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#### **Credit author statement**

Mireia Alcamí Muñoz: Conceptualization; Investigation; Data curation; Formal analysis; Roles/Writing — original draft. Jose Ignacio Priego-Quesada: Conceptualization, Methodology, Supervision, Formal analysis, Roles/Writing — original draft. Marc Gimeno-Raga: Conceptualization, Methodology, Supervision, Writing — review & editing. Alvaro Durán Lozano: Investigation, Writing — review & editing. Marina Gil-Calvo: Conceptualization, Methodology, Supervision, Writing — review & editing.

# Effect of fatigue strength exercise on anterior thigh skin temperature rewarming after cold stress test.

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# **Abstract**

Although dynamic thermography skin temperature assessment has been used in medical field, scientific evidence in sports is scarce. The aim of the study was to assess changes in anterior thigh skin temperature in response to a cold stress test after a strength exercise fatiguing protocol. Ten physically active adults performed a familiarization session and two strength exercise sessions, one with dominant and the other with nondominant lower limb. Participants performed bouts of 10 concentric and eccentric contractions of leg extensions in an isokinetic device until reaching around 30% of force loss. Infrared thermographic images were taken at baseline conditions and after the fatigue level from both thighs after being cooled using a cryotherapy system. ROIs included vastus medialis, rectus femoris, adductor and vastus lateralis. Skin temperature rewarming was assessed during 180s after the cooling process obtaining the coefficients of the following equation:  $\Delta Skin$  temperature =  $\beta_0 + \beta_1 * ln(T)$ , being  $\beta_0$  and  $\beta_1$  the constant and slope coefficients, respectively, T the time elapsed following the cold stress in seconds, and  $\Delta$ Skin temperature the difference between the skin temperature at T respect and the pre-cooling moment. Lower  $\beta_0$  and higher  $\beta_1$  were found for vastus lateralis and rectus femoris in the intervention lower limb compared with baseline conditions (p<0.05 and ES>0.6). Adductor only showed differences in  $\beta_0$  (p=0.01 and ES=0.92). The regressions models obtained showed that  $\beta_0$  and  $\beta_1$  had a direct relationship with age and muscle mass, but an inverse relationship with the number of series performed until 30% of fatigue (R<sup>2</sup>=0.8). In conclusion, fatigue strength exercise results in a lower skin temperature and a faster thermal increase after a cold stress test.

**Keywords**: dynamic thermography; infrared thermography; isokinetic exercise; thermal image.

#### **Abbreviations**

 $\Delta$ Skin temperature: the difference between the skin temperature at T respect with precooling moment.

 $\beta_0$ : Constant coefficient of the logarithmic equation obtained with the skin temperature data after the thermal cold stress test.

 $\beta_1$ : Slope coefficient of the logarithmic equation obtained with the skin temperature data after the thermal cold stress test.

ADD: adductor

**ADTC:** active dynamic thermography after cooling stress.

**NETD**: noise equivalent temperature difference.

RF: Rectus femoris

**ROI**: region of interest.

T: the time lasted after the cold stress in seconds

VL: Vastus lateralis.

VM: Vastus medialis.

#### 1. Introduction

Skin temperature is mainly the result of heat transfer from peripheral blood flow, core and environmental temperature to cutaneous surface, when there is not contact with other surfaces or convection currents (Ammer and Formenti, 2016). It can be measured by means of infrared thermography, a technique with several applications in the sports' field (Hillen et al., 2020), such as preventing and treating injuries (Gómez-Carmona et al., 2020; Hadžić et al., 2019; Hildebrandt et al., 2010), detecting delayed onset muscle soreness (Al-Nakhli et al., 2012), assessing the effect of garments on skin temperature responses (Fournet and Havenith, 2017), evaluating skin temperature after exercising in hot or cold environments (Fournet et al., 2013; Gerrett et al., 2015) or assessing the effect of aerobic or strength exercise (Pérez-Guarner et al., 2019; Weigert et al., 2018). In these applications, it is usual to apply infrared thermography in steady-state conditions before or after exercise, although another option is to assess skin temperature responses by active dynamic thermography.

Active dynamic thermography is used to examine the thermal response of a surface (e.g. skin surface) to an applied stimulus that can be either heating or cooling (Prindeze et al., 2018). It is a technique well-established in industrial applications and is gaining interest in medicine with the recent advances in cameras and image processing technologies (Prindeze et al., 2018). In the case of active dynamic thermography after cooling stress (ADTC), it has been applied in medical field such as diabetics assessment (Carbonell et al., 2019; Zeng et al., 2016), the detection of Raynaud pathology (Horikoshi et al., 2016), stenosis in the carotid artery (Saxena et al., 2020), infantile haemangiomas (Burkes et al., 2016), and breast cancer (Gonzalez-Hernandez et al., 2019; Sarigoz and Ertan, 2020), among others. The skin response after ADTC is related with the vasoconstriction produced during cooling by the cold stimulus, the capacity to resolve vasoconstriction of the participant having undergone cooling, or the heat present in deeper tissues than the skin (Burkes et al., 2016; Davey et al., 2013; Keramidas et al., 2014; Lahiri et al., 2016; Priego-Quesada et al., 2020). However, despite all the physiological information that ADTC can provide and its potential for assessing alterations of vascular function (Burkes et al., 2016; Carbonell et al., 2019; Zeng et al., 2016), its application in the sports' field is still scarce (Jose Ignacio Priego-Quesada et al., 2020).

The little evidence available on using ADTC in sport science includes Priego-Quesada et al. (2020) observation of a greater reduction of skin temperature after the application of a cold stress 24 hours after a marathon race. They suggested that this greater skin temperature reduction was due to a higher peripheral vasoconstriction produced by the muscle damage caused by the marathon (Priego-Quesada et al., 2020). Despite this initial study that analyzed the effect of muscle damage, it is unknown how the performance of physical exercise modifies skin temperature response after performing ADTC. This is important, as it would allow us to develop new applications of this type of test in sports science. Exercise increases the levels of endothelial nitric oxide, which can reduce blood vessel vasoconstriction (Percival, 2011), and therefore enhance the ability to resolve vasoconstriction after cooling. Moreover, although the increase of muscle temperature, due to the higher heat production resulting from exercise, can also passively increase heat transference during the rewarming phase after cooling, the heat transference via conduction is considered very slow and less important than skin blood flow (Cramer and Jay, 2016; Davey et al., 2013). We, therefore, expected a faster post ADTC rewarming following a fatigue and exercise strength test.

Thus, the aim of this study was to assess changes in anterior thigh skin temperature in response to a cold stress test after a strength exercise fatiguing protocol. We hypothesized that strength exercise may result in a faster skin temperature rewarming after applying cooling stress.

# 2. Material and methods

#### 2.1 Participants

Ten physically active adults (7 males and 3 females) participated voluntarily in this study (Table 1). Body mass, body fat percentage and muscle mass percentage were determined by bioelectrical impedance (Tanita BC-587, Tanita Corporation, Tokyo, Japan). All participants signed a written consent prior to the study, which complied with the Declaration of Helsinki. The ethics committee of the University of Valencia approved this study (Code: 1253395; Date of approval: 07 February 2020).

Table 1 near here

# 2.2 Experimental design

Several instructions were given to the participants before to start the study (Moreira et al., 2017; Priego Quesada et al., 2017), such as to avoid stimulant substances (alcohol, tobacco, coffee etc.) 12 h before the tests, heavy meals at least 2 h before the test, therapeutic treatments and strenuous exercise 24 h before the test and sprays or creams on the skin surfaces.

Each participant performed one familiarization session and two strength exercise sessions (Figure 1A) with one lower limb performing the exercise, while the other that did not perform the fatiguing exercise was used as a control (previously randomized). The second session was approximately one week after the previous one, changing the role of the lower limbs. Exercise sessions were composed by a warm-up protocol for both lower limbs, strength exercise in the isokinetic device for the corresponding lower intervention limb in each session, and infrared images after cold stress.

# Figure 1 near here

# 2.3.1 Warm-up protocol

Prior to each exercise session, the participant performed a warm-up protocol. The warm-up started with undertaking exercise for three minutes on a cycle ergometer (Bicicleta Spinning SPV, Gridinglux S.L, Madrid, Spain), at an effort perception of 3 to 5 on the Borg's scale of 10 points (Buckley and Borg, 2011). After that, on an isokinetic device (Genu Plus®, EasytTech S.r.l, Florence, Italy), participants performed two bouts of five repetitions of concentric and eccentric contractions at a speed of 120°/s, first with the control lower limb and then with the intervention one. Body position was adjusted in the isokinetic device to 90° of knee flexion and 75° of hip flexion (Souron et al., 2018). This position was recorded so that it could be maintained during the rest of the study. Once established, leg extension was performed with a perception of effort of 5-7 on the Borg's scale (Buckley and Borg, 2011).

# 2.3.2 Strength exercise protocol

After warming up, participants performed the exercise protocol only with the intervention lower limb. A series of 10 repetitions with 1 minute rest on the isokinetic device were performed, until achieving a force loss of 30% compared with the first exercise bout (Paulsen et al., 2012; Sánchez-Medina and González-Badillo, 2011). To determine the percentage of fatigue, the mean force production (N\*m) for each series, generated between the second and third repetition, was calculated. For each series, the mean of the first series was subtracted until determining that the decrease in force had reached 30%. The mean force production between the second and third repetition was used as a reference, given that in the first repetition the participant did not reach the maximum peak of force required. A range of 3% upper and lower of established force loss was accepted. Immediately after the exercise protocol, infrared thermography measurements were taken in steady state conditions and after applying cold stress.

# 2.3.3 Infrared thermography after cold stress test

On the familiarization day (after anthropometric and body composition parameters were taken) and on the intervention days (once finished the strength exercise protocol), a cold stress protocol was carried out. In a randomized order, one day the dominant limb was used as the intervention limb (performing the fatigue exercise) and the non-dominant as a control, and the other day, the non-dominant limb was the intervention limb while the other limb was used as a control. A cold stress protocol was carried out always on both limbs during the same session, first in the limb used as the intervention limb and then in the control limb.

A cryotherapy device (GameReady GRPro 2.1; CoolSystems Inc) using an assembled thigh band was used to perform thigh cooling. Temperature of cooling was set at 0-3°C with moderate pressure and in supine position with the trunk slightly raised and the lower limb to be cooled fully extended. After 3' of cooling, participants were asked to take off the thigh band and remain standing in front of a 2x2.5m matt black panel. Thermal images of the anterior part of the thigh were taken at 15, 30, 45, 60, 90, 120, 150 & 180 s after the cooling process in a room at a temperature of  $23.0 \pm 1.1$ °C and  $41.1 \pm 6.4$ % relative humidity.

Skin temperature was measured using a Flir E60bx camera (Flir E60bx, Flir Systems Inc.) with a resolution of 320x240 pixels, with measurement uncertainty of  $\pm 2^{\circ}$ C or 2%

and noise equivalent temperature difference (NETD) <0.05°C. It was located 1 m away from the participant, with the lens parallel to the thigh. The infrared camera was turned on 10 min before each measurement to ensure its electronic stabilization. Participants wore short sportswear and the thermograms were captured with the thighs uncovered. Reflected temperature of the room was measured according to the standard method (ISO, 20084-1, 2008). This data was introduced into the camera set up. Calibration of the camera was checked before the study using a black body (BX-500 IR Infrared Calibrator, CEM, Shenzhen, China). Emissivity was set at 0.98. ROIs included vastus medialis (VM), rectus femoris (RF), adductor (ADD) and vastus lateralis (VL) (Figure 1B). The thermographic imaging in sports and exercise medicine checklist (Moreira et al., 2017) was used to check that important methodological aspects related with infrared thermography measurement were followed.

The mean temperature of each ROI during the rewarming process was assessed using ThermaCAM Researcher Pro (ThermaCAM<sup>TM</sup> Researcher Pro 2.10, FLIR Systems AB, Sweden) analysis software. The temperature obtained after the exercise protocol was subtracted from the one obtained before the cold stress test to assess the  $\Delta$ Skin temperature. Data was compiled on an excel sheet, where a logarithmic equation for mean  $\Delta$ Skin temperature of each ROI, measurement moment and intervention lower limb was adjusted. Constant ( $\beta_0$ ) and slope ( $\beta_1$ ) coefficients for each body region at baseline conditions and for the intervention and control lower limb were obtained using the following logarithmic equation (Priego-Quesada et al., 2020):

$$\Delta$$
Skin temperature variation =  $\beta_0 + \beta_1 * \ln(T)$ 

T being the time elapsed after the cold stress in seconds, and  $\Delta$ Skin temperature the difference between the skin temperature at T compared with the pre-cooling moment

# 2.3.4 Statistical analysis

The statistical analysis was performed using SPSS v.20 (SPSS Statistics, IBM, Chicago, USA). The normality of the different variables ( $\beta_0$  and  $\beta_1$  of each ROI) was verified using the Shapiro-Wilks test, obtaining a normal distribution (p>0.05), so that parametric tests could be carried out. For each logarithmic coefficient of the study ( $\beta_0$  and  $\beta_1$ ) an intra-subject repeated measures ANOVA test was performed with two factors: lower limb condition factor (baseline, intervention and control), and body region factor (vastus medialis, rectus femoris, adductor and vastus lateralis). As a post

hoc test, a Bonferroni correction was carried out for pairwise comparisons. The level of significance was established at  $\alpha$ =0.05. Finally, the Cohen effect size (ES) was calculated for the significant differences found in the pairwise comparisons (d), and were classified as small (0.2-0.5), moderate (0.5-0.8) or large (>0.8). All results are presented as mean values  $\pm$  standard deviation (SD).

Finally, stepwise multiple linear regressions were performed to see whether other factors could be influencing the variation of  $\beta_0$  and  $\beta_1$ . For this reason,  $\beta_0$  and  $\beta_1$  were used as predictive variables, and the inputs of the models were: the other coefficient of the logarithmic equation, age, sex, condition (baseline, intervention or control lower limb), dominance (dominant or non-dominant lower limb), muscle mass, body fat, and the number of series performed until 30% of force loss and body region (vastus medialis, rectus femoris, adductor and vastus lateralis). Final models were then adjusted to retain only variables yielding p-values <0.05. For the models obtained, the coefficient of each variable of the equation, the percentage of the variance explained by the model ( $R^2$ ), and the significance value of the model were provided.

#### 3. Results

As presented in table 2, lower  $\beta_0$  were found for vastus lateralis, rectus femoris and adductor in the intervention lower limb compared with baseline conditions. In addition, higher  $\beta_1$  are showed for vastus lateralis and rectus femoris in the intervention lower limb when compared with baseline conditions; however, no differences in  $\beta_1$  were found for adductor. Vastus medialis showed no differences, neither in  $\beta_0$  nor in  $\beta_1$  between baseline conditions and intervention lower limb. Regarding the comparison between control and intervention, only rectus femoris showed higher  $\beta_0$  and  $\beta_1$  in the control lower limb, while the other muscles presented no differences. No differences were found in  $\beta_0$  nor in  $\beta_1$  in any of the muscles studied on comparing control lower limb and baseline conditions. Figure 2 graphically represents the participants' data and the logarithmic equations obtained.

Table 2 near here

Figure 2 near here

Table 3 shows the regressions models obtained using multiple linear regressions analysis. Both the  $\beta_0$  and  $\beta_1$  show direct relation with age and muscle mass, but present an inverse relationship with the number of series performed until 30% of fatigue.  $\beta_0$  also shows an inverse relationship with condition (baseline condition, control or intervention lower limb).

#### Table 3 near here

#### 4. Discussion

The aim of this study was to assess skin temperature changes in different regions of the anterior thigh in response to a cold stress test following a strength exercise fatiguing protocol. The main results were that higher  $\beta_0$  and  $\beta_1$  were found for vastus lateralis and rectus femoris in the intervention lower limb comparing with baseline conditions (coefficients obtained with the cold stress test before performing exercise), with no differences in any of the regions assessed between control (lower limb that did not perform exercise, assessed after the fatigue exercise) and baseline conditions. Moreover, the regression models obtained by multiple linear regression analyses showed that, generally,  $\beta_0$  was directly related to age and muscle mass, and inversely related to constant coefficient, series, condition and  $\beta_1$ . On the other hand,  $\beta_1$  was directly related with age and muscle mass, but inversely related with constant coefficient, series and  $\beta_0$ .

Generally, in healthy participants, a peripheral vasoconstriction is produced during cold stress by the cold stimulus, and a vasodilation on finishing the cold application to return to baseline values (Lahiri et al., 2016; Sawasaki et al., 2001). It has been shown that skin temperature rewarming following cold application depends mainly on skin blood flow, but it also can occur passively from the environment and from deeper tissues (Davey et al., 2013). While slower rewarming on finishing the cold stress has been observed for pathologies with a dysfunction in the blood circulation such as diabetes, Raynaud pathology or during simulating pathology conditions by the occlusion of the forearm (Carbonell et al., 2019; Horikoshi et al., 2016; Saxena et al., 2018; Zeng et al., 2016), faster rewarming has been observed for other pathologies such as cancer or haemangiomas (Burkes et al., 2016; Gonzalez-Hernandez et al., 2019; Sarigoz and Ertan, 2020). It is important to mention that these previous studies assessed different

body regions from the ones we explored in the current study. We hypothesized that strength exercise may result in a faster skin temperature rewarming after the application of cooling stress. This hypothesis proved to partially true for vastus lateralis and rectus femoris, where higher  $\beta_1$  was found in the intervention lower limb compared to baseline conditions. The higher  $\beta_1$  may be a result of an increase in levels of endothelial nitric oxide after exercise (Percival, 2011), which could enhance the ability to resolve vasoconstriction after cooling. Moreover, although muscle temperature increases through the higher heat production resulting from exercise, so enhancing heat conduction from the muscle tissue to the skin (González-Alonso, 2012), this is considerably slow process and of less importance than skin blood flow (Cramer and Jay, 2016; Davey et al., 2013), but could also have an influence on the higher  $\beta_1$  obtained in the results. While  $\beta_1$  is related with the slope of thermal recovery,  $\beta_0$  is more related with the ability to reduce skin temperature after cold stress, and therefore the skin peripheral vasoconstriction level (Leijon-Sundqvist et al., 2015). When performing demanding exercise with high physiological stress, muscles could present a baseline state of peripheral vasoconstriction to guarantee muscles blood supply (Priego-Quesada et al., 2019; Simmons et al., 2011), leading to lower  $\beta_0$  coefficients as shown in vastus lateralis, rectus femoris and adductor.

Although it is completely speculative to suppose that the effects of the different muscles would be reflected in differences in the skin temperature of the body areas analyzed, the role of the different muscles during the exercise performed could explain the differences observed between regions. Regarding  $\beta_1$  in the adductor, no differences were observed between conditions. This could be due to the involvement of this muscle in the exercise performance, it being more suitable for stabilization activities (Jeno and Schindler, 2020), requires less blood supply during the activity, and results in less muscle temperature increment. The same results were obtained for vastus medialis, which showed no changes in  $\beta_0$  or in  $\beta_1$ . This could be explained by its lower neuromuscular activity, this muscle being the weakest and most vulnerable muscle of the extensor mechanism (Fox, 1975), so having lesser involvement in the exercise.

When comparing control and intervention lower limbs, body regions in general showed no differences in  $\beta_0$  and  $\beta_1$ . This could be due to the state of peripheral vasoconstriction of the intervention lower limb, which could also be influencing the control limb, as seen in a study conducted by Priego-Quesada et al. (2020). Another explanation may be the

effect of contralateral muscles during strength training, where the neural signal unfolds, stimulating commissural interneurons on the spinal cord, and activating contralateral motoneurons (Escamilla-Galindo et al., 2017). This could explain an increased temperature in control lower limb. In rectus femoris, however, differences were observed between control and intervention lower limb. One possible explanation could be the greater amount of effort of the rectus femoris in the exercise, overcoming the possible temperature equalization with its contralateral counterpart. When seated, the rectus femoris is one of the weaker extensors of the knee (Murdock and Agyeman, 2019), needing more effort to perform the exercise. Finally, it is important to consider that both lower limbs performed the warm-up before the unilateral fatiguing exercise and were cooled sequentially, which could also influence the skin temperature response of the control lower limb, resulting in no differences between both conditions. Future studies should corroborate the results by cooling both lower limbs simultaneously and with no warm-up of the control lower limb.

Previous studies have suggested the TAR index (tissue activity ratio) using ADTC as a parameter to detect the presence of stenosis in the carotid artery or to predict the postoperative necrosis risk after breast reconstruction surgery (Saxena et al., 2020, 2019a). TAR is calculated at each pixel and is a dimensionless ratio of the rewarming rate to the cooling rate (Saxena et al., 2019b, 2020). Saxena et al. observed higher TAR values for control participants than patients with different grades of stenosis or patients who develop a necrosis after breast reconstruction surgery (Saxena et al., 2020, 2019a). For reasons of the method used in the present study, thermograms could not be obtained until 15 seconds after the end of cooling, and TAR values cannot be obtained using the raw data. However, using the logarithmic equations obtained, an estimation can be made, obtaining higher mean values at baseline (VL 0.86, RF 0.71, ADD 0.81, VM 0.72) than in control (VL 0.63, RF 0.57, ADD 0.61, VM 0.64) and intervention (VL 0.68, RF 0.62, ADD 0.65, VM 0.64) conditions. These values cannot be compared with previous studies as different regions were analyzed. However, they do show that the comparisons behave in a similar way to the differences in coefficients, showing clearly different values between the baseline condition and the intervention and control conditions, and also that an intervention results in a reduction of these values, which is line with the findings of previous studies (Saxena et al., 2020, 2019a).

We found some factors that could affect the rewarming process after the cold stress test. It is important to mention that because of the sample size assessed in this study, results should be considered with caution, and the observed relationships should be understood as indications for future lines of research. Our linear regression analysis shows that a greater amount of muscle mass resulted in a higher  $\beta_0$  and  $\beta_1$ . Higher muscle mass percentages imply higher metabolic heat production (Falk, 1998) which could create a higher thermal gradient between muscle and skin, resulting in a faster rewarming process. This is also in agreement with other studies, where trained participants had a higher and faster skin temperature increase after strength exercise (Escamilla-Galindo et al., 2017; Formenti et al., 2013). The number of series performed until reaching the 30% of force loss, however, is inversely related with  $\beta_0$  and  $\beta_1$ . The higher number of series performed implies more time for the muscles to exercise, which could increase the exercise demands and, therefore, the peripheral vasoconstriction explaining this association (Priego-Quesada et al., 2019; Simmons et al., 2011). The association obtained with age is in agreement with the higher skin temperatures in baseline state and the higher capacity of thermal response during exercise in younger participants observed by previous studies (Ferreira et al., 2008; Petrofsky et al., 2006). However, it is important to mention that in our study only young participants were assessed and the intensity of this associations could be increased in a sample size with a higher age variability. Finally, the association observed with the condition is in agreement with the differences observed between conditions in the logarithmic coefficients. However, it is interesting to note that the association was only observed for  $\beta_0$  and not  $\beta_1$ , which may suggest that exercise primarily affects the vasoconstricted state, and it is this reduction in temperature which affects thermal response. This would be in accordance with the associations observed between both coefficients, and also observed in a previous study (Priego-Quesada et al., 2020). This explanation should be verified by future studies.

Our study shows that performing exercise affects skin thermal parameters after a cold stress test. Further studies in this line should analyze whether muscles also respond after cold stress, by changing the variables, such as exercise intensity, the modality of the exercise performed or the level of fatigue reached. If the results were to coincide with our study, which shows changes in the post-cold test warm-up following fatiguing exercise, this type of protocol could be useful, either in scientific research or field

applications, as this would provide greater information, in a more straightforward way, on the participant's skin blood flow response.

Some limitations were noted while undertaking this research. The sample was not equally distributed between men and women, having a lower sample size for women, which did not allow us to study the sex effect in detail. Future studies with greater sample size and greater variability in their characteristics (ages, anthropometrical, fitness status, etc.) would improve the generality and statistical power of the results. Moreover, future studies could consider, with the aim of improving the calculations of the effect of the cold stress test, the body surface area cooled and the skinfolds of the region (Ng and Sudharsan, 2001). Also, although the presence of sweat during the fatigue strength exercise was unlikely, it may have affected the temperature responses of the skin. Finally, we analyzed skin rewarming after a fatigue strength exercise of regions in the thigh surface: future studies could use ADTC for other types of exercise or body regions, such as those of smaller muscle groups.

#### 5. Conclusion

The results of the present study suggest that fatigue strength exercise results in a lower skin temperature and a faster thermal increase after cold stress test. These results open the door to further research should analyze the application of this type of test for studying the effects of physical exercise and fatigue.

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# Figure captions

**Figure 1.** A) experimental protocol. B) ROIs for the assessment; four reference segments were drawn: A-axis draws the central axis of the thigh starting from the greater trochanter to the patella upper pole; B-axis, goes from the pubis to A-axis; C-axis, crosses A-axis horizontally right in the middle; finally, D-axis, delimits the upper pole of the patella. ROIs were selected as follows: adductor (1), was shaped as a right-angled isosceles triangle whose thighs were approximately long as axis-B; vastus lateralis (2), rectus femoris (3) and vastus medialis (4), started from axis C with the same width. After, 3 remains as a rectangle, while 2 and 4 were shaped accordingly to the anatomy of each participant and 3 edges.

**Figure 2.** A) Mean (middle line) and SD (upper and lower lines) of skin temperature response in each region of interest before and after cooling stress test. B) Curves of the logarithmic equations obtained that represent the skin temperature response in each condition (baseline, control and intervention conditions), for all the regions studied (adductor, rectus femoris, vastus lateralis, vastus medialis). Logarithmic equation was:  $\Delta$ Skin temperature =  $\beta_0 + \beta_1 * \ln(T)$ , being  $\beta_0$  and  $\beta_1$  the constant and slope coefficients, respectively, T the time lasted after the cold stress in seconds, and  $\Delta$ Skin temperature the difference between the skin temperature at T respect with pre-cooling moment.

# **Tables**

Table 1. Characteristics of participants

Age	$27 \pm 4$
Body mass (kg)	$67.5 \pm 11$
Height (cm)	$173 \pm 13$
Body fat (%)	$14 \pm 7$
Muscle mass (kg)	$57.6 \pm 11.2$
N° of series of 10 repetitions until 30% force loss	$23 \pm 9$

**Table 2.**  $\beta_0$  and  $\beta_1$  logarithmic coefficients of vastus lateralis (VL), rectus femoris (RF), adductor (ADD) and vastus medialis (VM) on comparing baseline conditions vs. intervention lower limb (B/I); control vs. intervention lower limb (C/I); and control vs. baseline lower limb conditions (C/B). Logarithmic equation was:  $\Delta$ Skin temperature =  $\beta_0 + \beta_1 * \ln(T)$ ,  $\beta_0$  and  $\beta_1$  being the constant and slope coefficients, respectively, T the time elapsed following the cold stress in seconds, and  $\Delta$ Skin temperature, the difference between the skin temperature at T compared with pre-cooling moment.

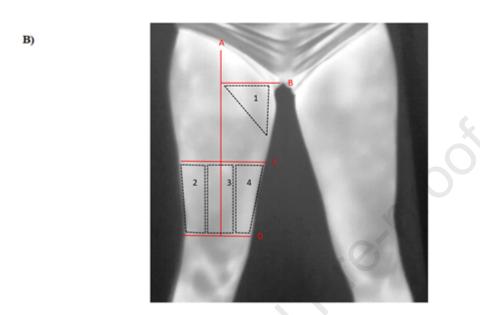
	$oxed{eta_0}$							
	$(\text{mean} \pm \text{sd})$			p (ES)				
	Baseline	Control	Intervention	B/I	C/I	C/B		
VL	$-4.7 \pm 2.21$	$-6.47 \pm 3.35$	$-7.97 \pm 4.63$	0.04 (0.96)	0.55	0.14		
RF	$-5.58 \pm 3.70$	$-6.97 \pm 3.57$	$-9.06 \pm 3.63$	0.00 (0.95)	0.01 (0.58)	0.31		
ADD	$-5.30 \pm 4.05$	$-7.10 \pm 2.74$	$-8.79 \pm 3.56$	0.01 (0.92)	0.27	0.07		
VM	$-6.18 \pm 3.31$	$-6.96 \pm 3.22$	$-8.47 \pm 4.21$	0.13	0.49	1.00		
	β1							
	$(\text{mean} \pm \text{sd})$			p (ES)				
	Baseline	Control	Intervention	B/I	C/I	C/B		
VL	$0.78 \pm 0.35$	$0.78 \pm 0.35$	$1.05 \pm 0.49$	0.03 (0.64)	0.08	0.52		
RF	$0.76 \pm 0.38$	$0.76 \pm 0.38$	$1.09 \pm 0.41$	0.01 (0.84)	0.00 (0.84)	1.00		
ADD	$0.83 \pm 0.35$	$0.83 \pm 0.35$	$1.10 \pm 0.40$	0.73	0.15	1.00		
VM	$0.86 \pm 0.33$	$0.86 \pm 0.33$	$1.05 \pm 0.54$	0.22	0.49	1.00		

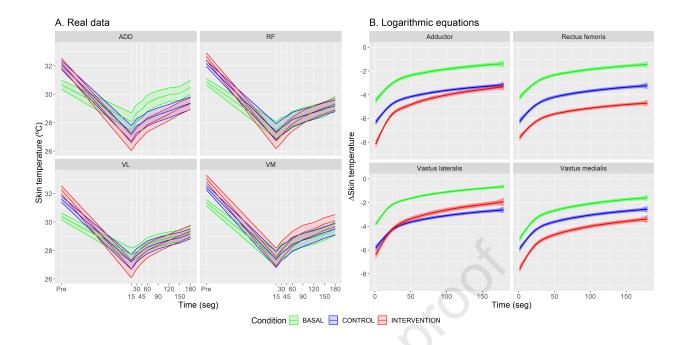
Table 3. Regression models obtained by multiple linear regressions analyses using variations in the logarithmic coefficients  $\beta_0$  and  $\beta_1$ . The inputs of the models were: the other coefficients of the logarithmic equation, age, sex, condition (baseline, intervention or control), dominance (dominant or non-dominant), muscle mass, body fat, number of series performed until 30% of force loss and the body region (vastus medialis, rectus femoris, adductor and vastus lateralis).

Regression models obtained						
Variable	Coefficient [CI95%]	R <sup>2</sup> (p-value)				
	βο					
Constant	-3.77 [-5.901.64]					
Age	0.08 [0.01 - 0.15]					
Muscle mass	0.06 [0.04 - 0.08]	0.04 (0.00)				
Series	-0.06 [-0.080.04]	0.84 (0.00)				
Condition	-0.48 [-0.740.22]					
β1	-7.12 [-7.626.60]					
	β1					
Constant	-0.26 [-0.53 - 0.00]					
Age	0.01 [0.01 - 0.15]					
Muscle mass	0.01 [0.00 - 0.01]	0.80 (0.00)				
Series	-0.00 [-0.010.00]					
β0	-0.12 [-0.00 - 0.02]					

*Note.* In the equation of the regression model obtained for  $\beta 0$  and  $\beta 1$ , the coefficient of the baseline condition is multiplied by 1, by 2 in the case of control lower limb and by 3 in the intervention lower limb.







# **Highlights**

- Greater skin temperature (Tsk) decrease was observed by cold stress test (CST) after exercise
- Faster Tsk rewarming after CST was observed in the exercised lower limb
- Age, muscle mass and exercise series could affect Tsk response during and after CST