*International Journal of Sports Physiology and Performance*, 2020, 15, 1467-1475
https://doi.org/10.1123/ijspp.2019-0963


Human Kinetics
ORIGINAL INVESTIGATION

# Effect of a Marathon on Skin Temperature Response After a Cold-Stress Test and Its Relationship With Perceptive, Performance, and Oxidative-Stress Biomarkers

**Jose Ignacio Priego-Quesada, Alejandro Pérez-Guarner, Alexis Gandia-Soriano, Fran Oficial-Casado, Carlos Galindo, Rosa M. Cibrián Ortiz de Anda, José David Piñeiro-Ramos, Ángel Sánchez-Illana, Julia Kuligowski, Marco A. Gomes Barbosa, Máximo Vento, and Rosario Salvador Palmer**

***Context:*** Although skin-temperature assessment has received much attention in recent years as a possible internal-load measurement, scientific evidence is scarce. ***Purpose:*** To analyze baseline skin temperature and its rewarming through means of a cold-stress test before and after performing a marathon and to study the association between skin temperature and internal/external-load measurements. ***Methods:*** A total of 16 runners were measured 48 and 24 h before and 24 and 48 h after completing a marathon. The measurements on each day of testing included urine biomarkers of oxidative stress, pain and fatigue perception, skin temperature (at baseline and after a cold-stress test), and jump performance. ***Results:*** Reduced jump performance ($P < .01$ and effect size [ES] = 0.5) and higher fatigue and pain perception were observed 24 h after the marathon ($P < .01$ and ES > 0.8). Although no differences in baseline skin temperature were observed between the 4 measuring days, posterior legs presented lower constant ($P < .01$ and ES = 1.4) and higher slope ($P = .04$ and ES = 1.1) parameters in the algorithmic equations fitted for skin-temperature recovery after the cold-stress test 24 h after the marathon than on the day before the marathon. Regressions showed that skin-temperature parameters could be predicted by the ratio of ortho-tyrosine isomer to phenylalanine (oxidative stress biomarker) and body fat composition, among others. ***Conclusions:*** Although baseline skin temperature was not altered 24 or 48 h after a marathon, the application of cold stress after the marathon would appear to be a good method for providing information on vasoconstriction and a runner's state of stress.

***Keywords:*** infrared thermography, thermal image, running, exercise, dynamic thermography, recovery

Monitoring internal and external load is important for athletes, sport, and medical staff in order to improve training schedules and athletes' adaptation and performance and to reduce the risk of injury and nonfunctional overreaching.[1,2] Although cardiovascular, biochemical, and psychological parameters are usually measured to determine internal load, all of them present one limitation or another, such as high interindividual and intraindividual differences, response variability, economical cost, or being too time consuming.[2,3]

Skin temperature assessment has attracted attention in recent years as a possible internal load measurement.[4–6] Although some sports staff may be currently assessing baseline skin temperature for the study of asymmetries related with injuries,[7] it is unknown if this outcome could help to provide information about internal load. Muscle damage and tissue inflammation resulting from training and competition could increase muscle temperature and be reflected on skin temperature.[7,8] In addition, skin blood flow alterations due to changes in autonomic nervous system activity could affect skin temperature as a result of the relationship between the 2 outcomes.[9]

Despite the rational explanation of the possible effect of competition and training on baseline temperature, scientific studies on this topic are scarce, and the results are contradictory. Increases in skin temperature 24 h after biceps curl exercises[10] or 24 h after playing a competitive soccer match[4] have been observed. These studies also observed correlations of skin temperature with delayed muscle onset soreness[10] and creatine kinase.[4] However, other studies did not observe any effect on skin temperature after an exercise protocol to induce calf damage[6] or a half marathon competition,[5] and no associations with other internal load measurements such as creatine kinase, countermovement jump, or delayed muscle onset soreness were observed. In the latter study,[5] the authors proposed measuring a more strenuous competition, such as a marathon, and to assess the application of a thermal cold stress test (dynamic thermography).

Dynamic thermography consists of applying thermal stress (eg, cooling or heating) to the skin in order to alter deeper structures and to analyze the response of vascularization. In the case of cooling, as this produces vasoconstriction and subsequent vasodilation during rewarming, alterations of vascular function may be evaluated using this test.[11,12] Although this protocol has been used in medicine for different vascular pathologies,[13–15] its possible application in sport science with a healthy population is largely unexplored.

This study had 2 objectives. First, to analyze baseline skin temperature and its rewarming after applying a cold stress test, both before and after performing a marathon competition, and second, to study the association between skin temperature data and other

Priego-Quesada and Oficial-Casado are with the Research Group in Sport Biomechanics (GIBD), Dept of Physical Education and Sports, and Priego-Quesada, Pérez-Guarner, Gandia-Soriano, Galindo, Cibrián Ortiz de Anda, and Salvador Palmer, the Biophysics and Medical Physics Group (GIFIME), Dept of Physiology, University of Valencia, Valencia, Spain. Piñeiro-Ramos, Sánchez-Illana, Kuligowski, and Vento are with the Neonatal Research Unit, Health Research Inst La Fe, Valencia, Spain. Vento is also with the Div of Neonatology, Hospital Universitario y Politécnico La Fe, Valencia, Spain. Barbosa is with the Physical Education Dept, Universidade Federal de Lavras, Lavras, Brazil. Priego-Quesada (j.ignacio.priego@uv.es) is corresponding author.

internal (oxidative stress and fatigue/pain perception) and external load measurements (jump performance and time performed at the marathon). Based on a previous study,[5] it was hypothesized that baseline skin temperature would not be altered after a marathon but that the response to a cold stress test would be different following the marathon than before.

# Methods

## Subjects

A total of 16 recreational runners volunteered to participate in the study (3 women and 13 men; age 40 [10] y, body mass 72.3 [11.5] kg, height 1.77 [0.05] m, body fat percentage 15.4% [6.0%], and training frequency of 4.8 [1.7] sessions/wk). Inclusion criteria involved participation in the marathon competition and a history of running training schedule of at least 3 running sessions/wk in the past year. Exclusion criteria were the development of any injury or disease during the month prior to the competition or not finishing the marathon. All participants signed a written consent form, and the study was approved by the University of Valencia ethics committee. In order to control the factors that can affect skin temperature, 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 12 h before the assessments. The participants confirmed their compliance with all these instructions on each assessment day.

## Design

Participants performed the Valencia Trinidad Alfonso Marathon (Valencia, Spain) on December 2, 2018, with a time of 229.4 (27.1) min, and a rate of perceived exertion of 15.6 (2.8) points (20-point Borg scale). The experimental design included 4 d 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 had performed the marathon. Each participant undertook the 4 tests at the same time of day, and all measurements were taken in the morning (between 6 AM and 11 AM) with the aim of minimizing the effect of the circadian rhythm. The measurements on each day of testing included collecting and recording urine samples, assessing pain and fatigue perception, registering skin temperature (baseline and cold stress test), and jump performance. About 1 wk before starting the experimental phase, participants visited the laboratory to receive the urine containers, to be trained for the jump test, and to receive all the instructions related to the experiment.

## Methodology

The participants filled the urine containers with the first secretion in the morning of each measurement day and took the container to the laboratory in ice-cooled bags. Once the samples arrived at the laboratory, they were stored at −80°C until the analysis.

Phenylalanine (Phe), para-tyrosine (*p*-Tyr), ortho-tyrosine (*o*-Tyr), meta-tyrosine (*m*-Tyr), 3-nitrotyrosine ($3NO_2$-Tyr), 3-chlorotyrosine (3Cl-Tyr), 8-oxo-2′-deoxyguanosine (8OHdG), and 2′-deoxyguanosine were assessed in urine samples employing a previously validated ultraperformance liquid chromatography coupled to the tandem mass spectrometry method.[16] Briefly, the samples were centrifuged at 16,000 *g* and 4°C for 10 min, and 90 µL of each supernatant were diluted with 10 µL of aqueous solution of isotopically labeled internal standards (ie, phenylalanine-d5, 2′-deoxyguanosine-$^{13}C^{15}N$, and p-tyrosine-d2 at 10 µM). These processed samples were directly injected into the ultraperformance liquid chromatography coupled to tandem mass spectrometry method system. Internal standard calibrations with pure compounds were undertaken on the same measurement day. Further details concerning the method parameters can be found in Kuligowski et al.[16]

Fatigue and pain perception were measured using a 150-mm visual analog scale.[17] Participants reported their perception from different body sites.[5] The scales were labeled from the left as "absence of fatigue/pain" to the right as "highest fatigue/pain imaginable."

Skin temperature was assessed using an infrared thermal camera (E-60, sensor array size of 320 × 240; FLIR Systems, Inc, Wilsonville, OR). A thermographic imaging in sports and exercise medicine checklist was used to certify that all the important aspects of the thermographic protocol were attended.[18] The camera was turned on 10 min before the measurements, and it was positioned 1.5 m from the participant. Participants stood in an upright resting position (men wearing underpants and women in shorts and sports bras), following 10 min of thermal adaptation to the room temperature.[19] After this time, the baseline images were recorded. Then, the preferred lower limbs of the participants were cooled for 3 min using an electronic cryotherapy system (Game Ready GRPro 2.1; CoolSystems Inc, Concord, CA), while the participants were lying supine (Figure 1A). The system was set to the lowest temperature (between 0°C and 3°C) and with moderate pressure from the device. After that, thermal images of the participants' lower limbs were taken 30, 60, 120, and 180 s after finishing the cold stress protocol (Figure 1B).

Environmental room conditions were 23.2°C (0.4°C) and 29% (4%) of relative humidity (no differences between tests). The mean environmental outdoor temperatures (and maximum inside the parenthesis) were 14°C (20°C), 14°C (20°C), 16°C (22°C), and 18°C (24°C) for the $test_{-48}$, $test_{-24}$, $test_{+24}$, and $test_{+48}$, respectively.

The mean temperature of 8 regions of interest (ROIs) of the full body (Figure 1C) was obtained for baseline images using thermography software (ThermaCAM Researcher Pro 2.10 software; FLIR Systems, Inc) and considering an emissivity of 0.98. For images after the cold stress protocol, 4 ROIs of the lower limbs were measured: anterior knee, posterior knee, anterior leg, and posterior leg. Thigh ROIs were not considered because the cryotherapy system did not cover in all cases the same proportion of the region surface.

Jump performance was measured using the countermovement jump test. From this test, jump height was obtained using a Chronojump platform (model DIN-A3; Chronojump-Boscosystem, Barcelona, Spain). Before data collection, participants performed a warm-up consisting of 15 to 20 squat exercise repetitions and joint mobility.[5] They were instructed to jump as high as possible, and participants performed 5 repetitions of the countermovement jump, with a rest interval of 30 s between them, and the mean of the best 3 highest jumps was used for analyzing jump height.

## Statistical Analysis

Data are reported as mean (SD) with 95% confidence intervals (95% CIs) of the differences between conditions. The normality of the different variables was assessed using the Shapiro–Wilk test ($P > .10$). Repeated-measures analysis of variance with Bonferroni post hoc test were applied for all the variables in order to assess the differences between the measurement days ($test_{-48}$ vs $test_{-24}$ vs $test_{+24}$ vs $test_{+48}$). For skin temperatures, the same approach, with one additional factor (preferred lower limb), was employed. To assess the cold stress test for each participant, on each measurement

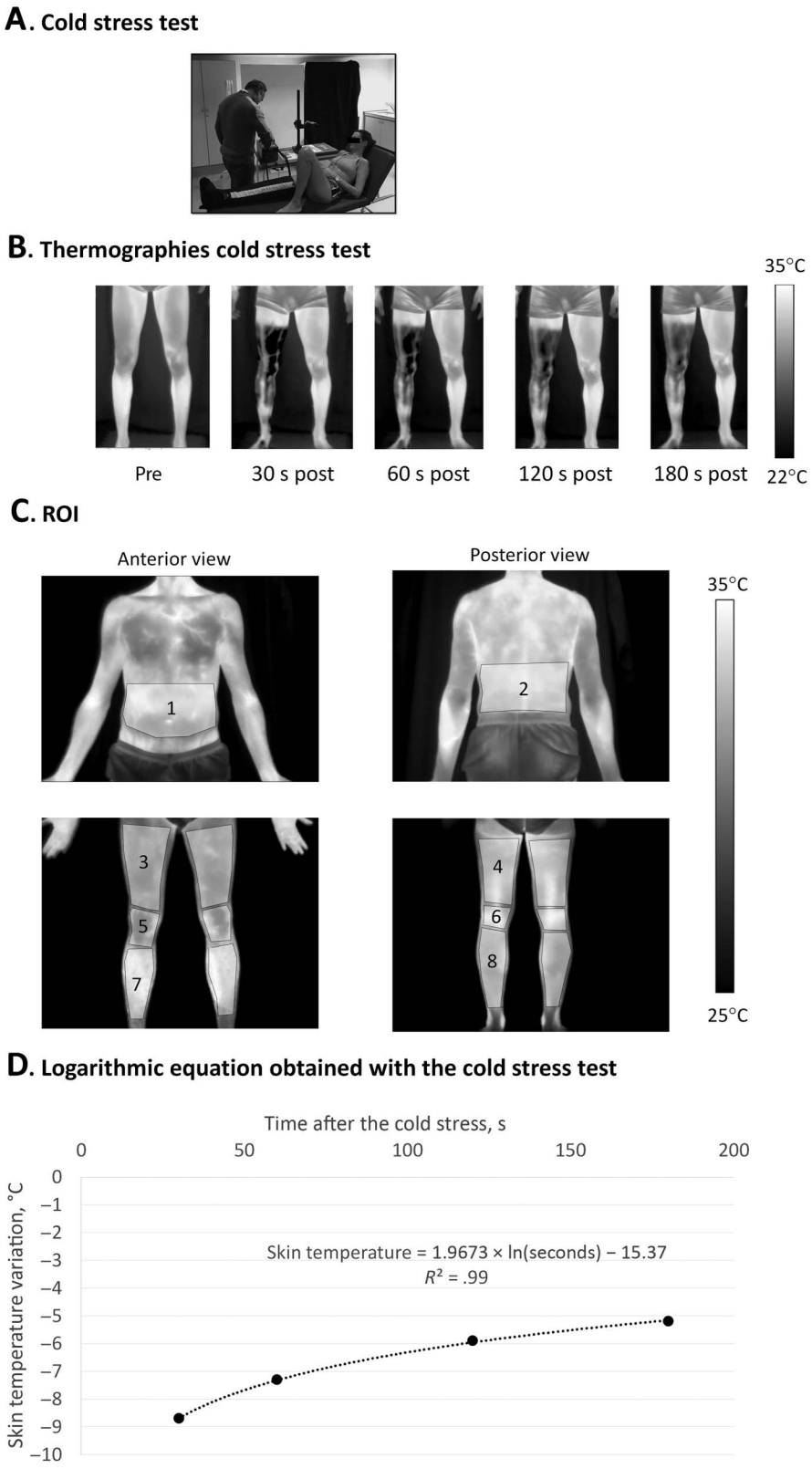


**Figure 1** — (A) Cold-stress protocol. (B) Examples of the thermographies of one participant after the cold stress protocol. (C) The ROIs for baseline thermography measurements: (1) abdominal, (2) lumbar back, (3) anterior thigh, (4) posterior thigh, (5) anterior knee, (6) posterior knee, (7) anterior leg, and (8) posterior leg. (D) The logarithmic equation obtained with the cold-stress test in the anterior leg. ROI indicates regions of interest.

day and for each ROI, a logarithmic equation was adjusted and the corresponding coefficients (ie, $\beta_0$ and $\beta_1$) were obtained. A logarithmic function of skin temperature versus time was employed (Figure 1D), with an average $R^2$ of all participants and measurement days of .94 (.08), .96 (.05), .98 (.02), and .98 (.02), for anterior knee, posterior knee, anterior leg, and posterior leg, respectively. Differences between measurement days in these parameters were then assessed using repeated-measures analysis of variance. The significance level was set at $P$ value < .05. Cohen effect sizes (ES) were calculated and classified as small (0.2–0.5), moderate (0.5–0.8), or large (>0.8). Finally, using variations of baseline skin temperature and logarithmic equation coefficients as prediction variables, stepwise multiple linear regressions were performed. The $\Delta_{24}$ was considered as the difference between the day after and the day before the marathon, and $\Delta_{48}$ as the difference between the second day after and the day before the marathon for all the parameters assessed. Inputs of the models were age, body fat percentage, body mass index, training frequency, time performed at the marathon, rate of perceived exertion at the marathon, variations of the oxidative stress biomarkers, countermovement jump, and the corresponding region of fatigue and pain perception. 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.

## Results

The percentage of nondetection of each oxidative stress biomarkers measured was Phe (0%), *p*-Tyr (0%), *o*-Tyr (50%), *m*-Tyr (55%), $3NO_2$-Tyr (38%), 3Cl-Tyr (64%), 8OHdG (0%), and 2'-deoxyguanosine (77%). Therefore, the *o*-Tyr/Phe and $3NO_2$-Tyr/*p*-Tyr ratios were analyzed, as they were the ratios of biomarkers with an acceptable percentage of data above the detection limits. No differences were observed for protein damage ($P = .09$ for *o*-Tyr/Phe and $P = .26$ for $3NO_2$-Tyr/*p*-Tyr; Figure 2A and 2B) between days 4 and 6 of the 16 participants presented higher values the day after than the day before the marathon for *o*-Tyr/Phe and $3NO_2$-Tyr/*p*-Tyr, respectively. A reduced jump performance ($test_{-48}$ vs $test_{+24}$: 95% CI, −3.2 to −0.7 cm, $P < .01$, ES = 0.4; $test_{-24}$ vs $test_{+24}$: 95% CI, −3.9 to −0.6 cm, $P < .01$, ES = 0.5; Figure 2C) and higher fatigue and pain perception in all body regions were observed after the marathon ($test_{-24}$ vs $test_{+24}$, eg, overall fatigue and pain—fatigue: 95% CI, 1.3 to 7.0 cm, $P < .01$, ES = 1.5; pain: 95% CI, 2.0 to 6.1 cm, $P < .001$ and ES = 1.6). In all the body regions, the highest fatigue/pain ratings were found in the lower limbs following the marathon (Figure 3).

With regard to skin temperature, there were no differences between the preferred and nonpreferred limb ($P > .25$). For this reason, this factor was not considered in the analysis of variance for the presentation of results. In most of the ROIs (7/8 of the ROIs), no differences of baseline skin temperature were observed between the 4 d of measurement ($P > .12$; Figure 4).

Although no differences were obtained between the days in the regression equation parameters of the cold stress test in the regions of anterior knee, posterior knee, and anterior leg ($P > .10$; Table 1), posterior leg presented higher $\beta_1$ coefficient 1 d after the marathon compared with 2 d before (95% CI of the differences, 0.0 to 0.8, $P = .04$, ES = 1.1) and lower $\beta_0$ coefficient than the days before ($test_{+24}$ vs $test_{-48}$: 95% CI, −5.6 to −1.0, $P < .01$, ES = 1.4; $test_{+24}$ vs $test_{-24}$: 95% CI, −4.3 to −0.4, $P = .02$, ES = 1.0).

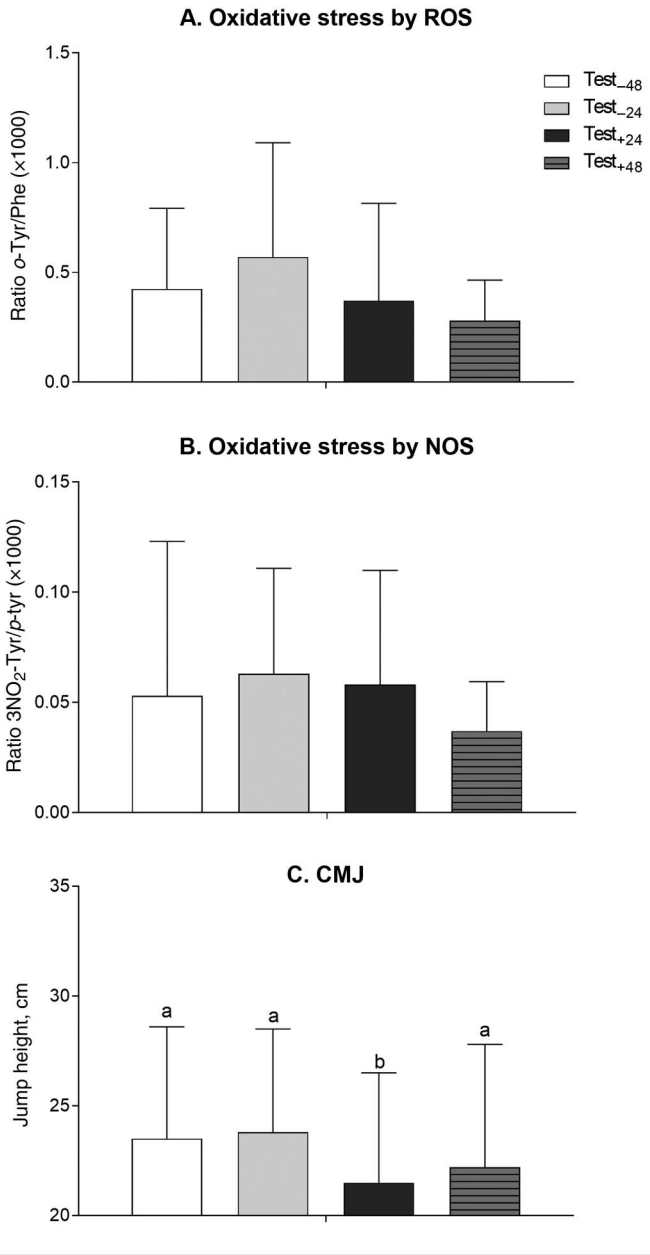


**Figure 2** — Mean (bars) and SD (vertical lines) of (A and B) oxidative stress biomarkers and (C) CMJ test 48 h before ($test_{-48}$), 24 h before ($test_{-24}$), 24 h after ($test_{+24}$), and 48 h after ($test_{+48}$) the marathon. Different letters identify differences between the measurements ($P < .05$; alphabetical order was used to reflect the quantity of the values a > b). CMJ indicates countermovement jump; 3NO2-Tyr, 3-nitrotyrosine; NOS, nitric oxide synthase; o-Tyr/Phe, ratio of ortho-tyrosine isomer to phenylalanine; *p*-Tyr, para-tyrosine; ROS, reactive oxygen species.

Table 2 shows the multiple linear regressions obtained between variations in baseline skin temperature ($\Delta T_{24}$ and $\Delta T_{48}$) and other outcomes of the study. Variable explained at most $\Delta T_{24}$ times was the $\Delta_{24}$ (*o*-Tyr/Phe) with a negative relationship. However, $\Delta T_{48}$ was explained in most of the models, having a positive relationship with body fat.

In addition, Table 2 also shows the multiple linear regressions obtained between the variation of the $\beta_0$ coefficient of the thermal cold stress logarithmic equations and the other outcomes of the study. The table only shows the regression of the $\beta_0$ coefficient

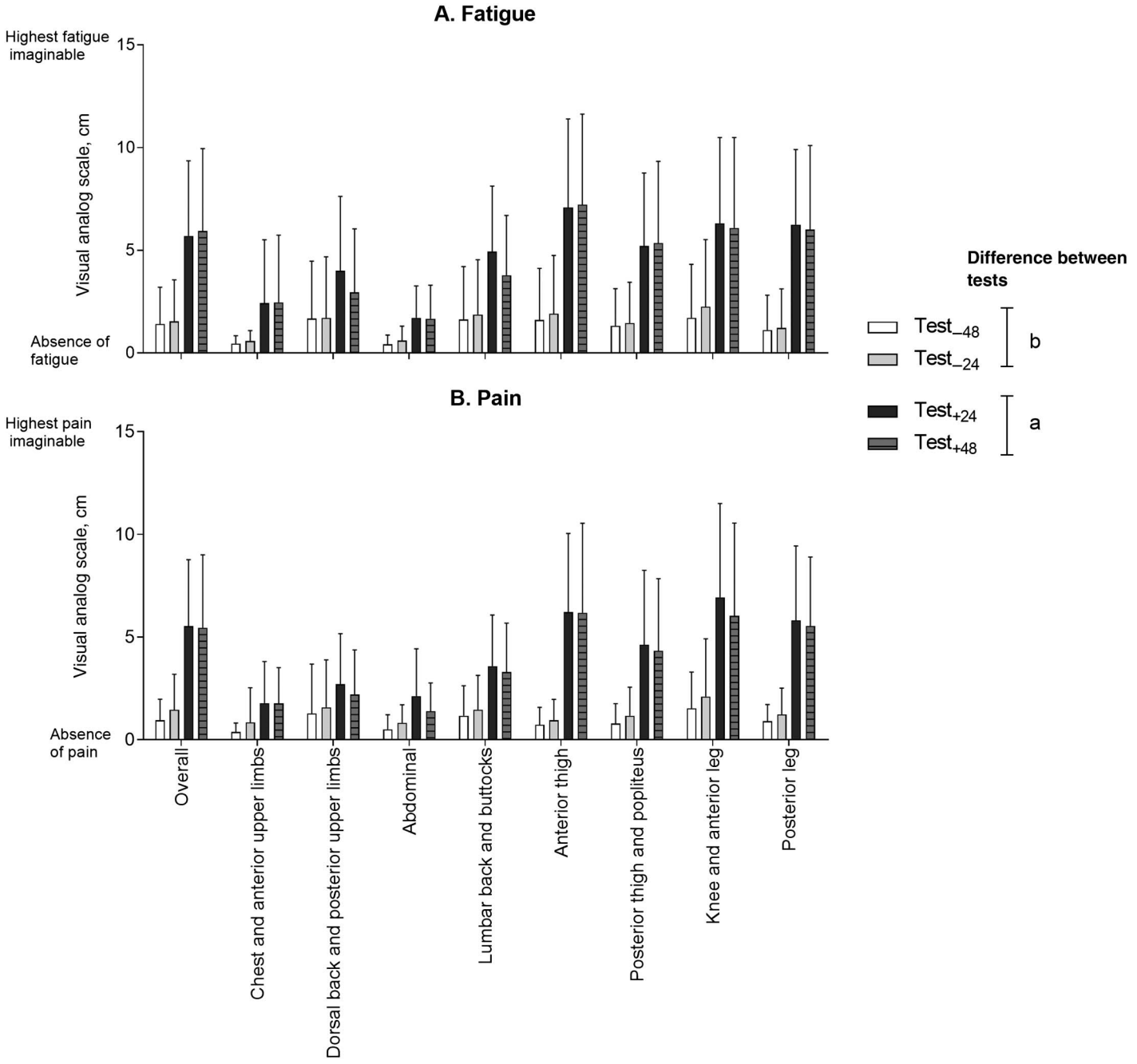


**Figure 3** — Mean (bars) and SD (vertical lines) of perceived fatigue and pain 48 h before ($test_{-48}$), 24 h before ($test_{-24}$), 24 h after ($test_{+24}$), and 48 h after ($test_{+48}$) the marathon. The 15-cm mark indicated the highest fatigue/pain imaginable. All the regions of interest presented higher ratings after the marathon than before (a > b at the legend; $P < .05$).

because the same results were observed for the $\beta_1$ coefficient, due to the high inverse relationship observed between them ($R^2 = .75$, .83, .82, and .71, for anterior knee, posterior knee, anterior leg, and posterior leg, respectively). The most frequent inverse relationship observed was between the $\Delta_{24}$ variation of the $\beta_0$ coefficient of the logarithmic equations and body fat percentage.

## Discussion

The aims of this study were to determine skin temperatures at baseline and after a cold stress protocol before and after a marathon, and to study the relationship between skin temperature data and other internal and external load measurements. The main findings were that although baseline skin temperatures did not present differences between days, the logarithmic regressions associated with the rewarming of the skin temperature of the posterior leg, after the cold stress protocol, presented a lower $\beta_0$ and a higher $\beta_1$ coefficient than before the marathon. Furthermore, baseline skin temperatures were mainly inversely related with protein damage recovery (variation in *o*-Tyr/Phe) 24 h after the marathon and 48 h after the marathon, directly related with body fat percentage. Variation 24 h after the marathon of the $\beta_0$ coefficient of the logarithmic equations was directly related with protein damage

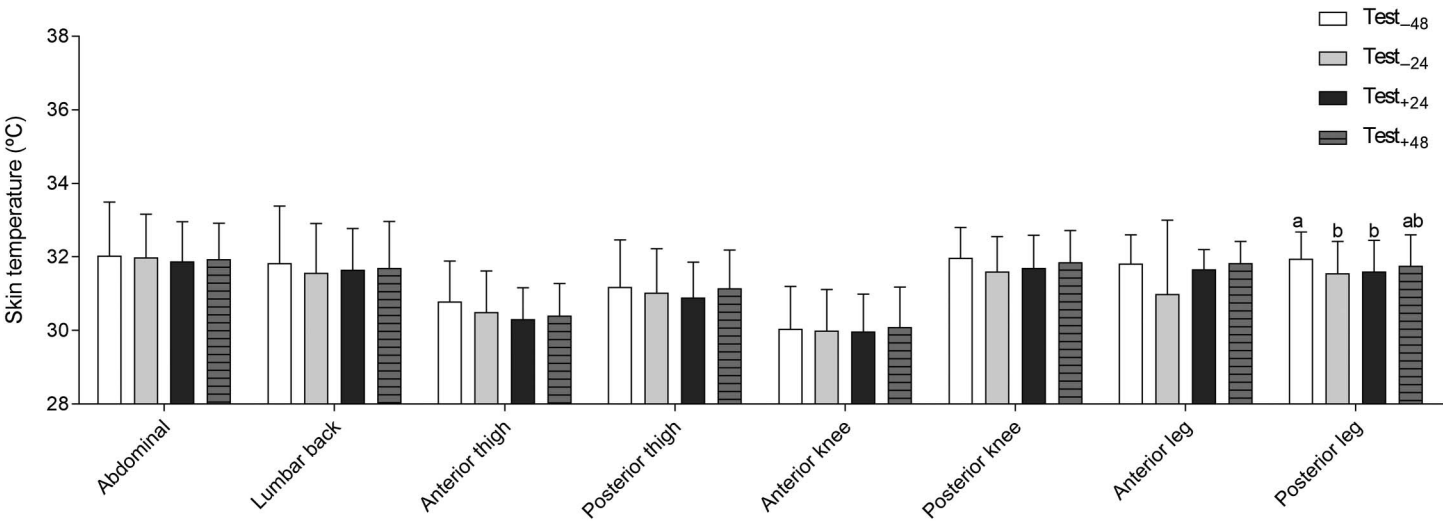


**Figure 4** — Mean (bars) and SD (vertical lines) of the baseline skin-temperature measurements 48 h before ($test_{-48}$), 24 h before ($test_{-24}$), 24 h after ($test_{+24}$), and 48 h after ($test_{+48}$) the marathon. Different letters identify differences between the measurements ($P < .05$; alphabetical order was used to reflect the quantity of the values a > b).

**Table 1 Parameters of the Logarithmic Equations Obtained With the Cold-Stress Test: Skin Temperature = $\beta_0 + \beta_1 \times$ ln (Time After the Cold-Stress Test in Seconds)**

| Region of interest | $\beta_0$ Mean (95% CI) | | | | $\beta_1$ Mean (95% CI) | | | |
|---|---|---|---|---|---|---|---|---|
| | $Test_{-48}$ | $Test_{-24}$ | $Test_{+24}$ | $Test_{+48}$ | $Test_{-48}$ | $Test_{-24}$ | $Test_{+24}$ | $Test_{+48}$ |
| Anterior knee | −8.9 (−10.0 to −7.7) | −9.3 (−10.5 to −8.2) | −10.3 (−11.7 to −8.9) | −10.1 (−11.5 to −8.7) | 1.2 (1.0 to 1.3) | 1.2 (1.1 to 1.3) | 1.3 (1.1 to 1.5) | 1.3 (1.1 to 1.4) |
| Posterior knee | −13.4 (−14.8 to −12.1) | −14.0 (−15.6 to −12.3) | −14.5 (−16.4 to −12.6) | −13.6 (−14.5 to −12.7) | 1.7 (1.5 to 1.9) | 1.9 (1.7 to 2.2) | 1.9 (1.6 to 2.2) | 1.8 (1.6 to 1.9) |
| Anterior leg | −9.5 (−10.3 to −8.7) | −9.8 (−10.6 to −9.0) | −11.4 (−13.0 to −9.9) | −10.0 (−10.9 to −9.0) | 1.2 (1.1 to 1.3) | 1.2 (1.2 to 1.5) | 1.4 (1.1 to 1.6) | 1.2 (1.1 to 1.4) |
| Posterior leg | $-16.8^{a}$ (−18.0 to −15.6) | $-17.8^{a,b}$ (−19.0 to −16.6) | $-20.2^{b}$ (−21.5 to −18.8) | $-18.2^{a,b}$ (−19.5 to −17.0) | $2.1^{b}$ (1.9 to 2.2) | $2.3^{b}$ (2.1 to 2.6) | $2.5^{a}$ (2.3 to 2.7) | $2.3^{a,b}$ (2.1 to 2.4) |

Abbreviation: CI, confidence interval. Note: Differences between days in the parameters were obtained in the posterior leg region ($P < .05$; alphabetical order was used to reflect the quantity of the values a > b). One equation was obtained for each measurement day: 48 h before ($test_{-48}$), 24 h before ($test_{-24}$), 24 h after ($test_{+24}$), and 48 h after ($test_{+48}$) the marathon.

recovery (variation in *o*-Tyr/Phe) and inversely related with body fat percentage and variation of jump performance.

Although performing a marathon resulted in a reduction in jump performance and a higher perception of fatigue and pain in the days following the competition, the results of this study show that baseline skin temperature did not increase 24 and 48 h after the marathon. These results were in agreement with several previous studies[5,6] but opposed to other investigations.[4,10,20] One possible explanation for the absence of any alteration of skin temperature could be that the location of muscle damage or inflammation may not be close to the skin.[5,6] In addition, it is important to mention that studies with a continuous follow-up of skin temperatures after competition and training are required in order to know when the peak in skin temperature occurs. Although some internal load measures such as creatine kinase or delayed onset muscle soreness presented their peaks 24 and 48 h after the competitions,[21] it is still unknown when the peak in skin temperature occurs.

Another explanation is that skin temperature depends on many factors (environmental conditions, skin blood flow, hydration/nutrition, etc)[22,23] and its effect on deep tissues may not be enough to alter skin temperature. In this sense, a recent study that observed increments of skin temperature during a training camp suggested that controlling all these factors could be an important aspect in this type of study.[20] Priego-Quesada et al[20] also suggested that training, including high external workloads without causing muscle damage, pain, and high physiological stress, can lead to increases in baseline temperatures, while the opposite situation could result in a baseline state of peripheral vasoconstriction leading to increased muscle vasodilation. This possible explanation could be understood as a curvilinear inverted *U* relationship between internal load and skin temperature responses. Some data that may support this

**Table 2 Regression Models Obtained by Multivariate Stepwise-Regression Analyses Using Variations in Baseline Skin Temperature ($\Delta T_{24}$ and $\Delta T_{48}$) and the $\beta_0$ Coefficient of the Logarithmic Equation Obtained From the Thermal Cold Stress as the Response Variables and as Inputs for Other Outcomes of the Study**

| Region of interest | Variable | Coefficient (95% CI) | $R^2$ (*P*) |
|---|---|---|---|
| $\Delta T_{24}$ | | | |
| Abdominal | Constant | −1.40 (−2.64 to −0.15) | .28 (.04) |
| | Age | 0.03 (0.00 to 0.06) | |
| Lumbar back | $\Delta_{24}$ (*o*-Tyr/Phe) | −1.59 (−2.64 to −0.53) | .51 (.01) |
| | Body fat percentage | −0.10 (−0.20 to −0.01) | |
| Anterior thigh | Constant | −0.49 (−0.90 to −0.08) | .41 (<.01) |
| | $\Delta_{24}$ (*o*-Tyr/Phe) | −1.74 (−2.93 to −0.55) | |
| Posterior thigh | Constant | −0.33 (−0.64 to −0.01) | .34 (.02) |
| | $\Delta_{24}$ (*o*-Tyr/Phe) | −1.14 (−2.05 to −0.23) | |
| Anterior knee | $\Delta_{24}$ (*o*-Tyr/Phe) | −1.21 (−2.28 to −0.15) | .30 (.03) |
| Posterior knee | Constant | −0.76 (−1.51 to −0.00) | .57 (<.01) |
| | Age | 0.03 (0.01 to 0.04) | |
| | $\Delta_{24}$ (CMJ) | 0.10 (0.02 to 0.18) | |
| Anterior leg | No variable was included | | |
| Posterior leg | No variable was included | | |
| $\Delta_{24}$ ($\beta_0$) of the equations of the thermal cold stress | | | |
| Anterior knee | Constant | −7.93 (−9.12 to −6.75) | .37 (.01) |
| | $\Delta_{24}$ (*o*-Tyr/Phe) | 4.63 (1.18 to 8.09) | |
| Posterior knee | Constant | −10.00 (−13.37 to −6.63) | .28 (.04) |
| | Body fat percentage | −0.22 (−0.43 to −0.02) | |
| Anterior leg | Constant | −8.45 (−10.04 to −6.88) | .66 (<.01) |
| | Body fat percentage | −0.13 (−0.22 to −0.04) | |
| | $\Delta_{24}$ (CMJ) | −0.46 (−0.71 to −0.21) | |
| Posterior leg | Constant | −12.97 (−15.50 to −10.43) | .46 (<.01) |
| | Body fat percentage | −0.25 (−0.40 to −0.10) | |
| $\Delta T_{48}$ | | | |
| Abdominal | No variable was included | | |
| Lumbar back | No variable was included | | |
| Anterior thigh | Constant | −3.81 (−6.11 to −1.51) | .62 (<.01) |
| | Body fat percentage | 0.18 (0.08 to 0.27) | |
| | $\Delta_{48}$ (fatigue anterior thigh) | −0.09 (−0.16 to −0.01) | |
| Posterior thigh | Constant | −2.13 (−3.54 to −0.73) | .46 (<.01) |
| | Body fat percentage | 0.10 (0.04 to 0.16) | |
| Anterior knee | Constant | −3.66 (−6.94 to −0.37) | .30 (.03) |
| | Body fat percentage | 0.16 (0.02 to 0.30) | |
| Posterior knee | Constant | −2.01 (−3.50 to −0.51) | .44 (<.01) |
| | Body fat percentage | 0.10 (0.03 to 0.16) | |
| Anterior leg | Constant | −2.30 (−4.53 to −0.07) | .29 (.03) |
| | Body fat percentage | 0.11 (0.01 to 0.20) | |
| Posterior leg | No variable was included | | |
| $\Delta_{48}$ ($\beta_0$) of the equations of the thermal cold stress | | | |
| Anterior knee | No variable was included | | |
| Posterior knee | No variable was included | | |
| Anterior leg | Constant | −3.48 (−6.78 to −0.18) | .26 (.04) |
| | $\Delta_{48}$ (CMJ) | 0.15 (0.01 to 0.29) | |
| Posterior leg | No variable was included | | |

Abbreviations: CI, confidence interval; CMJ, countermovement-jump test; *o*-Tyr/Phe, ratio of ortho-tyrosine isomer to phenylalanine; $\Delta_{24}$, difference between the day after and the day before the marathon; $\Delta_{48}$, difference between the second day after and the day before the marathon; $\Delta T_{24}$, skin-temperature difference between measurements 24 h after and 24 h before marathon; $\Delta T_{48}$, skin-temperature difference between measurements 48 h after and 24 h before marathon.

idea were the inverse relationship observed 24 h after the marathon in some of the body regions between variation of skin temperature and protein damage by oxidative stress measured by *o*-Tyr/Phe. This relationship may show that the participants with a higher oxidative stress or lower recovery rate present higher reductions or lower increases in skin temperature and vice versa. Results of the cold stress protocol also support this idea. Further studies are necessary to explore these possible explanations.

The positive relationship observed in most of the body regions, 48 h after the marathon, between skin temperature variation and body fat percentage may support the previous explanation. The sample used in this study presented a body fat percentage between normal and low values of the population (5%–21%). Participants with lower percentages of body fat therefore have higher muscle percentages. The muscle damage produced by the marathon could increase the time necessary for recovery of these participants while maintaining lower skin temperatures due to higher peripheral vasoconstriction levels.

The application of the cold stress protocol induces an activation of the skin sympathetic nerve activity resulting in a higher vasoconstriction, and after the withdrawal of the test, vasoconstriction is reduced with the aim of increasing skin blood flow and recovery to the normal values.[11,12] The first variable that can be analyzed is the level of decrease in skin temperature after the cold stress test, which in our results is the $\beta_0$ coefficient of the logarithmic equations. Zeng et al[13] observed a higher decrease of skin temperature after the cold stress test in diabetic patients. In this study, the lower value of the $\beta_0$ coefficient and therefore higher decrease of skin temperature after the cold stress protocol were observed the day after the marathon in the posterior leg region. This lower $\beta_0$ coefficient could be associated with a higher vasoconstriction resulting from a higher activation of the skin sympathetic nerve activity due to a higher stress.[24] This idea is in agreement with the observed direct relationship between the variation of the $\beta_0$ coefficient of the logarithmic equations and the protein damage by oxidative stress measured by *o*-Tyr/Phe. This means that participants with lower recovery in their oxidative stress presented an increment in their $\beta_0$ coefficient of the logarithmic equation after the marathon. Therefore, this relationship supports the idea that the lower $\beta_0$ coefficient of the logarithmic equation after the marathon is due to the muscle damage and physiological stress produced by the competition, which results in a higher vasoconstriction. The negative relationship observed between the variation of the $\beta_0$ coefficient of the logarithmic equations and the body fat percentage also supports the previous idea commented on that participants with lower body fat and therefore higher muscle mass presented higher muscle damage or physiological stress.

Slow recovery rate was observed after a cold stress protocol in pathological populations, such as diabetics[13,15] or patients with Raynaud disease.[14] This lower recovery rate is commonly associated with a dysfunction of the peripheral vasodilation capacity.[13,15] Although a higher $\beta_1$ coefficient was observed on the day after the marathon in this study in the posterior leg, it is important to mention that an inverse relationship between the $\beta_0$ and the $\beta_1$ coefficient was observed ($R^2 > .7$). This is in agreement with previous results with healthy population that showed that greater vasoconstriction response was followed during recovery by a greater temperature rise.[11] The greatest $\beta_1$ as a result of a lower $\beta_0$ coefficient was consistent with a healthy population,[11] and therefore, our results could suggest that after a marathon, there is no decrease in the peripheral vasodilator capacity.

The measurement of other physiological parameters such as core and muscle temperature, skin blood flow, or autonomic nervous system activity would also help to interpret the results. Although only the results of the mean temperature of the ROIs have been shown, other parameters such as the maximum temperature or the average deviation of the ROIs were also analyzed, adding no value to what was already shown. These data have not been included in order to synthesize the information of the manuscript. However, other different analytical methods could be valuable, for example, the $T_{max}$ method[25] or measuring temperature recovery after thermal stress using a continuous thermographic video. Finally, a greater sample size with a higher number of women or with runners of different age and level could have provided an analysis of the effect of all these factors on the results. This increase in sample size could also modify the number of predictor variables obtained by the multivariate stepwise regression analyses.

## Practical Applications

The measurement of baseline skin temperatures every day before training to detect injuries associated with skin temperature asymmetries is one of the suggested applications of infrared thermography in sport field.[7] Those who are conducting the assessments could doubt whether they can relate skin temperature peaks to muscle damage due to the external workload of the previous days. The results of this study, in accordance with the previous studies, reject the idea that a skin temperature peak will be related to muscle damage.[5,6] Therefore, the first practical advice is that when a skin temperature peak is observed, it cannot be assumed that it will be due to muscle damage. Paradoxically, it seems to be quite the opposite: the results of this and other studies seem to suggest that if there is damage, no temperature increases are observed.[5,6] This is in agreement with the results of the cold stress test: higher reductions in skin temperature in the posterior leg after the marathon suggest that greater damage causes greater peripheral vasoconstriction in the baseline state.

## Conclusions

Baseline skin temperature was not altered 24 and 48 h after a marathon due to the oxidative stress variability of the sample. Participants with lower recovery in their oxidative stress present lower skin temperatures. Finally, the application of a cold thermal stress after the marathon seems to be a method that provides valuable information on vasoconstriction and the stress state of the runner.

### Acknowledgments

The authors would like to thank NostrumSport company for providing this study with the Game Ready system. The authors acknowledge the technical support from the *Servei Central de Suport a la Investigació Experimental* at the University of Valencia. Also, the authors are grateful to all the runners for their voluntary participation in this study.

## References

1. Mujika I. Quantification of training and competition loads in endurance sports: methods and applications. *Int J Sports Physiol Perform*. 2017;12(suppl 2):S2-9–S2-17. doi:10.1123/ijspp.2016-0403
2. Halson SL. Monitoring training load to understand fatigue in athletes. *Sports Med*. 2014;44(2):139–147. doi:10.1007/s40279-014-0253-z
3. Saw AE, Main LC, Gastin PB. Monitoring the athlete training response: subjective self-reported measures trump commonly used objective measures: a systematic review. *Br J Sports Med*. 2016; 50(5):281–291. PubMed ID: 26423706 doi:10.1136/bjsports-2015-094758
4. de Andrade Fernandes A, Pimenta EM, Moreira DG, et al. Skin temperature changes of under-20 soccer players after two consecutive

matches. *Sport Sci Health*. 2017;13(3):635–643. doi:10.1007/s11332-017-0394-1
5. Pérez-Guarner A, Priego-Quesada JI, Oficial-Casado F, Cibrián Ortiz de Anda RM, Carpes FP, Palmer RS. Association between physiological stress and skin temperature response after a half marathon. *Physiol Meas*. 2019;40(3):034009–034019. PubMed ID: 30870816 doi:10.1088/1361-6579/ab0fdc
6. da Silva W, Machado ÁS, Souza MA, Kunzler MR, Priego-Quesada JI, Carpes FP. Can exercise-induced muscle damage be related to changes in skin temperature? *Physiol Meas*. 2018;39(10):104007. doi:10.1088/1361-6579/aae6df
7. Fernández-Cuevas I, Lastras JA, Galindo VE, Carmona PG. Infrared thermography for the detection of injury in sports medicine. In: Priego Quesada JI, ed. *Application of Infrared Thermography in Sports Science*. Biological and Medical Physics, Biomedical Engineering. Cham, Switzerland: Springer; 2017:81–109. http://link.springer.com/chapter/10.1007/978-3-319-47410-6_4.
8. Hildebrandt C, Raschner C, Ammer K. An overview of recent application of medical infrared thermography in sports medicine in Austria. *Sensors*. 2010;10(5):4700–4715. doi:10.3390/s100504700
9. Schlager O, Gschwandtner ME, Herberg K, et al. Correlation of infrared thermography and skin perfusion in Raynaud patients and in healthy controls. *Microvasc Res*. 2010;80(1):54–57. PubMed ID: 20144625 doi:10.1016/j.mvr.2010.01.010
10. Al-Nakhli HH, Petrofsky JS, Laymon MS, Berk LS. The use of thermal infra-red imaging to detect delayed onset muscle soreness. *J Vis Exp*. 2012;(59):3551. doi:10.3791/3551
11. Sawasaki N, Iwase S, Mano T. Effect of skin sympathetic response to local or systemic cold exposure on thermoregulatory functions in humans. *Auton Neurosci*. 2001;87(2):274–281. doi:10.1016/S1566-0702(00)00253-8
12. Lahiri BB, Bagavathiappan S, Nishanthi K, et al. Infrared thermography based studies on the effect of age on localized cold stress induced thermoregulation in human. *Infrared Phys Technol*. 2016;76:592–602. doi:10.1016/j.infrared.2016.04.023
13. Zeng S, Chen Q, Wang X, et al. Longer rewarming time in finger cooling test in association with HbA1c level in diabetics. *Microvasc Res*. 2016;107:72–75. PubMed ID: 27211911 doi:10.1016/j.mvr.2016.05.003
14. Horikoshi M, Inokuma S, Kijima Y, et al. Thermal disparity between fingers after cold-water immersion of hands: a useful indicator of disturbed peripheral circulation in Raynaud phenomenon patients. *Intern Med*. 2016;55(5):461–466. PubMed ID: 26935364 doi:10.2169/internalmedicine.55.5218
15. Adam M, Ng EYK, Tan JH, Heng ML, Tong JWK, Acharya UR. Computer aided diagnosis of diabetic foot using infrared thermography: a review. *Comput Biol Med*. 2017;91:326–336. PubMed ID: 29121540 doi:10.1016/j.compbiomed.2017.10.030
16. Kuligowski J, Torres-Cuevas I, Quintás G, et al. Assessment of oxidative damage to proteins and DNA in urine of newborn infants by a validated UPLC-MS/MS approach. *PLoS One*. 2014;9(4):e93703. PubMed ID: 24695409 doi:10.1371/journal.pone.0093703
17. Mündermann A, Nigg BM, Stefanyshyn DJ, Humble RN. Development of a reliable method to assess footwear comfort during running. *Gait Posture*. 2002;16(1):38–45. doi:10.1016/S0966-6362(01)00197-7
18. Moreira DG, Costello JT, Brito CJ, et al. Thermographic imaging in sports and exercise medicine: a Delphi study and consensus statement on the measurement of human skin temperature. *J Therm Biol*. 2017;69:155–162. PubMed ID: 29037377 doi:10.1016/j.jtherbio.2017.07.006
19. Marins JCB, Moreira DG, Cano SP, et al. Time required to stabilize thermographic images at rest. *Infrared Phys Technol*. 2014;65:30–35. doi:10.1016/j.infrared.2014.02.008
20. Priego-Quesada JI, Oficial-Casado F, Gandia-Soriano A, Carpes FP. A preliminary investigation about the observation of regional skin temperatures following cumulative training loads in triathletes during training camp. *J Therm Biol*. 2019;84:431–438. PubMed ID: 31466783 doi:10.1016/j.jtherbio.2019.07.035
21. Bird SR, Linden M, Hawley JA. Acute changes to biomarkers as a consequence of prolonged strenuous running. *Ann Clin Biochem*. 2014;51(2):137–150. doi:10.1177/0004563213492147
22. Fernández-Cuevas I, Bouzas Marins JC, Arnáiz Lastras J, et al. Classification of factors influencing the use of infrared thermography in humans: a review. *Infrared Phys Technol*. 2015;71:28–55. doi:10.1016/j.infrared.2015.02.007
23. Priego Quesada JI, Kunzler MR, Carpes FP. Methodological aspects of infrared thermography in human assessment. In: Priego Quesada JI, ed., *Application of Infrared Thermography in Sports Science*. Cham, Switzerland: Springer; 2017:49–79. http://link.springer.com/chapter/10.1007/978-3-319-47410-6_3
24. Leijon-Sundqvist K, Lehto N, Juntti U, Karp K, Andersson S, Tegner Y. Thermal response after cold-water provocation of hands in healthy young men. *Thermol Int*. 2015;25:48–53.
25. Formenti D, Ludwig N, Rossi A, et al. Is the maximum value in the region of interest a reliable indicator of skin temperature? *Infrared Phys Technol*. 2018;94:299–304. doi:10.1016/j.infrared.2018.06.017