Acute ischemic preconditioning does not influence high-intensity intermittent exercise performance

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This study evaluated the acute effect of ischemic preconditioning (IPC) on a high-intensity intermittent exercise performance and physiological indicators in amateur soccer players. Thirteen players (21.5 ± 2 yrs) attended 3 trials separated by 3-5 days in a counterbalanced randomized cross-over design: IPC (4x5-min occlusion 220 mmHg/reperfusion 0 mmHg) in each thigh; SHAM (similar to the IPC protocol but "occlusion" at 20 mmHg) and control (seated during the same time of IPC). After 6-min of each trial (IPC, SHAM or control), the players performed the YoYo Intermittent Endurance Test level 2 (YoYoIE2). The distance covered in the YoYoIE2 (IPC 867 ± 205 m; SHAM 873 ± 212 m; control 921 ± 206 m) was not different among trials (p = 0.10), furthermore, lactate concentration and rate of perceived exertion did not differ ($P > 0.05$) among protocols. There were also no significant differences in either mean heart rate (HR) or peak HR (p > 0.05) for both IPC and SHAM compared to control. Therefore, we conclude that acute IPC does not influence high-intensity intermittent exercise performance in amateur soccer players and that rate of perceived exertion, heart rate and lactate do not differ between the intervention IPC, SHAM and control.

1 **Acute ischemic preconditioning does not influence high-intensity intermittent exercise**

2 **performance**

4 Short Title: **Ischemic preconditioning and yoyo test**

6 Research Article

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25

26   **Abstract**

27   This study evaluated the acute effect of ischemic preconditioning (IPC) on a high-intensity

28   intermittent exercise performance and physiological indicators in amateur soccer players.

29   Thirteen players (21.5 ± 2 yrs) attended 3 trials separated by 3-5 days in a counterbalanced

30   randomized cross-over design: IPC (4x5-min occlusion 220 mmHg/reperfusion 0 mmHg) in each

31   thigh; SHAM (similar to the IPC protocol but "occlusion" at 20 mmHg) and control (seated

32   during the same time of IPC). After 6-min of each trial (IPC, SHAM or control), the players

33   performed the YoYo Intermittent Endurance Test level 2 (YoYoIE2). The distance covered in

34   the YoYoIE2 (IPC 867 ± 205 m; SHAM 873 ± 212 m; control 921 ± 206 m) was not different

35   among trials (p = 0.10), furthermore, lactate concentration and rate of perceived exertion did not

36   differ ($P > 0.05$) among protocols. There were also no significant differences in either mean

37   heart rate (HR) or peak HR (p > 0.05) for both IPC and SHAM compared to control. Therefore,

38   we conclude that acute IPC does not influence high-intensity intermittent exercise performance

39   in amateur soccer players and that rate of perceived exertion, heart rate and lactate do not differ

40   between the intervention IPC, SHAM and control.

41   **Key-words**: blood occlusion, physical training, ischemia, skeletal muscle, ergogenic.

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Introduction

The technique of ischemic preconditioning (IPC) alternates brief periods of occlusion and re-establishment (e.g., 3 to 5-min each) of muscle blood flow prior to exercise using a tourniquet applied in the proximal part of a limb (e.g., lower limbs) (Marocolo et al. 2016a) aiming to improve exercise performance. The ergogenic effects are still debatable (Marocolo et al. 2016a) especially because some study designs are limited (e.g., without placebo-control) (da Mota & Marocolo 2016) and the mechanism behind IPC as well as its supposed exercise enhancement has yet to be clarified. Potential mechanisms of IPC exercise enhancement are hyperemia (5-6 fold increase in muscle blood flow) during reperfusion (Libonati et al. 2001), attenuated ATP depletion, and increased phosphocreatine production/oxygen uptake during the reperfusion phase (Andreas et al. 2011). Additionally, IPC has been suggested to improve aerobic and anaerobic performance (Cruz et al. 2015; Incognito et al. 2016) mostly in high-intensity exercise in cyclic modalities (e.g., running, cycling, swimming).

Although one study (Gibson et al. 2015) evaluated the IPC effect in athletes with a history of participation in team sport and others in repeated sprint cycling (Patterson et al. 2015), no study has examined its effects on high-intensity intermittent exercise (i.e., specific to team sports). The YoYo intermittent endurance test level 2 (YoYoIE2) simulates high-intensity intermittent exercise similar to the activity seen in soccer, and is effective due to its simplicity and reproducibility (Bradley et al. 2011), and it is correlated with both oxygen uptake and citrate synthase activity in untrained men (Krustrup et al. 2015). Although Krustrup et al. (2015) stated that aerobic energy production plays a "more important role" for fatigue resistance in untrained subjects versus trained male soccer players during the YoYoIE2, their results showed also an important contribution of the glycolytic pathway in the YoYoIE2 (e.g. blood lactate ~11 mmol at 5-min of recovery). Since IPC seems to improve performance in exercise tests of predominantly lactic anaerobic and aerobic capacity (Incognito et al. 2016), the aim of this study was to evaluate the acute effect of IPC on YoYoIE2 performance and some physiological parameters in amateur soccer players. We hypothesized that IPC would increase the distance covered in YoYoIE2, because this test for our specific sample (amateur players) would be suitable to recruit predominantly both the glycolytic and aerobic systems and, therefore, meet the ergogenic potential from IPC (Cruz et al. 2015; Incognito et al. 2016).

Materials and Methods

Thirteen male amateur soccer players (21.5 ± 2 yrs, 173 ± 5 cm, 69.7 ± 6 kg, 12.6 ± 5 body fat %) volunteered for this study. The players participated in 2.7 ± 0.5 h.wk^{-1} of training and 1.3 ± 0.2 matches per week and playing experience of 11 ± 4 yrs. Inclusion criteria were: a) soccer playing experience more than 5 yrs, b) no smoking history during the last year, c) absence of any cardiovascular or metabolic disease, d) systemic blood pressure lower than 140/90 mmHg and no use of antihypertensive medication, e) no use of creatine supplementation, anabolic steroids, drugs or medication with potential effects on physical performance (self-reported) and g) no recent musculoskeletal injury. This study was approved by the local institutional Ethical Committee for Human Experiments (Federal University of Triangulo Mineiro - 993.636/2015) and was performed in accordance with ethical standards in sports science research (Harriss & Atkinson 2015). In addition, all subjects signed an informed consent form. Based on prior research (Bradley et al. 2011), a sample size less than 13 (i.e., n between 6 and 9) was sufficient to detect a significant ($p < 0.05$) difference among playing positions ("group") in the YoYoIE2 (main dependent variable). Also, a study using a similar IPC protocol (independent variable) and physical test duration, reported significant statistical effects with a sample size of 12 individuals (Cruz et al. 2015). Thus, to counteract any potential drop out, a sample of 13 subjects was included for this study.

Experimental design of the study

Subjects attended the laboratory five times (with 3 to 5 days in-between), for initial screening and anthropometric measurements and for familiarization with the equipment and the test. In the 3rd, 4th and 5th visits, a randomized crossover assignment (IPC, SHAM and control) was adopted and the YoYoIE2 was carried out after 6-min of each trial (IPC, SHAM or control). All YoYoIE2 tests were conducted by the same experienced researcher in a constant environment ($23 \pm 2°$ C; Humidity: 75 ± 4 %) at the same time of the day (9:00 - 11:00 h). To prevent the possibility of a placebo (positive) effect (Marocolo et al. 2017; Marocolo et al. 2016b; Marocolo et al. 2016c), all subjects were informed that all conditions could improve performance. Also, in order to prevent nocebo (negative) effects the subjects were informed that IPC and SHAM would cause absolutely no harm, despite discomfort related to the maneuvers (Ferreira et al. 2016).

109   Additionally, the tester was blinded for which protocol (i.e. IPC, SHAM or control) the subjects

110   had undergone before. Also the subjects were kept blinded in relation to performance and other

111   indicators until the end of the research, i.e., no information about distance covered (the audio of

112   speed and level of YoYoIE2 was in unknown language), HR and lactate. Coffee (or caffeine

113   products), tea, and alcohol intake was prohibited as well as strenuous exercise for 48 hours

114   before testing (Incognito et al. 2016).

115

116   *Ischemic preconditioning protocol*

117   The IPC maneuver consisted of four cycles of 5-min occlusion (220 mmHg) and 5-min

118   reperfusion (no pressure) in each thigh (total duration 40-min), using a pneumatic tourniquet

119   (komprimeter Riester®, Jungingen, Germany) administered at the subinguinal region of the

120   thighs. We applied this specific IPC protocol for the following reasons: a) several studies have

121   successfully explored the ergogenic effects of IPC on exercise performance with the same

122   protocol (Cruz et al. 2015; Jean-St-Michel et al. 2011; Marocolo et al. 2016a; Patterson et al.

123   2015); b) at least three ischemia-reperfusion cycles are necessary to protect against skeletal

124   muscle infarction and endothelial dysfunction after prolonged periods of imposed ischemia

125   (Pang et al. 1995) and c) it is considered safe and well tolerated in both patients and healthy

126   volunteers (Gonzalez et al. 2014). The occlusion and reperfusion phases were conducted

127   alternately between the thighs, with subjects remaining seated (knee at 90º angle). The

128   effectiveness of occlusion in the IPC session was controlled by auscultation of the arteries

129   around the ankle (Loenneke et al. 2012). In the SHAM protocol, an external pressure of 20

130   mmHg was administered, as proposed in previous studies (Foster et al. 2011; Marocolo et al.

131   2016a). For the control condition, the subjects remained seated for a respective period of time.

132   Although a consensus does not exist with regards to the time between the last IPC cycle and the

133   exercise testing, ranging from 5-min to 90-min in most studies with exercise performance

134   (Marocolo et al. 2016a), the IPC has been shown to improve exercise performance within 45-min

135   of the final cuff inflation (Bailey et al. 2012; Patterson et al. 2015). Thus, in the current study,

136   the warm-up to the YoYoIE2 was performed after 6-min of each trial (i.e. IPC, SHAM or

137   control).

138

139   *Perceived recovery status*

140 Before each session, all subjects indicated a score on a perceived recovery scale (Laurent et al.
141 2011), (from 0 "very little recovered, feeling extremely tired" to 10 "very well recovered, feeling
142 with great energy"), about their relative physical recovery to make sure the player was in the
143 same recovery condition before the trials. If a player scored 4 or less (somewhat recovered) for
144 his recovery status, he was excluded from that day's session and invited to wait until the next day
145 to perform the session.

146

147 *Yo-Yo Intermittent Endurance Test Level 2 (YoYoIE2):*

148 After 6-min of recovery from each trial (IPC, SHAM or control) the warm-up for the YoYoIE2
149 began. The warm-up consisted of performing the first three running bouts of the YoYoIE2
150 followed by a period of lower-extremity stretching. All players were familiar with the YoYoIE2
151 because they used this test on regular basis for fitness evaluation purpose. The YoYoIE2 consists
152 of a repeated 2 x 20-m shuttle run at progressively increasing speed stages (initial speed ~12
153 km.h-1), guided by specific audio (5-s to recovery in a marked 2.5 x 2 m area behind the
154 finishing line). Cessation of the test was assessed by failure to reach the finish line by the tone on
155 two occasions(Bradley et al. 2011). The YoYoIE2 is a reproducible, sensitive tool associated
156 with soccer match performance and can differentiate intermittent exercise performance of several
157 standards and is therefore highly suggested for soccer players (Bradley et al. 2014; Bradley et al.
158 2012). For consistency, the subjects performed the YoYoIE2 in groups of three or four (always
159 the same group) and they received similar verbal encouragement during the tests.

160

161 *Heart rate (HR), rate of perceived exertion (RPE) and blood lactate concentration:*
162 HR was monitored throughout the entire YoYoIE2, by an individual RS800CX heart monitor
163 (Polar Electro®, Kempele, Finland). After the YoYoIE2, the player indicated (individually to
164 prevent influence from other player) a score for his RPE via CR-10 Borg scale in order to
165 determine the subjective intensity of the session. This scale ranges from 0 to 10, where 0 is
166 "nothing at all" and 10 is "very very hard (maximal)" (Borg 2007; Borg & Kaijser 2006). Blood
167 samples (25 µL) were collected from the fingertip 4-min post YoYoIE2 using a lancet, and the
168 lactate concentration was measured via a valid (Fell et al. 1998) portable lactate analyzer
169 (Accutrend® Plus system, ROCHE, Basel Switzerland).

170

171   *Statistical analysis*

172   The Shapiro-Wilk test was applied to verify the normal distribution of the data. For between-

173   protocol analysis, one-way analysis of variance (ANOVA) for repeated measures was conducted,

174   followed by post-hoc Tukey's test or nonparametric ANOVA (Friedman test) followed by a

175   post-hoc Dunn's test was performed. Only for the distances covered in the YoYoIE2 we

176   calculated the effect size (ES; Cohen d) to determine the meaningfulness of the difference

177   (practical relevance), classified as: trivial (<0.2), small (> 0.2-0.6), moderate (> 0.6-1.2), large (>

178   1.2-2.0) and very large (> 2.0) as recommended (Batterham & Hopkins 2006). The significance

179   level was set at 0.05. The software used for data analysis was GraphPad® (Prism 6.0, San Diego,

180   CA, USA).

181

182   **Results**

183   Before the trials, the perceived recovery scores were not different ($P = 0.1$) among the protocols

184   (IPC = 6.9 ± 1.7, SHAM = 7.7 ± 1.5 and control = 7.7 ± 0.7). RPE and lactate concentrations did

185   not differ after the trial among all protocols. Mean HR and peak HR presented no difference (p =

186   0.17 and p = 0.06, respectively) among the three trials (Table 1).

187   **Table 1 near here**

188   The distance covered in the YoYoIE2 did not differ ($P = 0.10$; ES = 0.16) among the protocols

189   (Figure 1). The mean distances were: IPC 867 m ($SD = 205$), SHAM 873 m ($SD = 212$) and

190   control 921 m ($SD = 206$). The effect sizes were 0.26 (small) IPC vs control, 0.2 (trivial) SHAM

191   vs control and 0.03 (trivial) IPC vs SHAM.

192   **Figure 1 near here**

193   Figure 2 shows the individual change in distances covered by each player in the YoYoIE2. It

194   should be noted that only three players (~23%) performed better after SHAM and four (~30%)

195   after IPC, while the others virtually covered the same distances or even shorter distances than

196   under control conditions.

197   **Figure 2 near here**

198

Discussion

200 The purpose of this study was to evaluate the acute effect of IPC on YoYoIE2 performance and
201 related physiological indicators in amateur soccer players. Our main findings are that acute IPC
202 did not influence performance in the YoYoIE2. In addition, IPC did not influence perceived
203 exertion, blood lactate concentrations (after the test) or either mean or peak HR in comparison
204 with SHAM and control interventions.

205

206 Our current study is the first to evaluate the IPC effect on a high-intermittent performance test
207 that involves changing of direction, and acceleration/deceleration (YoYoIE2), all of which is
208 important for team sports, specifically soccer (Bradley et al. 2011; Paul et al. 2016; Varley &
209 Aughey 2013). The distance covered in the YoYoIE2 did not differ significantly among the three
210 conditions, showing no influence (i.e., neither positive nor negative), as well as no practical
211 relevance (i.e.; trivial effect sizes) of acute IPC on this sort of test and population. The distance
212 covered in the current study in the control conditions (935 ± 232 m) is higher than that reported
213 for untrained men (665 ± 271 m) but lower than highly trained soccer players (2027 ± 298 m)
214 (Krustrup et al. 2015). Since the scores of perception of recovery were similar before all of the
215 three trials (i.e., players starting under same conditions), basic conditions for comparison of
216 different protocols were ensured.

217

218 According to a recent systematic review (Incognito et al. 2016), the most consistent benefit of
219 IPC would be an improvement in exercise tests with the prevalence of energy coming from
220 glycolytic and aerobic sources. The YoYoIE2 has been designed to stimulate both anaerobic and
221 aerobic pathways; however despite this, IPC had no effect on intermittent exercise lasting ~6-
222 min. These same authors have argued that there are IPC responders (i.e. improvements in
223 performance) and non-responders (i.e., no effects on performance) phenotypes, due to large
224 between-subject variability of results and, therefore, they recommend prudence in the
225 interpretation of mean group changes in exercise performance due to IPC (Incognito et al. 2016).
226 In other words, individual analysis should also be performed to more thoroughly assess the
227 potential ergogenic effects of IPC on exercise performance. Analyzing the individual responses

228   of YoYoIE2 in the current study, we found only three and four players better in SHAM and IPC
229   protocols, respectively. These results suggest that acute IPC does not enhance high-intensity
230   intermittent exercise performance for the majority of the players. Considering that the same acute
231   IPC protocol (i.e., 4 X 5 cycles of occlusion/reperfusion) improved endurance performance
232   (~8%) with exercise time duration very close to ours (~6-min) in recreational cyclists (Cruz et al.
233   2015), we may speculate that the nature of YoYoIE2; i.e., intermittent maximal test, including
234   acceleration and deceleration, changing of directions, important eccentric actions involved,
235   might counterbalance the effect following acute IPC. Future studies should explore this
236   hypothesis more deeply by including other kinds of YoYo test (e.g., recovery level I).

237

238   Recent evidence suggests the possibility of a placebo (positive) or nocebo (negative) effect
239   (Ferreira et al. 2016; Marocolo et al. 2015), but in the current study, this potential bias was
240   controlled by three methods: first, all subjects were informed that both maneuvers could improve
241   performance (SHAM and IPC) and none of them could harm performance; second, we kept the
242   tester blinded in relation to the previous protocol (i.e., the physical tester did not know if the
243   player had performed IPC, SHAM or control) and third, no information about distance covered,
244   HR and lactate were provided.

245

246   The RPE is used in studies as an essential and valid indicator of exercise intensity, in addition to
247   the measurement of traditional physiological variables (Borg & Kaijser 2006). In our study, RPE
248   was checked immediately after each test and reached high values, suggesting near maximal
249   internal intensity in the tests. However, RPE did not differ in any condition. Considering that IPC
250   effects could have any motivational basis on exercise performance (Marocolo et al. 2015;
251   Marocolo et al. 2016b; Marocolo et al. 2016c) the register of the RPE for evaluating
252   psychophysical parameters would assist in intensity measures. Our current results showed that
253   IPC did not affect the RPE and it is consistent with previous studies: after sprints series
254   compared to SHAM (Gibson et al. 2015) and after a five-kilometer race to the control
255   intervention (Bailey et al. 2012).

256

257   Heart rate was measured continuously during the test, and neither peak nor mean HR differed
258   among interventions (IPC, SHAM and CON). One study showed a shortening QT interval during

259   exercise, without any RR interval changes after IPC intervention (Caru et al. 2016). Conversely,

260   in another study (Clevidence et al. 2012) significantly higher values for the same parameters in

261   cyclists were found after IPC intervention. For these authors, the HR increase could be explained

262   by a decrease in compensation to a reduced stroke volume and therefore cardiac output at the

263   time of the IPC maneuver, as previously described (Iida et al. 2007). However, our values of

264   covered distance were not different among interventions; nine and eight subjects covered a little

265   shorter distance after SHAM and IPC, respectively, compared to control, which could explain, at

266   first glance, no difference in values of peak and mean HR during the YoYoIE2 following IPC

267   and SHAM interventions.

268

269   In our study, blood lactate concentrations are in line with the literature for YoYoIE2 (Krustrup et

270   al. 2015) in similar subjects (i.e., untrained) confirming also the significant anaerobic

271   contribution to ATP supply. Another study showed that IPC generated lower blood lactate

272   concentration at submaximal intensity during an incremental running test (Bailey et al. 2012),

273   but in the current study we did not find any difference among IPC, SHAM and control

274   conditions. This difference is probably due to the type of exercise, since in the present study we

275   applied an intermittent maximal test.

276

277   There are several limitations to the present study: the absence of measurements to understand the

278   mechanisms underpinning the IPC/SHAM maneuvers, as well the YoYoIE2, such as muscle

279   blood flow, muscle oxy/deoxyhemoglobin, oxygen uptake, enzyme activities, serum nitric oxide,

280   and partial oxygen/carbon dioxide pressures. Also, considering that reactive oxygen species play

281   an important role in both IPC (Zhu & Zuo 2013) and exercise (He et al. 2016), measurements of

282   reactive oxygen species for both IPC and exercise test would be interesting. Our sample size (n

283   = 13) was relatively small, and it may generate a type II error. However, it is higher than one

284   study that applied the same IPC protocol and found statistically significant effects from IPC in

285   cyclists performing similar duration of maximal exercise (Cruz et al. 2015). Lastly, despite our

286   control to avoid potential placebo and nocebo effects, we recognize the limited ability to control

287   any psychological factors that might impact performance.

288

289   **Conclusion**

290   Our results demonstrate that acute ischemic preconditioning does not influence the performance

291   of amateur soccer players during the YoYoIE2 test and does not alter the rate of perceived

292   exertion, heart rate (peak and mean) and blood lactate among IPC, SHAM and control. However,

293   as we have not tested the effectiveness of chronic IPC protocols (e.g., for several days or weeks),

294   nor different IPC protocols (e.g., 3 X 3-min occlusion/2-min reperfusion) nor other types of

295   YoYo tests (e.g., recovery level I) and mechanistic measurements (e.g., oxygen uptake, reactive

296   oxygen species, muscle blood flow), these could be a future direction.

297

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Figure 1

Figure 1

The distance covered in the YoYoIE2 test for the trials. IPC = ischemic preconditioning.

Values are mean ± standard deviation (n = 13).
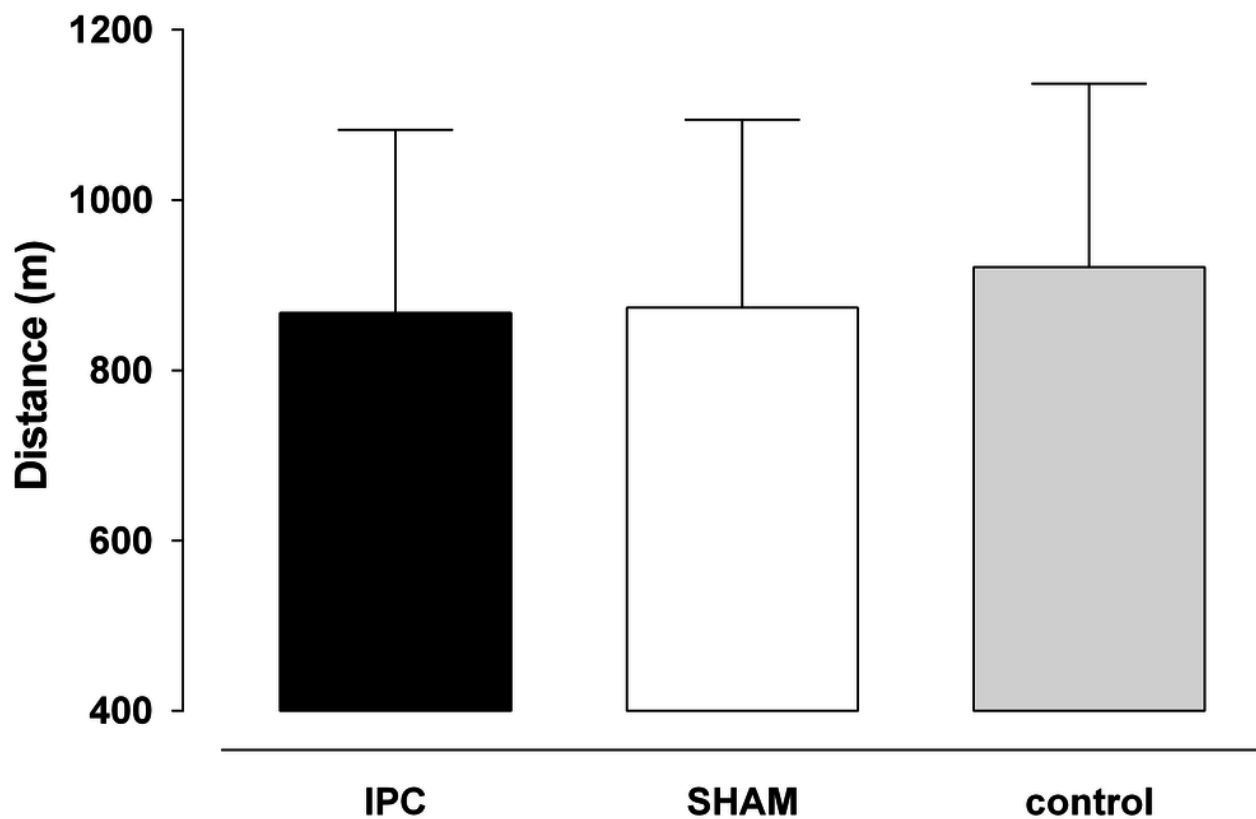
Figure 2

Figure 2

Individual changes with SHAM (A) and ischemic preconditioning - IPC (B) on the distance covered in the YoYoIE2 test. Each black line represents different players (n = 13). The horizontal continuous line represents the mean difference of each intervention from control.
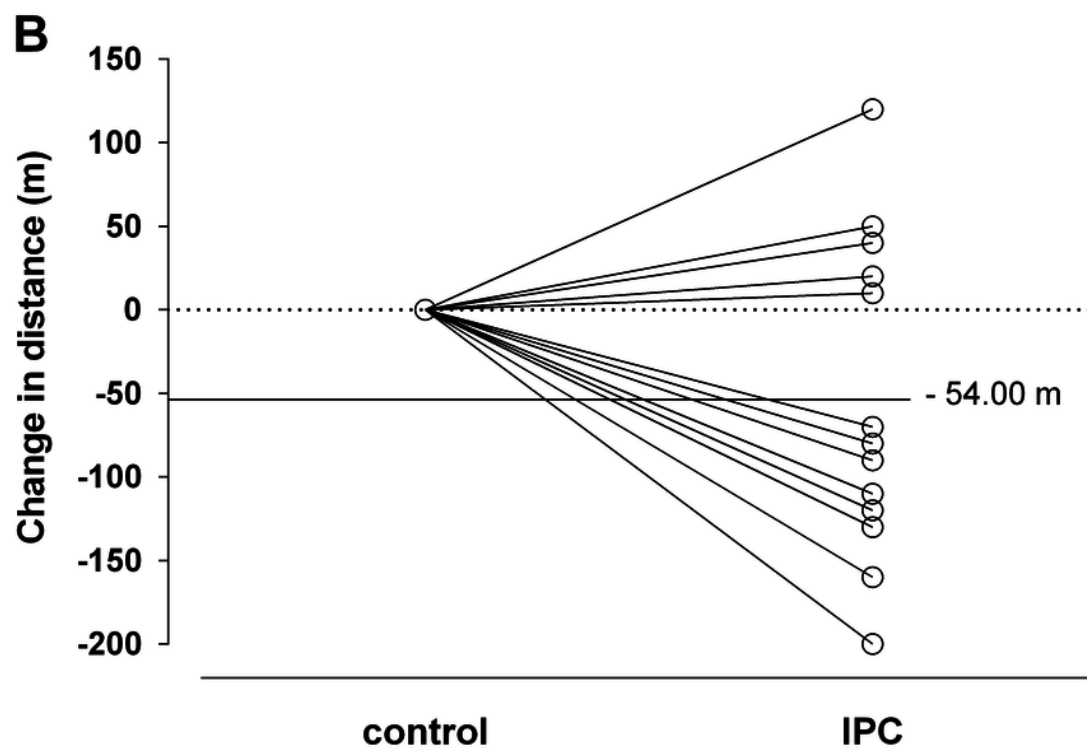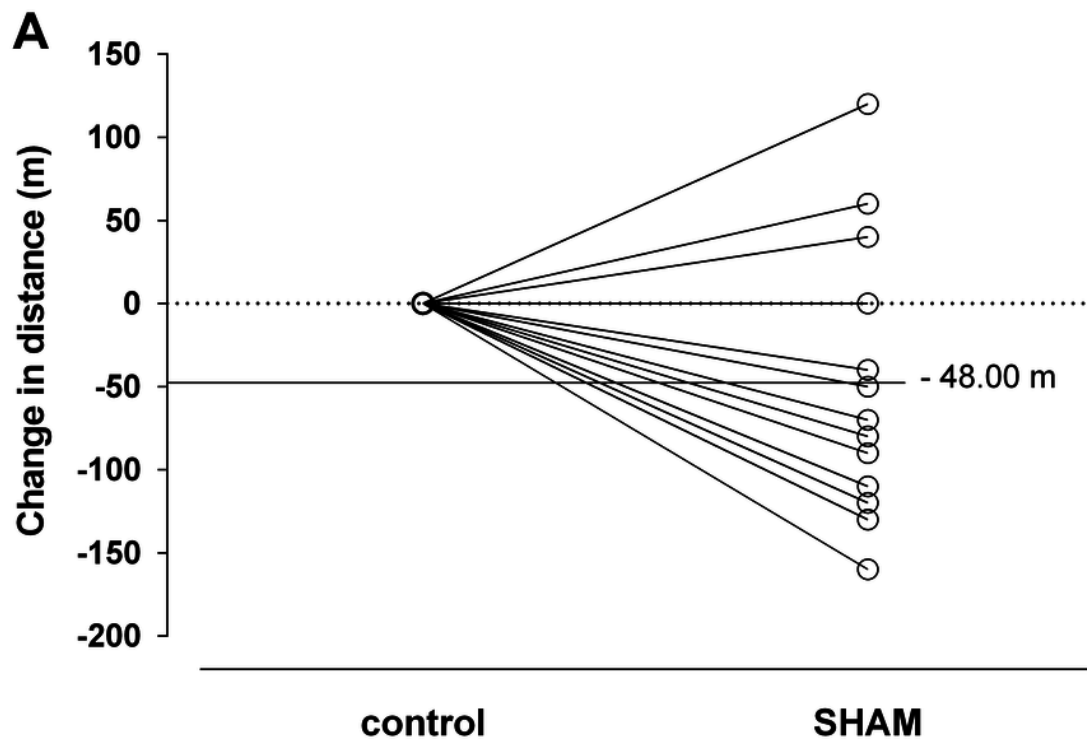
Table 1: Rate of perceived exertion, blood lactate concentration, mean and peak heart rate for the trials.

Values are mean ± standard deviation; (n = 13); HR = heart rate; IPC = ischemic preconditioning, SHAM = placebo IPC.

Variable	IPC	SHAM	control	p value
Rate of perceived exertion	9.0 ± 1.1	8.7 ± 1.3	8.8 ± 0.9	0.69
Blood lactate (mmol.L^{-1})	11.1 ± 2.7	11.4 ± 3	12.7 ± 2.4	0.19
HR mean (bpm)	169 ± 7	169 ± 5	171 ± 6	0.17
HR peak (bpm)	189 ± 5	189 ± 5	191 ± 4	0.06