The effect of ischaemic preconditioning on central motor output and contractile properties in healthy males following fatiguing isokinetic exercise

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Abstract

Ischaemic preconditioning (IPC) is a procedure in which tissue is exposed to brief, alternating periods of ischaemia and reperfusion. IPC has been shown to delay the development of fatigue, which is suggested to be caused by changes in central motor output; however, this remains speculative. We hypothesised that IPC would delay the development of fatigue by changing the central motor output, resulting in a smaller inhibition of corticospinal excitability, voluntary activation and contractile properties. Ten healthy males completed a fatiguing protocol consisting of 90 isokinetic contractions of the dorsiflexor muscles following treatment of either IPC (4x5 min 250 mmHg) or sham (4x5 min 20 mmHg) on two occasions separated by a minimum of seven days. Supramaximal stimulation of the common peroneal nerve was utilized to examine voluntary activation (VA) and potentiated twitch torque (PT) during maximal voluntary contractions at baseline, preceding the fatigue protocol (pre) and after the fatigue protocol (post). Transcranial magnetic stimulation was utilized to examine corticospinal excitability at baseline, pre and post. Tissue oxygenation using near-infrared spectroscopy and surface electromyographic activity was measured during the intervention.

Across conditions, the maximal torque was significantly decreased by 49.8% from baseline and 48.2% from pre compared to post ($p < 0.001$) but no difference was found between the conditions ($p = 0.981$). VA and PT showed no significant differences. Corticospinal excitability was decreased at post measurements compared to baseline (all intensities, p's ≤ 0.048) and pre (intensities 110-140% resting motor threshold, p's ≤ 0.02). The fatigue protocol was divided into 10 intervals as a mean power output of three contractions at each interval throughout the protocol. Power output during the fatigue protocol decreased from interval 1 to interval 6-10 (p's ≤ 0.04), from interval 2 to interval 3-10 (p's ≤ 0.026) and from interval 3 to interval 4 and 6-10 (p's ≤ 0.048). The oxygenation during the fatigue protocol showed no differences for oxygenated or deoxygenated haemoglobin between conditions or interval, but total haemoglobin was higher for IPC than sham ($p = 0.003$) and total haemoglobin rose during the fatigue protocol.

These findings indicate that IPC did not delay the development of fatigue nor alter central motor output. Thus, IPC may not have important implications for central motor output during fatiguing exercise.

Keywords: Tibialis anterior, TMS, NIRS, EMG, CSE.

Introduction

Ischaemic preconditioning (IPC) is a procedure in which tissue is exposed to brief, alternating periods of ischaemia and reperfusion. In 1989, it was discovered that IPC can cause a delay of lethal cell injury in cardiac muscle (Murry et al., 1989) and since then, IPC has proven to be a universal phenomenon which can protect various organs and tissues including the heart, liver, brain, kidney and skeletal muscles (Stokfisz et al., 2017). Recently, IPC has been introduced to performance contexts because the method has been shown to enhance vasodilation and improve metabolism (Pang et al., 1995; Pang et al., 1997). The underlying mechanisms are not yet uniformly understood, and the results from investigations of the effects of IPC on performance are conflicting.

IPC has mainly been investigated in whole-body exercise and in different types of exercise with various metabolic demands (Cocking et al., 2019). When investigating similar exercise modalities, contradictory results have been found. For example, IPC has been shown to enhance the time to complete a 5 km running trial where participants were 2.3% faster after IPC compared to sham (Bailey et al., 2012). The authors suggested that IPC had a protective effect against the acute decrease in endothelial function, which is normally seen after strenuous exercise. On the other hand, a similar study also investigated a 5 km time trial but did not find any effect of IPC on the time to completion (Tocco et al., 2014). There were some differences in the methods of the two studies, including the training status of the participants (highly trained vs. moderately trained) and location of the study (laboratory settings vs. the field). Indeed, these results are in opposition to one another, and the same conflicting results are present when investigating repeated cycling sprints (Patterson et al., 2015; Gibson et al., 2015). Increases in peak and mean power during the first three sprints after IPC have been found when participants performed 12 sprints of six seconds each (Patterson et al., 2015), while no effect of IPC was seen on peak and total power in a sprint session consisting of five sprints of six seconds (Gibson et al., 2015). These results suggest that across different exercise modalities, a consensus of the effect is yet to be established. At the same time, different mechanisms behind the possible effects of IPC has been investigated and discussed.

One of the mechanisms investigated in IPC is oxygen delivery to muscle tissue. Muscle tissue oxygenation and blood flow can be investigated using near-infrared spectroscopy (NIRS) (Yokoi et al., 2014). Following IPC, oxygenation of working muscles is increased, which was found to improve cycling sprint performance (Patterson et al., 2015). However, greater oxygenation of the working muscles following IPC is not beneficial for all performance, such as during a sustained maximal leg exercise performed with the quadriceps muscles (Halley et al., 2018). The fact that greater oxygenation did not improve performance was explained with the choice of exercise (Halley et al.,

2018). Isometric contractions can impede arterial blood flow to the muscle through sustained increases in intramuscular pressure (Halley et al., 2018). On the basis of this, the authors reproduced their study but this time with isokinetic maximal knee extensions. However, they did not find an enhanced oxygenation of working muscles nor enhanced performance following IPC (Halley et al., 2019). This is notable, and it seems that establishing a consensus on how IPC affects oxygenation of the muscles is difficult.

An area that has gained interest in the field of IPC is whether IPC can affect fatigue during performance. Fatigue results from a complex interaction between central and peripheral mechanisms (Gandevia, 2001). When fatigued, motor evoked potentials (MEPs) induced by transcranial magnetic stimulation (TMS) are depressed (Ross et al., 2007; Gruet et al., 2013). Furthermore, voluntary activation (VA) and potentiated twitch torque (PT) are both reduced (Ross et al., 2007). This suggests that fatigue is attributable to both a disturbance of contractile properties within the muscle and central motor output to the muscle.

Recent research has demonstrated that IPC can delay the development of fatigue and it was proposed that IPC might affect central motor output (Barbosa et al., 2014; Crisafulli et al., 2011; Paradis-Deschênes et al., 2016; Cruz et al., 2015). This proposal originates from studies that observed a higher surface electromyography (EMG) signal (Cruz et al., 2016) and attenuation in the reduction of rates of force development and relaxation times (Barbosa et al., 2014) during fatiguing exercise-to-failure. The increased time-to-task failure was suggested to be caused by the possibility that IPC could lower the sensitivity of the body to fatigue signals (Crisafulli et al., 2011).
During fatiguing exercise, motoneuronal excitability is inhibited which indicates that afferent muscle feedback exerts an inhibitory influence on the central motor output during fatigue (Weavil et al., 2016). It was suggested that IPC may shift the threshold, at which the system terminates exercise, by desensitising afferent muscle feedback and thereby increase central motor output and delay the development of fatigue (Crisafulli et al., 2011). However, this remains speculative and is yet to be investigated further.

If IPC influences central motor output and delays the development of fatigue (Cruz et al., 2015), central motor output could be investigated further by measuring corticospinal excitability, muscle contractility and VA. A change in corticospinal excitability or VA following IPC would indicate a change in the central motor output, whereas a change in PT of the muscle would indicate a change in the contractile properties of the muscle (Gandevia, 2001; Ross et al., 2007). The investigation of these would help gain a better understanding of the underlying mechanisms and loci following IPC.

Only a few studies have investigated the effect of IPC on an isolated muscle in combination with fatigue but no effect was found from IPC (Halley et al., 2018; Halley et al., 2019). When investigating full-body exercise many factors can affect the results such as pulmonary oxygen uptake, cardiovascular responses and blood metabolic accumulation (Tanaka et al., 2016). Using an isolated muscle group might be a good alternative for avoiding these confounding effects (Christian et al., 2014).

The aim of this study was to investigate the effect of IPC on the development of fatigue during repeated isokinetic contractions of the dorsiflexor muscles. Mechanisms of fatigue were investigated using measures of central motor output, maximal torque, and contractile properties of the tibialis anterior (TA) muscle. Oxygenation of the TA muscle was measured during the IPC protocol and fatigue protocol using NIRS.

Based on previous work and suggestions, we hypothesised that if IPC affects central motor output, it would result in a smaller inhibition of the corticospinal excitability, VA and contractile properties of the TA after the fatigue protocol. Furthermore, we hypothesised that if IPC had an effect, the participants would be able to produce more power, or delay the attenuation of power throughout the fatigue protocol following IPC compared to sham.

Methods

Participants

Ten healthy males participated in the study (mean ± SD: 25.8 ± 1.4 years, 182.7 ± 7.2 m, 87.2 ± 11.0 kg, 8.3±3.0 hours of physical activity per week). At the time of the study, all participants were non-smokers and free of any known physical or neurological disorders. Participants gave their written consent prior to participation. Approval for the study was given by the Scientific Ethics Committee for Nordjylland (Reference No. N-20170081)**.** Participants refrained from caffeine (12h), alcohol (24h) and strenuous exercise (48h) before the test days (Halley et al., 2018).

Experimental design

The participants attended to the laboratory on three occasions; two test days and a preliminary session where they were familiarised with the procedures used in the study. The experimental design for the two test days is illustrated in figure 1.

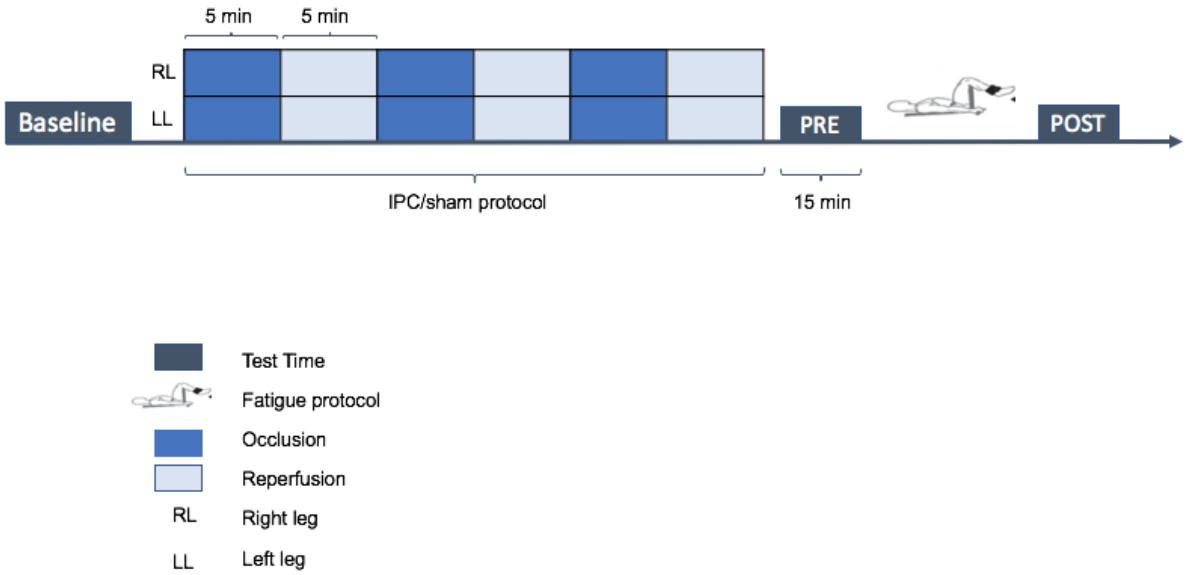

Figure 1: Experimental design

Familiarisation day

The familiarisation session started with two minutes of bilateral cuff occlusion on the upper thighs (see section: Ischaemic preconditioning). Then the participants were familiarised with electrical stimulation of the common peroneal nerve (see section: Peripheral nerve stimulation). The participants then performed maximal voluntary contractions (MVC) of the ankle dorsiflexors in a dynamometer with and without a supramaximal stimulation (125% M_{max}) (see section: Neuromuscular testing). The participants were familiarised with the supramaximal stimulation to ensure that they were not afraid of the stimulation which otherwise could affect the MVC outcome. To familiarise the participants to the fatigue protocol, they performed five dynamic contractions of the ankle dorsiflexors at 90 degrees/s (see section: Fatigue protocol). In addition, the participants were familiarised with the procedures of TMS (see section: Transcranial magnetic stimulation). Finally, the participants' weight and height were measured.

Test days

The study was a counterbalanced crossover study, where the participants attended two experimental sessions comprising two different interventions: IPC and sham. The two test days were separated with a minimum of seven days and were performed at the same time of the day (± 2 hours) to avoid any diurnal effects. From familiarisation day to the first test day there was a minimum of 48 hours. On both test days, the participants were prepared for testing with EMG electrodes, stimulation electrodes and NIRS (see figure 2A). Measurements were carried out before the intervention (baseline), after the IPC/sham protocol and prior to the fatigue protocol (pre) and immediately after

the fatigue protocol (post). At every time point, TMS measures were carried out before neuromuscular testing (MVC and peripheral nerve stimulation) except at post where TMS was performed after neuromuscular testing.

Dynamometer setup

Participants performed dynamic and isometric contractions with the ankle dorsiflexors. During the experiment, the participants were lying in a supine position in a dynamometer (Computer Sports Medicine Inc., Humac Norm testing and rehabilitation system, model 502140, USA) having their right foot placed in a footplate using velcro straps to secure the foot (see figure 2B). The participants' hip was flexed at a 120° angle (full hip extension = 180°) and the knee fixed in a 90° angle (full knee extension = 180°), which was maintained by a knee brace. The leg was positioned such that the axis of rotation of the ankle joint was aligned with that of the dynamometer. Straps with velcro were strapped around the thigh and hips to isolate the ankle joint and avoid unwanted movements. When positioned in the dynamometer, the participants were instructed to hold on the to the handles beside their hips. The anatomical zero (ankle joint flexed at a 90° angle) and the total range of motion of the ankle (maximal dorsiflexion to maximal plantarflexion) was measured in the dynamometer. The dynamometer was calibrated for each participant's limb weight to account for gravitational effects on torque. The fatigue protocol and all neuromuscular testing (except TMS) were performed in the dynamometer. When performing the TMS measurements, the participants were placed in a chair in an upright position.

Surface electromyography

Surface EMG activity was recorded with Ag/AgCl electrodes (20mm Neuroline 720; Ambu, Ballerup, Denmark) from the right TA muscle. The electrodes were used to assess corticospinal excitability, VA and the contractile properties. The EMG electrodes were placed on the skin on the lower part of the TA muscle. The ground electrode was placed on the skin on the bony surface on the anterior border of TA (see figure 2A). The skin was shaved and disinfected prior to applying the electrodes. Surface EMG was recorded with a sample rate of 2000 Hz, and amplified (x1000) and filtered (band-pass, 5 Hz to 1 kHz).

Figure 2: A) The placement of surface EMG electrodes, NIRS apparatus and stimulation electrodes. B) Experimental setup in the dynamometer. C) Cuff placement during IPC/sham protocol.

Transcranial magnetic stimulation

In order to measure changes in corticospinal excitability following the protocols, MEPs were recorded from the surface EMG of the TA muscle and were elicited with a MAGSTIM 200 Mono Pulse stimulator (Magstim Company Limited, Whitland, Wales, UK) through a figure-of-eight cone coil (110mm diameter). The optimal location for stimulation on the scalp was determined by delivering 30-50% maximal stimulation output through the coil at multiple locations in the general area of representation of the right TA muscle (Devanne et al., 1997). The location was marked with a permanent marker on the scalp to replicate the placement throughout the experiment. The resting motor threshold (RMT) was determined at the minimal intensity that induced at least five out of ten MEP's with an amplitude above 50 μV (Rossini et al., 2014). At all time points, a recruitment curve was collected with six stimuli delivered at each intensity of 90%, 100%, 110%, 120%, 130% and

140% of RMT. The order of the intensities was randomised (Möller et al., 2009). TA surface EMG was recorded from -150 ms to 300 ms relative to the TMS stimulation time at 0 ms. The size of the MEP was measured as the peak-to-peak MEP amplitude.

Peripheral nerve stimulation

Stimulation of the right CPN was applied using a NoxiTest isolated peripheral stimulator (IES 230; SMI, Aalborg, Denmark). The self-adhesive stimulation electrodes (DJO Brands, 32 mm, ref: 42205 Leadwire, USA) were placed on the skin overlying the deep branch of the right CPN with the cathode proximal (see figure 2A). The correct placement for the electrodes was where the largest M-wave in TA was observed. Palpation of soleus and peroneal muscles was performed during stimulation trials to ensure the absence of muscle contractions elicited by the stimulation. To establish the maximal M-wave (M_{max}), stimuli were delivered with interstimulus intervals of 15 seconds starting at 10 mA and progressively increased with increments of 5 mA until a plateau was seen in the peak-to-peak amplitude of the M-wave. The stimulation intensity for eliciting M_{max} was noted and multiplied with 125% to set the intensity for the supramaximal stimulation (Todd et al., 2004).

Neuromuscular testing

The participants performed isometric MVCs of the dorsiflexors, where some were coupled with supramaximal nerve stimulation (125% of M_{max}) of the CPN. The MVCs were performed in a 30° ankle angle (plantarflexed from anatomical zero). Torque and surface EMG were collected in a software program (Mr Kick III, Knud Larsen, Aalborg) with a sampling frequency of 2000 Hz.

The participants were instructed to press down with the heel and pull up with the toes during the MVC. Furthermore, the participants were instructed to perform the MVC as fast and forcefully as possible for 4 seconds and to keep pushing through the supramaximal stimulation. The supramaximal stimulation was applied during the MVC, approximately 1-1.5 seconds from onset, and 4-5 seconds post the MVC at rest (Merton et al., 1954). The supramaximal stimulation is used to investigate voluntary activation of the muscle (Merton et al., 1954).

Prior to the MVC, the participants performed six submaximal contractions of the dorsiflexors (two at 25%, two at 50% and two at 75% of the maximal torque) to warm up the muscles and get accustomed to the movement (Halley et al., 2019). Strong verbal encouragement was provided during all MVCs. For baseline measurements a total of four MVCs were performed; two with and two without supramaximal nerve stimulation, with the order randomized (Halley et al., 2019). This was done to ensure that the MVCs were similar despite the nerve stimulation. A coefficient of variation of 0.13% was recorded for baseline MVCs. In the pre measurements, two MVCs with supramaximal stimulation were performed and at the post measurement only one MVC was performed. Participants

rested one minute between MVCs (Frigon et al., 2007). The reason that only one MVC was performed at the post measurement was based on a pilot study where TA had time to recover before the second MVC and therefore the post measurement would be affected by the recovery time.

Near-infrared spectroscopy

Oxygenation of the right TA muscle was recorded with a NIRS apparatus which consisted of an Oxymon system with a probe attached (Mk III, Artinis Medical Systems, Elst, NL). The Oxymon consisted of three channels transmitting light in the NIRS. The channels transmitted light in two wavelengths (764 and 860 nm). A receiver optode was secured to the probe with a source-detector distance of 40 mm. Data were sampled at 2 Hz and the Oxymon system was calibrated before each session. NIRS data were collected for tissue oxyhaemoglobin (O2Hb), deoxyhaemoglobin (HHb) and total haemoglobin (tHb) equal to the sum of HHb and O2Hb and a surrogate indicator of changes in blood volume (Van Beekvelt, et al., 2001). The NIRS device was placed on the muscle belly of the TA and was fastened with tape to reduce movement of the apparatus and to avoid any light into the device (see figure 2A). Prior to applying the NIRS device, the skin was shaved and disinfected. The NIRS recording started after the baseline measurement and ran throughout the rest of the experiment. An oxygenation baseline measure was recorded two minutes prior the first occlusion.

Ischaemic preconditioning

The IPC and sham protocols were performed with the participants lying in a supine position with inflatable pressure cuffs (61 cm tourniquet cuff, VBM, Sulz am Necker, DE) attached around the upper thighs (see figure 2C). The cuffs were inflated with handheld manometers to a pressure of 250 mmHg (IPC) or 20 mmHg (sham) for four five-minute periods on both legs, each followed by a reperfusion period of five minutes (Paixao et al., 2014). The total time for both the IPC and sham protocol was 40 minutes. To avoid any placebo effect, the participants were notified that the purpose of the study was to investigate the effects of two different cuff pressures on fatigue.

Fatigue protocol

The fatigue protocol was performed in the dynamometer. The protocol consisted of 90 concentric MVCs at a nominal angular velocity of 90°/s. This protocol was chosen based on previous work (Lanza et al., 2004) and pilot testing. The participants were instructed to press down the heel and pull up with the toes during the MVC. Furthermore, the participants were instructed to perform the concentric dorsiflexor contractions as fast and forceful as possible and with the largest range of motion as possible throughout the dynamic protocol. In the eccentric phase they were instructed to

relaxe while the dynamometer returned the ankle joint to the start position. Verbal encouragement was provided during the protocol which lasted 2.98 ± 0.80 min.

Data analysis

All data were analysed offline.

Corticospinal excitability

For each participant, the mean MEP amplitudes of the six stimuli at each intensity of TMS were calculated for both the IPC and sham sessions at all time points. The amplitudes were normalised to the maximal amplitude of the MEP at the baseline measurement and the means of the normalised values were calculated across all participants.

Maximal torque during maximal voluntary contractions

The maximal torque was measured as the peak value during the MVC subtracted with the baseline immediately before the MVC. The baseline was measured as the mean of an interval of 100 ms immediately before the MVC onset.

Potentiated twitch

The PT was measured as the peak amplitude during the first 200 ms from stimulation onset of the PT subtracted with the baseline immediately before the stimulation. The baseline was a mean of 100 ms.

The velcro strap securing the foot in the footplate had loosened during the fatigue protocol and the dynamometer was unable to measure the PT after the protocol. Due to this complication, the PT at the post measurement was therefore excluded from the data analysis (see appendix 1).

Neuromuscular testing

VA was calculated from the following equation:

$VA = 100 - (d * (F_{sup} / MVC_{peak} / PT) * 100$, where d is the difference between the mean torque for 100 ms immediately before the stimulation (F_{sup}) and the peak torque in the 100 ms following the stimulation, MVC_{peak} is the peak torque from the beginning of the contraction to immediately before the stimulation and PT is the peak amplitude of PT (Strojnik & Komi, 1998).

Post measurement of VA was excluded from the analysis due to missing PT data. Therefore, only two timepoints (baseline and pre) were used for further analysis on VA.

Oxygenation

Due to technical errors, data from five participants were excluded from the analysis. Furthermore, only data from channel one was used for data analysis due to a noisy signal in channels two and three (see appendix 2).

During the two minutes oxygenation baseline, a mean value of the 30th-90th second was used for further analysis for each of the three oxygenation parameters.

The O2Hb, HHb and tHb values during the IPC/sham period were averaged across the four occlusion periods. The fatigue protocol was divided into ten intervals in order to analyse alterations in O2Hb, HHb and tHb throughout the protocol. The relative change from oxygenation baseline was used for analysis.

Power during the fatigue protocol

The mean across all concentric dorsiflexion contractions during the fatigue protocol was calculated. In addition, the data were divided into ten intervals during the protocol. Each interval was calculated as a mean of three contractions. The intervals were contractions 1-3, 9-11, 19-21, 29-31, 39-41, 49-51, 59-61, 69-71, 79-81 and 88-90.

Due to a technical error in data extraction, two participants' data were excluded from the analysis.

Statistical analysis

A three-way repeated measures analysis of variance (rmANOVA) was conducted with the MEP amplitude as the dependent variable and three independent variables; condition (IPC and sham), time (baseline, pre and post) and stimulation intensity (90%, 100%, 110%, 120%, 130% and 140% RMT).

Separate two-way rmANOVAs were conducted to analyse PT, MVC_{peak} and VA values, respectively. The dependent variable was the PT, MVC_{peak} or VA value and the independent variables were condition (IPC and sham) and time (baseline, pre and post).

To analyse the oxygenation during the IPC/sham protocol, the mean of the values during occlusion were compared to the oxygenation baseline value by three two-way rmANOVAs. The oxygenation parameter value was the dependent variable (O2Hb, HHb, tHb) and the independent values condition (IPC and sham) and time (baseline and occlusion). A test was conducted for each oxygenation parameter.

Three separate two-way rmANOVAs were conducted for each of the oxygenation parameters to analyse the oxygenation during the fatigue protocol. The oxygenation parameter value was the

dependent variable (O2Hb, HHb, tHb) and the independent variables were the condition (IPC and sham) and interval (1-10).

To analyse the power during the fatigue protocol, a two-way rmANOVA was conducted to analyse the intervals with the dependent variable being the power output and the independent variables being the condition (IPC and sham) and interval (1-10).

Mauchly's test of sphericity was used on all ANOVAs to determine whether the assumption of sphericity was violated and a Greenhouse-Geisser correction was used when this violation occurred. If any two- or three-way interactions were found, a simple main effects analysis was conducted. The Holm-Bonferroni post hoc test was applied to determine the locality of the significant differences (Holm, 1979). Differences with a probability of < 0.05 were considered significant.

Data are presented as mean ± standard deviation (SD).

Results

Normal distribution

According to the Shapiro Wilk's test, all data were normal distributed.

Maximal torque during maximal voluntary contractions

The two-way ANOVA did not show any interaction (condition*time) effect ($p = 0.595$). There was no main effect of condition ($p = 0.981$) (Table 1). The ANOVA revealed a main effect of time ($p < 0.001$). When applying Maucley's Test of Sphericity, the Greenhouse-Geisser correction showed a main effect of time ($F_{(1.135,\ 10.217)} = 121.4$, $p < 0.001$). At the post measurement (1942.3 ± 760.2 Nm), there was a significant decrease in MVC from both baseline (3866.9 ± 1014.7 Nm) and pre (3750.2 ± 958.7 Nm, *p's* < 0.001) witch correspond to a decreased by 49.8% and 48.2% from baseline and pre, respectively. There was no difference between baseline and pre ($p = 0.131$). (see figure 3).

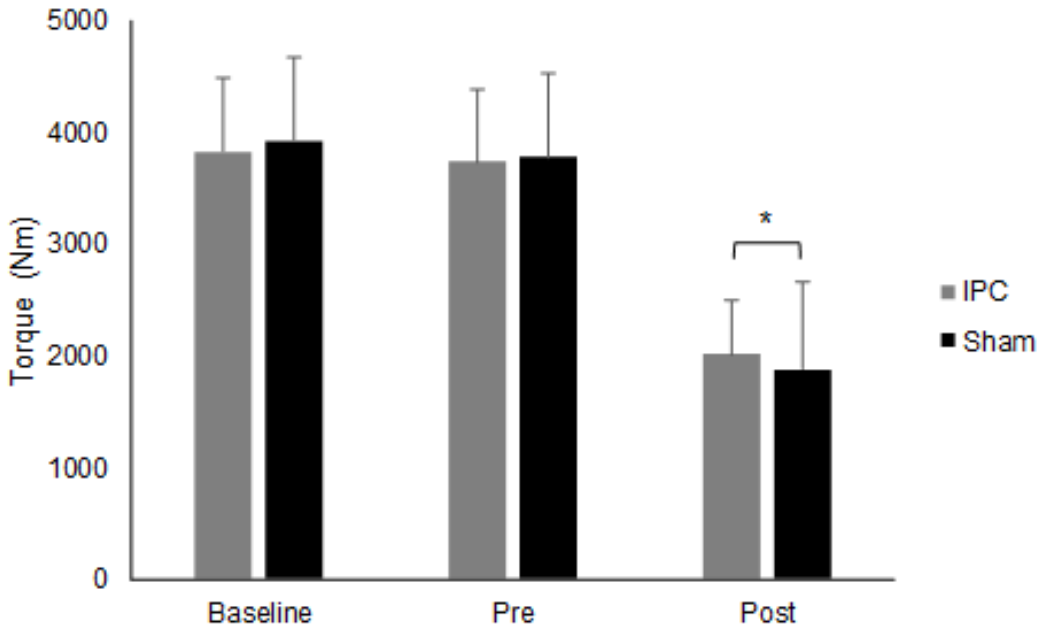

*Figure 3: Maximal torque during maximal voluntary contractions for each condition at baseline, pre and post. * represents a significant difference ($p < 0.05$) from baseline and pre measures. Data are presented as mean + SD.*

	MVC (Nm)		VA (%)		PT (Nm)	
	IPC	**Sham**	**IPC**	**Sham**	**IPC**	**Sham**
Baseline	3816.2 ± 687.3	3917.5 ± 768.0	99.3 ± 1.5	100.3 ± 3.1	995.6 ± 385.7	973.8 ± 407.4
Pre	3731.2 ± 640.8	3769.1 ± 762.8	100.4 ± 1.9	98.2 ± 4.0	828.5 ± 352.3	970.9 ± 398.8
Post	2007.7 ± 481.4*	1876.9 ± 774.6*				

*Table 1: Torque, VA and PT data at baseline, pre and post. * represents a significant difference ($p < 0.05$) from baseline and pre. Data are presented as mean ± SD.*

Neuromuscular testing

Voluntary activation

In measurements of VA, there were no condition*time interaction nor any main effects of time or condition (p's ≥ 0.053) (see table 1).

Potentiated twitch

There was no condition*time interaction nor any main effects of time or condition ($p \geq 0.081$) for PT (see table 1).

Corticospinal excitability

For the MEPs, the ANOVA showed no three-way interaction (p = 0.799). The ANOVA revealed a two-way interaction of time*intensity ($F_{(10, 90)}$ = 6.123, p < 0.001), but not for either condition*intensity nor condition*time (p = 0.675 and p = 0.692, respectively). No main effect was found for condition (p = 0.506). To further analyse the time*intensity interaction, we conducted a simple main effects analysis. There were no differences between baseline and pre (p's ≥ 0.366). The analysis showed that at all six intensities, the MEP amplitudes at the post measurement were decreased compared to baseline (p ≤ 0.048) and at intensity 110% RMT (p = 0.004), 120% RMT (p = 0.02), 130% RMT (p = 0.009) and 140% RMT (p = 0.003) the MEP amplitudes were also decreased at post compared to pre measurement (see figure 4) (See appendix 3 for raw data from one participant).

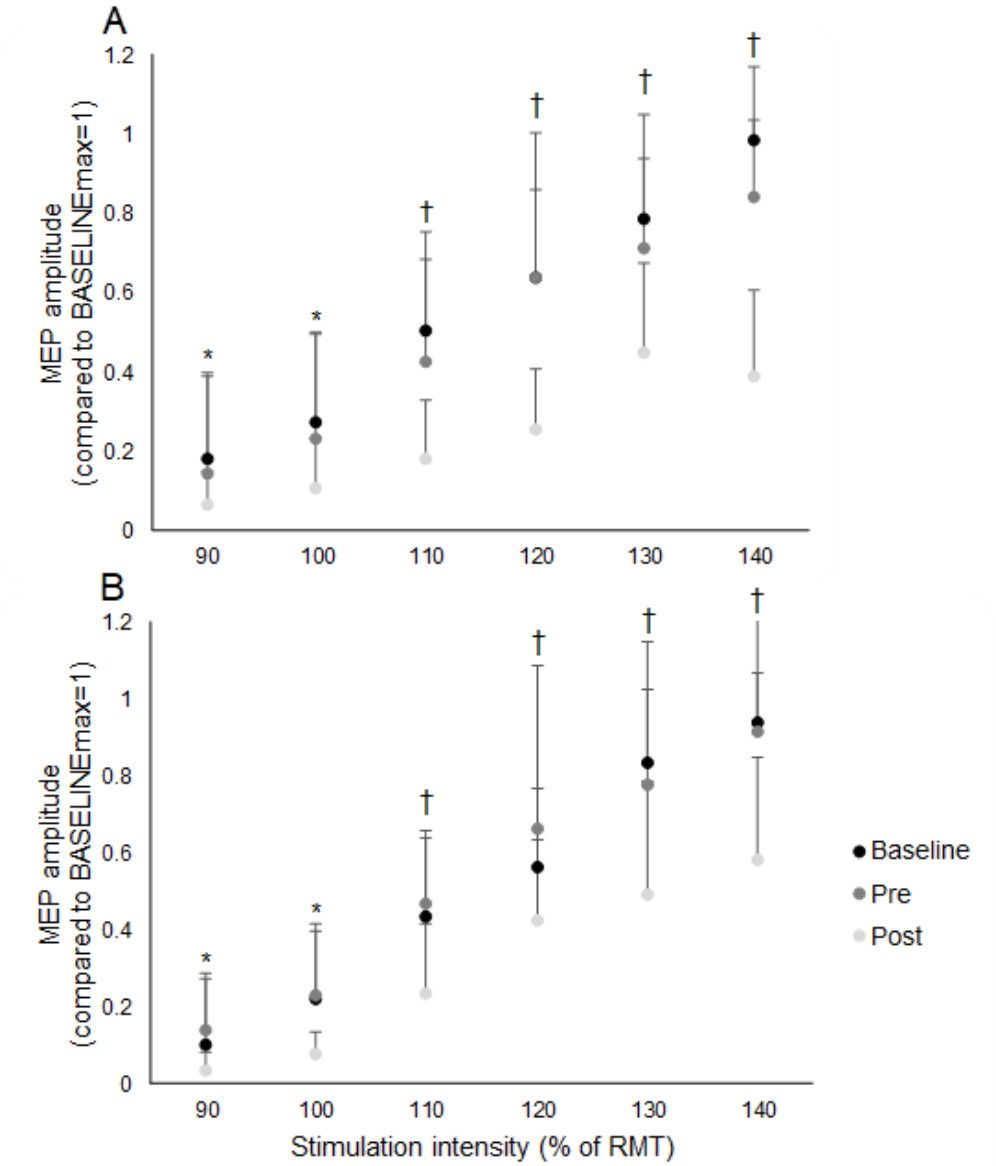

*Figure 4: A) MEP amplitudes for the IPC condition at each stimulation intensity are presented at baseline, pre and post. B) MEP amplitudes for the sham condition at each stimulation intensity are presented at baseline, pre and post. * represents a significant difference ($p < 0.05$) from baseline to post measures. † represents a significant difference ($p < 0.05$) from baseline and pre to post. Data are presented as mean + SD.*

During the fatigue protocol

Power

In the two-way ANOVA, no two-way interaction (condition*interval) was found ($p = 0.993$) and no main effect of condition ($p = 0.839$). The ANOVA showed a main effect of interval ($F_{(9, 63)} = 27.508$, $p < 0.001$) and the post hoc analysis revealed that the mean power in interval 1 was higher than interval 6-10 (p's ≤ 0.04). Interval 2 was higher than interval 3-10 (p's ≤ 0.026). Interval 3 was higher than

interval 4 and 6-10 (p's ≤ 0.048). There were no significant differences between the rest of the intervals (see figure 5).

Oxygenation

During the fatigue protocol, the two-way ANOVA showed no interaction nor main effects in either HHb (p's ≥ 0.178) or O2Hb (p ≥ 0.086) (see figure 5). For the tHb, there were no interaction effects (p=0.107), but there was a main effect of condition ($F_{(1, 4)}$ = 40.707, p = 0.003), where the tHb during the sham condition (-12.1 ± 4.2 AU) was smaller than during the IPC condition (5.0 ± 4.2 AU). The ANOVA also showed a main effect of interval ($F_{(9, 36)}$ = 8.089, p < 0.001). The post hoc test revealed that the tHb was lower in interval 2 than in interval 5 and 8 (p = 0.007 and p = 0.049, respectively) (see figure 5) (see appendix 4 for raw data).

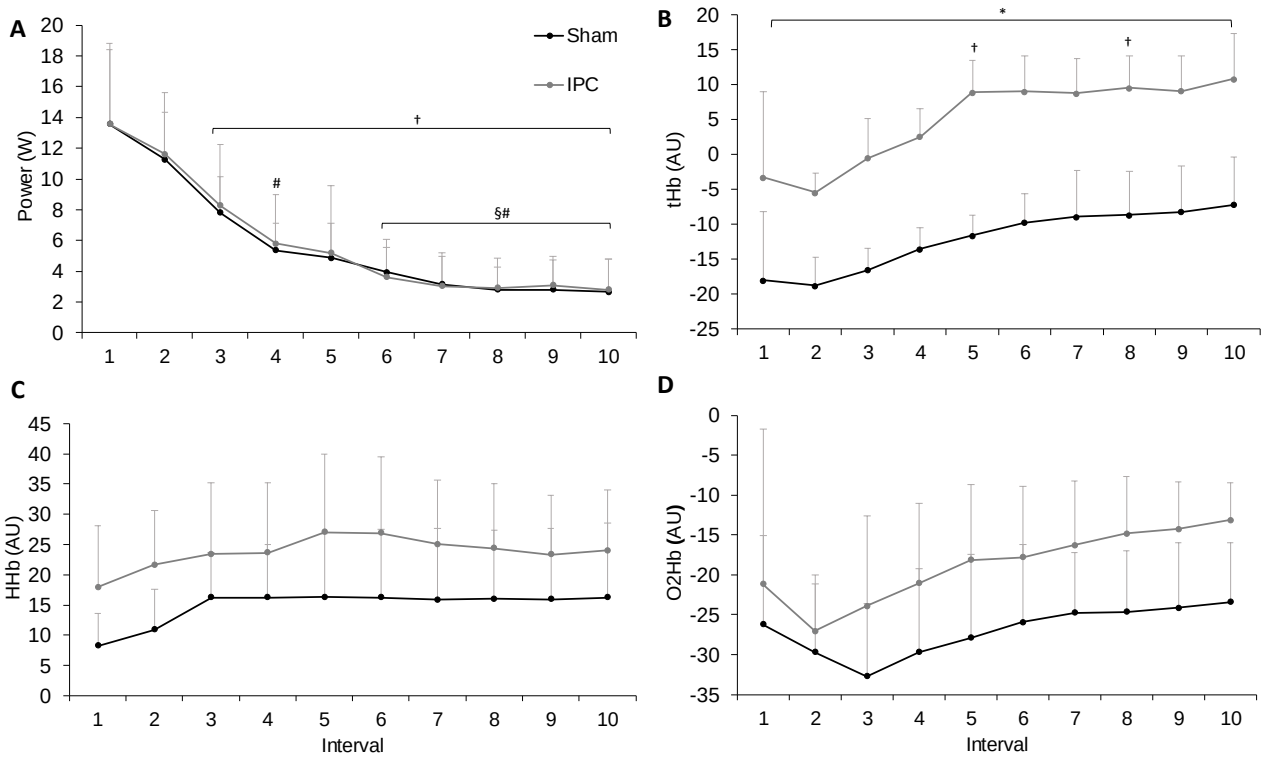

*Figure 5: A) Power during the fatigue protocol for each condition throughout the ten intervals. B) tHb values for each condition throughout the ten intervals. C) HHb values for each condition throughout the ten intervals. D) O2Hb values for each condition throughout the ten intervals. * represents a significant difference (p < 0.05) between conditions. § represents a significant difference from interval 1. † represents a significant difference from interval 2. # represents a significant difference from interval 3. Data are presented as mean + SD.*

Oxygenation during the IPC/sham protocol

During the 5 minutes occlusion periods in the IPC protocol, the two-way ANOVA of O2Hb showed a significant interaction (condition*time) ($F_{(1, 4)}$ = 17.371, p = 0.014). Post hoc analysis showed that O2Hb decreased by 13.8 ± 7.1 AU from baseline during the IPC condition (p = 0.018) and no differences in the sham condition (p = 0.543). The two-way ANOVA for HHb showed a two-way interaction for condition*time ($F_{(1, 4)}$ = 23.704, p = 0.008). Post hoc analysis showed that HHb increased by 22.5 ± 9.8 AU from baseline (p = 0.010) during the 5 minutes occlusion in the IPC protocol and decreased by 1.9±1.3 AU from baseline (p = 0.046) during the 5 minues periods in the sham protocol. Furthermore, the two-way ANOVA for tHb also showed a condition*time interaction ($F_{(1, 4)}$ = 15.493, p = 0.017). The post hoc analysis revealed that tHb increased by 8.7±5.0 AU (p = 0.025) for the IPC protocol and no changes in the sham protocol (p = 0.343) (see appendix 4 for raw data).

Discussion

The purpose of this study was to investigate the effect of IPC on the development of fatigue during repeated maximal isokinetic contractions of the dorsiflexor muscles. Based on previous work and suggestions (Barbosa et al., 2016; Crisafulli et al., 2011; Cruz et al., 2016), we hypothesised that if IPC delay the development of fatigue by changes in central motor output, it would result in a smaller inhibition of corticospinal excitability, VA and contractile properties of the TA after the fatigue protocol. Furthermore, we hypothesised that if IPC had an effect on fatigue, the participants would be able to produce more power, or delay the attenuation of power throughout the fatigue protocol following IPC compared to sham. Our findings showed that IPC did not affect corticospinal excitability or power output during the fatigue protocol. We were not able to analyse the VA or contractility properties of TA after the fatigue protocol due to equipment complications. However, speculations of what might have occurred are discussed below.

Fatigue

The participants' maximal torque was drastically decreased after the fatigue protocol, which is in accordance with previous studies who have used a similar fatigue protocol (Lanza et al., 2004). No difference in maximal torque between conditions were present, which suggests that the groups were equally fatigued after the fatigue protocol. This is in alignment with another study investigating the effect of IPC on fatigue after a dynamic isokinetic fatigue protocol performed with the quadriceps muscles, where IPC did not have any effect on the development of fatigue (Halley et al., 2019).

In the present study, fatigue is inherent in the changes in corticospinal excitability since the amplitudes of the MEPs were decreased from baseline and pre to post measurements for both conditions. This is in accordance with other studies investigating changes in corticospinal excitability after fatiguing exercise (Ross et al., 2007; Gruet et al., 2013). The authors speculate that the changes in corticospinal excitability results from the descending drive not contributing maximally and that the descending drive was insufficient to activate the motoneuronal pool optimally (Ross et al., 2007). One explanation was changes in the properties of corticospinal neurons or input to corticospinal neurons that might reduce descending output from the motor cortex. Another explanation was that the efficacy of output from the motor cortex to produce force was reduced by less responsive motoneurons or changes in muscle contractile properties (Ross et al., 2007).

IPC can delay the development of fatigue during all out exercise, which is suggested to be caused by changes in central motor output (Barbosa et al., 2015; Crisafulli et al., 2011; Paradis-Deschênes et al., 2016; Cruz et al., 2015). These studies reported a higher surface EMG during cycling sprints (Cruz et al., 2015) and a smaller decrease in rate of force development during a fatiguing handgrip exercise (Barbosa et al., 2015). Since higher surface EMG and changes in rate of force development can be can be an expression of changes in the central motor output (Barbosa et al., 2015; Cruz et l., 2015), we expected an alteration in central motor output from IPC compared to sham, where the corticospinal excitability would be less inhibited after the fatigue protocol. However, there was no difference in corticospinal excitability between the two conditions, which suggests that IPC did not affect the central motor output. Thus, the ability to activate the muscle through the corticospinal pathway seems to be independent of condition.

To further investigate central motor output, measurements of VA were carried out. However, since there were no post measurements included in the analysis, it was not possible to examine the effect of IPC on this parameter. We expected a smaller decrease in VA following the IPC protocol compared to sham, because IPC is further suggested to desensitise afferent muscle feedback and delay the development of fatigue (Crisafulli et al., 2011). But as discovered with corticospinal excitability, there might not have been any difference between conditions for VA either.

We had no PT results at the post measurement either. Halley et al. (2019) also investigated the effect of IPC on a dynamic fatigue protocol and found no differences between conditions in the measures of VA and PT at any time points. They did not see any changes in the VA throughout the experiment. However, PT was decreased after the dynamic fatigue protocol (Halley et al., 2019). This indicates that the fatigue was peripheral of origin. Because of the similarities between the present study and Halley et al. (2019), it is likely that the fatigue would develop in a similar way and a decrease in PT at the post measurement would have been discovered. This would further indicate

that the changes from fatigue occurs at the contractile level of the muscle. However, due to missing post measurements this is only speculations.

Power during the fatigue protocol

In the present study we found no effect of IPC on the power output throughout the fatigue protocol. This is in opposition to findings from cycling sprints, where IPC increased mean power during the first three sprints lasting 6 seconds each (Patterson et al., 2015). The present study distinguishes itself from the one by Patterson et al. (2015) by being a different exercise modality. The cycling sprints require several muscle groups, whereas the present study focused on the dorsiflexor muscles. The authors speculated that the increase in mean power during the first three sprints might result from increased ATP production from anaerobic sources, increasing the energy contribution in the early stage of the protocol (Patterson et al., 2015). If this is the case, the power throughout the fatigue protocol in the present study should show similar results, with increased power in the early intervals during the fatigue protocol following IPC because the dynamic contractions rely to a great extent on ATP contribution produced from anaerobic sources. However, no change of power was discovered and the effect of IPC on cycling sprints might result from other factors which apply to whole-body exercise, such as pulmonary oxygen uptake, cardiovascular responses, and blood metabolic accumulation (Tanaka et al., 2016). Furthermore, it is suggested that IPC has no effect on exercise under a certain duration threshold of approximately five seconds (Gibson et al., 2015). Even though the fatigue protocol in the present study lasted approximately three minutes, a single dynamic contraction lasted only one second during the protocol and might therefore not exceed the duration threshold. This might explain why we did not see any effect of IPC in the power output.

Oxygenation during the fatigue protocol

During the fatigue protocol, a difference between conditions was found for tHb. Although tHb only represents the tissue oxygenation level, these results may indicate that IPC mediated increases in blood flow during the fatigue protocol (Lucero et al., 2018). Since an increased blood flow is needed to remove the metabolites contributing to fatigue, which is accumulated in muscle tissue during fatiguing exercise, higher blood flow should enhance recovery from metabolic acidosis; that is to say reduce muscle fatigue (Yuka Yokoi et al., 2013). Despite greater tHb during the fatigue protocol, the IPC group was not able to delay the development of fatigue or delay the attenuation of power throughout the fatigue protocol compared to sham.

Furthermore, the increase in oxygen could result in a reduced muscle and blood lactate accumulation because of a decreased lactate production, which suggests that a greater amount of the ATP consumption would be covered by aerobic processes (Stellingwerff et al., 2005). Thus, we would

expect an increased oxygen utilization, represented by an increase in HHb. However, there was no increase in HHb and thereby no enhanced oxygen utilization for the IPC group and the possibility for removal of metabolites contributing to fatigue in TA during the fatigue protocol was the same for both conditions.

As mentioned previously, literature on how IPC affects oxygenation to working muscles has contradictory results in terms of whether IPC causes an increased oxygenation to the working muscles (Halley et al., 2019; Barbosa et al., 2016; Patterson et al., 2015) and/or whether the muscles are capable of using the increased oxygenation during exercise (Halley et al., 2018; Barbosa et al., 2016). The fact that a greater oxygenation did not affect a sustained maximal leg exercise was explained with the choice of exercise and the fact that isometric contractions can impede arterial blood flow to the muscle through sustained increases in intramuscular pressure (Halley et al., 2018). However, trying to reproduce the study with an isokinetic leg exercise did not show any increased oxygenation (Halley et al., 2019). The results in the present study could be an indication that an isokinetic exercise will not result in a greater utilization of oxygen, as Halley et al. (2018) proposed.
A closer look at the results from IPC found during an all-out dynamic handgrip exercise shows that exercise was prolonged for an IPC group compared to sham and, furthermore, that deoxygenation levels were higher after the end of exercise for the IPC group (Barbosa et al., 2016). The authors suggested that the greater deoxygenation indicates that oxygen uptake was proportionally larger than oxygen delivery; in other words, a greater oxygen extraction had occurred due to IPC (Barbosa et al., 2016). However, the authors still cannot exclude that the higher deoxygenation levels could be merely a consequence of the longer time to task failure and they suggest further investigation (Barbosa et al., 2016). In the present study, we did not see a greater deoxygenation during the fatigue protocol for the IPC group or a delay in the development of fatigue. Despite the different exercise modality, our results could suggest that the increased deoxygenation during the all-out handgrip exercise was due to the increased time to task failure, and not the fact that IPC had caused greater oxygen extraction.

Methodological considerations and limitations

IPC

The IPC protocol used in the present study was a typical 4*5 minutes of bilateral occlusion with five minutes of reperfusion in between. The purpose of the occlusion was to restrict blood flow to the lower legs. There was a significant lower level of O2Hb during the five minutes of occlusion in the IPC protocol compared to the corresponding five minutes of the sham protocol. This indicates that

the pursued effect of the occlusion occurred. The fact that IPC did not have an effect can thus not be a result of flaws in the IPC protocol.

Post measurements

The velcro strap securing the foot in the footplate had loosened during the fatigue protocol and the dynamometer was unable to measure the PT after the protocol (see appendix 1). Therefore, the post measurements for VA and PT were excluded from the study. This affects the validity of the present study as we were unable to investigate the contractile properties and VA following fatigue and can therefore only speculate on the possible outcomes.

Oxygenation

The NIRS apparatus is easily affected by light and it is of large importance that there are no gaps between the skin and the device. However, due to the shape of the TA muscle it was challenging to avoid external light, which was probably an explanation to why the signal on two out of three channels were disrupted by noise (see appendix 2). Using values from only one channel can affect the validity of the data, therefore a mean of three channels would have been more optimal.

Furthermore, there were issues in the data files resulting in the discarding of five participants' data in the analysis. The results in O2Hb and HHb might have been affected by the number of participants, since the graphs in figure 5C and 5D could indicate a difference between conditions and over time. If all ten participants' data were included the power of the statistical analysis would have been higher and we might have been able to see differences in the O2Hb and HHb as with the tHb. This would further support the validity of the oxygenation data.

Isolated muscle contractions

The choice of isolated muscle contractions was based on the fact that when investigating full-body exercise, many factors can affect the results (Tanaka et al., 2016). Using an isolated muscle group is therefore a good alternative for avoiding these confounding effects (Christian et al., 2014). Investigate the TA muscle was chosen because a lower intensity of stimulation is necessary to fully activate the TA muscle, compared to for example quadriceps, since the peripheral stimulator equipment was maximally able to deliver stimulation intensities of 100 mA.

Fatigue in the TA muscle may not be a limiting factor in a general performance context. However, investigating fatigue in the TA is seen in a multitude of studies (Lanza et al., 2004; Ross et al., 2007; Reid et al., 1993). Therefore, investigating whether IPC would have an effect on central motor output and muscle contractility in the TA after a fatiguing exercise could provide necessary knowledge to further research on the effects of IPC and was therefore chosen for the present study.

Conclusion

Our findings indicate that IPC does not delay the development of fatigue nor alter central motor output after fatiguing exercise despite a greater tHb following IPC. Our results provide novel information indicating that IPC does not alter corticospinal excitability.

The present study had some limitations due to the missing post measurements and further research involving the central motor output is therefore necessary to elucidate the effect of IPC and the mechanisms behind it.

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Appendix

Appendix 1: Torque and EMG data from a MVC with superimposed stim and a resting stim from one participant at baseline and post.

Appendix 2: Raw O2Hb data from the three channels throughout the experiment from one participant.

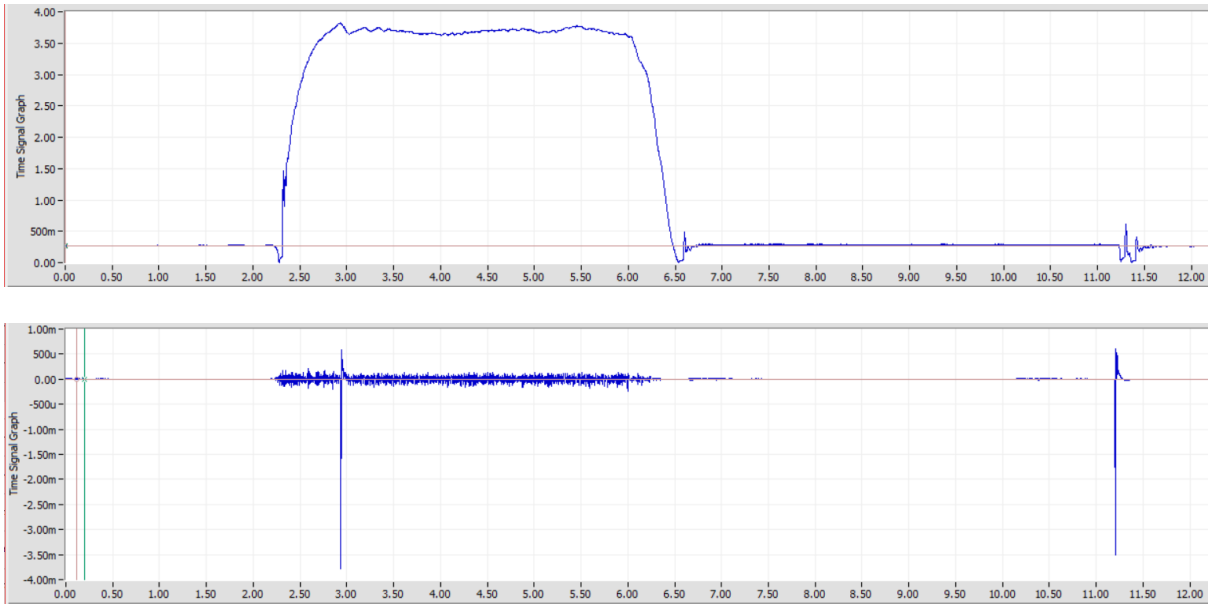
Appendix 3: Raw MEP data from one participant for the IPC and the sham protocol respectively.

Appendix 4: O2Hb, HHb and tHb throughout the experiment from one participant for the IPC and the sham protocol, respectively.

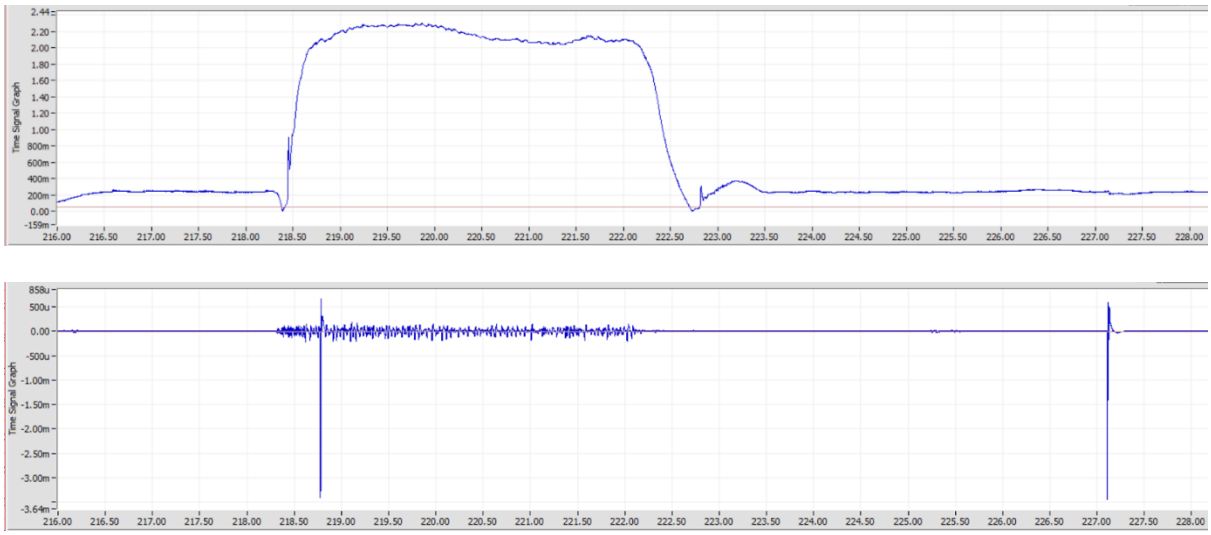
Appendix 1

Raw time*force graph (top) and time*EMG amplitude graph (bottom) of a MVC with superimposed stimulation and resting stimulation for a baseline trial (A) and a post test (B). The large artifacts in the EMG signal correspond to the time of stimulation.

A



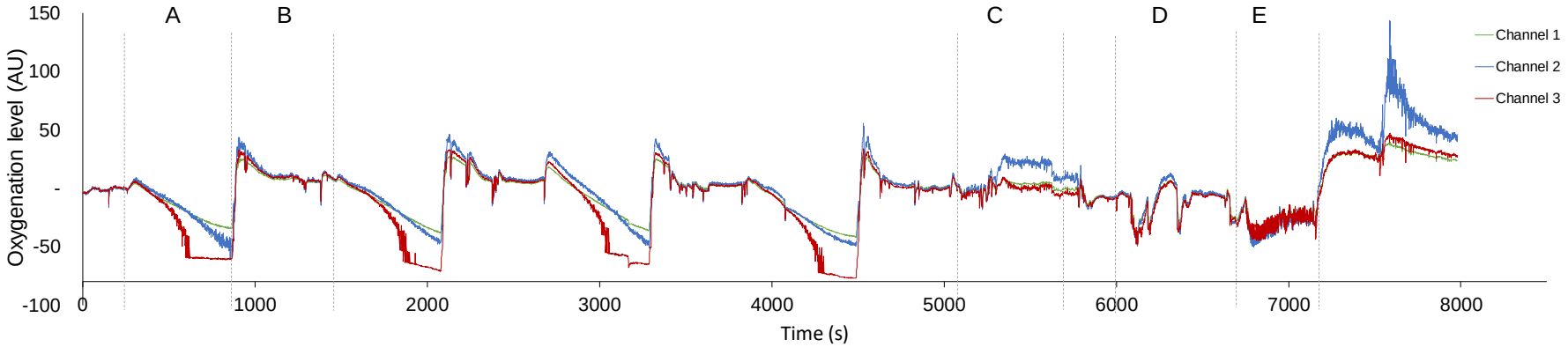
B



Appendix 2

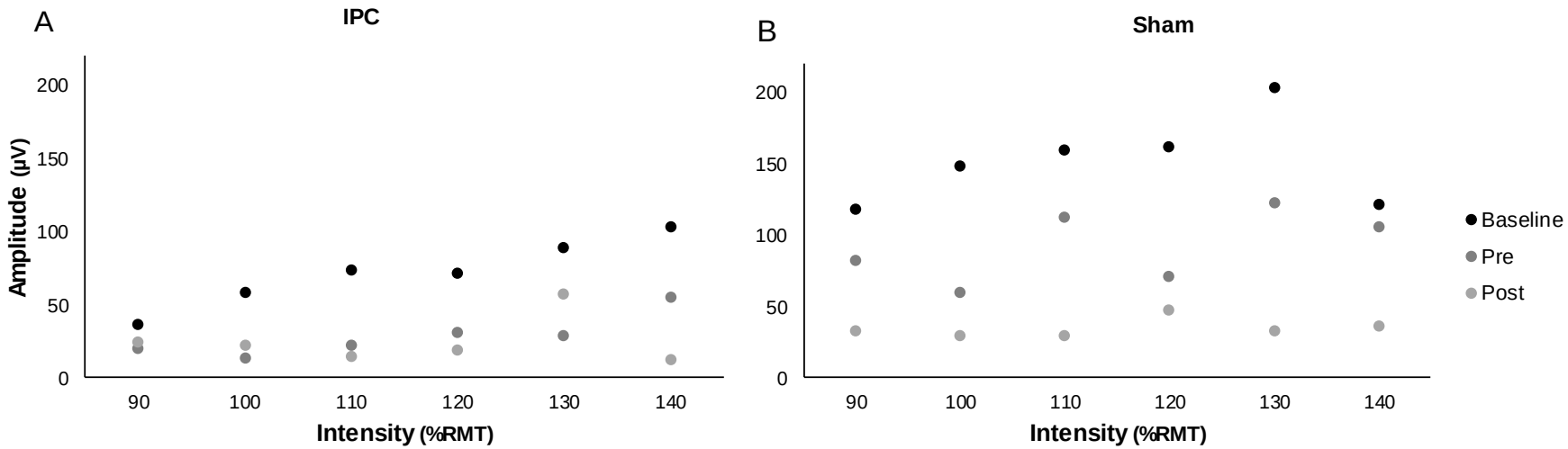
O2Hb data from the three channels from the NIRS device.

A: occlusion B: reperfusion C: TMS D: MVC E: fatigue protocol.



Appendix 3

MEP recruitment curve for IPC (A) and sham (B) at baseline, pre and post at the 6 intensities (90, 100, 110, 120, 130 and 140%)



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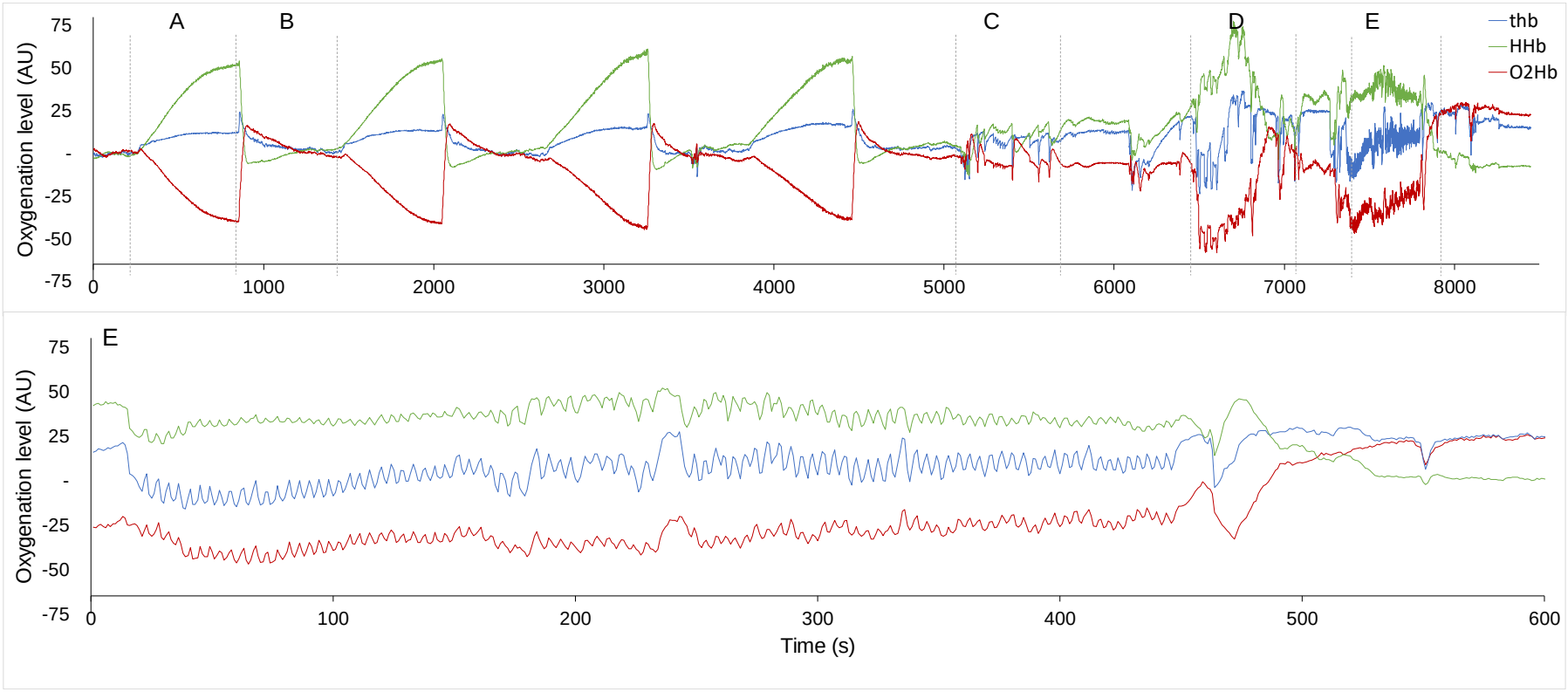
Appendix 4

O2Hb, HHb and tHb data throughout the experiment for the IPC (I) and the sham (II) protocol.

I)

A: IPC occlusion B: reperfusion C: TMS D: MVC E: fatigue protocol



II)

A: Sham occlusion B: reperfusion C: TMS D: MVC E: fatigue protocol