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How to cite

PLACE, Nicolas et al. Muscle fatigue: from observations in humans to underlying mechanisms studied in intact single muscle fibres. In: European journal of applied physiology, 2010, vol. 110, n° 1, p. 1–15. doi: 10.1007/s00421-010-1480-0

This publication URL: https://archive-ouverte.unige.ch/unige:25209
Publication DOI: 10.1007/s00421-010-1480-0

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Clinical and Experimental Pharmacology and Physiology (2009) **36**, 334–339 doi: 10.1111/j.1440-1681.2008.05021.x

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

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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 $[Ca^{2+}]$ 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.

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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.

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 $[Ca^{2+}]$ ($[Ca^{2+}]_i$; 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

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 muscles[9–12] 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 Gandevia[3]). 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 ago[17] 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 $[Ca^{2+}]_i$. 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 brevis[18] 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 $[Ca^{2+}]_i$ measurement during the fatigue run and from the comparison of force–$[Ca^{2+}]_i$ 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 voluntary[20–23] or electrically evoked[24] 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 pollicis[31] 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] dorsiflexors[33] and abductor pollicis brevis[34] 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 $[Ca^{2+}]_i$, 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 $[Ca^{2+}]_i$ 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 $[Ca^{2+}]_i$ (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 $[Ca^{2+}]_i$. Finally, in Phase 3, there is a rapid decline of both tetanic force and $[Ca^{2+}]_i$ attributed to the combined effect of reduced tetanic $[Ca^{2+}]_i$ and decreased myofibrillar Ca^{2+} sensitivity. The time-course of changes in the force–$[Ca^{2+}]_i$ 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

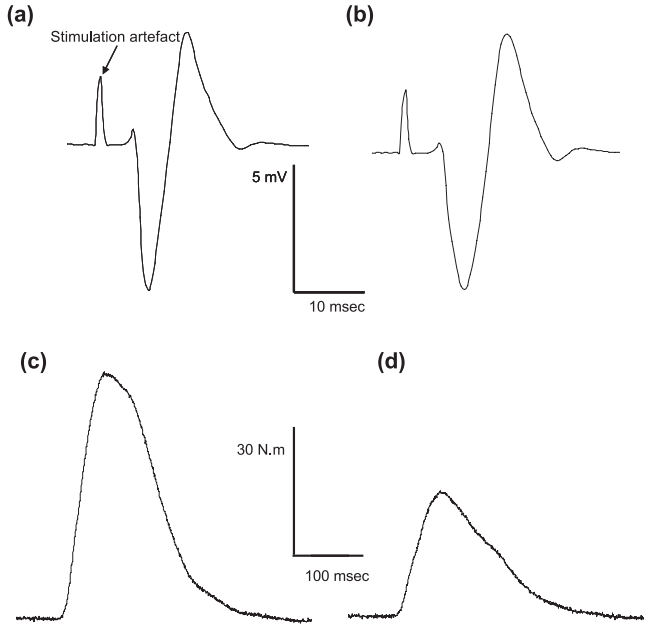

Fig. 1 Original M-wave (a,b) recordings from vastus lateralis muscle and associated muscular twitch (c,d) measured in knee extensors in response to a single supramaximal electrical stimulation of the femoral nerve evoked before (a,c) and after (b,d) prolonged isometric contraction of the knee extensor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

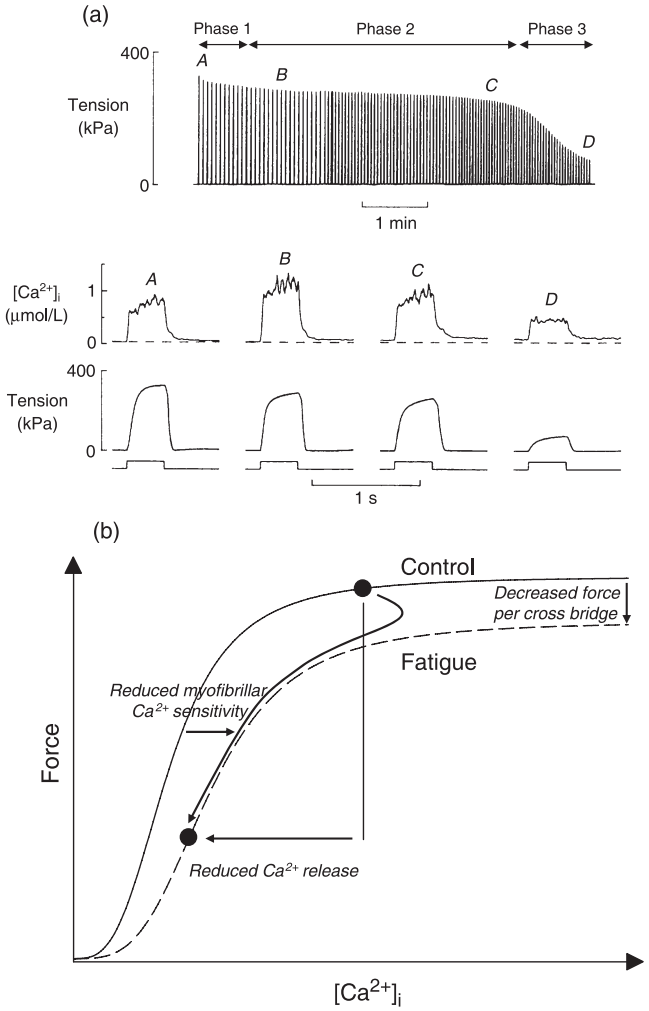

Fig. 2 (a) Original records of force and $[Ca^{2+}]_i$ obtained in a mouse single muscle fibre during a fatigue run. The upper panel shows a continuous force record, in which each vertical line represents a 350 msec tetanus at 100 Hz. The middle and lower panels show $[Ca^{2+}]_i$ (measured with Indo-1) 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]

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+}–Pi 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 Sjøgaard[51] and Clausen[52]). 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* frog[53] 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 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

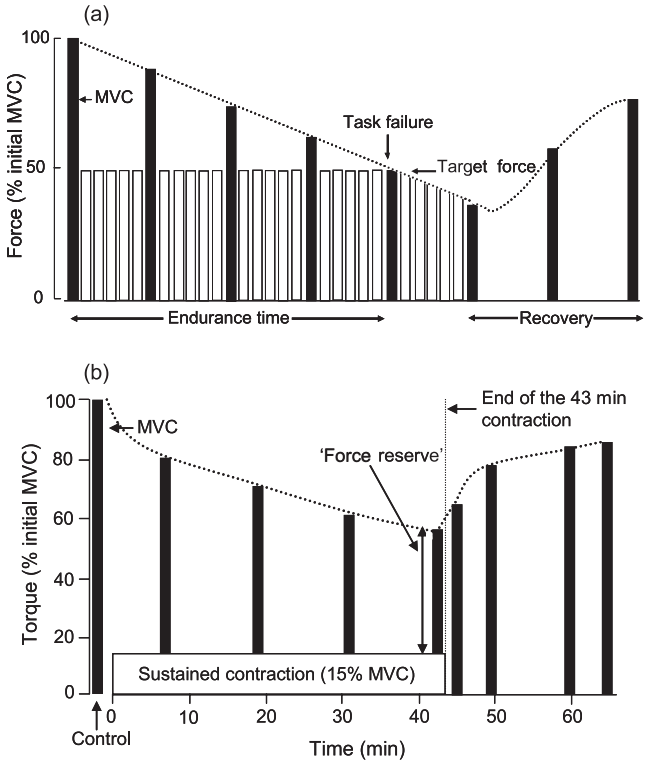

Fig. 3 An illustration of the role of central fatigue during fatiguing contractions performed by humans. The repeated intermittent contractions (a) resulted in very limited central fatigue, whereas continuous low intensity contraction (b) induced large neural adaptations. Maximal voluntary contractions (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 Søgaard *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), Søgaard *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 fatigue[59] 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, Søgaard *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.

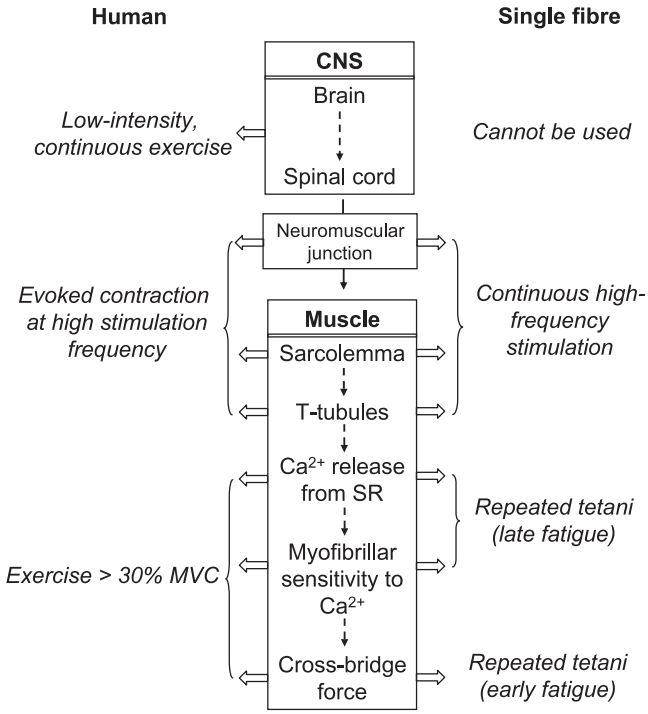

Fig. 4 Schematic representation of the potential sites (middle part) and their role in the fatigue development in humans (left part) and single muscle fibres (right part). Flanking descriptions indicate at which stage of fatigue each level is affected. CNS, central nervous system; MVC, maximal voluntary 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 Ca^{2+} 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 contraction[66] 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 Ca^{2+} 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.

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