

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

PLACE, Nicolas, *et al.*

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.

PLACE, Nicolas, *et al.* Muscle fatigue: from observations in humans to underlying mechanisms studied in intact single muscle fibres. *European journal of applied physiology*, 2010, vol. 110, no. 1, p. 1-15

DOI : 10.1007/s00421-010-1480-0

PMID : 20419312

Available at:

<http://archive-ouverte.unige.ch/unige:25209>

Disclaimer: layout of this document may differ from the published version.



Proceedings of the Australian Physiological Society Symposium:
EC Coupling and Fatigue

**MECHANISMS OF FATIGUE INDUCED BY ISOMETRIC
CONTRACTIONS IN EXERCISING HUMANS AND IN
MOUSE ISOLATED SINGLE MUSCLE FIBRES**

Nicolas Place, Joseph D Bruton and Håkan Westerblad

Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

SUMMARY

1. Muscle fatigue (i.e. the decrease in muscle performance during exercise) has been studied extensively using a variety of experimental paradigms, from mouse to human, from single cell to whole-body exercise. Given the disparity of models used to characterize muscle fatigue, it can be difficult to establish whether the results of basic *in vitro* studies are applicable to exercise in humans.

2. In the present brief review, our attempt is to relate neuromuscular alterations caused by repeated or sustained isometric contraction in humans to changes in excitation–contraction (E-C) coupling observed in intact single muscle fibres, where force and the free myoplasmic $[Ca^{2+}]_i$ can be measured.

3. Accumulated data indicate that impairment of E-C coupling, most likely located within muscle fibres, accounts for the fatigue-induced decrease in maximal force in humans, whereas central (neural) fatigue is of greater importance for the inability to continue a sustained low-intensity contraction. Based on data from intact single muscle fibres, the fatigue-induced impairment in E-C coupling involves: (i) a reduced number of active cross-bridges owing to a decreased release of Ca^{2+} ; (ii) a decreased sensitivity of the myofilaments to Ca^{2+} ; and/or (iii) a reduced force produced by each active cross-bridge.

4. In conclusion, data from single muscle fibre studies can be used to increase our understanding of fatigue mechanisms in some, but not all, types of human exercise. To further increase the understanding of fatigue mechanisms in humans, we propose future studies using *in vitro* stimulation patterns that are closer to the *in vivo* situation.

Key words: $[Ca^{2+}]_i$, muscle force, muscular twitch, M-wave, task failure.

INTRODUCTION

Skeletal muscles contract to produce the force necessary in everyday life, but cannot contract continuously without impairment in performance (i.e. they fatigue). The mechanisms underlying the decrease in muscle performance have been the focus of many experiments using different experimental paradigms, from the cellular level to whole-body exercise. However, the application of basic results from isolated single cells or whole muscles to the exercising human is not easy. Indeed, *in vitro* experiments can appear to be quite far from physiological conditions, which can limit their application to humans. But the isolation of single processes thought to play a role in fatigue is difficult with studies performed *in vivo* and, thus, the need for isolated muscle studies is evident. Therefore the aim of the present brief review is to link studies of muscle fatigue in humans *in vivo* with findings from electrically stimulated intact single muscle cells.

Different models have been used to study fatigue (muscle *in vivo*, isolated muscle, isolated single fibre and skinned fibre); the description of all these models is not the focus of the present review and the reader is referred to Allen *et al.*¹ for a recent review. Here, we have chosen to focus on the intact single muscle fibre model because it allows one to: (i) follow changes in force generation in response to action potentials (as occurs in voluntary contraction); and (ii) to gain insights into alterations in excitation–contraction (E-C) coupling (i.e. from action potential propagation to cross-bridge interaction) by measuring simultaneously changes in force and free myoplasmic $[Ca^{2+}]_i$ ($[Ca^{2+}]_i$; see below). To allow a comparison between the two models and thus to limit any exercise-induced difference due to the task dependency of fatigue,² we chose to focus on fatigue induced by isometric contractions. We first describe how fatigue is classically quantified in the two different models and then discuss the underlying mechanisms of muscle fatigue in these two models in order to clarify the cause of the fatigue development in humans.

**QUANTIFICATION OF FATIGUE IN
EXERCISING HUMANS AND IN MOUSE
INTACT SINGLE MUSCLE FIBRES**

Human

The extent of neuromuscular fatigue is classically quantified by the decrease in the maximal voluntary contraction (MVC) force.³ In

Correspondence: Nicolas Place, Department of Physiology and Pharmacology, Section of Muscle Physiology, von Eulers väg 8, Karolinska Institute, S-171 77 Stockholm. Email: nicolas.place@ki.se

Presented at the AuPS Symposium EC Coupling and Fatigue, Newcastle, December 2007. The papers in these proceedings were peer reviewed under the supervision of the AuPS Editor. The papers are being published with the permission of AuPS and were initially published on the AuPS website <http://www.aups.org.au>

Received 29 March 2008; revision 27 April 2008; accepted 13 May 2008.

practice, MVCs are generally performed before and immediately after a fixed duration or open-ended task and the force loss observed is considered as the extent of fatigue. The use of open-ended exercises, such as submaximal isometric contraction performed until exhaustion, provides another index of muscle fatigue: the time to voluntary exhaustion or endurance time.

In order to gain insights into the underlying mechanisms of the decreased force-generating capacity, voluntary and electrically evoked contractions coupled with surface electromyography (EMG) have been used. These tools are classically used to distinguish between central (neural) as opposed to peripheral (muscular) adaptations. In practice, a percutaneous stimulation is applied on the motor nerve to bypass the brain and spinal cord command and elicit a compound muscle action potential (M-wave) associated with a mechanical twitch. The change in the amplitude of these two responses is then used to assess peripheral changes, from neuromuscular propagation to cross-bridge interaction.⁴⁻⁷ The contractility of a muscle group is typically investigated by comparing tetanic force evoked at low (approximately 20 Hz) and high (approximately 80 Hz) stimulation frequencies before and immediately after exercise.⁸ However, these repeated electrical stimulations of the motor nerve can be painful and their clinical application is therefore limited. The assessment of central fatigue is generally performed measuring EMG activity of the working muscles⁹⁻¹² and with the twitch interpolation technique (an extra electrical stimulus superimposed on a voluntary contraction).^{3,13-15} More recently, transcranial stimulation of the motor cortex has also been used to specifically assess the level of supraspinal fatigue (for a review, see Gandevia³). Non-invasive nuclear magnetic resonance spectroscopy or invasive muscle biopsy techniques can also be used to investigate metabolic changes associated with muscle fatigue.¹⁶

Intact single fibre

The use of mammalian intact single cells was introduced 20 years ago¹⁷ and consists of manual dissection of a mouse single cell (diameter approximately 30 μm) with tendons kept on both ends. This allows force recording once the preparation is transferred to the experimental chamber and electrically stimulated. The fibre can be loaded with fluorescent indicator for measurements of Mg^{2+} , H^+ or Ca^{2+} . This model allows assessment of E-C coupling, because any change in force can be related to changes in $[\text{Ca}^{2+}]_i$. However, considerable training is required before being able to dissect viable fibres on a regular basis.

In single fibre studies, fatigue has generally been induced by repeated tetani of 300–600 msec duration with a duty cycle comprising between 0.1 and 0.5; the experiment is usually stopped when tetanic force drops 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 approximately 50–100 in the fast-twitch mouse flexor digitorum brevis¹⁸ to several hundreds in limb muscle fibres from fatigue-resistant soleus.¹⁹ In practice, most experiments are performed on the flexor digitorum brevis because single fibre isolation is much easier than in soleus or extensor digitorum longus. The origin of the force impairment (decreased sarcoplasmic reticulum (SR) Ca^{2+} release, reduced myofibrillar Ca^{2+} sensitivity and/or decreased force per cross-bridge) can be detected from $[\text{Ca}^{2+}]_i$ measurement during the fatigue run and from the comparison of force– $[\text{Ca}^{2+}]_i$ relationships obtained before, during and after the stimulation period.

FATIGUE INDUCED BY ISOMETRIC CONTRACTIONS

Human

Sustained or repeated isometric contractions are commonly used to characterize muscle fatigue because: (i) experiments are relatively easy to perform; (ii) one can induce selective fatigue in the muscle group of interest; and (iii) the measure of endurance time constitutes a reliable index of muscle fatigability.

Repeated voluntary²⁰⁻²³ or electrically evoked²⁴ submaximal isometric contraction of various muscle groups (plantar flexors, dorsiflexors, knee extensors, adductor pollicis) at intensities of 30–50% MVC have been used to characterize neuromuscular fatigue. These studies show that reduced force in evoked contractions at task failure is accompanied by a well-preserved central activation and limited, if any, change in M-wave properties, suggesting that the decreased force-generating capacity after exercise was due to altered E-C coupling. Other studies used sustained continuous submaximal (20–66% MVC) contraction and found that muscle excitability, as measured by the M-wave properties of the muscle being used, was unchanged after exercise performed with knee extensors,^{15,25-28} elbow flexors,^{29,30} adductor pollicis³¹ or plantar flexors.³² However, using similar voluntary exercise, others found a decreased M-wave amplitude in the first dorsal interosseous,⁵ plantar flexors,^{14,32} dorsiflexors³³ and abductor pollicis brevis³⁴ at intensities of 25–65% MVC. It should be noted that although changes in intracellular action potential amplitude, duration or conduction velocity that occur during fatigue^{35,36} will affect M-wave properties, the effect of these changes on force production is uncertain.¹ At any rate, it appears that: (i) a reduction in the amplitude of the mechanical twitch (twitch force) can be observed without any impairment in M-wave parameters,^{15,25,26,29,30} and (ii) alterations in twitch force are greater than changes in M-wave,^{5,7} indicating that most of the peripheral impairment induced by sustained submaximal isometric contractions is located within muscle fibres (Fig. 1). Some authors have even suggested that voluntary exhaustion is exclusively dependent on intramuscular processes, because a parallel decrease in MVC and electrically evoked force has been found during repeated submaximal contractions.^{21,22}

Intact single fibre

The concomitant measure of force and $[\text{Ca}^{2+}]_i$, which is possible in intact single cells, allows one to delve deeper into the underlying intracellular mechanisms that limit human performance. When repeated maximal tetani are applied to a single muscle fibre, the fatigue process occurs in three phases.^{37,38} Figure 2a shows these three consecutive phases of fatigue with typical force and $[\text{Ca}^{2+}]_i$ recorded at different times during the repeated contractions. Phase 1 consists of an initial fast decline of tetanic force by 10–20% associated with an increased tetanic $[\text{Ca}^{2+}]_i$ (first five to 10 tetani) reflecting a reduction in cross-bridge force-generating capacity. Phase 2 is a relatively long period of slow decline in force and $[\text{Ca}^{2+}]_i$. Finally, in Phase 3, there is a rapid decline of both tetanic force and $[\text{Ca}^{2+}]_i$, attributed to the combined effect of reduced tetanic $[\text{Ca}^{2+}]_i$ and decreased myofibrillar Ca^{2+} sensitivity. The time-course of changes in the force– $[\text{Ca}^{2+}]_i$ relationship during repeated tetani are shown in Fig. 2b.

To date, the underlying mechanisms of altered SR Ca^{2+} release and reduced myofibrillar sensitivity to Ca^{2+} are not fully understood. Nevertheless, it has now been established that acidosis has limited

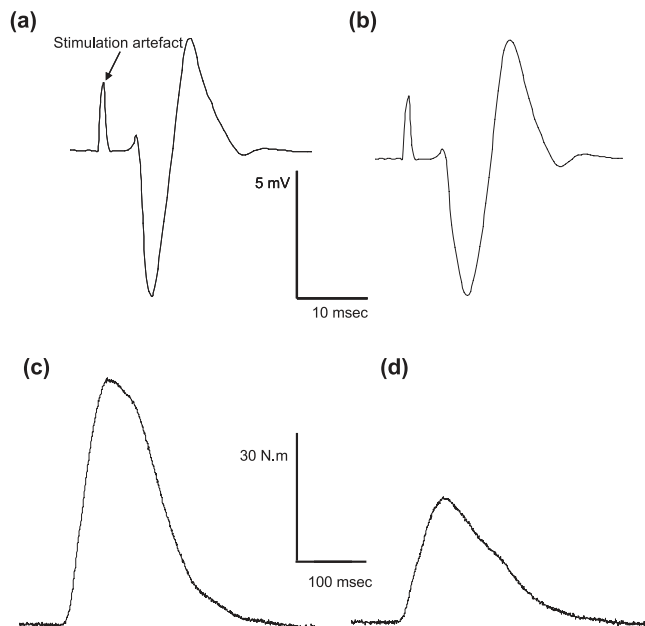


Fig. 1 Original M-wave (a,b) recordings from vastus lateralis muscle and associated muscular twitch (c,d) measured in knee extensors in response to a single supramaximal electrical stimulation of the femoral nerve evoked before (a,c) and after (b,d) prolonged isometric contraction of the knee extensor muscles. Note the marked reduction in twitch force with only little effect on the M-wave (slight increase in peak-to-peak duration).

effects on the development of fatigue at physiological temperature,^{39,40} but reduces the rate of force relaxation.¹⁸ Among the other potential mechanisms that have been considered, increased production of inorganic phosphate (P_i) and reactive oxygen species (ROS), which occur with the increased energy consumption during exercise, seem likely candidates. The rise in P_i resulting from the breakdown of phosphocreatine is thought to play a role in the early $[Ca^{2+}]_i$ increase observed in Phase 1, possibly by inhibiting SR Ca^{2+} pumps.^{41,42} Similarly, the late decrease in $[Ca^{2+}]_i$, reported in Phase 3 can be attributed to SR Ca^{2+} - P_i precipitation,⁴³⁻⁴⁵ reducing the releasable pool of Ca^{2+} in the SR, although this hypothesis is still the subject of debate.⁴⁶ Reactive oxygen species have been suggested to alter myofibrillar sensitivity to Ca^{2+} rather than SR function.^{47,48} However, a recent study using mice overexpressing the mitochondrial enzyme superoxide dismutase 2 suggests that superoxide ions and their byproducts can alter SR Ca^{2+} release.⁴⁹ Furthermore, recent human data suggest that ROS may also affect membrane excitability.⁵⁰

One way in which Ca^{2+} release from the SR can be reduced is to have a smaller action potential and thus decreased opening of the SR Ca^{2+} channels (for detailed review, see Allen *et al.*¹). A physiological consequence of exercise is an increase in interstitial K^+ , especially in the narrow t-tubular space, which is widely believed to impair action potential transmission, especially in the deeper portions of the t-tubules (for reviews, see Sjøgaard⁵¹ and Clausen⁵²). A lower $[Ca^{2+}]_i$ in the central part of the fibre compared with the surface has been measured during continuous high-frequency stimulation in fibres from *Xenopus* frog⁵³ and mouse.⁵⁴ This Ca^{2+} gradient was absent if, instead of continuous stimulation, repeated short tetani were used^{53,54} or the stimulation frequency was reduced.⁵³ In humans, a decrease in motor unit firing rate occurs during sustained MVC,^{55,56} limiting action potential generation to the minimum required for maximum

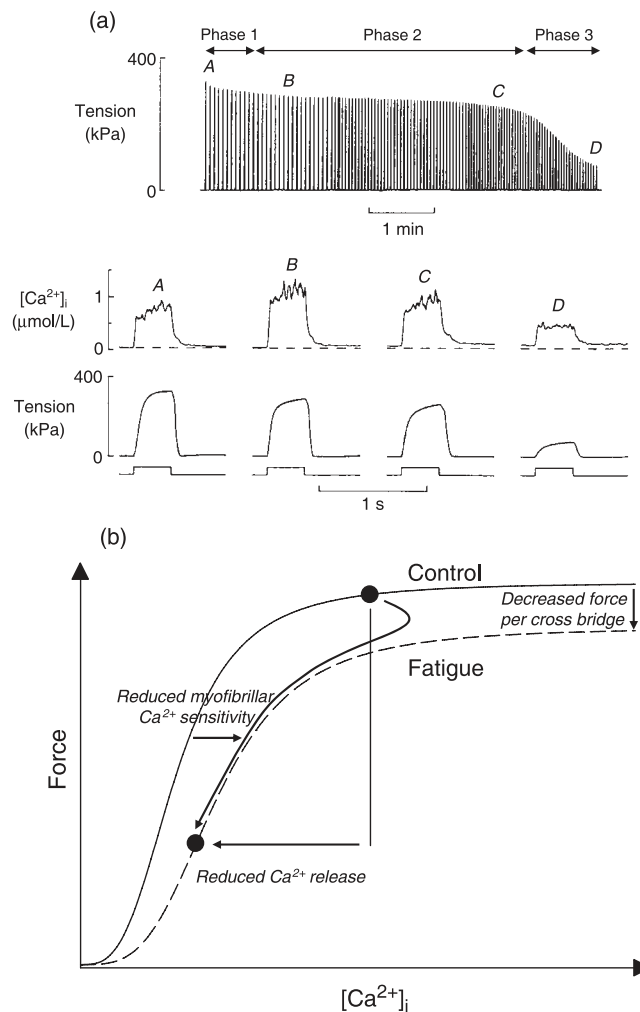


Fig. 2 (a) Original records of force and $[Ca^{2+}]_i$ obtained in a mouse single muscle fibre during a fatigue run. The upper panel shows a continuous force record, in which each vertical line represents a 350 msec tetanus at 100 Hz. The middle and lower panels show $[Ca^{2+}]_i$ (measured with Indo-1) and force records from selected tetani. (b) Change in the force- $[Ca^{2+}]_i$ relationship with fatigue. The continuous line is typically obtained by applying tetani of different frequencies under control conditions, whereas the dashed line represents the pattern observed during fatigue elicited by repeated tetani. The different causes of excitation-contraction (E-C) coupling failure are noted and the arrow between the two filled circles indicates the time-course of changes during fatigue. Redrawn from Westerblad and Allen.³⁸

force production (the core of the 'muscle wisdom' hypothesis proposed by Marsden *et al.*⁵⁷), which prevents failure of action potential propagation over the sarcolemma.^{58,59}

In summary, data obtained from humans suggest that processes located distal to the sarcolemma play a major role in fatigue. Intact single muscle fibre experiments show changes in Ca^{2+} handling and myofibrillar function that can explain the impaired contractile function in fatigue.

What about adaptations within the central nervous system?

Although it is clear that a large part of the decrease in MVC can originate from the muscle itself, some studies show that central

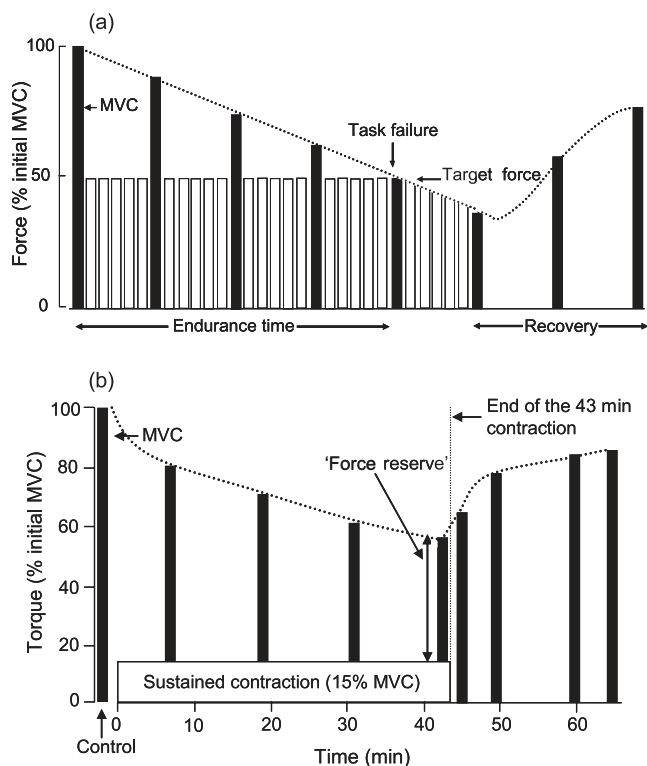


Fig. 3 An illustration of the role of central fatigue during fatiguing contractions performed by humans. The repeated intermittent contractions (a) resulted in very limited central fatigue, whereas continuous low intensity contraction (b) induced large neural adaptations. Maximal voluntary contractions (MVC; black bars) were performed during the course of both tasks. The dotted lines indicate the time-course of the decrease in maximal force-generating capacity during exercise. (a) Redrawn with permission from Bigland-Ritchie and Woods;⁵⁹ (b) redrawn with permission from Sogaard *et al.*³⁰

fatigue is involved in fatigue induced by continuous submaximal contraction,^{14,30,60,61} even if controversies still exist about the concept of central fatigue.⁶² The presence of extra force induced by an interpolated twitch at task failure has been interpreted as the inability to optimally recruit all the motor units, despite maximal effort;¹⁴ however, this method has been challenged recently because intramuscular processes have been shown to contribute to the increase in extra force production observed with fatigue.⁶³ Another piece of evidence in favour of a neural limitation is that at failure of a submaximal isometric contraction, the target force could be maintained if the muscles were instead electrically stimulated transcutaneously.⁶⁰ Immediately after this electrical stimulation, which constituted a period of recovery for the central nervous system, subjects were again able to voluntarily sustain the submaximal target force.⁶⁰ At task failure, EMG activity has been reported to be lower than maximal, indicating central fatigue,^{14,33} with some possible contribution of: (i) impaired neuromuscular propagation;⁵ and (ii) overlapping of positive and negative phases of motor unit potentials ('signal cancellation') leading to an underestimation of the motor units activity.⁶⁴ Using a low-force continuous-contraction protocol (15% MVC for 43 min), Sogaard *et al.*³⁰ recently showed development of central (supraspinal) fatigue, as assessed by transcranial magnetic stimulation during the submaximal contraction, which was simultaneous with peripheral alterations, as measured with twitch force changes. These authors estimated that supraspinal fatigue accounted for approximately 40% of the total torque loss.

Figure 3 illustrates the consequence of central fatigue development during exercise; the intermittent isometric contractions (50% MVC) presented in Fig. 3a resulted in very limited central fatigue⁵⁹ and task failure occurred when MVC force decreased below the target force. In contrast, the development of central fatigue during a low-intensity continuous contraction (15% MVC) resulted in an inability to use the reserve of force available when perceived effort was disproportionately high (Fig. 3b); indeed, Sogaard *et al.*³⁰ reported a 'very large' perceived exertion at the end of the contraction despite subjects working well below their maximal force-generating capacity.

In summary, whereas intramuscular processes seem to limit the duration of prolonged isometric contractions performed at relatively high intensity, central factors contribute to the failure of maintaining continuous contractions at lower intensities (approximately 30% MVC or less).^{14,30,60,61}

SUMMARY AND FUTURE DIRECTIONS

The present brief review points out that factors within the muscle cells are important contributors to the fatigue-induced changes in muscular function in humans. Therefore, the cellular mechanisms of fatigue found *in vitro* with intact single fibres can be used to describe fatigue *in vivo*, at least for sustained continuous or intermittent submaximal isometric contractions performed at relatively high (> 30% MVC) intensity. Consequently: (i) a reduced number of active cross-bridges due to a decreased release of Ca^{2+} ; (ii) a decreased sensitivity of the myofilaments to Ca^{2+} ; and/or (iii) a reduced force produced by each active cross-bridge could explain a large part of the decrease in force-generating capacity observed during this kind of voluntary exercise to exhaustion in humans. To determine which of these mechanisms is involved *in vivo*, we investigated changes in neuromuscular function induced by a sustained submaximal isometric contraction performed in two different sessions with slight adjustments of either the muscle length or feedback type.^{7,15,65} In both sessions, a similar reduction in MVC and the same extent of central fatigue were observed immediately after the exercise, whereas electrically evoked twitch properties were differently altered.^{7,15,65} Furthermore, M-wave parameters were relatively well preserved. Because a reduction in force production per cross-bridge would affect twitch force and MVC similarly, we can thus speculate that a reduced Ca^{2+} sensitivity and/or a decreased Ca^{2+} release by the SR in response to a single shock are the major mechanisms of the reduced twitch force. Therefore, the mechanisms underlying the rapid drop in force in the single fibre (Phase 3) are likely to be similar to those that occur during a task performed until voluntary exhaustion in humans (Fig. 4). However, in humans, the central component of fatigue should not be ignored, because it is often involved in the fatigue process (see above). It seems that the relative importance of central adaptations increases when continuous, low-intensity exercise (< 30% MVC) is performed. This intensity-dependent origin of muscle fatigue is also likely to occur during sport activities; thus, the performance in a 400 m race (less than 1 min) would be limited by intramuscular processes mainly, whereas running a marathon (more than 2 h) would result in a greater extent of central fatigue. However, performance in endurance events is not only dependent on the neuromuscular system, but is also greatly influenced by cardiac and ventilatory processes, as well as technique, which makes the fatigue-induced decrease in performance difficult to monitor.

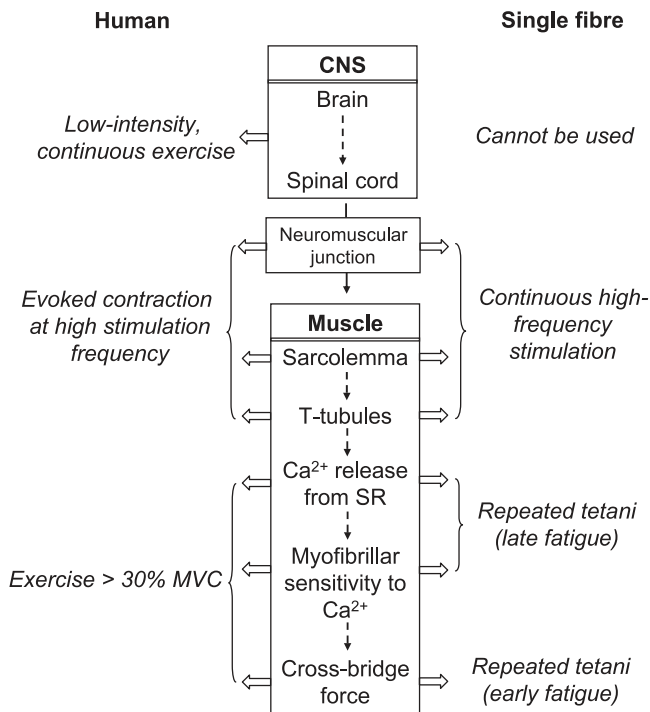


Fig. 4 Schematic representation of the potential sites (middle part) and their role in the fatigue development in humans (left part) and single muscle fibres (right part). Flanking descriptions indicate at which stage of fatigue each level is affected. CNS, central nervous system; MVC, maximal voluntary contractions.

It is clear that human and single-fibre models can gain from the other and help us better understand the fine tuning that occurs during whole-body exercise and recovery. On the one hand, it seems that the cellular mechanisms of force reduction are similar during repeated or sustained isometric contractions in humans and intact single cells and would then mainly involve changes in Ca²⁺ handling. Conversely, the complex process of voluntary exhaustion or increased perceived effort appears to rarely involve only peripheral mechanisms, but is also dependent on neural (both spinal and supraspinal) adjustments (Fig. 4). Clearly, here single fibres cannot be used to study the development of central fatigue. However, stimulation protocols of single fibres can be improved to gain further insights into the mechanisms of fatigue. For instance, it would be useful to induce fatigue in isolated muscle fibres with a pattern that mimics motor unit firing rate during exercise (i.e. use a high stimulation rate (up to 250 Hz) for 5–10 msec at the start of each contraction⁶⁶ and then decrease the stimulation rate to lower frequencies (10–30 Hz, as reported by Bellemare *et al.*⁵⁸) or progressively reduce the frequency for the remainder of the contraction). The assessment of changes in E-C coupling induced by this ‘close to *in vivo*’ stimulation pattern would then provide more realistic data about subtle adjustments that occur during the development of muscle fatigue in the intact animal.

In conclusion, the decrease in MVC induced by a prolonged isometric contraction can be mainly attributed to impairment within the muscle fibres and particularly to changes in Ca²⁺ handling. Similarly, task failure of a continuous or intermittent contraction performed at middle to high intensity (> 30% MVC) can be largely explained by E-C coupling failure, whereas central fatigue accounts for the inability to sustain a continuous low level of force.

ACKNOWLEDGEMENTS

This review was supported by the Swedish Research Council, the Swedish National Center for Sports Research and Funds of Karolinska Institutet. NP was supported by the Swedish Institute.

REFERENCES

- Allen DG, Lamb GD, Westerblad H. Skeletal muscle fatigue: Cellular mechanisms. *Physiol. Rev.* 2008; **88**: 287–332.
- Enoka RM, Stuart DG. Neurobiology of muscle fatigue. *J. Appl. Physiol.* 1992; **72**: 1631–48.
- Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. *Physiol. Rev.* 2001; **81**: 1725–89.
- Bigland-Ritchie B, Jones DA, Hosking GP, Edwards RH. Central and peripheral fatigue in sustained maximum voluntary contractions of human quadriceps muscle. *Clin. Sci. Mol. Med.* 1978; **54**: 609–14.
- Fuglevand AJ, Zackowski KM, Huey KA, Enoka RM. Impairment of neuromuscular propagation during human fatiguing contractions at submaximal forces. *J. Physiol.* 1993; **460**: 549–72.
- Kooistra RD, de Ruyter CJ, de Haan A. Muscle activation and blood flow do not explain the muscle length-dependent variation in quadriceps isometric endurance. *J. Appl. Physiol.* 2005; **98**: 810–16.
- Place N, Maffiuletti NA, Ballay Y, Lepers R. Twitch potentiation is greater after a fatiguing submaximal isometric contraction performed at short vs. long quadriceps muscle length. *J. Appl. Physiol.* 2005; **98**: 429–36.
- Millet GY, Martin V, Lattier G, Ballay Y. Mechanisms contributing to knee extensor strength loss after prolonged running exercise. *J. Appl. Physiol.* 2003; **94**: 193–8.
- Lloyd AJ. Surface electromyography during sustained isometric contractions. *J. Appl. Physiol.* 1971; **30**: 713–19.
- Fallentin N, Jorgensen K, Simonsen EB. Motor unit recruitment during prolonged isometric contractions. *Eur. J. App. Physiol. Occup. Physiol.* 1993; **67**: 335–41.
- Hunter SK, Ryan DL, Ortega JD, Enoka RM. Task differences with the same load torque alter the endurance time of submaximal fatiguing contractions in humans. *J. Neurophysiol.* 2002; **88**: 3087–96.
- Rochette L, Hunter SK, Place N, Lepers R. Activation varies among the knee extensor muscles during a submaximal fatiguing contraction in the seated and supine postures. *J. Appl. Physiol.* 2003; **95**: 1515–22.
- Merton PA. Voluntary strength and fatigue. *J. Physiol.* 1954; **123**: 553–64.
- Löscher WN, Cresswell AG, Thorstensson A. Excitatory drive to the α -motoneuron pool during a fatiguing submaximal contraction in man. *J. Physiol.* 1996; **491**: 271–80.
- Place N, Martin A, Ballay Y, Lepers R. Neuromuscular fatigue differs with biofeedback type when performing a submaximal contraction. *J. Electromyogr. Kinesiol.* 2007; **17**: 253–63.
- Sapega AA, Sokolow DP, Graham TJ, Chance B. Phosphorus nuclear magnetic resonance: A non-invasive technique for the study of muscle bioenergetics during exercise. *Med. Sci. Sports Exerc.* 1987; **19**: 410–20.
- Lännergren J, Westerblad H. The temperature dependence of isometric contractions of single, intact fibres dissected from a mouse foot muscle. *J. Physiol.* 1987; **390**: 285–93.
- Bruton JD, Lännergren J, Westerblad H. Effects of CO₂-induced acidification on the fatigue resistance of single mouse muscle fibers at 28°C. *J. Appl. Physiol.* 1998; **85**: 478–83.
- Bruton J, Tavi P, Aydin J, Westerblad H, Lännergren J. Mitochondrial and myoplasmic [Ca²⁺] in single fibres from mouse limb muscles during repeated tetanic contractions. *J. Physiol.* 2003; **551**: 179–90.
- Bigland-Ritchie B, Cafarelli EV, Vøllestad NK. Fatigue of submaximal static contractions. *Acta Physiol. Scand.* 1986; **556** (Suppl.): 137–48.
- Bigland-Ritchie B, Furbush F, Woods JJ. Fatigue of intermittent submaximal voluntary contractions: Central and peripheral factors. *J. Appl. Physiol.* 1986; **61**: 421–9.

22. Vøllestad NK, Sejersted OM, Bahr R, Woods JJ, Bigland-Ritchie B. Motor drive and metabolic responses during repeated submaximal contractions in humans. *J. Appl. Physiol.* 1988; **64**: 1421–7.
23. Baker AJ, Kostov KG, Miller RG, Weiner MW. Slow force recovery after long-duration exercise: Metabolic and activation factors in muscle fatigue. *J. Appl. Physiol.* 1993; **74**: 2294–300.
24. Duchateau J, Hainaut K. Electrical and mechanical failures during sustained and intermittent contractions in humans. *J. Appl. Physiol.* 1985; **58**: 942–7.
25. West W, Hicks A, McKelvie R, O'Brien J. The relationship between plasma potassium, muscle membrane excitability and force following quadriceps fatigue. *Pflügers Arch.* 1996; **432**: 43–9.
26. Plaskett CJ, Cafarelli E. Caffeine increases endurance and attenuates force sensation during submaximal isometric contractions. *J. Appl. Physiol.* 2001; **91**: 1535–44.
27. Gondin J, Guette M, Jubeau M, Ballay Y, Martin A. Central and peripheral contributions to fatigue after electrostimulation training. *Med. Sci. Sports Exerc.* 2006; **38**: 1147–56.
28. Place N, Matkowski B, Martin A, Lepers R. Synergists activation pattern of the quadriceps muscle differs when performing sustained isometric contractions with different EMG biofeedback. *Exp. Brain Res.* 2006; **174**: 595–603.
29. Bilodeau M, Henderson TK, Nolte BE, Pursley PJ, Sandfort GL. Effect of aging on fatigue characteristics of elbow flexor muscles during sustained submaximal contraction. *J. Appl. Physiol.* 2001; **91**: 2654–64.
30. Sjøgaard K, Gandevia SC, Todd G, Petersen NT, Taylor JL. The effect of sustained low-intensity contractions on supraspinal fatigue in human elbow flexor muscles. *J. Physiol.* 2006; **573**: 511–23.
31. Ljubisavljevic M, Milanovic S, Radovanovic S, Vukcevic I, Kostic V, Anastasijevic R. Central changes in muscle fatigue during sustained submaximal isometric voluntary contraction as revealed by transcranial magnetic stimulation. *Electroencephalogr. Clin. Neurophysiol.* 1996; **101**: 281–8.
32. Kuchinad RA, Ivanova TD, Garland SJ. Modulation of motor unit discharge rate and H-reflex amplitude during submaximal fatigue of the human soleus muscle. *Exp. Brain Res.* 2004; **158**: 345–55.
33. Lévênez M, Kotzamanidis C, Carpentier A, Duchateau J. Spinal reflexes and coactivation of ankle muscles during a submaximal fatiguing contraction. *J. Appl. Physiol.* 2005; **99**: 1182–8.
34. Duchateau J, Balestra C, Carpentier A, Hainaut K. Reflex regulation during sustained and intermittent submaximal contractions in humans. *J. Physiol.* 2002; **541**: 959–67.
35. Lännergren J, Westerblad H. Force and membrane potential during and after fatiguing, continuous high-frequency stimulation of single *Xenopus* muscle fibres. *Acta Physiol. Scand.* 1986; **128**: 359–68.
36. Balog EM, Thompson LV, Fitts RH. Role of sarcolemma action potentials and excitability in muscle fatigue. *J. Appl. Physiol.* 1994; **76**: 2157–62.
37. Westerblad H, Allen DG. Changes of myoplasmic calcium concentration during fatigue in single mouse muscle fibers. *J. Gen. Physiol.* 1991; **98**: 615–35.
38. Westerblad H, Allen DG. The contribution of $[Ca^{2+}]_i$ to the slowing of relaxation in fatigued single fibres from mouse skeletal muscle. *J. Physiol.* 1993; **468**: 729–40.
39. Pate E, Bhimani M, Franks-Skiba K, Cooke R. Reduced effect of pH on skinned rabbit psoas muscle mechanics at high temperatures: Implications for fatigue. *J. Physiol.* 1995; **486**: 689–94.
40. Westerblad H, Bruton JD, Lännergren J. The effect of intracellular pH on contractile function of intact, single fibres of mouse muscle declines with increasing temperature. *J. Physiol.* 1997; **500**: 193–204.
41. Dahlstedt AJ, Katz A, Westerblad H. Role of myoplasmic phosphate in contractile function of skeletal muscle: Studies on creatine kinase-deficient mice. *J. Physiol.* 2001; **533**: 379–88.
42. Duke AM, Steele DS. Mechanisms of reduced SR Ca^{2+} release induced by inorganic phosphate in rat skeletal muscle fibers. *Am. J. Physiol. Cell Physiol.* 2001; **281**: C418–29.
43. Fryer MW, Owen VJ, Lamb GD, Stephenson DG. Effects of creatine phosphate and P_i on Ca^{2+} movements and tension development in rat skinned skeletal muscle fibres. *J. Physiol.* 1995; **482**: 123–40.
44. Westerblad H, Allen DG. The effects of intracellular injections of phosphate on intracellular calcium and force in single fibres of mouse skeletal muscle. *Pflügers Arch.* 1996; **431**: 964–70.
45. Fryer MW, West JM, Stephenson DG. Phosphate transport into the sarcoplasmic reticulum of skinned fibres from rat skeletal muscle. *J. Muscle Res. Cell Motil.* 1997; **18**: 161–7.
46. Steele DS, Duke AM. Metabolic factors contributing to altered Ca^{2+} regulation in skeletal muscle fatigue. *Acta Physiol. Scand.* 2003; **179**: 39–48.
47. Andrade FH, Reid MB, Allen DG, Westerblad H. Effect of hydrogen peroxide and dithiothreitol on contractile function of single skeletal muscle fibres from the mouse. *J. Physiol.* 1998; **509**: 565–75.
48. Moopanar TR, Allen DG. Reactive oxygen species reduce myofibrillar Ca^{2+} sensitivity in fatiguing mouse skeletal muscle at 37°C. *J. Physiol.* 2005; **564**: 189–99.
49. Bruton JD, Place N, Yamada T *et al.* Reactive oxygen species and fatigue-induced prolonged low-frequency force depression in skeletal muscle fibres of rats, mice and SOD2 overexpressing mice. *J. Physiol.* 2008; **586**: 175–84.
50. McKenna MJ, Medved I, Goodman CA *et al.* N-Acetylcysteine attenuates the decline in muscle Na^+ , K^+ -pump activity and delays fatigue during prolonged exercise in humans. *J. Physiol.* 2006; **576**: 279–88.
51. Sjøgaard G. Potassium and fatigue: The pros and cons. *Acta Physiol. Scand.* 1996; **156**: 257–64.
52. Clausen T. Na^+ - K^+ pump regulation and skeletal muscle contractility. *Physiol. Rev.* 2003; **83**: 1269–324.
53. Westerblad H, Lee JA, Lamb AG, Bolsover SR, Allen DG. Spatial gradients of intracellular calcium in skeletal muscle during fatigue. *Pflügers Arch.* 1990; **415**: 734–40.
54. Duty S, Allen DG. The distribution of intracellular calcium concentration in isolated single fibres of mouse skeletal muscle during fatiguing stimulation. *Pflügers Arch.* 1994; **427**: 102–9.
55. Bigland-Ritchie B, Johansson R, Lippold OC, Smith S, Woods JJ. Changes in motoneurone firing rates during sustained maximal voluntary contractions. *J. Physiol.* 1983; **340**: 335–46.
56. Rubinstein S, Kamen G. Decreases in motor unit firing rate during sustained maximal-effort contractions in young and older adults. *J. Electromyogr. Kinesiol.* 2005; **15**: 536–43.
57. Marsden CD, Meadows JC, Merton PA. 'Muscular wisdom' that minimizes fatigue during prolonged effort in man: Peak rates of motoneuron discharge and slowing of discharge during fatigue. *Adv. Neurol.* 1983; **39**: 169–211.
58. Bellemare F, Grassino A. Force reserve of the diaphragm in patients with chronic obstructive pulmonary disease. *J. Appl. Physiol.* 1983; **55**: 8–15.
59. Bigland-Ritchie B, Woods JJ. Changes in muscle contractile properties and neural control during human muscular fatigue. *Muscle Nerve* 1984; **7**: 691–9.
60. Löscher WN, Cresswell AG, Thorstensson A. Central fatigue during a long-lasting submaximal contraction of the triceps surae. *Exp. Brain Res.* 1996; **108**: 305–14.
61. Smith JL, Martin PG, Gandevia SC, Taylor JL. Sustained contraction at very low forces produces prominent supraspinal fatigue in human elbow flexor muscles. *J. Appl. Physiol.* 2007; **103**: 560–8.
62. Marcora S. Is peripheral locomotor muscle fatigue during endurance exercise a variable carefully regulated by a negative feedback system? *J. Physiol.* 2008; **586**: 2027–8.
63. Place N, Yamada T, Bruton JD, Westerblad H. Interpolated twitches in fatiguing single mouse muscle fibres: Implications for the assessment of central fatigue. *J. Physiol.* 2008; **586**: 2799–805.
64. Keenan KG, Farina D, Maluf KS, Merletti R, Enoka RM. Influence of amplitude cancellation on the simulated surface electromyogram. *J. Appl. Physiol.* 2005; **98**: 120–31.
65. Place N, Martin A, Lepers R. Changes in neuromuscular function after tasks involving control of EMG versus torque feedback of the same duration. *Neurosci. Lett.* 2006; **394**: 37–41.
66. Hennig RL, Lømo T. Firing patterns of motor units in normal rats. *Nature* 1985; **314**: 164–6.