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1 **Partial-body cryostimulation does not impact peripheral microvascular responsiveness**
2 **but reduces muscular metabolic O₂ consumption (mVO₂) at rest.**

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22 **Abstract.**

23 This study aimed to investigate the effect of partial-body cryostimulation (PBC) on
24 microvascular responsiveness and muscular metabolic O₂ consumption rate (mVO₂). Twenty
25 healthy young adults (ten males and ten females) underwent post-occlusive reactive
26 hyperaemia (PORH) test at the flexor digitorum superficialis area before and after a 3-min
27 PBC session and a 3-min control session. Using near-infrared spectroscopy, occlusion and
28 reperfusion slopes were calculated: oxyhaemoglobin ([HbO₂]) decrease rate ([HbO₂] slope 1),
29 deoxyhaemoglobin ([HHb]) increase rate ([HHb] slope 1), [HbO₂] increase rate ([HbO₂] slope
30 2), and [HHb] increase rate ([HHb] slope 2). Using HbO₂ kinetics during the occlusion, mVO₂
31 was also calculated to characterize myocytes' metabolic O₂ consumption. HbO₂ slope 1 value
32 was lower after PBC than before PBC (-0.15 ± 0.08 vs -0.24 ± 0.11 s⁻¹; respectively; P < 0.05)
33 in male participants only. A lower [HHb] slope 1 was also observed after PBC compared to
34 before PBC (0.18 ± 0.10 vs 0.24 ± 0.16 s⁻¹; P < 0.05) with no interaction for sex categories.
35 mVO₂ was significantly lower after PBC than before (pre values 14.75 ± 3.94 vs 18.47 ± 5.73
36 $\mu\text{MO}_2\text{Hb}\cdot\text{s}^{-1}$; respectively; P < 0.01) with no interaction between sex categories. No changes
37 in the calculated slope 2 were observed. These findings suggest that a single session of PBC
38 reduces the muscular metabolic O₂ needs at rest; however, it does not alter the vascular ability
39 to provide O₂ to the myocytes.

40

41 **Keywords:** Cryotherapy, Cryostimulation, Vascular responsiveness, Muscular oxygenation,

42

43 **Introduction**

44 Over the last decades, cryostimulation has gained popularity in sports medicine as a recovery
45 technique for athletes or an adjunct treatment to relieve symptoms from various chronic
46 diseases [2]. A growing number of studies have shown the beneficial effects of whole-body
47 cryotherapy/cryostimulation (WBC) and partial-body cryotherapy/cryostimulation (PBC) on
48 physiological mechanisms such as oxidative stress [8,35], inflammation [1,9,36], and cardiac
49 autonomic control [15,20,21,39,41]. Such physiological responses to cryotherapy result from
50 thermoregulatory processes triggered by skin temperature reduction [29]. Feedforward
51 mechanisms, initiated by the brain's thermoregulatory center, lead to sympathetic stimulation
52 of peripheral blood vessels and vasoconstriction [6]. Consequently, blood volume is
53 redirected towards the core, increasing central blood pressure and decreasing heart rate
54 through autonomic modulations [32].

55 Another proposed hypothesis concerning the beneficial physiological effects of
56 cryostimulation is that changes in muscle vascularization, such as a reduction of muscle blood
57 flow, might participate in the decrease of inflammatory responses [30,40]. Muscular blood
58 flow following a WBC session has previously been studied, especially in lower limb muscles.
59 It has been reported that a WBC session reduces superficial femoral artery blood flow, as well
60 as vastus lateralis and gastrocnemius oxygenation, immediately after the cold exposure [24].
61 However, it also demonstrated superior muscle oxygenation in rugby players up to 20 minutes
62 after the WBC session compared to the baseline pre-exposure levels [31]. This increased
63 oxygenation following cold exposure is still not fully understood but might be linked to
64 vascular responsiveness mechanisms such as nitric oxide (NO) dependent vasodilation
65 regulated by endothelial function. Although the literature is scarce about the effect of WBC
66 on endothelium functions, previous research reported a beneficial impact of WBC on
67 endothelium inflammatory parameters in various populations [19,20]. NO production capacity
68 was also increased in a healthy older population after a 24 WBC exposure[21]. This greater
69 muscle oxygenation after exposure to cold may also be due to a greater vasodilatory effect
70 than before exposure, also called the 'Hunting effect' [7,19]. To date, the extent of these
71 results seems contradictory, and the effects of cryostimulation on muscular microcirculatory
72 hemodynamic responses are poorly understood. Although the FMD technique is the gold
73 standard measurement of vascular responsiveness, it cannot assess the downstream
74 microvascular function. Therefore, the microvascular function is classically studied through
75 near-infrared spectroscopy (NIRS) by monitoring the reperfusion slope after a vascular

76 occlusion [24–26]. Previous studies have already shown a correlation between FMD and the
77 reperfusion slope assessed by vascular occlusion combined with NIRS [24,27] providing
78 evidence for the reliability of this technique to assess endothelial function at the
79 microcirculatory level.

80 .

81 Several studies have already evaluated that microvascular perfusion and muscle metabolic
82 activity were reduced following cold-water immersion (CWI) protocols [17,18,42]. However,
83 physiological responses to cold water immersion and WBC are not comparable and there still
84 are significant research gaps concerning the effect of PBC on said variables, as well as a need
85 to investigate whether sex differences impact microvascular and metabolic responses
86 following PBC interventions.

87 Therefore, this study aimed to investigate the effect of a 3-min session of PBC at -150 °C on
88 the microvascular responsiveness of the flexor digitorum superficialis muscle, blood pressure,
89 skin temperature, and perceived sensation to cold in young, healthy male and female adults.

90

91 **Materials and Methods**

92 **Participants**

93 Twenty healthy male and female volunteers participated in this study (see Table 1 for
94 participants' characteristics). All participants were young (mean age: 22.3 ± 3.3 years),
95 physically active, and unaccustomed to PBC or cold exposure. Height (m), Body mass (kg),
96 and BMI (kg/m^2) were significantly higher ($p < 0.01$) in male participants compared to female
97 participants. As recommended by the working group of cryotherapy/cryostimulation from the
98 International Institute Refrigeration [3], volunteers presenting contraindications to cold
99 exposure such as hypertension, coronary disease, arrhythmia, circulatory disorders, Raynaud's
100 phenomenon (decreased blood flow to the extremities), cold allergies, pulmonary disease, and
101 obstruction of the bronchus caused by cold exposure were excluded. Only female participants
102 under monophasic contraception were included in the study to control the effects of
103 oestrogens and progesterone in women. Before each experimental session, volunteers were
104 expected to follow pre-experimental criteria such as restraining from physical exercise,
105 caffeine, and alcohol 24h before the session. All volunteers were given an in-depth
106 explanation of the protocol and experimental sessions (including risks and benefits). During

107 the first visit, participants provided written consent and anthropometrics characteristics were
108 measured. The study protocol was approved by local Ethics committee (CERSTAPS, number:
109 2019-26-05-34).

110 *Experimental design*

111 The experimental design is presented in Figure 1. This study was a randomized controlled
112 crossover experiment consisting of two experimental sessions separated by one week and
113 conducted at the same time of the day. Volunteers were randomly assigned to a control
114 session (CONT, where the volunteers stood 3-min in a cryo-cabin at ambient temperature) or
115 to a treatment session (PBC session, where the volunteers stood 3-min at -150°C) for the first
116 session. They switched conditions for the second experimental session. Both experimental
117 sessions were organized in Figure 1 in a counterbalanced fashion for the control and
118 cryotherapy sessions. Upon arrival at the laboratory, male participants were asked to remain
119 in their undergarments while female participants wore their two-piece swimsuits. Every
120 participant was given a bathrobe to be worn between exposures.

121 **Experimental measurements**

122 *Partial body cryotherapy*

123 The PBC session consisted of a 3-min cold exposure in a cryo-cabin (Cryojet, ICE MINI,
124 Paris, France) at -150°C. Following the pre-cooling phase of the cryo-cabin set at -70 °C,
125 participants were asked to enter the cryo-cabin wearing only a swimsuit (women) or
126 underwear (men), gloves, socks, and slippers. Participants were also asked to remove all
127 metallic jewellery to avoid cold-related injuries. The temperature of the cryo-cabin was set at
128 -150°C for the experimental session and remained stable throughout the entire duration of the
129 3-min exposure. During the control condition, participants were asked to follow the exact
130 instructions. However, the cryostimulation cabin was set at room temperature.

131 *Vascular responsiveness*

132 Microvascular responsiveness was assessed through the NIRS technique during a post-
133 occlusive reactive hyperemia test. Oxyhemoglobin ([HbO₂]) and deoxyhemoglobin ([HHb])
134 values were recorded continuously during the post-occlusive reactive hyperemia test. The
135 NIRS probe was placed on the muscle belly of the flexor digitorum superficialis. It was
136 placed 5 cm away from the medial epicondyle toward the distal insertion. A tightened elastic
137 strap secured the probe to reduce movement and external light intrusion. NIRS data were

138 continuously recorded during the test at a 10Hz frequency. A manual pneumatic cuff was
139 placed around the arm, approximately 5 cm above the elbow, to perform the occlusion. The
140 post-occlusive reactive hyperemia test lasted 9 minutes. It consisted of 1-minute baseline
141 measurements, a 5-min occlusion with cuff pressure set at 250mmHg, and a 3-min
142 reperfusion. The variation of [HbO₂] and [HHb] values obtained during the occlusion and the
143 beginning of the reperfusion phase represent a linear response allowing slope calculations and
144 characterization of microvascular responsiveness (Figure 2). As previously described, the rate
145 of decrease in [HbO₂] Slope 1 is an indication of the O₂ consumption rate, whereas the rate of
146 increase in [HbO₂] slope 2 characterizes the microvascular responsiveness [13]. O₂
147 consumption can also be estimated through [HHb] variation since venous and arterial outflow
148 are blocked during the occlusion. [HHb] variations can therefore be explained by changes in
149 O₂ consumption, allowing the use of [HHb] slope 1 to characterize the O₂ consumption rate.
150 [HHb] Slope 2 is also used as an indicator of microvascular responsiveness as the decrease
151 rate of [HHb] is impacted by the vascular motricity of capillaries.

152 Muscular metabolic O₂ consumption during arterial occlusion can also be calculated from the
153 [HbO₂] slope decrease. [HbO₂] concentration variations are expressed in μM.s⁻¹ and
154 converted to mlO₂.min⁻¹.100g⁻¹ using the mVO₂ equation:

$$(mVO_2 = Abs(((\Delta O_2 Hb * 60) / (10 * 1.04)) * 4) * 22.4 / 1000)$$

155 Where 1.04 kg.L⁻¹ represents muscle density, 22.4 L is the molar volume of gas, and 1:4 is the
156 molecular ratio between Hb and O₂.

157 *Skin temperatures*

158 Using a thermal camera Fluke Tis65 (FLUKE Corp, Washington), skin temperatures of the
159 head, left and right arms and trunk were recorded immediately before and after the PBC
160 exposure or control condition. The thermal camera emissivity was set to 0.98 which is the
161 recommended level for the assessment of skin temperature by thermal imaging. The Fluke
162 connect software (FLUKE Corp, Washington) calculated average temperatures by area.
163 Participants were asked to remain standing in the anatomical position approximately 3 meters
164 away from the thermal camera during the capture of the thermogram.

165 *Perceptual measures*

166 Subjective measures for thermal perception were assessed through a visual analogue scale
167 (VAS). Immediately before and after the PBC session or control session, participants' thermal

168 perception was assessed and recorded using an 11 points VAS from 0 to 10 (0 = neutral, 2 =
169 slightly cool, 4 = cool, 6 = cold, 8 = very cold, 10 = unbearably cold) (Lundgren et al. 2014).

170 **Statistical analysis and data distribution tests**

171 Data were presented as mean \pm standard deviation (SD). Data normality was evaluated by the
172 Shapiro-Wilk test. A 3-way analysis of variance (Condition [CONT VS PBC] x sex [Male VS
173 Female] x Time [Pre VS Post]) for repeated measures was performed to analyze the effect of
174 cryotherapy, contingent upon sex, on vascular responsiveness, muscular O₂ consumption rate,
175 HRV parameters, blood pressure, skin temperature, and cold perception. When a significant
176 interaction between Time, Condition, or Sex was found, post-hoc analysis was performed
177 using the Tukey HSD test. For all statistical analyses, alpha was set at $p < 0.05$ to establish
178 significance.

179 **Results**

180 *Skin temperatures*

181 Skin temperatures of the right and left arms, head, and trunk are displayed in Figure 3. Before
182 the PBC exposure, all skin temperatures were similar between the control and PBC group.
183 The ANOVA revealed a significant interaction between time and condition for the right and
184 left arm and the head ($P < 0.01$). Skin temperatures significantly decreased Post-PBC for the
185 right arm (Pre-PBC: 33.32 ± 1.74 vs Post-PBC: 23.67 ± 3.33 °C, $P < 0.01$), left arm (Pre-PBC:
186 32.89 ± 2.06 vs Post-PBC: 24.07 ± 3.07 °C, $P < 0.01$), and head (Pre-PBC: 33.10 ± 2.02 vs
187 Post-PBC: 29.87 ± 3.45 °C, $P < 0.01$) resulting in significantly lower skin temperatures for the
188 right and left arm and for the head following *post-PBC* compared to Post-control. A
189 significant interaction between time, condition, and sex were also found for trunk skin
190 temperatures ($P < 0.05$). Trunk skin temperature significantly decreased *post-PBC* both in
191 males (*pre-PBC*: 34.75 ± 0.91 vs *post-PBC*: 25.70 ± 1.18 °C, $P < 0.01$) and females (*pre-PBC*:
192 32.48 ± 1.49 vs Post-PBC: 21.31 ± 3.94 °C, $P < 0.01$) and significant lower skin
193 temperature values were observed *post-PBC* compared to Post-control. After the PBC
194 session, trunk skin temperature was also lower in the female group compared to the male
195 group (*post-PBC* female: 21.31 ± 3.94 vs *post-PBC* male: 25.70 ± 1.18 °C, $P < 0.01$).

196 *mVO₂*

197 Data for mVO₂ are presented in Figure 4. Baseline values for mVO₂ were identical between
198 the control and PBC groups. The ANOVA revealed a significant interaction between time and

199 condition ($P < 0.01$). Post-hoc analyses indicated a significantly lower value of mVO_2 *post-*
200 *PBC* compared to *pre-PBC* value (*pre-PBC*: 18.47 ± 5.73 vs. *post-PBC*: 14.75 ± 3.94
201 $\mu\text{MO}_2\text{Hb}\cdot\text{s}^{-1}$, $P < 0.01$)

202 *Slopes [HbO₂]*

203 Data of [HbO₂] slopes 1 and 2 are presented in Figure 5.

204 The ANOVA revealed a significant interaction between time, condition, and sex. Post-hoc
205 analyses revealed a significant reduction of [HbO₂] slope 1 after the PBC session only in male
206 subjects (*pre-PBC*: -0.24 ± 0.11 vs *post-PBC*: -0.15 ± 0.08 s^{-1} , $P < 0.05$). Slope 2 was not
207 significantly different between condition and time of measurements.

208 *Slope [HHb]*

209 Data of [HHb] slopes 1 and 2 are presented in Figure 5.

210 A significant interaction between time and condition ($P < 0.01$) was found, and post-hoc
211 analyses revealed statistical differences between groups. A significant reduction of the slope 1
212 in *post-PBC* compared to *pre-PBC* (*pre-PBC*: 0.24 ± 0.16 vs *post-PBC*: 0.18 ± 0.10 s^{-1} , P
213 < 0.05) was found with no interaction between sex categories. Slope 2 was not significantly
214 different between condition and time of measurements.

215 *Thermal sensation*

216 Thermal sensation data are presented in Figure 6. Baseline values were not statistically
217 different between the control and PBC groups, and no effect of sex was found in cold thermal
218 sensation ratings. The ANOVA revealed a significant interaction between time and condition
219 ($P < 0.01$), and post-hoc analyses showed a significantly higher perception of cold *post-PBC*
220 when compared to *pre-PBC* and *post-control* (*post-PBC*: 8.25 ± 0.64 vs *pre-PBC*: 0.80 ± 1.00
221 vs *post-control*: 1.25 ± 1.07 ; $P < 0.01$ for each comparison).

222

223 **Discussion**

224 This study aimed to investigate the effects of partial-body cryostimulation (PBC) on
225 microvascular responsiveness and muscular metabolic O₂ consumption rate (mVO_2). Using a
226 randomized controlled crossover design, the effects of a 3-min PBC session at -150 °C were
227 tested on microvascular responsiveness of a superficial muscle, muscular metabolic O₂

228 consumption, skin temperature, and perceived cold sensation in young, healthy male and
229 female subjects. The main results of this study are a reduction of [HHb] slope 1 and mVO_2
230 after a PBC session, both in male and female participants. Moreover, a PBC session only
231 reduced [HbO₂] slope 1 in male participants.

232 *Microvascular responsiveness and metabolic consumption*

233 In our male participants, *post-PBC* slope 1 of [HbO₂] was lower than *pre-PBC* slope 1 of
234 [HbO₂], demonstrating that cryostimulation induced a lower oxygen consumption in these
235 subjects. However, this phenomenon was not observed in our female participants.
236 Nonetheless, while [HbO₂] slope 2 was similar under control and PBC conditions for both
237 sexes, a lower value was observed in females compared to men in both conditions. This result
238 highlights that men exhibit a higher reperfusion capacity than women, with or without
239 previous cryotherapy exposure. While gender differences in reactive hyperaemia have already
240 been highlighted, with females showing reduced endothelial reactivity compared to men [22],
241 our study, to our knowledge, is the first to highlight a between-sex difference in vascular
242 responsiveness in healthy young adults.

243 Moreover, our study revealed that cryostimulation affects other parameters of microvascular
244 function independently of the participant's gender. PBC impacted [HHb] response during the
245 occlusion phase, as demonstrated by a reduced [HHb] slope 1 following PBC. In addition,
246 mVO_2 was reduced *post-PBC* compared to *pre-PBC*, indicating a lower O₂ metabolic
247 consumption induced by cryotherapy. These results are consistent with previous studies that
248 have established that cold exposure leads to immediate peripheral vasoconstriction and blood
249 flow restriction [12]. Yanagisawa et al. (2007) reported that tissue oxygenation decreased
250 during cooling. The total Hb drop was mainly due to [HbO₂] decline [42]. Also, Stanley et al.
251 (2014) observed that cold water immersion slowed VO_2 on-kinetics, leading to increased
252 anaerobic contribution during high-intensity intermittent exercise compared to the control
253 condition [37].

254 *Skin temperature and cold perception*

255 Skin temperature was significantly lower in male and female participants following the PBC
256 session. Although both males and females displayed significant reductions in trunk skin
257 temperature following PBC, female participants had significantly lower trunk skin
258 temperature than male participants. This particular result highlights a difference in response to
259 cryotherapy between sexes, a phenomenon that has already been reported. Hammond et al.

260 (2014) observed that skin temperature decrease was more significant in females than males
261 after a 2-min WBC session [14]. They also found a negative association between the
262 percentage of skin temperature variation after cryotherapy and body fat %. Indeed, apart from
263 the effect of *sex per se*, differences in body composition could partially explain differences
264 observed in skin temperature after cold exposure. Although we do not have data on body fat
265 % in our participants, we assume that females had a higher fat composition than male subjects
266 (as women generally usually present 14% more body fat than men [5]). Therefore, our
267 findings support the hypothesis that individuals with higher adiposity significantly reduce
268 skin temperature after cooling [4,23,38]

Specific responses in males compared to women

269 Our results confirm that the physiological responses to cold differ between men and women.
270 Indeed, as mentioned above, women seem to have a greater drop in skin temperature than men
271 at an equivalent single dose. This decrease in temperature, probably due to the difference in
272 body composition, will not have the same impact on physiological responses to cold. Authors
273 have reported that hormonal responses differ between men and women [34]. The question
274 then arises whether the duration of 3 minutes is the optimal duration for men and women.
275 According to some authors [28], the duration for women should be shorter than for men. Our
276 results demonstrated that an equivalent dose of cryotherapy could have different effects
277 depending on the participant's anthropometric characteristics. These confirm that
278 cryostimulation should not be a one-size-fits-all approach and that more efficient results will
279 be yielded from a more individualized approach.

280 *What is the implication of the responses obtained in the recovery process?*

281 Following the physical activity, a lower vascularization induced by cryostimulation at the
282 skeletal muscle level may be involved in the recovery process. Several studies demonstrated a
283 faster and more effective recovery from exercise-induced muscle damage following a WBC
284 session. Improvements in recovery have been characterized by an attenuation of DOMS,
285 muscle swelling, and pain [10,11,16]. Such improvements may be linked to a localized
286 reduced blood flow following PBC. Moreover, since reduced muscle vascularization leads to
287 reduced O₂ consumption by muscles, it is plausible to assume that there is an associated
288 reduction in the production of reactive oxygen species. Consequently, oxidative stress would
289 be reduced, representing one of the mechanisms involved in the improvements observed for
290 muscle recovery following cryostimulation [26,33]. With that said, further research is needed
291 to determine the potential effect of cryotherapy on reducing the production and release of free
292 radicals by the muscles. Future studies would be interesting to understand the possible

293 implication of the vascular mechanisms observed in this study and the effects of cryotherapy
294 on post-exercise recovery of power qualities, marked recently by the meta-analysis of Moore
295 and al. (2022) [27].The results of this study must be interpreted in the light of certain
296 experimental considerations. The NIRS technique used in the protocol to assess microvascular
297 reperfusion after the occlusion should be coupled with FMD techniques for a better
298 understanding of the endothelial function. However, FMD is a highly operator-dependent
299 measure, and the time to set up the measurement may vary between sessions and be
300 incompatible with the experimental time points.

301 **Conclusion**

302 This study provides novel data concerning the effect of PBC on peripheral microvascular
303 physiology. Our main finding is that a PBC session does not alter the microvascular
304 vasodilation ability both in male and female subjects. These results suggest that a PBC session
305 does not change the ability of the capillaries to provide O₂ to the muscles. The second
306 noteworthy finding of this study is that a PBC session reduces the O₂ consumption of
307 peripheral muscles at rest, especially in male participants. This could be explained by a
308 reduction of muscle's metabolic rate at rest. The results provided in this study, in their
309 globality, provide original information concerning the effect of PBC on peripheral vascular
310 physiology.

311

312 **Disclosures**

313 No conflicts of interest, financial or otherwise, are declared by the authors

314 **Author contributions**

315 DT, BD, OD conceived and designed the research protocol; DT, BD, OD performed
316 experiments; analyzed data; interpreted results of the investigation; DT, OD prepared the
317 figures; DT, BD, WD, JL, OD, RM,CAC drafted the manuscript; DT, BD, WD, JL, OD,
318 RM,CAC edited and revised the manuscript; DT, BD, WD, JL, OD, RM,CAC approved the
319 final version of manuscript

320

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475 **Figures**

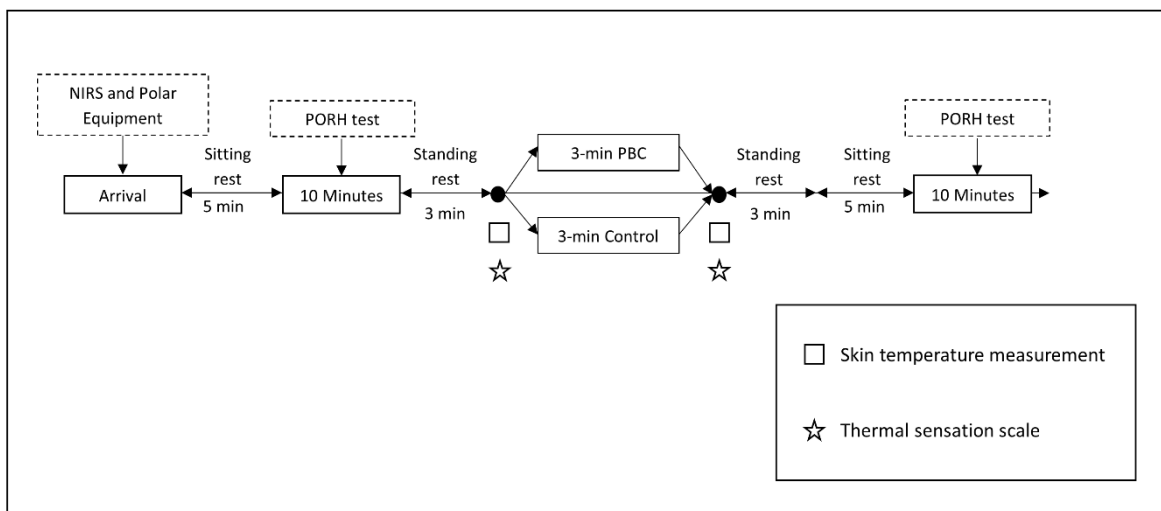
476 **Figure 1**

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483 **Figure 2**

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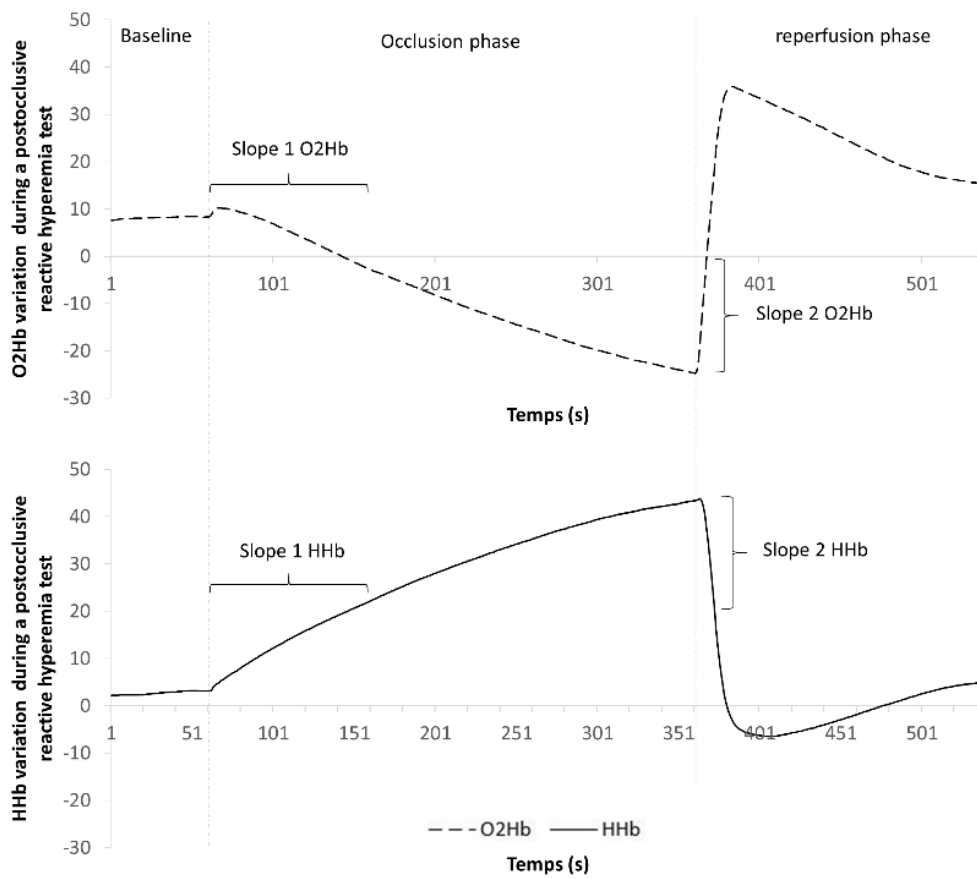
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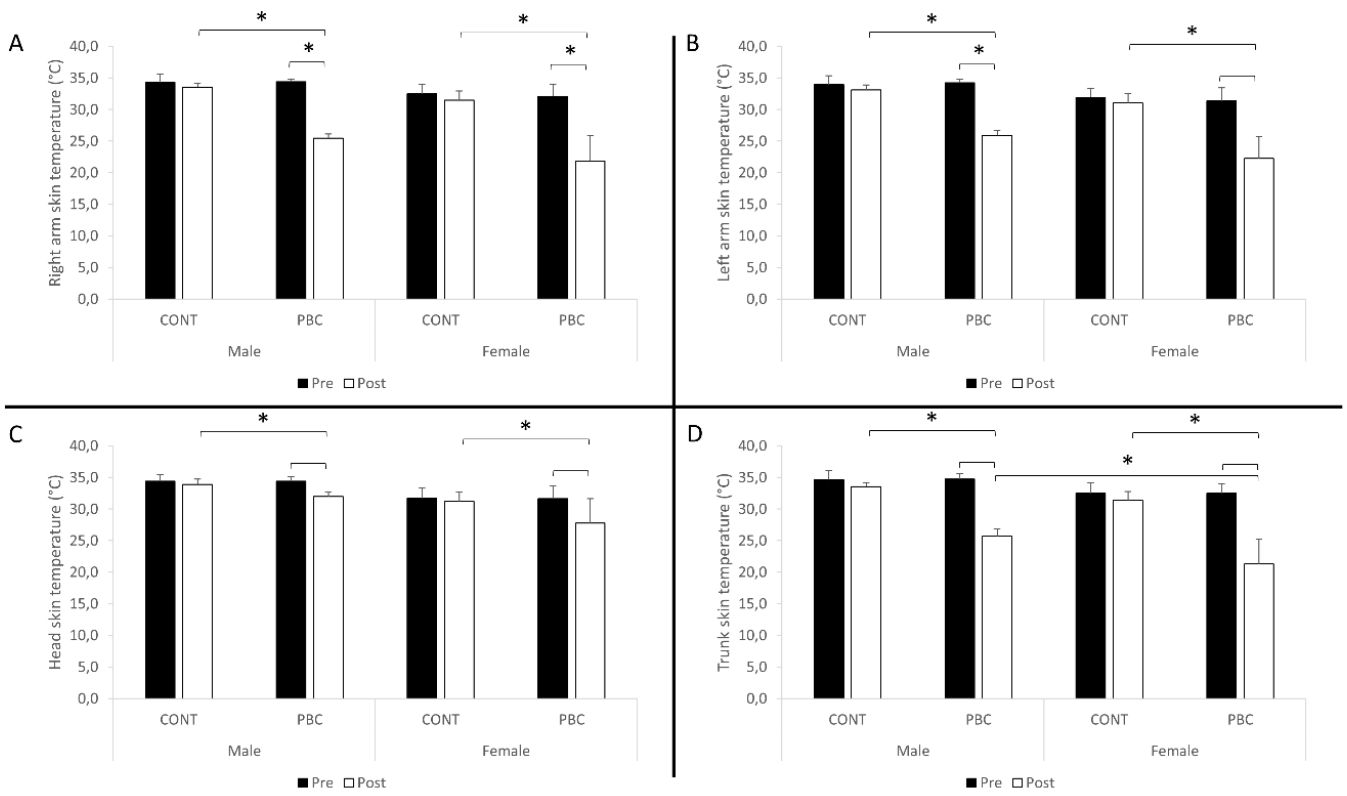
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492 **Figure 3**

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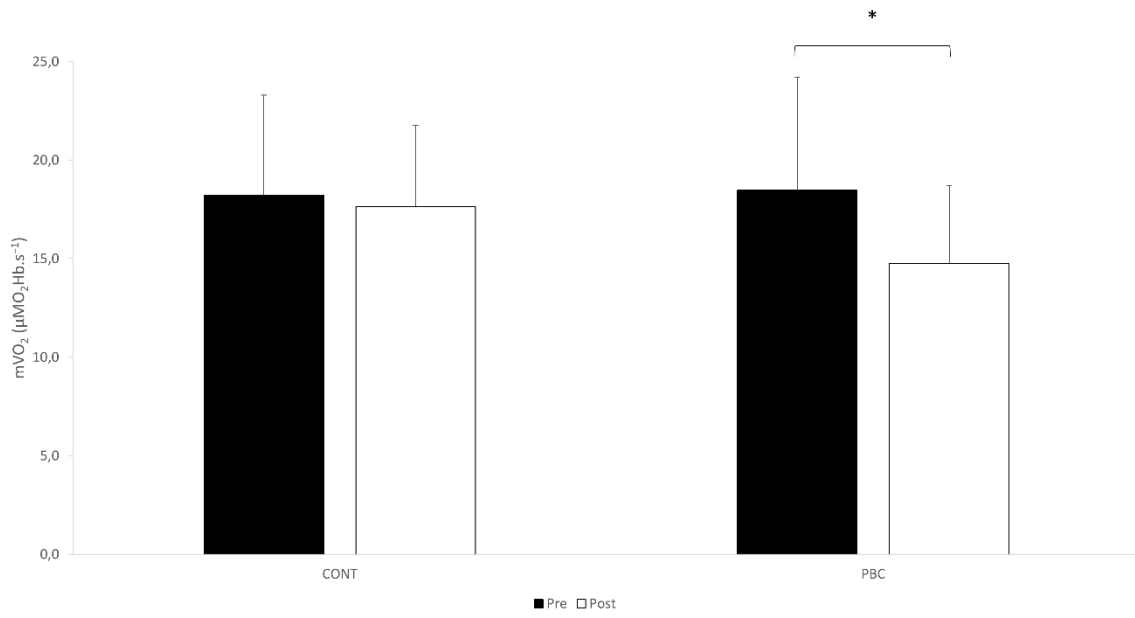
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500 **Figure 4**

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504 **Figure 5**

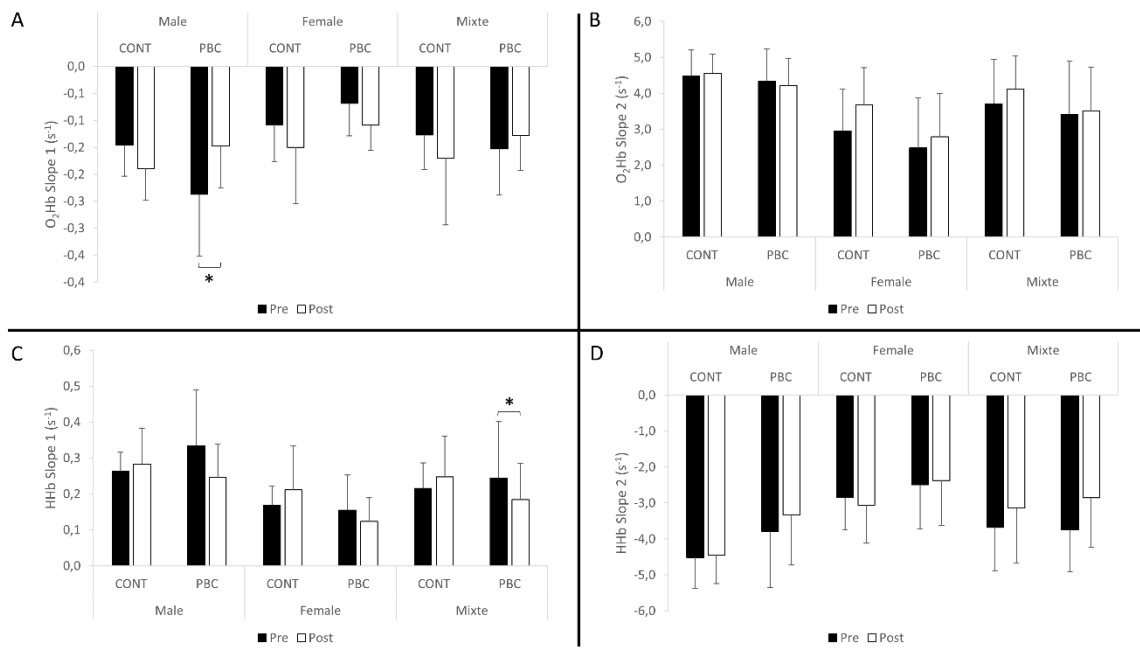
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510 **Figure 6**

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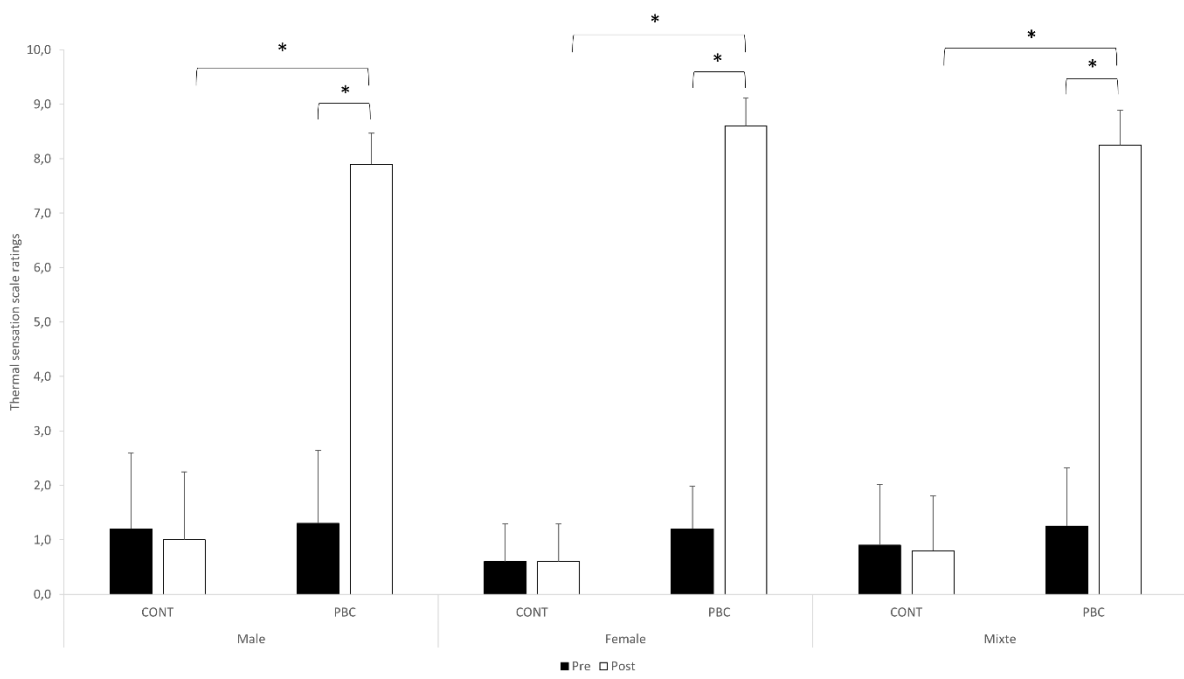
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518 **Captions**

519 **Figure 1:** Experimental protocol

520 **Figure 2:** Presentation of slope 1 and slope 2 during a post-occlusive reactive hyperemia test.

521 **Figure 3:** Right and left arm (panel A and B), trunk (panel C), and head (panel D)
522 temperature before and after a PBC session for male and female participants. * Significant
523 ($P<0.05$)

524 **Figure 4:** $m\dot{V}O_2$ before and after experimental conditions. * Significant ($P<0.05$)

525 **Figure 5:** $[HbO_2]$ slope 1 (panel A) and slope 2 (panel B), $[HHb]$ slope 1 (panel C) and slope
526 2 (panel D) before and after PBC session for male and female participants. * Significant
527 ($P<0.05$)

528 **Figure 6:** Thermal sensation rating before and after PBC session in male and female
529 participants. * Significant ($P<0.05$)

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531 **Tables**

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Table 1 : Participant characteristics

	Age (Years)	Height (m)	Weight (Kg)	BMI (Kg/m ²)
Males	23.10 ± 4.51	1.78 ± 0.09 *	81.00 ± 12.88 *	25.55 ± 2.36 *
Females	21.50 ± 1.27	1.65 ± 0.60	58.26 ± 8.49	21.40 ± 2.41

Data are presented mean ±SD; * significant difference between male and female p<0.05

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