THERMAL RESPONSES INDUCED BY NITROGEN AND FORCED CONVECTION BASED PARTIAL-BODY CRYOSTIMULATION

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Abstract

Partial Body Cryostimulation (PBC) involves a very cold air flow directed to the body of subjects with minimal clothing. PBC is performed in a rapid timeframe, inside an on-purposed designed cryo-cabin. Recently, cryo-cabins have been built with different energy systems, however a validation study on relative thermal responses is missing. This study was aimed at comparing thermal responses following a PBC in an electrically powered cryo-cabin based on forced convection or into a standard nitrogen-fueled cryo-cabin. In a randomized crossover fashion, thirty-six subjects (F=20; M=16) underwent both cryo-exposures lasting 150 s each. Thermal responses were assessed before and immediately after completing each PBC session. Mixed model analysis of variance revealed a significantly colder temperature after electric PBC in all the body regions (except for the thighs) with respect to a standard nitrogen based PBC. Moreover, a significant lower thermal discomfort was perceived at the end of electric PBC as compared to that one felt following standard PBC. For the first time, the safety and thermo-effectiveness of an electric cryo-cabin based on forced convection was ensured. This methodology can be viable for practitioners of PBC and clinicians.

Key words: cryostimulation, cryo-cabin, thermal imaging, thermal discomfort.

1. INTRODUCTION

Partial-body cryostimulation (PBC) is a local, brief, cold exposure with the head of a participant outside a specifically designed cryo-cabin. Conversely to PBC, whole-body cryostimulation (WBC) is performed in a cold room and may involve several participants all exposed to extremely cold air temperatures. In PBC, only one participant is involved and the regions of the head and the neck are excluded from the cryo-treatment. Skin temperature, blood pressure, heart rate, heart rate variability, thermal and comfort sensations during PBC and WBC were addressed by few articles (Hausswirth et al., 2013; Louis et al., 2015). Both technologies provided benefits on athletes (Bouzigon et al., 2021) and general population (Bouzigon et al., 2016). Technically speaking, PBC offers a couple of advantages with respect to WBC: it could be more easily moved on a sport venue (gym, pitch, etc.) and allows the user to maintain the neck and the head outside the device. This is crucial for those who have had previous episodes of claustrophobia. While, to date, the most prevalent model of PBC unit present on the market needs liquid nitrogen to generate extreme low temperatures, in the last ten years numerous manufacturers have built cryo-chambers that can be cooled electrically, without the use of nitrogen.

In challenging periods like ours, characterized by climate changes (World Health Organization, 2021) and energetic crisis, finding eco-sustainable solutions in the industry of PBC devices should be conceivable. Specifically, new generations of cryo-cabins powered by renewable energy such as electricity might be explored instead of those based on liquid nitrogen, limiting costs and with equal, or possibly superior, effects.

A further argument in favor to switching to the electricity instead of nitrogen, is the safety. In fact, nitrogen-powered cryo-cabins can expose to the possible risks of hypoxia (Carrard et al., 2017) and skin diseases (Greenwald et al., 2018; O’Connor et al., 2018; Selfe et al., 2014). In addition, an electrically powered cryo-cabin can be more ecological with respect to liquid nitrogen due to breakdown process production of this latter, its storage and transportation. Lastly, as suggested by Bouzigon and colleagues (2017), a wind chill generated by a forced convection over a participant placed inside a cabin may diminish the boundary effect phenomenon (Schlichting and Gersten, 2017), allowing the device to be more efficient.

In 2022, a new model of cryo-cabin based on forced convection (eCabin, CTN, Helsinki, Finland), was launched. The way to cool down the inner of this model of cryo-cabin is based on six fans that generate very cold air: two sets of three fans are placed vertically (at 83 cm, 130 cm, and 173 cm from the floor) in front of the others, permitting to homogeneously decrease the skin temperature of the user's front and back regions.

As also explained by Bouzigon et al. (Bouzigon et al., 2017), the thermo-adequacy of a singular cryo-session, is typically evaluated by assessing the delta of the skin temperature measured throughout the cryostimulation. Thus, an essential method to validate a cryostimulation device is measuring the subject's skin temperature drop before and after a single session. A simple validation might consist in comparing this temperature decrease measured post-stimulation in differently powered cryo-cabins.

To our best knowledge this is the first study concerning the effects of an electric fueled PBC on skin temperature. Specifically, the thermal responses registered after a single treatment of PBC (Fonda et al., 2014) in a standard (De Nardi et al., 2021b) nitrogen based cryo-cabin were compared to a PBC session performed in the forced convection based cryo-cabin. We hypothesized no major differences between the two cryo-cabins stimulations, particularly the skin temperature measured after the two cryo-exposures (either in the electric or in the standard nitrogen-based model) would be comparable.

2. METHODS

2.1 PARTICIPANTS

Beforehand the study, the sample-size was calculated using a commercial software package (G * Power 3.1.9.2), setting the statistical power of 0.95, the level of probability of 0.05 and an effect size *f* of 0.25. The software determined that thirty-six subjects would be the minimal number of participants for the study purpose. All subjects were examined by a qualified medical doctor to avoid any contraindication to very-low temperatures exposure. Participants were explained about the scope of the study and signed a written consent prior to be enrolled. The recruited participants were free to abandon the study at any time, without any specific justifications. Subjects were also asked not to drink alcohol and hot drinks, nor smoke in the twenty-four hours preceding the study. The Ethics Committee of the University of Milan approved the study.

2.2 STUDY DESIGN

The design of this trial was conceived as a randomized-crossover one. Therefore, each subject performed both two cryo-exposures (nitrogen- and electric-based) in a randomized order as determined by a computer algorithm.

The protocol consisted of two consecutive sessions of PBC with a washout interval of three weeks between each session. The time of both cryo-sessions was the same (from 16:00 to 18:00). An open cryo-cabin (De Nardi et al., 2021a) was utilized for the PBC session, whilst for the other session was utilized a forced convection based cryo-cabin.

Participants were asked to arrive at the venue 30 min before the experiments, where they must await sitting on a bench to acclimate to the room temperature (20.0 ± 0.5 °C) with minimal clothing of shorts, woolen socks, and wooden clogs, as a globally accepted safety protocol. Subjects were asked to indicate their height, because both devices are equipped with a mobile lift which allows to adjust the height of each participant, enabling the user to expose the whole body to the cold air up to the shoulders, leaving the region of the head and the neck outside (Fig.1).

During a session in the nitrogen based cryo-cabin, subjects were exposed to extremely cold temperatures, as proposed by Fonda and coauthors (Fonda et al., 2014) for 150 seconds at a temperature ranging from -130 to -170 °C, as shown by the display of the device, (Cryomed Pro, Criomed, Ltd, Kherson, Ukraine). The manufacturer set up that every time the temperature inside the cabin (measured at the nitrogen nozzle) rose above -130°C, the device would have blown three-second-long injections of nitrogen. An expert operator supervised all operation and asked the participants to keep walking on the identical spot without interruption during the session. Upon completion of the PBC, participants were required to exit rapidly from the cryo-cabin (within 10 s).

Essentially the same protocol was applied during a session in forced convection based cryo-cabin, with the following changes: each subject, according to the manufacturer's recommendations, wore a neck warmer in addition to other equipment used for the nitrogen based cryo-cabin. The standard parameters suggested by the manufacturer were chosen for this study: a duration of 150 s with a temperature of -25.4 ± 0.5 °C and a wind speed of 17.0 m/s. The inner temperature of the empty cabin, before each session, was controlled with the six thermocouples inside the device (Fig. 1B).

2.3 MEASUREMENT OF THE SKIN TEMPERATURE AND THERMAL DISCOMFORT

Thermal images of the whole body were collected before and after each exposure (strictly within one minute after the end of the treatment) (Fig. 2) utilizing a thermal imaging camera

(E54, Flir Systems, Danderyd, Sweden) in agreement with the normative requirements of infrared medical imaging (Costello et al., 2012; Ring and Ammer, 2015, 2012). Participants were also asked not to talk, eat, drink, rub the skin or do anything else that might potentially affect the skin temperature. The procedure for acquiring thermal images was the same as previously reported (De Nardi et al., 2021b). Sixteen regions of interest were likewise analyzed as previously studied (De Nardi et al., 2021b).

Thermal discomfort was rated by the subjects at the end of each PBC session using a 10-cm visual-analogue scale, where 0 = "not feeling cold in any way" and 10 = "feeling unbearably cold"(De Nardi et al., 2021a; Fonda et al., 2014; Leon et al., 2008) .

2.4 STATISTICAL ANALYSIS

Data were shown as mean ± standard deviation. Visual inspection of the Gaussian curve was controlled by skewness and kurtosis indexes, both within the conventional cut-off (Groeneveld and Meeden, 1984). Normality, homogeneity and sphericity were checked using Shapiro-Wilk, Levene, and Mauchly test, respectively.

An analysis of variance (ANOVA) mixed model with repeated measurements was performed as to body regions' temperature, and thermal discomfort. This model was selected to take into consideration the intrinsic (and uncontrolled) inter-variability of the subjects. The variability among the subjects was always accounted as a random factor.

In particular:

- the effects of type of cryostimulation (nitrogen and electric), positioning of body surfaces (front and rear), time (pre and post cryostimulation), gender (male and female), and the relative interactions were analyzed. For all these models, the dependent variable was the temperature.

- Subjects' *thermal discomfort* perceived at end of each PBC trial was evaluated with a two-way ANOVA with effects of cryo-exposure (nitrogen, electric), and gender (male, female). Post-hoc pairwise comparisons were performed by utilizing Bonferroni's test.

For all analyses, an alpha p-value of < 0.05 was intended as statistically significant.

Partial eta squared (η_p^2) effect sizes (ES) were determined and interpreted using the following cutoffs: small effect, $\eta_p^2 \leq 0.03$; medium/moderate effect, $0.03 < \eta_p^2 < 0.10$; large effect, $0.10 \leq \eta_p^2 < 0.20$; very large effect, $\eta_p^2 \geq 0.20$ (Cohen, 2013, 1988) .

All statistical analyses were executed with GraphPad Prism 9.4.0 (San Diego, CA, USA) and the Statistical Package SPSS version 28 for Mac (Armonk, NY, USA; IBM Corp.).

3. RESULTS

3.1 PARTICIPANTS

One male subject had to withdraw prior the completion of the study, therefore he was excluded from the analysis. A total of thirty-six participants (females = 20; males = 16) were recruited for the study with following characteristics: age, 43.1 ± 12 years; height 1.71 ± 0.08 m; body weight 68.5 ± 11.3 kg and BMI 23.2 ± 3.1 kg/m² (mean ± SD).

3.2 BODY SURFACES PROFILES OF THE TEMPERATURE: THERMAL IMAGES OUTPUTS

There was a significant interaction position x time x cryostimulation ($F_{(1, 8)} = 15.536$, $P < 0.001$, $\eta_p^2 = 0.320$) with very large effects of gender ($F_{(1, 8)} = 15.536$, $P < 0.001$, $\eta_p^2 = 0.232$), position ($F_{(1, 8)} = 36.446$, $P < 0.001$, $\eta_p^2 = 0.525$), time ($F_{(1, 8)} = 878.569$, $P < 0.001$, $\eta_p^2 = 0.964$), and the type of cryo-exposure ($F_{(1, 8)} = 78.504$, $P < 0.001$, $\eta_p^2 = 0.704$). Globally, over all body regions the temperature was perceived as significantly lower after electric PBC with respect to standard PBC, except for the thighs (right, P = 0.238; left, P = 0.296). As to gender pairwise comparisons, the temperature was significantly colder in the female subjects over all the body regions except for the trunk (upper: P = 0.98; lower: P = 0.181). Likewise, concerning the position comparison, a significantly lower temperature was measured following electric PBC in all body regions except for the trunk (upper: P = 0.94; lower: P = 0.193). A graphical representation of the temperature measurements, before and after each cryostimulation, taken over the principal body regions (arms, legs, trunks), is offered in Figure 3 for all subjects. The same output is partitioned per gender, in Table 1.

3.3 THERMAL DISCOMFORT

The ANOVA two-way disclosed a significant effect of cryostimulation ($F_{(1, 33)}$ = 8.166, P = 0.0073, η_p^2 = 0.907) with a lesser thermal discomfort perceived after electric PBC (Bonferroni's, P = 0.004). No effect of gender ($F_{(1, 33)}$ = 0.315, P = 0.578, η_p^2 = 0.06), neither an interaction cryostimulation x gender ($F_{(1, 33)}$ = 2.631, P = 0.114, η_p^2 = 0.004) were found. Mean VAS values of thermal discomfort, including those differentiated per gender, are reported in Table 2.

4. DISCUSSION

In this study, differences in skin temperature and thermal discomfort were evaluated after a single PBC session performed in two cryo-cabins: one in an open cryo-cabin cooled using liquid nitrogen, and one in a closed-top cryo-cabin based on forced convection. These study-results were comparable to those obtained by Bouzigon and colleagues (2017) who investigated the effects of a new model of cryo-chamber incorporating forced convection. Not only our hypothesis was confirmed but we also registered a significantly colder temperature after electric PBC in all the body regions (except for the thighs) with respect to following a standard nitrogen based PBC. Moreover, our study revealed a significant lower thermal discomfort perceived after forced convection PBC as compared to that one felt following standard PBC. For the first time, it was demonstrated that an electric cryo-cabin based on forced convection can be successfully utilized in the field of cryostimulation.

These findings demonstrated that the electric cabin led to a more homogeneous cooling of the skin of the entire body rather than the nitrogen-based one. In fact, as shown in Fig. 3, skin temperature values over the different body regions were tightly more accurate in the forced convection-based cabin rather than in the nitrogen based one. A possible reason for such a difference could not be attributed to the covered top of the forced convection-based cryo-cabin. In fact, this was confirmed in a previous study (De Nardi et al., 2021b) in which a very wide range of skin temperature excursions were registered, for example from chest and thighs, both for the standard opened PBC, and for the cryo-cabin covered with an insulating lid. In that study, both cryo-cabins were nitrogen fueled.

Instead, the present study data suggest that the variety of the skin temperature measured from each body region might be attributable to the presence of the gaseous nitrogen inside the cabin. This lends support to a previous study of Savic and colleagues (2013) who investigated the inner temperature of an empty cryo-cabin. They used the same cryo-cabin utilized for this research, and they showed that the nitrogen leads to reach substantial differences over measurement sites because of the position of the nozzle from which the nitrogen is injected into the cabin. In a traditional cryo-cabin, this nozzle is placed, on the average, at the height of the trunk (See Fig.1). For this simple reason, the lower parts of the cryo-cabin are colder than the upper. It is noteworthy that cold air has higher density then warm air. Therefore, a traditional cryo-cabin could not be able to generate a homogeneous drop of the skin temperature for each different body regions.

Conversely, a forced convection technology, because of the fans placed both in the front (n = 3) and in the back (n = 3) of the subject (see Fig. 1), leads to a similar and homogenous cooling of the skin regardless of the body region sites. These findings are comparable to those of Bouzigon et al. (2017) who investigated the effects of a forced convection cryo-chamber. They attributed the refrigerating capacity of the new device to the ability of the wind chill to extract heat from the body. According to the authors (Osczevski and Bluestein, 2005), the heat loss procured by the wind chill model is greater than that one by the simple air flow. Analogous to the literature, our results may encourage the use of forced convection technologies when the aim is the homogeneity of the cooling for most of the body surfaces. Furthermore, it is well-known that standard PBC devices are designed to reach low temperatures by using nitrogen gases, which are directly in contact with the user inside the cabin. As for the standard protocol, the subject must have the head out of the cabin so to remain as much far as possible from the free nitrogen vapors. This is an important safety rule during a PBC session because the exposure to free nitrogen gases could potentially lead to the risk of asphyxia (EIGA, 2018). Moreover, the body of literature is plenty of cases of skin diseases due to use of liquid nitrogen. A participant of the study conducted by Mackenzie O'Connor and coauthors suffered for a cold burn injury due to a malfunctioned nozzle which sprayed liquid nitrogen directly on his skin. Also, Selfe et al. in 2014 documented that a Samoan rugby player reported superficial skin burn bilaterally on the region of the thighs after a nitrogen-based WBC. On the contrary, in our study no adverse episodes were reported, even though we recorded skin temperatures very close to zero degrees in a couple of subjects, which in theory could be hazardous for the skin. In general, liquid nitrogen it must be handled with caution as it can cause severe injuries or frostbite due to its extremely low temperature. Moreover, switching to the electricity may represent a more ecological option than the nitrogen one, given the use of renewable sources to produce electricity on a side, and the breakdown of the process of production, storage, and transportation of liquid nitrogen on the other one (Burkhart and Burkhart, 2014).

Remarkably, as reported in table 2, the thermal discomfort after the forced convection based cryo-cabin is lower than in the nitrogen one. This depicts an ideal condition for the new technology: the average skin temperature diminishes although this thermo-decrement remains homogeneous over different body regions. These lower epidermic temperature values were accompanied with a lower thermal discomfort as compared to those achieved following a standard nitrogen powered PBC.

As a limitation of the study, the gender representation was not numerically equal (i.e., 20 females vs 16 males). However, this aspect should be negligible as properly weighed into the analysis.

In conclusion, these results indicated that cryo-cabins based on forced convection are thermo-effective and can be successfully utilized in the field of cryostimulation. The electrically powered equipment employed for a PBC based on forced convection met the safety standards and reached colder skin temperature over the majority of the body regions.

Figure Captions

Figure 1. Schematic representation of the two cryo-cabins (1A, forced convection based "electric"; 1B, nitrogen fueled).

Figure 2. Measurements of the skin temperature before and immediately after a standard nitrogen fueled PBC session (A, front; B, back) or a forced convection based, "electric" PBC (C, front; D, back).

Figure 3. Values of skin temperature before (pre) and immediately after (post) cryo-sessions, taken in eight body regions: in the front (A, nitrogen; B, electric) and in the back (C, nitrogen; D, electric). Data are presented as mean.

Table legend

Table 1. Male and females' skin temperature measured over the main body regions before and after the two different cryostimulations (nitrogen-, electric). Data are presented as mean ± SD.

Table 2. Thermal discomfort (VAS) at the end of the two different cryostimulations (nitrogen, electric).

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