**The effect of acute and repeated ischemic preconditioning on recovery following exercise-induced muscle damage**

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**Running Title**: Ischemic Preconditioning and muscle damage

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**Abstract**

**Objectives:** The aim of this investigation was to determine if acute or repeated applications of ischemic preconditioning (IPC) could enhance the recovery process, following exercise induced muscle damage (EIMD). **Design:** Randomized control trial. **Methods:** Twenty-three healthy males were familiarised with the muscle damaging protocol (five sets of 20 drop jumps from a 0.6 m box) and randomly allocated to one of three groups: SHAM (3 x 5 min at 20 mmHg), Acute IPC (3 x 5 min at 220 mmHg) and Repeated IPC (3 days x 3 x 5 min at 220 mmHg). The indices of muscle damage measured included creatine kinase concentration ([CK]), thigh swelling, delayed onset muscle soreness, counter movement jumps (CMJ) and maximal voluntary isometric contraction (MVIC). **Results:** Both acute and repeated IPC improved recovery in MVIC versus SHAM. Repeated IPC led to a faster MVIC recovery at 48 h (101.5%) relative to acute IPC (92.6%) and SHAM (84.4%) ($P < 0.05$). Less swelling was found for both acute and repeated IPC *vs.* SHAM ($P < 0.05$) but no group effects were found for CMJ, soreness or [CK] responses ($P > 0.05$). **Conclusion:** Taken together, repeated IPC can enhance recovery time of MVIC more than an acute application, and both reduce swelling following EIMD, relative to a SHAM condition.

**Key Words: muscle function, ischemia, vascular occlusion, delayed onset muscle soreness, eccentric exercise.**

## Introduction

Exercise induced muscle damage (EIMD), a typical response to eccentric or unaccustomed exercise, results in structural damage within the muscle, including disruption to the sarcomere and dysfunction of excitation-contraction coupling[1] and an increased inflammatory cascade[2]. These changes result in reduced muscle force production and function, increased muscle swelling, delayed onset muscle soreness (DOMS) and increased appearance of muscle proteins in the blood in the days following exercise[3]. These symptoms are exacerbated between 24 - 72 h post exercise, gradually decreasing to baseline thereafter, but may be attenuated with recovery strategies[3].

Ischemia is a phenomenon of inadequate blood supply and, thus, oxygen delivery to meet the metabolic demands of the organs and tissues[4,5]. Such prolonged interruptions to the blood supply of organs and tissues may result in ischemia reperfusion (IR) injury[4,5]. IR injury underpins the damage caused by pathologies, surgery, and organ transplantations, which are complicated by interruption of the blood supply to tissues, resulting in cellular dysfunction, apoptosis and cell death[4]. Therefore, strategies have been developed to improve tissue tolerance to ischemia or reduce the damage caused by IR injury. Furthermore, the responses to IR injury are similar to those observed following EIMD, namely increased intracellular calcium concentrations and an increase in appearance of muscle proteins in the blood and cytokine markers such as creatine kinase ([CK]), lactate dehydrogenase and interleukin-6[5].

Ischemic conditioning (IC), a process that typically applies short (5 min) intermittent periods of vascular occlusion, followed by reperfusion, has been shown to protect cardiac and skeletal tissue from metabolic and contractile damage, caused by IR injury, if applied before (pre-conditioning [IPC]) or following (post-conditioning [ICPost]) an ischemic event[6]. These brief cycles of ischemic conditioning prime the targeted tissue and bestow protection for future IR stress[7] or reduce ischemic stress post injury[8]. Indeed, the application of IC has been beneficial in reducing post-operative knee surgery pain[9] and oxidative stress following knee surgery[10].

Recently, IC has been shown to accelerate the recovery process following strenuous exercise[11-14]. Possible mechanisms explaining these ergogenic effects may include increased blood flow[15], reduction in oxidant generation[10], elevated adenosine levels[16] and/or reducing the inflammatory response[17] . However, the results for the use of IPC or ICPost EIMD are inconclusive. ICPost has shown some potential, as evidenced by improved recovery in functional measures of athletic performance, including repeated sprint ability and jump height, 24 h following several different ischemic interventions[12] and cycling performance[11]. Furthermore, Page et al.[14] demonstrated that ICPost could improve recovery of muscle force production, DOMS and reduce [CK] following an EIMD protocol. Alternatively, the application of IPC applied before exercise has received little attention regarding recovery from exercise and the findings are inconsistent. Franz et al.[13] demonstrated that IPC applied before EIMD exercise resulted in attenuated responses of [CK], DOMS and muscle force properties. In contrast Northey et al.[18] did not observe any benefits of IPC applied before a resistance exercise protocol on recovery.

Based on the current evidence, the application of IPC before strenuous exercise and its impact on recovery is inconclusive. Whilst acute application of IPC may provide protection and thus help recovery this is not always the case. In clinical populations acute IPC is attenuated in preventing ischemic injury[7] whereas if the application of IPC is repeated over a number of days then protection is conferred[19]. Therefore, it appears that an increased 'dose' of IPC may overcome some of the issues associated with exposure to a single bout of IPC. Research from our own lab suggests that repeated application of IPC across a number of days enhances skeletal muscle adaptations (skeletal muscle oxidative capacity and enhanced microvascular blood flow), independent of exercise[15], which has been supported elsewhere[20,21] and could explain observed improvements in exercise performance[22]. Therefore, the application of repeated IPC may be beneficial in augmenting the recovery process following exercise.

The aim of this investigation was to determine if single day or repeated applications of IPC prior to EIMD could enhance the recovery process. It was hypothesised that repeated IPC would attenuate the markers of EIMD during recovery in healthy recreational males to a greater extent than acute IPC and a control group.

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85 **Methods**

86

87 Twenty-three healthy males (age, 23 ± 3 years; height, 180± 7cm; body mass, 81.3 ± 10.3 kg; activity
88 time, 7.2 ± 3.1 h/week) volunteered to participate in the study. All participants were physically active
89 (1-3 x per week of resistance training and team sports training), non-smokers and free of any
90 medications that would preclude their participation in strenuous exercise. No participants had
91 previously used or had knowledge of IPC. Before testing, permission for the study was granted by the
92 local university ethics committee, all participants gave their informed consent and were informed on
93 the nature of the study.

94

95 Participants completed 5-8 visits and attended a familiarisation trial approximately one week before
96 testing. During visit 1, participants were familiarised with the EIMD protocol and given specific
97 instructions for how to perform a drop jump; however, they did not practice the exercise protocol to
98 reduce the repeated bout effect. The repeated bout effect is the adaptative process, that attenuates the
99 signs and symptoms of EIMD, following a second bout of exercise of similar magnitude.[3] They were
100 also familiarised with the muscle soreness scale and muscle function tests. After visit 1, participants
101 were randomly assigned into one of three intervention groups in a single-blind experimental design:
102 SHAM (3 x 5 min at 20 mmHg), Acute IPC (3 x 5 min at 220 mmHg) and Repeated IPC (3 days of 3 x
103 5 min at 220 mmHg). During visit 2, participants completed the intervention procedure measuring
104 baseline parameters of muscle damage, including [CK], thigh swelling, DOMS, counter movement
105 jumps (CMJ) and maximal voluntary isometric contraction (MVIC). Fifteen minutes following the
106 intervention, participants performed the EIMD protocol. Immediately post EIMD indices of muscle
107 damage were measured again and then collected at 24, 48 and 72 h post EIMD.

108

109 To induce muscle damage, participants performed five sets of 20 repetitions of drop jumps from a 0.6
110 m box[23]. Participants, with their hands placed on their hips, were asked to step off the box with their

dominant leg and upon landing on both feet, jump maximally into the air, with two-minute rest period between sets. Verbal encouragement was given to participants throughout the exercise protocol.

Participants performed the SHAM/IPC protocol whilst the participant was lying in a supine position on an examination couch. All protocols consisted of 3 cycles of 5 min of bilateral occlusion of the upper thigh (SHAM – 20 mmHg, IPC – 220 mmHg) and 5 min reperfusion using a pneumatic tourniquet system (14.5 cm cuff width; Delfi Medical, Vancouver, Canada). For the Repeated IPC condition, the protocol was repeated for three sessions prior to EIMD protocol (-48 h EIMD, -24 h EIMD, immediately before EIMD). During Acute IPC, the protocol was applied immediately before EIMD. Participants then rested for 15 min following IPC before completing the EIMD protocol.

Plasma [CK] was determined from fingertip capillary blood samples. Approximately 300 μL of capillary whole blood was collected (Microcuvette® CB300, Sarstedt, Numbrecht, Germany) and was immediately placed in a refrigerated centrifuge (Mikro 220R D-78532, Tuttlingen, Germany) and spun at 3,500 rev/min for 10 min at 4 °C. The sample was immediately stored at -80 °C for analysis at a later date. All samples were analysed in duplicate, using a semi-automated clinical chemistry analyser (Randox RX Monza Randox, Crumlin, United Kingdom). The normal plasma [CK] ranges for this assay are 24-195 IU/L and the coefficient of variation (CV) from our laboratory was 2.3%.

Muscle swelling was measured on the dominant leg midway between the greater trochanter and the lateral epicondyle of the femur. Thigh circumference (TC) was measured in an anatomical position using an anthropometric tape measure (HaB Direct Southam Warwickshire). To ensure consistent measurements between testing days, the measurement site was marked with a semi-permanent pen and was carried out by the same experienced researcher who was blinded to the experimental conditions.

DOMS was assessed via 200 mm visual analogue scale (VAS). Participants stood in anatomical position with hands on hips and were asked to hold a half squat (90° knee angle). The far-left of the 200 mm

line represented ‘no pain’ while the far-right represents ‘extremely painful’. Participants were asked to mark their perceived soreness on the scale[23].

Each CMJ was performed with hands on hips to assess lower limb muscular power. After a standardised warm-up (five incremental sub-maximal CMJ efforts), three maximal CMJ efforts were performed separated by 60 s recovery. Participants stood on a portable electronic matt (FLS Electronics Ltd. Jump matt. Ireland) and dropped to a self-selected level (approximately a 90° knee angle) before jumping maximally. Jump height (cm) was automatically calculated from the equation: $h=g \cdot t^2 /8$ (where h is the jump height in metres; g is gravitation acceleration [9.81 $m \cdot s^{-2}$]; t is the flight time in seconds). The maximal value was recorded and used for evaluation. This has been quantified to reduce the CV to < 5%[24].

*Maximal Voluntary Isometric Contractions (MVIC)*

Knee extension peak torque of the dominant leg was measured via a digital strain gauge (MIE Digital Myometer; MIE Medical Research Ltd. Leeds). Participants were seated in a standardised position with arms folded across their shoulders and both hips and knees flexed at 90º, measured prior to each contraction via a goniometer to minimise error (Bodycare Products, Warwickshire, UK). Straps were placed across the torso and hips to prevent any unwanted movement. Participants were required to extend their knees as hard as possible for 3 s. As per the CMJ test, measures for MVIC followed a warm-up consisting of five incremental sub-maximal knee extension efforts before commencing three maximal efforts separated by 60 s recovery, with the maximal value was recorded and used for evaluation (CV to < 5%)[24].

All data are reported as means ± SD. To account for inter individual variation all variables have been presented as a percentage change relative to baseline and presented in addition to absolute values. Differences between conditions and trials for variables measured were analysed using a repeated two-way ANOVA (Treatment, 3 x Time, 5), with time as the within-subjects factor and treatment as the

between-subject factor. A bonferonni *post-hoc* pair-wise comparison was calculated following significant interactions. All data were analysed using SPSS 21 (SPSS Inc., Chicago, IL) with statistical significance set at $p < 0.05$.

**Results**

There were no baseline differences between groups for MVIC (N) ($P > 0.05$). Expressed as a percentage of baseline values, there was an effect of time for MVIC ($P < 0.05$) and an interaction between time and group ($F_{(8,80)} = 2.31$, $P = 0.027$), with higher ($P < 0.05$) values demonstrated in the repeated IPC group at 24-h, 48-h and 72-h (90.4 ± 7.0 %, 101.5 ± 8.4 % and 103.1 ± 4.7 %, respectively) compared to the sham (81.4 6.7 %, 84.4 ± 7.0 % and 89.7 ± 7.1 %, respectively). The acute IPC group was also higher ($P < 0.05$) than SHAM at 24 h, 48 h and 72 h (91.2 ± 9.7 %, 92.6 ± 12.7 % and 99.1 ± 10.7 %, respectively) but was lower than the repeated IPC at 48 h ($P < 0.05$) (Figure 1).

There were baseline differences in TC between groups ($P < 0.05$). As a percentage of baseline values, there was an effect of time for thigh circumference ($P < 0.05$) and an interaction between time and group ($F_{(8,80)} = 4.1$, $P = 0.003$), with higher ($P < 0.05$) values demonstrated in the repeated IPC group at 24 h compared to acute IPC (101.8 ± 1.4 % *vs.* 100.4 0.7 %, respectively). There were no other pairwise differences between acute IPC and repeated IPC at any subsequent time-point. By 48 h (100.2 ± 0.5 % *vs.* 101.3 ± 0.6 %, respectively) and 72 h (99.5 ± 0.8 % *vs.* 100.9 ± 0.7 %, respectively), the repeated IPC group were lower than SHAM. Similarly, the acute IPC group was lower ($P < 0.05$) than the SHAM at 24 h (100.4 ± 0.7 %) and 48 h (100.8 ± 1.1 %) (Figure 2).

As a percentage of baseline values, there was an effect of time for CMJ ($P < 0.001$) but no interaction between time and group ($F_{(8,80)} = 1.49$, $P = 0.216$) (Figure 3). Similarly, there was an effect of time ($P < 0.05$) for the VAS scores but no interaction with group ($F_{(8,80)} = 0.67$, $P = 0.711$). [CK] responses were also affected by time ($P < 0.05$) but no interactions were found with group ($F_{(8,80)} = 0.77$, $P = 0.473$) (Figures 4-5).

## Discussion

Our aim was to examine the dose-response of a single and repeated application of IPC preceding a bout of EIMD, to facilitate recovery. We observed that both acute and repeated IPC enhanced recovery in MVIC versus SHAM. Importantly, repeated application of IPC facilitated a faster recovery than acute IPC (Figure 1). In addition, we observed lower levels of swelling in the 48-72 hr following EIMD for both acute and repeated IPC relative to SHAM but no differences were noted between the different IPC protocols in other indices of muscle damage including CMJ, muscle soreness or [CK] responses. Taken together, these findings lend support to the theory that both acute and repeated IPC may provide faster recovery of force and reduced swelling following EIMD, however repeated IPC provides greater recovery of MVIC than acute IPC alone.

The current study demonstrated that repeated IPC was more effective at maintaining MVIC relative to acute IPC and SHAM. This was evidenced by a MVIC returning to baseline levels 48 h post-exercise, relative to a 72 h time-course in the acute IPC condition. However, the acute IPC condition facilitated better maintenance of MVIC at all time points with respect to the SHAM condition. These findings support previous observations, where acute IPC[13] or ICPost[12] before or following an EIMD protocol maintained the contractile properties of the muscle and recovery of MVIC, respectively. Therefore, we further the current understanding by showing that either acute or repeated IPC could help to rapidly re-establish baseline levels of muscle strength following strenuous exercise among athletes, the effect of which is heightened with repeated applications. This could be particularly important during competitive periods of tournaments or seasons, which are characterised by clustered match and training schedules and, therefore, require interventions that facilitate player readiness.

EIMD occurs as a result of primary and secondary damage. The reduced force production observed in the days following eccentric damage, denoted as the primary phase, is caused by disruption of the contractile and non-contractile apparatus, followed by membrane damage and subsequent excitation-contraction coupling dysfunction[1]. After the primary phase, movement of $Ca^{2+}$ into the cytoplasm

causes further damage initiating an inflammatory cascade which stimulates tissue repair mechanisms and facilitates muscular adaptation[2]. IPC has historically been investigated in a clinical context, whereby it has been shown to confer protection from ischemic reperfusion (I/R) injury[4], which has similar properties to that observed during EIMD. This metabolic and contractile damage observed following IR injury, is similar to that seen in EIMD, namely increased intracellular calcium concentrations[25] and an increase in appearance of muscle proteins in the blood and cytokine markers such as [CK], lactate dehydrogenase and interleukin-6[5]. Preliminary work using an animal model showed that acute IPC can improve skeletal muscle function, assessed via electromyography, following a prolonged ischemic insult[26]. This early work by Phillips et al.[26] proposed that IPC could enhance function in damaged tissue. A possible mechanism by which IPC could attenuate the decline observed in force production is a change in $Ca^{2+}$ metabolism. Franz and colleagues[13] demonstrated acute increases in muscle stiffness, which they suggest may demonstrate a post-exercise potentiation, induced by modified $Ca^{2+}$ responsiveness and stiffness of the muscle's contractile properties.

Following EIMD, a loss of muscle force is observed, which returns to baseline within 72 hrs[3]. Peripheral mechanisms such as excitation-contraction coupling dysfunction[1] explain some of the changes observed with muscle force following EIMD, however central factors may play a role. Contractile properties are impaired immediately and for several days post EIMD, in contrast, voluntary activation rates are impaired immediately following EIMD but not in subsequent days[27]. The mechanisms by which IPC exerts its effect are complex and remain to be fully elucidated. IPC may increase the excitability of motoneurons as evidenced by increasing EMG recruitment[28,29] and maximal force[30] alongside delaying neuromuscular fatigue[31]. In contrast, IPC has no effect on voluntary activation, evoked twitch torques and potentials[32]. Muscle torque complexity, which represents the ability to modulate motor output rapidly and accurately[33], is reduced for 24 h following EIMD[34]. IPC has been shown to blunt the loss of torque complexity fatigue [35] and may potentially explain the attenuation of muscle force loss following EIMD in the current study. Future research should aim to explain the central and peripheral factors related to the attenuation of force loss following IPC and investigate the neuromuscular system's adaptations, which may explain the greater attenuation following repeated IPC.

Minor changes in thigh circumference suggest that IPC may have reduced swelling 48-72 h following EIMD. However, a notable increase occurred at 24 h following EIMD in the repeated IPC group. EIMD has been shown to alter vascular function, decreasing perfusion in the muscle. Specifically, increases in arterial stiffness[36], reductions in endothelial function[37] and altered capillary hemodynamics[38] have been reported. The application of repeated IPC has been shown to induce favourable changes in the vasculature[15,20,21,] it is, plausible that 3 days of repeated IPC may have conferred some positive vascular adaptations which facilitated increased perfusion in the 24 h following EIMD. The subsequent reduction in swelling could be related to the more rapid inflammatory response or a function of IPC's role in reducing reactive oxygen species (ROS) production, which typically initiates inflammatory processes[39]. In addition, IPC has also been shown to increase levels of adenosine and nitric oxide (NO), both of which are potent vasodilators[40].

Muscle soreness increased following EIMD, but there were no significant differences between conditions. This follows the typical pattern of EIMD whereby perception of soreness peaks between 24 - 48 h as a result of prostaglandin synthesis, which sensitise the afferent endings located within the muscle fibres[41]. Reductions in postoperative pain have been observed following IPC[9]; however, this was not supported by the current study. [CK] concentration, as an intramuscular indice of muscle damage, increased with time, peaking at 24 h following EIMD, which follows the identifiable time-course of muscle damage 24-48 h following an EIMD protocol[1]. However, there were no differences between conditions, suggesting that IPC did not appear to modify the [CK] response imposed by the protocol. However, it should be noted that the sensitivity in detecting [CK] and the presence of high and low responders, could explain these null effects considering the real functional decrements noted in the other tests in this study.

The aim of this study was to examine the dose-response of a single and repeated application of IPC preceding a bout of EIMD, to facilitate recovery. However, there are some potential limitations with this work. Firstly, we did not match the volume of IPC applications between conditions (Acute (3 x 5

min ~ 15 mins) vs Repeated (3 days x 3x5 min ~ 45 min), therefore the repeated IPC group experienced 45 mins of IPC vs 15 mins in the acute IPC group. This may explain the different recovery responses observed for MVIC in both conditions. Previous investigations into the optimal ergogenic IPC dose have shown no benefit of further applications in a single setting (4 x 5 min vs 8 x 5 min) before a cycling test[42], however future research should investigate if the same is observed for recovery protocols. Secondly, this study has investigated the use of acute and repeated IPC following an eccentric focussed EIMD protocol. This was chosen as it has previously successful in inducing muscle damage[23]. However, the recovery profile of EIMD may not be similar to more athletic events and exercise and thus future research should investigate this method in a more applied setting to see if the benefits confer between protocols.

**Conclusion**

In conclusion, IPC prevents force decrements and accelerates the recovery of MVIC following EIMD. Furthermore, repeated IPC was more effective than an acute administration, suggesting that the muscle may adapt to repeated IPC applications and thus reduce the damaging effects of IPC. Further work should examine the mechanisms behind this adaptation, including neuromuscular qualities as well as the impact in more applied sporting contexts.

**Practical Implications**

- Single and repeated applications of ischemic preconditioning, by a means of a tourniquet, can improve recovery of strength and reduce muscle swelling following exercise induced muscle damage.
- IPC may be applied following strenuous exercise and / or sports performance to help with faster recovery.

303 • IPC may also benefit athletes during competitive periods of tournaments or seasons, which are
304 characterised by clustered match and training schedules to facilitate player readiness.

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**Figures**

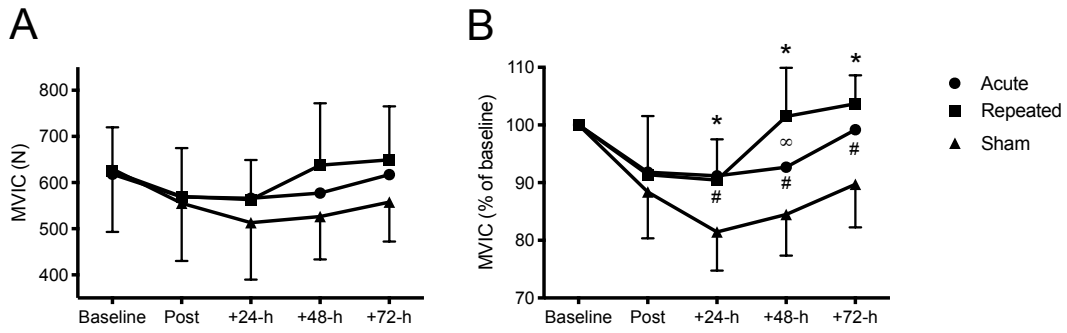


Figure 1. Maximal voluntary isometric contraction (MVIC) following an acute IPC, repeated IPC and sham intervention at baseline and following EIMD (A) averaged raw data, (B) shown relative to baseline expressed as 100%. Values are expressed as mean ± SD. * Indicates repeated IPC significantly different from SHAM # indicates acute IPC significantly different from SHAM, ∞ Indicates repeated IPC significantly different from acute IPC, $P < 0.05$.

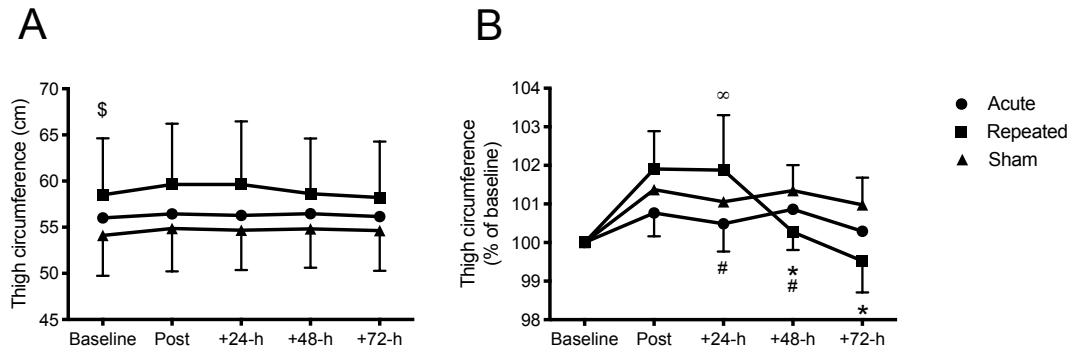


Figure 2. Thigh circumference measured following acute IPC, repeated IPC and SHAM intervention at baseline and following EIMD (A) averaged raw data, (B) shown relative to baseline expressed as 100%. Values are expressed as mean ± SD. * Indicates repeated IPC significantly different from SHAM # indicates acute IPC significantly different from SHAM, ∞ Indicates repeated IPC significantly different from acute IPC, $ represents a baseline difference across all raw data. $P < 0.05$.

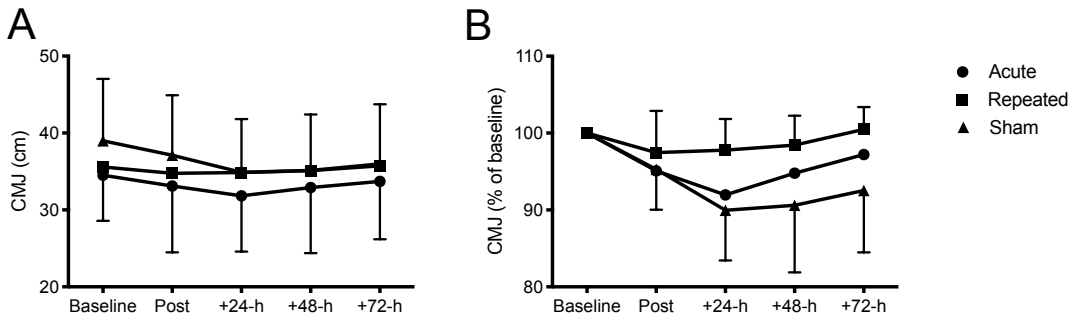


Figure 3. Countermovement jump (CMJ) following acute IPC, repeated IPC and SHAM intervention at baseline and following EIMD (A) averaged raw data, (B) shown relative to baseline expressed as 100%. Values are expressed as mean ± SD.

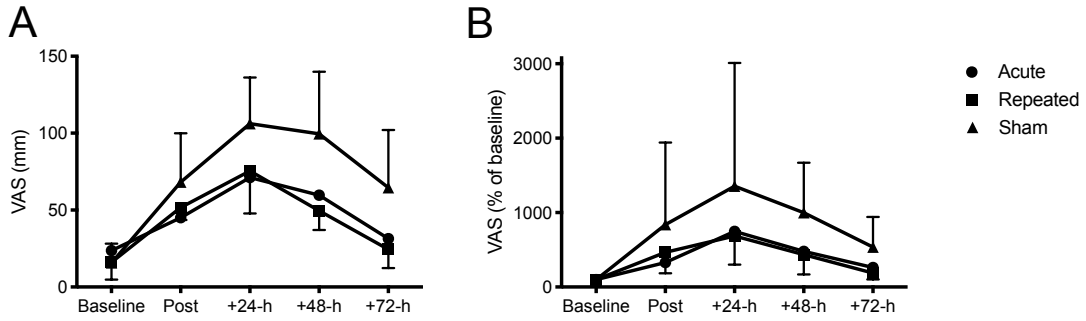


Figure 4. VAS scores following acute IPC, repeated IPC and SHAM intervention at baseline and following EIMD (A) averaged raw data, (B) shown relative to baseline expressed as 100%. Values are expressed as mean ± SD.

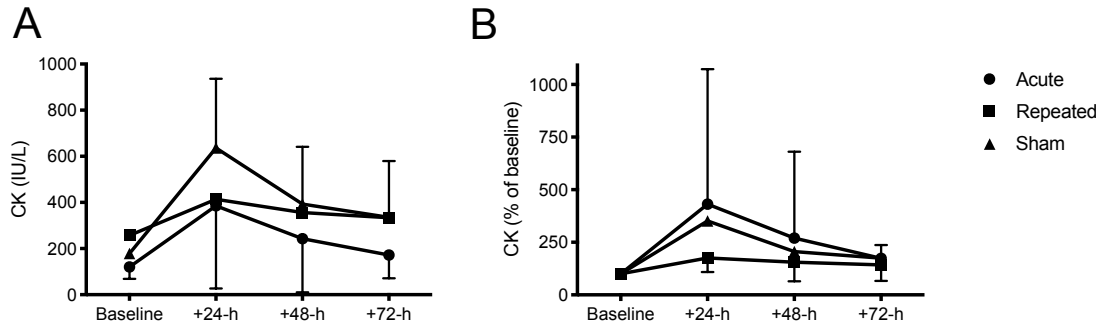


Figure 5. Creatine kinase ([CK]) following acute IPC, repeated IPC and SHAM intervention at baseline and following EIMD (A) averaged raw data, (B) shown relative to baseline expressed as 100%. Values are expressed as mean ± SD