

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

Stephen D Patterson¹, Rachael Swan¹, William Page¹, Moacir Marocolo², Owen Jeffries³, Mark Waldron^{4,5}

- 1. Faculty of Sport, Allied Health & Performance Sciences, St Marys University, London, UK.**
- 2. Department of Physiology, Federal University of Juiz de Fora, Juiz de Fora, Minas Gerais, Brazil.**
- 3. School of Biomedical, Nutritional and Sport Sciences, Newcastle University, Newcastle Upon Tyne, UK**
- 4. College of Engineering, Swansea University, Swansea, UK.**
- 5. School of Science and Technology, University of New England, NSW, Australia**

Running Title: Ischemic Preconditioning and muscle damage

Corresponding Author

Dr Stephen D Patterson

Faculty of Sport, Allied Health & Performance Sciences, St Marys University, Waldegrave Road, Strawberry Hill, Twickenham, UK.

Phone : +442082402357

Fax: +442082404212

Email: Stephen.Patterson@stmarys.ac.uk

1 **Abstract**

2 **Objectives:** The aim of this investigation was to determine if acute or repeated applications of ischemic
3 preconditioning (IPC) could enhance the recovery process, following exercise induced muscle damage
4 (EIMD). **Design:** Randomized control trial. **Methods:** Twenty-three healthy males were familiarised
5 with the muscle damaging protocol (five sets of 20 drop jumps from a 0.6 m box) and randomly
6 allocated to one of three groups: SHAM (3 x 5 min at 20 mmHg), Acute IPC (3 x 5 min at 220 mmHg)
7 and Repeated IPC (3 days x 3 x 5 min at 220 mmHg). The indices of muscle damage measured included
8 creatine kinase concentration ([CK]), thigh swelling, delayed onset muscle soreness, counter movement
9 jumps (CMJ) and maximal voluntary isometric contraction (MVIC). **Results:** Both acute and repeated
10 IPC improved recovery in MVIC versus SHAM. Repeated IPC led to a faster MVIC recovery at 48 h
11 (101.5%) relative to acute IPC (92.6%) and SHAM (84.4%) ($P < 0.05$). Less swelling was found for
12 both acute and repeated IPC vs. SHAM ($P < 0.05$) but no group effects were found for CMJ, soreness
13 or [CK] responses ($P > 0.05$). **Conclusion:** Taken together, repeated IPC can enhance recovery time of
14 MVIC more than an acute application, and both reduce swelling following EIMD, relative to a SHAM
15 condition.

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

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28 **Introduction**

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30 Exercise induced muscle damage (EIMD), a typical response to eccentric or unaccustomed exercise,
31 results in structural damage within the muscle, including disruption to the sarcomere and dysfunction
32 of excitation-contraction coupling¹ and an increased inflammatory cascade². These changes result in
33 reduced muscle force production and function, increased muscle swelling, delayed onset muscle
34 soreness (DOMS) and increased appearance of muscle proteins in the blood in the days following
35 exercise³. These symptoms are exacerbated between 24 - 72 h post exercise, gradually decreasing to
36 baseline thereafter, but may be attenuated with recovery strategies³.

37

38 Ischemia is a phenomenon of inadequate blood supply and, thus, oxygen delivery to meet the metabolic
39 demands of the organs and tissues^{4,5}. Such prolonged interruptions to the blood supply of organs and
40 tissues may result in ischemia reperfusion (IR) injury^{4,5}. IR injury underpins the damage caused by
41 pathologies, surgery, and organ transplantations, which are complicated by interruption of the blood
42 supply to tissues, resulting in cellular dysfunction, apoptosis and cell death⁴. Therefore, strategies have
43 been developed to improve tissue tolerance to ischemia or reduce the damage caused by IR injury.
44 Furthermore, the responses to IR injury are similar to those observed following EIMD, namely
45 increased intracellular calcium concentrations and an increase in appearance of muscle proteins in the
46 blood and cytokine markers such as creatine kinase ([CK]), lactate dehydrogenase and interleukin-6⁵.

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48 Ischemic conditioning (IC), a process that typically applies short (5 min) intermittent periods of vascular
49 occlusion, followed by reperfusion, has been shown to protect cardiac and skeletal tissue from metabolic
50 and contractile damage, caused by IR injury, if applied before (pre-conditioning [IPC]) or following
51 (post-conditioning [ICPost]) an ischemic event⁶. These brief cycles of ischemic conditioning prime the
52 targeted tissue and bestow protection for future IR stress⁷ or reduce ischemic stress post injury⁸. Indeed,
53 the application of IC has been beneficial in reducing post-operative knee surgery pain⁹ and oxidative
54 stress following knee surgery¹⁰.

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56 Recently, IC has been shown to accelerate the recovery process following strenuous exercise¹¹⁻¹⁴.
57 Possible mechanisms explaining these ergogenic effects may include increased blood flow¹⁵, reduction
58 in oxidant generation¹⁰, elevated adenosine levels¹⁶ and/or reducing the inflammatory response¹⁷.
59 However, the results for the use of IPC or ICPPost EIMD are inconclusive. ICPPost has shown some
60 potential, as evidenced by improved recovery in functional measures of athletic performance, including
61 repeated sprint ability and jump height, 24 h following several different ischemic interventions¹² and
62 cycling performance¹¹. Furthermore, Page et al.¹⁴ demonstrated that ICPPost could improve recovery of
63 muscle force production, DOMS and reduce [CK] following an EIMD protocol. Alternatively, the
64 application of IPC applied before exercise has received little attention regarding recovery from exercise
65 and the findings are inconsistent. Franz et al.¹³ demonstrated that IPC applied before EIMD exercise
66 resulted in attenuated responses of [CK], DOMS and muscle force properties. In contrast Northey et
67 al.¹⁸ did not observe any benefits of IPC applied before a resistance exercise protocol on recovery.

68
69 Based on the current evidence, the application of IPC before strenuous exercise and its impact on
70 recovery is inconclusive. Whilst acute application of IPC may provide protection and thus help recovery
71 this is not always the case. In clinical populations acute IPC is attenuated in preventing ischemic injury⁷
72 whereas if the application of IPC is repeated over a number of days then protection is conferred¹⁹.
73 Therefore, it appears that an increased 'dose' of IPC may overcome some of the issues associated with
74 exposure to a single bout of IPC. Research from our own lab suggests that repeated application of IPC
75 across a number of days enhances skeletal muscle adaptations (skeletal muscle oxidative capacity and
76 enhanced microvascular blood flow), independent of exercise¹⁵, which has been supported
77 elsewhere^{20,21} and could explain observed improvements in exercise performance²². Therefore, the
78 application of repeated IPC may be beneficial in augmenting the recovery process following exercise.

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

84

85 **Methods**

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87 Twenty-three healthy males (age, 23 ± 3 years; height, 180 ± 7 cm; 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 box²³. Participants, with their hands placed on their hips, were asked to step off the box with their

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

113

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

121

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

129

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

135

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

138 line represented ‘no pain’ while the far-right represents ‘extremely painful’. Participants were asked to
139 mark their perceived soreness on the scale²³.

140

141 Each CMJ was performed with hands on hips to assess lower limb muscular power. After a standardised
142 warm-up (five incremental sub-maximal CMJ efforts), three maximal CMJ efforts were performed
143 separated by 60 s recovery. Participants stood on a portable electronic matt (FLS Electronics Ltd. Jump
144 matt. Ireland) and dropped to a self-selected level (approximately a 90° knee angle) before jumping
145 maximally. Jump height (cm) was automatically calculated from the equation: $h = g \cdot t^2 / 8$ (where h is the
146 jump height in metres; g is gravitation acceleration [9.81 m·s⁻²]; t is the flight time in seconds). The
147 maximal value was recorded and used for evaluation. This has been quantified to reduce the CV to <
148 5%²⁴.

149

150 *Maximal Voluntary Isometric Contractions (MVIC)*

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

161

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

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

169

170 **Results**

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

178

179 There were baseline differences in TC between groups ($P < 0.05$). As a percentage of baseline values,
180 there was an effect of time for thigh circumference ($P < 0.05$) and an interaction between time and
181 group ($F_{(8,80)} = 4.1, P = 0.003$), with higher ($P < 0.05$) values demonstrated in the repeated IPC group
182 at 24 h compared to acute IPC ($101.8 \pm 1.4\%$ vs. $100.4 \pm 0.7\%$, respectively). There were no other
183 pairwise differences between acute IPC and repeated IPC at any subsequent time-point. By 48 h (100.2
184 $\pm 0.5\%$ vs. $101.3 \pm 0.6\%$, respectively) and 72 h ($99.5 \pm 0.8\%$ vs. $100.9 \pm 0.7\%$, respectively), the
185 repeated IPC group were lower than SHAM. Similarly, the acute IPC group was lower ($P < 0.05$) than
186 the SHAM at 24 h ($100.4 \pm 0.7\%$) and 48 h ($100.8 \pm 1.1\%$) (Figure 2).

187

188 As a percentage of baseline values, there was an effect of time for CMJ ($P < 0.001$) but no interaction
189 between time and group ($F_{(8,80)} = 1.49, P = 0.216$) (Figure 3). Similarly, there was an effect of time (P
190 < 0.05) for the VAS scores but no interaction with group ($F_{(8,80)} = 0.67, P = 0.711$). [CK] responses
191 were also affected by time ($P < 0.05$) but no interactions were found with group ($F_{(8,80)} = 0.77, P =$
192 0.473) (Figures 4-5).

193

194 **Discussion**

195

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

205

206 The current study demonstrated that repeated IPC was more effective at maintaining MVIC relative to
207 acute IPC and SHAM. This was evidenced by a MVIC returning to baseline levels 48 h post-exercise,
208 relative to a 72 h time-course in the acute IPC condition. However, the acute IPC condition facilitated
209 better maintenance of MVIC at all time points with respect to the SHAM condition. These findings
210 support previous observations, where acute IPC¹³ or ICP¹² before or following an EIMD protocol
211 maintained the contractile properties of the muscle and recovery of MVIC, respectively. Therefore, we
212 further the current understanding by showing that either acute or repeated IPC could help to rapidly re-
213 establish baseline levels of muscle strength following strenuous exercise among athletes, the effect of
214 which is heightened with repeated applications. This could be particularly important during competitive
215 periods of tournaments or seasons, which are characterised by clustered match and training schedules
216 and, therefore, require interventions that facilitate player readiness.

217

218 EIMD occurs as a result of primary and secondary damage. The reduced force production observed in
219 the days following eccentric damage, denoted as the primary phase, is caused by disruption of the
220 contractile and non-contractile apparatus, followed by membrane damage and subsequent excitation-
221 contraction coupling dysfunction¹. After the primary phase, movement of Ca²⁺ into the cytoplasm

222 causes further damage initiating an inflammatory cascade which stimulates tissue repair mechanisms
223 and facilitates muscular adaptation². IPC has historically been investigated in a clinical context,
224 whereby it has been shown to confer protection from ischemic reperfusion (I/R) injury⁴, which has
225 similar properties to that observed during EIMD. This metabolic and contractile damage observed
226 following IR injury, is similar to that seen in EIMD, namely increased intracellular calcium
227 concentrations²⁵ and an increase in appearance of muscle proteins in the blood and cytokine markers
228 such as [CK], lactate dehydrogenase and interleukin-6⁵. Preliminary work using an animal model
229 showed that acute IPC can improve skeletal muscle function, assessed via electromyography, following
230 a prolonged ischemic insult²⁶. This early work by Phillips et al.²⁶ proposed that IPC could enhance
231 function in damaged tissue. A possible mechanism by which IPC could attenuate the decline observed
232 in force production is a change in Ca²⁺ metabolism. Franz and colleagues¹³ demonstrated acute increases
233 in muscle stiffness, which they suggest may demonstrate a post-exercise potentiation, induced by
234 modified Ca²⁺ responsiveness and stiffness of the muscle's contractile properties.

235

236 Following EIMD, a loss of muscle force is observed, which returns to baseline within 72 hrs³. Peripheral
237 mechanisms such as excitation-contraction coupling dysfunction¹ explain some of the changes observed
238 with muscle force following EIMD, however central factors may play a role. Contractile properties are
239 impaired immediately and for several days post EIMD, in contrast, voluntary activation rates are
240 impaired immediately following EIMD but not in subsequent days²⁷. The mechanisms by which IPC
241 exerts its effect are complex and remain to be fully elucidated. IPC may increase the excitability of
242 motoneurons as evidenced by increasing EMG recruitment^{28,29} and maximal force³⁰ alongside delaying
243 neuromuscular fatigue³¹. In contrast, IPC has no effect on voluntary activation, evoked twitch torques
244 and potentials³². Muscle torque complexity, which represents the ability to modulate motor output
245 rapidly and accurately³³, is reduced for 24 h following EIMD³⁴. IPC has been shown to blunt the loss
246 of torque complexity fatigue³⁵ and may potentially explain the attenuation of muscle force loss
247 following EIMD in the current study. Future research should aim to explain the central and peripheral
248 factors related to the attenuation of force loss following IPC and investigate the neuromuscular system's
249 adaptations, which may explain the greater attenuation following repeated IPC.

250

251 Minor changes in thigh circumference suggest that IPC may have reduced swelling 48-72 h following
252 EIMD. However, a notable increase occurred at 24 h following EIMD in the repeated IPC group. EIMD
253 has been shown to alter vascular function, decreasing perfusion in the muscle. Specifically, increases
254 in arterial stiffness³⁶, reductions in endothelial function³⁷ and altered capillary hemodynamics³⁸ have
255 been reported. The application of repeated IPC has been shown to induce favourable changes in the
256 vasculature^{15,20,21}, it is, plausible that 3 days of repeated IPC may have conferred some positive vascular
257 adaptations which facilitated increased perfusion in the 24 h following EIMD. The subsequent reduction
258 in swelling could be related to the more rapid inflammatory response or a function of IPC's role in
259 reducing reactive oxygen species (ROS) production, which typically initiates inflammatory processes³⁹.
260 In addition, IPC has also been shown to increase levels of adenosine and nitric oxide (NO), both of
261 which are potent vasodilators⁴⁰.

262

263 Muscle soreness increased following EIMD, but there were no significant differences between
264 conditions. This follows the typical pattern of EIMD whereby perception of soreness peaks between 24
265 - 48 h as a result of prostaglandin synthesis, which sensitise the afferent endings located within the
266 muscle fibres⁴¹. Reductions in postoperative pain have been observed following IPC⁹; however, this
267 was not supported by the current study. [CK] concentration, as an intramuscular indice of muscle
268 damage, increased with time, peaking at 24 h following EIMD, which follows the identifiable time-
269 course of muscle damage 24-48 h following an EIMD protocol¹. However, there were no differences
270 between conditions, suggesting that IPC did not appear to modify the [CK] response imposed by the
271 protocol. However, it should be noted that the sensitivity in detecting [CK] and the presence of high
272 and low responders, could explain these null effects considering the real functional decrements noted
273 in the other tests in this study.

274

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

278 min ~ 15 mins) vs Repeated (3 days x 3x5 min ~ 45 min), therefore the repeated IPC group experienced
279 45 mins of IPC vs 15 mins in the acute IPC group. This may explain the different recovery responses
280 observed for MVIC in both conditions. Previous investigations into the optimal ergogenic IPC dose
281 have shown no benefit of further applications in a single setting (4 x 5 min vs 8 x 5 min) before a cycling
282 test⁴², however future research should investigate if the same is observed for recovery protocols.
283 Secondly, this study has investigated the use of acute and repeated IPC following an eccentric focussed
284 EIMD protocol. This was chosen as it has previously successful in inducing muscle damage²³. However,
285 the recovery profile of EIMD may not be similar to more athletic events and exercise and thus future
286 research should investigate this method in a more applied setting to see if the benefits confer between
287 protocols.

288

289 **Conclusion**

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

295

296 **Practical Implications**

297

- 298 • Single and repeated applications of ischemic preconditioning, by a means of a tourniquet, can
299 improve recovery of strength and reduce muscle swelling following exercise induced muscle
300 damage.
- 301 • IPC may be applied following strenuous exercise and / or sports performance to help with faster
302 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.

305

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Figures

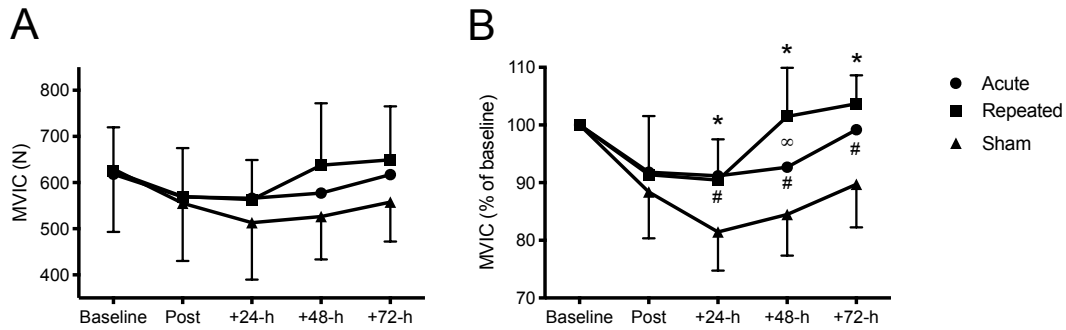


Figure 1. Maximal voluntary isometric contraction (MVIC) following an acute IPC, repeated IPC and sham intervention at baseline and following EIMD (A) averaged raw data, (B) shown relative to baseline expressed as 100%. Values are expressed as mean \pm SD. * Indicates repeated IPC significantly different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

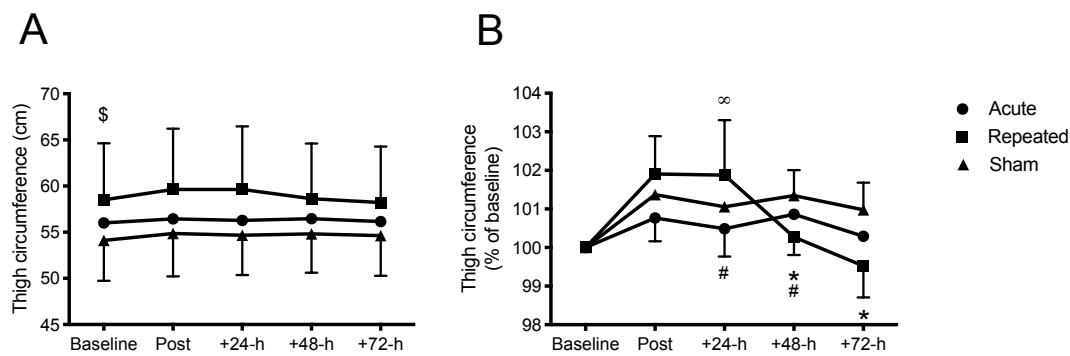


Figure 2. Thigh circumference measured following acute IPC, repeated IPC and SHAM intervention at baseline and following EIMD (A) averaged raw data, (B) shown relative to baseline expressed as 100%. Values are expressed as mean \pm SD. * Indicates repeated IPC significantly different from SHAM # indicates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

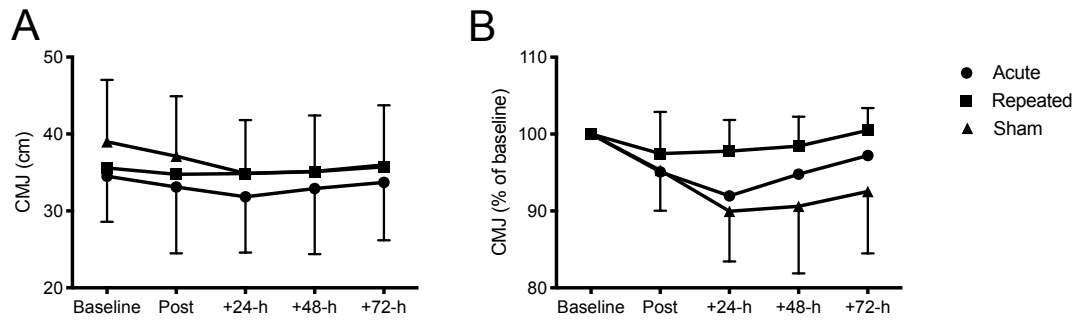


Figure 3. Counter movement jump (CMJ) following acute IPC, repeated IPC and SHAM intervention at baseline and following EIMD (A) averaged raw data, (B) shown relative to baseline expressed as 100%. Values are expressed as mean \pm SD.

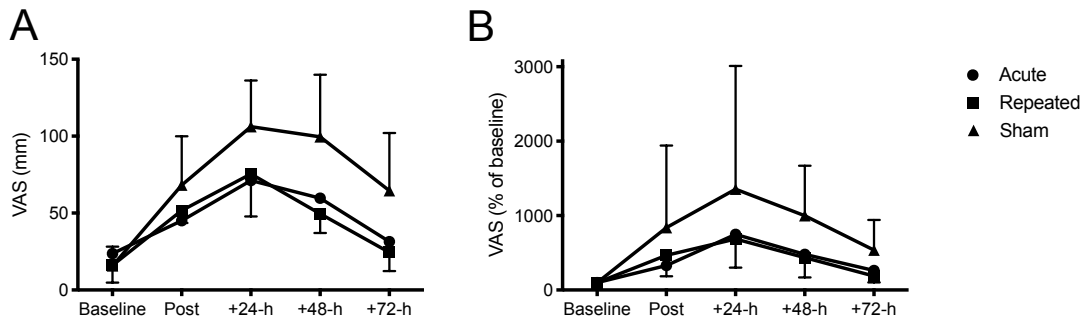


Figure 4. VAS scores following acute IPC, repeated IPC and SHAM intervention at baseline and following EIMD (A) averaged raw data, (B) shown relative to baseline expressed as 100%. Values are expressed as mean \pm SD.

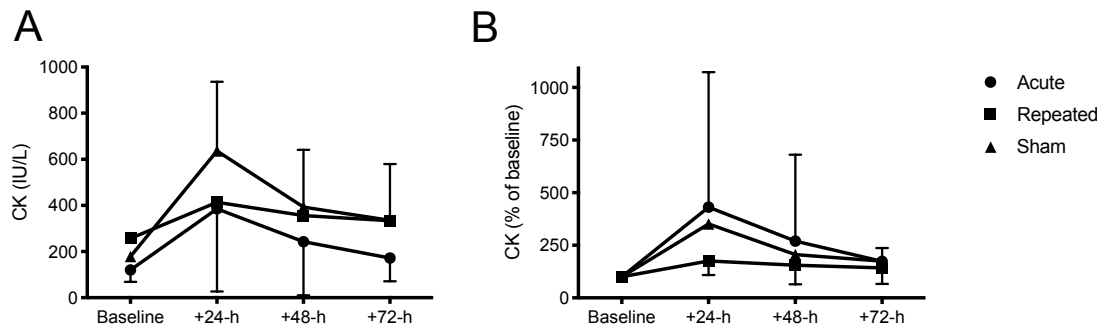


Figure 5. Creatine kinase ([CK]) following acute IPC, repeated IPC and SHAM intervention at baseline and following EIMD (A) averaged raw data, (B) shown relative to baseline expressed as 100%. Values are expressed as mean \pm SD

