR can accumulate substantial amounts of additional Ca^{2+} without evident effect,

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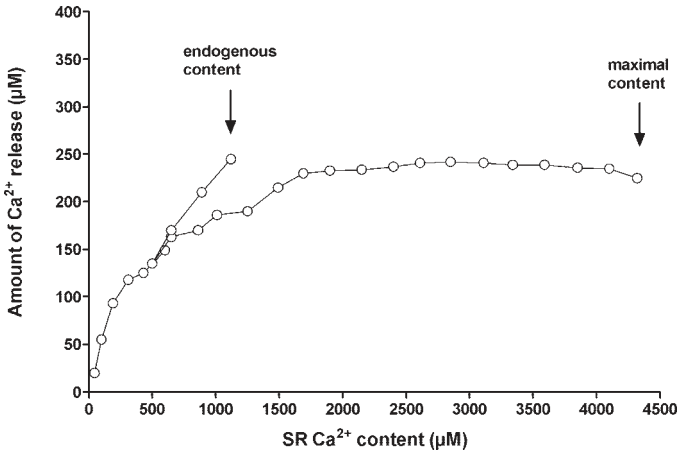

FIG. 5. Amount of Ca^{2+} released by single action potential (AP) stimuli versus sarcoplasmic reticulum (SR) Ca^{2+} content in a skinned fiber from rat extensor digitorum longus (EDL) muscle. The SR was progressively depleted of all its releasable Ca^{2+} in two sequences, first starting with the endogenous Ca^{2+} content and then a second time after reloading the SR to its maximal level. Ca^{2+} reuptake was blocked by an SR Ca^{2+} pump blocker during AP-induced release. The amount of Ca^{2+} released by an AP is virtually unchanged when the SR is loaded well above its endogenous content, but is decreased at lower content levels. Ca^{2+} is expressed in micromoles per liter fiber volume. [From Posterino and Lamb (360).]

whereas reduction of the amount of releasable Ca^{2+} in the SR adversely affects Ca^{2+} release and force responses.

C. Ca^{2+} Content Changes With Repeated Stimulation and With Exercise

SR Ca^{2+} content will change if there is a net influx or efflux of Ca^{2+} across the sarcolemma of a muscle fiber, and these changes in flux may affect fatigue development (64, 344, 494). Ca^{2+} may enter a muscle fiber via a number of different pathways (12), including the DHPRs (129, 260), store-operated Ca^{2+} entry (SOCE) (211, 251, 272), excitation-coupled Ca^{2+} entry (ECCE) (88, 220), Ca^{2+} leak channels (169, 216), and stretch-activated channels (SACs) (195, 448, 487). Experiments on ^{45}Ca uptake in frog single fibers found a "resting" Ca^{2+} influx of ~0.25 $pmol \cdot s^{-1} \cdot cm^{-2}$ of apparent surface area, and ~1 pmol Ca^{2+}/cm^2 on each twitch response (in 1.8 mM extracellular Ca^{2+}) (104). The latter equates to only ~0.7 μmol Ca^{2+}/kg wet wt on each AP, or ~0.07% of the resting SR Ca^{2+} content already present. Importantly, this is the unidirectional influx, not the net flux of Ca^{2+}. The rate of Ca^{2+} efflux in a fiber is also very low in resting fibers (time constant >2 h, Ref. 104), and low even with repeated activation, as is apparent from the large number of force responses that can be evoked in zero extracellular Ca^{2+} (18, 290). The combined effect of influx and efflux is that net Ca^{2+} flux is normally quite small.

Fast-twitch rat muscle stimulated in vitro at 40 Hz continuously or intermittently for a total of 30 s shows net uptake of 50–100 μmol Ca^{2+}/kg wet wt (183, 313), equivalent to ~5–10% of the Ca^{2+} already present in the SR endogenously and representing <5% of the available SR Ca^{2+} capacity. With continuous stimulation at 1 Hz for 2–4 h, fast-twitch rat muscles increase their Ca^{2+} content to ~2.5 times the endogenous level, whereas slow-twitch muscle shows little increase (183), consistent with the endogenous Ca^{2+} and maximum SR capacity observed in such muscle types (176, 440). Frog muscle fibers stimulated in vitro to fatigue increased SR Ca^{2+} content by ~10% (190). In contrast, fast- and slow-twitch muscle stimulated in situ in the rat with intermittent 100-Hz tetani for 5 min showed an ~40% decline in total Ca^{2+} content (286). Importantly, the latter study found no significant change in muscle Ca^{2+} content after rats had been swum to exhaustion (over ~5 min) (286). In humans running long distances, vastus lateralis muscle Ca^{2+} content increased by ~30% after a 20-km run, but was not significantly changed after a 10-km run (338). The above findings indicate that even though muscle, particularly fast-twitch fibers, can accumulate net Ca^{2+} with repeated stimulation, there in fact was relatively little change in muscle Ca^{2+} content during exercise except when it was very prolonged.

D. Mitochondrial Ca^{2+} Handling

Mitochondria in skeletal muscle fibers can take up cytoplasmic Ca^{2+} released from the SR during twitch and tetanic responses (68, 270, 383). Such Ca^{2+} uptake may stimulate mitochondrial ATP production, although it may not be essential (68). It has been frequently suggested that Ca^{2+} uptake by the mitochondria may play a significant role in overall Ca^{2+} movements in a fiber, particularly those mitochondria situated close to the SR (223, 401), with the effect possibly being more important in frog fibers than in mammalian fibers (68, 270). However, the mitochondria do not contain high-capacity Ca^{2+} binding proteins such as calsequestrin, and the absolute amount of Ca^{2+} contained within the mitochondria in frog fibers at rest, or after stimulation by either a single tetanus or to fatigue, is <2% of that contained within the SR (190, 408). In humans exercising for 1 h, the amount of Ca^{2+} in the mitochondria only increased by ~50% above the level in rested muscle (297). It should also be noted that the experiments in mammalian fibers reporting specific tunneling of SR Ca^{2+} to the mitochondria (223, 401) were performed with skinned fibers under conditions where the SR would have been loaded at near-maximal capacity, and this likely increased the extent of Ca^{2+} uptake by the mitochondria. In summary, the mitochondria appear to play at most a minor role in directly influencing overall Ca^{2+} movements during EC coupling.

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X. SHORTENING VELOCITY AND MECHANICAL POWER

Locomotion is generally driven by mechanical work generated by skeletal muscles, and the speed of locomotion consequently depends on the mechanical power that the muscles can produce. Mechanical power equals force times shortening velocity, and the mechanisms underlying fatigue-induced changes in isometric force production are not the same as those that affect the shortening velocity (8). This means that a decrease in maximum power output can depend mainly on decreased force production in some situations, whereas decreased shortening velocity can be of greater importance in other cases. Furthermore, decreased force production has a larger impact on the power output during movements requiring high forces, and reduced shortening velocity becomes more important as the speed of movement increases. The energy cost is considered to be higher during repeated contractions with shortening than during isometric conditions, and hence fatigue develops more rapidly when it is induced by shortening contractions (43, 102).

Fatigue-induced changes in shortening velocity can be described in terms of decreased maximal shortening velocity (i.e., velocity at zero load) and/or altered curvature of force-velocity relationship. Changes in maximal shortening velocity and curvature have been shown to develop with different time courses during fatiguing stimulation, which indicates different underlying mechanisms (228, 476).

Acidosis has classically been considered a major cause of the fatigue-induced decrease in shortening speed (8, 168). This conclusion was based on studies on frog muscle and mammalian skinned fibers studied at low temperature (10–15°C), where lowering pH to 6.2 causes a very substantial decrease in the $F_{Ca,max}$ (>50%) and in the maximum velocity of shortening (347). However, more recent data from intact mouse type II fibers studied at 32°C show no decrease in maximal shortening velocity under acidic conditions (478). Similar results were obtained in skinned mammalian type II fibers studied at 30°C, where decreasing pH from 7.0 to 6.2 had little inhibitory effect on maximum velocity of shortening and peak power (244, 347). A larger reduction (~34%) in peak power induced by lowering pH to 6.2 at 30°C was observed in type I fibers (244), but it is very doubtful that pH ever approaches such a low level in type I fibers in normal exercise.

Increased P_i decreases the myofibrillar capacity to generate force and impairs SR Ca^{2+} handling and is therefore regarded as a major cause of fatigue (see sect. VII*A*). However, the effect of increased P_i on maximum shortening velocity is very limited (99, 123, 348). Reduced ATP may result in decreased shortening speed, but ATP has to fall to very low levels (~0.5 mM) before this inhibitory effect becomes substantial (98, 164), and this will not occur in most types of fatigue (see sect. III). Decreased $[Ca^{2+}]_i$ results in decreased isometric force but has little effect on the maximal shortening velocity (150, 473).

Skinned fiber experiments have shown that ADP has a major inhibitory effect on maximal shortening velocity (99, 309). The free [ADP] in the myoplasm ($[ADP]_i$) has been estimated to increase to no more than ~300 μM in severely fatigued muscles, and this $[ADP]_i$ has little impact on the maximal shortening velocity (87). $[ADP]_i$ cannot be measured with standard biochemical methods because most ADP is bound to proteins and hence metabolically inactive (449). ^{31}P-NMR spectroscopy can measure free ADP, but $[ADP]_i$ in muscle is generally below the detection threshold and the time resolution is limited (198). Nevertheless, Hancock and co-workers (198, 199) were recently able to detect an increase in $[ADP]_i$ to ~1.5 mM in fatigued hindlimb muscles of mice deficient of adenylate kinase (AK). AK catalyzes the reaction: 2ADP → ATP + AMP, and muscles deficient of this enzyme will therefore display larger and more long-lasting increases in $[ADP]_i$ during fatigue than normal muscles. The unloaded shortening velocity of the gastrocnemius-plantaris-soleus muscle group of AK-deficient and wild-type mice was decreased to a similar extent during a series of repeated tetanic contraction (199). This would speak against a major role of increased $[ADP]_i$ in the decrease in shortening velocity during fatigue, but it should be noted that the muscle group used in these experiments has a mixed fiber type composition that will complicate the interpretation of the results.

Transient increases in $[ADP]_i$ have been suggested to occur during contractions, especially when the PCr store becomes depleted (177, 388). Accordingly, numerous studies have reported a substantial increase in IMP and a corresponding decrease in adenine nucleotides in fatigued muscles (225, 312, 390). The increase in IMP may be taken to support the occurrence of transient increases in $[ADP]_i$ during fatiguing contractions, because the formation of IMP is driven by an increase in $[ADP]_i$ and the flux would be very limited if $[ADP]_i$ remained low throughout fatigue.

Experiments were designed to test the effect of the putative $[ADP]_i$ transients on the maximal shortening velocity in single fast-twitch *Xenopus* frog and mouse muscle fibers (473, 477). The maximal shortening velocity was then first measured in a short (400 ms) tetanus. After a 2.4-s pause, it was again measured in a long (1,400 ms) tetanus, and finally after a 4-s pause in a short tetanus. The underlying assumption was that with limited PCr buffering, ADP would accumulate more in the long tetanus, and this effect would be rapidly reversed. Under control conditions, where the PCr buffering was intact and no transient increases in $[ADP]_i$ were expected, there was no difference in the maximal shortening velocity

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between the contractions. In the fatigued state, on the other hand, the maximal shortening velocity in the long tetanus was markedly lower than in the bracketing short tetani. Similar results were also obtained in unfatigued fibers where the CK reaction was inhibited by dinitrofluorobenzene (473, 477).

Results from fatigued frog and mouse muscles show a decreased curvature of the force-velocity relationship (31, 103, 476), which will limit the fatigue-induced decrease in maximum power output, whereas studies on human adductor pollicis muscles show an increased curvature (120, 228), which will further decrease the maximum power output. The mechanisms underlying these opposite results are uncertain, but they have been suggested to reflect differences in the temperature at which the experiments were performed (228). Recent studies on skinned rat fibers showed a marked decrease in the curvature of the force-velocity relationship as the temperature was increased from 15 to 30°C (123, 244). Furthermore, addition of 30 mM P_i, which has little effect on the maximum shortening velocity (see above), increased the curvature in both fast- and slow-twitch fibers when studied at 30°C but had no effect at 15°C (123).

In conclusion, fatigue involves changes in isometric force, maximal shortening velocity, and the curvature of the force-velocity relationship. These three factors have different underlying mechanisms, and they all affect the power output of fatigued muscles. Transient increases of $[ADP]_i$ appear to have a central role in the fatigue-induced decrease in maximal shortening velocity, whereas increased P_i or H^+ or decreased $[Ca^{2+}]_i$ have little impact on this parameter.

XI. SLOWING OF RELAXATION

Skeletal muscle fatigue is generally accompanied by a marked slowing of relaxation (Fig. 3*A*). This slowing may be beneficial during a prolonged isometric contraction where it increases fusion of the force output at lower stimulation frequencies, thus minimizing the force decline when the motor neuron firing rate decreases (52, 227). On the other hand, slowing of relaxation can limit performance during dynamic exercise where rapidly alternating movements are performed (8). It should also be noted that muscle cells can fatigue without any major slowing of relaxation; for instance, isolated slow-twitch fibers of mouse soleus muscles display no or very limited slowing during fatiguing stimulation (Fig. 3*B*) (67, 288).

Relaxation of skeletal muscle cells is a complex process that involves the following major steps: *1*) SR Ca^{2+} release stops, *2*) Ca^{2+} is taken up by the SR via ATP-driven pumps (myoplasmic buffers, such as parvalbumin, may contribute to the decrease in $[Ca^{2+}]_i$ in muscles that contain such buffers and provided they are not already saturated with Ca^{2+}), *3*) the resulting decline in $[Ca^{2+}]_i$ means the Ca^{2+} dissociates from troponin, and *4*) cross-bridge cycling ceases. Potentially, any of these steps could be slowed in fatigue and hence contribute to the slowing of relaxation. Currently available methods do not allow direct measurements of changes in the rate of each of these four steps during a fatigue experiment. In fact, it is doubtful whether any of the steps can be accurately measured during fatigue without interference from the other steps. To simplify this matter, we have developed techniques where simultaneous measurements of force and $[Ca^{2+}]_i$ (or shortening steps during relaxation) in single muscle fibers allow us to assess the relative contribution of changes in SR Ca^{2+} handling (*steps 1* and *2*; Ca^{2+} component) and myofibrillar function (*steps 3* and *4*; cross-bridge component) (465, 478). Using these techniques, we found a slowing of SR Ca^{2+} handling in mouse FDB fibers fatigued by repeated tetani, but this was counteracted by decreased myofibrillar Ca^{2+} sensitivity, and hence, the Ca^{2+} component appeared not to affect the rate of force relaxation (465). On the other hand, when using the same experimental approaches on easily fatigued (type 1) and fatigue-resistant (type 2) *Xenopus* frog fibers, we found that both the Ca^{2+} component and the cross-bridge component contributed significantly to the fatigue-induced slowing of relaxation (478). Similar experiments cannot be performed in humans, but a recent study using human adductor pollicis muscles in vivo compared changes in the force-velocity relationship and relaxation speed during a series of fatiguing contractions (228). The results showed a strong temporal correlation between an increased curvature of the force-velocity relationship and slowed relaxation, which indicates that altered cross-bridge function contributes to the slowing of relaxation in fatigued human muscles.

In conclusion, changes in SR Ca^{2+} handling and cross-bridge function, which have the potential to cause slowed relaxation, occur in parallel during induction of fatigue. As discussed above, the relative importance of the slowing of the Ca^{2+} component and the cross-bridge component to the observed reduction in relaxation speed depends on the species and muscle studied. In addition, the relative contribution of the Ca^{2+} versus the cross-bridge component undoubtedly depends on other factors, such as the temperature and the stimulation protocol employed to induce fatigue.

Numerous studies have correlated slowing of relaxation during fatigue with concurrent metabolic changes (8, 168). A large focus has been on acidosis, but like other aspects of fatigue [i.e., isometric force (sect. VII*B*) and shortening velocity (sect. X)], the inhibitory effect of acidosis on the relaxation speed of mammalian muscle becomes smaller as the temperature is increased towards physiological temperatures. Nevertheless, acidification of mouse FDB fibers at ~30°C (close to the ambient tem-

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perature of this superficially located muscle) caused a significant decrease in the rate of relaxation both in the unfatigued and the fatigued state (71, 478). Thus it appears that acidification contributes to the slowing of relaxation in fatigued mammalian muscle even at physiological temperatures, which agrees with in vivo human muscle results (77).

Changes in ATP, ADP, and P_i during fatigue will have a direct impact on both myosin and SR Ca^{2+}-ATPase, and hence, changes in these metabolites have classically been implicated in the fatigue-induced slowing of relaxation (118, 154). More recent studies show major effects of increased P_i on SR Ca^{2+} handling (sect. VII*A*), and changes in ADP and ATP also affect the SR function (sect. VII*C*). Moreover, experiments on skinned muscle fibers, where relaxation was induced by activation of the rapid Ca^{2+} chelator diazo-2, show that both increased ADP or P_i can slow relaxation by acting on the cross bridges (217, 319). Adenylate kinase limits the accumulation of ADP during fatigue; hence, fatigued skeletal muscles of AK-deficient mice show a large increase in free [ADP] (199). Fatigued AK-deficient muscles also show more slowing of relaxation during fatigue than a wild-type muscle (199). CK-deficient muscle fibers fatigue without any significant accumulation of P_i (422). At the onset of fatigue induced by repeated tetani, wild-type muscle fibers show decreased force production and a marked slowing of relaxation, and neither of these features are seen in CK-deficient fibers (106). Thus the lack of slowing of relaxation in these support an important role of P_i in this process.

In conclusion, increases in H^+, ADP, and P_i are likely metabolic factors causing a reversible slowing of relaxation in fatigued muscles. In addition, fatigue may also induce long-lasting structural changes in SR Ca^{2+} handling and/or myofibrillar proteins that might affect the rate of relaxation (see sect. VIII*D*).

XII. FACTORS COUNTERACTING FATIGUE

This review mainly deals with fatigue mechanisms that cause impaired muscle function during repeated activity. However, there are also mechanisms by which repeated activity improves muscle function, and this section deals with factors that can counteract fatigue. One example of this is the fatigue-induced slowing of relaxation that will increase fusion and hence isometric force production at low stimulation frequencies. This has been shown to have an important force-preserving effect during continuous voluntary contractions, where a reduction of the α-motor neuron firing frequency limits or prevents AP failure in the muscle fibers (sect. VI*C*). Decreased pH_i may also limit AP failure in fatigue by decreasing the Cl^- and hence the leak conductance (sect. VI*C*).

Muscle cells may take up water and swell during fatiguing exercise (403, 458). Muscle fiber swelling increases tetanic force production and maximum shortening velocity (151) and hence counteracts the opposite changes that occur in fatigue. Interestingly, the initial beneficial effects of creatine supplementation may be due to increased force production caused by water influx (321) (see sect. VII*A5*).

Submaximal force production is often increased after a brief period of muscle activity, and this phenomenon is called posttetanic potentiation (170, 366, 404). Posttetanic potentiation is generally larger in fast-twitch than in slow-twitch fibers (430). There is a correlation between this force potentiation and phosphorylation of the myosin regulatory light chain (RLC) (298, 317, 430). Furthermore, skinned fiber experiments have shown that myosin RLC phosphorylation increases force at submaximal, but not saturating, Ca^{2+} concentrations (353, 431). The increase in $[Ca^{2+}]_i$ during contractions initiates myosin RLC phosphorylation via a Ca^{2+}/calmodulin-dependent activation of skeletal muscle myosin light-chain kinase (skMLCK) (430, 495). In addition, it appears that in fatigued muscle other factors may also have a force-potentiating effect similar to that induced by myosin RLC phosphorylation (441), possibly including oxidation effects on the contractile apparatus (255).

The increase in $[Ca^{2+}]_i$ during contractions may also activate another Ca^{2+}-dependent kinase, calmodulin kinase II (CaMKII), which is known to regulate several proteins involved in SR Ca^{2+} handling. In fast-twitch fibers, CaMKII targets proteins involved in SR Ca^{2+} release, i.e., RyR, DHPR, and some of their associated proteins (97, 109). In slow-twitch fibers, CaMKII also acts on the SR Ca^{2+}-ATPase and phospholamban (110, 387). CaMKII has been shown to be activated in exercising humans (381). Injection of CaMKII inhibitory peptide into fast-twitch mouse FDB fibers resulted in an ~20% decrease in tetanic $[Ca^{2+}]_i$ in unfatigued fibers, and the extent of inhibition increased with repeated contractions (436). Thus the decline in $[Ca^{2+}]_i$ and force during fatigue might occur at a faster rate if these changes were not counteracted by Ca^{2+}-induced activation of skMLCK and CaMKII.

XIII. RECOVERY FROM FATIGUE

In this review we consider "recovery" to start immediately after the period of fatiguing stimulation. This means that the fatigue-induced impairment in muscle function does not necessarily have to improve during the initial part of the recovery period. In fact, the opposite is frequently observed. For instance, fast-twitch frog muscle fibers show a marked force decrease after the end of fatiguing stimulation, which has been named postcontractile depression (PCD) (475) and which will be discussed below.

Depending on the type of fatigue induced, restoration of force production after fatiguing stimulation has mark-

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edly different time courses. Force recovery after fatigue produced by repeated short tetani is generally completed within 30 min when tested at high frequencies, whereas the force at low-frequency stimulation may be markedly depressed for many hours. On the other hand, when fatigue is induced by continuous high-frequency stimulation, a marked force recovery occurs in the first seconds after cessation of fatiguing stimulation (sect. I*A* and Fig. 2). It has been proposed that this fast component of recovery over several seconds represents the diffusion of elevated K^+ from the t tubules (479). During long high-frequency tetani, reduced Ca^{2+} release was apparent in the center of a muscle fiber, consistent with K^+ accumulation depolarizing the t tubules. In addition, the time course of recovery of central Ca^{2+} release and force are both consistent with the time for diffusion of small ions out of the t tubule (326) (sect. I*C*).

A specific type of long-lasting lack of force recovery was originally observed by Edwards et al. (155) in human adductor pollicis muscles (155). In this type of delayed recovery from fatigue, tetanic force production is more depressed at low than at high stimulation frequencies (see sect. I*C*). It should be pointed out that a greater fractional force decrease at low compared with high stimulation frequencies (low-frequency weakness) is not restricted to situations with long-lasting lack of recovery. In fact, this is seen in all situations where the lower stimulation frequency is on a steeper part of the force-$[Ca^{2+}]_i$ relationship than the higher stimulation frequency (Fig. 6). Thus all situations where tetanic $[Ca^{2+}]_i$ and/or the myofibrillar Ca^{2+} sensitivity are reduced have the potential of showing low-frequency weakness. It can even be observed in unfatigued fibers as illustrated by experiments with inhibition of CaMKIII (436). In this situation, CaMKII inhibition causes a similar reduction of tetanic $[Ca^{2+}]_i$ at all stimulation frequencies, which results in a markedly lower force at low frequencies.

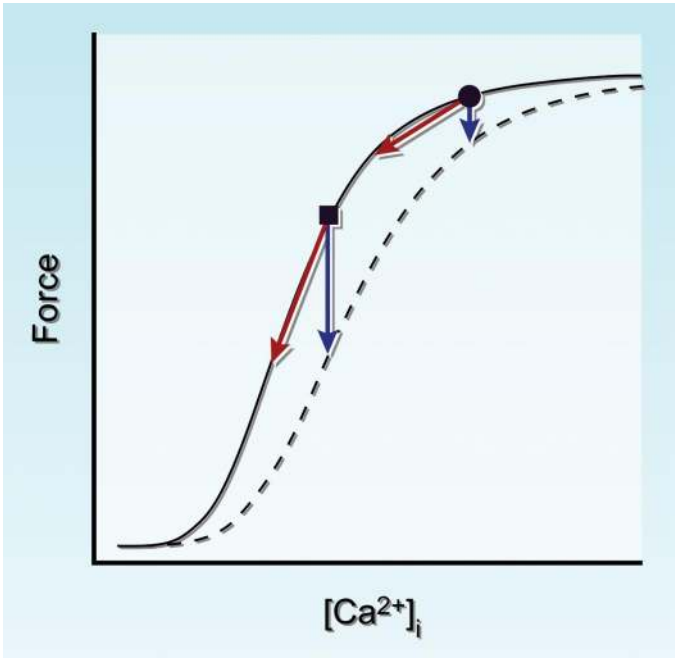

FIG. 6. Schematic showing mechanisms underlying a more marked force depression at low stimulation frequencies. Red arrows show what happens with a decrease in tetanic $[Ca^{2+}]_i$, and blue arrows with a decrease in myofibrillar Ca^{2+} sensitivity (indicated by dashed line). Note that the effect on force of both these changes is markedly larger when they originate from the steep part of the force-$[Ca^{2+}]_i$ relationship, i.e., at low stimulation frequencies.

A. Delayed Recovery From Fatigue

Following severe or prolonged exercise in humans, part of the overall force deficit can be due to changes in muscle function that persist for many hours (155, 210, 442). This deficit is not due to reduced amounts of ATP or CrP, nor to increases in associated metabolites, as these return close to resting levels within 15–60 min (22, 155, 210, 442). Furthermore, it is not due to changes in neuromuscular transmission or excitation of the sarcolemma, as the compound surface AP (M-wave) is unchanged in most circumstances (22, 155) (but see additional phenomenon of delayed tension reduction discussed below). A key feature of this long-term force reduction is that responses to low-frequency stimulation are reduced more than responses to high-frequency stimulation (e.g., 20 versus 50 or 100 Hz in humans). Some studies report that the response to high-frequency stimulation is virtually unchanged (155), whereas others report that it is reduced ~20% (210, 442). The latter has been interpreted as an additional fatigue mechanism (442) and described as postcontractile depression (PCD) to link it with a phenomenon described in frog single fibers (475) (see below), although it is quite likely that the reduction in force at high frequencies is due to the same processes as that at low frequencies. For instance, a large reduction in either SR Ca^{2+} release (Fig. 7*A*) or the Ca^{2+} sensitivity of the contractile apparatus (Fig. 7*B*) could result in some force reduction even with high-frequency stimulation, as well as a proportionately larger reduction in force to low-frequency stimulation.

A long-lasting force deficit has also been observed in vitro in both fast- and slow-twitch muscles (229) and in single fast-twitch mouse fibers (89, 474). Force was reduced ~10–20% at high frequencies and >50% at low frequencies and showed little or no recovery in the period 10–60 min after stimulation. Augmenting Ca^{2+} release with caffeine fully restored the force response to high-frequency stimulation (89, 229, 474), indicating that maximum force production by the contractile apparatus was unaffected. Tetanic $[Ca^{2+}]_i$ was measured in single mouse fibers, and the force deficit was found to be caused by a reduction in $[Ca^{2+}]_i$ at all frequencies of stimulation,

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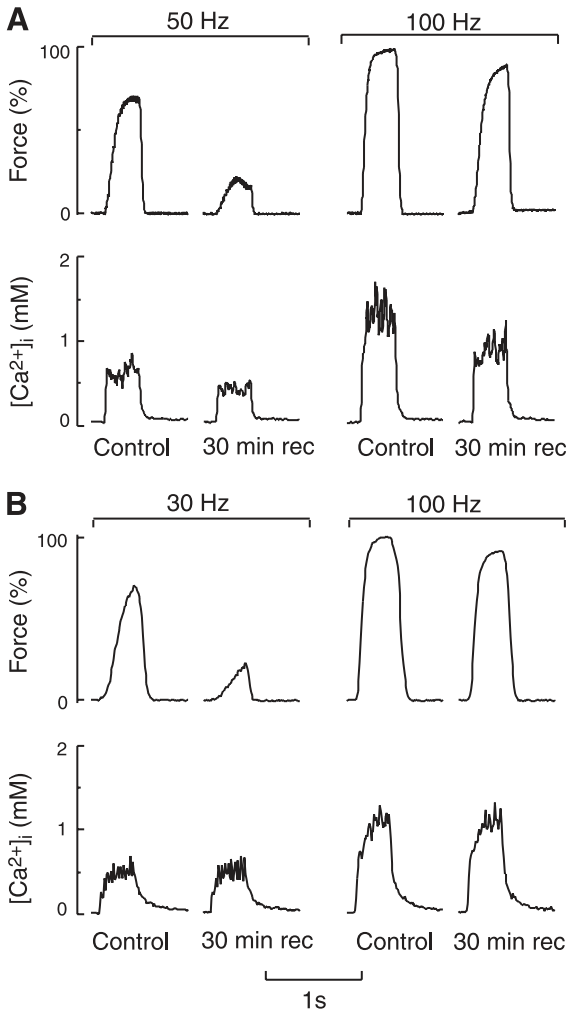

FIG. 7. Examples of decreased force production at low-frequency stimulation observed 30 min after fatigue induced by repeated tetani in a fast-twitch mouse (*A*) and rat (*B*) FDB fiber. Note two different mechanisms: the force decrease at 50 Hz in the mouse FDB fiber can be explained by a decreased $[Ca^{2+}]_i$ (*A*), and the force decrease at 30 Hz in the rat FDB fiber was not accompanied by any decrease in $[Ca^{2+}]_i$ (*B*). The fact that force at 100 Hz was similar before and after fatigue shows that maximal Ca^{2+}-activated force was little affected, and hence, the force decrease at low frequencies was due to a decrease in myofibrillar Ca^{2+} sensitivity. [*A* from Westerblad et al. (474); *B* from J. D. Bruton and H. Westerblad, unpublished observations.]

whereas there were no changes in Ca^{2+} sensitivity (474). The reduction in tetanic $[Ca^{2+}]_i$ was uniform throughout the single fiber, showing that it was not due to AP failure within the T system.

Another type of delayed force recovery, PCD, was originally observed after fatigue induced by repeated tetani in *Xenopus* fast-twitch fibers (475). PCD is manifested as a further decrease in tetanic force after cessation of fatiguing stimulation, which reaches a minimum after ~20 min of recovery when little or no tetanic force is produced. Thereafter, force starts to recover, eventually reaching the prefatigue level, but the force recovery generally takes much longer than the recovery of ATP and other metabolites which are completed within an hour (323). PCD is prolonged by mild mechanical stress, in particular by a brief (5 min) exposure to mildly hypotonic conditions following stimulation (69), and full recovery may then take ~16 h (269). PCD is caused by inhibition of SR Ca^{2+} release despite normal membrane potential and AP properties (219, 266, 283, 471, 475). The exact mechanism underlying PCD has not been revealed, but it seems to involve impaired mechanical coupling between the t-tubular voltage sensors (DHPR) and the SR Ca^{2+} release channels (RyR) (69, 72).

Yet another phenomenon of long-term reduction of tetanic responses has been observed in rat (264) and human (302) muscles. In this case, tetanic force first substantially recovers after the end of stimulation and then over the next 60 min declines markedly again before subsequently recovering once more. In contrast to PCD, the force loss is seemingly attributable to a loss of muscle excitation, as is apparent from the major reduction in the M-wave that occurs in parallel (264, 302). Although failure of transmission at the neuromuscular junction has not been ruled out, this effect is probably due to failure of the sarcolemmal AP. Given the long-lasting nature of the effect, it is more likely due to changes in ionic conductances (i.e., reduced Na^+ conductance or increased K^+ or Cl^- conductance) rather than to changes in ion concentration gradients. The reports to date of delayed tension reduction involved experiments in which the muscle was stimulated in situ by imposed stimulation of the nerve, either continuously for ~5 min (302) or intermittently for 15 min (264), and it is unclear whether or to what extent this effect happens after normal exercise in vivo. One study in exercising humans, however, has noted some cases of a loss in tetanic force to high-frequency stimulation that reached its maximum 1–2 h after the end of the exercise (155), which might be due to this phenomenon.

B. Prolonged Changes in the Contractile Apparatus

Chemically skinned fibers, obtained from whole muscles fatigued in vitro by intermittent tetani, have been used to examine whether there are changes in the contractile properties of fatigued fibers that persist after the cytoplasm is replaced with a standardized solution. These experiments showed no changes in maximum force production (111, 483). In fast-twitch rodent fibers, myofibrillar Ca^{2+} sensitivity was unchanged (111), whereas slow-twitch fibers showed an overall decrease in Ca^{2+} sensitivity (111). Experiments with frog twitch fibers showed a net increase in Ca^{2+} sensitivity after fatiguing stimulation (481, 483). The cause(s) of these changes has not been identified.

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C. Prolonged Reduction in Ca^{2+} Release

A number of studies have reported that Ca^{2+} release from isolated SR is reduced by ~20–40% following prolonged or intense exercise in humans (210, 284, 285) and rodents (161), although some studies found no change (111, 396) or a reduction in slow-twitch but not fast-twitch muscle (161, 221). It is unclear how long this effect persists after exercise, as this was only examined in one study to date, and the results were somewhat equivocal at the one recovery time examined (3.5 h) (210). Moreover, a major issue with such studies is that the Ca^{2+} release was triggered by nonphysiological means, such as by caffeine, chloro-*m*-cresol or the oxidizing agent Ag^{+}, and the rate of release was 30–1,000 times slower than occurs when activating Ca^{2+} release by the normal voltage-sensor mechanism. Given that voltage-sensor activation of Ca^{2+} release is frequently found to be unaffected by conditions that inhibit Ca^{2+} release in isolated SR channels (e.g., raised $[H^{+}]$, lactate, oxidation), the above findings cannot be taken as strong evidence that exercise-induced changes in the release channels themselves are the primary cause of either short-term or long-term reductions in Ca^{2+} release following exercise.

The long-term reduction in AP-induced Ca^{2+} release in isolated murine fibers (474) becomes more pronounced if the rise in $[Ca^{2+}]_i$ during the fatiguing stimulation is prolonged or pharmacologically augmented, and repeated fatiguing bouts have a cumulative effect (89, 92). Potentiating tetanic $[Ca^{2+}]_i$ also greatly prolongs PCD in *Xenopus* fast-twitch fibers (69, 70, 269). On the other hand, in a recent study fatigue was induced by repeated tetani in isolated mouse fibers exposed to *N*-benzyl-*p*-toluene sulfonamide (BTS), which inhibits cross-bridge force production (66). The long-term depression of SR Ca^{2+} release was no more marked in BTS-exposed fibers compared with control fibers despite the fact that the former experienced a markedly larger $[Ca^{2+}]_i$-time integral during induction of fatigue.

Experiments in skinned fibers with functional EC coupling show that coupling is disrupted in both mammalian and amphibian fibers if the cytoplasmic $[Ca^{2+}]$ is raised to tetanic levels (2–10 μM) for a prolonged period or to higher levels even briefly (254, 450). The disruption occurs irrespective of whether the Ca^{2+} is applied exogenously or released from the SR by triggering the normal release mechanism, and it can also be induced by triggering excessive Ca^{2+} release whilst the fibers are intact (254, 450). The effect is caused primarily by relatively high $[Ca^{2+}]$ near the release channels rather than by prolonged small rises in resting $[Ca^{2+}]$ (450). The reduced responses are not due to depolarization of the T system, or to dysfunction of the release channels, which can still be potently activated by direct stimulation. Instead, the effect appears to be due to the voltage sensors failing to properly activate the release channels, quite possibly because the triad junctions become distorted following the Ca^{2+} exposure (254). The elevated $[Ca^{2+}]$ appears to induce its effect via a pH-dependent enzymatic reaction that does not involve phosphorylation or oxidation (254), but the exact mechanism is uncertain.

It has frequently been suggested that Ca^{2+}-dependent proteolysis may play a role in muscle fatigue and damage (40, 41, 76, 182). Skeletal muscle contains the ubiquitous calcium-dependent neutral proteases μ-calpain and m-calpain, as well as a muscle-specific isoform, calpain-3 (186). Much of the ubiquitous calpain is freely diffusible, but some is normally bound at triad junction (182), and raising $[Ca^{2+}]$ within the physiological range increases calpain binding there (322, 367). Ca^{2+}-dependent uncoupling is prevented in toad skinned fiber by leupeptin, a calpain inhibitor, but only at low $[Ca^{2+}]$ when the uncoupling proceeds slowly (254, 451). Calpain inhibitors, however, were not found to prevent either long-duration fatigue in mouse fibers (89) or hypotonic-induced PCD in frog fibers (69). Calpain inhibitors such as leupeptin are quite poor at inhibiting in situ calpain-dependent proteolysis of structural proteins (141), unless used at very high concentration (241, 322, 451), so it still seems possible that they might play a role in the Ca^{2+}-dependent uncoupling. It is known, however, that Ca^{2+}-dependent uncoupling does not involve proteolysis of the DHPRs, RyRs, or another junctional protein, triadin (254). Interestingly, triad junction structure has been found to depend both on calpain-susceptible and calpain-resistant interactions (242), so Ca^{2+}-dependent disruption of triad structure and coupling may involve a number of different processes, leading to quite complex behavior of onset and recovery. Finally, neither μ-calpain nor calpain-3 was detectably activated by exhaustive sprint cycling in untrained human subjects or by prolonged cycling in trained subjects (320). Possibly calpain activation may only occur with relatively extreme exercise or when there is a damaging eccentric component.

In summary, the delayed recovery after fatigue is due primarily to a reduction in SR Ca^{2+} release, which may involve elevated $[Ca^{2+}]_i$ in some way causing impaired coupling between the t-tubular voltage sensors and the SR Ca^{2+} release channels, but the exact mechanisms involved remain uncertain.

XIV. BLOOD FLOW AND INTRACELLULAR Po_2

In intact muscles, the supply of O_2 depends on blood flow and the diffusion of O_2 from capillary blood across the interstitial space and into the muscle fiber. A long-debated issue is whether O_2 supply through this pathway is adequate or whether there are conditions in which O_2 supply is limited and contributes to reduced muscle per-

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formance and more rapid fatigue (429). It has already been noted that blood flow ceases in a continuous maximal contraction (33), and under these circumstances, failure of O_2 supply will presumably contribute to the rapid fatigue. The dramatic effect of preventing oxidative phosphorylation with cyanide is illustrated in Figure 1.

During intermittent contraction, blood flow recovers between contractions, and it might be assumed that autoregulation of the blood supply would match the blood flow to the overall metabolic needs of the muscle. However, there is evidence that this does not necessarily occur. For instance, simply raising the arm above the head accelerates the rate of fatigue of the adductor pollicis (490). This arises because the hydrostatic fall in arterial pressure in the hand is not matched by the fall in venous pressure presumably because the veins collapse. Thus a small fall in driving pressure occurs in the raised hand, which presumably reduces the blood flow.

Some athletes show a mild arterial hypoxemia during high-intensity exercise, and this is associated with a reduced performance (380). This performance decline is partly caused by peripheral fatigue and can be reduced by an increased inspired [O_2] and exacerbated by reduced inspired [O_2] (13, 215). Furthermore, blood doping and abuse of recombinant human erythropoietin have become an increasing problem in endurance sports because they enhance maximum oxygen uptake and improve aerobic performance (126, 156).

The development of a NMR method for measuring oxygenated versus deoxygenated myoglobin in muscle has allowed estimates of the muscle P_{O_2} and provided new insights into the diffusion of O_2 from capillaries to the muscle fibers. The P_{O_2} in resting capillaries and muscle are respectively 44 and 34 mmHg, whereas during exercise these values fall to 34 and 3 mmHg (376). The average value of 3 mmHg does not change in muscle from mild to severe exercise, suggesting that the diffusion gradient is maximal and that further increases in O_2 supply must come from increasing blood flow, opening new capillaries, etc. (377).

In summary, in intact individuals, supply of O_2 from capillaries to muscle fibers provides one limit to muscle performance during prolonged aerobic exercise.

XV. CONCLUDING REMARKS

It is widely accepted that fatigue has many causes and that in different physical activities both the mechanisms involved and their quantitative importance can vary. Consider the fatigue caused by voluntary repeated short tetani, which in a human can lead to a 50% reduction in electrically stimulated force (48). Of the 50% decline, we suggest that the early fall (phase 1 in Fig. 1), which is usually a ~10% reduction, is likely to be caused by a reduction in $F_{Ca,max}$ due to increased P_i. The remaining 40% has to be attributed to changes in activation, which include contributions from decreased Ca^{2+} sensitivity of the contractile proteins and reduced SR Ca^{2+} release. The reduced Ca^{2+} sensitivity could have contributions both from metabolites such as P_i and from the effects of ROS. The most likely causes for reduced SR Ca^{2+} release appear to be precipitation of Ca^{2+} phosphate in the SR with a contribution from reduced ATP and raised Mg^{2+}. Our interpretation of the evidence is that failure of the AP in the surface or the t-tubular membrane probably makes little contribution to the failure of SR Ca^{2+} release during repeated short tetani. If failure of the AP does contribute to fatigue, it is most likely to be during continuous maximal contractions when absence of perfusion enhances the K^+ accumulation, although slowing of the cortical firing rate, acid-induced reduction of the Cl^- conductance, acceleration of the Na^+-K^+ pump, and the large safety factor of the AP all help to minimize such failure. Figure 8 summarizes the mechanisms we believe to be important in fatigue.

The evidence for the above mechanisms comes largely from experiments on isolated animal muscle preparations. In intact muscle, the force reduction is easy to measure, and many of the metabolic changes can be estimated from NMR or muscle biopsies, but this information does not allow identification of the mechanisms involved. One promising new approach to study fatigue in intact animals is the use of genetically encoded calcium sensors that can provide Ca^{2+} signals from intact muscles (382, 434). This method could be used to assess the importance of failure of Ca^{2+} release in an intact, perfused model of fatigue. Another possibility would be if the target protein for the ROS-induced reduction in Ca^{2+} sensitivity was identified; this might allow assessment of this form of fatigue from a muscle biopsy. Other molecular targets, e.g., phosphorylation of the RyR (460), are also susceptible to this approach.

A major focus for the future must be to identify the mechanisms that contribute to human fatigue in various activities and particularly during disease processes. Increased fatiguability is reported in numerous clinical conditions; in fact, it has been claimed to be the most frequent presenting symptom in a primary healthcare setting (205). Fatigue, as reported by patients, would occur when the intended physical activity can no longer be continued or is perceived as involving excessive effort and discomfort. Thus the decreased fatigue resistance in many clinical conditions can be due to other factors than a reduced fatigue resistance of the muscle cells per se, and this is illustrated in Figure 9. In principle, the time until the intended force or power output no longer can be maintained depends on the interaction between the required force, the maximum force that the muscles can produce, and the endurance of the muscle cells. For instance,

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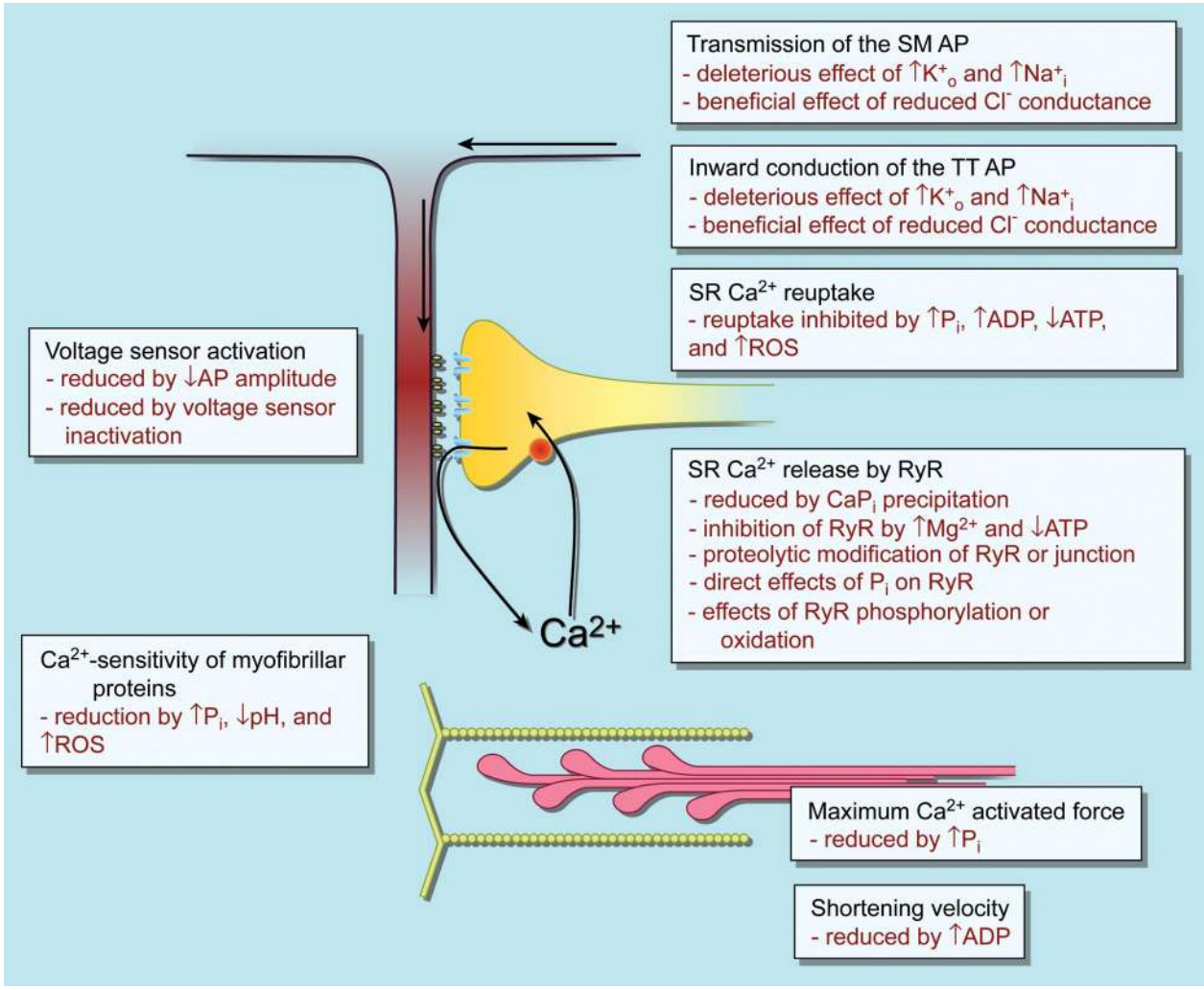

FIG. 8. Schematic diagram illustrating the major mechanisms that contribute to muscle fatigue. Heading in each box identifies subcellular function, and the subsequent list indicates cellular changes occurring during fatigue that influence the subcellular function. SM, surface membrane; TT, t tubule; SR, sarcoplasmic reticulum; AP, action potential.

obesity is associated with increased fatiguability during activities where counteracting gravity has a central role (e.g., walking and running), and this can be explained by an increase in the required force due to the increased body weight. On the other hand, obesity has little effect on the performance during activities where gravity is less important (e.g., swimming).

Numerous disorders like cancer cachexia, general inflammatory diseases, sepsis, burns, human immunodeficiency syndrome, chronic kidney failure, muscular dystrophies, as well as normal aging are associated with a loss of skeletal muscle tissue (291). The decreased muscle mass in these conditions results in a decreased capacity of muscles to generate force. Early fatigue development can then occur because muscles always have to work at a higher fraction of their maximal capacity, whereas the actual fatigue resistance of the muscle cells may not be affected.

There are also disorders where the fatigue resistance of the muscle cells is decreased, but this group appears rather small. Mutations in proteins involved in energy metabolism can result in a markedly increased fatiguability; for instance, patients with myophosphorylase deficiency cannot break down glycogen and show a markedly accelerated fatigue development (78). On the other hand, myoadenylate deaminase deficiency, which decreases the ability to deaminate AMP to IMP, has a relatively high incidence (~2%) in the general population and is only associated with limited changes in muscle performance (122, 336).

Patients with mitochondrial myopathies and deficient respiratory chain function may display severe muscle dysfunction (128). Intuitively, mitochondrial myopathy would lead to premature fatigue development due to an increased dependency on anaerobic metabolism during fatiguing stimulation. However, a recent study em-

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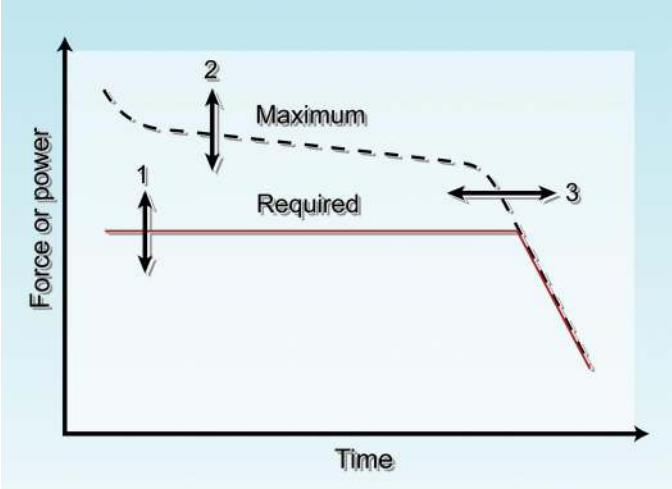

FIG. 9. Schematic to illustrate different mechanisms leading to exhaustion. Dashed line shows how the maximum force (or power) declines during repeated tetani. Solid red line indicates a submaximal force required for a particular activity. Exhaustion (failure to produce the required force) occurs at the intersection of the two lines. Increases and decreases in the required force (*arrow 1*) will cause earlier and later onset of exhaustion, respectively. Increases and decreases in the maximum force that the muscle can produce (*arrow 2*) will also change the time to exhaustion. Finally, changes in the intrinsic fatiguability of the muscle (*arrow 3*) will also change the time to exhaustion.

ploying a mouse model for mitochondrial myopathy, induced by skeletal muscle specific disruption of the gene for mitochondrial transcription factor A, showed no increase in the rate of fatigue development compared with wild-type controls, but the ability to generate force was markedly decreased in mitochondrial myopathy muscles (489). The unexpected lack of effect on fatigue development could be explained by an increased mitochondrial mass in mitochondrial myopathy muscle that compensated for the respiratory chain deficiency. Thus reduced mitochondrial ATP production might not be as critical for the pathophysiology of mitochondrial myopathy as previously thought.

Patients with congestive heart failure (CHF) frequently report decreased fatigue resistance, which intuitively would be related to an impaired O_2 delivery to the working muscles. However, there is a poor correlation between the decrease in fatigue resistance and the decrease in heart function (486). This suggests that CHF causes intrinsic defects in skeletal muscles, and numerous studies have reported important functional and biochemical changes in skeletal muscle cells associated with CHF (for recent reviews, see Refs. 202, 289, 330). These changes may affect fatigue development by acting both on myofibrillar function and on SR Ca^{2+} handling (287, 288, 372, 457).

In the clinical setting, a careful analysis of the muscle function is important for designing optimal strategies to treat patients with decreased fatigue resistance. As described above, increased fatiguability is frequently due to a general decrease in muscle force production rather than a decreased fatigue resistance of the muscle cells. This means that a strength training program to increase, or avoid further loss of, muscle mass may improve fatigue resistance more effectively than an endurance training program.

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GRANTS

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