Yngvild Gagnat

The cross-over effect of neuromuscular fatigue in the plantar flexors

Master's thesis in Human Movement Science

Trondheim, June 2015

NTNU

Faculty of Medicine

Department of Neuroscience

University of Nice Sophia Antipolis

Faculty of Sport Science

NTNU – Trondheim
Norwegian University of
Science and Technology

Abstract

Background Neuromuscular fatigue may be defined as an exercise-induced impairment in the ability to generate voluntary muscle force or power. This reduction in performance may occur during maximal or submaximal exercise, and the mechanisms contributing to this fatigue starts simultaneously with exercise. Fatiguability may be quantified by changes in maximal voluntary contraction (MVC) force and this muscular impairment of force capacity may be caused by peripheral or central mechanisms. Fatigue may not only affect the exercising muscles, but also other muscles not participating in the exercise. This is called a cross-over effect.

Aim The purpose of this study was to quantify neuromuscular fatigue in the plantar flexor muscles, and assess if there was a cross-over effect from the exercised leg to the contralateral, non-exercised leg. Underlying mechanisms in terms of peripheral and central fatigue was in addition investigated.

Method 13 participants, age 23.7 ± 1.9 years volunteered for this study, and attended for two testing sessions in the lab. Electromyography and force recordings were sampled. Measurements prior to (PRE) and after (POST) fatigue consisted of electrical stimulations and MVCs, and were conducted on both legs in counterbalanced order. At PRE stimulations at intensities eliciting maximal H-reflexes (H_{max}) and M-waves (M_{max}) amplitudes were given at rest and during four MVCs. The fatiguing tasks were performed on the right leg only (exercised leg) and consisted of three submaximal, isometric plantar flexions at 50% of MVC force, until exhaustion. The POST measurements consisted of stimulations at M_{max} intensity at rest and during MVC, followed by redetermination of H_{max} intensity if needed. Then stimulations at the given intensity was given at rest and during MVC.

Results The initial MVC force significantly decreased to after the fatiguing tasks, in both exercised and non-exercised leg, with about 18% and 8% respectively. One of the variable describing peripheral fatigue significantly decreased in both legs, although more pronounced in the exercised leg, with reductions of ~54% in the exercised leg and of ~15% in the non-exercised leg. There were no major changes in central parameters.

Conclusion The present study evaluating neuromuscular fatigue in the plantar flexors, found a cross-over effect to the contralateral, non-exercised leg. Peripheral mechanisms seem mainly to have attributed to the decrease in MVC force in the exercised leg, and to explain the effect of fatigue seen in the contralateral leg. Central mechanisms seems less to contribute.

Acknowledgement

First, I would like to thank my main supervisor at NTNU, Karin Roeleveld for giving me advice and guidance through this process. I would also like to thank my supervisor at the University of Nice, Serge Colson, for giving me a very educational semester, teaching me a whole lot about doing research, and also for all help and guidance writing this thesis.

Laura Gray, I am truly grateful for all your help in the lab! In addition, I would like to thank all the participants in this study.

I would like to thank my family for support and encouragement. Thanks to my fellow students for interesting discussions and the many social breaks during the year, and especially Tina and Astrid for making the stay in Nice fun and adventurous. Marit, this year wouldn't have been the same without you. Finally, thanks to Arve for having patience with me, and learning me syntax.

Abbreviations

H-reflex	Hoffman reflex
EL	Exercised leg
EMG	Electromyography
GM	Gastrocnemius medialis
GL	Gastrocnemius lateralis
cMVC	control MVC
MVC	Maximal voluntary contraction
M-wave	Compound muscle action potential
N	Newton
NEL	Non-exercised leg
POST	After fatiguing tasks
PRE	Prior fatiguing tasks
SD	Standard deviation
SOL	Soleus muscle
TA	Tibialis anterior
mV	millivolts
V-wave	First volitional wave

Content

Introduction

Intense, repeated or sustained contractions of the muscles will gradually reduce the ability to perform a given task (1). This decline in performance ability is known as neuromuscular fatigue and may be defined as an exercise-induced reduction in the ability to generate voluntary muscle force, due to extensive muscle activity (2–4). The fatiguing mechanisms occur during performance of both maximal and submaximal exercise, and include important physiological changes that happen before and during this eventual mechanical failure (5). Moreover, the neuromuscular system tries to compensate for this reduction in force capacity by implementing various muscle- and nervous-related mechanisms to avoid task failure and this may be seen as a tool for reducing the potential damaging effects of strenuous muscle exercise. As for submaximal tasks the nervous system tries to correct the muscle failure by increasing the drive and recruiting new motor unites (6, 7). The alterations of the neuromuscular system start simultaneously with exercise, and the fatigue becomes increasingly prominent until the muscle no longer is capable to perform the demanded task, however this decline in performance ability will mostly recover when the exercise stops (1, 6). The amount of fatiguability is often evaluated by changes in maximal voluntary contraction (MVC) force and time to exhaustion during a sustained contraction (2).

Neuromuscular fatigue may develop at different locations in the body, from the motor cortex and spinal cord to individual muscle fibers (8–10). Dependent on where the fatigue arises, it can be divided into peripheral and central sites. Peripheral fatigue results in impairment of the muscle's ability to respond to neural excitation and produce force, while central fatigue relates to failure of the central nervous system to elicit and drive motor neurons, and hence completely activate the muscle (1, 5, 8, 11). These different sites of fatigue may be quantified by changes in various responses to electrical stimulation of a peripheral nerve at different intensities (12). Peripheral fatigue involves alterations in neuromuscular transmission and excitation-contraction coupling failure, referring to the process where the action potentials binds to the cross-bridge cycles, which can be quantified by changes in the compound muscle action potential (M-wave) and mechanical twitch responses (6, 7, 13–17). The M-wave reflects the overall activation of the motoneuron pool and is visible on electromyography (EMG) recordings after supramaximal stimulation when muscle is at rest. The mechanical twitch responses can be measured as superimposed, unpotentiated or potentiated twitch depending on when the stimulation are given, either at peak MVC force, during rest, or just after an MVC, respectively. Both unpotentiated and potentiated twitch can be applied as measures of

peripheral fatigue, however, research has shown the latter to be a more reliable and sensitive quantification of peripheral fatigue, and hence the potentiated twitch will be considered in the present study (18, 19). Central fatigue has been associated with changes in the level of voluntary activation and in the reflex excitability of the α-motoneurons (5). The voluntary activation level may be quantified by implementing MVC force and the superimposed and potentiated twitch amplitudes in a formula (20, 21). The Hoffman reflex, also known as the H-reflex is another electrically induced response, and changes in the number of α-motoneurons recruited in the motoneuron pool, are reflected by changes in the reflex amplitude (22, 23). This reflex is a common tool for evaluating electrophysiological alterations that occur at the spinal level (24). The ratio of maximal H-reflex and M-wave amplitudes (H_{max}/M_{max}) can be used to evaluate the reflex inhibition of the motoneuron pool, and a decreased ratio indicates a reduction in motoneuron pool excitability (12, 16, 25, 26). The V-wave is an electrophysiological variant of the H-reflex recorded during an MVC and reflects the overall motoneuron rate coding and reflex excitability (27, 28). The H-reflex and V-wave recordings can be used as an approach to determine more precisely the sites mediating neural adjustments in response to the fatiguing tasks (29). Normalised to the respective M-wave (V/M_{sup}) the V-wave may quantify changes in the neural drive from the spinal α-motoneurons (28, 30).

The muscular impairment of force capacity caused by neuromuscular fatigue, may not only affect the exercising limb, but also the contralateral limb, not participating in the exercise (3, 4, 31). This process is known as a cross-over effect, and is related to the fact that activity of one muscle group may affect the performance of other muscle groups (31). When voluntary contractions are performed unilaterally, it may be associated with "unintentional" contractions in the contralateral homologous muscle (32). Cross-over effects of strength has been reported (5, 27, 33), however when it comes to the extent of the cross-over effect related to fatigue and the impact of the underlying mechanisms, the findings are vague and inconsistent (34, 35). Although fatigue has been evaluated both between contralateral, homologous muscles and between unrelated heterogeneous muscles, its most commonly evaluated between the former and hence the present study will focus on homologous muscles. When evaluating the cross-over effect in knee extensor muscles, there has been reported a decrease in force and voluntary activation in both exercised leg and non-exercised leg after performing unilateral, sustained exercise (3, 34, 36). While performing unilateral, intermittent exercise of the knee extensors or sustained exercise of the elbow flexors, it did not affect the respective contralateral limb's force (4, 37). Many factors may affect the different findings of fatigue and the effect on the non-

exercised limb, such as muscle group in action, experimental protocol in terms of duration, intensity and type of exercise and by the individual performing the exercise (7, 16). The contradictory results stated above, highlight the various affecting factors on neuromuscular fatigue and the cross-over effect

In daily life the influence of neuromuscular fatigue and the cross-over effect may have importance for functional activities, such as locomotion, balance, postural control and stabilisation of the body (3). These are all movements that demand coordination of limbs, and the cross-over effect of fatigue may hence be functionally important, maybe especially in the lower limbs as they are predominantly involved in such functionalities. The effect of cross-over has been evaluated in both upper and lower body limbs, and studies on the intrinsic hand muscles has shown minor effects (4, 31, 38), while using same protocol on the knee extensors the evidence of fatigue on the non-exercised limb is stronger (3, 34, 37, 39). As most research on fatigue and the cross-over effect in lower limbs has been performed on the knee extensors, there is a lack of research on the plantar flexors, which primarily consists of the Soleus (SOL), Gastrocnemius medialis (GM) and lateralis (GL) muscles. Although both central and peripheral fatigue have been quantified in exercised plantar flexor muscles (8, 22, 40), less is known about the cross-over effect, and the underlying mechanisms of this potential effect on the contralateral muscle has, to the authors knowledge, yet to be described.

The main aim of this study was to quantify neuromuscular fatigue in the plantar flexor muscles, evaluate if there was a cross-over effect from the exercised leg to the contralateral non-exercised leg, and to determine the sites of the fatigue in terms of central and peripheral mechanisms.

Methods

Participants

13 healthy men (n=9) and women (n=4) (age 23.7 ± 1.9 years; height 175.7 ± 7.2 cm; body mass 70.4 ± 9.2 kg; mean ± SD) volunteered to take part in this study. The participants were recreationally active and had a fitness score higher than 9.2 ± 1.6 of maximal score 15 according to the Baecke et al. (41) questionnaire. They did not report any neuromuscular disease or injuries in the lower limbs during the last six months. The study was conducted according to

the Declaration of Helsinki. Participants were informed about the procedure and provided informed written consent prior to enrolment in the investigation.

Experimental setup

All measurements were performed on both lower legs unilaterally. The fatiguing task was performed on the right leg only, hereafter called the exercised leg (EL). The participants were told to keep the left, non-exercised leg (NEL) relaxed during the fatiguing task. The participants were seated semi reclined with a hip angle of 105°, a knee angle of 120° and an ankle angle of 95° (180° fully extended) (Figure 1). The pelvis was secured with a waist belt and the arms were kept in front of the body. The body position was carefully maintained during the whole testing session, and head rotation during the test was proscribed in order to maintain constant cortico vestibular influences on the excitability of the motor neuron pool and limit afferent feedback from peripheral receptors.

Design of the study

Each participant visited the laboratory on three occasions. An initial session familiarized the participants with the equipment, experimental set-up and procedures. During this session they were asked to perform maximal voluntary isometric contractions (MVCs) of the plantar flexors of each leg as well as submaximal contractions of the EL with visual feedback until they felt comfortable with the equipment and the tasks. The participants were also accustomed to electrical stimulation of the tibial nerve and allowed the investigators to identify optimal stimulation intensities for each leg. The participants then came on two separate days (with at least three days in between) to perform the experimental procedure in which the order of the leg tested first (EL or NEL) was counterbalanced. The sessions were conducted at the same time of the day and the participants were asked to refrain from strenuous activity for 24 h before the experimental procedures.

Force measurements

A custom made ankle ergometer (42, 43) was used for the isometric force testing and the fatiguing task. The participants wore shoes that were bolted to the footplate of the ergometer and straps were placed around the foot to secure it firmly (Figure 2). A strain-gauged transducer

(model OMF06M, linear range 015kN, precision ± 0.5%, sensitivity 10mV/kN; OMICRO'N, Gambais, France) was connected to the footplate to measure ankle plantar flexion force production. A monitor provided subjects with visual feedback on the ankle force.

Figure 1. The position of the body through the experimental protocol

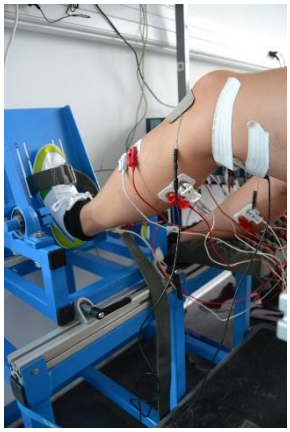

Figure 2. The ankle ergometer with foot strapping and surface electromyography electrodes

Surface electromyography

Bipolar surface electromyography (sEMG) electrodes (1cm diameter, 2cm inter-electrode distance) were placed on the soleus (SOL), gastrocnemius medialis (GM), gastrocnemius lateralis (GL) and tibialis anterior (TA) muscles of both legs according to the SENIAM recommendations (44). Prior the application of the sEMG electrodes, the skin was shaved, rubbed with sandpaper and cleaned with 70% alcohol. To ensure that the skin was properly prepared the impedance was measured with a required value of <3kΩ. The SOL electrodes were positioned approximately 2cm below the insertion of the gastrocnemii on the Achilles tendon. The GM, GL and TA electrodes were positioned on the bulk of the muscles. A common reference electrode was positioned on the patellarbone of the NEL. sEMG signals were amplified (×1000) with a bandwidth frequency ranging from 10 Hz to 500 Hz (MP 150 Biopac systems, Inc., Holliston, MA, USA) and recorded at a sampling rate of 2000 Hz. The electrode positions were marked with indelible ink to ensure that they were replaced exactly in the same

position at the different testing sessions. The sEMG and mechanical signals were digitized online and stored for analysis with commercially available software (Acqknowledge 4.1, Biopac systems, Inc., Holliston, MA, USA).

Electrical stimulation

The electrical stimulation of the plantar flexor muscles was induced using a constant current electrical stimulator (Model DS7AH, Digitimer, Hertfordshire, United Kingdom) delivering rectangular pulses of 1-ms duration. The tibial nerve was stimulated with a self-adhesive cathode (8-mm diameter, Ag-AgCl, Cotrôle Graphique Medical, Brie-Comte-Robert, France) firmly fixed with straps and taping. The optimal location of the stimulation site in the popliteal fossa was first determined using a hand-held ball electrode (0.5cm diameter). The anode (5x10cm Cefar-Compex, DJO France SAS, Mouguerrre, France) was placed on the patella tendon of the ipsilateral knee.

Experimental procedure

As general warm up the participants cycled for 5min at 50W, and as specific warm up of the plantar flexors they performed submaximal and maximal contractions in the ankle ergometer of EL and NEL. Then the testing procedure consisting of series of neuromuscular measurements prior to (PRE) and after (POST) the fatiguing task was realized (Figure 3). A recruitment curve was conducted to carefully determine the intensity to elicit maximal amplitudes of the H-reflex and M-wave responses of the SOL muscle at rest. The stimulation intensity started at 10mV and progressively increased by 2mA until the highest amplitude of the H-reflex (H_{max}) was observed in the ascending part of the recruitment curve (24). Stimulation intensity continued to increase with incremental steps of 10mA until a further increase in intensity did not resulted in greater amplitude of the M-wave and in the mechanical twitch response of the plantar flexor muscles. To ensure supramaximal stimulation to evoke M-wave at rest (M_{max}) and during MVC (M_{sup} and associated V-wave) the intensity was adjusted to 120% of the optimal intensity. The H_{max} and the M_{max} stimulation intensities ranged between 7.0 to 74.0 mA (mean of 32.5 ± 14 mA) and between 40.0 to 160.0 mA (mean of 109.0 ± 20.2 mA) respectively.

Following this the PRE measurements were carried out consisting in three stimulations at H_{max} intensity and three supramaximal stimulations (i.e. M_{max} intensity) delivered at rest and each interspaced by 5 seconds. Afterwards four MVCs of the plantar flexors of 5-s duration

were performed with 90 seconds of rest in-between. During two MVCs a stimulation at H_{max} intensity (MVC_{Hmax}) was delivered when the participants had reached a plateau (peak force) in order to measure the superimposed H-reflex (H_{sup}) and the M-wave at H_{sup} (M_{Hsup}). The two other MVCs were performed with supramaximal stimulation (MVC_{Mmax}), delivered over the MVC plateau to measure the superimposed M-wave (M_{sup}) and three seconds after the end of the contraction for the potentiated twitch. The MVCs were performed in a randomized order regarding the stimulation intensity delivered. The same procedure was conducted on both the EL and NEL and was counterbalanced across testing sessions and participants. Once PRE measurements were completed the participants performed the fatiguing task on the EL. Immediately at task termination the EL or the NEL of the participants was tested according to the randomisation procedure. On each lower leg, they performed a MVC with supramaximal stimulations during and after the contraction followed by three supramaximal stimulations delivered at rest. The intensity eliciting the maximal amplitude of the H-reflex was rapidly controlled and redetermined when necessary to match the shape and amplitude of the small M-wave obtained during the PRE tests, to ensure the same motoneuron pool recruitment (45). The experimental procedure ended with three stimulations delivered at rest at the redetermined H_{max} intensity and one MVC with a superimposed stimulation at this intensity. Strong verbal encouragement was provided by the experimenters throughout MVCs of the PRE and POST testing as well as during the fatiguing task

The fatiguing task consisted of three sustained submaximal isometric contractions performed at 50%, until exhaustion. The 50% force was calculated from the mean over the four MVCs performed on EL (control MVC; cMVC). The participants received real time visual feedback during the task. One-minute rest was provided between the contractions. Participants were not aware of the exhaustion criteria of each submaximal contraction defined as a decrease of 30% of the targeted force signal for more than three consecutive seconds.

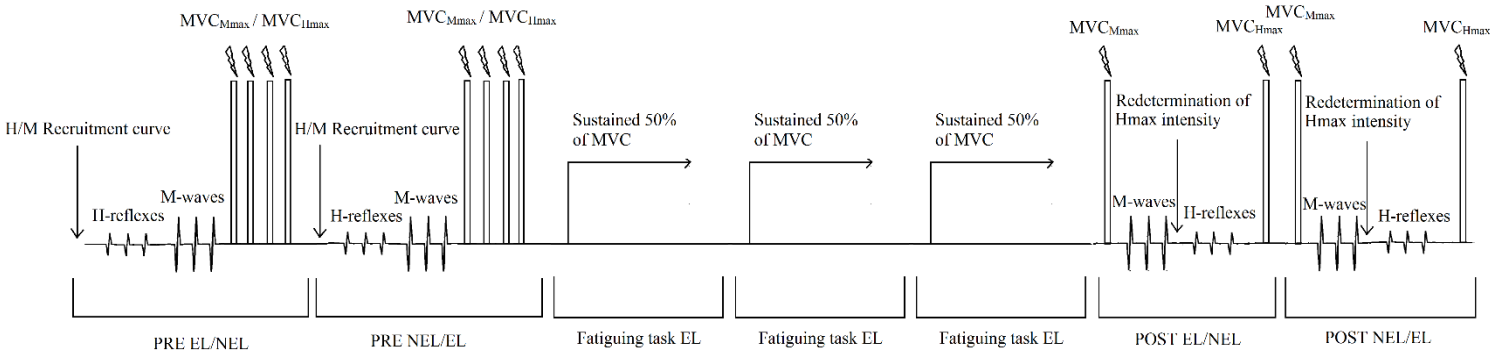

Figure 3. Experimental protocol.
Measurements prior to (PRE) and after (POST) fatigue consisted of electrical stimulations and MVCs, and were conducted on both legs in counterbalanced order. At PRE stimulations at different intensities eliciting H-reflex (H_{max}) and M-waves (M_{max}) were given at rest and during four MVCs. The fatiguing tasks were performed on the exercised leg only, and consisted of three isometric plantar flexions at 50% of MVC force, until exhaustion. The POST measurements consisted of stimulations at M_{max} intensity at rest and during MVC, followed by redetermination of the H_{max} intensity. The stimulations at the given intensity was given at rest and during MVC.
MVC, Maximal voluntary contraction

Data analyses

All outcomes were analysed with Acqknowledge 4.1, Biopac systems, Inc., Holliston, MA, USA.

Force data

The MVC_{Mmax} with highest peak force was considered for analysis. Peak force was measured from baseline to maximal force achieved prior stimulation and is reported in Newtons (N). Maximal voluntary activation level was calculated from the superimposed and potentiated twitch amplitude by the given formula (20):

$$\%\text{Voluntary activation} = (1\text{-superimposed twitch} \div \text{potentiated twitch}) \times 100$$

When stimulation was elicited slightly before or after real peak force, a correction was applied to the formula above (46):

$$\%\text{Voluntary activation} = (1\text{-(superimposed twitch} \times \text{force level just before stimulation} \div \text{MVC force)} \div \text{potentiated twitch)} \times 100$$

Superimposed and potentiated twitch was measured as peak-to-peak amplitude in response to supramaximal stimulation during the MVC and three seconds after, respectively (Figure 4).

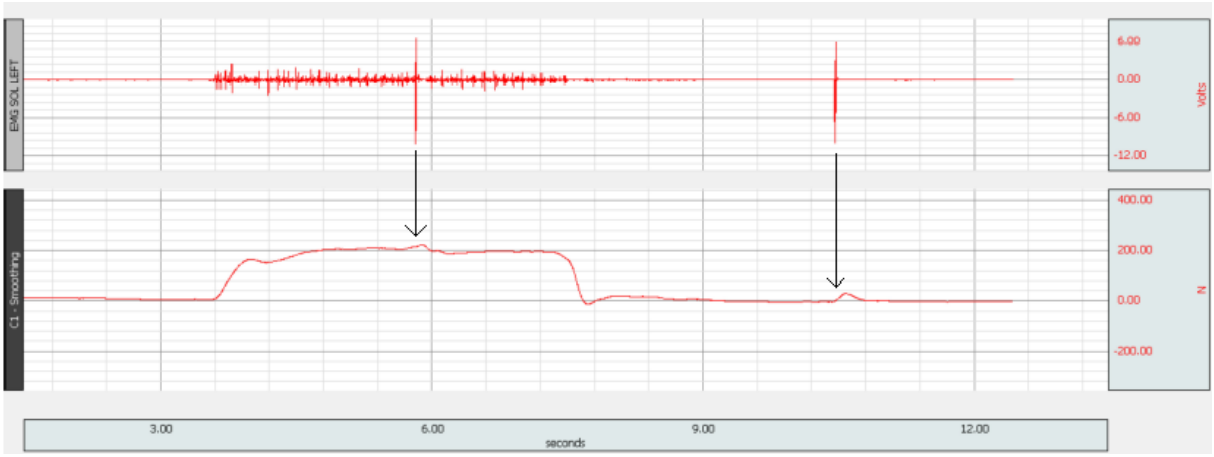

Figure 4. EMG and force recordings during an MVC.
Superimposed twitch as response to electrical stimulation at peak MVC, and potentiated twitch as response to stimulation at rest.
EMG, Electromyography; MVC, Maximal voluntary contraction

EMG data

Although EMG signals were recorded for both the SOL, GM, GL and TA muscles, only the recordings of the SOL muscle are analysed in the present study.

The SOL EMG activity associated with the three stimulations at H_{max} and M_{max} intensities were averaged for the analysis. Peak-to-peak amplitude were measured for the H-reflexes (H_{max}, H_{sup}) (Figure 5), small M-waves preceding H_{max} and H_{sup} (M_{Hmax} , M_{Hsup}), maximal M-waves (M_{max}, M_{sup}) (Figure 6) and V-wave, all expressed in millivolts (mV).

To insure that any changes in H_{max} were not due to changes at the muscle fiber membrane or neuromuscular junction, H_{max} were normalised to M_{max}, expressed as H_{max}/M_{max} ratio. The same was done for the superimposed recordings, i.e. H_{sup}/M_{sup} ratio, and the V-wave was normalised to the respective M_{sup}. M_{Hmax} and M_{Hsup} were respectively normalised to M_{max} and M_{sup} (M_{Hmax}/M_{max}, M_{Hsup}/M_{sup}), to insure that the same proportion of α-motoneurons was activated by the stimulation during PRE and POST tests.

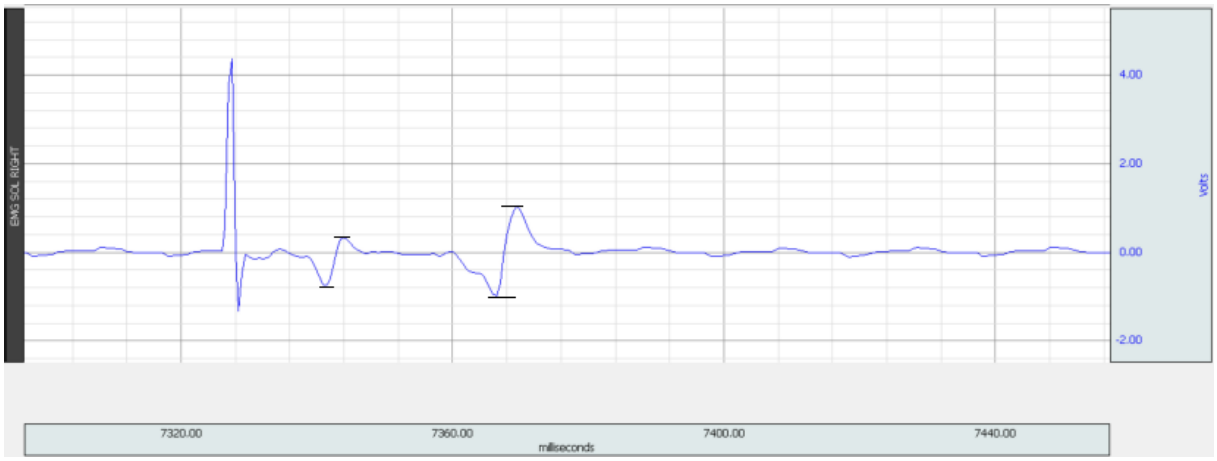

Figure 5. EMG recordings of electrical stimulation when muscle is at rest.
The first amplitude is the electrical stimulation, the second is the small M-wave (i.e. M_{Hmax}), and the third is the H-reflex (i.e. H_{max}).
EMG, Electromyography

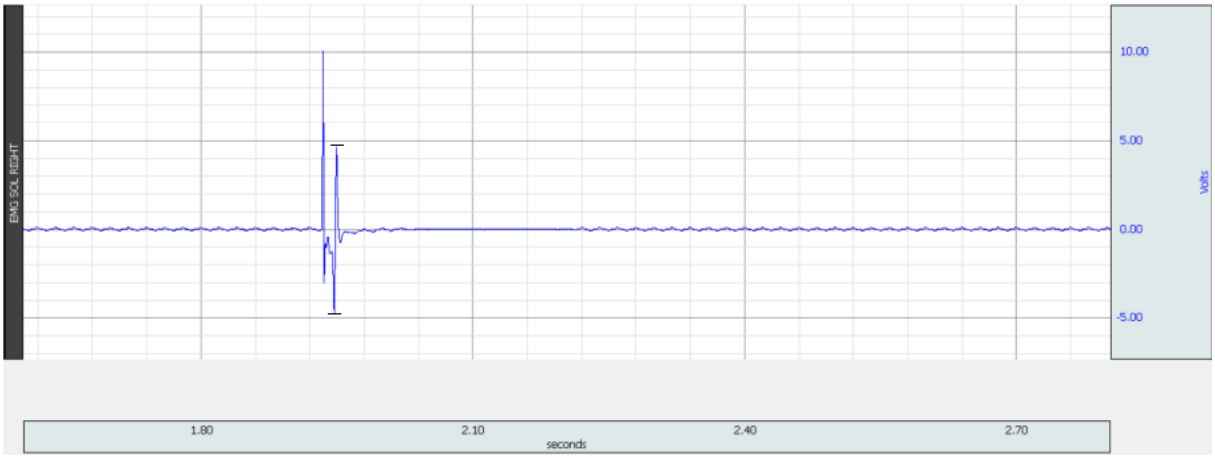

Figure 6. EMG recordings of supramaximal electrical stimulation when muscle is at rest.
The first amplitude is the electrical stimulation, the second is the M-wave (i.e. M_{max}).
EMG, Electromyography

Statistical analysis

The normality of the data was evaluated by using Shapiro Wilk tests and visual assessment. The non-normally distributed data was log-transformed to obtain normal distribution. A Paired

t-test was used to compare the baseline (PRE) measurements. Main effects leg; time, and interactions leg × time, were analysed using Mixed Models. Participants was set as random factor. A bonferrioni post hoc test was used to compare main effects and interactions.

All statistical analysis was conducted in IBM SPSS Statistics Version 21.0 (IBM Corp., NY, USA). For all analysis the significance level was set at $p \leq 0.05$. All variables are presented as mean ± standard deviation (SD). Objective measures of changes from PRE to POST are presented as an average relative percentage change $((POST - PRE) \div PRE \times 100)$. The respective relative standard deviations was calculated by the given formula:

$$SD_{Percentage\ change} = Percentage\ change \times \sqrt{((SD_{PRE} \div PRE)^2 + (SD_{POST} \div POST)^2)}.$$

Results

At baseline, there were no significant differences between the EL and the NEL, for any of the variables ($p>0.141$). There were neither significant differences between test day one and test day two ($p>0.096$). Hence, the percentage changes from PRE to POST and the results in the tables and figures will be presented as a mean over the two test days.

The M_{Hmax}/M_{max} and M_{Hsup}/M_{sup} ratios did not change between PRE and POST for neither legs ($p>0.393$), and was not significantly different from each other ($p>0.399$) which indicates that stimulus conditions were the same over the experimental protocol.

Fatiguing task

All of the participants were able to perform the three submaximal fatiguing tasks on both test days as described in the methods section, and the participants maintained the 50% of cMVC force as instructed. Time to exhaustion in the first fatiguing task significantly decreased over the three fatiguing tasks ($p<0.001$) (Figure 7). The average time to exhaustion at the third fatiguing task was reduced with 63 ± 34% from the first fatiguing task.

MVC

Figure 8 shows the peak force produced during MVC with the EL and NEL prior to and after the fatiguing tasks. The EL and NEL significantly reduced MVC force with time, with approximately 18 ± 6% and 8 ± 3% respectively ($p<0.001$ for the EL, $p= 0.046$ for the NEL) (Table 1). There was a trend to a leg × time interaction for MVC ($p=0.062$).

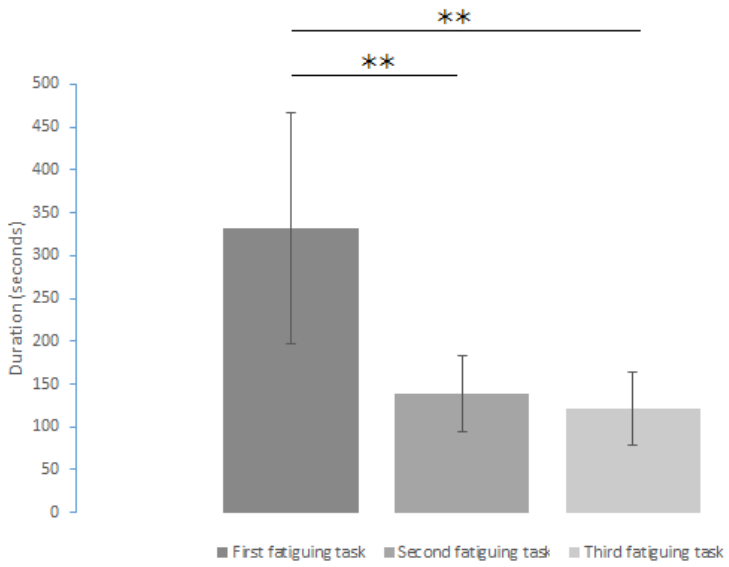

Figure 7. Time to exhaustion, given i seconds, for the three fatiguing tasks.
Significant differences between tasks **p≤0.005

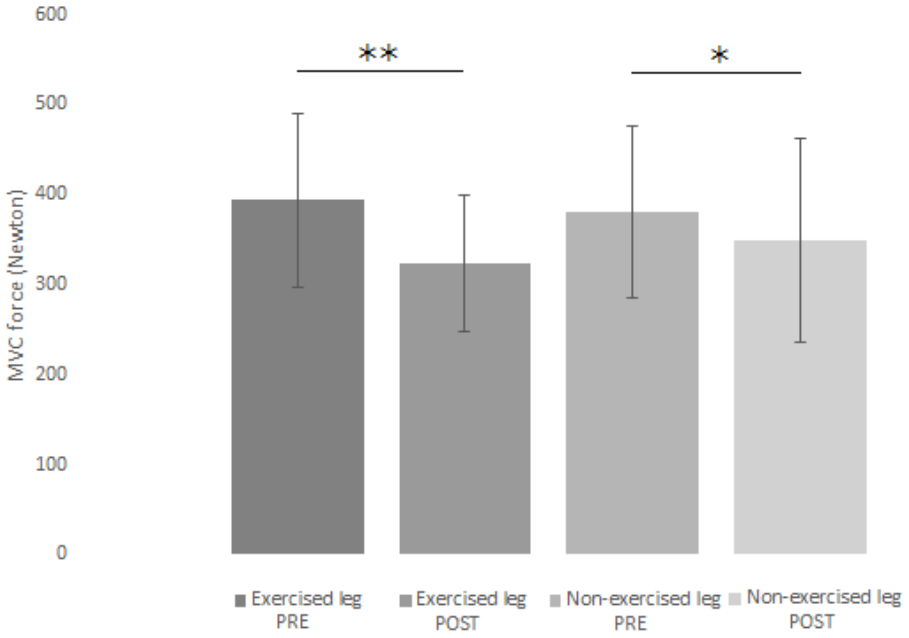

Figure 8. MVC force prior to (PRE) and after (POST) fatiguing tasks for the exercised leg and the non-exercised leg
Significant differences from PRE to POST *=p≤0.05, **=p≤0.005
MVC, Maximal voluntary contraction

Peripheral mechanisms

The potentiated twitch had initial values of approximately 58N in both the EL and NEL (Table 1). There was a reduction after the fatiguing tasks of ~54 ± 17% in the EL and of ~15 ± 5% in the NEL (both $p<0.001$). The interaction of leg × time was significant ($p<0.001$).

There were no significant changes from PRE to POST in M_{max} amplitude for the EL ($p=0.397$) or the NEL ($p=0.802$) (Table 2). The M_{sup} amplitude was reduced in both legs after the fatiguing tasks, ~23 ± 13% in the EL and of ~11 ± 6% in the NEL, but these changes were only significant for the EL ($p=0.039$, $p=0.262$ for the NEL) (Table 2).

Central mechanisms

The voluntary activation level as investigated by the twitch interpolation technique was about 89 ± 8% in the EL and 86 ± 10% in the NEL prior the fatigue tasks (Table 1). In both legs there were no significant changes from PRE to POST ($p=0.738$ for the EL, $p=0.151$ for the NEL).

The H_{max} and H_{sup} amplitudes showed no effect of the fatiguing tasks for neither the EL ($p=0.306$ and 0.872 respectively) nor the NEL ($p=0.263$ and 0.342 respectively) (Table 2).

The H_{max}/M_{max} ratio in the EL had non-significant changes from PRE to POST, with an increase of ~6 ± 3% ($p=0.494$), whereas the NEL had trend to a decrease with ~13 ± 8% ($p=0.063$) (Table 2). The H_{sup}/M_{sup} ratio had significant changes in the EL ($p=0.05$), however the variance of the change was quite high, with an increase of ~12 ± 11% (Table 2). For the NEL there was no significant changes ($p=0.315$).

The V-wave nor the V/M_{sup} had any significant changes from PRE to POST ($p=0.121$ and $p=0.156$ for the EL, $p=0.485$ and $p=0.156$ for the NEL, resepctively) (Table 2).

Table 1. MVC force, voluntary activation level and mechanical response, evoked by electrical stimulations. Values prior to (PRE) and after (POST) the fatiguing tasks for the exercised and the non-exercised leg.

	Exercised leg		Non-exercised leg	
	PRE	POST	PRE	POST
MVC force (N)	393.58 ± 97.03	323.87 ± 76.05**	380.27 ± 95.08	349.18 ± 112.69*
Voluntary activation (%)	88.51 ± 8.31	91.84 ± 15.37	85.25 ± 10.51	88.51 ± 7.96
Potentiated twitch (N)	58.42 ± 10.19	26.85 ± 7.2**	57.87 ± 14	49.28 ± 13.59**

Values presented as mean ± SD. Significant differences from PRE to POST *= $p\leq0.05$, **= $p\leq0.005$

MVC, maximal voluntary contraction; Potentiated twitch, mechanical response to supramaximal stimulation after MVC; N, Newton

Table 2. Reflex responses and ratios. Values prior to (PRE) and after (POST) the fatiguing tasks for the exercised and the non-exercised leg.

	Exercised leg		Non-exercised leg	
	PRE	POST	PRE	POST
M_{max} (mV)	8.64 ± 3.1	8.29 ± 2.76	8.87 ± 3.3	9.01 ± 3.54
M_{sup} (mV)	11.37 ± 4.72	8.75 ± 3.49*	11.89 ± 4.43	10.55 ± 4.65
H_{max} (mV)	3.38 ± 1.79	3.75 ± 1.83	3.5 ± 1.67	3.21 ± 1.97
H_{sup} (mV)	3.54 ± 2.13	3.61 ± 2.42	3.27 ± 2.6	3.38 ± 2.7
H_{max}/M_{max}	0.41 ± 0.17	0.44 ± 0.18	0.41 ± 0.14	0.35 ± 0.18
H_{sup}/M_{sup}	0.35 ± 0.26	0.40 ± 0.21*	0.26 ± 0.15	0.35 ± 0.26
V-wave (mV)	2.0 ± 1.5	1.6 ± 1.39	2.0 ± 1.42	2.15 ± 1.74
$V/_{Msup}$	0.22 ± 0.22	0.14 ± 0.09	0.17 ± 0.11	0.21 ± 0.15

Values presented as mean ± SD. Significant differences *= $p \leq 0.05$

M_{max}, maximal M-wave at rest; M_{sup}, maximal M-wave during voluntary contraction; H_{max}, maximal H-reflex at rest; H_{sup}, maximal H-reflex during voluntary contraction; V, maximal V-wave amplitude during voluntary contraction

Discussion

Main findings

The aim of this study was to assess changes in maximal voluntary force in the plantar flexors, evaluate if there was a cross-over effect from the exercised leg (EL) to the non-exercised leg (NEL), and investigate central and peripheral mechanisms of the cross-over. The main findings of this study were that sustained isometric contractions led to fatigue in both the EL and the NEL, which indicates a cross-over effect. The mechanisms behind this effect seems mainly to be attributed to peripheral changes, as potentiated twitch had great reductions in both legs, while the central mechanisms had less effect of time in both legs.

MVC force and the cross-over effect

The maximal voluntary contraction (MVC) force had an average reduction of approximately 18% for the EL, which is consistent with other studies of neuromuscular fatigue of the plantar flexors (8, 12, 16). The decrease in the present study is smaller than reported of others; reductions of ~55% (8) and ~29% (16) have been reported after maximal fatiguing tasks. For the NEL there was an average force reduction of approximately 8%. This decline of the contralateral limb is supported by other studies on fatigue and cross-over effect to contralateral muscles, showing reductions of 4%, 11%, 14% and 8% in the knee extensors (3, 34, 39) and of 9% in the intrinsic hand muscles (38).

Peripheral changes in exercised and non-exercised limb

In the present study, peripheral fatigue was quantified by changes in potentiated twitch and M-wave amplitude from PRE to POST fatiguing task. The significant reduction in potentiated twitch amplitude in both the EL and the NEL, to about 46% and 85% of initial values respectively are consistent with other studies reporting a reductions in the exercised knee extensors of ~12% and ~2% in the non-exercised (34), and of ~66% in exercised intrinsic hand muscle and ~20% in the non-exercised (38). A study evaluating differences between gender reported a ~58% reduction among the men's exercised knee-extensors and 56% among the women, and in the non-exercised contralateral legs reductions of 7% and 2% respectively (39).

The M_{max} amplitude in the EL remained stable from PRE to POST the present study, which is consistent with other studies of the plantar flexors (8, 12, 16, 26, 40, 47). Regarding the NEL and M_{max} amplitude, no changes from PRE to POST was found, which is similar to other studies evaluating the cross-over effect on knee extensors (3, 34).

These findings of reduced potentiated twitch in both legs, together with no changes in M_{max} amplitude, indicates that peripheral mechanisms and a failure in the excitation-contraction coupling, highly contributed to the fatigue, and also the cross-over effect (11, 16, 48–50).

However, since the reductions in potentiated twitch was three times greater than the reduction in MVC force in the EL, and almost twice the amount of the force decrements in the NEL, it might seem like there have been some other mechanisms compensating for the failure in contractile properties. This could be due to the central nervous system increasing neural drive, or another possible explanation could be activation of an antagonist muscle (i.e. Tibialis anterior muscle). The EMG recordings of the TA muscle was not analysed in the present study, hence the impact of a potential activation are difficult to assess.

Central changes in exercised and non-exercised limb

In this study, the comparison of PRE and POST levels of voluntary activation and various electrically induced responses quantified the central fatigue. The voluntary activation level, quantified by the twitch interpolation technique, did not change from PRE to POST in neither the EL nor the NEL, consistent with a study of the elbow flexors (4). But the results are in contrast to several other studies reporting a decrease in voluntary activation for both exercised and non-exercised limbs after fatiguing tasks (3, 34, 36, 38, 39).

In addition to voluntary activation, electrically induced responses may contribute to examine the central nervous system's adaptations to fatigue. There is not much known about potential alterations in the motoneuron level within the effect of cross-over, and for this the H-reflex is considered as a valid tool in explaining the reflex excitability (23, 28, 51). Results of the present study shows that neither the resting maximal H-reflex amplitude nor the H-reflex obtained during MVC, changed significantly from PRE to POST, in none of the legs. Although, there has previously been reported of reductions of the H-reflex in both exercised and contralateral non-exercised limb (51). The H-reflex normalised to the M-wave reflects the amount of motoneurons that are capable of being recruited from the entire motoneuron pool, as the H-reflex itself is an indirect estimate of the number of motoneurons recruited, and the M-wave represents the whole motoneuron pool (13). In the present study H_{max}/M_{max} ratio, i.e. the spinal excitability at rest, showed a nearly significant reduction in the NEL of ~13%, while the effect on the EL was in the opposite direction, although not significant. Other studies of fatigue have reported of significantly reduced H_{max}/M_{max} ratios in the exercised limb (12) or no changes (12, 25, 26)

The H_{sup}/M_{sup} ratio however, showed a significant increase of ~29% in the EL, whereas the NEL had no changes. The minor increase of spinal excitability at rest for the EL, is further increased during MVC. This could be the central nervous systems compensatory mechanism for the great peripheral failure, discussed in the section above, with increased neural drive to the motoneuron pool. For the NEL opposite findings was present as the spinal excitability decreased from PRE to POST at rest, while did not change during MVC.

There were no changes in the V-wave or the V/M_{sup} ratio from PRE to POST indicating no alterations in the level of efferent neural drive from the α-motoneurons during the MVCs (28, 52).

Even though most of the findings of central mechanisms on the fatigue and the effect of cross-over, are not statistically significant, they still may be functionally important, as they might act as compensatory factors to the peripheral failure. The central mechanisms may have resulted from peripheral feedback of a decreased function, and acted as a tool for reducing the failure in the peripheral mechanisms, at least in the EL as H_{sup}/M_{sup} ratio increased.

Methodological considerations

The present study have used a fatiguing protocol consisting of isometric submaximal contractions, until exhaustion. There are many differences among the variety of protocols, and

most of them consists of sustained maximal contractions. As for the main muscle investigated in the present study, the SOL muscle, a sustained submaximal contraction was more appropriate, as it consists mainly of slow twitch muscle fibres. Submaximal intensity was also chosen since it has shown to delay the recovering of the H-reflex after fatiguing tasks (26), which was one of the variables of interests for the present study.

Although for voluntary activation level, it could be questioned if the intensity was high enough, as the studies reporting decreased voluntary activation level in both exercised and non-exercised leg have used maximal fatiguing tasks. A study evaluating the differences of maximal versus submaximal fatiguing tasks on voluntary activation level in addition to the effect of cross-over, reported that maximal fatiguing task led to decreased voluntary activation level in both exercised and non-exercised intrinsic hand muscles, while submaximal fatiguing task only led to reduction in the exercised limb (38). In contrast, it is thought that when performing a continuous contraction at low levels (≤30% MVC) fatigue is mainly due to central mechanisms, whereas fatigue from contractions at higher intensities results from peripheral mechanisms (22) which could explain our findings.

As there are a lack of research on the cross-over effect on plantar flexors compared to the knee extensors, there is difficult to make a direct comparison between the different studies, although it may be assumed that the other studies could be related to the present study.

To ensure valid and reliable H-reflex measurements, the methodological requirements were carefully monitored, as the reflex is very sensible to body position, stimulation site, duration and frequency (13). Redetermination of the H_{max} intensity was done if needed for the POST measurements, and the small M-wave preceding the H-reflex was used as a control to ensuring same stimulus conditions.

There has been reported of differences in fatiguing mechanisms between gender, but it was beyond the scope of the present study to evaluate. Although, a quick statistical check did not reveal any major effect of gender on any of the variables.

Relevance of findings

As mentioned in the introduction, the cross-over effect may have importance for functional activities in daily life. The plantar flexor muscles are important for e.g. postural control and balance, and the fact that the NEL also get affected by the fatigue in EL, may seem like a defence mechanism to preserve equal conditions between the legs.

Further research

The present study recorded EMG signals from several muscles in the leg, i.e. the SOL, GM, GL and TA muscles, although only the recordings from the SOL muscle was analysed for this thesis. It would be interesting to analyse the other muscles as well, to evaluate potential co-activation and antagonist activation during the MVCs and the fatiguing tasks.

Conclusion

The present study reported of a decreased capacity in MVC force, after fatiguing tasks, which establish the presence of fatigue. In addition, this fatiguing experience was seen in both the exercised leg and the non-exercised leg, confirming a cross-over effect. As underlying mechanisms of this effect, it may seem as peripheral fatigue are the main cause to the reduction in MVC force. As the decrease in MVC force was less than the decrease in peripheral mechanisms, a compensatory mechanisms may have been involved. However, no centrally mediated mechanisms was revealed, hence it could be due to antagonist-activation or other factors not accounted for in the present study.

References

1. Allen DG, Lamb GD, Westerblad H. Skeletal muscle fatigue: cellular mechanisms. Physiol Rev. 2008;88(1):287-332.
2. Enoka RM, Duchateau J. Muscle fatigue: what, why and how it influences muscle function. J Physiol. 2008;586(1):11-23.
3. Rattey J, Martin PG, Kay D, Cannon J, Marino FE. Contralateral muscle fatigue in human quadriceps muscle: evidence for a centrally mediated fatigue response and cross-over effect. Pflugers Arch. 2006;452(2):199-207.
4. Todd G, Petersen NT, Taylor JL, Gandevia SC. The effect of a contralateral contraction on maximal voluntary activation and central fatigue in elbow flexor muscles. Exp Brain Res. 2003;150(3):308-13.
5. Gandevia SC. Spinal and supraspinal factors in human muscle fatigue. Physiol Rev. 2001;81(4):1725-89.
6. Janet L. Taylor SCG. A comparison of central aspects of fatigue in submaximal and maximal voluntary contractions. Taylor JL, Gandevia SC, editors2008 2008-02-01 00:00:00. 542-50 p.
7. Boyas S, Guevel A. Neuromuscular fatigue in healthy muscle: underlying factors and adaptation mechanisms. Ann Phys Rehabil Med. 2011;54(2):88-108.
8. Kennedy A, Hug F, Bilodeau M, Sveistrup H, Guevel A. Neuromuscular fatigue induced by alternating isometric contractions of the ankle plantar and dorsiflexors. J Electromyogr Kinesiol. 2011;21(3):471-7.
9. Taylor JL, Todd G, Gandevia SC. Evidence for a supraspinal contribution to human muscle fatigue. Clin Exp Pharmacol Physiol. 2006;33(4):400-5.
10. Cresswell AG, Loscher WN. Significance of peripheral afferent input to the alpha-motoneurone pool for enhancement of tremor during an isometric fatiguing contraction. Eur J Appl Physiol. 2000;82(1-2):129-36.
11. Enoka RM, Stuart DG. Neurobiology of muscle fatigue. J Appl Physiol (Bethesda, Md : 1985). 1992;72(5):1631-48.
12. Paasuke M, Rannama L, Ereline J, Gapeyeva H, Oopik V. Changes in soleus motoneuron pool reflex excitability and surface EMG parameters during fatiguing low- vs. high-intensity isometric contractions. Electromyogr Clin Neurophysiol. 2007;47(7-8):341-50.
13. Palmieri RM, Ingersoll CD, Hoffman MA. The hoffmann reflex: methodologic considerations and applications for use in sports medicine and athletic training research. J Athl Train. 2004;39(3):268-77.
14. Dulhunty AF. Excitation-contraction coupling from the 1950s into the new millennium. Clin Exp Pharmacol Physiol. 2006;33(9):763-72.
15. Kent-Braun JA. Central and peripheral contributions to muscle fatigue in humans during sustained maximal effort. Eur J Appl Physiol Occup Physiol. 1999;80(1):57-63.
16. Nordlund MM, Thorstensson A, Cresswell AG. Central and peripheral contributions to fatigue in relation to level of activation during repeated maximal voluntary isometric plantar flexions. J Appl Physiol. 2004;96(1):218-25.
17. Bigland-Ritchie B, Woods JJ. Changes in muscle contractile properties and neural control during human muscular fatigue. Muscle & nerve. 1984;7(9):691-9.
18. Kufel TJ, Pineda LA, Mador MJ. Comparison of potentiated and unpotentiated twitches as an index of muscle fatigue. Muscle & nerve. 2002;25(3):438-44.
19. Place N, Maffiuletti NA, Martin A, Lepers R. Assessment of the reliability of central and peripheral fatigue after sustained maximal voluntary contraction of the quadriceps muscle. Muscle & nerve. 2007;35(4):486-95.

20. Allen GM, Gandevia SC, McKenzie DK. Reliability of measurements of muscle strength and voluntary activation using twitch interpolation. Muscle & nerve. 1995;18(6):593-600.
21. Herbert RD, Gandevia SC. Twitch interpolation in human muscles: mechanisms and implications for measurement of voluntary activation. J Neurophysiol. 1999;82(5):2271-83.
22. Place N, Bruton JD, Westerblad H. Mechanisms of fatigue induced by isometric contractions in exercising humans and in mouse isolated single muscle fibres. Clin Exp Pharmacol Physiol. 2009;36(3):334-9.
23. Girard O, Racinais S, Micallef JP, Millet GP. Spinal modulations accompany peripheral fatigue during prolonged tennis playing. Scand J Med Sci Sports. 2011;21(3):455-64.
24. Grospretre S, Martin A. H reflex and spinal excitability: methodological considerations. J Neurophysiol. 2012;107(6):1649-54.
25. Loscher WN, Cresswell AG, Thorstensson A. Excitatory drive to the alpha-motoneuron pool during a fatiguing submaximal contraction in man. J Physiol. 1996;491 (Pt 1):271-80.
26. Kuchinad RA, Ivanova TD, Garland SJ. Modulation of motor unit discharge rate and H-reflex amplitude during submaximal fatigue of the human soleus muscle. Exp Brain Res. 2004;158(3):345-55.
27. Fimland MS, Helgerud J, Solstad GM, Iversen VM, Leivseth G, Hoff J. Neural adaptations underlying cross-education after unilateral strength training. Eur J Appl Physiol. 2009;107(6):723-30.
28. Aagaard P, Simonsen EB, Andersen JL, Magnusson P, Dyhre-Poulsen P. Neural adaptation to resistance training: changes in evoked V-wave and H-reflex responses. J Appl Physiol. 2002-06-01 00:00:00. 2309-18 p.
29. Upton AR, McComas AJ, Sica RE. Potentiation of "late" responses evoked in muscles during effort. Journal of neurology, neurosurgery, and psychiatry. 1971;34(6):699-711.
30. Duclay J, Martin A. Evoked H-reflex and V-wave responses during maximal isometric, concentric, and eccentric muscle contraction. J Neurophysiol. 2005;94(5):3555-62.
31. Zijdewind I, Zwarts MJ, Kernell D. Influence of a voluntary fatigue test on the contralateral homologous muscle in humans? Neurosci Lett. 1998;253(1):41-4.
32. Zijdewind I, Kernell D. Bilateral interactions during contractions of intrinsic hand muscles. J Neurophysiol. 2001;85(5):1907-13.
33. Hortobagyi T, Lambert NJ, Hill JP. Greater cross education following training with muscle lengthening than shortening. Med Sci Sport Exerc. 1997;29(1):107-12.
34. Doix AC, Lefevre F, Colson SS. Time course of the cross-over effect of fatigue on the contralateral muscle after unilateral exercise. PLoS One. 2013;8(5):e64910.
35. Zwambag DP, Brown SHM. The Effect of Contralateral Submaximal Contraction on the Development of Biceps Brachii Muscle Fatigue. Hum Factors. 2014.
36. Halperin I, Copithorne D, Behm DG. Unilateral isometric muscle fatigue decreases force production and activation of contralateral knee extensors but not elbow flexors. Appl Physiol Nutr Metab. 2014;39(12):1338-44.
37. Grabiner MD, Owings TM. Effects of eccentrically and concentrically induced unilateral fatigue on the involved and uninvolved limbs. J Electromyogr Kines. 1999;9(3):185-9.
38. Post M, Bayrak S, Kernell D, Zijdewind I. Contralateral muscle activity and fatigue in the human first dorsal interosseous muscle. J Appl Physiol (Bethesda, Md : 1985). 2008;105(1):70-82.
39. Martin PG, Rattey J. Central fatigue explains sex differences in muscle fatigue and contralateral cross-over effects of maximal contractions. Pflugers Arch. 2007;454(6):957-69.
40. Kawakami Y, Amemiya K, Kanehisa H, Ikegawa S, Fukunaga T. Fatigue responses of human triceps surae muscles during repetitive maximal isometric contractions. J Appl Physiol (Bethesda, Md : 1985). 2000;88(6):1969-75.

41. Baecke JA, Burema J, Frijters JE. A short questionnaire for the measurement of habitual physical activity in epidemiological studies. Am J Clin Nutr. 1982;36(5):936-42.
42. Simoneau EM, Billot M, Martin A, Van Hoecke J. Antagonist mechanical contribution to resultant maximal torque at the ankle joint in young and older men. J Electromyogr Kines. 2009;19(2):e123-31.
43. Billot M, Simoneau E, Van Hoecke J, Martin A. Coactivation at the ankle joint is not sufficient to estimate agonist and antagonist mechanical contribution. Muscle & nerve. 2010;41(4):511-8.
44. Hermens HJ, Freriks B, Disselhorst-Klug C, Rau G. Development of recommendations for SEMG sensors and sensor placement procedures. J Electromyogr Kines. 2000;10(5):361-74.
45. Rupp T, Girard O, Perrey S. Redetermination of the optimal stimulation intensity modifies resting H-reflex recovery after a sustained moderate-intensity muscle contraction. Muscle & nerve. 2010;41(5):642-50.
46. Strojnik V, Komi PV. Neuromuscular fatigue after maximal stretch-shortening cycle exercise. J Appl Physiol. 1998-01-01 00:00:00. 344-50 p.
47. Patikas DA, Bassa H, Kotzamanidis C. Changes in the reflex excitability during and after a sustained, low-intensity muscle contraction. Int J Sport Med. 2006;27(2):124-30.
48. Behm DG, St-Pierre DM. Effects of fatigue duration and muscle type on voluntary and evoked contractile properties. J Appl Physiol (Bethesda, Md : 1985). 1997;82(5):1654-61.
49. Neyroud D, Maffiuletti NA, Kayser B, Place N. Mechanisms of fatigue and task failure induced by sustained submaximal contractions. Med Sci Sport Exerc. 2012;44(7):1243-51.
50. Duchateau J, Hainaut K. Electrical and mechanical failures during sustained and intermittent contractions in humans. J Appl Physiol (Bethesda, Md : 1985). 1985;58(3):942-7.
51. Hortobagyi T, Taylor JL, Petersen NT, Russell G, Gandevia SC. Changes in segmental and motor cortical output with contralateral muscle contractions and altered sensory inputs in humans. J Neurophysiol. 2003;90(4):2451-9.
52. Gondin J, Duclay J, Martin A. Soleus- and gastrocnemii-evoked V-wave responses increase after neuromuscular electrical stimulation training. J Neurophysiol. 2006;95(6):3328-35.