Eur J Appl Physiol (2010) 110:1–15
DOI 10.1007/s00421-010-1480-0

REVIEW ARTICLE

Muscle fatigue: from observations in humans to underlying mechanisms studied in intact single muscle fibres

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Accepted: 7 April 2010 / Published online: 24 April 2010

Abstract Prolonged dynamic exercise and sustained isometric contractions induce muscle fatigue, as manifested by decreased performance and a reduction in the maximum voluntary contraction force. Studies with non-invasive measurements in exercising humans show that mechanisms located beyond the sarcolemma are important in the fatigue process. In this review, we describe probable cellular mechanisms underlying fatigue-induced changes in excitation–contraction (E–C) coupling occurring in human muscle fibres during strenuous exercise. We use fatigue-induced changes observed in intact single muscle fibres, where force and cellular Ca^{2+} handling can be directly measured, to explain changes in E–C coupling observed in human muscle during exercise.

Keywords Muscle fatigue · Human · Evoked force · Electromyography · Single muscle fibre · Ca^{2+} handling · Myofibrillar function

Introduction

Muscle fatigue refers to the decreased force/power generating capacity (Gandevia 2001) during and following prolonged or repeated muscle activity. Numerous factors influence muscle output during prolonged exercise, from oxygen transport capacity to metabolic substrate availability, from efferent motor command from the brain to contractile protein interaction within the muscle fibres. This review will highlight some of the probable mechanisms underlying muscle fatigue observed in exercising humans by using a translational approach. We will show that data obtained from single muscle fibres under tightly controlled physiological conditions are useful to understand acute changes in contractile function when people perform repeated or prolonged exercise. First, the techniques that can be used to study muscle fatigue in humans and in isolated single muscle fibres will be overviewed. Second, changes in muscle performance in humans will be summarized and discussed with respect to global activities (cycling, running) as well as during sustained contractions of isolated muscle groups. Last, we discuss how data from single muscle fibres have been used to confirm or exclude cellular mechanisms supposed to underlie fatigue in humans. This review is not meant to be all inclusive but rather to emphasize the cross-fertilization that is possible when data from single cells and whole animals are considered together. We focus on studies where fatigue-induced changes in isometric force have been assessed because most comparable studies have used this type contraction, but altered contractile speed is also briefly considered.

Communicated by Nigel Taylor.

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Methods to study fatigue-induced changes in excitation–contraction coupling in humans and in isolated single fibres

During exercise central motor areas activate the α-motoneurones, which in turn activates the skeletal muscle cells.

Excitation–contraction (E–C) coupling describes the different steps necessary to convert an action potential to cross-bridge formation in muscle cells. Each action potential generated at the neuromuscular junction propagates along the surface membrane of the muscle fibre and into the transverse tubules (t-tubules), where it triggers a transient release of Ca^{2+} from the sarcoplasmic reticulum (SR). The transient rise in myoplasmic free Ca^{2+} concentration ($[Ca^{2+}]_i$) allows formation of crossbridges and thus contraction. Reuptake of Ca^{2+} into the SR ends the cycle and the muscle relaxes.

In humans it is difficult to distinguish between different factors that may be responsible for the decreased force-generating capacity during fatiguing exercise, as changes may occur in parallel at a multitude of sites: in the neural drive, in the propagation of muscle fibre action potentials, in the contractile apparatus, in the blood flow, etc. Indirect, non-invasive techniques were developed to study fatigue in humans more than 30 years ago (Merton 1954; Bigland-Ritchie et al. 1978) and these are still being widely used. In humans, fatigue is usually quantified by measuring the reduction of the maximal voluntary contraction force performed under isometric conditions. As long as the force remains reduced compared with the pre-exercise period, a state of fatigue can be considered to exist. Voluntary evoked contractions coupled with measurements of force and surface electromyography (EMG) are used to localize the potential sites of fatigue along the neuromuscular system. Changes in the contractile properties of individual muscles have been studied with percutaneous electrical stimulation of the motor nerve or directly of the muscle thus bypassing the brain and spinal cord. For instance, a single supramaximal electrical stimulus (generally 120–150% of the stimulus that first evokes a contraction) elicits an action potential in muscle cells and force is generated. The resultant compound muscle action potential (M wave) and the maximal mechanical twitch response (peak twitch force, Pt) are recorded. A reduction in M-wave amplitude is interpreted as evidence of impaired neuromuscular transmission or action potential propagation (Bigland-Ritchie et al. 1982) and if Pt is reduced to a comparable extent, this probably indicates that the impaired force production is due to reduced muscle excitability (Zory et al. 2005). However, it should be noted that action potentials in muscle cells might have a large safety margin so that their amplitude can be greatly reduced before Ca^{2+} release from the SR and hence force are affected (Lännergren and Westerblad 1986; Balog et al. 1994; Allen et al. 2008). In contrast, a greater reduction in Pt than in the M-wave amplitude indicates that the alteration is distal to the sarcolemma and involves changes in Ca^{2+} release, myofibrillar Ca^{2+} sensitivity and/or force produced by active cross-bridges. Other methods such as the non-invasive nuclear magnetic resonance (NMR) imaging (Sapega et al. 1987), invasive muscle biopsy (Bergström et al. 1969) and blood samples are used to investigate ionic and metabolic changes associated with muscle fatigue. These techniques provide values at selected points in time and allow one to correlate ionic and metabolic changes with changes in force production.

The different methods used to assess E–C coupling function in studies of human exercise are summarized in Fig. 1. When scrutinizing these methods it becomes clear that they provide limited information about changes in E–C coupling during exercise and their underlying mechanisms as (1) Ca^{2+} handling cannot be directly assessed and (2) many other factors that cannot be precisely controlled influence the measures.

Ca^{2+} ions are essential for the conversion of the electrical stimulus to the mechanical response. Ideally, any attempt to characterize E–C coupling should include methodologies allowing force measurement combined with measurement of $[Ca^{2+}]_i$ and/or manipulations in $[Ca^{2+}]_i$. This can be done in isolated muscle fibres, where force and $[Ca^{2+}]_i$ can be measured in the intact cell. Various manipulations of cellular Ca^{2+} handling (e.g. partial inhibition of SR Ca^{2+} release or reuptake) and of energy metabolism that would be impossible or ethically unacceptable in humans can easily be performed in experiments on intact single fibres (Fig. 1).

Since the end of the 1980s, the model of intact single skeletal muscle fibres has been used to describe changes in mammalian muscle function related to exercise (Lännergren and Westerblad 1987). The first step is to manually dissect a single viable fibre. Fibres are most easily obtained from the fast twitch flexor digitorum brevis (FDB), but more recently fibres have also been isolated from the fast extensor digitorum longus and slow soleus muscles, allowing one to work with fibres having a large range of fatigability (Bruton et al. 2003; González and Delbono 2001). Tendons are kept on both ends of the fibre to permit force recording once the preparation is transferred to the experimental chamber and electrically stimulated. The fibre can be loaded with fluorescent indicators for measurements of Ca^{2+}, H^+ and other ions/molecules. Repeated tetani of 300–600-ms duration with a duty cycle between 0.1 and 0.5 are used to induce a fall in tetanic force (fatigue), usually to 40–50% of initial force. The number of tetani required to induce fatigue is largely dependent on the fibre's metabolic characteristics and can vary from $\sim$50 to 100 in the fast-twitch mouse flexor digitorum brevis (Westerblad and Allen 1991) to several hundreds in limb muscle fibres (Bruton et al. 2003; González and Delbono 2001).

An alternative to using isolated intact fibres is the usage of skinned fibres (whose sarcolemma has been removed):

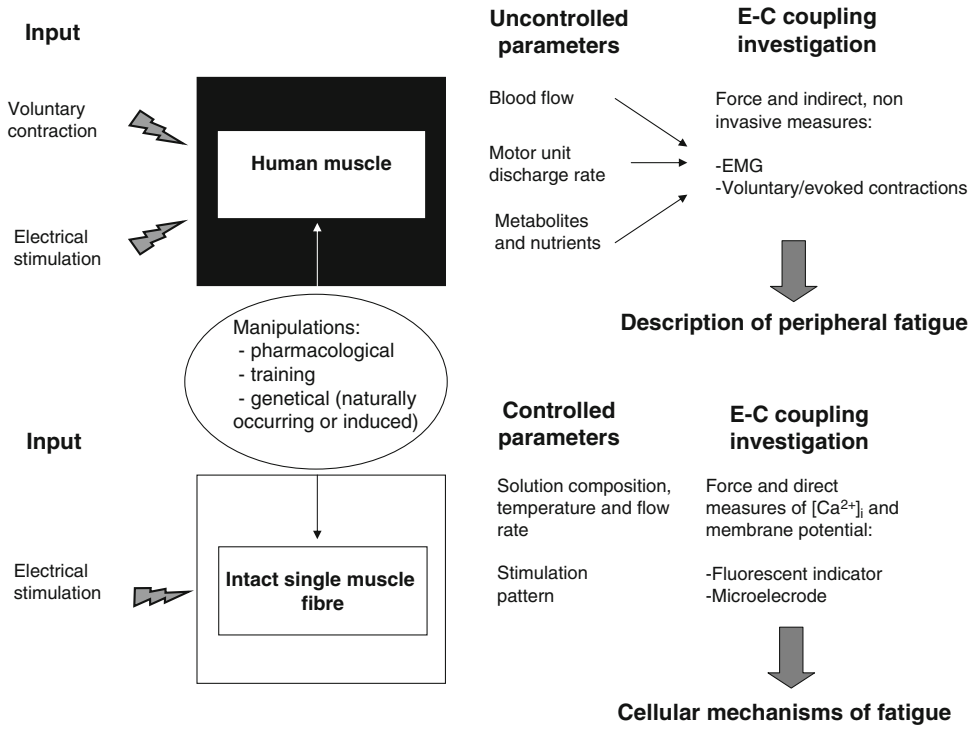

Fig. 1 Schematic diagram illustrating possibilities to study excitation–contraction (E–C) coupling in human muscles and in isolated single muscle fibres. In exercising humans, muscle can be considered as a *black box* where the outcome depends on several parameters which cannot be controlled and where direct measurements cannot be performed. Experiments on isolated muscle fibres allow more parameters to be controlled and more direct measurements can be performed

in skinned fibres the force response to changes in $[Ca^{2+}]$, metabolites, etc. can be studied simply by altering the composition of the bath solution, which in this preparation represents the intracellular milieu. Thus, skinned fibres can be used to assess the effect on force of individual factors such as $[Ca^{2+}]$, inorganic phosphate (Pi), Mg^{2+}, pH, etc. (see Lamb 2002 for a review). An advantage with the skinned fibre technique is that fibres can be obtained not only from animal muscles but also from biopsies obtained from human muscles (Widrick et al. 1998; Malisoux et al. 2006). In this context it should be noted that fully functional intact single fibres are difficult to obtain from human muscles. The potential problems with studying fatigue-induced changes in E–C coupling with the skinned fibre technique are that soluble cellular proteins are lost during the skinning procedure and the spacing between myosin and actin filaments is changed (Allen et al. 2008). Moreover, activation of the contractile apparatus is mostly performed by increasing $[Ca^{2+}]$ in the bath solution rather than by triggering action potentials, although action potential-induced contractions can be produced in mechanically skinned fibres with sealed t-tubules (Posterino et al. 2000).

Human muscles have been stimulated with electrical current pulses at different frequencies and the resulting force production plotted against the stimulation frequency (Fig. 2a). Functional changes in fatigued muscles will be reflected in altered force–frequency relationship but detailed information about the underlying changes in E–C coupling is not revealed. Intact single fibres have also been stimulated with electrical current pulses at different frequencies and they display a force–frequency relationship remarkably similar to that observed in human muscles. In single fibres it is possible to combine force recordings with measurements of $[Ca^{2+}]_i$. Force and $[Ca^{2+}]_i$ in contractions at different frequencies can then be used to construct force–$[Ca^{2+}]_i$ curves in the unfatigued state (Fig. 2b, black circles). The force–$[Ca^{2+}]_i$ curves obtained in this way are similar to those previously obtained in skinned fibres exposed to bath solutions with different $[Ca^{2+}]$ (e.g. Stephenson and Williams 1981). Force–$[Ca^{2+}]_i$ curves in intact fibres can also be obtained during fatigue by plotting force against $[Ca^{2+}]_i$ as fatigue progresses (Fig. 2b, white circles). Fatigue-induced changes in force production can then be analysed in terms of a generally decreased ability of cross-bridges to generate force (a general downward shift of the curve), decreased myofibrillar Ca^{2+} sensitivity (a rightward shift of the curve), a decreased SR Ca^{2+} release (leftward movement along the curve), or a combination of these (Fig. 2b).

Central versus peripheral fatigue

Classically, changes leading to impaired force generating capacity are described as "central" or "neural" when located prior to the neuromuscular junction, and as "peripheral" or "muscular" beyond the neuromuscular

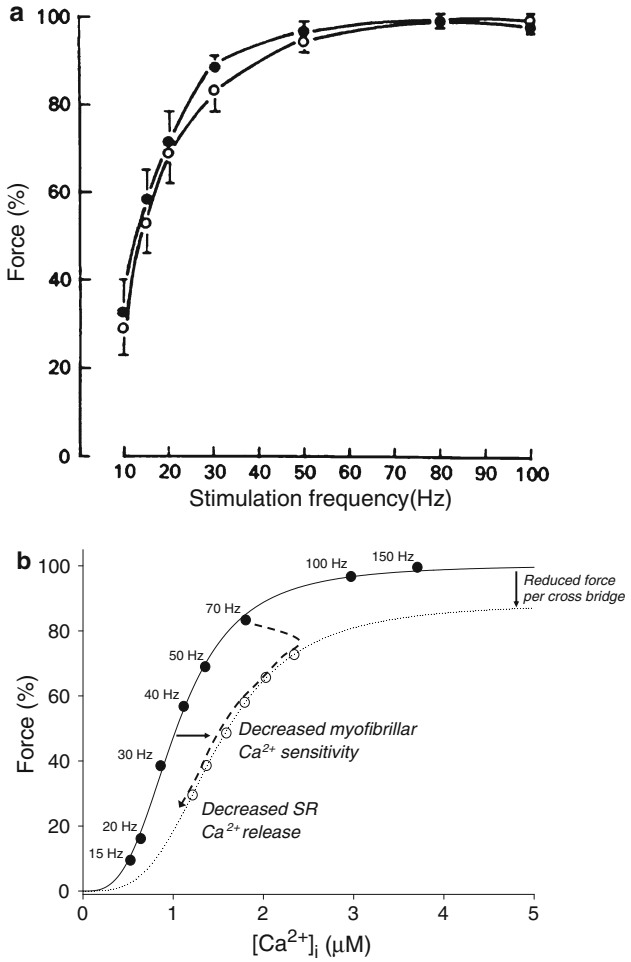

Fig. 2 **a** Force–frequency relationship is steep in human skeletal muscle (*black circles* quadriceps; *white circles* adductor pollicis). Reprinted and modified from Edwards et al. (1977) with the permission of Wiley Blackwell publisher. **b** Force–$[Ca^{2+}]_i$ relationship is also steep. Data points are obtained from an intact single mouse flexor digitorum brevis (FDB) fibre with 350-ms tetanic stimulations elicited at various frequencies (15–150 Hz) on a resting fibre (*black circles*) and at various times during repeated 70-Hz tetanic contractions, i.e. during the induction of fatigue (*white circles*). During fatigue force can proportionally decrease at all $[Ca^{2+}]_i$ due to a reduced force per cross-bridge (*vertical arrow*) and/or the force–$[Ca^{2+}]_i$ relationship can be shifted to the right due to decreased myofibrillar Ca^{2+} sensitivity (*horizontal arrow*). The *dashed line* illustrates the typical pattern during fatigue, which during the latter part of fatiguing stimulation also includes a decrease along the force–$[Ca^{2+}]_i$ relationship due to reduced SR Ca^{2+} release

junction. The decreased force-generating capacity can be thought of as consisting of central fatigue elements and peripheral fatigue elements. The assessment of the peripheral fatigue elements underlying task failure and/or reduced maximal voluntary contraction (MVC) force is done by monitoring M-wave, Pt and force production during tetanic stimulation performed at different frequencies (Merton 1954; Edwards et al. 1977; Millet and Lepers 2004). Central fatigue is classically identified with the twitch interpolation technique in humans (Merton 1954), where an electrical stimulus is given to the peripheral nerve during the plateau phase of a MVC. When this results in an increased force, it is concluded that the descending drive to the motoneurone is not maximal and that central fatigue exists. Recently, we applied this twitch interpolation technique to intact fatigued single muscle fibres and observed an increase in force during the plateau of an isometric contraction indicative of "central fatigue", which is obviously impossible in single fibres (Place et al. 2008). The explanation for the increased force lies in the sigmoidal shape of the force–$[Ca^{2+}]_i$ relationship. As fatigue develops, tetanic $[Ca^{2+}]_i$ decreases and fibres move to the steep part of the force–$[Ca^{2+}]_i$ relationship where a small increase in tetanic $[Ca^{2+}]_i$ has a very large effect on the developed force (see Fig. 3). Thus, an intracellular mechanism in the form of an increased tetanic $[Ca^{2+}]_i$ can account for the relative increase in the extra force generated by an interpolated twitch during fatigue (Place et al. 2008). As the twitch interpolation technique is used with force levels corresponding to MVC, paralleling the situation in single fibres, there is the likelihood that the extent of central fatigue can be overestimated with this technique (Place et al. 2008). This "peripheral contamination" of the measure of the extent of central fatigue may occur when all the motor units are recruited, i.e. when the force level is mainly modulated by changes in motor unit firing rate. The upper limit for motor unit recruitment is $\sim$ 85% MVC in most muscles and even less for smaller muscles (Duchateau et al. 2006) and above this level further increases in muscle force are essentially produced by increased discharge frequency of motor neurones.

In addition, any change in surface EMG activity may reflect changes in motor unit recruitment strategy by the CNS and/or peripheral changes, such as impairments in neuromuscular transmission or action potential propagation along the muscle fibres. Normalization of the integrated EMG signal to the M-wave amplitude or area is used to minimize peripheral contamination and hence enhance the sensitivity of this method to assess the level of central motor output. However, this method of assessing central fatigue should also be interpreted cautiously, since some inconsistencies in these measurements have been reported (Place et al. 2007a). Furthermore, α-motoneuron excitability can be assessed with the Hoffmann reflex (H reflex), where the Ia afferents are electrically stimulated which then activate the motor neurons; the EMG response (usually recorded on soleus muscle in humans) depends on the facilitation of the synaptic transmission and changes in pre- and post-synaptic inhibitions in the spinal cord (Schieppati 1987). H-reflex amplitude has been used in numerous studies attempting to assess change in the balance of the excitations and inhibitions received by the α-motoneurons

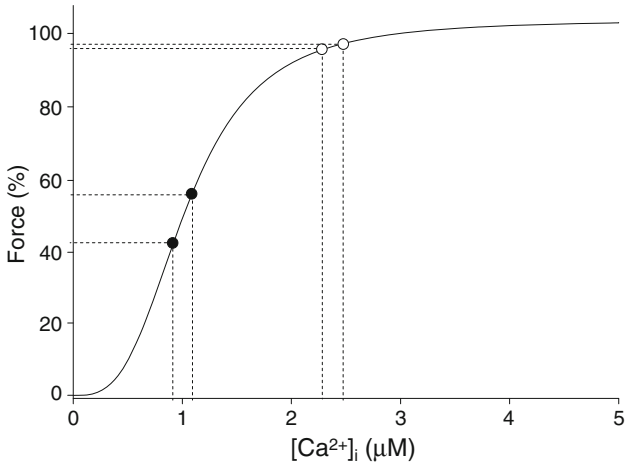

Fig. 3 The sigmoidal shape of the force–$[Ca^{2+}]_i$ relationship explains why a change in $[Ca^{2+}]_i$ on the steep part of the curve (resulting from low-frequency stimulation in unfatigued fibres or higher frequency stimulation in fatigued fibres, *black circles*) has much greater effects on force production compared to a similar change in $[Ca^{2+}]_i$ near or on the plateau of the curve (high frequency stimulation in unfatigued fibres, *open circles*). This sigmoidal relationship between force and $[Ca^{2+}]_i$ explains why (1) "central fatigue" can be observed in isolated single fibres, (2) post activation potentiation is important at non-saturated Ca^{2+} levels and non-existent at high relative force levels and (3) a small change in $[Ca^{2+}]_i$ in fatigued fibres results in large variations in force

during/after exercise (Place et al. 2009a; Duchateau et al. 2002; Löscher et al. 1996; Racinais et al. 2007), and no consensus has really emerged certainly because of different experimental conditions.

Assessment of sites of fatigue with sustained isometric contractions and with global dynamic exercise

Two types of prolonged exercise can be distinguished in vivo: (*i*) sustained contractions until voluntary exhaustion or for a given time period, which focus on a particular muscle group and are usually performed under isometric conditions, and (*ii*) global exercises, such as cycling, running or skiing, which involve a large muscle mass and are performed under dynamic conditions. In experiments where subjects undertake sustained isometric contractions, it is easy to record many parameters during the course of the experiment, as subjects are already installed in the appropriate isometric ergometer. In contrast, the assessment of the extent of muscle fatigue following running or cycling bouts is not as simple, as the subjects have to be moved from the treadmill/cycle ergometer to the isometric ergometer (which may take 1-3 min). The recovery process has already begun during this period and the extent of muscle fatigue may be underestimated.

One confounding factor in the measurement of the degree of muscle fatigue is the presence of post activation potentiation (PAP). PAP, or post-tetanic potentiation as it was first called (Walker 1951), is defined as the transient increase in muscle twitch or low-frequency tetanic force after a conditioning contractile activity (Sale 2002), which is usually a MVC. PAP is frequently used to characterize exercise-induced changes in contractile function, and it can also be used to illustrate the importance of taking the sigmoidal shape of the force–$[Ca^{2+}]_i$ relationship into account when assessing muscle function. PAP is observed only at non-saturating $[Ca^{2+}]_i$ as occurs during single muscle twitches, doublets (two stimulation pulses separated by ~10 ms) and low-frequency tetanic stimulation. Thus, it is observed when muscles are on the steep part of the force–$[Ca^{2+}]_i$ relationship, which means that it would be due to increased Ca^{2+} release from the SR and/or increased myofibrillar Ca^{2+} sensitivity (see Fig. 2b). PAP has been found to be important only in fast-twitch skeletal muscle fibres and the molecular mechanism underlying the phenomenon is suggested to be due to phosphorylation of the myosin regulatory light chains resulting in increased myofibrillar sensitivity to Ca^{2+} (Sweeney et al. 1993; Rassier and MacIntosh 2000). PAP has been found to be reduced after prolonged isometric (Place et al. 2006) or repeated dynamic (Klass et al. 2004) contractions, as well as after prolonged running exercise (Millet et al. 2003a), which might be interpreted as evidence of decreased myofibrillar Ca^{2+} sensitivity in these types of fatigue. However, a fatigue-induced decrease in SR Ca^{2+} release would also decrease PAP and the relative contribution of these two factors cannot be distinguished in experiments on exercising humans.

Table 1 summarizes results from studies that have measured M-wave amplitude and/or Pt before and immediately after prolonged isometric contractions performed at intensities ranging from 15 to 80% MVC. As can be seen, M-wave amplitude was unchanged in 10 out of 14 studies of fatigue, suggesting that impaired sarcolemmal excitability is not a prerequisite in human muscle fatigue (as evidenced by the reduced MVC force). On the other hand, the decrease in Pt was far greater than the M-wave changes in 6 of the 11 studies that reported both measures, which suggests impairment within the muscle fibres themselves. However, it must be noted that Pt is far from being a perfect measure of fatigue. For instance, whereas fatigue-induced changes tend to decrease Pt, this might be counteracted by PAP induced by the preceding muscle activity and hence the extent of peripheral fatigue might be underestimated (Rassier and MacIntosh 2000). In this context it is interesting to note that two studies in Table 1 actually show Pt potentiation associated with decreased MVC and minimal alterations in M-wave properties after

Table 1 Quantification of neuromuscular fatigue after prolonged isometric contractions measured in various muscle groups

Study	Fatiguing task	Muscle group	MVC change (%)	M-wave amplitude change (%)	Pt change (%)	Pd change (%)
West et al. (1996)	30% MVC for 3 min	Knee extensors	–	NS	−58%	–
Plaskett and Cafarelli (2001)	50% MVC, intermittent (15 s on/1.5 s off), ~1 min 30	Knee extensors	−34%	NS	−70%	–
Gondin et al. (2006)	20% MVC, ~8 min	Knee extensors	−25%	NS	NS	–
Place et al. (2005)	20% MVC, ~16 min	Knee extensors	−28%	−5%	32%	19%
Place et al. (2006)	EMG level corresponding to 20% MVC, ~3 min	Knee extensors	−28%	NS	NS	−11%
Place et al. (2007b)	EMG level corresponding to 40% MVC, ~4 min	Knee extensors	−18%	NS	−11%	−14%
Löscher et al. (1996)	30% MVC, ~6–9 min	Plantar flexors	–	NS	NS	–
Kuchinad et al. (2004)	Low intensity: 25% MVC, ~20 min high intensity: 42–66% MVC, ~3 min	Plantar flexors	Low intensity: −27% high intensity: −34%	Low intensity: −9% high intensity: NS	–	–
Place et al. (2009a)	EMG level corresponding to 80% MVC, ~2 min	Plantar flexors	−20%	NS	28%	NS
Lévénez et al. (2005)	50% MVC, ~5 min	Dorsiflexors	−41%	NS	–	–
Bilodeau et al. (2001)	35% MVC, ~3 min	Elbow flexors	–	NS	−40%	–
Søgaard et al. (2006)	15% MVC, 43 min	Elbow flexors	−42%	NS	−41%	–
Fuglevand et al. (1993)	20, 35 and 65% MVC, ~1, 4 and 9 min, respectively	First dorsal interosseous	−19 to −40%	−12 to −26%	−55 to −60%	–
Duchateau et al. (2002)	Low intensity: 25% MVC, ~8 min high intensity: 50% MVC, ~3 min	Abductor pollicis brevis	Low intensity: −34% high intensity: −26%	Low intensity: −20% high intensity: −10%	–	–

MVC maximal voluntary contraction, *Pt* peak twitch force or torque, *Pd* peak doublet, *NS* not significant, – not measured

exercise, illustrating the perpetual competition between fatigue and potentiation. Moreover, twitch and doublet forces will always be on the steep part of the force–$[Ca^{2+}]_i$ relationship, where small changes in many parameters can have large effects. Thus, prolonged isometric contractions frequently lead to impaired E–C coupling and decreased force production in muscle fibres, but the extent of the contractile impairment and the underlying mechanisms cannot be revealed by measuring Pt and M-wave amplitudes.

Muscle fatigue after prolonged global dynamic exercise is closer to real-life sports activity than sustained isometric contractions. In this case, the identification of the underlying mechanisms of muscle fatigue might help to optimize physical training and competition performance. As for sustained contractions, it appears that the degree of central fatigue is greater for bouts of long-duration exercise (>2 h) than for shorter, more intense bouts (for a review see Millet and Lepers 2004). The decrease in MVC force varies between ~10–30%, depending on the details of the task and on the delay between the end of exercise and the MVC measurement, which is introduced because the need for subjects to move to a new apparatus. In fact, the lack of delay in measuring MVC force after a sustained isometric contraction compared with the few minutes delay after global exercise might explain the slightly greater degree of force loss after isometric contractions (MVC force loss ~20–40%) in comparison with that with prolonged cycling or running. Table 2 summarizes changes in contractile function reported after prolonged running and cycling exercises of durations up to 8 h. About half the studies reported unchanged M-wave amplitude, whereas a majority (9 of 14) reported reduced Pt amplitude (Table 2). Low-frequency contractions or twitches evoked after global dynamic exercise may also be potentiated compared with before exercise (Table 2); however, these results seem dependent on the exercise type, duration and intensity and also the time since the end of the exercise and the measurement. For example, it has been shown that prolonged running (Place et al. 2004; Millet et al. 2002) and skiing exercises (Millet et al. 2003b) may transiently potentiate the Pt response of quadriceps muscle, whereas potentiation is not observed following prolonged cycling exercise lasting 30 min to 5 h (Lepers et al. 2000, 2001, 2002).

Cellular mechanisms underlying peripheral fatigue found in humans

Muscle biopsies, NMR and blood samples have been used to assess mechanisms underlying the impaired muscle performance during human exercise. These types of experiments generally produce correlations rather than directly revealing underlying mechanisms; fatigue-induced changes in a factor may correlate temporally and/or quantitatively with the impairment in contractile function, but it is seldom clear whether the factor causes the impairment or whether the correlation is coincidental. Nevertheless, these types of experiments have suggested factors that might play a central role in the impairment of contractile function during fatiguing exercise. The next section will show how single fibres can be used to identify the extent to which several factors contribute to fatigue. This will not be an exhaustive list but rather focussed on key changes that are widely considered to be involved in fatigue: (1) extracellular K^+ accumulation, (2) muscle acidosis, (3) Pi accumulation, and (4) reactive oxygen and nitrogen species.

(1) During repeated muscle activity, each action potential leads to an efflux of K^+, which increases the extracellular $[K^+]$, especially in the narrow t-tubules out of which it cannot easily diffuse. Thus, it is reasoned that a gradual rise in t-tubules $[K^+]$ would lead to depolarization and inactivation of Na^+ channels which in turn reduces SR Ca^{2+} release initially via a decrease in the amplitude of action potentials and subsequently complete failure of action potentials (Westerblad et al. 1990; Overgaard et al. 1999; Clausen and Nielsen 2007). Nevertheless, several pieces of evidence suggest that the depolarization induced by a rise in extracellular $[K^+]$ is not a major factor behind muscle fatigue in exercising humans (Jones 1996; Zhang et al. 2006). For instance, the force decrease observed in muscles kept in solutions with elevated $[K^+]$ under resting conditions can be counteracted by activation of the Na^+–K^+ pumps (Clausen et al. 1993; Clausen and Nielsen 2007). These pumps are activated during exercise and hence no marked effects of elevated extracellular $[K^+]$ on tetanic force were found during intermittent fatiguing tetanic stimulation of soleus whole muscle or single fibres (Zhang et al. 2006). Furthermore, the motor unit discharge rate in vivo is relatively low (~10–30 Hz) thus limiting the increase in extracellular $[K^+]$ (Bigland-Ritchie et al. 1983; Bellemare et al. 1983; Hennig and Lømo 1985) and may decrease further during fatiguing contractions (Bigland-Ritchie et al. 1983). Last, there is a resting leak Cl^- current in mammalian skeletal muscle cells that opposes depolarization. The intracellular acidosis, which often develops during vigorous exercise, decreases the Cl^- leak and thus facilitates the ability of Na^+ channels to generate action potentials (Nielsen et al. 2001; Pedersen et al. 2004, 2005). In conclusion, results on isolated muscle preparations are in accordance with the human data discussed earlier and show that impaired muscle excitability is not a prerequisite for fatigue induced by prolonged endurance exercise.

Table 2 Quantification of neuromuscular fatigue in various muscle groups after prolonged running and cycling exercises lasting 20 min to more than 8 h

Study	Fatiguing exercise	Muscle group	MVC change (%)	M-wave amplitude change (%)	Pt change (%)	Tetanic force change (%)
Davies and White (1982)	60 min level running 70% VO_2 max	Plantar flexors	−9%	–	−9%	P20: −18%
Millet et al. (2002)	65 km running race (511 min)	Knee extensors	−30%	NS	20%	–
Millet et al. (2003a)	30 km running race (189 min)	Knee extensors	−24%	−9%	−8%	P20: −10% P80: −9%
Place et al. (2004)	5 h running 55% MAV (treadmill)	Knee extensors	−28%	−34%	18%	P20: NS P80: NS
Gauche et al. (2006)	55 km running race (417 min)	Knee extensors	−37%	NS	NS	–
Racinais et al. (2007)	90 min running at first ventilatory threshold (treadmill)	Plantar flexors	−11%	−16%	−11%	–
Ross et al. (2007)	42 km running treadmill (208 min)	Dorsiflexors	−18%	NS	−35%	–
Skof and Strojnik (2006)	20 min running at anaerobic threshold (track)	Knee extensors	NS	–	−14%	P20: −21% P100: NS
Saldanha et al. (2008)	2 h running 75% VO_2 max (treadmill)	Plantar flexors	−17%	–	NS	–
Lepers et al. (2000)	2 h cycling 65% MAP (ergometer)	Knee extensors	−13%	VL: NS VM: −19%	−24%	–
Lepers et al. (2001)	30 min cycling 80% MAP (ergometer)	Knee extensors	−13%	NS	−20%	–
Lepers et al. (2002)	5 h cycling 55% MAP (ergometer)	Knee extensors	−18%	NS	−16%	–
Millet et al. (2003c)	140 km cycling race (278 min)	Knee extensors	−9%	–	NS	P20: NS P80: NS
Theurel and Lepers (2008)	33 min cycling with variations in power output (ergometer)	Knee extensors	−12%	−6.5%	−11%	–

MVC maximal voluntary contraction, *Pt* peak twitch, *Pd* peak doublet, *VL* vastus lateralis, *VM* vastus medialis, *NS* not significant, – not measured

(2) During intense exercise a significant fraction of the glycogen stores are broken down to lactate ions and H^+, resulting in an acidosis of ~0.5 pH units (Sahlin et al. 1976). Lactate has been shown by several different groups to have no major effect on force production and cannot be considered as a cause of the muscle fatigue (see Allen et al. 2008). There has been a lot of controversy as to whether muscle acidosis is a major factor in the development of muscle fatigue (see Fitts 1994). Indeed, data on skinned fibres, obtained at room temperature (i.e. ~20°C) showed that a reduction in pH decreased force and maximum velocity of shortening (Fabiato and Fabiato 1978; Metzger and Moss 1987; Chase and Kushmerick 1988), two processes commonly observed during fatigue. Muscle acidosis was thus promoted as the major mechanism underlying muscle fatigue. Later experiments conducted in both skinned (Pate et al. 1995) and intact single fibres (Westerblad et al. 1997a) at more physiological temperatures (~30°C) show that acidosis depressed tetanic force by less than 10% and did not accelerate the rate of fatigue development (Ranatunga 1987; Bruton et al. 1998, Westerblad et al. 1997a), but slightly reduced the rate of force relaxation (see below). These data agree with results obtained in exercising humans where the initial force recovery occurred without any simultaneous recovery of pH (Cady et al. 1989).

(3) During exercise, the breakdown of phosphocreatine results in a rise in the inorganic phosphate concentration ([Pi]) which has multiple effects inside muscle cells. Of particular interest to this review is the effect of [Pi] on tetanic force production. In skeletal muscle, resting [Pi] is about 1–5 mM but can rise to 30–40 mM during intense exercise (Cady et al. 1989). In permeabilised fibres, increasing [Pi] depresses tetanic force production (Cooke and Pate 1985; Coupland et al. 2001) which has been attributed to a decrease in the number of force-generating crossbridges (Caremani et al. 2008). However, while the force depression by [Pi] is marked at 20°C and lower temperatures, it is unlikely to contribute more than 15% to the force decline in the range of temperatures encountered during exhaustive exercise (Coupland et al. 2001). Moreover, a study on human muscles fatigued in vivo show a rapid initial decrease in [Pi], which was accompanied by little force decrease (Cady et al. 1989).

In addition, Pi might enter the SR during fatigue and it has been suggested that the rapid decline in tetanic $[Ca^{2+}]_i$ and force commonly observed towards the end of a series of 50–100 tetanic stimuli of intact single fibres (Westerblad and Allen 1991) could represent Ca^{2+}–Pi precipitation in the SR, thus reducing the free Ca^{2+} available for release from the SR and consequently muscle force (Fryer et al. 1995, 1997; Westerblad and Allen 1996).

Interestingly, brief bouts of exercise have been found to reduce [Pi] in skeletal muscle by up to 50% (Bruton et al. 1996, 1997). The consequence of this is a 5–10% increase in maximum tetanic force that persists for up to 15 min (Bruton et al. 1996, 1997). It would be exciting to optimize protocols to benefit from a low resting [Pi] in skeletal muscle at the start of exercise.

(4) The production of reactive oxygen and nitrogen species (hereafter called ROS) may increase during exercise, and there is widespread belief that these molecules play an important role in muscle fatigue. Infusion of the general antioxidant *N*-acetylcysteine has been shown to slow the onset of fatigue in well-trained humans (Reid et al. 1994; McKenna et al. 2006), but not in untrained subjects (Medved et al. 2003). However, Powers and Jackson (2008) noted that while some studies have reported beneficial effects of dietary supplements of antioxidants, others have noted no positive effects (Powers et al. 2004). The conflicting human and whole animal data suggests that the ROS status is tightly controlled and that simply swamping the tissues with one or more antioxidants overwhelms this balance. Indeed, recently it was reported that supplementary antioxidants may actually prevent the beneficial effects of exercise (Ristow et al. 2009). The use of single fibres has allowed us to determine that ROS affect several key steps in E–C coupling, although myofibrillar Ca^{2+} sensitivity appears to be most sensitive (Andrade et al. 1998, 2001; Moopanar and Allen 2005). Intriguingly, the effects of ROS were both time- and concentration-dependent. Thus, brief exposure to physiological levels of ROS resulted in augmented tetanic force while longer exposure times resulted in depressed tetanic force (Andrade et al. 2001). It is interesting to speculate that early in exercise the increased ROS production might help to maintain force but the longer the exercise proceeds, the longer the muscle fibres experience elevated ROS and then the deleterious effects of ROS will dominate. This suggests that ROS can affect recovery from fatigue (see below).

Several studies suggested that the production of superoxide may increase with temperature (van der Poel and Stephenson 2002; Edwards et al.2007), and the resulting oxidative stress was suggested to accelerate fatigue development in bundles of FDB fibres (Moopanar and Allen 2005). However, two groups have now reported that elevating muscle temperature about 6°C above that normally experienced by a muscle does not accelerate the onset of fatigue in intact single fibres (Reardon and Allen 2009; Place et al. 2009b).

To sum up, factors that tentatively have large impact on contractile function during human exercise have been identified from muscle biopsies, NMR recordings and blood samples obtained during exercise. The potential of these factors to affect force production and the mechanisms by which this can occur have then been studied in isolated single fibres. The results of these studies show that

increased [Pi] and possibly ROS can have important depressive effects on contractile function during fatigue, whereas increased extracellular [K^+] and decreased pH are less important.

Loss of power output

Athletic and everyday activities are generally performed using dynamic contractions, i.e. both lengthening and shortening of muscles. James et al. (1995) showed that the decrease in isokinetic force occurs more rapidly than the decrease in isometric force in human quadriceps muscle, suggesting that dynamic contractions require more energy than isometric contractions. Power output (force × velocity) is reduced with fatigue (de Ruiter et al. 1999; Jones et al. 2006), especially during high-intensity activities that require a high rate of contractions (Sargeant et al. 1981). Interestingly, the maximum power output of fatigued human muscle can be decreased more than expected from the changes in isometric force and maximum shortening velocity due to an increased curvature of the force–velocity relationship (Jones et al. 2006). Moreover, it was recently suggested that maximum power output from a limb is not obtained with all muscle operating at their individual peak power output (Wakeling et al. 2010), suggesting that an impaired coordination with fatigue can also reduce maximal power output during athletic activities. Thus, the assessment of isometric force cannot reveal all the mechanisms behind the reduced performance with fatigue and further mechanistic studies with dynamic contractions are clearly warranted, although these are generally more complicated to perform than studies under isometric conditions.

Force relaxation

Results from single fibres suggest that slowing of force relaxation is an important feature in fatigue of fast-twitch muscle fibres (Westerblad and Lännergren 1991; Westerblad and Allen 1994; Westerblad et al. 1997b), although the slowing might become less marked with increasing temperature (Westerblad et al. 1997a). In contrast, intact single fibres from the slow-twitch soleus muscle show very limited slowing in force relaxation (Bruton et al. 2003; Lunde et al. 2006; Place et al. 2009b). The slowing of force relaxation may, in principle, be caused by two mechanisms: slowing of SR Ca^{2+} pumping and/or slowing of cross-bridge cycling. The cross-bridge component appears to be the main factor involved in the fatigue-induced slowing of relaxation in mouse fast-twitch fibres (Westerblad and Allen 1993b; Westerblad et al. 1997b) and human adductor pollicis muscle (de Ruiter et al. 1999). Furthermore, in recent studies on human muscles the slowing of relaxation in the fatigued state was associated with altered cross-bridge cycling resulting in decreased power output and an increased curvature of the force–velocity relationship, which was suggested to reflect a reduction in the rate of cross-bridge attachment (Jones et al. 2006, 2009).

In exercising humans, the half-relaxation time of the twitch (measured before and immediately after exercise) is often used as a measure of slowing of force relaxation. Interestingly, studies showed no change (Booth et al. 1997; Place et al. 2004; Lepers et al. 2000; Gauche et al. 2006) or even a reduced half-relaxation time (Lepers et al. 2001; Millet et al. 2002) after prolonged dynamic exercises lasting 30 min–8 h. As slow-twitch fibres mainly contribute to prolonged exercise performed at submaximal intensity, results from single fibres are in accordance with human studies and indicate that fast-twitch fibres are preserved during this kind of exercise. On the other hand, slowing of relaxation is expected to be more important during intense exercise where performance is limited by the maximum speed at which repeated movements can be performed. For instance, any decrease in speed observed during a 400-m running race (Hanon and Gajer 2009) can be partly explained by the reduction in stride frequency. Figure 4 schematizes the effect of slowed muscle relaxation during vigorous exercise; even if muscles are still able to produce large amounts of force, the slowing in the rate

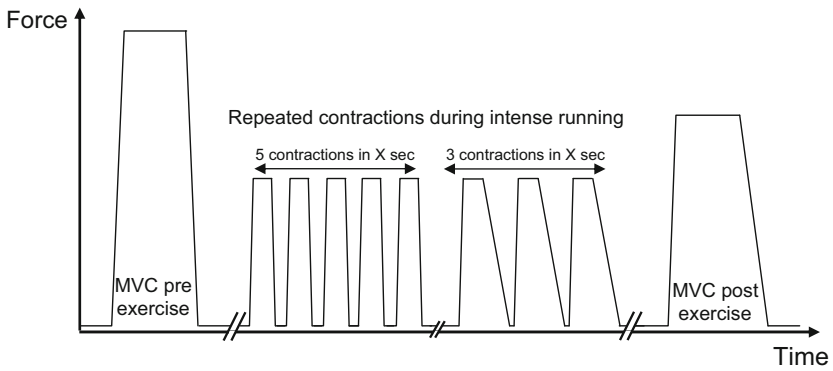

Fig. 4 A slowing of force relaxation may affect performance of intense exercise even if force generating capacity is relatively well preserved. In this example, running speed would be reduced to 3/5 of the original (assuming that the stride length remains constant). *MVC* maximal voluntary contraction

of relaxation limits stride frequency, which in turn affects performance.

A candidate for the reduced rate of force relaxation is acidosis in muscle cells, which certainly develops at a larger extent and has a much greater effect on muscle performance during a 400-m race (duration ~45–50 s) than a marathon race. Indeed, studies performed with intact single fibres show that the rate of force relaxation is reduced with acidosis (Bruton et al. 1998, Westerblad et al. 1997a, Westerblad and Allen 1993a, Westerblad and Lännergren 1991). However, acidosis is not the only mechanism and probably not the most important factor in the slowing of force relaxation (Cady et al. 1989). Results from mammalian muscle fibres show that processes located at the cross-bridge level (Westerblad et al. 1997b; Westerblad and Allen 1994) and probably involving Pi (Allen et al. 2008) contribute to the slowing of relaxation observed during the development of fatigue.

Recovery after fatiguing exercise

The full recovery of contractile function after fatiguing activities can be very slow, sometimes requiring days for full recovery. A markedly delayed force recovery was first described in human muscles and was most marked at low-stimulation frequencies and therefore called "low-frequency fatigue" (Edwards et al. 1977). Forces evoked at high-stimulation frequencies are relatively well preserved (Edwards et al. 1977) and thus this force deficit can be distinguished from the force deficit produced by muscle damage which would impair muscle force production at both low- and high frequencies. Importantly, these authors made the point that is often forgotten or ignored nowadays, that most voluntary contractions are maintained with motoneurones discharging at no more than ~30 Hz (Bigland-Ritchie et al. 1983; Bellemare et al. 1983). Thus, there is a very strong relationship between neurone discharge and force production (Fig. 2a). This has an important consequence in that during fatigue development and recovery, subjects can voluntarily counteract the force deficit by increasing motoneurone discharge rate. This increase in neural activity will lead to a perception of greater sense of effort (Carson et al. 2002). As shown in Fig. 3, a similar fatigue-induced reduction in tetanic $[Ca^{2+}]_i$ has a minor impact on force at a high-stimulation frequencies, whereas it has a substantial effect at lower discharge frequencies. Thus, force production is more sensitive to changes in $[Ca^{2+}]_i$ at the low-stimulation frequencies normally used by humans during exercise. This is also the situation for decreased myofibrillar Ca^{2+} sensitivity, which affects force much more on the steep part of the force–$[Ca^{2+}]$ relationship. On the other hand, a decreased ability of the cross-bridges to generate force will affect force equally at all stimulation frequencies.

The term "low-frequency fatigue" may be confusing as it has been also used to describe the force loss induced by low-frequency stimulation and recently an alternative description "prolonged low-frequency force depression" (PLFFD) has been proposed (Allen et al. 2008). In humans, PLFFD is usually assessed by comparing force (duration ~0.5 s) developed at low- (10–20 Hz) and high- (50–100 Hz) stimulation frequencies before and after exercise. For instance, a reduction in the ratio of mechanical responses to 20- and 80-Hz stimulation (P20/P80) would indicate the presence of PLFFD, i.e. a relatively greater loss of force at low-stimulation frequencies compared with higher ones. PLFFD has been reported after periods of isometric (Edwards et al. 1977), concentric/eccentric contractions (Dundon et al. 2008), and global exercise such as running (Edwards et al. 1977; Davies and White 1982; Martin et al. 2004).

The exact cause of PLFFD remains unclear but certain factors related to the induction of fatigue have been clearly ruled out. For example, all the metabolic disturbances in energy-rich phosphates and pH are reversed rather rapidly after the end of exercise (Edwards et al. 1977; Fitts 1994; Allen et al. 2008). Recent work suggests that ROS-induced modifications can be involved in PLFFD (Bruton et al. 2008). Figure 5 shows that the reduction in 30-Hz tetanic force obtained after a 30-min recovery period of repeated stimulations may be associated with lower or similar $[Ca^{2+}]_i$, depending on the Mn^{2+}-dependent superoxide dismutase (SOD2) activity, which converts superoxide to hydrogen peroxide. Thus, muscles of transgenic mice overexpressing SOD2 would have reduced superoxide and increased hydrogen peroxide levels (Silva et al. 2005). FDB fibres of SOD2-overexpressing mice displayed PLFFD due to decreased myofibrillar Ca^{2+} sensitivity. FDB fibres of wildtype mice, where superoxide levels are expected to be larger than in the SOD2 overexpressing fibres, also showed PLFFD, but in this case it was caused by impaired SR Ca^{2+} release (Bruton et al. 2008). Thus, it appears that hydrogen peroxide preferentially affects myofibrillar Ca^{2+} sensitivity, which is in accordance with experiments where hydrogen peroxide was applied to the bath solution (Andrade et al. 1998, 2001), whereas endogenously produced superoxide affects the SR Ca^{2+} release mechanism. Moreover, these findings again illustrate that both changes in SR Ca^{2+} release or myofibrillar Ca^{2+} sensitivity affect force more at low- than at high frequencies. In human muscle biopsies, PLFFD has been associated with reduced SR Ca^{2+} release (Hill et al. 2001) or impairment in Ca^{2+} uptake (Tupling et al. 2000). However, we are not aware of any studies on human muscle where the role of myofibrillar Ca^{2+} sensitivity in PLFFD was investigated.

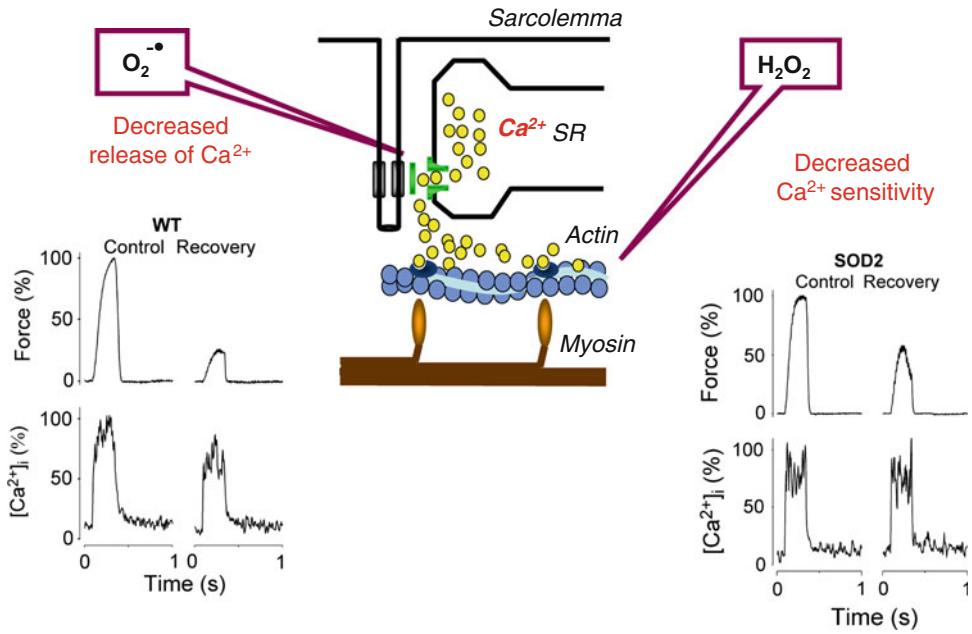

Fig. 5 Cartoon illustrating two possible mechanisms behind prolonged low-frequency force depression (PLFFD): decreased Ca^{2+} release from the sarcoplasmic reticulum (SR), which is observed in wild type (WT) mouse FDB fibres, and decreased myofibrillar Ca^{2+} sensitivity, which is observed in FDB fibres of mice overexpressing SOD2 (Bruton et al. 2008)

A phenomenon similar to PLFFD has also been found after eccentric exercise (Jones 1996; Martin et al. 2004; Warren et al. 1992). Eccentric exercise is associated with muscle damage (Fridén et al. 1983; Warren et al. 1992) and is outside the scope of the present article and we refer to reviews specifically addressing this topic (Proske and Morgan 2001; Byrne et al. 2004).

Conclusion

In summary, prolonged dynamic exercise and sustained isometric contractions induce muscle fatigue, as manifested by a reduction in the MVC force. Experimental results obtained from non-invasive measurements in exercising humans show that mechanisms located beyond the sarcolemma are important in the fatigue process. This means that impaired E–C coupling within the skeletal muscle fibres is an important cause of fatigue in exercising humans. The present review shows how data obtained in isolated single muscle fibres can be used to explain mechanisms underlying the decreased force production found in humans during various types of physical exercise.

Acknowledgments We thank the Swedish Research Council and the Swedish National Center for Sports Research for funding.

Conflict of interest statement The authors declare that they have no conflict of interest.

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