

1 **High-impact routines to ameliorate trunk and lower limbs flexibility in women**

2

3

4 **Abstract**

5 Several types of routines and methods have been experimented to gain neuro/muscular
6 advantages, in terms of overall range of motion, in athletes and fitness enthusiasts. The aim
7 of the present study was to evaluate the impact of different routines on trunk- and lower
8 limbs flexibility in a sample of young women. In a randomized-crossover fashion, eleven
9 subjects underwent to: hamstrings stretching [S]; hamstrings stretching plus whole-body
10 vibration [S + WBV]; partial-body cryotherapy [Cryo]; rest [Control]. Standing hamstrings
11 stretch performance and sit-and-reach amplitude resulted to be improved with [S + WBV]
12 compared to all other protocols ($p < 0.05$). [Cryo] ameliorated the active knee extension
13 performance with respect to all other interventions ($p < 0.05$). These flexibility
14 improvements were obtained without a loss in the trunk position sense proprioception.

15 These results represent the first evidence that a single session of either vibration or
16 cryotherapy can ~~ameliorate favourably impact on~~ flexibility without ~~losing the trunk~~
17 ~~position sense compromising~~ proprioception ~~in young women~~.

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20 **Keywords:** cryotherapy, whole-body vibration, standing hamstrings stretch

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22 **Abbreviations:** AKET, active knee extension test; ES, effect size; ICC, intraclass
23 correlation coefficient; PBC, partial body cryotherapy, ROIs, regions of interest; ROM,
24 range of motion; S, stretching; SHS, standing hamstrings stretch; SD, standard deviation,
25 TRE, trunk repositioning errors, WBC, whole-body cryotherapy; WBV, whole-body
26 vibration

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29 **Introduction**

30 A litany of studies has been repeatedly debating on different methods of stretching
31 to gain advantages in range of motion (ROM) and therefore in flexibility. However, it is
32 still controversial the extent by which a particular stretching routine might be effective in
33 relation to a specific sport rather than in the general population. For instance, it has been
34 previously shown that static stretching may be counterproductive immediately before
35 performing a strength/power sports discipline [1–3]. ~~In fact, P~~rolonged static stretching
36 can impair maximal muscle performance [4]. however these negative effects, owing to the
37 decrease of the muscle-tendon unit stiffness, are transient [5] and affect muscle
38 performance only marginally [6]. Other works have reported that either static or dynamic
39 stretching can increase flexibility by the same magnitude of change [7–10]. In the vast
40 majority of these studies, the stretching-induced effects were observed with the targeted
41 muscles, in isolation, rather than in comparison with contralateral muscles, i.e. as global
42 effects [11]. The effects of whole-body vibration (WBV) have been extensively
43 investigated [12–15], either as an adjunct to static stretching with the potential to enhance
44 retention of flexibility gains [16], or alone, to improve hamstring flexibility [17]. Short-
45 term WBV interventions have obtained improvements in flexibility and in other health-
46 related physical fitness indicators such as endurance, balance, and muscle strength, in
47 physically active adults and elderly [17, 18]. In essence, WBV transmits mechanical
48 oscillations of a given frequency, through a contact with the base of the platform, to the
49 human body. P-rior works [15, 19–21] have explained how the mechanical stimulus could
50 generate effects on the neuromuscular system. Issurin et al. [19] proposed that WBV would
51 facilitate flexibility by means of potential mechanisms such as: increase in pain threshold,

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52 elevation of blood flow and induced-relaxation of the stretched muscle. Sands et al. [20]
53 underlined that carrying out a stretching exercise during vibration could result in Golgi
54 tendon organs activation, inducing an autogenic inhibition of the vibrated muscle.

55 Moreover, acute whole-body cryotherapy has been successfully used to reduce
56 neural activation therefore affecting ROM and increasing sit-and-reach amplitude in men
57 and women [22]. Even when locally applied, cryotherapy has been shown to decrease nerve
58 conduction velocity along with an analgesic effect [23]. Basically, cryostimulation consists
59 in a short exposure to very cold air (from -130 to -170 °C) either in on-purpose designed
60 cryo-chamber (Whole-Body Cryotherapy, WBC) or cryo-cabin (Partial-Body Cryotherapy,
61 PBC) or vaporizers (local cryotherapy) [24]. Bleakely and Costello reported favourable
62 effects of cryotherapy on ROM [25], however cohesive results are lacking and therefore
63 more research protocols are warranted. The hypothesised mechanism is that cryostimulation
64 inhibits spinal excitability [23], hence decreases neural activity, allowing more elongation
65 of the muscle at a given load [22].

66 A variety of routines have been sought by athletes, fitness enthusiasts and general
67 population to earn specific advantages in muscular performance, in terms of ROM and/or
68 flexibility, or simply in order to meet prevention programs against osteo-arthro-muscular
69 injuries, which represent the major cause of sick leave and back pain [26–28].

70 In this context, the aim of this study was to explore the effects of different
71 interventions, including stretching, WBV and PBC on trunk/lower limbs flexibility, while
72 also taking into account for trunk proprioception in young women. The hypothesis was that
73 a single session of either or some of these interventions would improve the indicators of
74 flexibility.

75

76 **Materials & Methods**

77 **Research design**

78 Based on the *a priori* sample-sized determination (software package, G * Power 3.1.9.2),
79 with a statistical power of 0.8, a probability level of 0.05, an effect size f of 0.38 (which
80 corresponds to a $\eta^2 = 0.13$), eleven young adult women were enrolled for this study. The
81 present investigation was designed as a randomized-crossover trial. Using a restricted
82 blocks randomization (computer-generated sequence), the 11 participants were randomly
83 subjected to: 1) hamstrings stretching [S] in upright position; 2) S in conjunction with
84 whole-body vibration [S + WBV]; 3) PBC [Cryo]; 4) rest [Control]. All 11 participants
85 completed the 4 experimental trials, for which there were two within-group independent
86 variables, one with four conditions (intervention) and the other with two conditions (time:
87 pre- and post-interventions). To evaluate the flexibility responses to the interventions, three
88 dependent variables were examined: the ROM measured during the standing hamstring
89 stretch (SHS) test, sit-and-reach amplitude, and active knee extension test (AKET). In
90 addition, another dependent variable was registered as the absolute difference during the
91 trunk repositioning test: trunk repositioning error (TRE, Fig. 1). All the dependent variables
92 were measured prior and following each experimental condition (Fig. 1).

93 All subjects were examined by a qualified physician to exclude severe hypertension,
94 chronic low back pain, history of cardiovascular diseases, cold hypersensitivity (Raynaud's
95 syndrome) and history of previous fractures of bone lesions prior to performing any
96 contraindication to PBC and to WBVthe study. To minimize the effects of circadian
97 variation, measurements were consistently carried out at the same hour of the day (from
98 08:30 to 10:30). Subjects were also instructed to refrain from consuming alcohol, caffeine,
99 theine, hot drinks nor undertaking any stretching, weight-training, or arduous physical
100 activity in the day before and during testing days. In addition, subjects were also instructed
101 not to take medications or supplements during the study. All measurements were completed

102 by the principal investigator and recorded onto a separate data collection sheet. To avoid
103 potential bias, the investigator did not review the participants' measurements from the
104 previous days on which they completed the other interventions until they ~~complete~~ end of
105 the study. Rater was experienced with measurement protocol and the utilization of the
106 digital inclinometer and the Flex-Tester box.

107

108 [Figure 1 near here]

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110 ***Ethics statement***

111 The study protocol, including each aspect of the design, was approved by the
112 academic ethical board in accordance with the Declaration of Helsinki. All subjects were
113 given verbal and written information on the study and gave their written informed consent
114 to participate. The experimental protocol meets the ethical standards of the journal [29].

115

116 ***Procedures***

117 A diagram of the overall study-design is offered in Fig. 1.

118 On the day of the experiment, each participant arrived at the laboratory 30 min
119 before the session so to acclimate to the room temperature (22 ± 0.5 °C). They had to wear
120 loose fitted clothing such as a T-shirt and shorts, to prevent any restrictions in range of
121 movement [30]. After acclimatisation, a measurement training session was held to
122 standardize measurement protocol. Before each session movements were practiced three
123 times to diminish stretching and learning biases [31]. Hamstring and lower limb muscle
124 length tests were performed in the same order at each testing session for each participant.

125 *Tests*

126 Before placement of the digital inclinometer (Fabrication Enterprises Inc., White

127 Plains, NY, USA), the anatomical reference points were marked with a skin marker.
128 Reference points were on the mid-point of the anterior tibial crest, which was determined
129 by applying tape to measure the participant's tibia length from the medial malleolus to the
130 medial joint line of the knee [32] and at the level of the T4 spinous process [33].

131 *Standing Hamstrings Stretch (SHS) test* [34–36] To assess the maximum trunk and
132 lower limb flexibility. During the SHS test, the baseline digital inclinometer
133 (~~Fabrication Enterprises, White Plains, NY, USA~~) was used to measure the trunk
134 flexion. The manufacturer reports an accuracy of ± 0.5 degrees [37]. The participants
135 were asked to stand upright in a comfortable position and to flex slowly the trunk in the
136 sagittal plane without bending the knees, and hold this position for 3 s. After returning
137 to the neutral upright position, they were asked to ~~duplicate the previously attained~~
138 ~~position~~ perform once again the maximal anterior trunk flexion and held the position for
139 3 s. The mean of the two scores was used to assess the value of the SHS test. ~~The~~
140 ~~reliability of the standing hamstring stretch test was excellent ($\alpha = 0.990$).~~

141 *Sit-and-reach test* [38] Using a Flex-Tester box (Cranlea, Birmingham, UK),
142 participants were barefoot with legs fully extended and instructed to lean forward as far
143 as possible with the end position held for at least 2 s. The task was repeated two times.
144 The better of the two trials from each time point was taken for further statistical
145 analysis [22]. ~~The reliability of the sit and reach tests was excellent ($\alpha = 0.990$).~~

146 *Active Knee Extension Test (AKET)* [30] Participants were positioned supine on a
147 plinth so that the leg not being tested was flat on the plinth with the knee extended. A
148 strap was placed over the mid-thigh of this leg to eliminate any elevation of the limb.
149 An additional strap was positioned over the front of the participant's pelvis and around
150 the plinth to maintain the pelvis in a neutral position during hamstring measurements.
151 An iron apparatus was then placed on the plinth in line with the participant's anterior

152 superior iliac spine of the pelvis. The participant was asked to flex the hip of the tested
153 leg so that their thigh was touching the iron apparatus. The participant was then asked,
154 “straighten your leg at the knee as far as you can while maintaining your thigh touching
155 the iron apparatus” and held the end position for at least 2 s. The digital inclinometer
156 determined the angle of knee extension giving an indication of hamstring muscle
157 length [30]. Two measurements were taken for each leg and the mean of the two scores
158 was used to assess the value of the AKET. ~~The reliability of the AKET testing was~~
159 ~~excellent ($\alpha = 0.976$ for the left leg; $\alpha = 0.987$ for the right leg).~~

160 *Trunk repositioning* To assess the trunk position sense, as indicated by trunk
161 reposition errors (TRE), a digital inclinometer was held at the level of the T4 spinous
162 process. Participants, with eyes closed, flexed the trunk in the sagittal plane until the
163 position the rater wanted the individual to be and hold this position for 3 s and to
164 remember this position (position 1). After returning to the neutral upright position, they
165 were asked to duplicate the previously attained position. Participants indicated verbally
166 when they felt they had reached the angle and held their position for a count of 3 s
167 (position 2). The absolute difference in degrees between position 1 and 2 was defined
168 as the TRE [33]. Participants generated three scores and the mean of the scores
169 represents the TRE score. ~~The reliability of the trunk repositioning testing was~~
170 ~~excellent ($\alpha = 0.976$).~~

171 Interventions

172 The four above mentioned tests were performed before and immediately after each
173 condition: 1) [S]; 2) [S + WBV]; 3) [Cryo]; 4) [Control];

174 1) Subjects performing [S] were asked to stand upright in a comfortable position and
175 to flex the trunk in the sagittal plane and hold this position for 30 s. After returning
176 to the neutral upright position, they were asked to recover for 30 s and to repeat this

177 procedure for three times [39]: the total duration (SHS repetitions plus recovery)
178 was 150 s.

179 2) Similarly to [S], subjects performing [S + WBV] had to stand upright in a
180 comfortable position and to flex the trunk in the sagittal plane and hold this position
181 for 30 s over a vibration platform (Power Plate pro5; Power Plate International Ltd,
182 London, United Kingdom) set with a moderate (40Hz) frequency and a low (2-4
183 mm) amplitude. After returning to the neutral upright position, they were asked to
184 recover for 30 s and to repeat this procedure for three times [39]: the total duration
185 (SHS repetitions and recovery) was 150 s. The 30 s duration of each set over the
186 vibration platform can provide adequate safety for participants who engage in WBV
187 training for the first time [40].

188 3) During the PBC [Cryo], subjects completed a partial-body cryotherapy session of
189 150 s in a cryo-cabin (Space Cabin, Criomed, Ltd, Kherson, Ukraine). PBC session
190 allowed to expose participants to very low temperatures (from -130 to -170 °C, as
191 reported by the manufacturer). During the session subjects wore swimwear, a pair of
192 gloves, woollen socks and wooden clogs to prevent the occurrence of frostbite. The
193 head was not cryo-exposed. Participants were instructed to turn around continuously
194 in the cabin for the entire time of the session [41].

195 4) Under control condition [Control], subjects rested in sitting position for a period of
196 150 s between the two bouts of tests, upright against the back of a chair with feet
197 flat on the floor, in a room where the temperature was stabilized (22 ± 0.5 °C).

198 Skin temperatures of the ventral and dorsal regions of of interest (ROIs) were assessed by
199 means of a ThermoVision SC640 thermal imaging camera (Flir Systems, Danderyd,
200 Sweden) in accordance with the standard protocol of infrared imaging in medicine [42,43].
201 Thermal images were taken prior to each testing protocol. The camera, with the emissivity

202 set in the range of 0.97 to 0.98, was connected to a personal computer with appropriate
203 software (Thermacam Researcher Pro 2.10, version 5.13.18031.2002, Flir systems 2015,
204 Danderyd, Sweden). The camera was mounted on a tripod and positioned in a way to focus
205 on the hamstrings area. The distance between the camera and the ROIs was kept constant at
206 1m. A mean temperature was calculated by averaging the skin temperature recorded for the
207 ROIs.

208

209 *Statistical analysis*

210 The normality of the data distribution was assessed by Shapiro-Wilk test.

211 The test-retest reliability of the sit-and-reach test, AKET, SHS test, trunk
212 repositioning test were measured using an intraclass correlation coefficient (ICC,
213 Cronbach- α) and interpreted as follows: $\alpha \geq 0.9$ = excellent; $0.9 > \alpha \geq 0.8$ = good; $0.8 > \alpha$
214 ≥ 0.7 = acceptable; $0.7 > \alpha \geq 0.6$ = questionable; $0.6 > \alpha \geq 0.5$ = poor [44].

215 All data were presented as mean \pm standard deviation (SD). The flexibility
216 performances and trunk repositioning pre- and post-intervention, and the relative
217 thermographic measurements were analyzed with a mixed (time x intervention) repeated
218 measures analysis of variance (ANOVA). Post hoc pair-wise comparisons were conducted
219 utilizing Tukey's or Bonferroni's test when main or interaction effects were demonstrated.
220 The sphericity assumption was examined using Mauchly's test. Eta squared (η^2) effect sizes
221 (ES) were determined and interpreted using the following criteria: 0.01 = small; 0.06 =
222 medium; 0.14 = large [45]. For all analyses, a p value less than 0.05 was considered
223 statistically significant.

224 Analyses were carried out with the Statistical Package SPSS version 25 for Mac
225 (IBM Corp., Armonk, NY, USA), GraphPad Prism 5 (San Diego, CA, USA), and Excel
226 version 16.32 for Mac (Microsoft, Redmond, WA, USA).

227

228 **Results**

229 All demographic and anthropometric characteristics of the participants are offered in

230 Table 1. All women resulted to be normal weight, fit, and healthy, as assessed on physical
231 examination.

232

233 [Table 1 near here]

234

235 *Flexibility variables and proprioception*

236 *Standing Hamstring Stretch (SHS) test* Results, expressed as change from baseline,
237 revealed a main interaction ($F = 3.575$, $pP = 0.0254$, $\eta^2 = 0.153$) among the interventions
238 with a significant improvement in ROM after [S + WBV] with respect to [Control] (Tukey's
239 post-hoc, $pP < 0.05$; mean difference: -2.909, 95% CI: -5.599 to -0.2188) (Fig. 2A). The
240 reliability of the standing hamstring stretch test was excellent ($\alpha = 0.990$).

241 *Sit-and-reach* ANOVA showed a significant interaction among the interventions as to
242 the sit-and-reach amplitude of the women tested ($F = 3.688$, $pP = 0.0226$, $\eta^2 = 0.243$). In line
243 with the SHS results, the participants' sit-and-reach amplitude benefitted from [S + WBV]
244 as compared to [Control] (Tukey's post-hoc, $pP < 0.05$; mean difference: -2.136, 95% CI: -
245 4.067 to -0.2058) (Fig. 2B). The reliability of the sit-and-reach test was excellent ($\alpha = 0.990$).

246 *Active Knee Extension Test (AKET)* Outcomes, still expressed as change from baseline,
247 revealed a main effect among the interventions ($F = 4.694$, $pP = 0.0084$, $\eta^2 = 0.168$), with a
248 significant improvement in ROM after PBC [Cryo] with respect to [Control] (Tukey's post-
249 hoc, $pP < 0.05$; mean difference: 3.434, 95% CI: 0.4880 to 6.380) (Fig. 2C). The reliability
250 of the AKET testing was excellent ($\alpha = 0.976$ for the left leg; $\alpha = 0.987$ for the right leg).

251 *Trunk Repositioning Error* As to absolute difference during the repositioning test, a
252 main effect was not found (Fig. 2D). The reliability of the trunk repositioning testing was
253 excellent ($\alpha = 0.976$).

254 Overall performance data, emerging from the four routine protocols, are also reported
255 as pre- and post- intervention values in Table 2.

256

257 [Figure 2 near here]

258 [Table 2 near here]

259

260 ***Thermal responses***

261 Ventral skin temperature analysed by means of thermal images confirmed an effective
262 decrease procured by cryotherapy (Fig. 3). A significant interaction “time x treatment” was
263 documented ($F = 715.6$, $pP < 0.0001$, $\eta^2 = 0.906$). In detail, ventral temperature of both lower
264 limbs was significantly affected by PBC ($F = 146.2$, $pP < 0.0001$, $\eta^2 = 0.916$) and time ($F =$
265 845 , $pP < 0.0001$, $\eta^2 = 0.793$). As expected, post-hoc comparisons revealed that temperature
266 was significantly lower after PBC (13.7 ± 1.9 °C, Bonferroni’s, $pP < 0.0001$, Fig. 3) with
267 respect to all other post-intervention temperatures, which remained ~ 29-30 °C (Fig. 3).

268 [Figure 3 near here]

269

270 **Discussion**

271 In this study we investigated the impact of different routines to ameliorate trunk-,
272 lower limbs flexibility while considering trunk proprioception in a sample of young
273 women.

274 The present data showed that women gained a greater effect in SHS test and sit-and-
275 reach amplitude with [S + WBV] as compared to all other protocols. PBC allowed to

276 achieve a major benefit in the AKET with respect to the other routines. Overall, these
277 flexibility improvements were obtained without a loss in the trunk position sense
278 proprioception.

279 A broad literature entails a multitude of strategies and stretching protocols to obtain
280 specific advantages in flexibility [9, 10, 46, 47]. Nevertheless, only a few studies have
281 included cryo-exposures protocols to assess possible gains in ROM and proprioception
282 acuity [48, 49]. Proprioception can be defined as the awareness of the position of a joint or
283 of a particular body district in space. Although proprioception encompasses several
284 components, including kinesthesia, balance, reflexive joint stability, somatosensation, a
285 number of evidence-based indications suggest that the somatosensory system is affected
286 when very low temperature is applied to the muscles [48]. Increases in flexibility could be
287 due a reduced neural activation during cryo-exposure [22]. Cryo-induced pain limitation
288 may represent another potential mechanism responsible for these flexibility improvements.
289 Similarly, increases in pain thresholds have been reported in research articles studying
290 flexibility by means of whole-body vibration [19, 20].

291 Consistently with other studies focusing on the combination of stretching with
292 vibration [15,16], the present results confirmed the efficacy of WBV in augmenting
293 flexibility, acutely. ~~Differently from cryotherapy, it~~ It has been suggested that vibration
294 might procure increases in flexibility by enhancing the stretch reflex loop, through the
295 activation of the primary endings of the muscle spindles, which influences agonist muscle
296 contraction, whilst antagonists are simultaneously inhibited [50]. Furthermore, vibration
297 may also enhance flexibility by activating the Ia inhibitory interneurons of the antagonist
298 muscle, thus decreasing the braking force around a joint [51]. It has also been hypothesized
299 that the inhibition of the antagonist muscle, induced by vibration, is mediated by
300 supraspinal neuronal circuitry [52]. Finally, the vibratory stimulus of the Ia neural drive and

301 ~~proprioceptive loop may also replicate a warm-up effect by increasing pain threshold, blood~~
302 ~~flow and muscle elasticity augmenting muscle temperature and elasticity resulting from~~
303 ~~elevated blood flow~~ [19, 20]. However, these mechanisms remain putative and associated to
304 prolonged interventions and longer vibration applications than the subjects experienced in
305 the present study.

306 At the superficial level, skin temperature decreased significantly only after
307 cryostimulation as expected.

308 The results of this research provided the first and unique evidence that a single
309 session of either PBC or WBV can significantly and positively impact on flexibility without
310 compromising the proprioception capacity. This is of practical value for coaches and
311 trainers aiming to find routines to ameliorate trunk and lower limbs flexibility of female
312 athletes in sports disciplines where a high level of proprioception, in addition to the
313 flexibility, is required. That might be the case, for example, for ice skaters, divers and
314 gymnasts.

315 As a limitation, the present research was restricted to female subjects therefore
316 generalizations cannot be inferred and applied to a large spectrum of the population. This
317 latter problem is exacerbated by the small sample size. Promising data stemming from
318 previous works [22], along with a prevalent practice of gymnastics and skating among
319 females [53], justified a local mono-gender investigation. Beyond enrolling different
320 gender, further insights could be gathered by recruiting different athletes and diverse sport
321 practitioners. In addition, longitudinal studies may examine adaptive effects originating
322 from chronic modalities of training.

323 As a strength point, the experimental design in which all the tests were performed, in
324 all interventions, in the same subjects, enhanced the signal-to-noise ratio associated to this
325 study, permitting to achieve a clear-cut significance.

326 In light of the emerged findings, it is now sound that coaches can schedule PBC and
327 WBV sessions before a training session or a competition, such as in skating, diving, and
328 gymnastics, whereby adequate levels of both flexibility and proprioception are required.

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335 **Declaration of interest**

336 None.

337

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341

342 **Author Contributions** M.D.N., C.F. performed the studies. R.C. analyzed the data.

343 M.D.N., R.C. wrote the manuscript. M.D.N., C.F., P.R., A.L.T., R.C. contributed to the
344 discussion and reviewed the manuscript. M.D.N. designed the studies. M.D.N., R.C.

345 supervised the studies. All authors edited the manuscript. R.C. is the guarantor of this work

346 and, as such, had full access to all the data in the studies and takes responsibility for the

347 integrity of the data and the accuracy of data analysis.

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504

Captions

Table 1. Demographic and anthropometric characteristics of the women studied.

Table 2. Summary results of the participants performing different routine protocols (mean \pm SD)

Figure 1. Flow-chart of the study.

AKET, active knee extension test; Cryo, partial-body cryotherapy; S, stretching; SHS, standing hamstrings stretch; SD, standard deviation, TRE, trunk repositioning errors; WBV, whole-body vibration.

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Figure 2. Flexibility outcomes and proprioception

Graphical representation of the absolute difference in the three flexibility tests (A, B, C) and trunk position sense (D) compared to the baseline measurements. Whiskers box-plot: the hinges of the plot extend from the 25th to 75th percentiles. The whiskers go down to the smallest value and up to the largest one.

AKET, active knee extension test; Cryo, partial-body cryotherapy; S, stretching; SHS, standing hamstrings stretch; SD, standard deviation, TRE, trunk repositioning errors; WBV, whole-body vibration.

* $p < 0.05$

Figure 3. Thermal responses

Cryo, partial-body cryotherapy; S, stretching; WBV, whole-body vibration.

*** $p < 0.0001$

