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Modeling and prediction of exposure durations**

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1 Preliminary Study on the Effect of Sex on Skin Cooling Response during Whole Body 2 Cryostimulation (-110°C): Modelling and Prediction of Exposure Durations

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10

11 Abstract

12 In order to determine the required duration of whole-body exposure to extreme cold (-110°C) in
13 males and females for achieving the same cold-induced response, a mathematical model of skin
14 cooling kinetics was developed. This modeling is derived from the implementation of a new
15 experimental cryotherapy protocol to obtain continuous skin temperature maps over time. Each
16 three-minute whole-body cryostimulation session was divided into six incremental sessions of 30
17 seconds carried out over six consecutive days. Seventeen young, healthy subjects (8 males aged
18 22.6 ± 3.0 years and 9 females aged 23.7 ± 4.7 years) agreed to participate in this study. The
19 smallest sex-related difference in temperature was found in the trunk area (2.93°C after 3 minutes)
20 while the greatest temperature drop was found in the lower limbs (5.92°C after 3 min). The largest
21 temperature variation was observed between the trunk and the lower limbs, and peaked at 2.67°C in
22 males and 6.99°C in females. For both sexes, skin cooling kinetics showed a strong transient
23 exponential type decrease followed by linear regression behavior. It appeared that for achieving the
24 same cold-induced response, the required duration of cryostimulation is longer for males. **For
25 example, a trunk skin cooling of -12°C should be achieved in 125s for females against 170s for
26 males (+36% longer); for the lower limbs, the same skin cooling magnitude should be reached after
27 87s for females against 140s for males (+62% longer).**

28

29 **Keywords** Skin temperature, cooling ratio, sex effect, protocol duration

30 Abbreviations

31	ANOVA	Analysis of variance
32	C₀	Ratio of cooling
33	RH	Relative humidity
34	ROI	Region of interest
35	SFCCE	French Society of Whole-Body Cryotherapy
36	SD	Standard Deviation
37	Tr	rectal temperature
38	T_{sk}	Skin temperature
39	WBC	Whole-body cryostimulation

40

41 1. Introduction

42 Whole-body cryostimulation (WBC) is a physiotherapy method where patients are exposed to
43 extreme cold air (generally -110°C to -140°C) in minimal clothing, for a relatively short duration (2-4
44 min) [4, 17, 27]. The basic medical efficiency of WBC is related to a thermal stress that generates
45 vasoconstriction [9, 31] and stimulation of the thermal skin receptors by lowering skin temperature,
46 leading to an analgesic effect by slowing down nerve conduction [1]. WBC is commonly used as a
47 method to relieve pain and inflammation symptoms caused by various diseases, particularly those

48 associated with rheumatic conditions. It is recommended for the treatment of arthritis [19, 23]
49 fibromyalgia [3, 41] and ankylosing spondylitis [38]. WBC is also used by athletes to relieve muscle
50 soreness after exercise [2, 18, 37]. Whole-body cryostimulation has also been found to improve
51 sleep quality and insomnia [16, 25] as well as depression and anxiety disorders [35].

52 The basic premise of cryotherapy is that cold exposure activates thermosensitive skin receptors,
53 which would be the main cause for cryostimulation efficiency because it triggers regulatory
54 mechanisms aiming at maintaining a constant central temperature. Resulting changes in skin
55 temperature then become the key parameter on which the cryostimulation process is based. Past
56 studies dedicated to cryostimulation have used data collected post-WBC during the body warming
57 phase (e.g., biological markers, self-reported perceptions) [28]. Studies evaluating the temporal
58 dynamics of skin temperature have been done following a similar procedure [5, 8, 10, 12, 33, 36].
59 This was mainly due to the impossibility of documenting skin thermal variations occurring while
60 receiving cryostimulation, inside the chamber. However, the thermal and metabolic response as it
61 appears following cryostimulation is at least partly related to what happened in the course of the
62 cryostimulation session; in particular to the rate of skin cooling. The magnitude of skin tissue cooling
63 is known to determine WBC ability to trigger a meaningful analgesic effect and avoid adverse effects
64 [10]. The dynamics of skin temperature during WBC depends on two factors, the thermal gradient
65 between skin and external environment and tissues capacity to attenuate the heat losses from the
66 body's core to the skin [32]. Due to the paucity of data in the literature, it is currently not known
67 whether sex-related differences affect skin thermal cooling during WBC. The reasons for this lack of
68 data are linked to the narrowness of usual cryotherapy chambers (including the larger ones for 4 to 5
69 people) which does not allow the recommended distances for taking thermal images to be respected
70 [29]. More importantly, the extremely low temperatures are known to damage electronic equipment
71 like thermal cameras. To compensate for the impossibility of obtaining real-time monitoring of skin
72 temperatures, a new experimental protocol had to be developed.

73 The aim of this paper is to propose a new incremental time-step cooling protocol, making it possible
74 to evaluate the temporal dynamics of skin temperature (via thermal imaging) in males and females
75 while being exposed to extreme cold air. Our methodological innovation consists in collecting
76 temperature data before, during and immediately after the WBC session in order to obtain a more
77 complete picture of skin temperature variations. Experimentally-induced acute cooling responses can
78 be tracked over selected body areas of interest, but also over the whole body surface (excepted
79 head, neck, and areas covered by clothing). On the basis of these experimental data, a
80 mathematical model (statistical regression) can be constructed, that can be used in other studies
81 involving whole-body cold air exposure at -110°C in order to predict the needed WBC duration to
82 trigger a specific change in skin temperature. Because rectal temperature is considered as the gold
83 standard for core temperature, this measure is also included in our proposed procedure.

84 **2. Materials and methods**

85 **2.1. Participants**

86
87 Seventeen young, healthy subjects (8 males aged 22.6 ± 3.0 years and 9 females aged 23.7 ± 4.7
88 years) agreed to participate in this study and constituted the sample selection. Prior to the study and
89 to meet the inclusion criteria, participants were examined by a physician who confirmed that they had
90 no medical contraindications to WBC among those listed by the French Society of Whole-Body
91 Cryotherapy (SFCCE, Paris France). None of the participants had WBC experience prior to the
92 study. A summary of anthropometric characteristics of the participants can be seen in Table 1.
93 Female participants were asked to inform investigators on the stage of their menstrual cycle. Six
94 were in the follicular phase of their menstrual cycle while the other three were in the luteal phase.
95 Given that menstrual cycles are likely to alter core temperature [13], the menstrual cycle changes
96 might not be followed by parallel changes in skin temperature [42].

97
98 *****Table
99 here*****

101 Participants were also asked to refrain from consuming alcohol, caffeine or food 3 hours prior to the
102 cryostimulation session and from vigorous activities during the 6-day whole protocol duration. During
103 cryostimulation sessions, participants were wearing minimal clothing, gloves, socks, shoes, woolen
104 cap to protect ears and surgical mask to avoid any risk of cold burns to the respiratory tract. During
105 cold exposure, they had to walk slowly around the chamber.

106

107 **2.2. Study design**

108

109 The cooling duration used in this study (-110°C during 3 minutes) is typical of those found in practice
110 and reported in the literature [11-12, 21-22, 37]. In order to obtain continuous information over time
111 on skin cooling kinetics, the innovative protocol developed was based on a decomposition of each
112 three-minute session into six sessions of 30-seconds, conducted over six consecutive days,
113 according to the timing diagram in Table 2.

114 *****Table 2 here*****

115

116 To minimize any measurement bias, the sessions were scheduled at the same time each day for
117 each participant. For each session, the skin temperature over the entire front as well as the back of
118 the body was measured, before the session (baseline) and immediately post-WBC. Participants were
119 asked to take their rectal temperature in individual fitting rooms inside the laboratory before the
120 session (baseline) and immediately after the thermal images were obtained.

121

122

123 **2.3. Measurements**

124

125 Body composition of participants was assessed using a dual energy X-ray absorptiometry technology
126 (Horizon DXA system, Hologic, Marborough, USA). For this, all subjects were positioned in the
127 supine position with the arms along the body and then underwent three consecutive full-body scans
128 [20]. Skin temperature measurements took place in a laboratory adjacent to the cryotherapy
129 chamber -110°C (Mecotec, Germany). Because ambient temperature and humidity may affect the
130 skin temperature, the temperature and relative humidity of the laboratory adjacent to the
131 cryostimulation chamber were initially measured and found to be 21°C and 35% RH respectively
132 (Sensirion EK-H4, Switzerland). It can be specified that this laboratory temperature of 21°C is in
133 accordance with the recommendations of Ring and Ammer [34] advocating a controlled environment
134 temperature ranging from 18°C to 25°C to avoid any bias in the skin temperature measurement.
135 Rectal temperature was recorded after the participants self-inserted an electronic thermometer (PX-
136 TH519, Tex, Pelimex, Ingwiller, France) in the anal sphincter before and immediately after the cold
137 exposure. Skin temperatures were recorded in orthostatic position using a Variocam HD thermal
138 imaging camera 1024x768 (Jenoptik, Germany) in accordance with the standard protocol for infrared
139 imaging in medicine [29]. An emissivity factor of 0.98 for the human skin has been used to get
140 appropriate skin thermographs. The analysis of the thermal images was made possible with the use
141 of post-processing software (IRBIS 3.1 from InfraTec, Germany). Example of skin temperature
142 thermal image is presented in Figure 1 for a young woman. It is recalled that the thermal analysis
143 concerns both front and back sides of the body even if Figure 1 presents only front side.

144

145 *****Figure 1 here*****

146

147

148 **2.4. Data analysis**

149 The choice was made to analyze skin temperatures in three regions of interest (ROI), namely trunk,
150 upper limbs and lower limbs, as shown in Figure 2. In order not to bias the thermal results, a cutout
151 was made around the underwear, and more specifically around the females's bras, both front and
152 back for evaluating trunk skin temperature.

153 *****Figure 2 here*****

154

155 As this paper focuses on the presentation of a new WBC protocol and inferred modeling, we have
 156 deliberately limited the number of regions of interest to three, so as not to significantly increase
 157 processing times. The same principle developed in this article can be applied to more localized
 158 areas.

159 The identification of the regions of interest was done by means of a polygonal-type cutout (post-
 160 processing software IRBIS 3.1 from InfraTec, Germany), the number of segments chosen having to
 161 correspond as closely as possible to the exact anatomical shape of the periphery of these identified
 162 ROIs. The average skin temperature of each ROI is then a weighted average of the temperatures,
 163 quotient for each polygon of the weighted sum of the temperatures by the sum of the pixels. For
 164 upper and lower limbs, whatever the sex is, four polygons are necessary (back, front, left, right). For
 165 the trunk, two polygons are required for males while for females, between four and eight are
 166 necessary, depending on the shape of their bra straps. The average skin temperature of the whole-
 167 body surface is then the weighted average of the ROI skin temperatures. Obviously, what is called
 168 whole body surface is limited to the sum of the ROIs not taking into account the head and neck, and
 169 areas covered by clothing.

170 **2.5. Mathematical approach**

171 To predict the duration of whole-body cryostimulation at -110°C in males and females to achieve the
 172 same dose-response effect to cold, a mathematical model of skin cooling kinetics was developed in
 173 order to fit experimental data. This modelling can be used to obtain and extrapolate predictive skin
 174 temperature at any time by fitting experimental data. Assuming an exponential law during the initial
 175 transient [24] from $t \in [0; 30]$, followed by a linear one during the second part of the transient from $t \in$
 176 $[30; \beta]$, the time-dependent skin temperature can be expressed as:

$$177 \quad T_{SK}(t) = [Ke^{-(at)^n}]_{0 \rightarrow 30} + [At + B]_{30 \rightarrow \beta} \quad (1)$$

178 Where K, a, A and B are constants to be analytically determined using both thermal boundary
 179 conditions and a C¹- continuous junction between these two functions. The resulting analytical
 180 expression of the skin temperature is

$$181 \quad T_{SK}(t) = \left[T_{sk0} \left(\frac{T_{sk0}}{T_{sk1} + C_0(30 - \beta)} \right)^{-\left(\frac{t}{30}\right)^n} \right]_{0 \rightarrow 30} + [C_0 t + (T_{sk1} - C_0 \beta)]_{30 \rightarrow \beta} \quad (2)$$

$$182 \quad \text{With } n = \left(\frac{-30C_0}{[T_{sk1} + C_0(30 - \beta)] \ln\left(\frac{T_{sk0}}{T_{sk1} + C_0(30 - \beta)}\right)} \right) \quad (3)$$

183 T_{sk0} , T_{sk1} and C_0 are respectively the baseline skin temperature, the skin temperature at the end of
 184 the session and the rate of cooling, and β corresponds to the end of the session.

185

186 **2.6. Statistical analyses**

187
 188 All data are expressed as means and standard deviations. All statistical analysis were performed
 189 using both STATISTICA (data analysis software, version 13.0) and Excel (Microsoft Corporation,
 190 Nunes, 2015). Normality of raw data was checked in each group (males and females) using the
 191 Shapiro-Wilk and Kolmogorov-Smirnov tests. Five sex-by-time mixed analyses of variance (ANOVA)
 192 were performed to investigate changes in time with one between subject's variable (sex: male vs.
 193 female) and one within-subject variable (moment of measure: 0s, 30s, 60s, 90s, 120s, 150s, 180s)
 194 for rectal temperature and skin temperatures (trunk, upper limbs, lower limbs and whole body
 195 surface). Statistical significance was set at $p < 0.05$. Associations between body composition
 196 variables in Table 1 and changes in skin temperature were analyzed using Pearson's correlation
 197 coefficients.
 198

199 **3. Results**

200

201 **3.1. Rectal temperature**

202

203 For the present study, evolution in time of rectal temperature is shown in Figure 3 where the dashed
204 line represents the best fit regression linear line. In more detail, rectal temperature was found to vary
205 between $37.04 \pm 0.27^{\circ}\text{C}$ and $37.19 \pm 0.36^{\circ}\text{C}$ for males and from $37.39 \pm 0.20^{\circ}\text{C}$ to $37.47 \pm 0.26^{\circ}\text{C}$
206 for females. For this parameter, the ANOVA did not reveal any interaction between sex and time ($F_{(6, 90)} = 0.35, p = 0.91$). However during the cryostimulation protocol, rectal temperature in females was
207 higher than for males ($F_{(1, 15)} = 11.70, p = 0.004$). A positive linear correlation between rectal
208 temperature and time was noted, with Pearson's correlation coefficient $r = 0.79$ for males and $r =$
209 0.84 for females. Best-fit linear regression lines give a positive thermal gradient of $+0.04^{\circ}\text{C}/\text{min}$ for
210 females and $+0.07^{\circ}\text{C}/\text{min}$ for males during the protocol. For both males and females, bivariate
211 correlation analyses between body fat percentage and rectal temperature at different times of
212 measurement showed a moderate positive relationship ($0.312 < r < 0.669$). Also of interest were a
213 negative relationship of moderate size ($-0.639 < r < -0.442$) between the percentage of lean body
214 mass and rectal temperature at the different moments of measurement. Bivariate correlation
215 analyses between body mass index and rectal temperature at different measurement times showed
216 no relationship.
217

218
219
220

*****Figure 3 here*****

221
222

223 **3.2. Skin temperature of ROI and whole-body surface**

224

225 Figure 4 highlights the temporal evolution of skin temperature during the 3 reconstituted minutes that
226 the WBC session lasted at -110°C , in the same range $10^{\circ}\text{C} - 35^{\circ}\text{C}$. For all ROIs, the dashed curves
227 in Figure 4 correspond to the best fit mathematical predictive laws that can be deduced (Eq. 2)
228 where the different parameters in equations 2 and 3 are given in Table 3.

229
230

*****Table 3 here*****

231 For trunk skin temperature there was a significant interaction effect between sex and time
232 ($F_{(6,90)}=4.66, P<0.001$), a difference between sexes ($F_{(1,15)}=35.74, P<0.001$) and also a difference
233 over time ($F_{(6,90)}=397.65, P<0.001$). For upper limbs skin temperature there was a significant
234 interaction effect between sex and time ($F_{(6,90)}=8.47, P<0.001$), a difference between sexes
235 ($F_{(1,15)}=78.39, P<0.001$) and also a difference over time ($F_{(6,90)}=690.09, P<0.001$). For lower
236 limbs skin temperature there was a significant interaction effect between sex and time
237 ($F_{(6,90)}=10.42, P<0.001$), a difference between sexes ($F_{(1,15)}=168.12, P<0.001$) and also a
238 difference over time ($F_{(6,90)}=565.17, P<0.001$). Females's skin temperature was always lower than
239 that of males throughout the whole cooling in the different ROIs. For both males and females,
240 bivariate correlation analyses between body fat percentage and skin temperature at different
241 measurement times showed a large negative relationship ($-0.799 < r < -0.675$), whereas a large
242 positive relationship ($0.632 < r < 0.820$) was found between the percentage of lean body mass and
243 skin temperature at different times of measurement, as well as an overall large positive relationship
244 ($0.630 < r < 0.821$) between the percentage of body water content and skin temperature. The
245 bivariate correlation analyses between body mass index and skin temperature at the different
246 measurement times did not show any relationship.

247
248

*****Figure 4 here*****

249 Several observations can be drawn from these different temporal patterns. First of all, for participants
250 of both sexes and at whatever body area, the evolution of skin temperatures seems to follow the
251 same pattern over time: a significant drop in temperature during the first 30 seconds of the transient,
252 followed by a slower decrease, with a linear trend. This first sudden drop is linked to the
253 phenomenon of massive vasoconstriction coming with the sudden exposure to extreme cold. From

254 30 seconds onwards, the skin temperature at all body surface areas are best fit with linear models
255 which attained very high coefficients of determination (i.e., $0.982 < R^2 < 0.994$). The slopes of these
256 lines, denoted C_0 , correspond to the ratio of cooling and are given in Table 3. Figure 5 summarizes
257 the cooling ratio of the second linear phase, for $t > 30s$.

258 *******Figure 5 here*******
259

260 The average whole-body skin temperature is defined as the surface-weighted average of the skin
261 temperatures from the different ROIs. The following relations can be drawn:

262 For males, $T_{skin} = 18.7\% T_{Upper\ limbs} + 39.0\% T_{Lower\ limbs} + 42.3\% T_{Trunk}$ (4)

263 For females, $T_{skin} = 19.0\% T_{Upper\ limbs} + 47.9\% T_{Lower\ limbs} + 33.1\% T_{Trunk}$ (5)

264 Considering the ratio of the surfaces explored, the weight of the skin temperature of the lower limbs
265 (lowest temperatures) will be preponderant in females, whereas it is that of the trunk (highest
266 temperatures) which has priority in males. Figure 6 highlights the temporal pattern of skin
267 temperature during the 3 min long WBC ($-110^{\circ}C$).

268
269 *******Figure 6 here*******
270

271 As far as whole-body skin temperature is concerned, there was a significant interaction effect
272 between sex and time ($F_{(6,90)} = 16.68$, $p < 0.001$), a difference between sexes ($F_{(1,15)} = 171.35$, $p <$
273 0.001) and also a difference over time ($F_{(6,90)} = 870.02$, $p < 0.001$). The best-fitting model of linear
274 nature from 30 seconds onwards, was also found to have determination coefficients (R^2) close to 1
275 (0.994 for males and 0.996 for females).

276

277 4. Discussion

278 The results obtained from the development of a new study design made it possible to obtain precise
279 thermal kinetics in males and females, focusing on rectal and skin temperatures. A deduced
280 mathematical modeling has been developed in order to propose predictive patterns of different
281 durations of exposure to extreme cold depending on whether the subject is male or female. To date,
282 this has never been studied in the literature.

283

284 Some findings in the literature revealed slight discrepancies in the evolution of rectal temperature
285 from pre to post-WBC session. Westerlund et al. [43] indicated that mean rectal temperature did not
286 change during WBC, as confirmed by Zalewski et al. [44] who investigated core temperature
287 (measured from swallowed capsules) and found that this parameter remained unchanged
288 immediately after cryostimulation ($p > 0.05$) compared to baseline. Finally, Cuttel et al. [14] (see
289 Figure 1 p. 43) and Costello et al. [11] (see Figure 4 p. 6) even found a slight increased trend for
290 rectal temperature during similar cold air exposure at $-110^{\circ}C$. It should be noted, however, that the
291 latter authors observed a decrease in rectal temperature for immersion in $8^{\circ}C$ cold water. The
292 reason behind these apparently contradicting findings is related to the different modes of heat loss in
293 the latter case (different convective exchange coefficient, absence of radiative transfer in water). At
294 rest, females had slightly higher core temperatures than males, as revealed by Mackowiak et al. [30].
295 It is known that core temperature usually increases in response to skin cooling [24]. This is in
296 accordance with our observation (see Figure 3) that regardless of sex, rectal temperature rose
297 slightly during exposure to extreme cold. The distribution of raw data can be satisfactorily fitted with a
298 linear function (coefficient of determination $R^2 = 0.70$ for females and $R^2 = 0.63$ for males) of the
299 type:

300 $T_R(^{\circ}C) = 0.07(^{\circ}\frac{C}{min})t(min) + T_{R-Baseline}(^{\circ}C)$ for males and $T_R(^{\circ}C) = 0.04(^{\circ}\frac{C}{min})t(min) +$
301 $T_{R-Baseline}(^{\circ}C)$ for females.

302 For both females and males, this finding of a slight increase in rectal temperature is directly related
303 to the initial stimulation of cold receptors in the skin causing constriction of peripheral blood vessels
304 and a redirection of blood flow to the core. Similar trend of a positive rate of rectal temperature
305 $\Delta T_R/\Delta t$ has already been reported in the early adaptation phases of a study involving cold water
306 immersion [39]. If exposure to cold was to be prolonged over time, the strong thermal imbalance of
307 the body (heat loss greater than the internal metabolic intake of heat) would lead to body
308 hypothermia with a decrease in core temperature [6].

309 The skin is the interface between the organism and the external environment which contributes to
310 the dynamic homeostasis of the organism's internal environment, despite changing external
311 conditions. Thermal regulation takes place at the level of the posterior hypothalamus: in the event of
312 exposure to cold, the hypothalamus is stimulated leading to vasoconstriction of the peripheral
313 cutaneous arterioles directing the blood flow towards the deep venous system to form a heat shield
314 protecting the vital organs. This is certainly the reason why the internal temperature rises slightly
315 during this phase of vasoconstriction. Consequently, due to vasoconstriction, the role of thermal
316 insulation played by adipose tissue became preponderant to diminish heat losses. The skin, being
317 more insulated on its body inner side, then becomes more dependent on external conditions [26].
318 Percentage of BF is known to modulate thermogenic responses to extreme cold [39] and both males
319 and females with lowest BF percentage tended to have higher skin temperature for all measures at
320 all times [7]. In the present study, body fat (BF%) was $24.0 \pm 3.7\%$ for females versus $11.2 \pm 4.4\%$
321 for males ($p = 0.0001$). Because fat mass is positively related to tissue insulation [15], and since
322 females have more adipose tissue than males, they have a higher equivalent thermal resistance of
323 0.037 K/W compared to 0.027 K/W for males in a rest state [32]. The direct consequence is that their
324 skin temperature is much more influenced by environmental conditions, and therefore more abruptly
325 cools down during cold stress compared to males. With regard to body surface areas, it clearly
326 appears from Figure 4 that for both males and females, the trunk is the body area for which skin
327 temperature fell by the smallest amount. This is probably due to the heat shield effect that it provides
328 by protecting the vital organs, by blood return due to vasoconstriction. It is also in this area of body
329 that the smallest sex-related difference in temperature was found (i.e., 2.93°C after 3 minutes of
330 exposure to extreme cold). On the other hand, the lower limbs showed the greatest temperature
331 drop, with a maximum difference between the sexes of 5.92°C after 3 min. In males, the largest
332 temperature variation was between the trunk and the lower limbs, and peaked at 2.67°C after 3 min.
333 For females, the largest temperature variation was also between the trunk and the lower limbs, and
334 peaked at 6.99°C after 3 min. With regard to whole-body skin surface temperature (see Figure 6),
335 similar findings were reported between males and females, with the largest difference between
336 males and females peaking at 5.53°C after 3 min.

337 All these findings suggest that males and females adapt differently to extreme cold air exposure, with
338 females having much more heterogeneous skin temperature variations, depending on body surface
339 areas. This brings into question the current clinical practices in WBC centers which most often use
340 identical cryostimulation protocols for males and females. This issue was previously raised in
341 Cholewka et al. [8]. The amplitude of the cutaneous variation $\Delta T = T_{sk} - T_{sk_0}$ can be considered as a
342 key parameter in the evaluation of the effect of sex in cryostimulation. The analytical formulation of
343 the cutaneous thermal behavior (Eq. 2) makes it possible to easily predict the durations of protocols
344 which make it possible to reach this set point. Table 4 summarizes the different durations numerically
345 calculated in a range of cooling gradient ΔT from -8°C to -20°C , for the different body areas
346 considered in our study. It appears that in order to produce the same thermal skin response, protocol
347 durations in males have to be higher than those for females. For instance, to reach a same cooling
348 magnitude of -12°C in the trunk area, the duration of the protocol must be 36% longer in males
349 compared to females. This difference can reach 54% and even 62% for the upper limbs and lower
350 limbs respectively. It is necessary to specify that these percentages, which may be similar to
351 recommendations for use, could be adapted to the population of the present study only, and future
352 recommendations would mainly depend on body fat percentages. In addition, our analysis revealed
353 that the relative difference (E%) for the duration of protocols between males and females tends to
354 decrease as the desired temperature gradient increases.

355 *****Table 4 here*****

356 The development of a regression model has two major advantages. First, it can allow cryostimulation
357 researchers as well as therapists to easily know the thermal behavior of the skin during
358 cryostimulation sessions at any time. And on the other hand, this analysis can make it possible to
359 predict the duration of protocol, according to the sex and the zones of the body surface considered.
360 To the best of our knowledge, no systematic investigation has yet been performed to identify the
361 intervention dose needed to reach the same skin temperature response in males and females.

362

363 **5. Limitation**

364 Some limitations should be taken into consideration when interpreting our findings. First, even
365 though our sample size is roughly similar to what can be found in most published WBC studies it can
366 be said to be fairly "modest" to generalize findings. Consequently, the present study should be
367 considered as a preliminary investigation. Second, directly in relation to the limitation mentioned
368 above, body composition and sex did not appear as explicit input parameters in our regression
369 model. These variables were included in the calculation of C_0 cooling ratios. Further parametric
370 analyses including larger samples should make it possible to include these parameters as explicit
371 input variables in future statistical models. The accuracy of the model is linked, on the one hand, to
372 the number of experimental that allowed the construction of the regression laws (a total of 7
373 measurements points per situation, established every 30 seconds), and, on the other hand, to the
374 values of the regression coefficients deduced from these curves, which are very close to 1. The
375 criticism that could be made is that the number of healthy and young subjects is modest and that
376 coefficients of determination close to 1, which are our key mathematical reliability criterion, are based
377 on the experimental data from 8 male and 9 female subjects only. However, to confirm this, this
378 preliminary study would require a larger sample size. Deducted, a final limitation relates to the
379 applicability of our regression model to cold air exposures that would last more than 3 min. In spite of
380 these limitations, we do believe that the initial regression model resulting from our preliminary study
381 can be of great help in defining individualized WBC prescriptions.
382

383 **6. Conclusion**

384 Due to the extremely low (and potentially damaging to measurements devices) temperatures inside
385 the WBC chambers (-110°C), a new methodological protocol was developed to investigate the real-
386 time dynamics of skin cooling in healthy male and female participants. The aim of the present study
387 was threefold: (1) providing the scientific and medical communities with pre-, per-, and post-WBC
388 data on skin temperature dynamics at various body areas, (2) defining a regression model that would
389 allow the researchers/practitioners to estimate the average value of skin cooling at the various
390 stages of a typical WBC session (-110°C), and (3) highlighting sex-based disparities in skin
391 temperature response to cryostimulation.

392 Major findings can be summarized as follows. Females had slightly higher core temperatures than
393 males during WBC. For both males and females, a positive linear correlation between rectal
394 temperature and time of exposure was found (i.e., $r = 0.79$ for males and $r = 0.84$ for females).
395 Probably due to greater adipose tissue, the magnitude of skin temperature decrease was larger in
396 females, regardless of the body surface area studied (lower limbs, upper limbs, trunk or total surface
397 area). For all sexes and body areas, the dynamics of skin temperature followed two successive
398 transitional regimes: an initial exponential decline period (i.e., steep drop of skin temperature in the
399 first 30 seconds), followed by a linear-type behavior. Deducted regression laws suggested that to
400 achieve the same dose-response effect, the duration of the WBC protocol must be much longer in
401 males than in females (for example +36% to +62% depending on body area for a same skin cooling
402 magnitude of -12°C). However these results must be considered as a proof of concept and taken
403 with caution. Anybody using a WBC chamber should not blindly increase the WBC session duration
404 by 36 to 62% in males compared to females, the consequences could be dramatic. Protocols should
405 be carried out within exposure times no longer than 4 minutes at -110°C not to compromise the
406 health of the patients.

407 This preliminary study is the first to show that it seems necessary to differentiate in whole body
408 cryostimulation the exposure durations in males and females in order to achieve the same dose-

409 response effect and to propose individualized protocols. Limited to young and healthy subjects, it
410 requires, in perspective, on the one hand to be confirmed by a larger sample size and on the other
411 hand to be extended to other populations, such as obese populations.

412 **Ethics Statement**

413 No permission from the local ethics committee was obtained, since only biomedical/clinical studies
414 require this approval according to French law. However, the research participants provided written
415 informed consent and the study was conducted in line with the principles of the Helsinki Declaration
416 and its following amendments.

417 **Conflict of interests**

418 The authors report no conflict of interests regarding the publication of this paper.

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421 this study, as well as the physiotherapy students from the CHU of Reims who participated in this
422 study.

423

424 **Author contribution statement**

425 GP and BB conceived and designed research. FB and RE conducted experiments. FL contributed
426 statistical tools. GP, FB and BA analyzed data. GP wrote the manuscript. All authors read and
427 approved the manuscript.

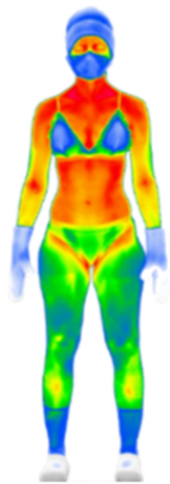
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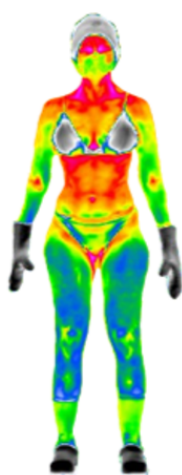
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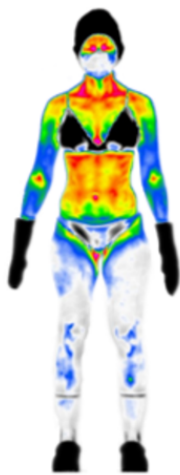
rest



30s



1min



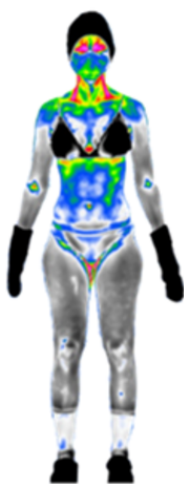
1min30



2min



2min30

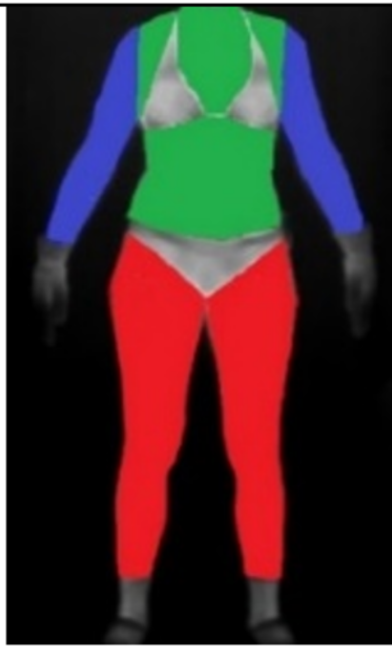


3min



Front side

Back side



Lower Limbs

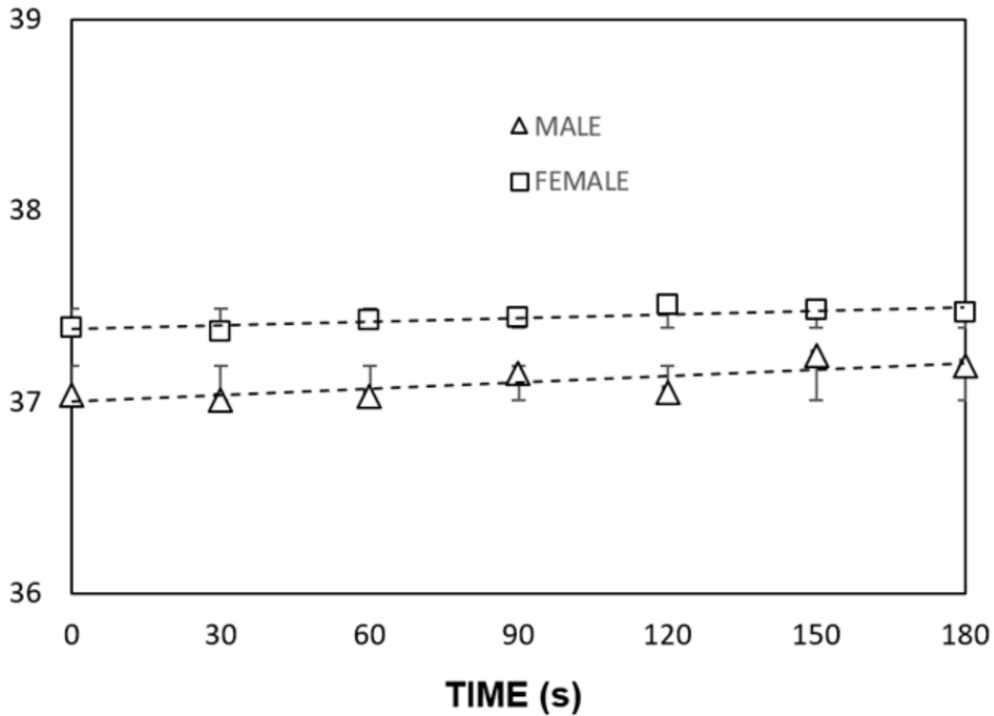


Upper Limbs

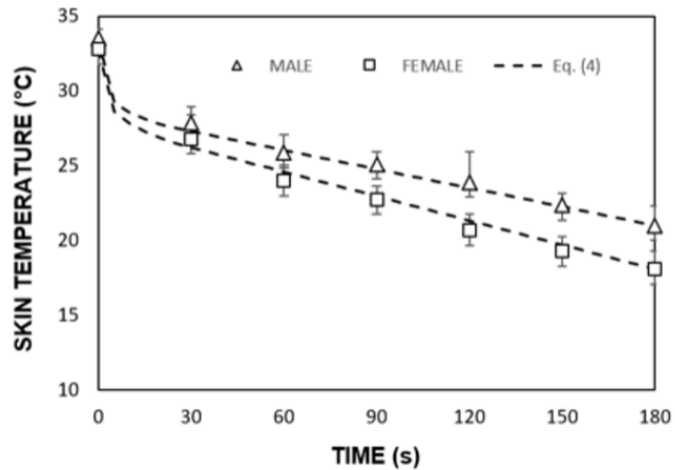


Trunk

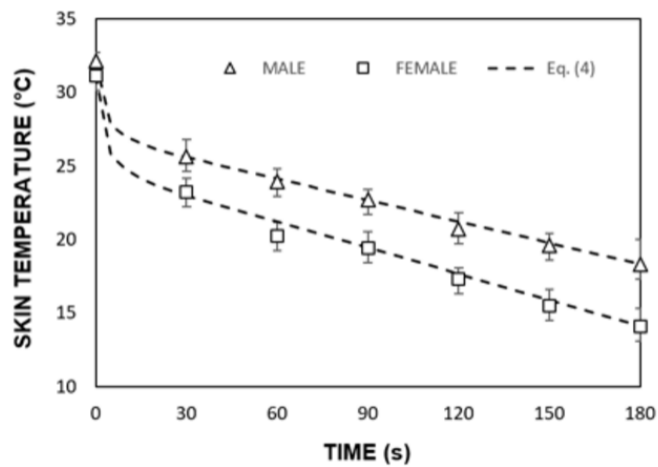
RECTAL TEMPERATURE (°C)



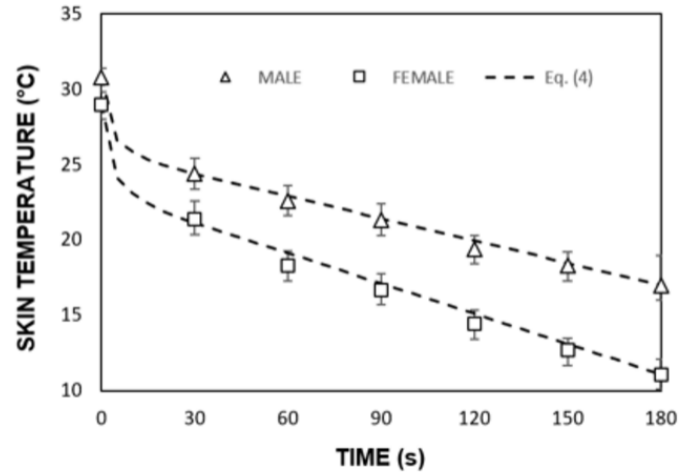
TRUNK

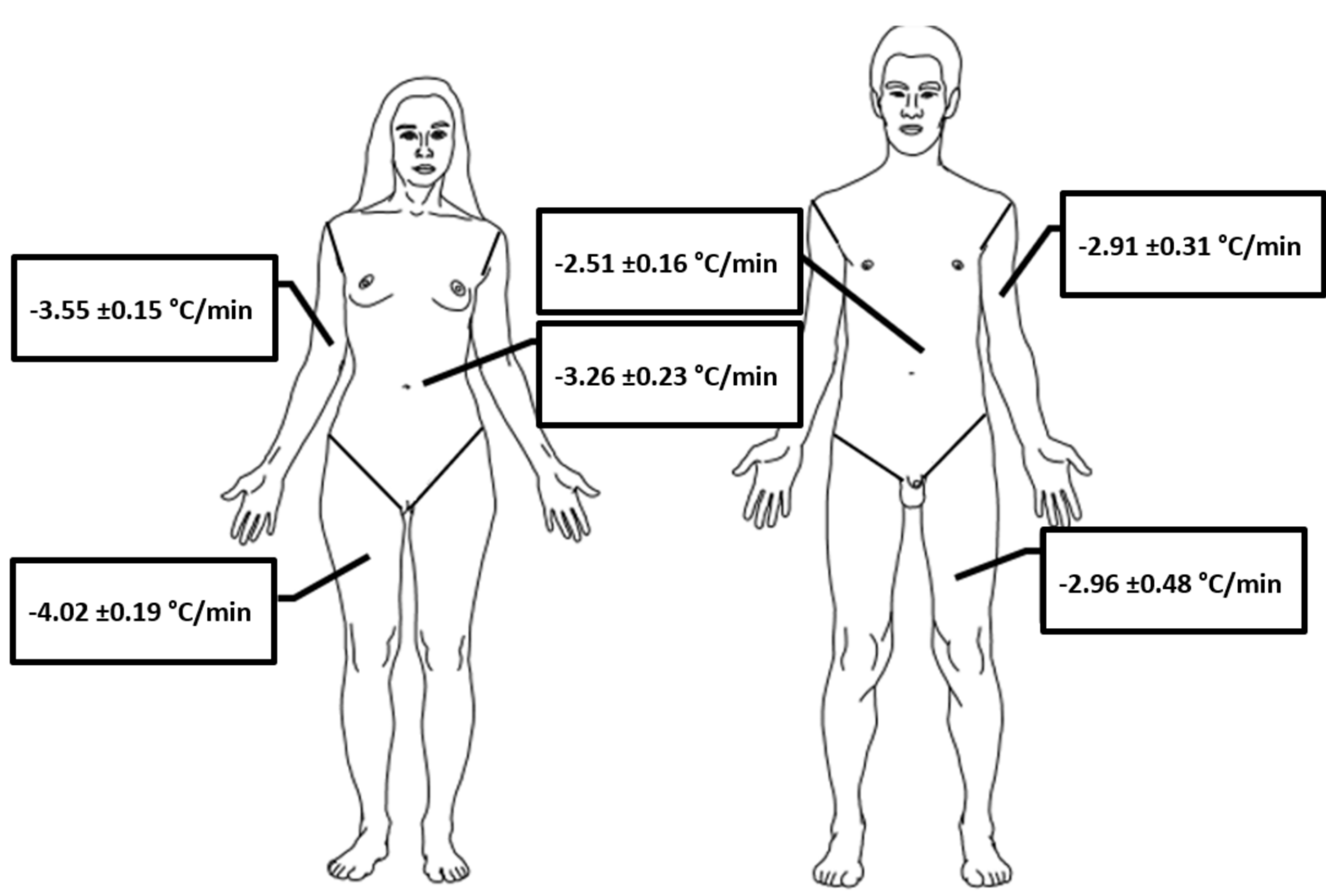


UPPER LIMBS



LOWER LIMBS





BODY

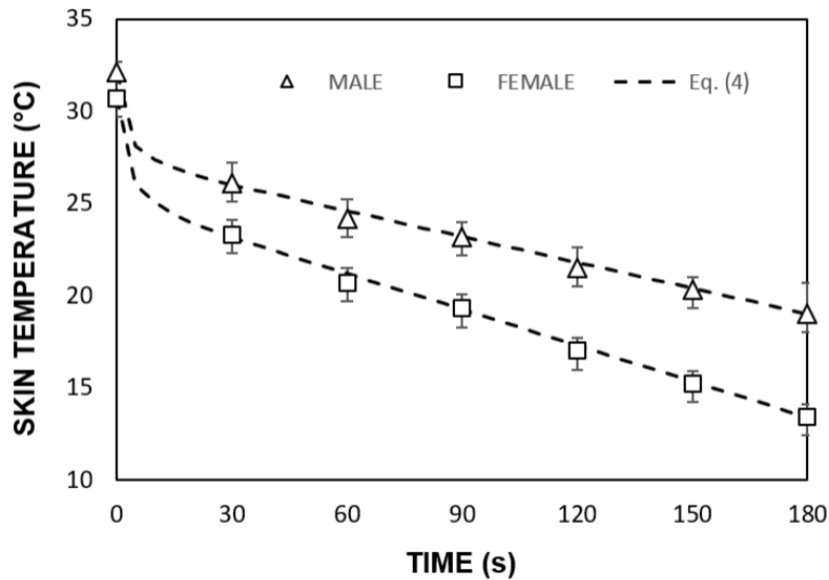


Table 1

	MALES (n=8)		FEMALES (n=9)		P Value
	Mean	SD	Mean	SD	
Height (cm)	181.5	5.6	167.7	7.2	^β 0.0005
Mass (kg)	74.2	7.5	60.9	4.7	^β 0.0004
Age (Years)	22.6	3.0	23.7	4.7	0.5982
Body Mass Index (kg/m²)	22.6	2.6	21.7	1.4	0.3859
Body Fat (%)	11.2	4.4	24.0	3.7	^β 0.0001
Body Lean Mass (%)	83.7	4.2	71.5	3.5	^β 0.0001
Body Fat Mass (kg)	8.5	4.0	14.6	2,4	^β 0.0016
Lean Mass (kg)	61.9	5.0	43.5	4.1	^β 0.0001
Body Surface Area (m²)	1.9	0.1	1.6	0.1	^β 0.0004

^β indicates a significant difference between sexes P≤0.01

Table 2

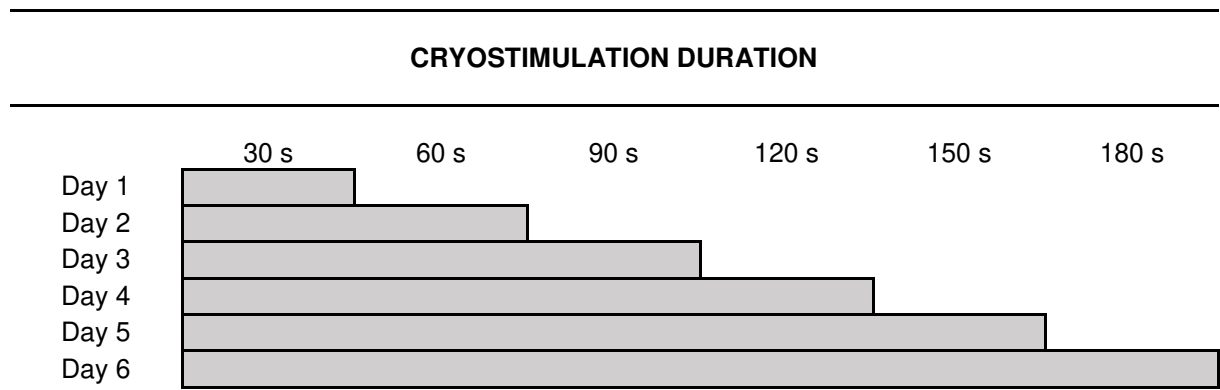


Table 3

	Trunk	Upper limbs	Lower limbs	Whole body surface
C₀ (°C/s) ; (SD)				
Females	- 0.0543 (0.0038)	- 0.0592 (0.0025)	- 0.0670 (0.0032)	- 0.0650 (0.0033)
Males	- 0.0419 (0.0026)	- 0.0485 (0.0052)	- 0.0493 (0.0080)	- 0.0467 (0.0053)
Tsk₀ (°C) ; (SD)				
Females	32.79 (0.53)	31.15 (0.53)	29.00 (0.41)	30.67 (0.44)
Males	33.53 (0.58)	32.11(0.63)	30.75 (0.64)	32.14 (0.63)
Tsk₁ (°C) ; (SD)				
Females	18.08 (1.26)	14.10 (1.17)	11.09 (1.03)	13.43 (0.75)
Males	21.01 (1.33)	18.34 (1.68)	17.01 (1.93)	18.96 (1.66)

Table 4

		Estimation of cryostimulation protocol duration (s)								
		Trunk			Upper limbs			Lower limbs		
Cooling gradient		Female	Male	E(%)	Female	Male	E(%)	Female	Male	E(%)
	-8°C	55	78	42	24	58	141	27	59	120
	-10°C	90	124	38	58	99	72	57	100	76
	-12°C	125	170	36	91	140	54	87	140	62
	-14°C	160	215	34	125	182	46	116	181	55
	-16°C	195	261	-	158	223	41	146	222	51
	-18°C	230	307	-	192	264	-	176	262	-
	-20°C	265	352	-	225	305	-	206	303	-