Abstract:

Purpose: Prolonged durations of ischemia followed by reperfusion are known to cause irreversible injury to tissues and organs. The damage by ischemia/reperfusion (I/R) is characterized by proinflammatory responses as well as increases in intracellular Ca^{2+} and reactive oxygen species (ROS), which cause damage to the cell membrane, which may lead to cell death. Ischemic preconditioning (IPC) is known to reduce infarct size following prolonged durations of ischemia followed by reperfusion. Damages caused by I/R have similarities to exercise-induced muscle damage (EIMD) since both are characterized by inflammation as well as increases in intracellular Ca^{2+} and ROS Therefore, the purpose of this study was to investigate whether IPC reduces known markers of EIMD following eccentric exercise.

Methods: Eleven healthy volunteers were stratified and randomized into two conditions (IPC and sham) in a crossover-design. Participants received an IPC (220 mmHg) or sham (20 mmHg) condition, consisting of 4x5 minute cuff inflations. The EIMD-protocol, consisting of 5 x 10 controlled eccentric contractions of the tibialis anterior (TA) muscle performed on a custom-built platform, was executed immediately following IPC/sham procedures. Muscle stiffness (using shear wave elastography), muscle thickness (using B-mode ultrasound), muscle strength (using maximal voluntary contraction test) and perceived pain (using numeric rating scale) were measured prior to IPC/sham procedures, immediately post-exercise as well as 24 and 48 hours post-exercise. Perceived pain was additionally assessed 72, 96, 120 and 144 hours post-exercise.

Results: Perceived pain increased significantly between trials for both conditions, compared to baseline. Perceived pain did not significantly differ between the conditions ($P > 0.05$). Both conditions increased in perceived pain until 48 hours post-exercise ($P = 0.005$). However, only the sham condition increased perceived pain 72 hours post-exercise ($P = 0.007$). Muscle thickness increased significantly between trials 24 hours ($P = 0.041$) and 48 hours ($P = 0.036$) post exercise. However, muscle thickness did not differ between conditions ($P = 0.734$) and had no significant interaction ($P = 0.851$). No significant changes in muscle stiffness (P-values ≥ 0.471) and muscle strength (P-values ≥ 0.15) were found.

Conclusion: The present findings indicate that IPC may reduce perceived pain following eccentric exercise of tibialis anterior (TA). No significant effects were observed in muscle stiffness, muscle swelling and muscle strength.

Ischemic preconditioning attenuates pain perception following eccentric exercise - a crossover-study

BENTZON, SABRINA MOSEGAARD; HANSEN, CASPER ØSTERGAARD; JOKUMSEN, PETER SØRENSEN & TER BEEK, FRANK.

Department of Health Science and Technology, Aalborg University, Denmark.

Introduction

It is well established that eccentric resistance training can provide increases in muscle mass and strength as well as offer eccentric specific adaptations, such as changes in neural activation patterns (Roig et al. 2008; Seger et al. 1998). Furthermore, eccentric resistance training provides superior results in overall strength and muscle mass gains compared to concentric resistance training (Roig, et al., 2008). However, eccentric contractions have been shown to cause greater amounts of exercise-induced muscle damage (EIMD) compared to concentric muscle contractions (Clarkson & Hubal, 2002), especially when unaccustomed (Nosaka & Newton, 2002). EIMD is ascribed to mechanical disruptions in the muscle fibers (Clarkson & Hubal, 2002), which are followed by inflammatory responses (MacIntyre et al., 1995). Beyond this, it has been found in EIMD studies using ultrasound elastography that muscle stiffness increases following eccentric resistance training (Niitsu et al., 2011). Furthermore, EIMD is accompanied by muscle soreness, muscle swelling and decreases in strength (Clarkson & Hubal, 2002; Proske & Morgan, 2001).

Muscle stiffness has been shown to increase immediately post-exercise and remain increased four days post-exercise (Howell et al., 1985). Increases in muscle stiffness following eccentric exercise are also supported by the literature assessing EIMD by ultrasound (Niitsu, et al., 2011). It has been argued that muscle stiffness caused by EIMD may be related to intracellular increases in Ca^{2+} (Howell et al., 1985; Proske & Morgan, 2001).

Following eccentric resistance training, an inflammatory response arises with the purpose of clearing the muscle of metabolic by-products in order to prepare for regeneration (Clarkson & Hubal, 2002). This process is accompanied by an influx of fluid and plasma proteins into cells, hereby inducing cell swelling and increases in muscle size (Clarkson & Hubal, 2002). Muscle swelling has been shown to occur gradually after eccentric exercise and peak four days post-exercise (Chleboun et al., 1998).

Furthermore, EIMD is usually accompanied with decreases in muscle strength (Sayers & Clarkson, 2001). This reduction in muscle strength may last 1-2 weeks with the greatest decrease 1-24 hours post-exercise (Clarkson & Hubal, 2002). The decrease in muscle strength is likely to be caused by the initial mechanical disruptions and subsequent events of inflammation and altered homeostasis, such as increased intracellular levels of Ca^{2+} (Sayers & Clarkson, 2001).

Moreover, EIMD is accompanied by delayed onset muscle soreness (DOMS), which typically lasts for 5-7 days while peaking 24-48 hours post-exercise (Clarkson & Hubal, 2002). Theories suggest that inflammation and edema may sensitize type III and IV afferent nerve endings, increasing perception of pain (Cheung et al., 2003). Furthermore, intracellular Ca^{2+} accumulation may inhibit mitochondrial adenosine triphosphate (ATP) regeneration, which inhibits ATP dependent transport of Ca^{2+} out of the cell (Cheung et al., 2003).

Therefore, bouts of eccentric resistance training may result in increased recovery time compared to concentric bouts (Newham et al, 1983), which may negatively affect performance and adherence to training (Franz et al., 2017a). Thus, several studies have investigated the effects of different methods of reducing the amount of EIMD following eccentric exercise (Chen et al. 2012; Sousa et al.,

2014). Evidence was found that ischemic preconditioning (IPC) reduced EIMD following a bout of eccentric resistance training of the elbow flexors (Franz et al., 2017b). This was determined by measuring creatine kinase (CK) levels, muscle contractile function, DOMS and muscle swelling, showing significant results for DOMS, muscle contractile function and CK levels for IPC compared to control.

IPC has shown to be useful in both medical studies and exercise studies (Incognito et al. 2016; Hausenloy & Lim, 2012). For instance, IPC has been shown to decrease infarct size in the rabbit's heart by 75% following 40 minutes of ischemia (Murry et al., 1986). Likewise, it was later found that IPC can protect skeletal muscles from ischemia/reperfusion injury (I/R injury) locally (Aktaş et al, 2015) and remotely (Addison et al., 2003). It is believed that this protective effect arises due to pro-inflammatory responses (Sullivan et al., 2009), a reduction in the development of metabolic stress (Van et al., 2008), as well as a reduction in Ca^{2+} accumulation and reactive oxygen species (ROS) production (Franz, et al., 2017a).

It is suggested that the inflammation and stress caused by I/R injury is similar to that caused by EIMD (Franz, et al., 2017a). Therefore, it was hypothesized that IPC would protect the skeletal muscles, not from the mechanical disruptions of eccentric contractions, but rather from the subsequent inflammation and homeostatic imbalance (Franz, et al., 2017a). Similarities in responses post I/R and post EIMD are also found, as in both cases, nuclear factor κB (NFKB) is activated with consequential increases in proinflammatory cytokines and chemokines (Memtsoudis et al., 2010; Xin et al, 2013). This, in turn, will cause further inflammation in the muscle (Hughes et al., 2007; Paulsen et al., 2012).

Based on the similarities between I/R-injury and EIMD presented in the introduction, we believe that IPC may reduce several indicators of EIMD, such as muscle stiffness, muscle swelling, pain and muscle strength. IPC has been proven to reduce the intracellular accumulation of Ca^{2+}, which is believed to increase muscle stiffness (Howell, et al., 1985), soreness (Cheung, et al., 2003), and reduce maximal force production (Sayers & Clarkson, 2001). Furthermore, IPC may reduce the activity of NFKB, thereby reducing inflammation, which is believed to reduce maximal force production (Sayers & Clarkson, 2001), muscle swelling (Clarkson & Hubal, 2002) and DOMS (Cheung, et al., 2003).

Therefore, the purpose of this study was to investigate the effects of IPC on EIMD in the tibialis anterior (TA) on parameters of muscle stiffness and muscle thickness using ultrasound, maximal force production using a maximal voluntary contraction (MVC) test and pain using a numeric rating scale (NRS).

We expect that IPC will reduce the occurrence of increased muscle stiffness, muscle swelling, DOMS and decreases in muscle strength, as well as decrease recovery time for each parameter.

MATERIALS AND METHODS

Participants. Eleven healthy participants volunteered for the present study (for participant characteristics, see Table 1). Prior to the investigation, the participants gave their written, informed consent. All participants reported to not having performed any resistance training of the dorsiflexor muscles within the past six months. The participants engaged in no vigorous physical activity 48 hours prior to any of the examinations. Moreover, resistance training and massage of the dorsiflexor muscles during the full test period were not allowed. Furthermore, intake of caffeine as well as any analgesic and anti-inflammatory medication was prohibited 48 hours prior to the first test day and throughout the full test period.

Table 1: Characteristics of participants.

	Age (yr)	Height (cm)	Weight (kg)
Male (n = 9)	25.7 ± 5.3	182.0 ± 8.0	87.5 ± 15.3
Female (n = 2)	25.5 ± 3.5	170.5 ± 3.5	73.00 ± 17.0

All values are means ± standard deviations.

Study design. A randomized crossover design was utilized to investigate the effects of IPC on EIMD in TA. All participants reported to the laboratory 6 times in total. The study consisted of two test periods split by a washout period of at least seven days (for study design, see Figure 1). Each test period consisted of 3 days of intervention and testing. The first day, the participants had their baseline measurements taken for muscle stiffness, muscle thickness, muscle strength and pain, followed by the IPC intervention. Subsequently, the participants underwent the EIMD protocol for TA, which again was followed by the same measurements as for the baseline measurements. The two following days, with 24 hours in between, measurements of muscle stiffness, muscle thickness, muscle strength and pain were taken again. Pain was assessed until the participants reached their baseline measurements.

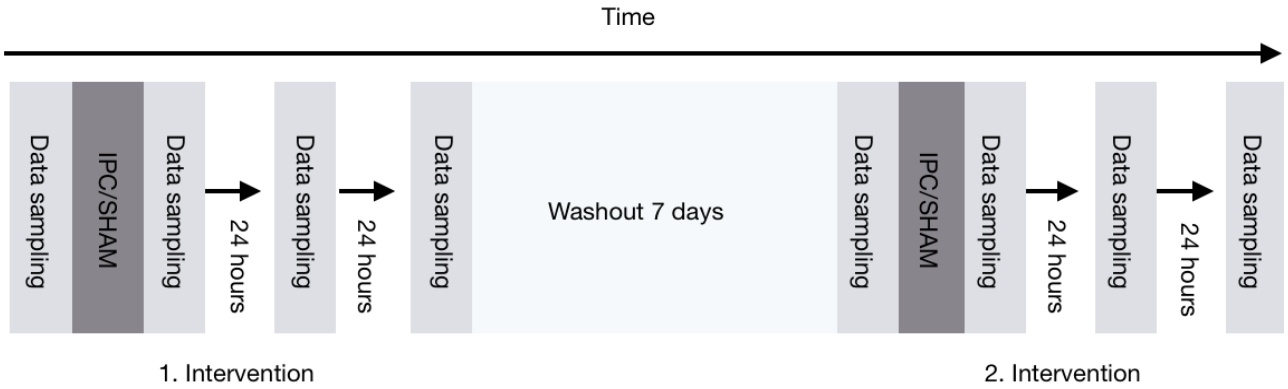

Figure 1. Study design. Data sampling = Muscle strength (maximal voluntary contraction), muscle stiffness (shear wave elastography), muscle thickness (B-mode ultrasound) and pain (numeric rating scale).

After the washout period, the crossover took place. Several precautions were taken in order to counterbalance the possibility of any prolonged effects of IPC, as well as of a contralateral repeated bout effect (CL-RBE) including any possible effect of dominant and non-dominant leg on the amount of EIMD. Therefore, half of the participants were treated with IPC during the first test period, and the other half were treated with a sham intervention first. Likewise, half of the participants received IPC and EIMD on their dominant leg, and the other half on their non-dominant leg (see Figure 2). These subconditions were constructed through randomization, stratified by gender.

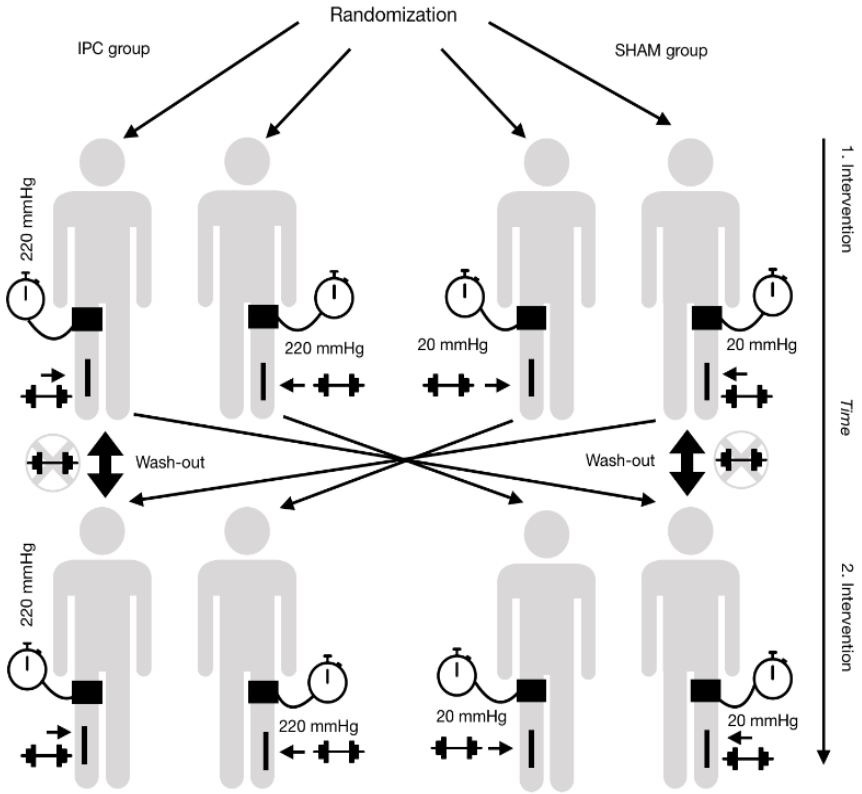

Figure 2. Overview of the crossover design of the present study.

Double blinding was strived for as much as possible. Therefore, the participants were informed that the effect of different cuff pressures was investigated. Blinding of researchers was strived for by assigning only one researcher to conduct the IPC intervention. This researcher had no influence on any of the measurements. The remaining researchers handled the execution of the EIMD protocol as well as MVC, NRS and ultrasound measurements.

Exercise induced muscle damage protocol. A custom-made 15 cm tall platform with a hinged footplate (25 cm) was used to induce muscle damage. The platform was placed 45 cm from a wall. The participants were instructed to position the lateral malleolus at the fulcrum, with toes facing towards the wall. The foot was secured to the footplate using a nylon Velcro® strap (2.5 x 20 cm). The participants were instructed to perform 5 sets of 10 repetitions of controlled plantar flexions. The participants were instructed to keep the knee extended during all repetitions. Participants' hands were placed on the wall in front of them for the sake of balance, and the opposite leg was used to return to starting point again (see Figure 3). A 60 beats per minute (BPM) metronome was used to help the participants control their plantar flexions. The participants were instructed to hold each contraction for 3 metronome beats (i.e. 3 seconds). If the plantar flexion could not be completed in a controlled manner for 3 repetitions in a row, the exercise was terminated. The number of sets and repetitions for each participant was noted during the first intervention for identical reproduction during the second intervention.

Figure 3. Illustrates the custom-built platform used for the exercise-induced muscle damage protocol.

IPC protocol. The IPC protocol was executed, with participants placed supine, using four cycles of 5 min ischemia, separated by five min reperfusion, with a total duration of 40 minutes. The pressure was applied using a sphygmomanometer (WelchAllyn Duraschock Silver®, DS-6501-300, New York City, NY, USA) and a 22 cm wide cuff (WelchAllyn Flexiport® Reusable Blood Pressure Cuff THIGH 13, New York City, NY, USA). The cuff was placed distally on participant's thigh with a pressure of 220 mmHg for IPC and 20 mmHg inflation for the sham procedure.

MVC test. Participants were placed supine on an examination table with legs stretched and arms crossed. The participant's foot was placed on a custom-made footplate, connected to a force transducer (SSM-AJ-1000, Interface, Scottsdale, AZ, USA). The signal was sampled at a frequency of 500 Hz and amplified 2000 times. The foot was fixated to the footplate at a 120° angle with a nylon Velcro® strap. Three MVCs were collected for each person per test. Each MVC was separated by 1 min rest. A specific warm-up was used, consisting of 14 submaximal dorsiflexor contractions of approximately 50% maximal force lasting five seconds, separated by 10 seconds. This was followed by three submaximal contractions of approximately 75% maximal force for three seconds separated by 30 seconds. The warm-up was implemented before each MVC test.

Muscle stiffness and muscle swelling. A LOGIQ® S8 ultrasound system was used to collect muscle stiffness and swelling data, using the 9L transducer. 10 muscle stiffness measurements were taken with shear wave elastography (SWE) for each test, and the five most homogenous measurements, selected through visual inspection, were used. The scanned muscle area was a fixed 1.2 x 0.88 cm rectangular area which was placed just beneath the superficial fascia of TA. A frequency of 10 MHz was used for the measurements. The scanner depth was specifically adjusted to the size of the participant's TA. The ultrasound transducer was placed on the muscle belly of TA, at one third of the length

from the head of the fibula to the medial malleolus. Marks were placed to assure that all measurements on the same leg were taken at the exact same spot.

Muscle thickness measurements, obtained through the LogiQview® mode, were used for evaluation of muscle swelling. One image was taken of the entire TA muscle (from the head of the fibula to the medial malleolus). On the image, the muscle belly was determined at one third of the total distance from the head of the fibula (see Figure 4). On this location, three measurements were taken, one on the center of the muscle belly and one measurement 2.5 cm to each side of the center. The mean of these three measurements was used as data. Foot angle and position were relaxed to minimize TA activity. However, ankle joint angle was measured at each test to assure that the same angle was used for each measurement.

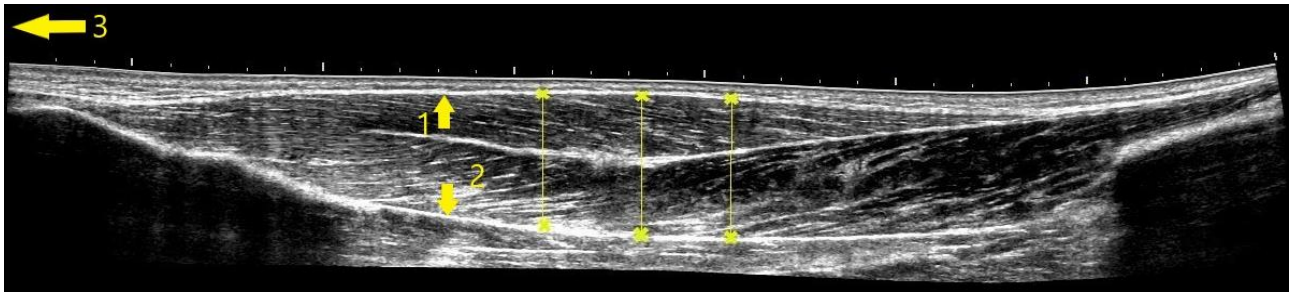

Figure 4. Shows a LogiQview of a participant's tibialis anterior. The yellow lines indicate the location of the measurements of muscle thickness. 1. The superficiel fascia of tibialis anterior. 2. The deeper lying fascia of tibialis anterior. 3. The proximal direction of the lower leg.

Pain. An NRS scale ranging from 0-10 was used to evaluate the participants' pain in TA during dorsiflexor activation before the EIMD protocol, after the EIMD protocol and the following seven days after the intervention. The participants were informed that zero corresponds to no pain, and 10 corresponds to worst imaginable pain.

Statistical analyses. Data were analyzed using SPSS (IBM CORP, version 25.0, Armonk, NY). The statistical significance level was set to an alpha-value of 0.05.

The parametric results of the study (muscle swelling, muscle strength and muscle stiffness) are presented in mean values with standard deviations and the following statistical information: *F*-value(degrees of freedom), *P*-value. The Shapiro-Wilk test was used to calculate and check the normality of the data and all parametric data were normally distributed. A two-way repeated measures analysis of variance (ANOVA) was applied to calculate the main effects of trials (Pre, Post0, Post24, Post48), conditions (IPC, sham) and interaction (trial x condition). If the assumption of sphericity was not fulfilled, Greenhouse-Geisser corrected degrees of freedom were applied. Post-hoc Bonferroni corrections were applied to counteract the problem with multiple comparisons.

The non-parametric results (pain) are presented as median values with interquartile range and *P*-value. The Friedman test was applied to check for significant differences between trials (Pre, Post0, Post24, Post48) in both interventions (IPC, sham) separately. If any difference was found, a post-hoc test was conducted through the Wilcoxon signed rank test. For the comparison between trials (Pre, Post0, Post24, Post48), Pre was compared to the remaining trials. For the comparison between intervention, Pre (IPC) was compared to Pre (Sham), Post0 (IPC) was compared to Post0(Sham), Post24 (IPC) was compared to Post24 (Sham), and Post48 (IPC) was compared to Post48 (Sham) using the Wilcoxon signed rank test. The Holm-Bonferroni correction was applied to counteract the multiple comparison error. The alpha-values were set according to the Holm-Bonferroni calculations and the number of comparisons (Holm, 1979).

Results

Pain. The participants in both interventions reported no pain on the NRS at the baseline trial (see Figure 5). A Friedman test was performed on both conditions separately, both showing a significant difference ($P < 0.001$). A post-hoc analysis, the Wilcoxon signed rank test, was applied, which showed that the participants reported significantly increased pain perception for IPC condition 24 hours ($P = 0.004$) and 48 hours post exercise ($P = 0.007$). Sham reported significantly increased pain perception immediately after exercise ($P = 0.005$) and 24 hours ($P = 0.003$), 48 hours ($P = 0.005$) and 72 hours ($P = 0.007$) post exercise. NRS data did not statistically differ between conditions (all P-values ≥ 0.046). However, none of the P-values were significant due to the Holm-Bonferroni corrected, which set the significance level at 0.007 for the lowest P-value.

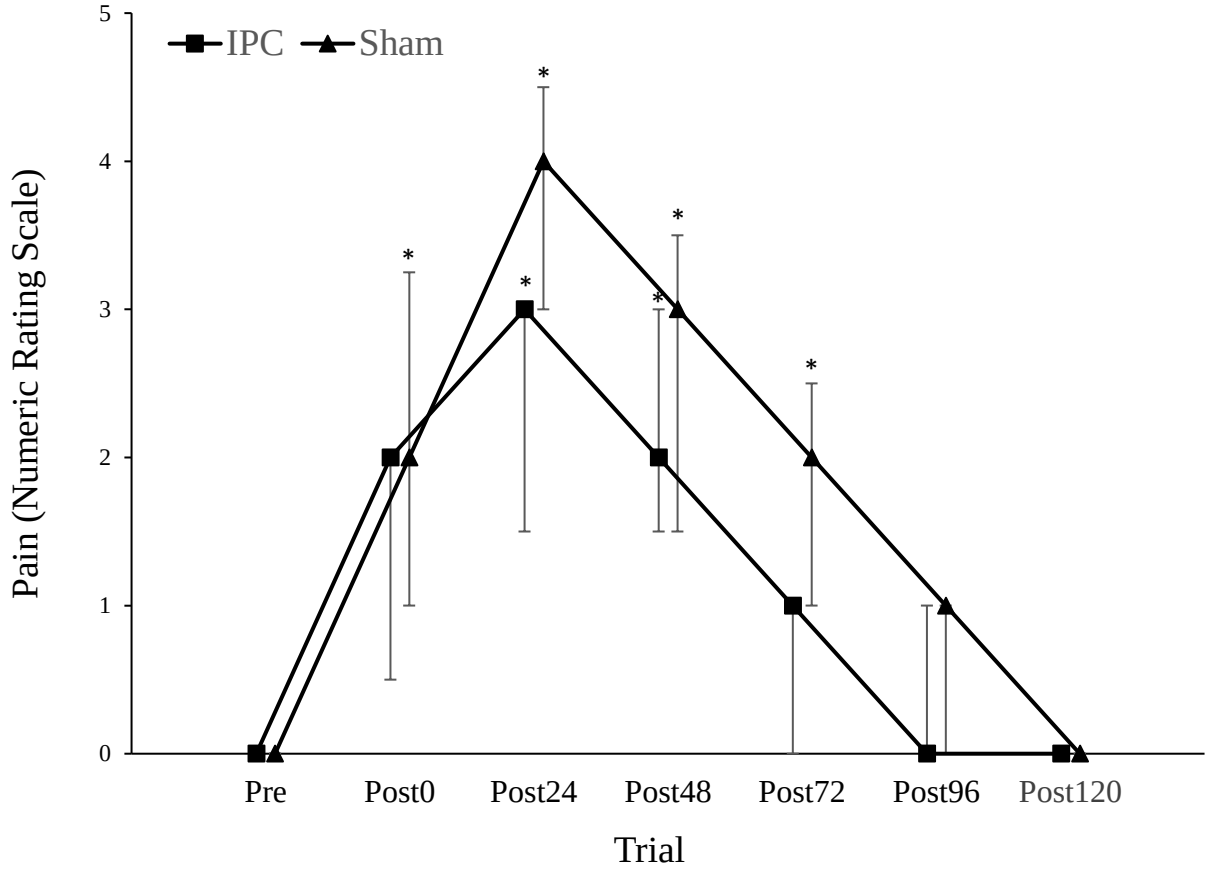

Figure 5. All values are medians ± interquartile range.
**Significantly different from baseline, i.e. Pre (P < 0.05)*
Pre, pre-exercise; Post0, immediately post-exercise; Post24, 24 hours post-exercise; Post48, 48 hours post-exercise.

Muscle thickness For muscle thickness results, see Table 2 and Figure 6. Muscle thickness differed significantly between trials $F(3,30) = 18.218$, $P < 0.001$. The post-hoc analysis showed that significant increases in muscle thickness were found 24 hours ($P = 0.041$) and 48 hours ($P = 0.036$) post-exercise compared to baseline. However, muscle thickness did not significantly differ between condition $F(1,10) = 0.122$, $P = 0.734$, nor was there any interaction between trial and condition $F(3,30) = 0.155$, $P = 0.818$. For the interaction, Mauchly's Sphericity could not be assumed ($P = 0.044$), and therefore a Greenhouse-Geisser correction was applied.

Table 2: Indicators of muscle damage after exercise induced muscle damage (EIMD)

	Pre	Post0	Post24	Post48
Muscle stiffness (kPa)				
IPC	26.93 ± 6.40	24.88 ± 6.71	26.07 ± 8.09	27.50 ± 6.99
Sham	24.62 ± 6.98	23.74 ± 5.75	29.18 ± 7.41	27.83 ± 5.44
Muscle thickness (mm)				
IPC	28.20 ± 2.85	29.10 ± 3.25	30.30 ± 2.87*	30.0 ± 2.68*
Sham	28.10 ± 2.51	29.30 ± 2.82	30.50 ± 3.28*	30.2 ± 3.49*
MVC (N)				
IPC	216.48 ± 29.50	199.20 ± 32.84	186.14 ± 48.36	196.46 ± 54.91
Sham	209.37 ± 30.50	200.47 ± 37.32	198.94 ± 43.17	203.74 ± 56.68

All values are means ± standard deviations.
**Significantly different from baseline, i.e. Pre (P < 0.05).*
Pre, pre-exercise; Post0, immediately post-exercise; Post24, 24 hours post-exercise; Post48, 48 hours post-exercise.

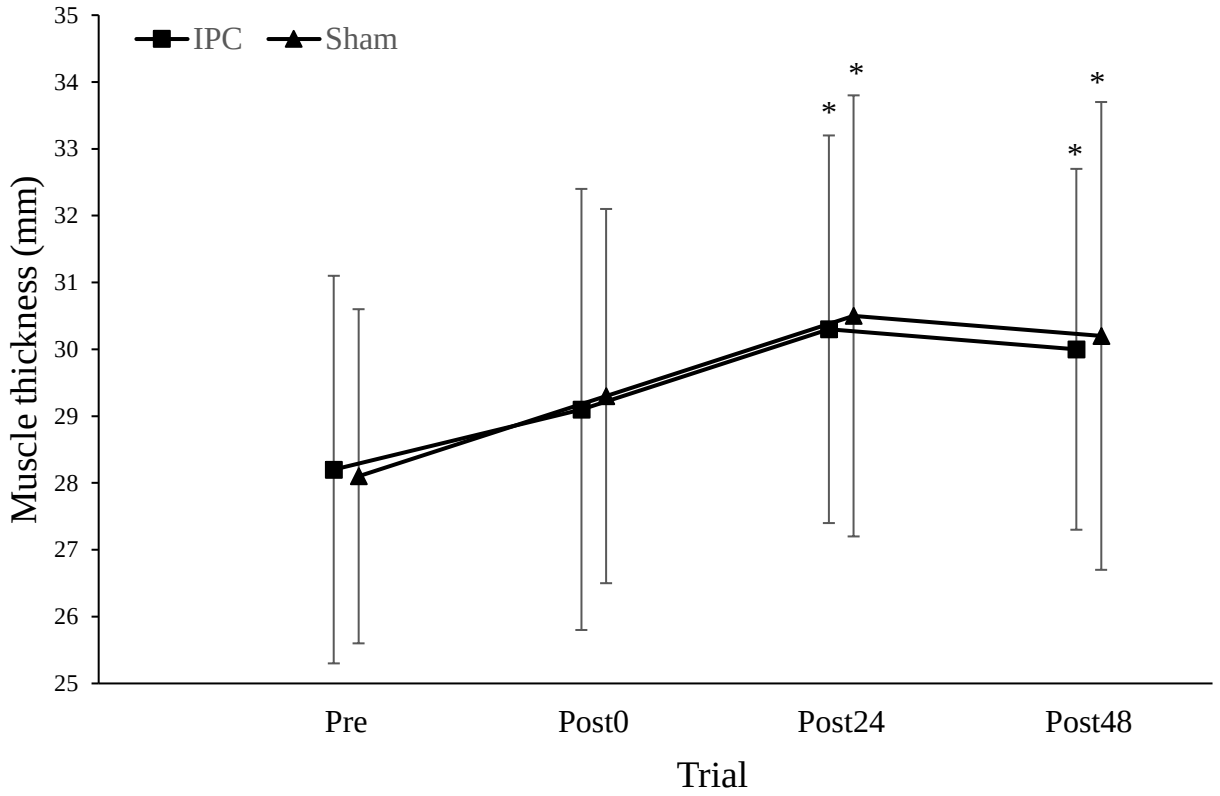

Figure 6. All values are means ± standard deviations.
**Significantly different from baseline, i.e. Pre (P < 0.05).*
Pre, pre-exercise; Post0, immediately post-exercise; Post24, 24 hours post-exercise; Post48, 48 hours post-exercise.

Muscle stiffness. For muscle stiffness results, see Table 2. Muscle stiffness did not significantly differ between trials F(3,30) = 2.356, P =0.92, conditions $F(1,10) = 0.561$, $P = 0.471$, nor was there any interaction between trial and condition $F(3,30) = 2{,}464$, $P = 0.82$.
Muscle strength. For muscle strength results, see Table 2. MVC force production measurements did not significantly differ between trials F(3,30) = 1.904, $P = 0.15$, interventions $F(1,10) = 0.082$, $P = 0.781$, and there was no interaction between trial and condition $F(3,30) = 0.458$, $P = 0.714$.

Discussion

The aim of this study was to investigate the possible effect of IPC on EIMD with focus on pain, muscle swelling, muscle stiffness, loss of muscle strength using a crossover design. The main finding was that the IPC condition caused quicker recovery from increased perceived pain following eccentric exercise. IPC showed no significant effect on muscle swelling, muscle stiffness and muscle strength as a result of EIMD.
Pain. The significant main effects of trials for both interventions showed that sham caused significant increases in perceived pain for 24 hours longer than IPC. Pain levels during sham intervention were significantly increased immediately after eccentric exercise as well as 24, 48 and 72 hours after, while pain levels were only significantly increased for IPC 24 and 48 hours post-exercise. This is in agreement with a recent finding by Franz et al. (2017b), who showed significant increases in pain 24, 48 and 72 hours after eccentric exercise for the control group compared to baseline, and only significant increases in pain 24 and 48 hours after eccentric exercise for the IPC group. Therefore, IPC may be able to reduce the perception of pain following unaccustomed eccentric exercise.

However, there has been no research to investigate and explain the underlying mechanisms. One theory of DOMS is that the muscle cell is infiltrated with proinflammatory substances and fluid (Cheung et al., 2003). It has previously been argued that cell swelling following EIMD occurs because of Na^+ accumulation in the cell caused either by increased permeability of the cell membrane or because the ATP-dependent Na^+ extrusion is slower than passive Na^+ influx (Chleboun et al., 1998). This Na^+ accumulation creates an osmotic pressure, which transports fluid through the cell membrane into the cell (Cheung et al., 2003). This swelling is argued to cause pain if type III and type IV afferent neurons from the muscle are active (Cheung et al., 2003). It is argued that macrophages are involved in the synthesis of prostaglandin E2 (Smith, 1991), which sensitizes afferent nerve endings and play an important role in increased nociception during inflammation (Mense, 1981). The increased sensitivity may, in combination with cell swelling, result in an increased sensation of pain during movement that would normally not produce pain (Cheung et al., 2003; Proske & Morgan, 2001).
Similarly to EIMD, ischemia also causes the accumulation of Na^+ and Ca^{2+} in the cell, which results in intracellular ion accumulation and cell swelling, which may result in membrane ruptures, perhaps leading to cell death (Murphy & Steenbergen, 2008). Evidence was previously found that IPC may have the effect of maintaining ion homeostasis in later ischemic periods by preventing the inhibition of the ATP-dependent Na^+/K^+ pump, presumably through cellular neuroprotection caused by increased adenosine levels (de Souza Wyse et al., 2000). Furthermore, IPC was demonstrated to decrease cell swelling in the rabbit's heart, with a possible explanation being the maintained activity of Na^+/K^+-ATPase (Diaz et al., 2003).

Therefore, it is possible that IPC may be able to improve ion homeostasis following eccentric exercise, which may reduce cell swelling and nociception. However, whether or not muscle swelling is the cause of increased pain in this study is unknown.

Nevertheless, despite the significant increases in muscle thickness from baseline compared to 24 and 48 hours post-exercise, the lack of a significant interaction between IPC and sham conditions indicates that IPC's attenuating effect on perceived pain cannot be ascribed to differences in muscle swelling.

It is well established that nerve growth factor (NGF) plays an important role in sensitization of nociceptors as a result of EIMD, causing hyperalgesia (Lee et al., 2008; Murase et al., 2010). Studies have shown that NGF is upregulated by a number of different cytokines, including Interleukin-1β (IL-1β) (Woolf et al., 1994; Woolf et al., 1997), which has been shown to increase as a result of EIMD (Pournot et al., 2011). Furthermore, evidence has been found that IPC suppresses cytokine expression (Hausenloy & Lim, 2012), such as IL-1β (Brzozowski et al., 2004). This suggests that IPC may be able to reduce proinflammatory cytokine expression following eccentric exercise and, therefore, reduce the production of NGF, which is correlated with hyperalgesia. This could be the reason why IPC condition resulted in a reduced recovery time for perceived pain.

Muscle thickness. Muscle thickness measurements did not significantly differ between conditions. This is comparable to the findings of a recent study that did not find a difference in arm circumference for the IPC condition following eccentric exercise of the elbow flexors compared to the control group (Franz et al., 2017b). However, the present study does not provide any evidence that IPC has an effect on decreasing muscle swelling following eccentric exercise.

Nevertheless, a significant main effect of trials was found in the present study, with significant increases in muscle thickness at 24 and 48 hours post-exercise compared to baseline, yet with no significant increases immediately after eccentric exercise. This is in agreement with a previous study by Bowers et al. (2004), which similarly did not find any significant muscle swelling immediately following eccentric exercise of the quadriceps muscles. Furthermore, peak swelling has been found to occur 24 hours post-exercise (Proske & Morgan, 2001), which is also the case in the present study.

Muscle swelling and pain have been argued to be indirect markers of inflammation (Smith, 1991). Therefore, increases in muscle thickness can be interpreted as an indirect marker of the inflammatory processes involved in reparation and regeneration of the damaged muscle following EIMD (Clarkson & Hubal, 2002). This suggests that the EIMD protocol has induced EIMD regardless of condition.

MVC. No significant declines were found in muscle strength measured by MVC following eccentric exercise. Nevertheless, the occurrence of DOMS and muscle swelling in the present study indicates that EIMD occurred for both conditions (Clarkson & Hubal, 2002). Decreases in maximal force production have previously been argued to be among the best indicators of EIMD (Warren et al., 1999; Paulsen et al., 2012). Muscle strength was measured using MVC, which is argued to be a reliable method for assessment of EIMD (Morton et al., 2005). It is likely that one or several familiarization sessions could have increased the reliability of the MVC test as a tool for assessing the degree of muscle damage. A study showed that 3-4 sessions were needed in order to achieve similar measurements with no further increases in force production for young women during one repetition maximum (1RM) knee extensions (Ploutz-Snyder & Giamis, 2012). Knee extension is arguably comparable to ankle dorsiflexion, both being single-joint movements. Therefore, familiarization sessions may have prevented strength increases (Ploutz-Snyder & Giamis, 2012) and may have contributed to an increased reliability of the MVC test as a means of EIMD assessment.

Furthermore, a few of the participants encountered difficulties during the maximal isometric dorsiflexions. These participants consistently flexed their knees during the contraction, which may have led to additional muscle groups contributing to the total maximal force. Therefore, familiarization could have been beneficial for proper execution of the dorsiflexion MVC test.

However, familiarization may have had the effect of attenuating EIMD from eccentric contractions (Chen et al., 2013). Chen et al. (2013) found evidence that two maximal isometric contractions of the elbow flexors reduced EIMD in response to 30 maximal isokinetic eccentric contractions two and four days after the maximal isometric contractions. Furthermore, a recent study provided knowledge that two maximal voluntary contractions of the elbow flexors in one arm reduced the

EIMD in the contralateral elbow flexors following 30 maximal eccentric contractions 1-2 days after the isometric contractions (Chen et al., 2018).

Furthermore, maximal eccentric exercise of the elbow flexors on one arm have shown to decrease EIMD following eccentric exercise in the contralateral arm 14 days later (Emberger et al., 2011). This was taken into account in the present study by counterbalancing the crossover design. Thus, half of the participants received IPC as the first intervention, while the other received sham, so the CL-RBE would affect the conditions equally.

Therefore, because EIMD was the main focus of the study, it was decided that the possible attenuations in EIMD accompanying familiarization were to be avoided.

Muscle stiffness. Muscle stiffness measurements, collected through SWE, did not statistically differ between trials or conditions. Furthermore, no interaction was found between trials and conditions. Despite the lack of significance, a tendency towards a different development for the two conditions was seen after 24 hours (IPC: -3.19%; sham: + 18.54%) and 48 hours (IPC: 2.12% sham: 13.03%) following EIMD-protocol.

Previous studies have shown increases in muscle stiffness immediately after eccentric exercise (Niitsu et al. 2011), and 1 hour post-exercise after eccentric exercise (Lacourpaille et al., 2014), with further muscle stiffness increases for several days before returning to baseline (Niitsu et al., 2011; Lacourpaille et al., 2014). However, the findings for muscle stiffness in the present study immediately after eccentric exercise are not in agreement with the aforementioned studies. The results of the present study show a tendency towards decreases in muscle stiffness following eccentric exercise for both conditions. This finding may receive some support from a study that shows a correlation between temperature and muscle fiber stiffness, with decrease in muscle fiber stiffness at higher temperatures (Sapin-de Brosses et al., 2010). This study explains that muscle fiber stiffness is strongly affected by temperature (Sapin-de Brosses, et al., 2010). Therefore, the decrease in muscle stiffness immediately after eccentric exercise may be due to increases in the temperature of TA as a result of the EIMD protocol.

Nevertheless, there may be, although not significant, a slight tendency of increased muscle stiffness for the sham condition at 24 and 48 hours post-exercise compared to the IPC condition. This may indicate that IPC could decrease muscle stiffness following eccentric exercise. However, no definite conclusions can be drawn from this study.

Conclusion. The present study found that the application of IPC prior to eccentric exercise of TA reduced the perceived pain as a result of EIMD. No significant effects of IPC were observed for muscle stiffness, muscle swelling and muscle strength. However, the similar muscle swelling between conditions indicates that the differences in perceived pain between conditions was not caused by differences in muscle swelling. Therefore, this may implicate that IPC reduces the proinflammatory gene expression and thereby NGF production, resulting in decreased perception of pain.

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References

Addison, P. D., Neligan, P. C., Ashrafpour, H., Khan, A., Zhong, A., Moses, M., ... & Pang, C. Y. (2003). Noninvasive remote ischemic preconditioning for global protection of skeletal muscle against infarction. *American Journal of Physiology-Heart and Circulatory Physiology*, *285*(4), 1435-1443.

Aktaş, E., Atay, Ç., Deveci, M. A., Arikan, M., Toğral, G., & Yildirim, A. (2015). Impact of oxydative stress on early postoperative knee functions and muscle injury biochemical markers. Can we create an ischaemic preconditioning effect in sequential ischaemic surgical procedures?. *Acta orthopaedica et traumatologica turcica*, *49*(4), 387-93.

Bowers, E. J., Morgan, D. L., & Proske, U. (2004). Damage to the human quadriceps muscle from eccentric exercise and the training effect. *J Sports Sci*, *22*(11-12), 1005-14.

Brzozowski, T., Konturek, P. C., Konturek, S. J., Pajdo, R., Kwiecien, S., Pawlik, M., ... & Pawlik, W. W. (2004). Ischemic preconditioning of remote organs attenuates gastric ischemia–reperfusion injury through involvement of prostaglandins and sensory nerves. *European journal of pharmacology*, *499*(1-2), 201-213.

Chen, H. L., Nosaka, K., Pearce, A. J., & Chen, T. C. (2012). Two maximal isometric contractions attenuate the magnitude of eccentric exercise-induced muscle damage. *Applied Physiology, Nutrition, and Metabolism*, *37*(4), 680-689.

Chen, T. C., Chen, H. L., Lin, M. J., Chen, C. H., Pearce, A. J., & Nosaka, K. (2013). Effect of two maximal isometric contractions on eccentric exercise-induced muscle damage of the elbow flexors. European journal of applied physiology, 113(6), 1545-1554.

Chen, T. C., Lin, M. J., Chen, H. L., Lai, J. H., Yu, H. I., & Nosaka, K. (2018). Muscle damage protective effect by two maximal isometric contractions on maximal eccentric exercise of the elbow flexors of the contralateral arm. *Scandinavian journal of medicine & science in sports*, *28*(4), 1354-1360.

Cheung, K., Hume, P. A., & Maxwell, L. (2003). Delayed onset muscle soreness. *Sports Medicine*, *33*(2), 145-164.

Chleboun, G. S., Howell, J. N., Conatser, R. R., & Giesey, J. J. (1998). Relationship between muscle swelling and stiffness after eccentric exercise. *Medicine and science in sports and exercise*, *30*(4), 529-535.

Clarkson, P. M., & Hubal, M. J. (2002). Exercise-induced muscle damage in humans. *American journal of physical medicine & rehabilitation*, 81(11), 52-69.

de Souza Wyse, A. T., Streck, E. L., Worm, P., Wajner, A., Ritter, F., & Netto, C. A. (2000). Preconditioning prevents the inhibition of Na+, K+-ATPase activity after brain ischemia. *Neurochemical Research*, *25*(7), 971-975.

Diaz, R. J., Armstrong, S. C., Batthish, M., Backx, P. H., Ganote, C. E., & Wilson, G. J. (2003). Enhanced cell volume regulation: a key protective mechanism of ischemic preconditioning in rabbit ventricular myocytes. *Journal of molecular and cellular cardiology*, *35*(1), 45-58.

Emberger, M., Koller, J., Laimer, M., Hell, M., Oender, K., Trost, A., et al. (2011). Original article. *Journal of the European Academy of Dermatology and Venereology*, *25*(2), 227-231.

Franz, A., Behringer, M., Nosaka, K., Buhren, B. A., Schrumpf, H., Mayer, C., ... & Schumann, M. (2017a). Mechanisms underpinning protection against eccentric exercise-induced muscle damage by ischemic preconditioning. *Medical hypotheses*, *98*, 21-27.

Franz, A., Behringer, M., Harmsen, J. F., Mayer, C., Krauspe, R., Zilkens, C., & Schumann, M. (2017b). Ischemic Preconditioning Blunts Muscle Damage Responses Induced by Eccentric Exercise. *Medicine and science in sports and exercise*, *50*(1), 109-115.

Hausenloy, D. J., & Lim, S. Y. (2012). Remote ischemic conditioning: from bench to bedside. *Frontiers in physiology*, *3*, 27.

Holm, S. (1979). A simple sequentially rejective multiple test procedure. *Scandinavian Journal of Statistics*, *6*(2), 65-70. Retrieved from http://www.jstor.org/stable/4615733

Howell, J. N., Chila, A. G., Ford, G., David, D. & Gates, T. (1985). An electromyographic study of elbow motion during postexercise muscle soreness. *Journal of Applied Physiology* 58, 1713–1718.

Hughes, S. F., Hendricks, B. D., Edwards, D. R., Bastawrous, S. S., Roberts, G. E., & Middleton, J. F. (2007). Mild episodes of tourniquet-induced forearm ischaemia-reperfusion injury results in leukocyte activation and changes in inflammatory and coagulation markers. *Journal of Inflammation*, *4*(1), 12.

Incognito, A. V., et al. (2016). The effects of ischemic preconditioning on human exercise performance. *Sports Medicine, 46*(4), 531.544.

Lacourpaille, L., Nordez, A., Hug, F., Couturier, A., Dibie, C., & Guilhem, G. (2014). Time-course effect of exercise-induced muscle damage on localized muscle mechanical properties assessed using elastography. *Acta Physiologica*, *211*(1), 135-146.

Lee, T. H., Yang, J. T., Ko, Y. S., Kato, H., Itoyama, Y., & Kogure, K. (2008). Influence of ischemic preconditioning on levels of nerve growth factor, brain-derived neurotrophic factor and their high-affinity receptors in hippocampus following forebrain ischemia. *Brain research*, *1187*, 1-11.

MacIntyre, D. L., Reid, W. D., & McKenzie, D. C. (1995). Delayed muscle soreness. *Sports Medicine*, *20*(1), 24-40.

Memtsoudis, S. G., Della Valle, A. G., Jules-Elysse, K., Poultsides, L., Reid, S., Starcher, B., Ma, Y., & Sculco, T. P. (2010). Perioperative inflammatory response in total knee arthroplasty patients: impact of limb preconditioning. *Regional anesthesia and pain medicine*, *35*(5), 412-416.

Mense, S. (1981). Sensitization of group IV muscle receptors to bradykinin by 5-hydroxytryptamine and prostaglandin E2. *Brain research*, *225*(1), 95-105.

Morton, J. P., Atkinson, G., MacLaren, D. P., Cable, N. T., Gilbert, G., Broome, C., McArdle, A., & Drust, B. (2005). Reliability of maximal muscle force and voluntary activation as markers of exercise-induced muscle damage. *European journal of applied physiology*, *94*(5-6), 541-548.

Murase, S., Terazawa, E., Queme, F., Ota, H., Matsuda, T., Hirate, K., et al. (2010). Bradykinin and nerve growth factor play pivotal roles in muscular mechanical hyperalgesia after exercise (delayed-onset muscle soreness). *Journal of Neuroscience*, *30*(10), 3752-3761.

Murphy, E., & Steenbergen, C. (2008). Mechanisms underlying acute protection from cardiac ischemia-reperfusion injury. *Physiological reviews*, *88*(2), 581-609.

Murry, C. E., Jennings, R. B., & Reimer, K. A. (1986). Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation*, *74*(5), 1124-1136.

Newham, D. J., Mills, K. R., Quigley, B. M., & Edwards, R. H. T. (1983). Pain and fatigue after concentric and eccentric muscle contractions. *Clinical science*, *64*(1), 55-62.

Niitsu, M., Michizaki, A., Endo, A., Takei, H., & Yanagisawa, O. (2011). Muscle hardness measurement by using ultrasound elastography: a feasibility study. *Acta radiologica*, *52*(1), 99-105.

Nosaka, K., & Newton, M. (2002). Concentric or eccentric training effect on eccentric exercise-induced muscle damage. *Medicine & Science in Sports & Exercise*, *34*(1), 63-69.

Paulsen, G., Mikkelsen, U. R., Raastad, T., & Peake, J. M. (2012). Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise? *Exercise immunology review*, *18*, 42-97.

Ploutz-Snyder, L. L., & Giamis, E. L. (2001). Orientation and familiarization to 1RM strength testing in old and young women. *The Journal of Strength & Conditioning Research*, *15*(4), 519-523.

Pournot, H., Bieuzen, F., Louis, J., Fillard, J. R., Barbiche, E., & Hausswirth, C. (2011). Time-course of changes in inflammatory response after whole-body cryotherapy multi exposures following severe exercise. *PloS one*, *6*(7), e22748.

Proske, U., & Morgan, D. L. (2001). Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. *The Journal of physiology*, *537*(2), 333-345.

Roig, M., O'Brien, K., Kirk, G., Murray, R., McKinnon, P., Shadgan, B., & Reid, D. W. (2008). The effects of eccentric versus concentric resistance training on muscle strength and mass in healthy adults: a systematic review with meta-analyses. *British journal of sports medicine*, *43*(8), 556-568.

Sapin-de Brosses, E., Gennisson, J. L., Pernot, M., Fink, M., & Tanter, M. (2010). Temperature dependence of the shear modulus of soft tissues assessed by ultrasound. *Physics in Medicine & Biology*, *55*(6), 1701.

Sayers, S. P., & Clarkson, P. M. (2001). Force recovery after eccentric exercise in males and females. *European journal of applied physiology*, *84*(1-2), 122-126.

Seger, J. Y., Arvidsson, B., & Thorstensson, A. (1998). Specific effects of eccentric and concentric training on muscle strength and morphology in humans. *European journal of applied physiology and occupational physiology*, *79*(1), 49-57.

Smith L. L. (1991) Acute inflammation: the underlying mechanism in delayed onset muscle soreness? *Medicine and science in sports and exercise*. 23(5):542-51.

Sousa, M., Teixeira, V. H., & Soares, J. (2014). Dietary strategies to recover from exercise-induced muscle damage. *International journal of food sciences and nutrition*, *65*(2), 151-163.

Sullivan, P. J., Sweeney, K. J., Hirpara, K. M., Malone, C. B., Curtin, W., & Kerin, M. J. (2009). Cyclical ischaemic preconditioning modulates the adaptive immune response in human limb ischaemia–reperfusion injury. *British Journal of Surgery*, *96*(4), 381-390.

Van, M., Olguner, Ç., Koca, U., Şişman, A. R., Muratli, K., Karci, A., Mavioğlu, Ö, & Kilercik, H. (2008). Ischaemic preconditioning attenuates haemodynamic response and lipid peroxidation in lower-extremity surgery with unilateral pneumatic tourniquet application: a clinical pilot study. *Advances in therapy*, *25*(4), 355-366.

Warren, G. L., Lowe, D. A., & Armstrong, R. B. (1999). Measurement tools used in the study of eccentric contraction-induced injury. *Sports medicine*, *27*(1), 43-59.

Woolf, C. J., Allchorne, A., Safieh-Garabedian, B., & Poole, S. (1997). Cytokines, nerve growth factor and inflammatory hyperalgesia: the contribution of tumour necrosis factor α. *British journal of pharmacology*, *121*(3), 417-424.

Woolf, C. J., Safieh-Garabedian, B., Ma, Q. P., Crilly, P., & Winter, J. (1994). Nerve growth factor contributes to the generation of inflammatory sensory hypersensitivity. *Neuroscience*, *62*(2), 327-331.

Xin, L., Hyldahl, R. D., Chipkin, S. R., & Clarkson, P. M. (2013). A contralateral repeated bout effect attenuates induction of NF-κB DNA binding following eccentric exercise. *Journal of Applied Physiology*, *116*(11), 1473-1480.