Association between physiological stress and skin temperature response after a half marathon

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Abstract

Objective: The objective of this study was to determine the association between skin temperature response and the physiological stress after a half marathon. Approach: Seventeen runners were measured 48 h before, 24 h before, 24 h after and 48 h after completing a half marathon. The measurements on each day of testing included blood markers (creatine kinase [CK] and glutamate oxaloacetate transaminase [GOT]), perception of pain and fatigue (using a visual analogue scale), skin temperature (using infrared thermography), and jump performance (using countermovement jump test). Main results: CK ($p<0.001$ and ES=2.1), GOT ($p=0.04$ and ES=1.3), and perception of fatigue and pain ($p<0.001$ and ES>1.0) increased 24 h after the half marathon, whereas jump performance decreased ($p<0.01$ and ES=0.4). No increase of skin temperature was observed in the tests after the competition and no regression model was able to predict physiological stress using skin temperature. Only a bivariate correlation was observed between the 24 h variation (pre - 24h) of CK and the skin temperature of the posterior upper limb ($p=0.04$ and $r= 0.5$), and between the 48 h variation (pre – 48h) of pain perceived and the skin temperature of the knee ($p<0.01$ and $r= 0.6$). Significance: In conclusion, follow-up on basal skin temperatures does not seem to be an adequate method to detect physiological stress after a half marathon. In line with the observed results, we recommend caution when interpreting peaks in basal skin temperature in field sports assessments.

Keywords: infrared thermography; thermal image; running; exercise; fatigue; recovery.

Abbreviations

ΔCK_{24}: creatine kinase difference between measurement 24 h post half marathon and the measurements 24 h and 48 h before half marathon (average of both).

ΔCMJ_{24}: jump height difference, in the countermovement jump test, between measurement 24 h post half marathon and the measurement 24 h before half marathon.

ΔGOT_{24}: glutamate oxaloacetate transaminase difference between measurement 24 h post half marathon and the measurements 24 h and 48 h before half marathon (average of both).

Δ$overallfatigue_{24}$: difference in perception of overall fatigue between measurement 24 h post half marathon and the measurements 24 h and 48 h before half marathon (average of both).

Δ$overallpain_{24}$: difference in perception of overall pain between measurement 24 h post half marathon and the measurements 24 h and 48 h before half marathon (average of both).

ΔT_{24}: skin temperature difference between measurement 24 h post half marathon and the measurements 24 h and 48 h before half marathon (average of both).

CK: creatine kinase.

CMJ: counter Movement Jump test.

ES: effects sizes.

GOT: glutamate oxaloacetate transaminase.

ROI: region of interest.

$test_{-48}$: test 48 h before the half marathon competition.

$test_{-24}$: test 24 h before the half marathon competition.

test$_{+24}$: test 24 h after the half marathon competition.

test$_{+48}$: test 48 h after the half marathon competition.

VAS: visual analogue scale.

1.Introduction

Skin temperature is the result of heat transfer from deep tissues to the skin surface (Ammer and Formenti 2016). When there is no contact with other surface or exposition to convection streams, skin temperature is mainly dependent on skin blood flow, core temperature and environmental temperature being possible to be assessed by infrared thermography (Ammer and Formenti 2016). Measuring skin temperature through infrared thermography to detect injury in professional athletes has been gaining in popularity (Fernández-Cuevas *et al* 2017). This application is commonly based on the assessment of the thermal symmetry between body sides assuming that asymmetries higher than 0.5-0.7 ºC could be associated with dysfunction of the musculoskeletal system (Fernández-Cuevas *et al* 2017, Hildebrandt *et al* 2010, Vardasca *et al* 2012). Furthermore, the relationship between training load and changes in skin temperature was suggested as another application to investigate (Fernández-Cuevas *et al* 2014, Priego Quesada and Vardasca 2017).

To identify the relationship between skin temperature response and training response requires observing athletes performing periodic assessments. Hence, a follow-up analysis of the basal temperatures is necessary to identify changes in thermal characteristics in response to external and internal loads resulting from training and competition. An increase in the load during training or competition can lead to muscle damage and tissue inflammation (Dupuy *et al* 2018, Silva *et al* 2018). Such conditions are known to some extent to increase muscle temperature, which may be reflected as changes in skin temperature (Fernández-Cuevas *et al* 2017, Hildebrandt *et al* 2010, Ring and Ammer 2012). Skin temperature was also related with skin blood flow

(Cheuvront *et al* 2010, Schlager *et al* 2010), which can be sensitive to exercise intensity (Stöcker *et al* 2018).

Although the theoretical association between these variables seems simple to explain, available literature lacks evidence on the relationship between physiological stress induced by exercise, and changes in basal skin temperature. Al-Nakhli et al. (2012) reported an increase in the skin temperature of the exercised body region 24 h after 4 sets of 25 repetitions of bicep concentration curls. In soccer players, two consecutive matches resulted in higher lower limb skin temperature with a weak association (r = 0.2-0.4) with muscle damage markers (de Andrade Fernandes *et al* 2017). A recent study found a poor relationship between asymmetry of skin temperature and asymmetry of muscle effort, which may reflect the difficulty involved in correlating skin temperature with training intensity (Trecroci *et al* 2018).

Here, we set out to determine the association between basal skin temperature response and physiological stress in trained athletes. We assessed the effect of completing a half marathon on different physiological stress markers and on basal skin temperature. Running a half marathon increases levels of oxidative stress, muscle damage, cardiac stress, liver injury markers, and pain perception (Lippi *et al* 2011, Vassalle *et al* 2018, Withee *et al* 2017). We hypothesized that 24 h and 48 h after a half marathon, basal skin temperature of the lower limbs would be higher than before competition, and this thermal response would be related to the athlete’s level of physiological stress.

2.Methods

2.1.Participants

Seventeen recreational runners (6 women and 11 men) with age 41 ± 6 years old, body mass 66.5 ± 10.0 kg, height 1.74 ± 0.10 m, body fat percentage 14.0 ± 6.1%, muscular percentage 40.4 ± 4.5%, body mass index 21.7 ± 1.5 kg/m^2, and training frequency 5.9 ± 1.9 sessions/week participated in the study. Inclusion criteria involved participation in the half marathon competition and a history of running training of at least 4 sessions/week in the past year. Exclusion criteria were the development of any injury or disease during the three months previous to the competition or not finishing the half marathon competition. Necessary data to recruit participants following criteria were obtained using a previous online questionnaire. Three participants were not considered for the criteria of running training frequency and two participants for the injury/disease criteria. To participate in the study, all participants signed a written consent form. This research protocol is in accordance with the Declaration of Helsinki and was approved by the University of Valencia Ethics Committee (approval H1510573609785). In order to reduce skin temperature variability, participants were instructed to avoid smoking, drinking alcohol, caffeine, or other stimulant beverages, large meals, ointments, cosmetics, sunbathing, physiotherapy treatments and high intensity physical activity in the 12 h before the assessments. The participants confirmed the compliance of each of the instructions on each day of measurement.

2.2.Procedures

In order to assess if a physiological stress after a competition could result in higher skin temperature, we selected a half-marathon competition. The competition was the World Half Marathon Championship in Valencia, Spain, organized by the IAAF on March 24th 2018. Participants performed the half marathon competition in 101.1 ± 14.1 min. The experimental design included 4 days of testing performed 48 h before ($test_{-48}$), 24 h before ($test_{-24}$), 24 h after ($test_{+24}$) and 48 h after ($test_{+48}$) the participants performed the half marathon. Participants were measured two days before the competition with the aim of both obtaining their normal range of values for several parameters (skin temperature and blood parameters) and training in the procedures (e.g. jump test). Two days after the half marathon, participants were measured in order to determine their physiological responses to the competition. In order to minimize the effects of the circadian rhythm (Marins *et al* 2015), each participant performed the 4 tests at the same time of day and all measurements were performed in the afternoon (between 3pm and 8pm). The measurements from each day of testing included blood markers, perception of pain and fatigue, skin temperature, and jump performance. Figure 1.A and 1.B depict the experimental design.

2.3.Blood markers

Creatine kinase (CK) and glutamate oxaloacetate transaminase (GOT) were measured using a Reflotron® Plus System (F. Hoffman-Roche Ltd., Basel, Switzerland) operated according to the manufacturer's instructions. Both markers were determined from blood samples collected from the finger using reactive strips (Reflotron® CK ref.11126695202 and Reflotron® GOT ref.10745120202).

2.4.Fatigue and pain perception

Perception of fatigue and pain were measured using a 150-mm visual analogue scales (VAS) (Mündermann *et al* 2002). The scales were labeled from the left as "lowest fatigue/pain imaginable" to the right as "highest fatigue/pain imaginable". Fatigue and pain were measured taking different body sites into consideration: 1) overall, 2) chest and anterior upper limbs, 3) dorsal back and posterior upper limbs, 4) abdominal, 5) lumbar back and buttocks, 6) anterior thigh, 7) posterior thigh, 8) knee and anterior leg and 9) knee and posterior leg.

2.5.Skin temperature

Skin temperature was determined using an infrared thermal camera (E-60, 320x240 pixels, Flir Systems Inc., Wilsonville, OR, USA) with noise-equivalent temperature difference (NETD) <0.05 °C, and measurement uncertainty of ±2°C or 2%. Before starting the study, the calibration of the camera was checked using a black body (BX-500 IR Infrared Calibrator, CEM, Shenzhen, China). In order to ensure the quality of the thermography assessment, a TISEM checklist was used to certify that all the important aspects were attended to (Moreira et al 2017) and all the images were taken by the same trained thermography technician (Level I thermographer accredited by the Infrared Training Center). The camera was turned on 10 min before taking the images in order to ensure its stabilization and positioned 1.5 m from the participant, perpendicular to the body region of interest (ROI). Thermal images were recorded after 10 min of the participant resting so as to adapt to the room temperature (Marins et al 2014), with male participants standing up and wearing underpants and women in shorts and sports bras. The room was adequately conditioned for infrared thermography to be used, taking into account the following aspects: area absent of sunlight and airflow; an anti-reflective panel placed behind the participants; only the thermography technician and the participant in the measurement space; and no electronic equipment near the measurement space. Reflected temperature was measured according to the standard method ISO 18434-1:2008 and introduced into the camera setup. Room temperature and relative humidity were input into the camera setup for every thermographic measurement using a thermohygrometer (digital thermohygrometer, TFA Dostmann, Wertheim-Reicholzheim, Germany). Environmental room conditions were 23.2 ± 0.1ºC and 20 ± 1% of relative humidity (no differences between tests). The maximum environmental outdoor temperatures were 18ºC, 22ºC, 18ºC and 18ºC for the test-48, test-24, test+24 and test+48, respectively.

The average temperature, the maximum temperature and the standard deviation of 10 ROIs of the full body (Figure 1.C) were obtained using thermography software (Thermacam Researcher Pro 2.10 software, FLIR, Wilsonville, Oregon, USA) and considering an emissivity of 0.98 (Steketee 1973). For the ROIs of the upper and lower limbs, the averages of both sides were considered because no differences were observed between them ($p>0.05$). In addition, mean skin temperature was calculated using the modified equation of Newburg-Spealman (mean= 0.34*abdomen + 0.15*posterior forearm + 0.33*posterior thigh + 0.18*posterior leg) (Choi *et al* 1997).

FIGURE 1 NEAR HERE

2.6.Jump performance

The Counter Movement Jump test (CMJ) was performed to evaluate the lower limbs power. CMJ performance was recorded using a Chronojump platform (model DIN-A3, Chronojump Bosco-System®, Barcelona, Spain). The $test_{-48}$ was used to familiarize the participants with the CMJ technique. For data collection, participants performed a warm-up consisting of 15-20 squat exercise repetitions and joint mobility. They were instructed to jump as high as possible using the following technique: 1) hold a start position from a standing posture with the hands placed at the hips in order to minimize the influence of arms, 2) to perform a quick semi-squat movement reaching 90º of knee flexion, and 3) to perform the fastest possible upward movement to jump as high as possible and land on their toes. Participants performed 5 repetitions of the CMJ, with a rest interval of 30 seconds between them (Guglielmo *et al* 2009). The average of the best 3 highest jumps was used for analyzing jump height (Guglielmo *et al* 2009).

2.7.Statistical analysis

Statistical analysis was performed using the software SPSS 21.0 (IBM Armonk, New York, USA). Data are reported as mean ± SD with 95% confidence intervals of the differences between conditions (CI95%). The normality of the variables was checked using the Shapiro-Wilk test ($p>0.05$). Repeated measures ANOVA with the test factor time ($test_{-48}$ vs. $test_{-24}$ vs. $test_{+24}$ vs. $test_{+48}$) were applied for all the variables in order to assess the differences between the measurement days. When significance was found, the Bonferroni post hoc test was applied to identify the differences. The significance level was set at 0.05. Cohen's effect sizes (ES) were computed and classified as small (ES 0.2–0.5), moderate (ES 0.5–0.8) or large (ES>0.8) (Cohen 1988).

Different approaches were taken to evaluate the potential of thermal data in estimating the physiological stress of the athletes after competition. Variations in average skin temperature (ΔT_{24}, ΔT_{48}) and the other parameters measured (e.g. ΔCK_{24}, ΔGOT_{24}, etc.), between pre- (average of test$_{-48}$ and test$_{-24}$, although in the case of the CMJ variable only test$_{-24}$ was considered as the test$_{-48}$ was considered to be a familiarization test) and post-competition were determined. Then, the correlation between thermal variations and the variations of the other measurements of the study was verified using the Pearson correlation test. Furthermore, an elastic-net penalized linear regression model was used to predict the variables CK_{24} and CK_{48} using the thermal variables and several covariates (age, sex, body fat percentage, and training frequency). As the variability of stress suffered by the participants may be masking results, *K*-means cluster analysis was performed to group participants according to their stress level based on the variables that were affected by the half marathon. Student-t tests were used to evaluate the differences between clusters on demographic, physiological and thermal variables. Again, an elastic-net penalized logistic regression model was used to predict the cluster using the thermal variables and the same covariates as the linear model. R-Studio software was used for the elastic-net penalized regressions.

3.Results

3.1.Effects of the half marathon

Half marathon performance resulted in increased blood markers of CK and GOT in $test_{+24}$ ($test_{-24}$ vs. $test_{+24}$: CK CI95%[291.6, 922.9 U/L] $p<0.001$ and ES=2.1; GOT CI95%[1.1, 79.0 U/L] $p=0.04$ and ES=1.3; Figure 2.A), reduced jump performance ($test_{-24}$ vs. $test_{+24}$: CI95%[-2.7, -0.5 cm] $p<0.01$ and ES=0.4, Figure 2.C), and higher perception of fatigue and pain in all the body regions ($test_{-24}$ vs. $test_{+24}$, e.g. overall fatigue and pain: fatigue CI95%[3.0, 6.3 cm] $p<0.001$ and ES=2.0; pain CI95%[1.5, 5.7 cm] $p<0.001$ and ES=1.7). Among all the body regions, higher average ratings of fatigue/pain were found in the lower limbs (Figure 3).

FIGURE 2 NEAR HERE

FIGURE 3 NEAR HERE

Average skin temperature increased in the posterior upper limb and in the anterior leg ($test_{+24}$ vs. $test_{-48}$: posterior upper limb CI95%[0.3, 1.3ºC] $p<0.001$ and ES=0.9; anterior leg CI95%[0.2, 1.3ºC] $p<0.01$ and ES=1.0) after the half marathon (Figure 4. A). However, average skin temperature increased in the day before the half marathon for most of the ROIs ($test_{-48}$ vs. $test_{-24}$: mean skin temperature CI95%[0.0, 0.9ºC] $p=0.03$ and ES=0.6; anterior upper limb CI95%[0.1, 1.1ºC] $p<0.01$ and ES=1.1; posterior upper limb CI95%[0.4, 1.3ºC] $p<0.001$ and ES=0.9; anterior thigh CI95%[0.2, 1.4ºC] $p<0.01$ and ES=0.7; knee CI95%[0.5, 1.6ºC] $p<0.001$ and ES=0.8; anterior leg CI95%[0.5, 1.3ºC] $p<0.001$ and ES=0.9; posterior leg CI95%[0.2, 1.1ºC] $p<0.01$ and ES=0.6). Similar results were observed for maximum skin temperature (Figure 4.B). No differences were observed between the measurement days on the standard deviation of each ROI (Table 1).

FIGURE 4 NEAR HERE

TABLE 1 NEAR HERE

3.2.Prediction of physiological stress

A bivariate correlation analysis showed that the only relationships observed between the skin temperature response and other variables were found for ΔCK_{24} and the ΔT_{24} of the posterior upper limb ($p=0.04$ and $r= 0.5$), and between $\Delta overallpain_{48}$ and the ΔT_{48} of the knee ($p<0.01$ and $r= 0.6$). No significant linear regression model was obtained to predict the ΔCK_{24} or the ΔCK_{48} using the skin temperature variations as predictor variables.

For the *k*-means clustering analysis, ΔCK_{24}, ΔGOT_{24}, ΔCMJ_{24}, $\Delta overallfatigue_{24}$, and $\Delta overallpain_{24}$ were used as inputs. Subsequently, two clusters were obtained and were denominated as Higher and Lower stress (Table 1). No significant logistic regression model was able to predict the cluster using the ΔT_{24} of the different ROIs as predictor variables.

TABLE 2 NEAR HERE

4.Discussion

In this study, the aim was to investigate the association between skin temperature and physiological stress variables before and after completing a half marathon competition. Our main findings were that the half marathon resulted in physiological stress, as shown by the increases in different variables (blood markers, jump performance and perception of fatigue and pain). However, physiological stress was not accompanied by basal skin temperature changes. Furthermore, it was not possible to determine a regression model that predicts the stress of the runner using skin temperature variables.

The analysis of all the variables confirmed the presence of stress in response to the competition. Mainly 24 h after the half marathon there was an increase of CK and GOT markers, reduction in jump performance and increase in the perception of fatigue and pain in all the body regions, especially the lower limbs. These stress effects resultant of the physical effort are in agreement with previous studies (Lippi *et al* 2011, Rousanoglou *et al* 2016, Withee *et al* 2017).

Different approaches were employed to explore the effect of the half marathon on skin temperature, but none of them showed that skin temperature is related with runners' stress: no peak of skin temperature was observed in the tests after the competition and no regression model (linear or logistic) was able to predict the variation in the different measurements of CK or the cluster with higher stress. The possible effect of the half marathon on greater muscle damage and inflammation may be counteracted for by the multifactorial dependence of the skin temperature (Fernández-Cuevas *et al* 2015, Priego Quesada *et al* 2017). In addition, muscle damage and inflammation may not be close to the skin, and therefore not have a significant effect on skin temperature. As an example of this, false negatives have been observed in the application of infrared thermography in the detection of breast cancer when the tumour is not superficial and the breast has a low vascularization (Vardasca 2016). Therefore, some studies have used thermal stress protocols (for example, by applying cold), to analyze recovery, which is more influenced by alterations in the deeper tissues (Burkes *et al* 2016, Fernández-Cuevas *et al* 2017, Kolacz *et al* 2017). As observing basal skin temperatures is not enough to provide information about the assimilation of training loads, future studies should analyze whether the application of a thermal stress protocol could provide valuable information about physiological stress, inflammation and post-competition muscle damage.

In addition to the average skin temperature, we determined other measures for each ROI. The maximum temperature was determined by the maximum value observed within the ROI, which could be more sensitive to changes related to blood flow and inflammation (Ludwig *et al* 2014, Formenti *et al* 2018). The main limitation of this variable was that was calculated by a single pixel, and some researchers proposed other estimation from the average of the 125 warmest pixels in the ROI (called Tmax method) which presents less noise (Formenti *et al* 2018). On the other hand, the standard deviation within the ROI was determined because it represents the temperature distribution within the ROI and could be affected by physical exercise (Ammer and Formenti 2016). These analyses conduced to the same outcomes from average temperature supporting the overall results.

It is important to mention the difference observed between test$_{-48}$ and test$_{-24}$ on skin temperature in most of the ROIs. Given that we had provided instructions to the participants on reducing their skin temperature variability, one possible explanation of the increase in temperature that occurred in test$_{-24}$ could be the result of the 4ºC increase in the environmental temperature of the city recorded on that day. Another possible explanation is the activation of the sympathetic activity of the autonomic nervous system due to the nerves caused on the day before the competition (Formenti and Merla 2017). The differences observed in skin temperature two days before the competition is an important result since it highlights the complexity of practically applying the analysis of athletes' basal temperatures. The interpretation of the results in a sports club is complicated because an increase in the skin temperature could also be due to environmental, psycho-physiological causes or other factors, and not only to the training workloads of the previous sessions.

Some results were observed such as the increase in skin temperature of the posterior upper limb and in the anterior leg compared with 48 h before the half marathon, correlations observed between ΔCK_{24} and the ΔT_{24} of the posterior upper limb, and between $\Delta overallpain_{48}$ and the ΔT_{48} of the knee, or the differences between cluster on abdominal skin temperature. Some of these results had a logical direction of association and could provide future research directions. Correlation between ΔCK_{24} and the ΔT_{24} of the posterior upper limb could be interpreted as a result of an increase of skin blood flow on the recovery in a region that it is not affected by muscle damage. In the case of the knee, this region is very susceptible to suffering inflammation after the repetition of impacts over long running distances, which could explain the observed association. However, it is important to be cautious because these results are isolated and may be the result of chance. Future studies should explore whether they can be replicated.

CK isoenzymes of muscles are more specific for measuring muscle damage than CK in plasma, and this can be considered as a limitation of our study. However, we decided to measure CK in plasma in order to better reproduce the measurements that were performed in the field of application. Another limitation of the study was the low number of women, which did not allow an analysis of sex differences.

5.Conclusion

Following up basal temperatures does not seem to be an adequate method to detect the stress produced by a half marathon. Furthermore, skin temperature responses to a half marathon were not able to predict physiological stress markers in runners. In line with the observed results, we recommend caution when interpreting peaks in basal skin temperature in field sports assessments.

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Declarations of interest: none

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Figures and captions

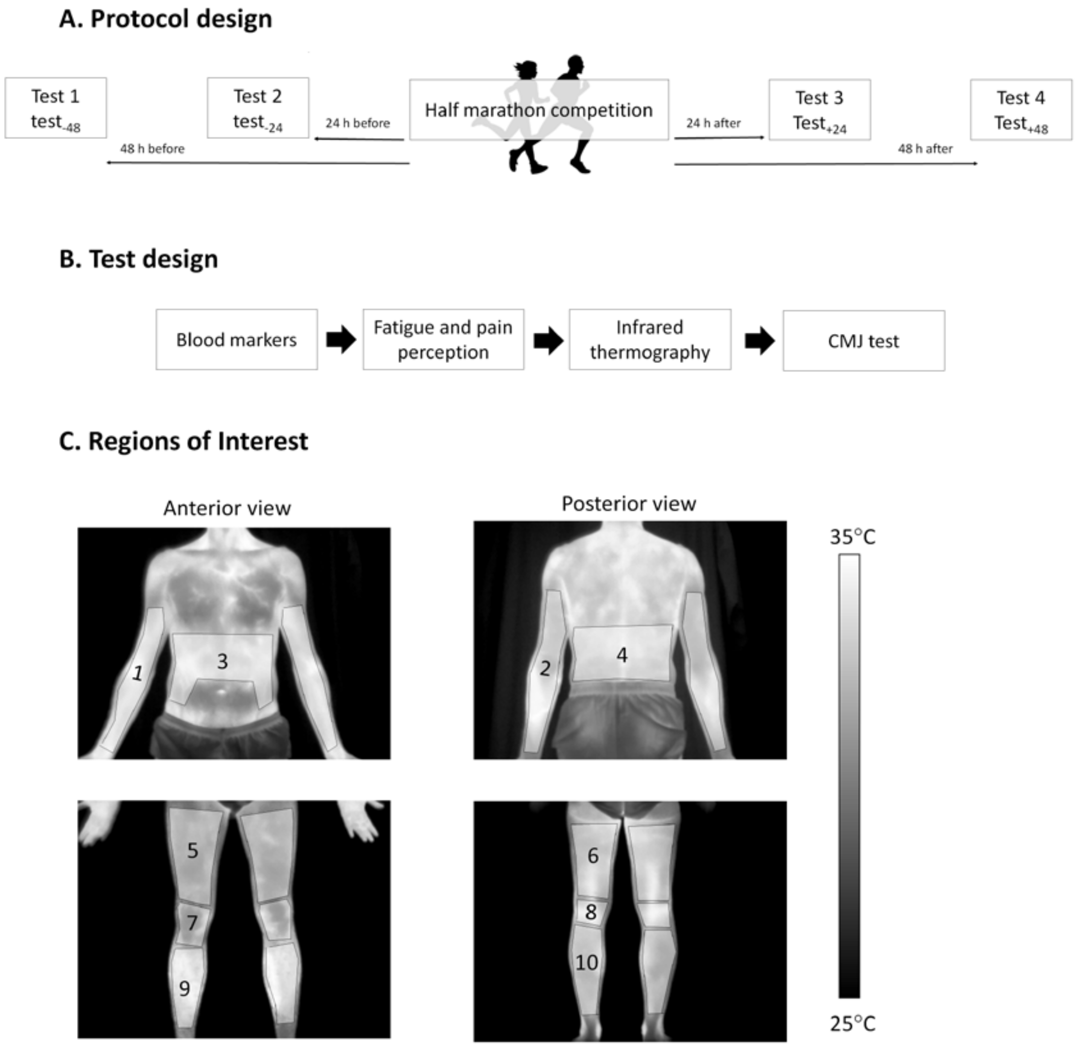


Figure 1. Schematic representation of the experimental design (A), the order in which each test was performed on each day of assessment (B), and the regions of interest for thermography measurements (C): 1) anterior upper limbs, 2) posterior upper limbs, 3) abdominal, 4) lumbar back, 5) anterior thigh, 6) posterior thigh, 7) knee, 8) popliteus, 9) anterior leg, and 10) posterior leg.

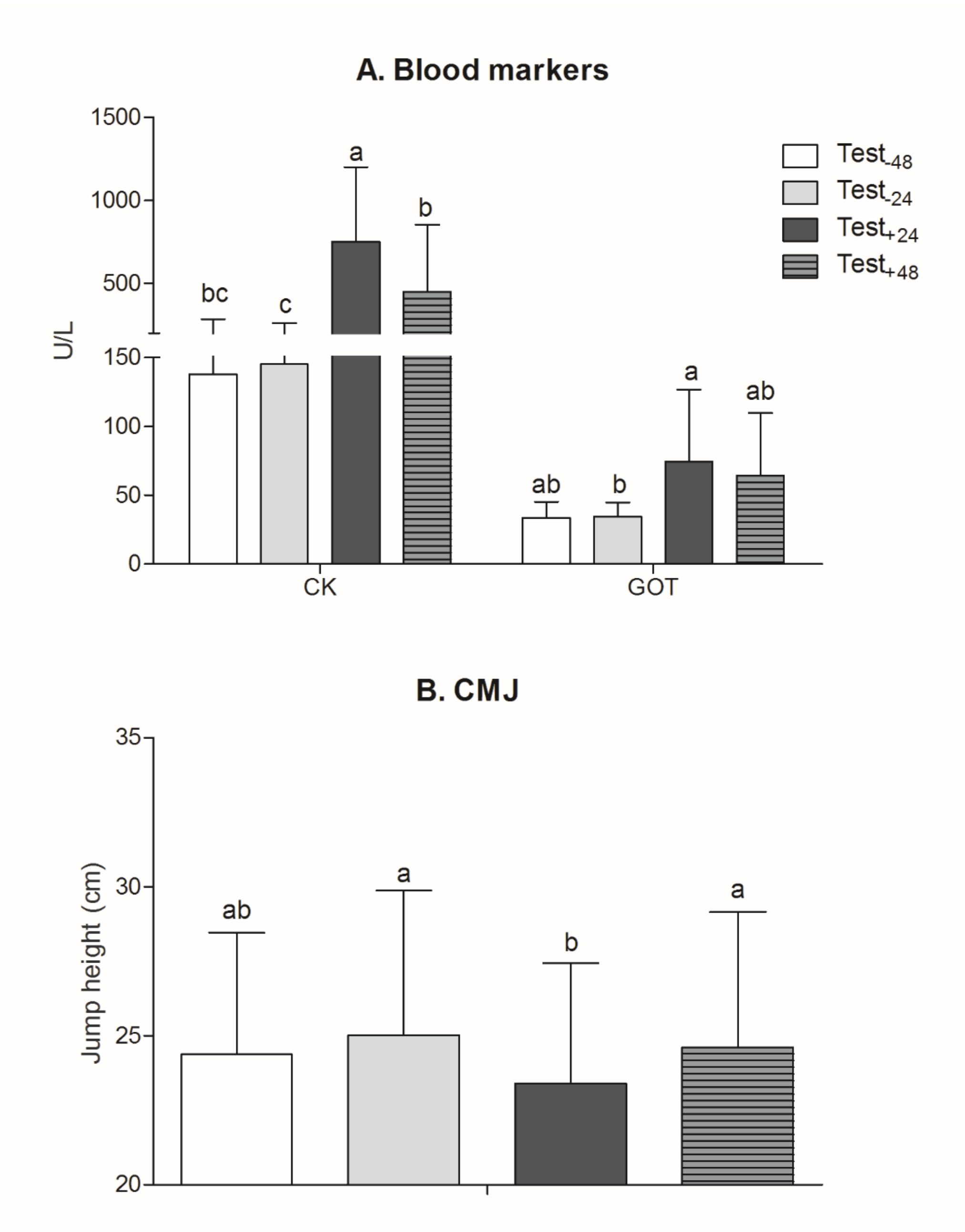


Figure 2. Mean (bars) and standard deviation (vertical lines) of the different measurements of blood markers (A; creatine kinase [CK] and glutamate oxaloacetate transaminase [GOT]), and countermovement jump test (B), 48 h before ($test_{-48}$), 24 h before ($test_{-24}$), 24 h after ($test_{+24}$) and 48 h after ($test_{+48}$) the half marathon. Different letters identify differences between the measures ($p<0.05$; alphabetical order was used to reflect the quantity of the values a>b>c).

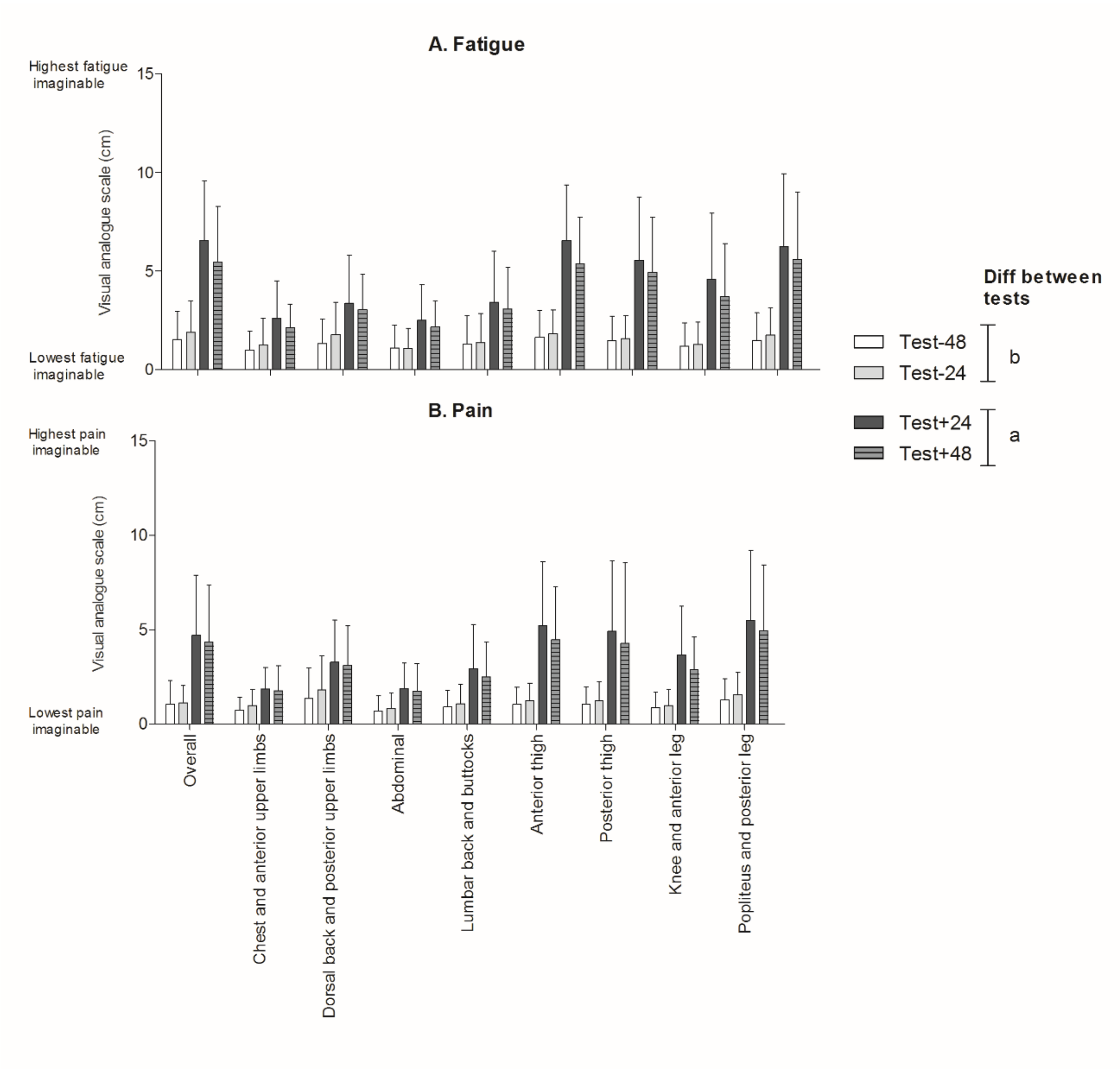
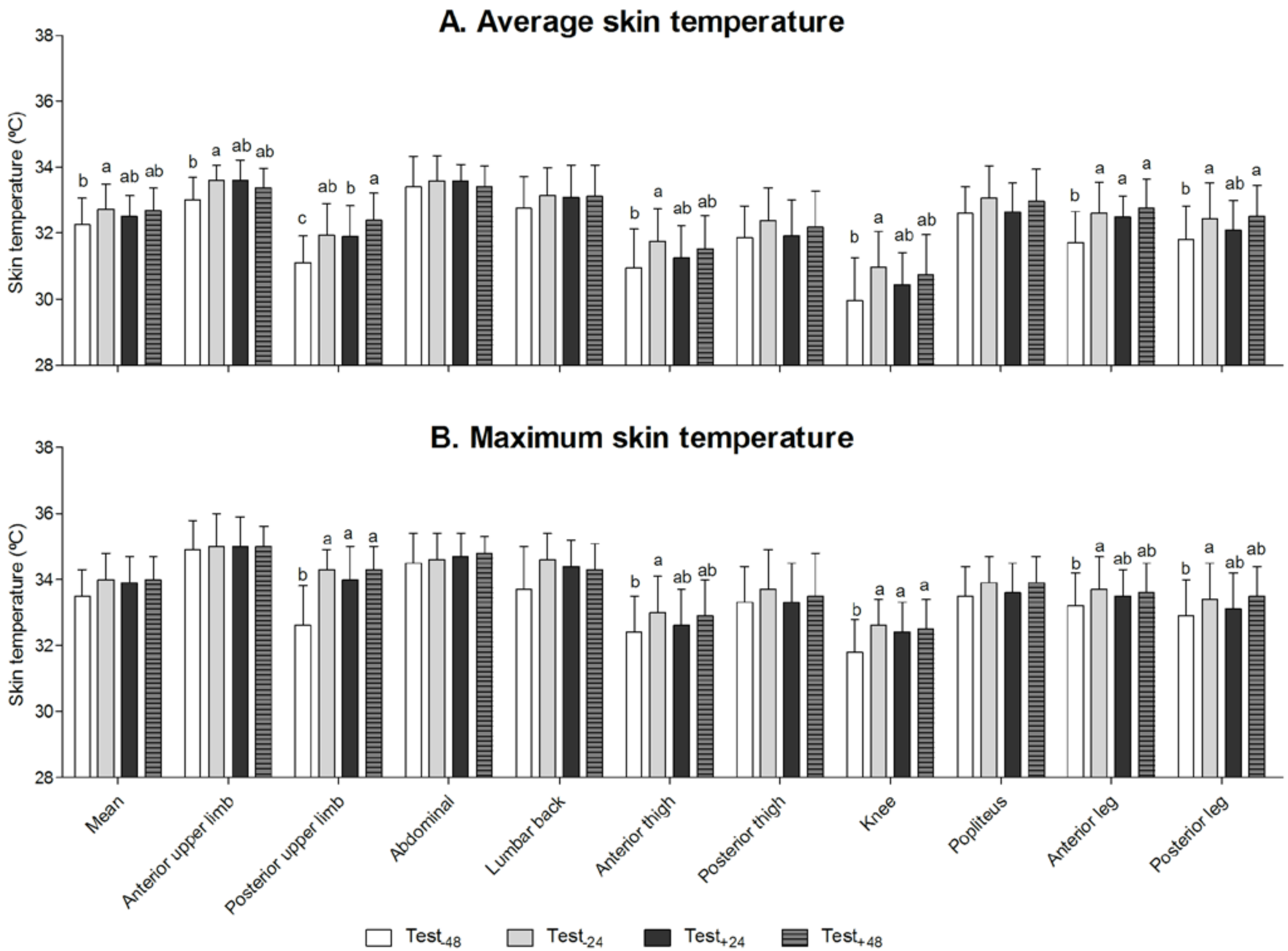


Figure 3. Mean (bars) and standard deviation (vertical lines) of perceived fatigue (A) and pain (B), 48 h before (test$_{-48}$), 24 h before (test$_{-24}$), 24 h after (test$_{+24}$) and 48 h after (test$_{+48}$) the half marathon. 15 cm was the highest fatigue/pain imaginable. All the regions of interest presented higher ratings after the marathon than before (a>b at the legend; $p<0.05$).



A. Average skin temperature
B. Maximum skin temperature
Skin temperature (ºC)
38
36
34
32
30
28
Mean
Anterior upper limb
Posterior upper limb
Abdominal
Lumbar back
Anterior thigh
Posterior thigh
Knee
Popliteus
Anterior leg
Posterior leg
Test-48
Test-24
Test+24
Test+48

Figure 4. Mean (bars) and standard deviation (vertical lines) of the average (A) and maximum (B) skin temperature measurements, 48 h before ($test_{-48}$), 24 h before ($test_{-24}$), 24 h after ($test_{+24}$) and 48 h after ($test_{+48}$) the half marathon. Different letters identify differences between the measures ($p<0.05$; alphabetical order was used to reflect the quantity of the values a>b>c).

Tables

Table 1. Mean ± SD of the standard deviation within each region of interest in the four measurement moments: 48 h before ($test_{-48}$), 24 h before ($test_{-24}$), 24 h after ($test_{+24}$) and 48 h after ($test_{+48}$) the half marathon.

Region of interest	$Test_{-48}$	$Test_{-24}$	$Test_{+24}$	$Test_{+48}$
Anterior upper limb	0.6 ±0.2ºC	0.6 ±0.1ºC	0.6 ±0.2ºC	0.6 ±0.1ºC
Posterior upper limb	0.9 ±0.2ºC	0.8 ±0.2ºC	0.9 ±0.2ºC	0.8 ±0.2ºC
Abdominal	0.4 ±0.1ºC	0.4 ±0.1ºC	0.4 ±0.1ºC	0.4 ±0.1ºC
Lumbar back	0.6 ±0.2ºC	0.5 ±0.2ºC	0.5 ±0.2ºC	0.5 ±0.1ºC
Anterior thigh	0.5 ±0.2ºC	0.5 ±0.2ºC	0.5 ±0.2ºC	0.5 ±0.1ºC
Posterior thigh	0.6 ±0.2ºC	0.5 ±0.2ºC	0.6 ±0.2ºC	0.5 ±0.2ºC
Knee	0.6 ±0.1ºC	0.6 ±0.1ºC	0.7 ±0.1ºC	0.6 ±0.2ºC
Popliteus	0.5 ±0.1ºC	0.5 ±0.1ºC	0.6 ±0.2ºC	0.5 ±0.1ºC
Anterior leg	0.6 ±0.2ºC	0.5 ±0.1ºC	0.6 ±0.2ºC	0.6 ±0.1ºC
Posterior leg	0.4 ±0.1ºC	0.4 ±0.1ºC	0.4 ±0.2ºC	0.4 ±0.1ºC

Note: No differences were observed between measurement days ($p>0.05$).

Table 2. Mean ± SD of the groups obtained using the *K*-means cluster analysis, denominated as Higher and Lower stress clusters.

Variables	Higher stress Mean ± SD	Lower stress Mean ± SD	Higher vs. Lower stress	
			CI95%	p (ES)
Participant characteristics				
Nº participants and sex	3 females 5 males	3 females 6 males	----	----
Age (years)	39.6 ± 7.3	42.8 ± 3.9	-9.1, 2.8	0.28 (0.6)
Body fat (%)	15.7 ± 6.8	13.0 ± 5.3	-3.5, 9.0	0.36 (0.5)
Half marathon time (min)	104.8 ± 15.7	97.9 ± 12.6	-7.8, 21.5	0.32 (0.5)
Cluster´s inputs				
ΔCK_{24} (U/L)	1076.6 ± 178.9	248.6 ±59.0	661.5, 994.4	***<0.001 (7.0)***
ΔGOT_{24} (U/L)	69.7 ± 69.2	17.7 ± 18.4	0.7, 103.1	***0.04 (1.2)***
ΔCMJ_{24} (cm)	-1.1 ± 1.5	-2.0 ± 1.6	-0.7, 2.5	0.25 (0.6)
$\Delta overallfatigue_{24}$ (cm)	4.2 ± 2.4	5.4 ± 2.2	-3.5, 1.3	0.33 (0.6)
$\Delta overallpain_{24}$ (cm)	4.1 ± 3.0	3.2 ± 3.2	-2.4, 4.1	0.58 (0.3)
Average skin temperature variations (ΔT_{24}) (ºC)				
Mean	0.2 ± 0.5	-0.2 ± 0.3	-0.0, 0.8	0.07 (1.0)
Anterior upper limb	0.5 ± 0.7	-0.1 ± 0.6	-0.1, 1.2	0.12 (0.8)
Posterior upper limb	0.7 ± 0.6	0.1 ± 0.5	-0.0, 0.27	0.05 (1.0)
Abdominal	0.4 ± 0.8	-0.3 ± 0.5	0.0, 1.4	***0.04 (1.1)***
Lumbar back	0.3 ± 1.0	-0.1 ± 0.4	-0.4, 0.4	0.26 (0.6)

Anterior thigh	-0.3 ± 1.0	-0.2 ± 0.5	-0.9, 1.0	0.74 (0.2)
Posterior thigh	-0.2 ± 0.7	-0.3 ± 0.5	-0.6, 0.3	0.79 (0.1)
Knee	-0.1 ± 1.2	-0.1 ± 0.5	-0.9, 1.0	0.96 (0.0)
Popliteus	-0.2 ± 0.8	-0.3 ± 0.4	-0.6, 0.3	0.79 (0.1)
Anterior leg	0.3 ± 1.0	0.2 ± 0.3	-0.6, 0.9	0.67 (0.2)
Posterior leg	0.0 ± 0.8	-0.2 ± 0.4	-0.4, 0.3	0.50 (0.4)

Note: Δ_{24} for each variable is the difference between measurement 24 h post half marathon (test+24) and the measurements 24 and 48 h before half marathon (average of test$_{-48}$ and test$_{-24}$; but in the case of ΔCMJ_{24} only test$_{-24}$ was taken into account, given that test$_{-48}$ was considered to be a familiarization test). Abbreviations: CK: creatine kinase; GOT: glutamate oxaloacetate transaminase; CMJ: jump height in the countermovement jump test; Overall fatigue: perception of overall fatigue using 15-cm visual analogue scale; Overall pain: perception of overall pain using 15-cm visual analogue scale.