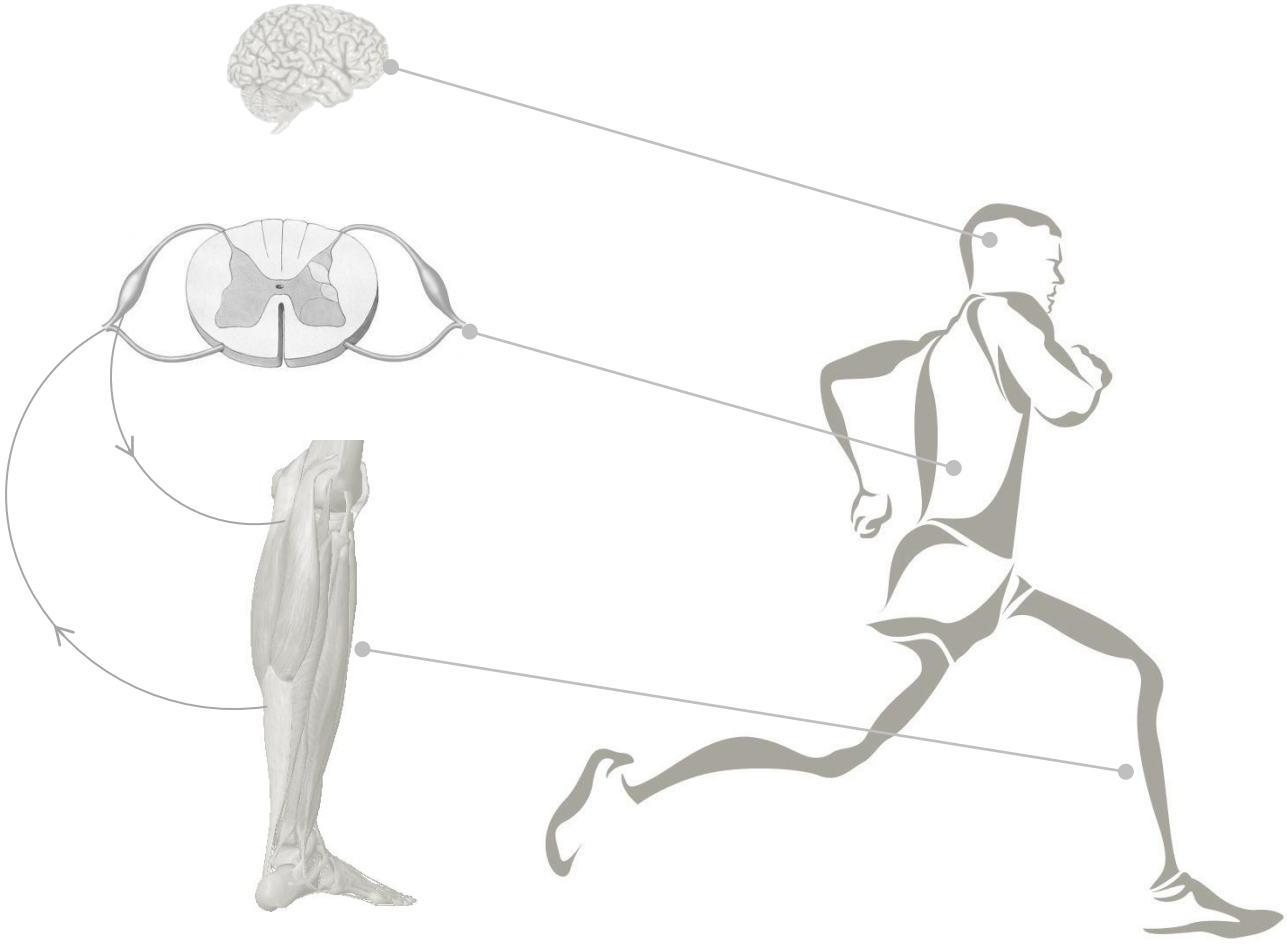
Neuromuscular Alteration in the Human Soleus Muscle During and After a Fatiguing 800 m Run



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Abstract

A progressive speed decline during maximal 800m running might be related to limitations of the neuromuscular system. Previously, neuromuscular fatigue has been measured post exercise, but neuromuscular modulation during running to fatigue has never been investigated. 8 male competitive, middle-distance runners performed two submaximal and one maximal 800m run on a treadmill with electrically evoked soleus H-reflexes, maximal M-waves (Mmax) and V-waves every 2 sec at ground contact. Isometric voluntary contractions at 50, 75 and 100% were performed pre and post running with transcranial magnetic stimulation and electrically evoked twitches to assess maximum voluntary contraction (MVC) motor evoked potentials (MEP), silent periods (SP), voluntary activation (VA) and rest twitches (RT). The results showed an decline of MVC -9.7±9.6% (P=0.049), VA -9.3±7.3% (P=0.026) and RT -28.5±15.7% (P=0.004) and an increase of MEP 7.4±4.8% (P=0.037) and SP 7.3±4.8ms (P=0.014) post running (all P<0.05). During running an increase in H-reflex 18.1±12.8% (P=0.043) and V-wave 23.9±13% (P=0.038) and an Mmax decrease of -16.06±2.13% (P=0.001) were found from the first epoch of the run compared to the last. Stride-time and rmsEMG was unchanged. The decreased RT and Mmax showed that peripheral fatigue was occurring during 800m of maximal running but the increased H-reflex and V-wave suggests that central factors increased excitability or drive to maintain power output. Thus central mechanisms were possibly not limiting to 800m running. However, decline in MVC and VA post running suggests that central factors were fatigued but the discrepancy might be attributed to the difference in contraction modus.

Keywords – Middle-distance running, Transcranial magnetic stimulation, Peripheral electrical stimulation, H-reflex, V-wave, Central/Peripheral fatigue, Triceps surae, Twitch interpolation technique.

Preface

The following master's thesis was developed and conducted at Aalborg University (AAU) and at University of Western Sydney (UWS) by group 1035 during the masters-program in Sports Science at AAU. The thesis has been developed and conducted during a one year period and includes work equivalent to 60 ECTS points.

Initially, the authors of the thesis spent three months in Sydney to gain knowledge of international research and to be familiarized with methods of testing neuromuscular alteration and other physiological measures. The initial aim was to develop and conduct a pilot study in Sydney for investigating 800m running fatigue, but upon arrival the local supervisors requested help to develop and conduct a study with a different scope with similar methodology. At UWS the authors of this thesis obtained experience and gained knowledge of neuromuscular testing of the human triceps surae. The study concerned the neurophysiological modulation to fatigue during intermittent isometric contractions of the triceps surae with chemical loading of acid and base. The study was conducted in collaboration with the sport and exercise science program at UWS. The main supervisors were Dr. Jason Siegler, Dr. Simon Green and PhD student at the Janet Taylor group; David Kennedy. The project idea came from UWS, but the development of protocol, conduction of

the study and analysis of results were performed by the authors of the present thesis. The study is currently in preparation for a submission in Journal of Applied Physiology and a manuscript draft is presented in the appendix.

Upon the return to AAU, the gained knowledge from UWS was applied in the initial study concerning neuromuscular alteration to 800m running and fatigue. This project has been based on the authors' own ideas as have the protocol development and conduction of the study. The project was performed at Aalborg University with supervision from Dr. Natalie Mrachaz-Kersting and Dr. Michael Voigt.

Acknowledgements
The authors would like to thank Dr. Natalie Mrachaz-Kersting and Dr. Michael Voigt for their supervision and guidance throughout the development of the thesis.

Furthermore, we would like to thank the academic staff at UWS for their help and hospitality during the internship in Sydney, Australia.

Finally, we would like to thank the athletes for their participation in the study.

Reader guidelines

Initially, the reader will be introduced to the overall problem and theoretical background. This is followed by a presentation and discussion of the results leading to the final conclusion and perspectives.

References are made by use of the Harvard method. Author and date are cited when used in the text. A detailed alphabetic reference list is presented at the end of the report.

The appendix presents the manuscript for the study conducted at the University of Western Sydney. Furthermore, the ethical application for the current study, allowing the authors to conduct experimental research on human subjects has been attached. This was approved by the local ethics committee in Region Nordjylland prior to testing. Finally a Danish summary of the project has been attached to meet formal requirements.

Content

Introduction

800 m of running is an Olympic track and field discipline which consists of 2 laps on a standard 400 m cinders track.

The demand for sustained high velocity running in this discipline induces decreasing speed, even at maximal effort, as shown by Thomas et al. 2004 in figure 1. During a maximal pace attempt (not tactical), the velocity drops progressively from start to finish. The decreasing velocity may be the result of fatigue, caused by a conglomerate of physiological factors – all limiting performance. These include cardiopulmonary and psychological limitations (Brandon 1995) but possibly also undiscovered neurophysiological factors.

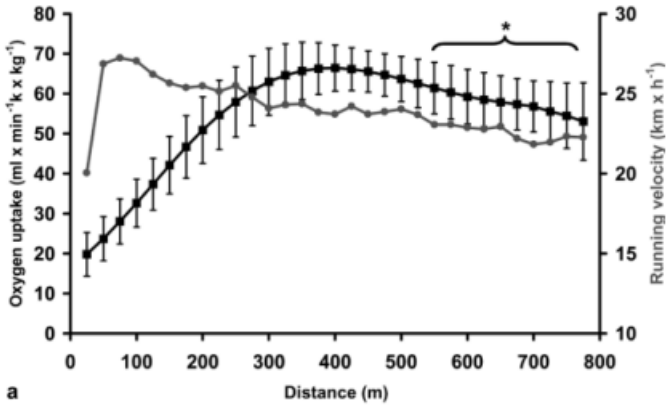


Figure 1: Displays a decrease in oxygen uptake (black line) and speed (grey line) during an 800 m run as investigated by Thomas et al 2004.

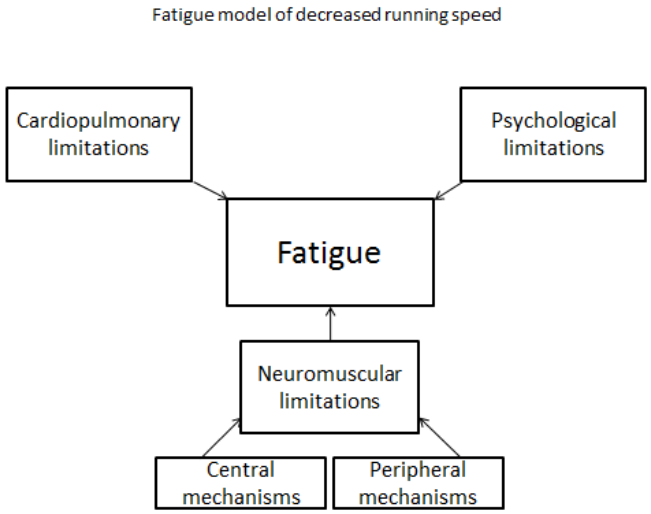


Figure 2 – Simplified model of general limiting factors during fatiguing exercise

In figure 2, a fatigue model has been constructed to illustrate which factors might limit 800m running performance. Cardiopulmonary limitations have been investigated in middle distance running (Brandon 1995) (Camus 1992), but Ross, Levitt and Reik 2001argued that neuromuscular factors might also be limiting to high intensity running. However, they did not have direct data to support this. Therefore further investigation on this topic could illuminate previously undiscovered performance limitations.

Taylor and Gandevia 2008 defined neuromuscular fatigue as "*any exercise-induced decrease in maximal voluntary force or power produced by a muscle or muscle group"*. This decrease may be caused by either *central* or *peripheral* factors as shown in Fig. 2. *Central fatigue* refers to progressive exercise-induced processes happening proximal to the neuromuscular junction (e.g. spinal or supraspinal), reducing voluntary activation of the working muscles (Taylor and Gandevia 2008). *Peripheral fatigue* can be defined as exercise-induced processes at or distal to the neuromuscular junction which leads to a reduction of contractile force (Taylor and Gandevia 2008). The contribution of either mechanism remains elusive and contradictory. Investigation of neuromuscular mechanisms includes various measurements such as the H-reflex, V-wave, transcranial magnetic stimulation (TMS) and peripheral electrical stimulation (Gandevia, et al. 1996), (Nordlund, Thorstensson and Cresswell 2004), (Walton, Kuchinad, et al. 2002), (Iguchi and Shields 2011), (Vila-Chã, et al. 2012). Results from these studies suggest that central mechanisms such as presynaptic inhibition, supraspinal control, spinal excitability and III/IV afferents may influence the neuromuscular modulation to fatigue. The studies have been performed during sustained maximal isometric contractions (Butler, Taylor and Gandevia 2003), sustained submaximal contractions (Levenez, et al. 2008), intermittent maximal contractions (Nordlund, Thorstensson and Cresswell 2004), post cycling exercise (Amann, et al. 2011) and post prolonged running exercise (Racinais, Girard, et al. 2007). Many of the obtained results vary considerably with a predominant lack of concord, probably due to differences in method and considerable task sensitivity of the nervous system (Nybo and Nielsen 2001) (Bilodaeu 2006).

Furthermore, studies have been conducted using isometric muscle contractions in highly controlled environments anomalous to actual sport settings. These findings might not reflect actual neurophysiological modulations to fatigue during ballistic dynamic contractions such as running - contractions and rest periods are markedly different, there is a different level of activation and also aspects from the cardiopulmonary systems are different and may alter the response.

It is methodically hard to obtain neuromuscular results during actual running and multiple factors such as movement artifacts and various inhibitions affect the measurements. Capaday & Stein 1987 and Simonsen & Dyhre-Poulsen 1999, performed reliable H-reflex measurements of the human triceps surae during running, thus providing a measure of the excitability of the motoneuron pool and of inhibitory mechanisms. However, these measurements were not recorded during fatiguing contractions and in addition the H-reflex provides no interpretation of possible supraspinal mechanisms (Racinais, Girard, et al. 2007) – a mechanism which is often discussed (S. C. Gandevia 2001). The V-wave has recently been introduced and may provide a reliable measure of cortical drive (Aagaard, et al. 2002), (Solstad, et al. 2011). It is at the same time possible to measure during actual running (Simonsen, Alkjær og Raffalt 2012). To the knowledge of the authors, the H-reflexes and V-waves have never been measured during running to fatigue. Such measurements combined with pre/post measures of suprapsinal factors utilizing twitch interpolation technique and transcranial magnetic stimulation (TMS) may provide novel information of the neuromuscular factors involved.

During running, the triceps surae provides propulsive force production and balance while being easily accessible to stimulation of the tibial motor nerve. Therefore, this muscle group holds valuable information regarding central and peripheral fatigue in high intensity running.

The aim of this study was to assess neurophysiological control and modulation to fatigue in a setting with high transferability to sport. This included measurements of both H-reflexes and V-waves evoked in the triceps surae during 800 m running to fatigue in, high level athletes, combined with TMS and twitch interpolation technique measured pre and post.

We hypothesized that 800 m running would induce neuromuscular fatigue comprising peripheral factors distal to the neuromuscular junction and central mechanisms through decreased motoneuron excitability and central drive.

Theory

This chapter constitutes the theoretical foundation of the study. It contains a description of the outcome measures and the mechanisms possibly influencing these. The chapter is based upon scientific articles and reviews.

The H-reflex

The H-reflex is a widely utilized tool in the investigation of the spinal pathways. It is based on the work by Hoffmann, P. in the 1950s to investigate the monosynaptic connections from Ia afferent nerve fibers to spinal motoneurons. It is elicited by electrical stimulation of the Ia afferents, resulting in monosynaptic excitation of α-motoneurons. The pathways and technique is shown in Fig. 3. It is the electrical analogue of the monosynaptic stretch reflex – though it bypasses the muscle spindles and the fusimotor drive, which may affect the sensitivity of the Ia afferents (Knikou 2008).

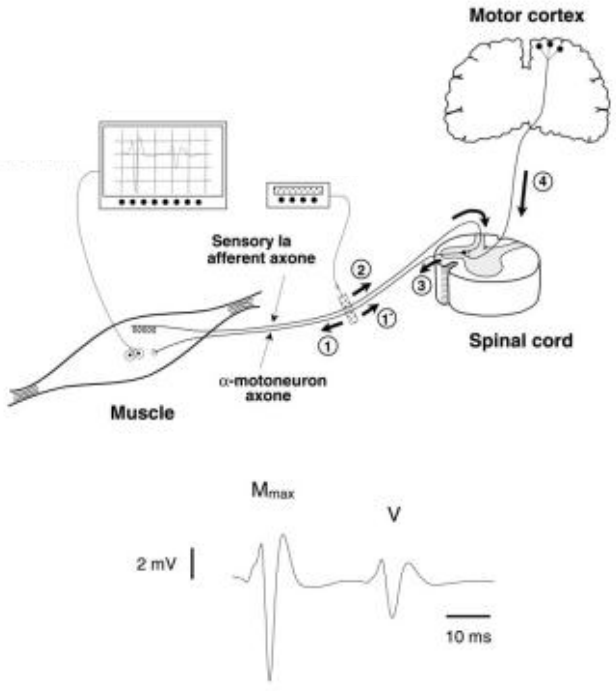


Figure 3 – Displays the aspects and pathways involved when eliciting a H-reflex or V-wave (Aagaard, et al. 2002). 1. Antidromic and orthodromic current from stimulation flowing through the axon. 2. Ia afferent signals running towards the spinal cord. 3. H-reflex or V-wave travelling towards the muscle. 4. Shows central drive.

The H-reflex can be elicited in almost all muscles given that the peripheral nerve is accessible. The electrical stimulation of the afferent nerve results in two responses in the homonymous muscle, depending on the stimulus intensity. First an M-wave is evoked, which is a short latency direct motor response from the stimulation of the motor axons and second the H-reflex previously described will appear as shown in fig. 4. (Zehr 2002).

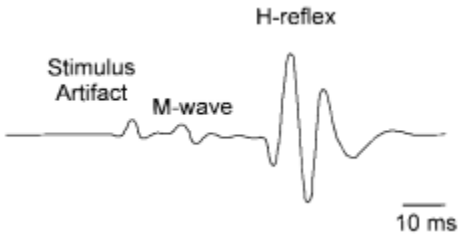


Figure 4 – Illustration of the evoked potentials when stimulating for the H-reflex. Initially a stimulation artefact, followed by a direct muscle response, the M-wave and finally the H-reflex response can be seen as indicated (Misiaszek 2003).

At low stimulus intensity, only the H-reflex is evoked because of the lower threshold for activation in the Ia fibers compared to the motor axons. At higher intensities, the M-wave appears and at still higher intensities the H-reflex progressively declines whereas the M-wave continues to increase until plateau (defined as the Mmax). The decline in the H-reflex amplitude is due to antidromic signals propagating through the motoneuron axon towards the cell body which cancels out the ortodromic action potentials from the reflex in the same motor axons. This means that at very high stimuli intensities, only the M-wave is visible (Knikou 2008). This relationship is shown in fig. 5.

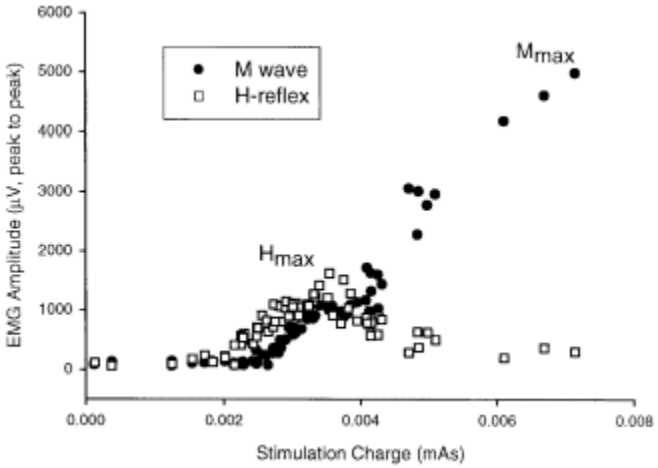


Figure 5 – An illustration of the correlation between stimulation intensity and the M-wave and H-reflex responses (Zehr 2002).

The H-reflex should be elicited at the ascending part of the curve. This is to avoid oligosynaptic inputs, Ib and recurrent inhibitory pathways affecting the response (Pierrot-Deseilligny, et al. 1981). Furthermore, the M-wave should remain stable between 25±10% of Mmax during dynamic

contractions to make sure that a constant number of motor nerve fibers and Ia afferents are excited (Simonsen and Dyhre-Poulsen 1999).

It is suggested that the H-reflex is a measure of motoneuron pool excitability. However, conclusions should be drawn cautiously due to pre- and postsynaptic inhibitory events and supraspinal influence.

One of these inhibitory mechanisms is the post-activation depression which occurs at the presynaptic terminal between the Ia afferents and the α-motoneurons (Knikou 2008). It derives from previously activated Ia afferents influencing the H-reflex amplitude. The inhibitory effect diminishes during voluntary activation. However, it is dependent of the inter-stimulus interval (Knikou 2008).

Furthermore, the various afferent inputs received from the joints, muscles, tendons and skin need to be controlled by way of inhibition in order for a motor task to be successfully executed. This inhibition also occurs at the presynaptic terminal between the Ia afferent and the α-motoneurons and is called presynaptic inhibition (Knikou 2008). It plays an important role in muscle synergy, smooth execution and movement patterns during various locomotor tasks. It results in primary afferent depolarization and is caused by gamma-aminobutyric (GABA) synapses which reduce the presynaptic impulse – leading to a decreased liberation of excitatory transmitters, thereby reducing the monosynaptic transmission. The inhibition is controlled by descending tracts and interneurons and is readily capable of modulating the H-reflex amplitude, regardless of excitation levels (Knikou 2008). Thus, an extensive amount of consideration is needed when interpreting H-reflex data.

During movement, additional control of agonist and antagonist muscles is needed. This is caused by an Ia inhibitory interneuron, excited by corticospinal, rubrospinal and vestibulospinal tracts as well as by flexion reflexes and group II, III and IV muscle afferents (Knikou 2008). It is called reciprocal inhibition and is able to reduce the H-reflex whenever the antagonistic muscles contract. Contrary, Ib afferents from golgi tendon organs, which are sensitive to active and non-active force at the muscle-tendinous junction, participate in the inhibition of motoneurons projecting to synergists and further facilitate motoneurons projecting to antagonists (Knikou 2008). This inhibition reverses during loading to Ib facilitation (Knikou 2008). It is suggested that the mechanism is important for regulating muscle stiffness during movement (Knikou 2008) and also in its contribution to timing between different phases of locomotion.

Other inhibitory neurons such as the renshaw cell, located in the ventral horn medial to the motor nuclei, affect neural control as well through recurrent inhibition. The renshaw cells are excited by axon collaterals from motoneurons and inhibit the α-motoneurons projecting to the same or synergistic muscles (Knikou 2008). They are further influenced by activity in segmental afferents and connect with γ-motoneurons, interneurons mediating reciprocal inhibition, other renshaw cells and descending tracts. It has been suggested that recurrent inhibition functions as a stabilizing feedback mechanism to reduce sensitivity of neurons – reducing their discharge frequency to a given input (Knikou 2008). Consequently recurrent inhibition affects selection of appropriate muscle synergy patterns during movement and acts as a gain regulator of motor output in weak/strong contractions (Knikou 2008).

Finally, as fatigue occurs there is an increase in muscle pain, hypoxemia, ischemia, bradykinin, potassium, phosphate and lactacte. Small diameter, III/IV afferents are sensitive to such increases

along with mechanical and thermal events, and as a result exert presynaptic inhibition on reflex responses (Walton, Kuchinad, et al. 2002) (S. C. Gandevia 2001). The effects of III/IV afferents during fatigue are still controversial and have been stated to initially reinforce muscle contraction through fusimotor activation followed by a later disfacilitation, acting at both spinal and supraspinal levels (S. C. Gandevia 2001).

Accordingly much attention should be paid to the contribution of the various inhibitory and facilitatory mechanisms when interpreting h-reflex data.

The V-wave

During voluntary motor efforts an electrophysiological variant of the H-reflex can be measured - the V-wave. It is elicited by the use of supramaximal electrical stimulus which elicits action potentials in all Ia afferents and α-motor axons. Similar to the H-reflex an M-wave is evoked and recorded as the Mmax. Additionally due to the large stimulation intensity, antidromic signals will propagate towards the spinal cord. These collide with orthodromic signals originating from descending voluntary activation resulting in cancellation of the two signals. This allow for the H-reflex to pass to the muscle, where it is recorded as the V-wave as shown in fig. 3 (Aagaard, et al. 2002). Both the H-reflex and V-wave are affected by common neural mechanisms. However, the H-reflex is more sensitive to motoneuron excitability and presynaptic inhibition and V-wave is more sensitive to supraspinal input to the motoneural pool (Vila-Chã, et al. 2012). An increase in v-wave response reflects an increase in antidromic collision due to increased efferent motoneural output (Upton, McComas og Sica 1971), which means that at greater efferent output more antidromic cancellation will occur, and hence, greater V-waves will appear. Thus combined, the H-reflex and V-wave may provide important information in the investigation of neural modulation to fatigue.

Peripheral supramaximal stimulation

Alternatively, peripheral supramaximal stimulation during a maximal voluntary contraction can be utilized when investigating central fatigue – eliciting a superimposed twitch. When stimulation is applied to the nerve of an active muscle during a voluntary contraction, the motor units that have not already been recruited present a twitch response in force (Shield and Zhou 2004). As the neural drive to the muscle increases, fewer motor units are available for recruitment and the twitch response diminishes, and will be "undetectable" during full muscle activation, as shown in figure 6.

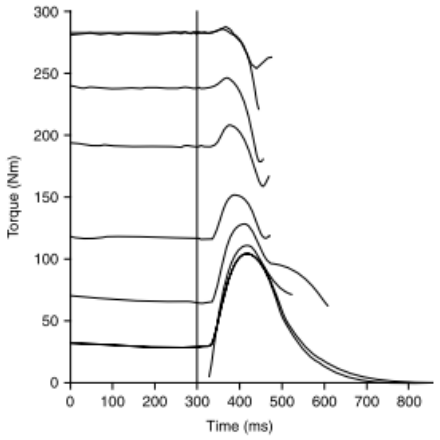


Figure 6 – Displays the inverse relationship between twitch force and voluntary force output (Shield og Zhou 2004).

Merton 1954 found a negative linear relationship between twitch force and voluntary force and also stated that full muscle activation could be achieved. Later studies using more sensitive techniques found that this was not always the case (Shield and Zhou 2004).

The quantification of muscle activation using this technique can be difficult and has been subject to some controversy (Shield and Zhou 2004). The technique used is called the twitch interpolation technique and is quantified by expressing the interpolated twitch as a percentage of the twitch during rest. The resting twitch should be evoked between 1.5-5 sec after the voluntary activation to ensure that both twitches are equally potentiated (Shield and Zhou 2004). Some studies have found a nonlinear relationship between twitch force and voluntary force – both concave upward and asymptotic (Shield and Zhou 2004). These discrepancies in findings have led to the thought that voluntary activation may not be evaluated by the single interpolated twitch ratio. It might be influenced by collisions between antidromic and orthodromic potentials and spinal effects on the subsequent silent period, resulting in diminishing force responses (Greatest influence between 40-80% MVC) (Shield and Zhou 2004). Accordingly, overestimation of voluntary force will occur when scaling the superimposed twitches to resting twitches.

Despite controversy and discrepancy, the twitch interpolation technique remains a valid and reproducible measure of voluntary activation (L. J. Taylor 2008) and problems can be minimized using proper experimental technique.

Using the twitch interpolation technique, it is not possible to deduce where, proximal to the stimulation site the neural modulation takes place. For this purpose, transcranial magnetic stimulation can be used to provide additional knowledge cortical excitability and central drive.

Transcranial magnetic stimulation

Transcranial magnetic stimulation (TMS) was originally developed by Barker and colleagues in 1985, and is another way to investigate the neural modulation to fatigue at cortical level. By utilizing induction to transport electrical current across resistive layers into the brain, it induces alterations in the electrical environment of neurons, causing them to fire (Wassermann and Zimmermann 2012). Figure 7 illustrates a typical TMS setup where the subject is provided with visual feedback of force production while EMG is recorded and the TMS coil is placed over the motor-cortex.

The stimulus evokes multiple descending volleys in the corticospinal neurons, direct D-waves and indirect I-waves, which is dependent on the stimulus intensity and the excitability of cortical cells. Stimulation over the motor cortex induces short-latency excitatory responses in many muscles and is called motor evoked potentials (MEPs) (Taylor and Gandevia 2001). These are dependent on the transmission through relevant excitatory and inhibitory interneurons and on the excitability of the motoneuron pool.

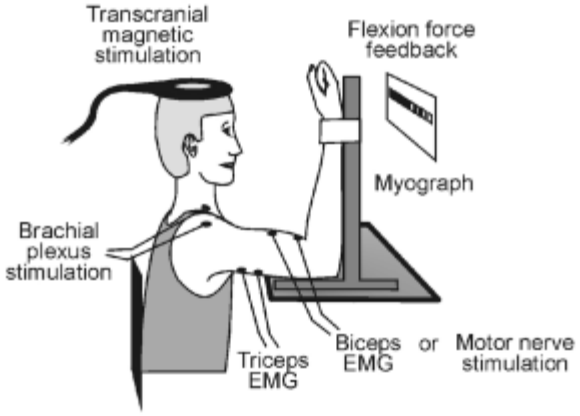


Figure 7 – Examble of a setup utilizing transcranial magnetic stimulation during contractions of the biceps brachii (Todd, J.L. and Gandevia 2003).

These aspects need to be taken into account when interpreting modulations in the cortex (Taylor and Gandevia 2001).

When stimulating during voluntary contractions, the MEP is followed by a silent period, where the electromyogram amplitude is near zero, sometimes for more than 200 ms. The silent period (SP) may represent different aspects such as inhibition of central drive, reduced motoneuron excitability and intracortical inhibition. Moreover, the MEPs are much larger compared to relaxation, due to increased excitability in both corticospinal neurons and motoneurons and also increased output from the motor cortex (Taylor and Gandevia 2001).

Method

The following methodical approach was developed to investigate some of the above mechanisms during 800 m running and fatigue.

Subjects

Experiments were performed on n=8 highly trained healthy, competitive, middle distance male runners [age 27± 4 (mean, SD) yr, body height 175 ± 4.7 cm, body mass 66.8 ± 7.2 kg] who volunteered to participate in the study.
The subjects gave written informed consent after being properly informed of the experimental setup and conditions. The local ethics committee approved all procedures (N-20130013), and the study was conducted according to the Declaration of Helsinki.

Experimental setup

The subjects reported for testing on two separate test days for an 800 m speed test on a 400 m tartan track and 800 m running on a treadmill, respectively.

The initial 800 m speed test estimated the subjects' average speed during an actual 800 m run, which was used during 800 m treadmill running. All subjects went through individual warm-up prior to the test. The mean speed of the maximal run was 22.2 ± 0.6 km/h, with a speed drop equivalent to 5.7±1 sec from the first 400m to the last. The subjects were encouraged verbally during the entire run and split times were provided every 200m.

800 m treadmill running was tested at three different speeds. The subjects were strapped in a safety harness attached above the treadmill.
Pre and immediately post 800 m treadmill running the subjects performed six sets of isometric maximal voluntary contractions (MVC), receiving peripheral electrical stimulation (PES) and transcranial magnetic stimulation (TMS). Each set consisted of 4 contractions – 100%, 50%, 75% and 100% respectively, and each set was spaced by 60sec. The method was previously tested and proved reliable ((b) Sidhu, Bentley og Carrol 2009). Subjects were seated in a custom built rig, legs and feet in a 90 degree angle – their dominant foot positioned on a footplate, with an imbedded force transducer (Kistler, Germany). Subjects were further strapped down above their knees to isolate plantar flexion movement and to avoid movement artifacts. Prior to testing, the subjects had several familiarization contractions at different force levels.

800 m running protocol

Speeds during 800 m treadmill running were calculated as % of maximal speed, which was tested prior. Three different speeds were performed on a treadmill (Technogym, Runrace HC1200, Italy) for 800 m – 50 % and 75 % of 800 m maximal speed and at maximal speed with 1% incline (Jones and Doust 1996). Subjects were given at least 10 min of rest between running trials. To ensure that the subjects were maximally exerted, they were instructed to run at least 800m or as long as they could maintain speed. This modification was used since treadmill running might be slightly easier than track running and to make sure that subjects were maximally exerted.

For MVCs, plantar flexion of the dominant foot, were performed pre and immediately post 800 m running, seated in a custom built rig. Subjects practiced performing a MVC prior to the test and were given at least 1 min of rest between MVC trials. Visual feedback was provided (Follow Me, Knud Larsen, SMI, AAU). A conceptual illustration is shown in Fig. 8.

Guidance and strong verbal encouragement were given throughout the protocol.

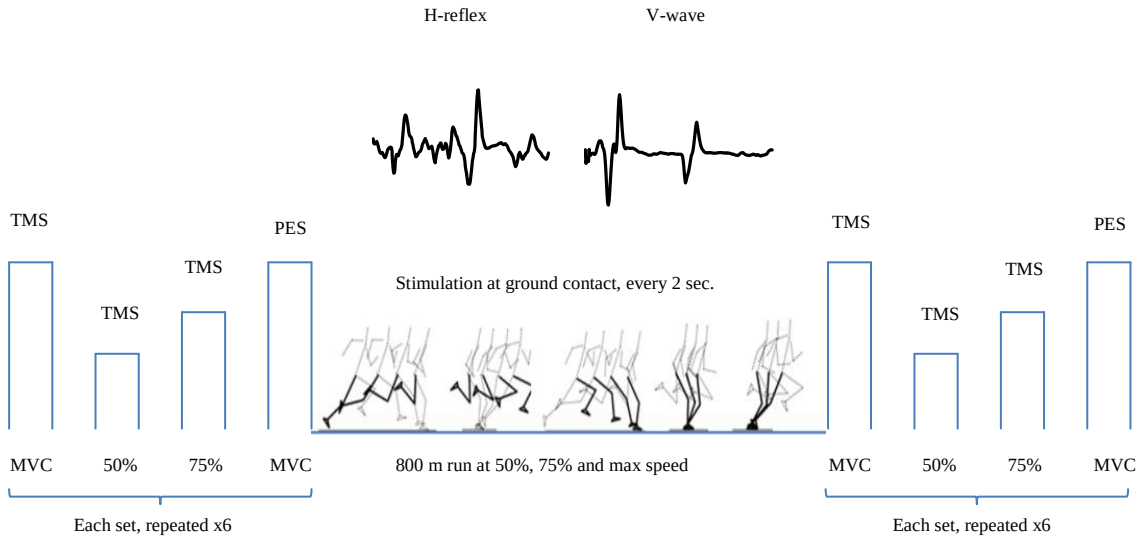


Figure 8 – A conceptual illustration of the methododical approach

Stimulation procedures

The electrical stimulations consisted of 1 ms square pulses delivered by a constant current electrical stimulator (Isolated Stimulator, NoxiTest; IES 230). All electrical stimulations were elicited to the tibial nerve through a cathode (Pals® Platinum Pre-gelled Ø=3.2cm, Axelgaard Manufacturing) placed in the popliteal fossa and an anode (Pals® Platinum Pre-gelled 5×9cm, Axelgaard Manufacturing) placed at the iliac crest. Probing for detection of optimal cathode position was performed initially. First Mmax during standing was found by increasing the stimulation intensity until a peak plateau was found in the M-wave amplitude. Then stimulation during running should fit the following criteria: Stimulation intensity should evoke an M-wave that was 25±10 % of the Mmax and the second stimulation intensity, in the stride 2 sec after, should be supramaximal – 2 times the

Mmax stimulation intensity found while standing. To find an initial H-reflex stimulation intensity, a running input/output (I/O) curve was performed at max velocity. This was done by delivering 20 stimuli divided in 10 classes of varying intensity. The intensity was set to stimulate in 10 classes following a logarithmic scale. The intensity chosen for further appliance was found by inspecting M-wave amplitudes and corresponding H-reflex amplitudes in the I/O curve. The class where the M-wave fitted the criteria, and the H-reflex was on the ascending recruitment curve was chosen. Finally, electrical stimulation was elicited consecutively at two different intensities every 2 sec during 800 m running - triggered at 0 % of ground contact by a footswitch attached mid foot under the subjects' shoe. The first stimulation evoked the H-reflex while the second stimulation evoked the V-wave.

The six MVC sets pre and post 800 m running consisted of four contractions of 100%, 50% 75% and 100%, respectively. During the initial three contractions, 100%, 50% and 75%, transcranial magnetic stimulation (TMS) (Magstim 200^2, The Magstim Company, Camarthenshire, UK) was elicited, using a figure of eight coil (Double Cone Coil, The Magstim Company, Camarthenshire, UK), during the max plateau in force to induce motor evoked potentials (MEP) and force twitches. Initially, a hotspot was found by locating the position where MEP's, stimulating with lowest possible intensity, could be measured in the Soleus and Gastrocnemius, while evoking a small MEP response in the tibialis anterior. When the hotspot was found, the location was marked directly on the subjects scalp with a permanent marker. Next, stimulator output was determined by altering the output to an intensity eliciting MEP areas >50% of Mmax area in the soleus and gastrocnemicus and MEP areas <10% of Mmax in the tibialis anterior. The Mmax area was found by peripheral electrical stimulation during a 50% contraction sitting in the custom build rig and by using the same procedure as previously described during standing. The stimulator output fitting our criteria was used throughout the protocol. Subjects were given a minimum of 10 sec rest between each contraction and TMS, and 1min rest between each set.

During the last 100% contraction in each set a supramaximal peripheral electrical stimulation was elicited to induce superimposed twitches. These were also elicited to the tibial nerve using the same stimulation sites and electrodes as during running – however, with a stimulation intensity of 120 % of the Mmax found seated during a 50% contraction. The stimulation was elicited during peak plateau of the MVC and 5sec after during a rest period to evoke a rest twitch.

EMG recordings

Surface electromyography (EMG) was measured at the lateral soleus, medial gastrocnemius and tibialis anterior. Measurements were conducted by use of bipolar surface electrodes (Neuroline, 720 Ag/AgCl, Medicotest) with an inter-electrode distance of 20 mm on all muscles investigated. The soleus electrodes were placed at $2/3^{rd}$ of the line between the tip of the fibula to the lateral malleolus and the gastrocnemius electrodes were placed medially in the fiber direction on the muscle belly. The tibialis anterior electrodes were placed at $1/3^{rd}$ on the line between the tip of the fibula and the tip of the medial malleolus. A ground electrode was placed on the tibial bone. All electrode locations had undergone proper skin preparation (shaved, abraded, alcohol) and electrodes were fixated by use of medical tape. The EMG was amplified by 1000 and band-pass filtered between 10 and 1000 Hz, with a sample rate of 5000Hz.

Force measurements

Force was measured by a force transducer located under a footplate and all force signals were sampled at 5000Hz. The force transducer were calibrated prior to all tests.

Data analysis

Force and VA measurements

MVC force was measured in Newton [mean N 100ms pre stimulation – baseline N] and normalized to highest prefatigue MVC.

Voluntary activation (VA) measured by peripheral electrical stimulation was analyzed by use of the twitch interpolation technique. Force responses elicited by the supramaximal electrical stimulations were measured during contractions (Superimposed Twitch (ST): Mean force 100 ms prior to the stimulation artifact, subtracted from peak evoked twitch-force within 200 ms after the stimulation artifact) and at the following rest periods (Resting twitch (RT): Peak evoked twitch-force within 200 ms after the stimulation artifact). VA was then calculated as: VA (%) = [1-(ST/RT)]×100. This was the same for the TMS force measurements, although the rest twitch was calculated by linear regression of the TMS-evoked force twitch measurements during 50%, 75% and 100% contractions, respectively.

Electrical stimulation

Amplitudes of evoked waves were measured as EMG peak to peak. For M-waves and Mmax cursors were placed at 5ms and 25ms post stimulation and at 30ms and 60ms post stimulation for the H-reflex and V-wave. Mean M-waves, H-reflexes and V-waves were normalized to the mean Mmax measured during the same epoch. Data from the run was divided into 8 epochs where the mean from the first and last epoch was compared. H-reflexes with M-waves not within a range of 25±10% of Mmax were excluded.

EMG root mean square

Background EMG root mean square (rmsEMG) was measured for soleus, gastrocnemius and tibialis anterior 20ms prior to each stimulation and was normalized to the Mmax measured during the first epoch of the run. rmsEMG of MVC pre and post the run was found 100ms pre stimulation and normalized to Mmax found during a 50% contraction.

MEP and Silent periods

MEP was quantified as integrated area between cursors at the beginning and end of the evoked potentials (between ≈ 20-60ms post stimulation). Area was used, not peak to peak, due to the multiphasic nature of soleus MEPs and they were normalized to the Mmax area found during 50% contraction.

Silent periods were quantified by visual inspection as ms from following criteria: Start = Stimulation onset. MEP onset defined as deflection positive or negative above volitional EMG. Zero-Crossing of baseline an 0V after MEP. End = Return of visual EMG was defined as positive or negative deflection associated with resumed EMG. This method was developed by (Damron, et al. 2008) and they showed that visual inspection of silent periods was more reliable between test days than mathematical detection.

Statistical analysis

All data was recorded with Mr Kick III*preview* (v. 2.9) and stored on a personal computer (Fujitsu Siemens, Esprimo). Analysis of data was performed post hoc in Mr Kick III*preview* (v. 2.9) and Excel 2010 (Microsoft) except for the footswitch data which was analyzed in MatLab (Version 7.8.0.347 (R2009a)). Statistical analysis of all data was performed with IBM SPSS 20.

All data are presented as mean±sd, and paired T-test (2 tailed) was applied to evaluate statistical significance with significance level set to $P<0.05$. Further MANOVA was used to detect statistical significance of H-reflex, V-wave and rmsEMG between speeds. All parameters were tested for Gaussian distribution by the Shapiro-Wilk test. All measures had a Gaussian distribution except for the V-wave. Despite of this, parametric tests were applied to all measures since more subjects will be added continuously.

Results

This chapter presents the obtained results. Below is an overview of the subjects who participated in the study, the exclusions that were made and reasons for why. This is followed by a section on force measurements and RPE to assess neuromuscular fatigue. Then measurements illustrating the origin of fatigue are shown. This includes measurements during the actual running tasks, i.e. H-reflexes, V-waves and M-wave and measurements Pre/Post running, i.e. VA, RT, MEP and SP.

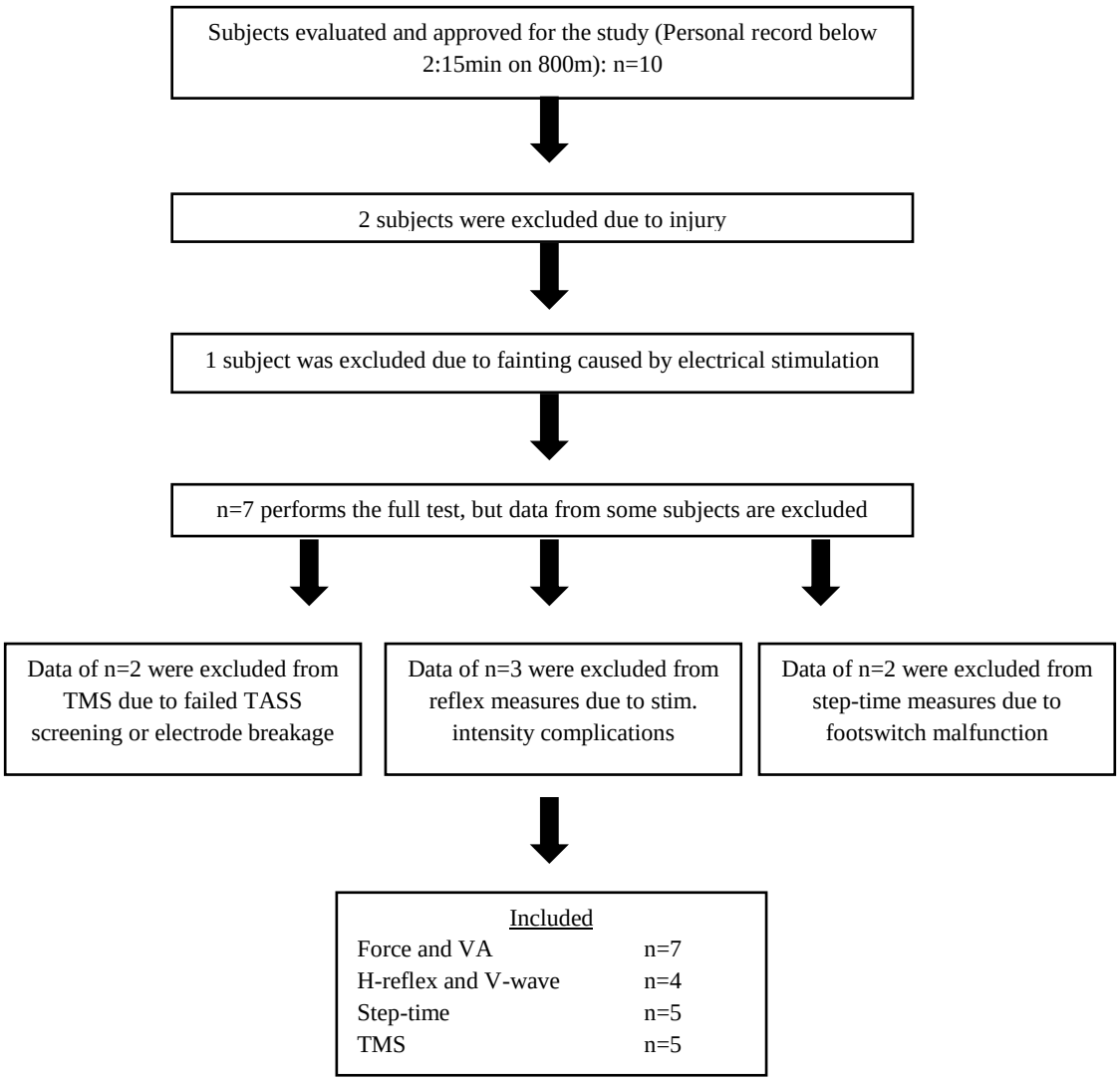


Figure 9 – The exclusions that were made for each individual measure and the reasons for why.

Neuromuscular fatigue

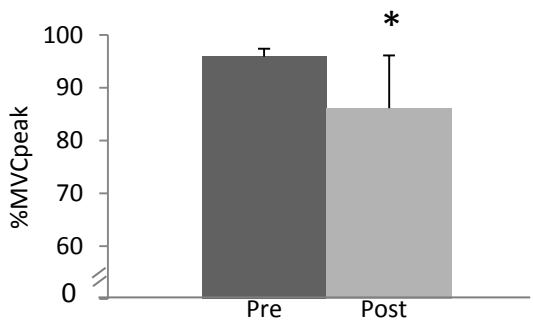


Figure 10 - Mean of 5 highest MVC force pre and post running normalized to prefatigue. Black represents Pre measurements and grey represents post measurements. (n=7)

The mean MVC peak force measurements obtained pre and immeditely post the maximal run showed a signicant decrease in force, -9.7 ± 9.6% (n=7, P = 0.049; Fig 10).

The mean Borg rating for 7 subjects obtained for every 100m of the 100%, 75% and 50% run showed clear difference between speeds (Fig 11). The Borg rating for the 50% run was stable between a rating of 8 and 9 which is defined as "very light". The 75% run was executed between 11 and 13 which are defined as "Fairly Light" and "somewhat hard" on the scale. However in the 100% run, subjects reported an initial rating of 13 which progressed to 20 defined as "maximal exertion" during the last epoch of the run.

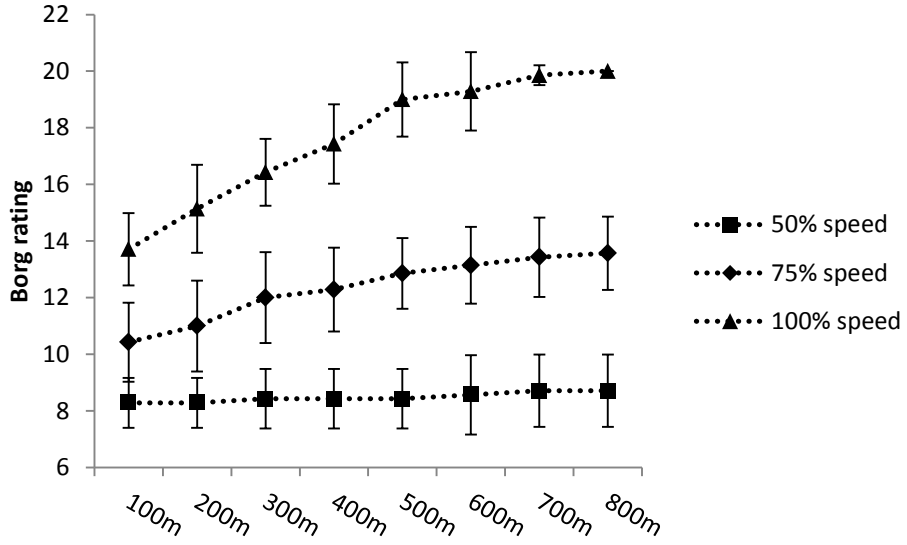


Figure 11 – Mean Borg Rating obtained every 100m during 50% run, 75% run and 100% run. Triangle represents the 100% run, diamond represents the 75% run and square represents the 50% run. (n=7)

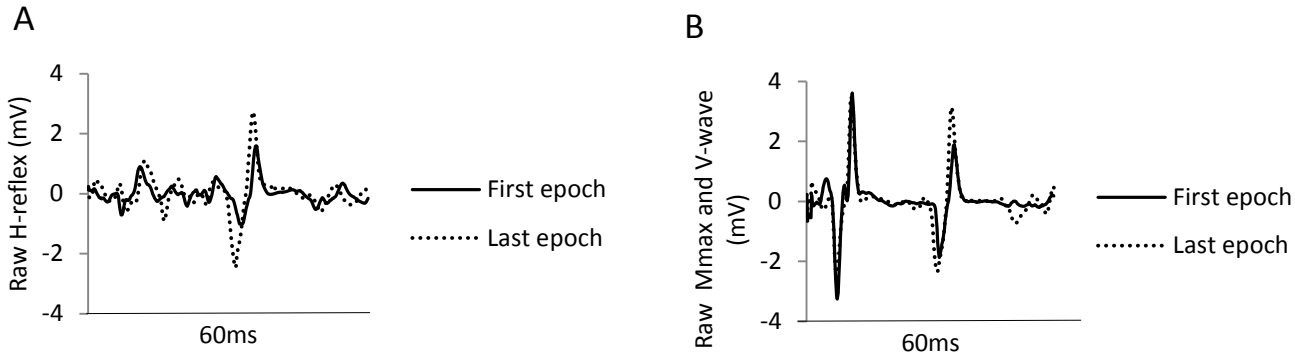


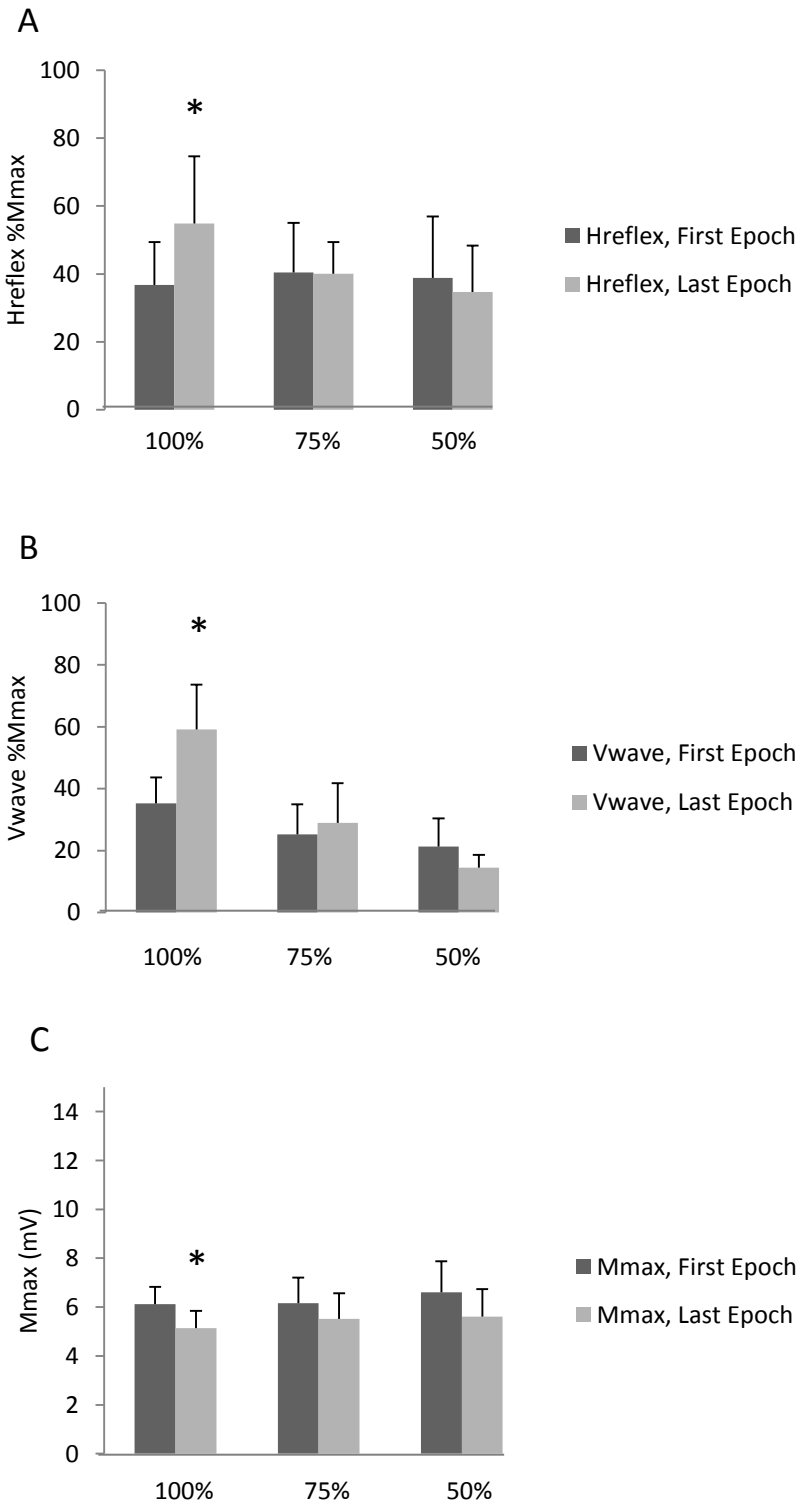
Figure 12 - Representative H-reflex (A) and V-wave (B) responses from the first to the last epoch for one subject. Full line represents the first epoch and dashed line represents the last epoch.

H-reflex and V-wave measurements were obtained for all subjects at 50%, 75% and 100% speeds. The modulation from the first and last epoch of the runs were compared. Fig 12A and 12B illustrate raw H-reflex and V-wave amplitudes for one subject, representative for the sample.

The mean H-reflex amplitudes from the first to the last epoch for all the subjects, n=4, showed a significant increase during the 100% run, 18.13 ± 10.7% (P = 0.043; Fig 13A). The corresponding M-wave also showed a small but significant increase, 7.9 ± 2.7% (P = 0.009). However the values were within our criteria for acceptable M-waves, 25±10% of Mmax. V-wave modulations during the same epochs at the 100% run, also showed a significant increase, 23.9 ± 1% (P = 0.038; Fig 13B). The corresponding Mmax showed a significant decrease, -16.1 ± 2.1% (P = 0.001; Fig 13C). See also table 1 for individual subject data and means.

	Mmax (mV)					Hreflex (%Mmax)					Vwave (%Mmax)				
	First Epoch		Last Epoch		Diff.	First Epoch		Last Epoch		Diff.	First Epoch		Last Epoch		Diff.
1	5.1	±0,3	4.2	±0,3	-1.0	49.7	±11,2	73.6	±16,8	23.8	31.1	±2,9	73.9	±8,3	42.9
2	6.8	±0,4	5.9	±0,5	-1.0	45.3	±15,2	66.7	±16,1	21.3	47.9	±8,3	67.9	±26,4	20.0
3	6.1	±0,4	5.2	±0,2	-0.9	25.0	±5,1	50.0	±5,8	25.1	31.3	±7,1	52.9	±3,8	21.6
4	6.4	±0,4	5.3	±0,2	-1.1	26.9	±1,5	29.2	±2,7	2.3	30.9	±15,1	42.1	±9,3	11.2
Avg.	6.1	±0,4	5.1	±0,3	-1.0	36.7	±8,2	54.9	±10,3	18.1	35.3	±8,4	59.2	±11,9	23.9

Table 1 - Data from 4 subjects from the first and last epoch during the 100% run. The epochs comprise 12.5% of the data-points in either the first or latter part of the run. Mmax, H-reflex and V-wave data are displayed for each subject with the appertaining standard deviations and difference. The overall means are also shown.



The size of the H-reflex amplitude was significantly dependent on treadmill speed (P = 0.05; Fig 13A).

The size of the V-wave amplitude was also dependent on treadmill speed (P=0.01; Fig 13B).

The Mmax results revealed significant changes during the 100% run only (P = 0,001), and not during the submaximal runs, 50% and 75% (Fig 13C). Furthermore, no change was found between the 100%, 75% and 50% runs in either epoch (Fig 13C). M-wave results showed significant increase during the 100% run (P=0,009), and not during the submaximal runs.

Voluntary activation (VA) measured during MVC pre and immediately post 100% running and quantified by use of the twitch interpolation technique showed a significant decrease, -9.3 ± 7.3% (n=7, P = 0.026; Fig 14A) and the appertaining resttwitches (RT) also showed a significant decrease, -28.5 ± 15.7% (n=7, P = 0.004; Fig 14B).

Figure 13 - All plots show mean data from 4 subjects from the first (Black) to the last (Grey) epoch during their 100%, 75% and 50% run. The epochs comprise 12.5% of the data-points in either the first or latter part of the run. * signifies significant statistical difference between epochs (P < 0.05). A: H-reflex amplitude normalized to Mmax within the same epoch from the first to the last epoch, during the 100% run, 75% run and 50% run. B: V-wave amplitude normalized to Mmax within the same epoch from the first to the last epoch, during the 100% run, 75% run and 50% run. C: Mmax modulation normalized to Mmax within the same epoch (mV) from the first to the last epoch, during the 100% run, 75% run and 50% run.

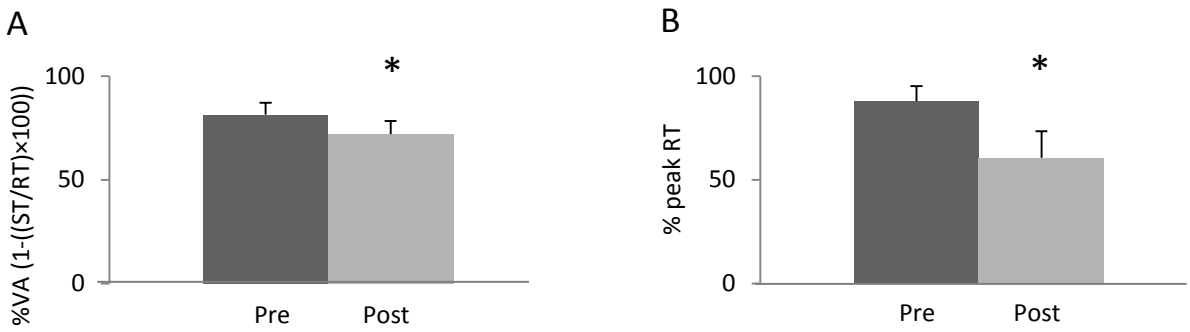


Figure 14 - . A: Voluntary activation pre and post running – Calculated by way of the twitch interpolation technique. B: Rest twitches normalized to prefatigue rest twitch. Black represents Pre measurements and grey represents post measurements (n=7).

MEP and SP measurements were obtained pre and post running during 50%, 75% and 100% contractions. Fig. 15 display raw MEP data pre and post during 50% contraction for one subject, representative for the sample.

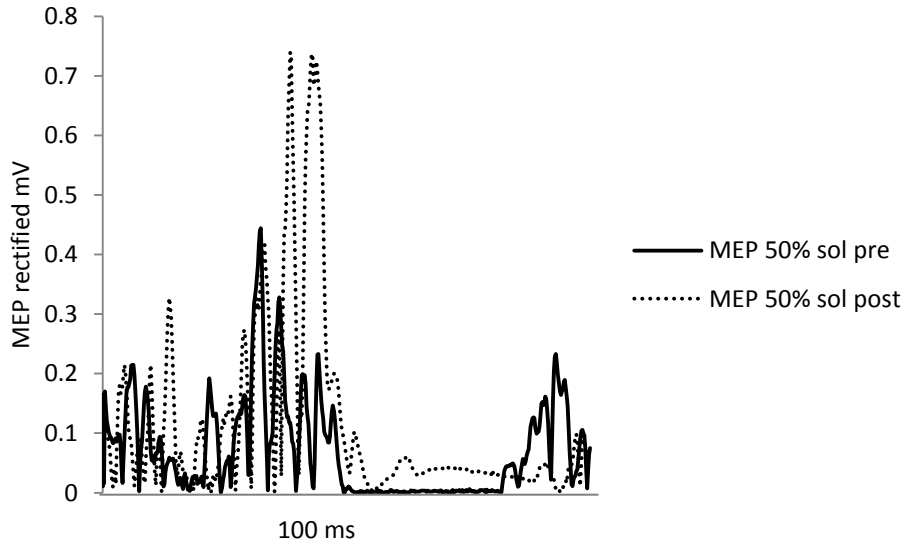


Figure 15 - Representative MEP, pre and post running for one subject. Full line represent pre measurements and dotted line represent post measurements (n=1)

MEP area and silent periods were measured for 5 subjects and showed no significant difference from pre to post during 100% and 75% contractions but a significant increase in both parameters during 50% contractions, MEP increase 7.4 ± 4.8% ($P = 0.037$), SP increase 7.3 ± 4.8ms ($P = 0.014$; Fig 16A, 16B).

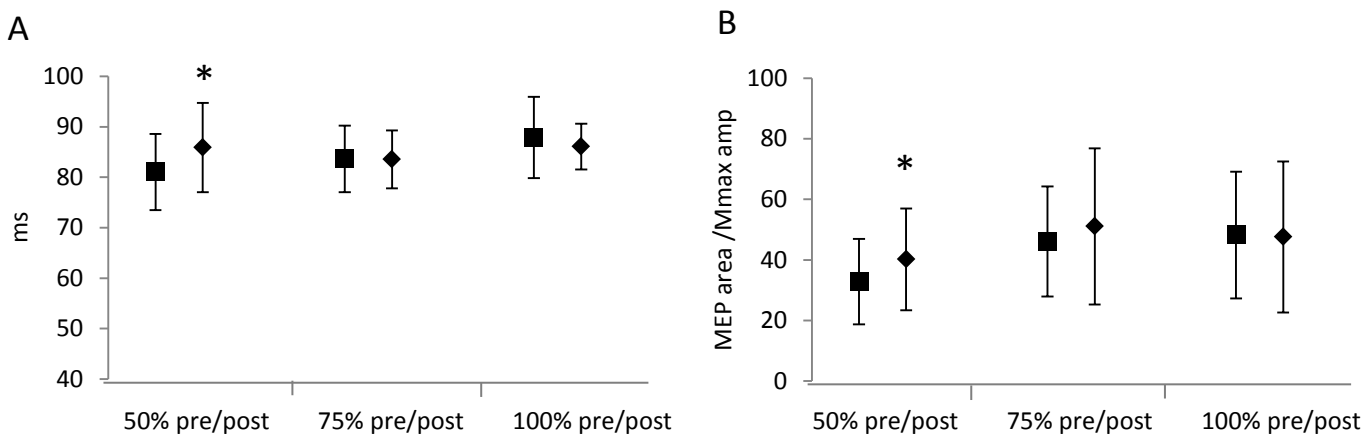


Figure 16 - A: Soleus silent periods at 50, 75 and 100% contractions. B: Soleus MEP area at 50, 75 and 100% contractions normalized to Mmax peak to peak from sitting position. Square represent pre measurements and diamond represent post measurements. (n=5)

The underlying rmsEMG during the first and last epoch in the 100%, 75% and 50% run is shown in Table 2. The results showed no significant change from the first to the last epoch during either of the runs (n=4).

	Soleus (%Mmax)					Gastrocnemius (%Mmax)					Tibialis anterior (%Mmax)				
	First Epoch		Last Epoch		Diff.	First Epoch		Last Epoch		Diff.	First Epoch		Last Epoch		Diff.
100% run	4.4	±0,4	3.5	±0,6	-1.0	12.2	±7,1	10.1	±6,4	-2.1	1.1	±0,6	0.9	±0,7	-0.1
75% run	3.4	±0,6	2.7	±0,8	-0.7	6.3	±2,4	5.8	±2,4	-0.5	0.9	±0,5	0.7	±0,4	-0.2
50% run	2.5	±0,7	2.0	±0,5	-0.5	4.4	±2,1	3.6	±2,2	-0.8	1.0	±0,6	0.6	±0,1	-0.4

Table 2 - rmsEMG for the soleus, gastrocnemius and tibialis anterior from the first to the last epoch, during 50% run, 75% run and 100% run. The epochs comprise 12.5% of the data-points in either the first or latter part of the run (n=4).

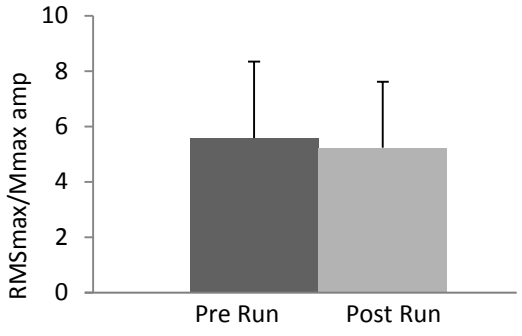


Figure 17 - : Mean of 5 highest MVC RMS normalized to prefatigue. Black represents Pre measurements and grey represents post measurements. (n=7)

No significant change was measured in the mean rmsEMG during MVC pre and immediately post (Fig 17).

Stride time for 4 subjects showed no significant difference from the first to the last epoch in either of the runs. There was a significant difference when comparing the 50% run, 75% run and 100% run (P = 0.05; Fig 18).

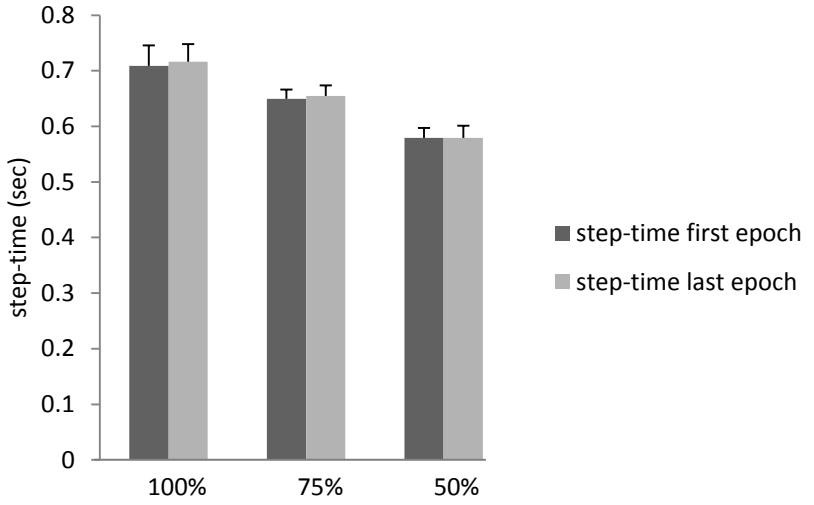


Figure 18 - Stride time in seconds from the first to the last epoch during 50% run, 75% run and 100% run. Black represents the first epoch and grey represents the last (n=4).

Discussion

This study was designed to test the hypothesis that high level athletes running 800 m at maximal velocity would induce neuromuscular fatigue and that central factors would modulate at the end of the run. MVC force decreased (P=0.049) and VA decreased (P=0.026) post running. During running the H-reflex and V-wave increased (P=0.043, P=0,038), respectively. To the authors' knowledge this is the first study to show increased central excitability and central drive during 800 m running to fatigue, accompanied by a decrease in MVC and VA post running.

Neuromuscular fatigue

Figure 10 shows a decline in maximal voluntary force indicating that neuromuscular fatigue as defined by Taylor et al. 2008, was present as a result of the 800 m run. There was an overall decline for n=7 (P = 0.049) and this observation was in line with previous studies investigated post 5000m running (Girard, et al. 2012) and post 400m sprints (Tomazin, et al. 2012) who found MVC decline of 27% (P<0.001) and 13.8 (P<0.001) respectively.

The subjects in the current study were instructed to continue running until exhaustion. During the last epoch of the run RPE evaluated every 100 m showed that the subjects had reached maximal exertion i.e. a Borg rating of 20 (Fig 11). During this part of the run a decline in speed has been recorded during actual 800 m running (Thomas, et al. 2004). Due to these findings the last epoch during 800 m running was of main interest. It should be noted that speed remained constant during this study due to methodical considerations, however the results does show that neuromuscular fatigue did occur as a result of 800m running. Various sites within the neuromuscular system may cause the decrease in neuromuscular output - one of them being peripheral mechanisms. These will be discussed first, followed by a discussion of the spinal and supraspinal mechanisms.

Peripheral fatigue

Similar to previous studies twitches evoked during rest decreased after running (P=0.004) suggesting that peripheral fatigue was evident (Girard, et al. 2012) (Tomazin, et al. 2012). A decrease in Mmax response, equivalent to 16.3% further supports this notion. Studies have shown similar effects during fatiguing contractions (Duchateau, Balestra and Carpentier 2002) (Fuglevand, et al. 1993) (Girard, et al. 2012). During high intensity running sarcolemma excitability is possibly reduced by metabolic perturbation and this effect deteriorates the excitation-contraction coupling (Tomazin, et al. 2012). The decrease may be attributed to changes in neuromuscular transmission and muscle fiber action potentials (Fuglevand, et al. 1993). The Na^+/K^+ pump may not be able to reaccumulate the K^+ to the muscle cells, resulting in excessive extracellular K^+ concentrations (Clausen, et al. 1998). These concentrations cause muscle membrane depolarization reducing excitability at the sarcolemma (Clausen, et al. 1998). Other muscular parameters such as inorganic phosphate, H^+ accumulation and pH may influence Ca^{++} sensitivity, and excitation-contraction coupling during fatigue as well (Perrey, et al. 2010). In the current study we expected peripheral factors to contribute to fatigue but we were also interested to investigate if central mechanisms play a role. In the following paragraphs it will be discussed how both spinal and supraspinal pathways may be affected by fatigue in order to understand their contribution to the development of fatigue during 800m running.

Spinal modulation

The H-reflex amplitude increased from the first epoch to the last during the fastest 800 m run (P=0.043). Interestingly the same modulation was not found during submaximal 800 m running speeds, and this supports the idea that H-reflex modulation occur as the result of maximal exertion and fatigue (Fig. 13A). This is to the authors' knowledge the first study to show an increase in central mechanisms during actual running to fatigue.

Previous studies have shown a decrease in H-reflex amplitude during fatiguing contractions (Racinais, Girard, et al. 2007), (Kuchinad, Ivanova and Garland 2004), (Duchateau, Balestra and Carpentier 2002), (Girard, et al. 2012). The decrease in H-reflex amplitudes was attributed to recurrent inhibition (Kukulka, Moore and Russell 1986), III/IV afferents (Garland 1991), presynaptic inhibition and supraspinal control (Rudomin and Schmidt 1999). Contrarily, the results of the current study in support of few other studies found a fatigue-related H-reflex increase (Löscher, Cresswell and Thorstensson 1996) (Nordlund, Thorstensson and Cresswell 2004). It is plausible that when peripheral factors fatigue central mechanisms such as an increase in alpha motoneuron excitability, act as a compensation to maintain force output (Löscher, Cresswell and Thorstensson 1996). The increase in excitability can be ascribed to increased central drive and decreased inhibitory mechanisms to recruit silent homonymous motoneurons (Löscher, Cresswell and Thorstensson 1996).

One of the plausible mechanisms increasing spinal excitability might be decreased presynaptic inhibition. This might be explained by decreased GABA-mediated primary afferent depolarization or decreased homosynaptic post activation depression (Nordlund, Thorstensson and Cresswell 2004). The liberation of neurotransmitter in the Ia afferent terminals are controlled by primary afferent depolarization caused by GABA synapses. Activation of these synapses causes an efflux of Cl^- ions decreasing the action potential in the afferent terminals decreasing Ca^{++} influx (Rudomin and Schmidt 1999). Furthermore, during non-fatigued repeated activation of the Ia afferents the neurotransmitter release are controlled by interneurons to provide subsequent stabile excitatory post synaptic potentials (Hultborn, Illert, et al. 1996). During fatigue supraspinal control might overwrite these presynaptic inhibitions. This control might act to increase Ia afferent input to the α-motoneuron resulting in an increased excitability. However, the role of these mechanisms during voluntary contractions and fatigue remains unclear (Knikou 2008).

Another possible mechanism acting on the increased spinal excitability was the III and IV afferents. 800 m running has been shown to induce significant metabolic changes (Thomas, et al. 2004). Therefore, it was expected that III and IV afferents might reduce excitability as a result of the metabolic change. It has previously been suggested that III and IV afferents were inhibiting at both supraspinal (Amann, et al. 2011) and spinal levels (Garland 1991). Therefore, the increase in H-reflex amplitude was surprising and may suggest that the feedback from III/IV afferent projections may be increasing spinal excitability as previously shown by Martin et al. 2008. Further attenuation of Ib inhibition from golgi tendon organs sensitive to changes in tendon-load together with an increase in fusimotor drive have been shown during III and IV afferent firing - further increasing spinal excitability.

Also, Recurrent inhibition may have an opposite effect than previously suggested (Duchateau, Balestra and Carpentier 2002) decreasing its inhibition during fatigued dynamic contractions.

Recurrent inhibition is mediated through the Renshaw cell which is influenced by numerous reflex pathways and supraspinal control (Mazzocchio, Rossi and Rothwell 1994), (Knikou 2008). It has been stated to act as a gain regulator providing controlled motor output during weak contractions, but high muscle force output during strong contractions (Hultborn and Pierrot-Deseilligny 1979) (Knikou 2008). Therefore, during fatigue a depression of recurrent inhibition might increase spinal excitability (Kukulka, Moore and Russell 1986).

The discrepancy of H-reflex modulation to fatigue found in previous studies might be related to measurements performed post exercise in resting muscles and thus, possibly not reflecting spinal modulation during dynamic contractions to fatigue. It was evident that the H-reflex amplitude did not decrease and that reflex inhibition of the motoneuron pool was not a contributing factor to fatigue as previously suggested (Garland 1991) (Girard, et al. 2012). Instead spinal mechanisms might compensate for peripheral fatigue, however this needs further investigation. One single mechanism cannot account for the increase, and it is likely that multiple mechanism act in concert to regulate motor drive.

Since spinal and supraspinal factors are interconnected, both factors need consideration. The following discussion will elaborate on the supraspinal influence.

Supraspinal modulation

There was a significant increase in V-wave amplitude when comparing the first and last epoch of the 800 m run (P=0,038), suggesting an increase in central drive as maximal exertion and fatigue occur. The V-wave reflects changes in central drive but is at the same time subjected to the same inhibitory/facilitatory mechanisms as the H-reflex (Vila-Chã, et al. 2012). Therefore interpretation should be made with caution (Simonsen, Alkjær and Raffalt 2012).

The increased V-wave amplitude was larger than the H-reflex amplitude which indicated an increased central drive (although the difference was not statistically significant). Furthermore the direct muscle responses showed a significant decrease in Mmax and stable M-waves relative to our criteria, 25±10% of Mmax. The results indicate that central factors are altering as fatigue sets in, by increasing central drive. A possible explanation could be to compensate for fatigued peripheral factors in order to maintain force output (Taylor and Gandevia 2008) possibly controlled by intial facilitatory input from III/IV afferents. This increase in central drive was unexpected since previous studies utilizing the twitch interpolation technique, have found the central drive to decrease post fatiguing exercise – suggesting central mechanisms to be performance inhibiting by decreasing recruitment and/or firing frequency by way of the same III/IV afferents (Lattier, et al. 2004) (Girard, et al. 2012) (Tomazin, et al. 2012) (Racinais, Girard, et al. 2007). In line with these studies a similar decrease in VA was found in the current study during MVCs pre/post (P=0,026). This discrepancy might be explained by a complex multifaceted influence by the III/IV afferents possibly interpreted by higher cortical areas such as the basal ganglia (S. C. Gandevia 2001). Further research is needed concerning their contribution. The discrepancy might be further explained by an initial increase in central drive to compensate for peripheral fatigue shown by the increase in V-wave but might ultimately reach a threshold where it is no longer possible to maintain motor output – hence the decrease in VA measured post exercise during MVC. Alternatively, measuring during static MVC

might be significantly different from dynamic submaximal contractions. Furthermore, a MVC might reflect conscious effort – where measurements obtained during running possibly reflects spinal regulation. The soleus muscle has weak cortical projections and is largely controlled by spinal regulation. Therefore, conscious efforts (i.e. MVC) might not reflect modulations happening at "automated" dynamic locomotor contractions.

Additional investigation of supraspinal modulation to fatigue showed increases in MEP area and SP length. In line with previous findings (Fernandez-del-Olmo, et al. 2013) (Iguchi and Shields 2011) MEPs and SPs were found to increase during a 50% contraction ($P<0.037$), but not at 75% and 100% contraction. This suggests that cortical excitability might not be able to increase during strong contractions after short duration fatiguing exercise. A possible explanation for why MEP during MVC did not increase is that maximal excitability may already have been achieved pre running (Sidhu, Bentley and Carrol 2009a). Further cortical excitability may also not alter after short intense locomotor exercise as suggested previously (Sidhu, Bentley and Carrol 2009a), (Fernandez-del-Olmo, et al. 2013).

The mechanism responsible for the increase during weak contractions has been shown during fatigue and is possibly mediated through a decrease of motor cortex inhibition through GABAa and b receptor activation (Benwel, Mastaglia and Thickbroom 2007). Alternatively the submaximal contractions were performed at the same absolute force pre and post running, which might explain the increased cortical excitability, reflecting a compensatory mechanism to reach the target force in the fatigued muscle (Fernandez-del-Olmo, et al. 2013).

Increasing silent periods during fatigue have been suggested to reflect an impaired voluntary excitation from centers "upstream" of the primary motor cortex and might explain the increased SP in the current study (Benwel, Mastaglia and Thickbroom 2007) (S. C. Gandevia 2001). Additionally, a decreased TMS VA have previously been reported (Sidhu, Bentley and Carrol 2009a) suggesting that central cortical fatigue does account for a large portion of post exercise decreased MVC (Sidhu, Bentley and Carrol 2009a). This is in line with the decreased VA in the current study possibly controlled by input from higher cortical areas mediated by key central nervous system transmitters, e.g. serotonin and dopamine, related to conscious effort and exercise (S. C. Gandevia 2001), (Davis 1995).

The current study signifies the need for a high correlation between measurements and the setting of interest since significant differences apply between static/dynamic, submaximal/maximal contractions. Future research should focus on a uniform approach in the investigation of neuromuscular fatigue

Methodical considerations

By means of the current methodology it was possible to obtain acceptable measurements of central mechanisms during dynamic contractions and fatigue. M-waves were used to ensure that the H-reflex was stimulated on the ascending recruitment curve during the run. The criteria for acceptable M-waves were amplitudes with a maximum of 35% Mmax as used by previous investigators (Capaday and Stein 1987) (Edamura, Yang and Stein 1991) (Simonsen and Dyhre-Poulsen 1999). Despite the fact that M-waves were within the criteria the M-waves had a statistical significant increase from the first to last epoch, which may compromise the validity of the H-reflex results. An increase in M-wave

could signify an increased stimulation effect resulting in an increased H-reflex. Variations in the M-wave may occur due to muscle geometry, electrode movement or change of subliminal fringe of the α-motoneurons.

Significant M-wave variance could be observed in some subjects and data points outside of the criteria were excluded resulting in the exclusion of entire trials. This resulted in a low number of subjects which makes definitive conclusions difficult. Use of stimulation intensity correcting software should be applied in future studies (Simonsen and Dyhre-Poulsen 1999).

Some of the observed variance may also be due to the dynamic nature of treadmill running. The influence of movement artifacts are significantly greater compared to controlled static movements. Careful preparation and fixation of EMG electrodes and wires were performed to keep artifacts to a minimum. Another aspect was that subjects may have changed their running technique as the run progress resulting in changes of muscle geometry, muscle, electrode distance. Although this possibility cannot be excluded, stride-times did show a very consistent pattern throughout all trials – suggesting similar technique.

The maximal voluntary contractions measured pre/post running similarly posed some challenges. MVC of the triceps surae is an abnormal movement which few people have tried to perform. This entailed thorough familiarization to attain a valid MVC from the subjects. Limited MVC familiarization was performed in the current study due to methodical considerations which should be taken into account. Further familiarization of the different measuring techniques, such as electrical stimulation and TMS, would also be preferred in future studies, to create a safe testing environment for the subjects. However it should be noted that careful guidance and information were given throughout the protocol to achieve this.

Furthermore, the MVC rig itself should be modified in future studies, to provide back support and strapdown for the subjects during MVC. This would limit movement artifacts and provide more stable force measurements. This would also make TMS measurements more reliable, making it easier to place the coil in the same position throughout the trial. Further rigidity and sensitivity should also be introduced to improve twitch force measurements.

It is possible that some subjects experienced fatigue performing the pre/post MVCs. However the 1 minute rest between contractions was allocated to avoid fatigue. This approach is commonly used in the literature (Sidhu, Bentley and Carrol 2009b) (Todd, J.L. and Gandevia 2003) (Fernandez-del-Olmo, et al. 2013). Also subject motivation may have been limited affecting both performance and concentration. Strong verbal encouragement was given throughout the protocol.

The TMS evoked force was not used for analysis. The method developed by Todd et al. 2003 and proven reliable by Sidhu et al. 2009b was applied pre and post 800m maximal running. This method did not produce linearity of twitches induced by TMS and the dispersion of twitch-sizes had large variation hence this method was not included for further analysis. The explanation for why this technique did not work is caused by the low motor-threshold of TA on the motorcortex compared to soleus (Brouwer and Ashby 1990). When TMS is applied the TA contracts more or as much as the soleus, and being antagonistic this means that no or a negative twitch appears (S. C. Gandevia 2001).

Furthermore H-reflex and V-wave measurements obtained from the gastrocnemius could not be used with the current methodology. This was due to the difference in motor unit thresholds between the soleus and gastrocnemius – making it necessary to stimulate each muscle at individual intensities (Simonsen, Alkjær and Raffalt 2012). Results from the gastrocnemius would have been interesting to investigate given that the soleus muscle is comprised by mainly slow-twitch muscle fibers being very resistant to fatiguing interventions. Hence alterations may only be limited. Muscles characterized by fast muscle fibers, such as the gastrocnemius, may have shown more pronounced fatigue and a different response to fatigue (Kawakami, Amemiya, et al. 2000).

Conclusion

In conclusion, 800m running in elite athletes induced neuromuscular fatigue since MVC significantly decreased and RPE increased to maximal exertion. This was likely due to peripheral factors such as decreased sarcolemma excitability and impaired excitation-contraction coupling. To maintain force output throughout the run, central mechanisms possibly counteract peripheral fatigue. This was evident by increases in both cortical and spinal excitability possibly through decreased inhibitory mechanisms and also by an increased central drive. This was shown by increased MEP, H-reflex and V-wave amplitudes. Post measures revealed a decreased central drive during MVC suggesting that some central fatigue did occur. However, such limitations did not apply during running – probably due to the significant difference between dynamic/static and submaximal/maximal contractions.

The findings contradict previous studies, however in these, assessment of fatigue occurred during a static situation, after the performance had been completed. In the current study, fatigue was assessed both during dynamic and static conditions revealing that fatigue may be specific under these conditions. Future studies aiming to investigate the peripheral and central factors contributing to fatigue should thus be performed in dynamic situations.

Perspectives

The results in the present study contradict the existing neuromuscular fatigue related literature (Duchateau, et al. 2002), (Garland, et al. 1991). The main difference being that measurements performed in this study, was done both during and post actual running, and not during rest or pre/post fatiguing isometric contractions. This increases the transferability of the results considerably and makes the results applicable to sport settings. The underlying mechanisms responsible for the central and peripheral modulations need to be tested during and not post the intervention of interest –these mechanisms could be presynaptic inhibition, recurrent inhibition and III/IV afferents. This may elucidate how the central mechanisms are being controlled and how they respond during fatiguing exercise.

One of the major limitations of the current study was that treadmill speed was constant throughout the fatiguing run. The constant speed meant that the force output was equally constant throughout the run, possibly limiting the amount of fatigue measureable and also, to some extent, forcing the subject to continue exertion. It is plausible that the decline in speed observed during actual 800m running, mainly is contributed to psychological, and peripheral factors, despite central mechanisms being able to continue and even increase performance. Treadmills capable of alternating running speed continuously according to the effort of the subject have been developed (Tomazin, et al. 2012). Measurements on such a treadmill would allow further information of the fatiguing mechanisms as

force output decrease. Further appliance of movement analysis would possibly illuminate how central modulations are related to speed decrements and also how decreased inhibitory mechanisms affect neuromuscular control.

The measurements in this study indicate that central factors are not limiting, thus training in sports should primarily focus on improving psychological, cardiopulmonary and muscular factors. Another important observation was the specificity of the neuromuscular modulation between different muscles, movement types and contraction intensities influences measurement outcomes. Therefore an interesting perspective would be to study how other populations such as sedentary individuals, world class athletes, power trained and endurance trained compare to the results. Also other different modes of exercise might differentiate considerably – such as cycling, prolonged running or sprints.

Due to the increasing optimization and competition in the world of sports, athletes are using legal ergogenic agents and new training interventions to enhance performance. This includes overspeed training and plyometric training. It is unclear how these methods affect central mechanism during actual exercise, but this kind of training have been shown to enhance especially sprinting capabilities and neuromuscular function (Ross, Leveritt and Riek 2001), (Markovic and Mikulic 2010). The same applies for consumption of various vitamins, caffeine, L-carnitine, taurine and sodium bicarbonate (Carr, Hopkins and Gore 2011). The study presented in the appendix interestingly indicates a delayed onset of central fatigue with the consumption of sodium bicarbonate during isometric submaximal contractions.

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Appendix 1 - Manuscript Draft

Neuromuscular fatigue during intermittent isometric contractions and the effect of acid/base ingestion

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Poulsen MK, Nielsen NPB. Neuromuscular fatigue during intermittent isometric contractions and the effect of acid/base ingestion. In preparation for review June 2013 – This study investigated the effect of Acid/Base ingestion on endurance and central mechanisms. Eight healthy male subjects participated in the study, each performing submaximal intermittent plantar flexion contractions to fatigue during either acid, base and placebo ingestion. Force recordings and electromyography were measured throughout the fatiguing protocol and voluntary activation was measured during MVC every minute. The data showed significant reduced time to failure during the acid trial (-11%, P<0,05) and decreased voluntary activation for all ingestions during the last part of the protocol (7,4%, 16,22% and 11,09% respectively P<0,05). No change was found between trials. These observations indicated that central mechanisms may be limiting performance during intermittent submaximal contractions. However induced acidosis or alkalosis through oral ingestion do not have any effect on voluntary activation during fatigue.

Intermittent, voluntary activation, acid/base, central drive

Introduction

Neuromuscular fatigue has been extensively investigated previously (S. C. Gandevia 2001) and due to its complexity, it can be difficult to generate reliable results with definitive conclusions. Merton 1954 found an inverse relationship between the amplitude of superimposed twitches during contraction, relative to the degree of muscle activation. This technique was called the twitch interpolation technique and has been used to asses voluntary activation during fatigue (Merton 1954). Previous findings during sustained isometric maximal contractions to fatigue suggest that central fatigue does not occur. Hence the central nervous system is able to maintain full muscle activation throughout the contraction, despite task failure (Merton 1954) (Bigland-Ritchie, Johansson, et al. 1983). Furthermore, no limitation of the neuromuscular transmission has been found either (Merton 1954) (Bigland-Ritchie, Kukulka and Lippold 1982) – indicating that fatigue occur peripherally due to processes within the muscle fibers. However, recent studies suggest that suboptimal central activation is likely to occur during fatiguing contractions (Taylor and Gandevia 2008). One discrepancy possibly accounting for the difference is that the studies vary in method and are measured on different muscles during different tasks. It cannot be assumed that the obtained results apply for all types of muscles and exercises (Bigland-Ritchie, Furbush and Woods 1986) (Iguchi and Shields 2011). Furthermore, much research has been focusing on sustained contractions (Butler, Taylor and Gandevia 2003) (Levenez, et al. 2008) (Merton 1954). Nybo & Nielsen 2001 suggested a significant difference in the development of central fatigue between sustained and intermittent exercise tasks. In an intermittent exercise task, no difference in voluntary activation was found, whereas in prolonged sustained contractions voluntary muscle activation failed. This was confirmed by Bilodeau 2006 contradicting previous findings (Bigland-Ritchie, Johansson, et al. 1983), (Merton 1954). Studies further differentiate, by measuring during submaximal or maximal contractions. This has been shown to affect neurophysiological responses to fatigue as well (Taylor and Gandevia 2008). This augments the need for further investigation.

Neuromuscular fatigue studies have often been performed with isometric contractions, and the

results have been discussed in relation to actual exercise.

However, marked differences arise when neuromuscular fatigue is investigated in static body positions. One of such is the metabolic difference from isolated contractions to whole body exercise, where in the latter there is a more pronounced perturbation of pH, intracellular H+ and HCO_3. (Churchward-Venne, Kowalchuk and Marsh 2010). It has previously been shown that small diameter afferents significantly influence the neurophysiological response to fatigue (Garland 1991). The response can be related to a decrease in α-motoneuron pool excitability as a result of reflex inhibition brought on by sensory input from III/IV afferents in the fatigued muscle. The III/IV afferents are sensitive to chemical agents such as bradykinin, phosphate H+ and lactate (Kniffki, Mense and Schmidt 1978). These chemical agents are apparent during fatigue along with changes in the metabolism pH balance towards a more acidic environment. Consequently, it would be favorable to introduce chemical loading to manipulate intramuscular pH to an intermittent isometric fatigue protocol, in order to investigate if facilitation or inhibition becomes evident.

Further research is needed in the investigation of neuromuscular fatigue given the variety of the results obtained utilizing different methods and measuring on different muscles. This necessity is further increased when attempting to compare and apply the results to actual exercise. The aim of the study was to investigate neuromuscular fatigue of m. Gastrocnemicus and m. Soleus during an intermittent submaximal fatigue trial with acid, base and placebo loading.

We hypothesized that the intermittent plantar flexion trial would induce neuromuscular fatigue, and that chemical loading would alter the time course and magnitude of neuromuscular fatigue.

Method

Subjects

Experiments were performed on 8 healthy male subjects who volunteered to participate in the study [age 23.6 ±1.5 (SD) yr, body height 179.3± 6.2cm, body mass 81.1 ± 11.9kg].

The subjects gave their voluntary written consent after being properly informed of the experimental setup and conditions. The local ethics committee approved all procedures, and the study was conducted according to the Declaration of Helsinki.

Experimental setup

The subjects were tested on three separate test days spaced at least 48 hours apart. The loading consisted of either $NaHCO_3$ [0.3 $g*kg^{-1}$], NH_4Cl [0.2 $g*kg^{-1}$] or placebo [0.3 $g*kg^{-1}$].

During testing, the subjects were lying supine on a custom built rig - their left foot strapped to a vertical footplate, with an imbedded force transducer. The subjects were further strapped down on both shoulders to minimize movement and were instructed to keep their upper body still throughout the experiment.

Before the fatigue protocol the subjects performed three maximal voluntary contractions (MVC) spaced at least 1 minute apart to establish highest MVC and 55% of MVC. These values were used on all test days. To establish stimuli intensity, Mmax was determined on each test day. Subjects were given at least 5 min of rest before the fatigue protocol was initiated.

Prior to testing, all subjects went through a familiarization protocol on a separate day to improve performance. All equipment were calibrated prior to the experiment.

Blood sampling and loading procedures

The subjects consumed either $NaHCO_3$ [0.3 $g*kg^{-1}$], NH_4Cl [0.2 $g*kg^{-1}$] or placebo(CaCO3) [0.3 $g*kg^{-1}$]. The solution was sealed in soluble gelatin pills which were administered in three segments 90 min, 60 min and 30 min prior to the initiation of the fatigue protocol. The loadings were randomized and double blinded.

Two baseline capillary blood samples were obtained prior to loading and again immediately prior the fatigue protocol. All blood samples were obtained aseptically using capillary finger sticks, and were collected in a balanced 95 µL blood gas capillary tube. To arterialize capillary blood and increase blood flow to the sampling site, the hand was heated using a heating pad. The capillary blood samples were immediately capped and placed on ice after sampling until further analysis using a clinical blood gas analyzer (ABL700 Radiometer, Copenhagen).

Fatigue protocol

The subjects repeatedly performed bouts of one MVC, followed by 9 submaximal contractions at 55% of MVC. Due to practical aspects the left foot was used for measurements. Each contraction was maintained for three seconds followed by a three second rest period. This procedure was continued until task failure (defined as three submaximal contractions <55%). One MVC was performed immediately after this point. A metronome provided audio signal to guide the timing of contractions, additionally, verbal instructions were also applied. Visual feedback of the 55% contraction threshold was presented by a monitor and strong verbal encouragement was given throughout the fatigue protocol.

Stimulation procedures

Electrical stimulation was elicited 1.5 sec into submaximal contractions and at the following rest period, every third and eighth contraction throughout the bouts, and at the peak plateau during all MVC's. The electrical stimulation was elicited to the tibial nerve and with stimulation intensity of 150% of Mmax. Mmax was found during rest by increasing the stimulation intensity until a peak was found in the m-wave amplitude. All electrical stimulations were elicited through a cathode placed in the popliteal fossa and an anode placed just proximal to the patella – both with conductive gel and consisted of 1ms square pulses delivered by a constant current electrical stimulator (Digitimer, constant current stimulator, model DS7A, Herefordshire, England).

EMG recordings

Surface electromyography (sEMG) was measured at the medial soleus and medial gastrocnemius. Measurements were conducted by use of bipolar surface electrodes (Silver/silver chloride, Maxensor; Medimax Global, Sydney, Australia) with an interelectrode distance of 20 mm on the soleus and 70 mm on the gastrocnemius. The soleus electrodes were placed at $2/3^{rd}$ of the line between the medial condylis of the femur to the medial malleolus and the gastrocnemius electrodes were placed on the muscle belly and at the tendomuscular junction, respectively. A ground electrode was placed on the tibial bone. All electrode locations had undergone proper skin preparation (shaved, abraded, alcohol) and electrodes were fixated by use of regular tape. The EMG was recorded using an ML138 Bio Amp and converted through a 16-channel A-D converter (ADI instruments, Analog Digital Instruments, Sydney, Australia) and band-pass filtered between 10 and 1000 Hz, with a sample rate of 1000 Hz.

Force measurements

Force was measured by a force transducer and all force signals were sampled at 1000 Hz.

Voluntary activation (VA) was analyzed by use of the twitch interpolation technique. Force responses elicited by the supramaximal electrical stimulations were measured during contractions (Superimposed Twitch (ST) – mean force 50 ms prior to the stimulation artifact substracted from peak force within 200 ms after the stimulation artifact) and at the following rest periods (Resting twitch (RT) - peak force within 200 ms after the stimulation artifact). VA was then calculated as: VA (%) = [1-(ST/RT)] *100.

Statistical analysis

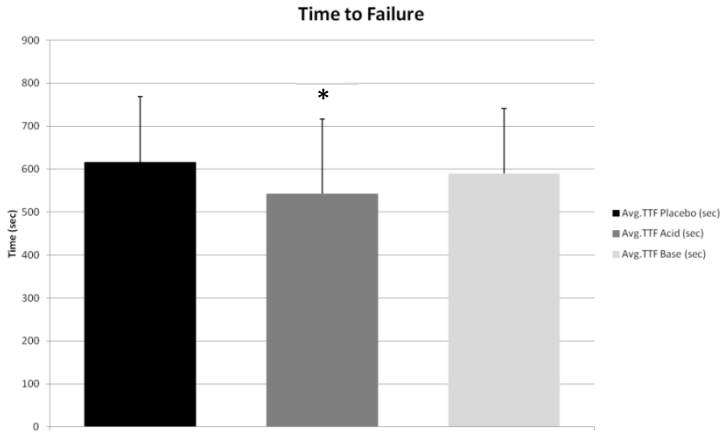
The force and EMG were recorded with LabChart (V. 7, ADI Instruments, Sydney, Australia). To test for significant difference between acid, base and placebo, a MANOVA was performed with a significance level of $P<0.05$. When measurements within a trial were tested for pre-post interaction repeated measures ANOVA was used with a significance level of $P<0.05$.

Results

This chapter will present the results obtained for the intermittent fatigue trial and includes data from MVC's in the acid, base and placebo trials respectively. The outcome measures were force, time to failure, voluntary activation (VA).

Time to failure

The following figure 1 displays the average time to failure during acid (543s±173,24s), base (589,5s±151,03s) and placebo (616.86s±151,35s) ingestion. A significantly shorter time to failure was found in the acid trial compared to the placebo trial (11% decline, $P < 0,05$). No significant change was found between the other trials.



Figur 1 – Time to failure for acid, base and placebo trials respectively in seconds. Black represents placebo, Grey represents acid and Lightgrey represents Base. * Signifies statistical difference between trials.

Blood samples

Table 1 shows an average of the blood values obtained pre ingestion and pre exercise. Statistical significant difference was found during both acid and base loading in pH, HCO3, BE and H+ ($P < 0,05$). No significant change was found in the placebo trial.

	Acid					
	Pre ingestion, Avg.		Pre exercise, Avg.		Difference	
pH	7,389	±0,035	7,299	±0,062	0,09	***
HCO3(mmol/l)	23,681	±2,129	18,975	±2,8	4,706	***
BE (mmol/l)	-0,906	±2,567	-6,575	±3,678	5,669	***
H+ (mmol/l)	40,95	±3,44	50,794	±8,226	-9,844	***
Na+ (mmol/l)	142,875	±3,227	144,188	±16,481	-1,313	
K+ (mmol/l)	4,644	±0,343	4,813	±0,453	-0,169	
Cl- (mmol/l)	107	±4,383	108,313	±15,802	-1,313	
PCO2	39,494	±1,831	38,963	±3,017	0,531	
PO2	80,044	±5,812	80,831	±10,625	-0,787	

	Base					
	Pre ingestion, Avg.		Pre exercise, Avg.		Difference	
pH	7,394	±0,025	7,449	±0,05	-0,055	*
HCO3(mmol/l)	24,013	±1,49	28,469	±3,047	-4,456	**
BE (mmol/l)	-0,513	±1,77	4,675	±3,332	-5,188	**
H+ (mmol/l)	40,394	±2,392	35,806	±4,361	4,588	*
Na+ (mmol/l)	143,313	±4,574	144,938	±3,234	-1,625	
K+ (mmol/l)	4,688	±0,459	4,35	±0,225	0,338	*
Cl- (mmol/l)	103,5	±7,176	102,5	±4,309	1	
PCO2	39,625	±0,968	41,8	±3,118	-2,175	
PO2	82,694	±4,323	74,056	±4,421	8,638	***

	Placebo					
	Pre ingestion, Avg.		Pre exercise, Avg.		Difference	
pH	7,403	±0,022	7,413	±0,031	-0,01	
HCO3(mmol/l)	24,638	±1,304	25,731	±2,167	-1,093	
BE (mmol/l)	0,244	±1,518	1,519	±2,546	-1,275	
H+ (mmol/l)	39,594	±2,022	38,775	±2,921	0,819	
Na+ (mmol/l)	141,688	±2,59	141,313	±2,329	0,375	
K+ (mmol/l)	4,6	±0,443	4,563	±0,572	0,037	
Cl- (mmol/l)	106,125	±4,494	104,75	±6,205	1,375	
PCO2	39,981	±1,355	45,781	±12,933	-5,8	
PO2	88,494	±12,82	77,638	±9,034	10,856	

Table 1 - The blood values obtained pre ingestion and pre exercise for all trials. (P < 0,005 ***, P < 0,01 **, P < 0,05 *)

Force

Figure 2 and table 2 show the average MVC force plateau post 30ms of stimulation for acid base and placebo trials for all subjects (n=8). Table 2 shows both raw (N) and normalized (%) data and has been divided into 25% of TTF sweeps. The data has been normalized to peak Maximal Voluntary Contraction (MVC) during the familiarization trial. The data shows a similar and steady decline in force to the point of failure – an overall decline of acid 25,32%, base 25,98% and placebo 30,25%, respectively. All trials start significantly below 100% of MVC, 89,71%, 88,97% and 85,89%, respectively, and ends at 64,39%, 62,99% and 55,64%. The decline in force was statistically significant ($P < 0,05$). No interaction was found between trials.

Voluntary activation

Figure 3 presents average voluntary activation in % for all trials, derived by use of the twitch interpolation technique. Data has been divided into 25% of TTF sweeps in order to compare subjects with differences in time to failure. There were no significant differences from the time-slices 0-25% to 25-50% in either of the trials - trials were constant in acid 90.66 – 90.64%, base 95,32-94,5% and placebo 91.08 – 91.65%. Subsequently from 25-50% to 50-75% a drop occurred in all trials although not statistically significant. However, in the last time sweep 75-100% level of voluntary activation in acid were 83,26%, base, 79,1%

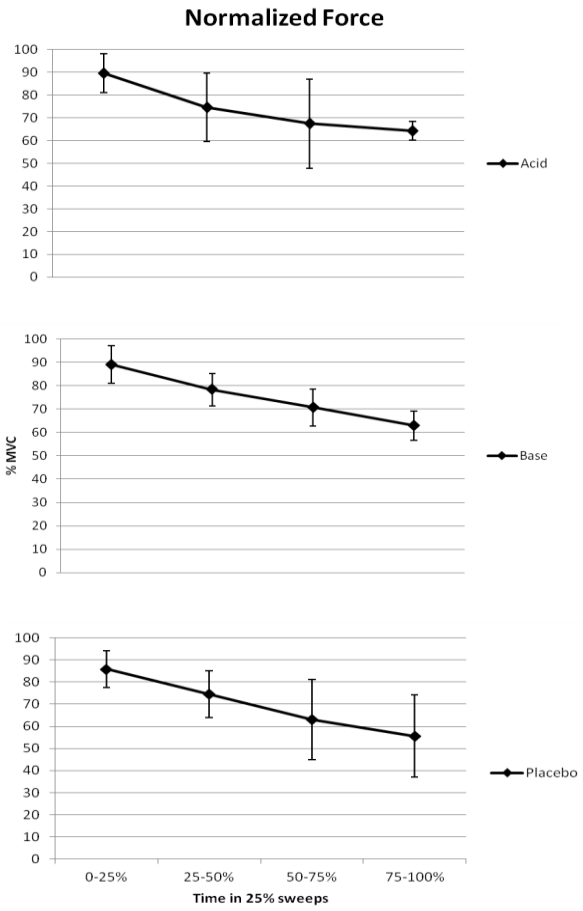


Figure 2 - Normalized force in 25% sweeps. Normalized to MVC peak during familiarization trials. The decline of acid 25,32%, base 25,98% and placebo 30,25% was statistically significant ($P < 0,05$). No difference between trials.

Force (N)

	0-25%		25-50%		50-75%		75-100%	
Acid	1184,3	±205,2	820,4	±494,6	825,7	±367,4	846,6	±107,0
Base	1169,7	±160,4	1032,6	±160,4	932,7	±166,3	827,6	±111,1
Placebo	1131,2	±176,9	977,6	±147,8	803,9	±333,2	726,8	±301,1

Normalized Force (%)

	0-25%		25-50%		50-75%		75-100%	
Acid	89,7	±8,6	74,6	±15,1	67,4	±19,6	64,3	±4,1
Base	88,9	±8,0	78,3	±6,8	70,6	±7,8	62,9	±6,2
Placebo	85,8	±8,2	74,5	±10,5	63,1	±18,1	55,6	±18,6

Table 2 - Raw and normalized force in 25 % sweeps. Normalized to MVC peak during familiarization trials.

and placebo 79,9% which were statistically different from 0-25% in all trials – a decline of 7,4%, 16,22% and 11,09% respectively ($P < 0,05$).

Rest twitches

Table 3 shows rest twitch data obtained post every MVC. Table 3 contains both raw and normalized data. The data has been normalized to Peak MVC obtained during the familiarization trial. A statistical significant change was found in all trials ($P < 0,05$).

All trials display very similar patterns in voluntary activation and no significant difference was found between trials.

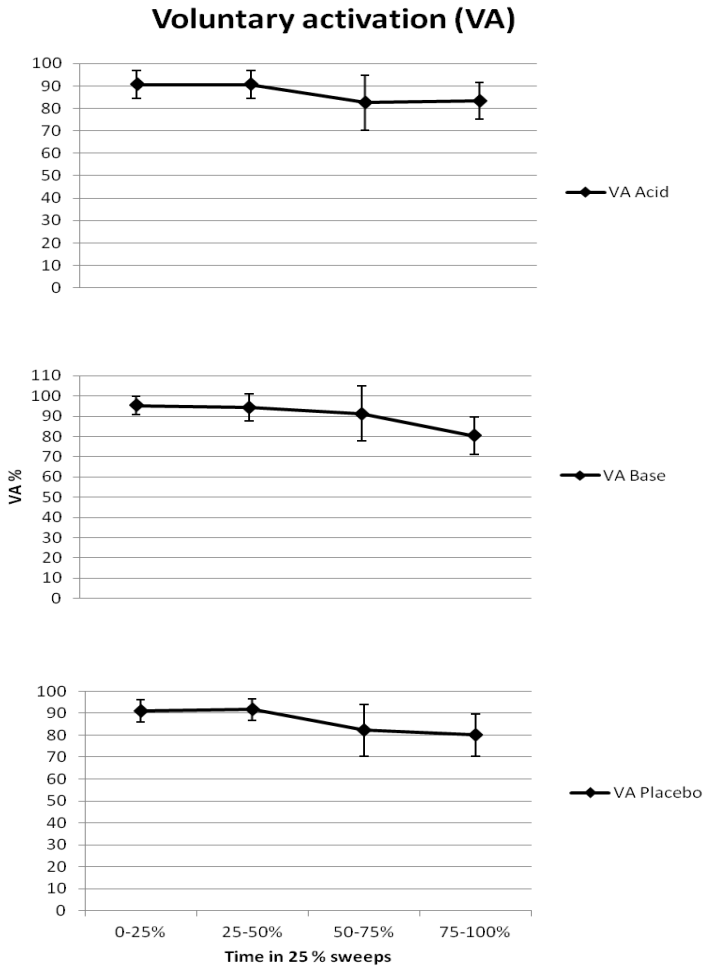


Figure 3 - %Voluntary activation (VA) in 25% sweeps for the acid, base and placebo trial respectively. Calculated by way of the twitch interpolation technique. * signifies significant statistical difference

Table 3 - Raw and normalized rest twitches. Normalized to MVC peak during familiarization trial. * indicates statistical significant difference between time sweeps ($P < 0,05$). Data has been divided into 25% sweeps.

Rest twitch (N)

	0-25%		25-50%		50-75%		75-100%	
Acid	142,46	±30,11	137,04	±22,67	127,93	±24,69	121,42	±24,91
Base	133,20	±30,86	125,57	±28,73	110,43	±24,15	111,50	±26,17
Placebo	132,23	±26,77	129,02	±22,22	119,08	±19,91	116,51	±26,34

Normalized Rest twitch

	0-25%		25-50%		50-75%		75-100%	
Acid	10,91	±2,45	10,53	±2,09	9,82	±2,20	9,31	±2,08
Base	10,18	±2,42	9,57	±2,16	8,42	±1,79	8,50	±1,94
Placebo	10,12	±2,14	9,84	±1,65	9,09	±1,49	9,26	±2,06

Discussion

The results of this study showed that sites proximal to the neuromuscular junction are involved during fatiguing intermittent isometric contractions. Exploiting the twitch interpolation technique it was shown that activation of the soleus and gastrocnemius was impaired when subjects fatigued. Additionally the study showed that loading with $NaHCO_3$ [0.3 $g*kg^{-1}$], NH_4Cl [0.2 $g*kg^{-1}$] or placebo [0.3 $g*kg^{-1}$] had no impact on voluntary activation nor peripheral fatigue – suggesting that the various loading had no effect during intermittent isometric contractions in isolated muscles.

Neuromuscular fatigue

The intermittent protocol did induce neuromuscular fatigue under all conditions illustrated through the significant decline in MVC force (Acid 25,32%, base 25,98% and placebo 30,25%) ($P < 0,05$). The magnitude of force decline is in accordance with other studies (Bigland-Ritchie, Furbush and Woods 1986). The soleus being a fatigue resistant muscle consisting of mainly slow twitch fibers and different methodology might explain discrepancies in force decline between studies.

Peripheral fatigue

As expected the rest twitches significantly decreased as MVC force declined in all trials – indicating some contribution of peripheral fatigue. This include factors at or distal to the neuromuscular junction such as e-c coupling failure and decreased sarcolemma excitability (Taylor and Gandevia 2008).

Central fatigue

In addition central fatigue was apparent in all trials, illustrated by the statistical significant reduction in interpolated twitch response from the first time sweep 0-25% to the last 75-100% – Decrease in voluntary activation in acid 7,4%, base 16,22% and placebo 11,09% ($P < 0,05$). Previous investigators did not find central aspects to be limiting during fatigue (Merton 1954). However, recent studies show similar results (Kawakami, Amemiya, et al. 2000) (McKenzie, et al. 1992) (Gandevia, et al. 1996). The obtained result indicated that the central drive was suboptimal and did not activate the motoneuron pool optimally - hence performance might be impaired by central factors. The explanations for this might be changes at the motoneuron pool, such as the response of motoneurons to inputs, recurrent inhibition and inhibition from III/IV afferents acting on both spinal and supraspinal levels. Further investigations are needed to elucidate some of these factors.

Similar to other studies it was found that the subjects were unable to fully activate the triceps surae even in the unfatigued state, acid 90,66%±4,93%, base 95,32%±4,41% and placebo 91,08%±6,13% in the first 0-25% sweep (Kawakami, Amemiya, et al. 2000) (Gandevia, et al. 1996) (Shield and Zhou 2004). Also the onset of decreased voluntary activation as the fatiguing task progress appear to be late, with no statistically significant decrement until the last 75-100% sweep. This is comparable with other studies (Bilodaeu 2006) (Gandevia, et al. 1996). This decrement is accompanied with a significant increase in variation, suggesting that control of the motor output to the triceps surae muscles becomes less stable as fatigue sets in. The variation more than doubled (see table 2) (Bilodaeu 2006) (Gandevia, et al. 1996).

Chemical loading

The metabolites associated with fatigue such as H+, pH and lactate were shown to be significantly different between the acid, base and placebo trials (Table 1). This had a significant impact on time to failure during the acid trial. Time to failure decreased significantly from placebo to acid ingestion (≈11%) but no significant difference was observed with ingestion of base. As suggested by Churchwald-Venne et. al 2009 the decrease in time to failure during acid ingestion might explained by attenuation of intracellular lactate transport via the membrane bound monocarboxylat transporter and thereby a decrease in the intracellular pH causing problems with actin myosin coupling and leading to premature fatigue of the muscle fibers (Churchward-Venne, Kowalchuk and Marsh 2010).

Carr et. al. 2011 showed a smaller but similar decline in time to failure with acid ingestion 1.6%±1.9% while showing an increase in time to failure with base ingestion 1.7%±2%. The differences between results might be explained by the whole body exercise used in Carr et. al 2011, compared to the isolated isometric contractions in the current study. Isolated isometric contractions might not be challenging to the cardiopulmonary system limiting the impact of blood metabolite manipulation.

An additional cause for the decrement in performance were subject discomfort. Four out of eight subjects vomited just prior to test start under acid ingestion, which might have influenced the subjects' individual motivation and comfort.

Although this study showed a significant difference in performance during acid ingestion, no significant change was found in voluntary activation between trials. A decrease in voluntary activation was evident in all trials however no statistical difference was found between loadings which were a surprising result. Amann et. al. 2011 suggested that III/IV afferents play a vital role in regulating VA, since blockade of these afferents resulted in a decreased performance by not inhibiting the motor drive during cycling, and thus fatigue occurred 70% faster because of excessive activation. In the current study, all conditions showed a VA decrement to the same level at task failure (83,26%±9,63%, 79,1%±9,13% and 79,99%±8,17%) despite introduction of chemical blood buffering agents. The data indicated that the decrease in VA during base was slightly delayed. At 50-75% of the performance time the VA was 8.8% and 9.1% higher than in the acid and placebo trial respectively – although not statistically significant.

Overall the changes in intracellular metabolites did not change enough to make the afferent feedback reduce VA. The changes during whole body exercise are much greater than what can be induced by chemical manipulation. Also the lack of difference in VA between trials in this study may be explained by discrepancies in the fatigue protocol or muscle type. Intermittent fatigue protocols allow for an augmentation in the removal of metabolites compared with sustained fatigue protocols (Babault, et al. 2005). Also the fatigue resistant soleus muscle may respond differently compared to other muscles (S. C. Gandevia 2001) and whether the muscle is a flexor or extensor (Martin, et al. 2006). Intensity of the fatiguing contractions has also been shown to have an effect (Miller, et al. 1993).

Conclusion

Conclusively the results show that peripheral factors and sites proximal to the neuromuscular junction combined are involved during fatiguing intermittent isometric contractions although not affected by loading of either $NaHCO_3$ [0.3 $g*kg^{-1}$], NH_4Cl [0.2 $g*kg^{-1}$] or placebo [0.3 $g*kg^{-1}$].

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Appendix 2 - Ethical approval (in Danish)

Projekttitel
Neurofysiologiske ændringer under løb og træthed.

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Forsøgsprotokol

Baggrund

Muskeltræthed og de begrænsende faktorer inden for fysisk aktivitet er et fundamentalt og omdiskuteret emne i den sportsvidenskabelige verden. Til trods for en stor mængde videnskabelig litteratur og forskning er de faktiske mekanismer, der ligger bag, stadig ikke fastlagt.
Muskeltræthed inkluderer både centrale samt perifere mekanismer – de centrale er den manglende evne, induceret af aktivitet, til frivilligt at aktivere en muskel fuldt ud, og de perifere er musklens manglende evne til at producere kraft *(S. C. Gandevia 2001)*.
I nyere tid er interessen for de centrale neurofysiologiske mekanismer steget væsentligt. For at undersøge disse har især H-refleksen, transcranial magnetisk stimulering (TMS) og twitch-force været hyppigt anvendt med succes *(Iguchi and Shields 2011) (S. C. Gandevia 2001) (Racinais, Girard, et al. 2007) (Walton, Kuchinad, et al. 2002) (Misiaszek 2003).*
Det menes, at mekanismer som presynaptisk inhibition, co-kontraktion, post-aktiveringsdepression, muskulær adaptation, eller sensitivitet af gruppe III og IV kemisk sensitive afferenter kan indvirke på moduleringen af den neurofysiologiske muskelkontrol (Misiaszek 2003) (Zehr 2002) (Knikou 2008) (Kuchinad, Ivanova and Garland 2004) (Garland, Griffin and Ivanova 1997) (Garland, Enoka, et al. 1994) (Bigland-Ritchie, Furbush and Woods 1986). Ikke desto mindre kendes de faktiske årsager og mekanismer stadig ikke og det er stadig ukendt om og hvor i systemet moduleringen finder sted. Af disse årsager er yderligere forskning på området nødvendig for at få en endelig afklaring (Walton, Kuchinad, et al. 2002) (Kuchinad, Ivanova and Garland 2004).

Det er ydermere kendt, at mange af de bagvedliggende mekanismer afhænger af kontraktionstype og muskelspecificitet. Studie-typerne varierer og har været udført under flere forskellige typer af kontraktioner; vedvarende voluntær maksimal kontraktion (Butler, Taylor and Gandevia 2003), vedvarende submaksimal kontraktion (Levenez, et al. 2008) og intermitterende submaksimal kontraktion (Nordlund, Thorstensson and Cresswell 2004). Få studier har været udført under dynamiske kontraktioner, og alle med fokus på skridtcyklus - ikke på træthed (Simonsen and Dyhre-Poulsen 1999) (Edamura, Yang and Stein 1991). Samtidig er også flere forskellige muskler blevet brugt til undersøgelse af moduleringen, hvilket yderligere opfordrer til mere forskning på området, da hver muskel vil respondere forskelligt.

Derfor vil denne undersøgelse forsøge at afklare nogen af de ovenstående usikkerheder vha. en dynamisk kontraktionsprotokol, nærmere bestemt løb. Både soleus-musklen og gastrocnemius-musklen, er i dette tilfælde interessant pga. deres fordelagtige position og innervering, og samtidig spiller de en essentiel rolle under løb ved høj hastighed, da de genererer kraft ved hvert skridt under plantar fleksion af foden (Martini og Nath 2009). Samtidig har reflekserne og nervesystemet muligvis betydning, da de kan være bestemmende for kraftproduktion, tendo-muskulær stivhed og elastisk energilagring samt kontrol og ekscitabilitet under løb. (Ross, Leveritt and Riek 2001). En afklaring heraf kan være behjælpelig under træning samt forberedelse og optimering af den menneskelige præstationsevne. På baggrund af den manglende enighed omkring de bagvedliggende mekanismer samt stor variation i valg af metode og kontraktions- og muskeltyper er der begrundelse for en undersøgelse (Iguchi and Shields 2011).

Formål

Formålet med dette studie er at undersøge den neurofysiologiske modulering under løb og træthed i m. soleus og m. gastrocnemius samt m. tibialis anterior.

Hypoteser

H-refleksens amplitude moduleres og bliver mindre mod slutningen af et maximal eller udtrættende løb som et resultat af udmattelse og inhiberende signaler.

V-wave amplituden moduleres og bliver mindre mod slutningen af et maximal eller udtrættende løb som et resultat af udmattelse og mindre central aktivering

TMS målingerne moduleres og bliver mindre efter et maximal eller udtrættende løb som et resultat af central træthed.

Twitch-force målinger vil forøges efter et maximal eller udtrættende løb, som et resultat af central træthed.

Forsøgspersoner

Til forsøget rekrutteres 60 forsøgspersoner.

Inklusion

- Raske mænd og kvinder mellem 18 og 50 år

Eksklusion

- Addiktiv eller tidligere addiktiv adfærd defineret som misbrug af hash, opioder eller andre euforiserende stoffer
- Tidligere neurologiske, muskuloskeletale eller psykiske sygdomme
- Bærer af smitsomme sygdomme
- Graviditet
- Manglende evne til at samarbejde
- Lever ikke op til "Transcranial magnetic stimulation adult safety screen (TASS)" testen. Hvis der svares positivt på svarene i TASS-testen, vil der blive udført en klinisk vurdering af sikkerheden i forbindelse med personens eventuelle deltagelse i forsøget.

Rekruttering

- Forsøgspersoner vil blive rekrutteret ved hjælp af opslag i relevante idrætsklubber/ organisationer (opslag vedlagt denne forsøgsprotokol).

Metoder

Studiet vil inkludere en gruppe trænede løbere og kontrolgruppe der har lignende fysisk tilstand men som ikke er trænede løbere. Minimum 48 timer før laboratorietesten gennemfører testpersonen et

testløb til udmattelse på en tartan 400m løbebane ved maksimal hastighed. Denne test vil være bestemmende for løbebåndshastigheden under forsøget. Denne træningssession varer 30-60min.

På selve dagen for forsøgssessionen må personen ikke indtage mad eller læskedrikke 1 time før forsøgets start, dog er indtagelse af vand tilladt. Ved ankomst til laboratoriet vejes og måles forsøgspersonen. Herefter klargøres hud til påføring af elektromyografi(EMG)-elektroder på tibialis anterior, den mediale soleus – og gastrocnemius muskel på det højre ben. Elektroder (Ambu Neuroline 720) placeres over musklerne, som findes vha. palpering under plantar og dorsal fleksion. Der placeres yderligere stimuleringselektroder (katode: PALS Platinum pre-gelled, Ø=3,2cm; anode: PALS platinum pre-gelled, 5×9cm) ved den tibiale nerve. Herudover vil en opmåling af testpersonens hoved finde sted – for at fastslå lokaliteten af stimulering. Desuden påføres en fodkontakt midt under forsøgspersonens skosål. Efter fastgørelse og inspektion af elektrodeplacering varmer testpersonen op ved at løbe mindst 1,5 km ved selvvalgt hastighed på et løbebånd (Technogym, Runrace HC1200).

Når personen er varmet op, tilsluttes en elektrisk stimulator til stimuleringselektroderne, og opsamlingselektroderne tilsluttes en forstærker samt A/D converter og videre til en personlig computer, som gemmer data. Stående modtager forsøgspersonen elektrisk stimulering (Isolated Stimulator, NoxiTest, IES 230) ved stigende milliampere, indtil et plateau af input/output-kurven findes. Denne milliampere-intensitet fordobles, og personen får et par enkelte stimuleringer ved denne intensitet for tilvænning. Herudover vil testpersonen modtage stimulering på motor cortex ved hjælp af TMS. Først findes det optimale stimuleringssted(hotspot), det sted med størst EMG amplitude på m. soleus og m. gastrocnemius. Herefter findes den motoriske tærskel ved at forøge stimulerings intensiteten indtil fem ud af 10 stimuleringer ligger over 50 µV. TMS stimuleringen vil derefter være 120% af den motoriske tærskel.

Personen vil blive fastgjort i en stol designet til maksimale kontraktioner af m. soleus og m. gastrocnemius. Her vil testpersonen udføre maksimale kontraktioner, hvor twitch-force måles ved hjælp af elektrisk stimulering og TMS. Denne procedure vil blive foretaget både før og efter selve løbetesten.

Personen påføres herefter en sele, som er monteret i loftet over et løbebånd, og stiller sig på fodpladerne i siden af løbebåndet. Hastigheden øges til den tidligere målte personlige fart, og personen starter med flyvende start en kort test for bestemmelse af korrekt stimuli- intensitet under løb. Stimuleringen gives randomiseret i 10 % trin af den fundne stimuli-intensitet ved hvert skridt. Der tages i alt 20 skridt. Herefter stopper forsøgspersonen og den stimuleringsintensitet, der skal anvendes ved videre test, beregnes ud fra data fra de 20 skridt. Hvis utilstrækkelige resultater er opnået, justeres stimuleringsintensiteten og proceduren gentages(denne metode vurderes som forsvarlig grundet pilot studier).

Når korrekt stimuli-intensitet er fundet, accelereres løbebåndet til den personligt målte hastighed og testen igangsættes. Under testen stimuleres personen ved 0 % af hvert skridt og igen supramaximalt 60ms efter.

Der står på alle tidspunkter en testleder klar ved stopknappen på løbebåndet, og ved mindste udsagn fra forsøgspersonen afbrydes testen.

Under testen opsamles EMG data, via programmet MrKick II, fra hele løbet som efterfølgende vil blive analyseret statistisk.

Forsøgssessionen varer 1½ time.

Risici, bivirkninger og ulemper

Risici og ulemper forbundet med løb på en løbebane

Ved løvfald eller regn kan banen være glat, hvormed risikoen for at falde i sving øges.

Risici og ulemper forbundet med løb på et løbebånd

Der er altid en risiko for at snuble, mens man går eller løber på et løbebånd, hvorved personen risikerer at falde. Denne faldrisiko er fjernet ved at anvende en sikkerhedssele, der er fastgjort i loftet, og som bæres af alle forsøgspersoner. Der er et nødstop på gangbåndet, som forsøgspersonen samt testleder nemt kan trykke på.

Risici og ulemper forbundet med optagelse af EMG

Måling af EMG med overfladeelektroder kan betragtes som ufarlig, og der er ingen kendte risici eller ulemper forbundet med denne metode. Der kan dog forekomme en let rødme i huden i forbindelse med placering af elektroderne på huden, men dette er ufarligt og vil hurtigt fortage sig.

Risici og ulemper forbundet med elektrisk stimulation af perifere nerver

Stimuleringen af motoriske nervefibre med elektriske pulser med en længde på op til 1ms, der benyttes i dette projekt, kan betragtes som ufarlig. Der er ingen kendte risici ved disse elektriske stimuli, men de kan blive opfattet som smertefulde. Stimulationerne, der benyttes i dette projekt, er sammenlignelige med de stimulationer, der benyttes i andre protokoller i klinisk neurofysiologi (såsom bestemmelse af nerveledningshastigheder). Erfaringen fra disse undersøgelser er, at de ikke opfattes som værende direkte smertefulde men kan opfattes som ubehagelige af raske forsøgspersoner eller patienter.

Risici og ulemper forbundet med TMS

TMS med enkelte pulser, som gives med de nævnte intervaller (>5s), betragtes som et sikkert og brugbart værktøj til at studere forskellige aspekter af neurofysiologi hos både raske forsøgspersoner og patienter. TMS benytter magnetiske felter til at inducere en strøm i hjernen, som kan aktivere neuronerne. Disse stimulationer kan derved udføres, uden at der skal sendes en strøm igennem huden. Derved undgår man den smerte og det ubehag, der er forbundet med elektrisk stimulation af hjernen med elektroder anbragt på hovedbunden. Det har endvidere vist sig, at TMS er en relativ smertefri metode. De TMS-stimulatorer, der er i brug i dag og som benyttes i nærværende projekt, skaber et magnetfelt på op til 3.0 tesla (T), og de menes at aktivere kortikale neuroner i en dybde af 1,5-2 cm. Disse stimulationsintensiteter er sikre, så længe der kun gives enkelte pulser med intervaller på over 1 sekund imellem stimulationerne. Den anvendte stimulator er rent fysisk forhindret i at give stimulationer med et mindre interval end et sekund på grund af den måde, elektronikken er designet

på. Derudover vil den maksimale intensitet, som anvendes i projektet være maksimalt 120% af den motoriske tærskel. Den nuværende grænseværdi for sikker anvendelse af TMS er et minimalt interval på 1s imellem stimulationerne og en maksimal intensitet på 200% af den motoriske tærskel (Wassermann 1998). De anvendte stimulationsintervaller (minimum 5s, maksimum 7s) og maksimale stimulationsintensiteter (120% af motorisk tærskel) ligger derved inden for disse grænseværdier.

Der er ikke blevet rapporteret om bivirkninger med raske forsøgspersoner med denne form for TMS, siden den blev opfundet i 1985. De bivirkninger, der er blevet rapporteret, har været i forbindelse med højfrekvent TMS. På trods af den udstrakte brug af TMS (en søgning i PubMed med "Transcranial Magnetic Stimulation" eller "TMS" giver 6054 referencer, hvilket anslås at svare til ca. 60.000 til 90.000 forsøg med TMS) er der i 1998 på verdensplan rapporteret om syv epileptiske anfald med højfrekvent TMS (Angel, Jankowska og McCrea 2004). Der er ikke rapporteret om tilfælde af epileptiske anfald med den form for TMS, der benyttes i dette studie, hvorfor der er lav sandsynlighed for, at der opstår et epileptisk anfald i dette studie. At sandsynligheden ikke sættes til nul skyldes, at det er blevet påvist, at et epileptisk anfald kan opstå, hvis forsøgspersonen i forvejen har haft epileptiske anfald. Før forsøget vurderes forsøgspersonernes sygehistorie, og forsøgspersoner med tidligere epileptiske anfald udelukkes fra at deltage i forsøget. Dette er imidlertid baseret på forsøgspersonens tidligere erfaringer, og det kan derfor ikke udelukkes, at en forsøgsperson kan være disponeret for epileptiske anfald uden selv at være klar over det. Vi har derfor valgt at benytte de samme forholdsregler for forsøgene i dette projekt, som benyttes i forsøg med højfrekvent TMS. Det skal understreges, at der er konsensus blandt videnskabsfolk, der arbejder med TMS om, at der ikke er nogen risici forbundet med lavfrekvent TMS, hverken med hensyn til epileptiske anfald eller andre risici (Stokes 2005). De forholdsregler, der tages i forsøget, er følgende:

Før en forsøgsperson bliver udsat for TMS, vil han/hun blive bedt om at udfylde et spørgeskema (Adult Safety Screen Questionnaire, (Keel, Smith og Wassermann 2000)). Spørgsmålene i dette spørgeskema er rettet mod at undgå at inkludere personer med skader i hovedet, gravide kvinder eller kvinder, der kan være gravide, personer der selv har eller har familiemedlemmer med epilepsi, eller som selv har svær/kronisk hovedpine (hvis en person har hovedpine på tidspunktet for forsøget, vil forsøget blive aflyst, uanset om forsøgspersonen ellers ikke lider af svær/kronisk hovedpine).

Det personale, som udfører forsøgene, er trænet i at genkende symptomer på epileptiske anfald og i hvordan disse håndteres. (jvf. " Hvordan man forbereder sig og hvad man gør i forbindelse med et epileptisk anfald", som er vedhæftet denne ansøgning). Gennem hele forsøget vil personalet, der udfører forsøget, spørge til forsøgspersonens velbefindende for på den måde at kunne afbryde forsøget øjeblikkeligt, hvis forsøgspersonen skulle få det dårligt. I laboratorierne (inklusive det laboratorium forsøgene udføres i) er der en plakat, som beskriver håndteringen af epileptiske anfald.

Hvis der opstår et epileptisk anfald, vil det på forhånd være sikret, at der er en læge til stede på instituttet, når forsøgene udføres, og at der er lavet aftale med den pågældende læge om, at der vil blive ringet til lægens mobiltelefon, hvis det skulle ske, at en forsøgsperson får et epileptisk anfald. Lægen, der er lavet aftale med, ved hvilke forsøgspersoner, der bliver testet, og hvornår forsøget starter og slutter. I laboratoriet er der også adgang til en nødtelefon med direkte forbindelse til alarmcentralen og en nødhjælpskasse.

Derudover har de epileptiske anfald, der indtil nu kun er opstået med høj-frekvent TMS, været relativt milde med en varighed på 2 til 3 minutter og ingen med permanente skader. Det vurderes derfor, at der ikke er nogen nævneværdige risici for forsøgspersonerne i forbindelse med deltagelse i projekter med lavfrekvent TMS.

Der er ulemper forbundet med deltagelse i dette projekt. Det er blevet rapporteret, at TMS kan forårsage en midlertidig forøgelse af lydfølsomhed (Counter, et al. 1990) på grund af klikstøjen, der opstår, når der sendes strøm igennem den spole, der skaber magnetfeltet. I dette forsøg vil forsøgspersonerne derfor blive bedt om at anvende udleverede engangs-øreproppper for at beskytte ørene mod dette fænomen.

Muskler og nerver, der befinder sig tæt på spolen/stimulationsstedet (øjne og kæbemuskler), kan også blive aktiveret under TMS-stimulationerne. Dette kan blive opfattet som værende ubehageligt for forsøgsperson på trods af, at TMS kun sjældent opfattes som direkte smertefuldt. Det er derfor forventeligt, at forsøgspersonerne opfatter forsøgene i dette projekt som lettere ubehagelige. Udover at forsøgsleder løbende vil spørge ind til forsøgspersonens velbefindende, vil forsøgspersonerne også blive bedt om at rapportere alt ubehag forbundet med forsøget. Før hver forsøgsserie vil forsøgspersonerne blive gjort opmærksomme på, at de til enhver tid kan trække sig ud af forsøgene uden at give nogen begrundelse for dette, og at det ikke vil påvirke den kompensation, som de modtager.

Statistik

Effektparametre

De afhængige variable for projektet bliver beskrevet nedenfor.

(i) V-wave og h-refleks amplituder, vil blive målt som root mean square (RMS), og vil blive målt inden for en tidsramme på 40-55 ms efter elektrisk stimulering.
(ii) Twitches fra perifer stimulering under maksimale kontraktioner vil blive målt vha. twitch interpolation teknik.
(iii) TMS stimuleringen vil inducere såkaldte MEP's, der er et udtryk for hvor stærke forbindelser der er mellem hjerne og muskler.

Statistiske tests

Til at vurdere effekten af de enkelte forsøg i forhold til hinanden vil anerkendte statistiske metoder som parret t-test samt envejs og tovejs-ANOVA blive anvendt. Statistikken vil kun blive brugt til at vurdere, hvordan H-refleksen, V-waven, TMS og twitch-force afhænger af de valgte stimulationsparametre, udmattelse og løbe-forhold.

De gentagne faktorer vil være nervestimulation (intensitet) og tidspunkt i løbet.

For alle statistiske procedurer vil en p-værdi på p=0,05 blive brugt til at vise signifikans.

Styrkeberegning

I dette projekt ønsker vi at vurdere moduleringen af de neurofysiologiske baner i m. soleus og m. gastrocnemius under løb samt om de reaktioner, der observeres, varierer med forskellige neurale

forbindelser. I tillæg hertil observeres det, om disse forandringer varierer ved forskellige elektriske stimulationsveje.

Antallet af forsøgspersoner er beregnet ud fra bogen af Bausell og Li (2002). En analyse af data fra pilotforsøg har vist, at den gennemsnitlige H-refleks amplitude ligger cirka 0,35 standardafvigelser fra hinanden med en korrelation på cirka 0,80 for de foreslåede forsøgsbetingelser.

H-refleks amplituden er den primære effektparameter i dette forsøg.

Power for forsøget er sat til 0,80 og signifikansniveauet til $p \leq 0,05$. Ved at bruge tabellen til en to-sidet parret t-test er antallet af forsøgspersoner fundet til 28. Det betyder, at mindst 28 forsøgspersoner skal testes. Variansen i forsøget vil stige, hvis forsøgsgruppens responser er mere heterogen end de responser, der blev opnået i et pilotforsøg. For sikre mod stigninger i variansen ønskes det derfor at teste 30 forsøgspersoner. Derfor bliver det totale antal forsøgspersoner 60.

Etiske overvejelser

Undersøgelsen overholder de i Helsinki Deklarationen II nævnte forhold.
Undersøgelsesprotokollen sendes til Den Videnskabsetiske Komité for Region Nordjylland til godkendelse.

Studiet forudsætter inklusion af raske deltagere, som ikke har nogen nuværende sundhedsmæssige problemer. Dog vurderes interventionen at være minimal og uden risici for længerevarende følger af undersøgelsen.
Forsøgets gevinst vil være en metodisk stringent udarbejdet dokumentation for anvendelse af neurofysiologiske målinger på m. soleus, m .gastrocnemius og moduleringen heraf under dynamiske kontraktioner/løb. Andre studier, der gør brug af lignende metoder, vil kunne sammenlignes med de opnåede resultater. En forståelse af neurofysiologiske faktorer ved fysisk aktivitet op til udmattelse kan også medvirke til fremtidige bedre træningsmetoder for idrætsudøvere, der i sidste ende kan resultere i forbedret præstation og mindre risici for skader.
I og med at forsøget ikke er forbundet med betydelige risici og ulemper og da gevinsterne ved forsøget inkluderer fordele for forskning og sport vurderes det, at undersøgelsen er videnskabsetisk forsvarligt.

Forsikring

Forsøgspersonerne er dækket af Patientforsikringen.

Personlige data

Efter forsøget gemmes data og disse identificeres kun vha. et ID-nummer. Der vil ikke blive gemt data, der kan identificere hvilken person dataene stammer fra og data vil blive opbevaret på Aalborg universitet.

Forsøget anmeldes til Datatilsynet.

Økonomi

Projektet er startet på gruppens eget initiativ med støtte fra Det Sundhedsvidenskabelige Fakultet ved Aalborg Universitet. Desuden med støtte fra Natalie Mrachacz-Kersting, lektor og ph,d, ved Center for Sanse-Motorisk Interaktion (SMI), Aalborg Universitet.

Et beløb på kr. 500 fra Det Sundhedsvidenskabelige Fakultet er til rådighed ved projektets start til køb af elektroder, tape m.v.. SMI vil udlåne udstyr – dvs. forstærkere, A/D converter, elektrisk stimulator samt computer og software. Desuden betales deltagerhonorar af SMI.

Betaling til forsøgspersoner

Forsøgspersonerne modtager kr. 100 pr. time som betaling for deres deltagelse i forsøget. Beløbet er skattepligtigt og bliver derfor indrapporteret til Skat som B-indkomst.

Publicering af resultater

Projektets resultater vil blive forsøgt offentliggjort i anerkendte tidsskrifter uanset udfaldet af projektet.

Tidsplan

Projektet vil blive påbegyndt umiddelbart efter indhentelse af etisk godkendelse og forventes afsluttet indenfor 2 år.

Retningslinjer for mundtlig information og informeret samtykke

Indkaldelse af mulige forsøgspersoner

Når mulige forsøgspersoner kontaktes telefonisk med henblik på deltagelse i forsøget, skal følgende oplyses:

- At der er tale om en anmodning om deltagelse i et videnskabeligt forsøg
- Formålet med forsøget
- At det er frivilligt at deltage, og at forsøgspersonen når som helst kan trække sit tilsagn om deltagelse tilbage, uden at dette vil påvirke forsøgspersonens nuværende eller fremtidige behandling
- At forsøgspersonen har ret til betænkningstid, før der afgives samtykke til deltagelse i forsøget, og at forsøgspersonen har ret til at medbringe en bisidder, når den mundtlige information gives. Forsøgspersonen vil få udleveret skriftet "Forsøgspersonens rettigheder i et sundhedsvidenskabeligt forskningsprojekt", som indeholder oplysninger omkring tavshedspligt, aktindsigt og klageadgang
- At materialet "Deltagerinformation" fremsendes pr. brev til forsøgspersonen, således at denne kan få oplysninger om forsøget inden informationssamtalen.
- Til slut aftales et tidspunkt og sted for informationssamtalen

Når mulige forsøgspersoner kontaktes pr. brev med henblik på deltagelse i forsøget, skal alle ovenstående oplysninger gives og materialet "Deltagerinformation" inkluderes i det fremsendte.

Informationssamtalen

Til informationssamtalen reserveres et egnet lokale, f.eks. et mødelokale, hvor samtalen kan gennemføres uforstyrret. Der kan evt. serveres kaffe, te og/eller sodavand. Selve informationssamtalen skal afholdes af den projektansvarlige eller en seniorforsker, der har fået bemyndigelse til dette.

Samtalen skal indeholde følgende oplysninger/spørgsmål:

- Det er frivilligt at deltage, og forsøgspersonen kan når som helst trække sit tilsagn om deltagelse tilbage, uden at dette vil påvirke hans/hendes nuværende eller fremtidige behandling
- Forsøgspersonen har ret til betænkningstid før samtykket afgives, og forsøgspersonen har ligeledes ret til at medbringe en bisidder, når han/hun modtager den mundtlige information.
- Forsøgspersonen spørges, om han/hun ønsker, at der er en bisidder til stede.
- Formålet med forsøget oplyses, og det forklares, hvordan forsøget skal udføres. Der tages udgangspunkt i "Deltagerinformation", som forsøgspersonen har modtaget inden informationssamtalen.
- Forsøgspersonen spørges, om han/hun er sund og rask, og om han/hun er bærer af en smitsom sygdom.
- Forsøgspersonen spørges, om vedkommende er dansk statsborger. Hvis svaret er nej, spørges vedkommende, om han/hun har en gyldig arbejdstilladelse.
- Skriftet "Forsøgspersonens rettigheder i et sundhedsvidenskabeligt forskningsprojekt" udleveres, og det forklares, at skriftet indeholder oplysninger omkring tavshedspligt, aktindsigt og klageadgang.
  Forsøgspersonen spørges om, at han/hun har gennemlæst "Deltagerinformation". Hvis dette ikke er tilfældes, beder vi forsøgspersonen gennemlæse denne.
- Når det er sikret, at forsøgspersonen har gennemlæst deltagerinformationen, spørges forsøgspersonen, om han/hun har spørgsmål til forsøget?
  Herefter gives forsøgspersonen en demonstration i laboratoriet og div. måleudstyr og dets anvendelse i forsøget fremvises.
  Forsøgspersonen gøres opmærksom på, at han/hun har ret til betænkningstid, før samtykket afgives (vær opmærksom på, at Den Nationale Videnskabsetiske Komité anbefaler, at man så vidt muligt skal give 24 timers betænkningstid!).
- Forsøgspersonen gøres igen opmærksom på, at det er frivilligt at deltage, og at han/hun når som helst trække dit tilsagn om deltagelse tilbage, uden at dette vil påvirke nuværende eller fremtidige behandling.
- Forsøgspersonen oplyses om, at såfremt han/hun ikke ønsker at gøre brug af betænkningstiden, kan samtykket afgives herefter.
- Der aftales tidspunkt og sted for forsøgets afholdelse.
- Til slut informeres der om, hvem der er kontaktperson for projektet (det vises, at navnet fremgår af "Deltagerinformation"), og at denne person til hver en tid kan kontaktes, hvis der er yderligere spørgsmål.

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Appendix 3 - Thesis Summary (in Danish)

Neuromuskulære ændringer i den menneskelige soleusmuskel under og efter et udtrættende 800m løb

Under maximalt 800m løb falder farten fra start til slut og neuromuskulær træthed har muligvis en negativ indflydelse på præstationen. Neuromuskulær træthed inkluderer både centrale samt perifere mekanismer – de centrale er den manglende evne, induceret af aktivitet, til frivilligt at aktivere en muskel fuldt ud, og de perifere er musklens manglende evne til at producere kraft (Gandevia 2001). For at undersøge disse har især H-refleksen, transcranial magnetisk stimulering (TMS) og twitch-force været hyppigt anvendt (Iguchi and Shields 2011), (Gandevia 2001), (Racinais, et al. 2007), (Walton et al. 2002). Det menes, at mekanismer som presynaptisk inhibition, co-kontraktion, post-aktiveringsdepression, muskulær adaptation, eller sensitivitet af gruppe III og IV kemisk sensitive afferenter kan indvirke på moduleringen af den neurofysiologiske muskelkontrol (Misiaszek 2003), (Zehr 2002), (Knikou 2008), (Kuchinad, Ivanova and Garland 2004), (Bigland-Ritchie, Furbush og Woods 1986). Ikke desto mindre kendes de faktiske årsager og mekanismer stadig ikke og det er stadig ukendt hvor i systemet moduleringen finder sted.

Få studier har været udført med dynamiske kontraktioner, og alle med fokus på skridtcyklus - ikke på træthed (Simonsen and Dyhre-Poulsen 1999) (Edamura, Yang and Stein 1991). Soleusmusklen er interessant pga. dens fordelagtige position og innervering, og samtidig spiller den en essentiel rolle under løb ved høj hastighed, da den genererer kraft ved hvert skridt under plantar fleksion af foden. Samtidig har reflekserne og nervesystemet muligvis betydning, da de kan være bestemmende for kraftproduktion, tendo-muskulær stivhed og elastisk energilagring samt kontrol og eksitabilitet under løb (Ross, Leveritt and Riek 2001). Derfor vil denne undersøgelse forsøge at afklare nogen af de ovenstående usikkerheder vha. en løbeprotokol med måling af H-reflekser og V-bølger samt "traditionelle" neuromuskulære træthedsundersøgelser efter løbet i form af maksimal kraft udvikling, transkranial magnetisk stimulering (TMS) og twitch interpolation technique.

7 raske mandlige mellemdistance konkurrenceløbere deltog i studiet. Før laboratorietesten gennemførte hver testperson et maksimalt 800m løb på en udendørs bane. Den gennemsnitlige fart blev brugt på et løbebånd. Forsøgspersonerne løb to submaksimale og et maksimalt 800m løb på løbebåndet hvor H-reflekser, V-bølger og maksimale M-bølger (Mmax) blev elektrisk stimuleret i soleusmusklen under løbene hvert 2. sekund, styret af en fodkontakt. Før og efter løbene udførte testpersonerne isometriske plantarfleksioner på 50, 75 og 100% af maksimal voluntær kontraktion (MVC) med TMS og elektrisk stimulering. Ved denne del af protokollen måltes MVC, voluntær aktivering (VA), rest twitches (RT) motor evoked potentials (MEP) og silent periods (SP).

Resultaterne viste et fald af MVC -9.7 ± 9.6%, VA -9.3 ± 7.3% og RT -28.5 ± 15.7% samt en stigning i MEP 7.4 ± 4.8% og SP 7.3 ± 4.8ms efter løbene (alle $P<0.05$). Under det maksimale løb steg H-refleksen med 18.1 ± 12.8% og V-bølgen med 23.9 ± 13% samtidig faldt Mmax med -16.06 ± 2.13% fra den første epoke til den sidste (alle $P<0.05$). Skridttid og rmsEMG forblev uændret. Ved de submaksimale løb ændredes ingen af de nævnte faktorer.

Det maksimale 800m løb inducerede neuromuskulær træthed vist gennem faldet i MVC og VA. Denne observation er i overensstemmelse med tidligere løbe-studier (Girard et al 2012), (Tomazin et

al. 2012). Som forventet bestod en del af den neuromuskulære træthed af perifere muskel faktorer illustreret gennem et fald i RT og Mmax. Under løbet indikerede stigningen i H-reflex og V-bølger at spinale og supraspinale mekanismer forøgede voluntær nerve aktivering – muligvis for at kompensere for perifer træthed. Stigningen skete muligvis gennem reduceret presynaptisk inhibition samt øget cortikalt drive. Yderligere steg MEP og SP ved lavere kontraktioner, hvilket indikerede en øget cortikal excitabilitet. Begrundelsen herfor findes muligvis i at centralt drive kan kompensere for at perifere faktorer fungerer suboptimalt.

Nærværende studie viste at centrale nerve-faktorer ikke er begrænsende for et maksimalt 800m løb. Dog er stigningen i output fra centrale faktorer ved træthed under løbet i kontrast til faldet i central aktivering efter løbet. Forskellen i resultater skyldes muligvis at dynamiske og statiske kontraktioner skaber forskellige vilkår for måling af træthed og resultatet fra nærværende studie viser at fremtidige studier der vil undersøge neuromuskulær træthed i en dynamisk situation også er nødt til at anvende en dynamisk protokol for at finde applicerbare resultater.

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