# Hydration Status, Executive Function, and Response to Orthostatism After a 118-km Mountain Race: Are They Interrelated? 

Ignacio Martínez-Navarro, ${ }^{1,2}$ Oscar Chiva-Bartoll, ${ }^{3}$ Barbara Hernando, ${ }^{4}$ Eladio Collado, ${ }^{4}$ Vicente Porcar, ${ }^{4}$ and Carlos Hernando ${ }^{4}$<br>${ }^{1}$ Department of Physical Education and Sports, University of Valencia, Valencia, Spain; ${ }^{2}$ NISA Sports Health, 9 de Octubre Hospital, Valencia, Spain; ${ }^{3}$ Department of Teaching Music, Arts and Body Expression, University of Valencia, Valencia, Spain; and ${ }^{4}$ Jaume I University, Castellón, Spain


#### Abstract

Martínez-Navarro, I, Chiva-Bartoll, O, Hernando, B, Collado, E, Porcar, V, and Hernando, C. Hydration status, executive function and response to orthostatism after a $118-\mathrm{km}$ mountain race: are they interrelated? J Strength Cond Res 32(2): 441-449, 2018-The present study aimed to explore whether blood pressure (BP) and heart rate (HR) variability (HRV) responsiveness to orthostatism, jointly with executive function (EF) performance, was diminished after an ultra-endurance mountain race. Besides, we wanted to assess whether hydration status was related to either performance or the abovementioned alterations. Fifty recreational ultra-endurance athletes participating in the Penyagolosa Trails CSP115 race ( 118 km and a total positive elevation of $5,439 \mathrm{~m}$ ) were evaluated before and after the competition. The HRV and BP were measured in response to an orthostatic challenge. The EF was evaluated using the color-word interference task of the Stroop test. Body mass (BM) and urine specific gravity (USG) changes were used to assess hydration status. The HRV and BP responsiveness to orthostatism was diminished after the race. Besides, a significant BM loss of $3.51 \pm$ 2.03\% was recorded. Conversely, EF and USG showed no significant changes from prerace to postrace. Eventually, BM loss was inversely related to finishing time ( $r=-0.34$ ) and postrace orthostatic HR and EF were positively associated ( $r=0.60$ ). The USG and BM loss appear to provide different insights into hydration status, and our results challenge the well-established criteria that BM losses $>2 \%$ are detrimental to performance. Coaches are advised to consider athletes' performance level when interpreting their BM changes during an ultra-endurance competition. Similarly, coaches should be


[^0]aware that increased vulnerability to orthostatism is a common phenomenon after ultra-endurance races, and diminished HR responsiveness to orthostatism could constitute a practical indicator of EF worsening.

KEY Words ultra-endurance, heart rate variability, Stroop test, urine specific gravity, performance

## INTRODUCTION

The acute effects of ultra-endurance races are the main point of an increasing number of research studies, encompassing fields such as cardiac hemodynamics, inflammation, muscle damage, sleep management, cognitive performance, central and peripheral fatigue, or hydration status. Indeed, this latter and its influence on performance during endurance exercise has been the object of an intense debate in the literature $(17,32,40)$, and it remains a matter of concern for ultraendurance coaches. Similarly, management of increased vulnerability to orthostatic challenges and cognitive performance worsening during ultra-endurance races are relevant to ultra-endurance coaches' practice ( $10,18,22,29,30,33$ ). However, previous literature does not offer studies providing a joint assessment of those 3 fields (i.e., hydration status, orthostatic tolerance, and cognitive performance) after an ultra-endurance event. Such approach would enable to examine whether ultrarunners who display greater end-of-exercise body mass (BM) losses are prone to increased vulnerability to orthostatic challenges or cognitive performance worsening or whether responsiveness to orthostatism and cognitive performance are interrelated.

Exercise-induced dehydration has been demonstrated to alter baroreflex sensitivity and contribute to orthostatic intolerance under a laboratory setting (i.e., 90 minutes cycling at $55 \% \dot{V}_{2}$ peak wearing water-impermeable plastic garments) (7). However, as far as we are aware, only one study has previously assessed heart rate (HR) variability (HRV) and blood pressure (BP) responsiveness to orthostatism, jointly with hydration status, after a competitive
ultra-enduranceevent (i.e., mountain marathon) (33). Although they did not attempt to find possible associations between HRV and BP responses to orthostatism and postrace hydration status, the authors concluded that differences in hydration status were not responsible for the reduction in orthostatic tolerance, inasmuch as urine specific gravity (USG) did not change from prerace to postrace. In a similar manner, only Mahon et al. (30) have previously assessed a possible relationship between cognitive performance impairment and dehydration after an ultra-endurance event. Contrary to the authors' expectations, they failed to find significant differences in a choice reaction time test as a function of hydration status (i.e., USG).

Therefore, our first purpose was to examine the effect of an ultra-endurance event on executive function (EF), which it is assimilated as the orchestra director regarding cognitive processing (14), on one hand and BP and HRV responses to orthostatism, on the other hand. We were also interested in assessing whether EF, orthostatic tolerance, and hydration status after an ultra-endurance event may keep any relationship. Eventually, our aim was also to broaden previous findings in relation to the role played by dehydration regarding the achievement of best performance. Our study hypothesis was that athletes would show diminished BP and HRV responsiveness to orthostatism and their EF would be impaired after the race. We also hypothesized that orthostatic intolerance and EF worsening would be interrelated. Eventually, our third hypothesis was that faster runners would display a greater end-ofexercise BM loss.

## Methods

## Experimental Approach to the Problem

This research was carried out at the Penyagolosa Trails CSP115 race in 2015 (May 9th-10th). The track consisted of 118 km , starting at an altitude of 40 m and finishing at $1,280 \mathrm{~m}$ above the sea level, with a total positive and negative elevations of 5,439 and $4,227 \mathrm{~m}$, respectively. Temperature and humidity were recorded at the start, at 2 midpoints during the race ( 72.3 and 91.1 km ), and at the finish line. The EF, jointly with HRV and BP responsiveness to orthostatism, was assessed in the afternoon the day before the race and within 30 minutes after race completion. Hydration status was estimated in duplicate from USG and from changes in BM. The USG was measured from a first-morning-void urine sample (the day of the race) and the first-postrace-void urine sample. The BM was measured within 1 hour before race started and immediately after crossing the finishing line. Participants were informed to avoid caffeine and exercise in the 12 hours before prerace testing. Participants were also informed not to consume any large meal in the previous 4 hours. During postrace evaluation, participants were allowed to drink but not eat. Finishing time was considered as an independent variable.

## Subjects

Fifty recreational ultra-endurance athletes ( 44 men and 6 women) were recruited to participate in the study. Selected athletes were required to have previously completed at least one ultramarathon $(>60 \mathrm{~km})$. A questionnaire was used to collect demographic information and training and competition history. All athletes considered the Penyagolosa Trails CSP115 as their main competitive goal of the season. The characteristics of the sample are presented in Table 1. All subjects were informed of the benefits and risks of the investigation before signing an institutionally approved informed consent document. They were also allowed to withdraw from the study at will. The investigation was conducted according to the Declaration of Helsinki, and it was approved by the Research Ethics Committee of the University Jaume I of Castellon.

## Procedures

Orthostatic challenge consisted of 8 minutes of supine bed rest followed by 7 minutes in an upright freestanding posture $(43,44)$. To limit the effect of the skeletal muscle pump, subjects were instructed not to make any major muscle contractions at supine and standing postures. Beat-to-beat HR was recorded continuously using a Polar RS800 HR monitor together with a Polar Wearlink Wind electrode transmitter

TABLE 1. Sample main characteristics (mean $\pm$ SD).*

|  | $n=50$ |
| :--- | :---: |
| Age (y) | $22-61$ |
| Body mass (kg) | $71.53 \pm 9.25$ |
| Height $(\mathrm{cm})$ | $170.9 \pm 6.1$ |
| $\mathrm{BMI}\left(\mathrm{kg} \cdot \mathrm{m}^{-2}\right)$ | $24.43 \pm 2.36$ |
| Years since first | $3.27 \pm 2.91$ |
| ultramarathon $(>60 \mathrm{~km})$ |  |
| Ultramarathon $(>100 \mathrm{~km})$ races | $\%$ |
| $\quad$ before event |  |
| 0 | 22.4 |
| 1 | 20.4 |
| 2 | 10.2 |
| 3 | 16.3 |
| 4 | 6.1 |
| 5 | 4.1 |
| $>5$ | 20.4 |
| Average weekly sessions | $4.61 \pm 1.10$ |
| Average weekly | $\%$ |
| training volume (h) | 42.9 |
| $<12$ | 34.7 |
| $12-15$ | $16.3-7.17$ |
| $16-20$ | 6.1 |
| $>20$ | $65.81 \pm$ |
| Average weekly training | 27.16 |
| volume (km) |  |

*BMI = body mass index.
(Polar Electro, Kempele, Finland), after application of conductive gel as recommended by the manufacturer. This instrument has been previously validated for the accurate measurement of RR intervals in young and middle-aged men $(12,53)$. Systolic BP (SBP) and diastolic BP (DBP) were measured after 2 minutes of supine bed rest and after assuming the upright freestanding posture, using an automatic digital BP monitor (Model M6-IT; OMRON, Kyoto, Japan) with the cuff at heart level. Orthostatic hypotension $(\mathrm{OH})$ was defined as a drop of $\geq 20 \mathrm{~mm} \mathrm{Hg}$ SBP or $\geq 10 \mathrm{~mm} \mathrm{Hg}$ DBP on standing (42). Respiratory rate was not controlled to not interfere in athletes' recovery, although they were asked to avoid irregular respiration. Normal respiratory rate does not result in significantly different HR-derived indices compared with controlled breathing (4).

RR intervals were transferred to Polar Pro Trainer 5 software (Polar Electro) and then analyzed using Kubios HRV Analysis Software 2.0 (The Biomedical Signal and Medical Imaging Analysis Group, University of Kuopio, Finland). The whole analysis process was carried out by the same researcher to ensure consistency. Artifacts were identified and corrected according to manufacturer's recommendations (47), and only those recordings with $<1 \%$ of artifacts were considered. According to previous studies in the field $(43,44)$, analyses were performed on $R R$ intervals recorded between the third and eighth minute supine and between the 9 th and 14th minute standing. The following indices were obtained: mean HR , the $S D$ of normal RR intervals (SDNN) as a measure of overall variability and the root-mean-square difference of successive normal RR intervals (RMSSD) as a measure of vagal modulation (48), the short-term scaling exponent ( $4-11$ beats, $\alpha 1$ ) from detrended fluctuation analysis to estimate sympathovagal balance and fractal correlation properties (52), and sample entropy (SampEn) to provide an indication of the complexity of the time series under these circumstances (35). Time domain indices were chosen instead of spectral indices because of its greater intraindividual reproducibility (1).

Executive function was measured using the color-word interference task of the Stroop test, which it is considered a test of response inhibition (i.e., measures the ability to suppress an overlearned response) (16). The task consists of 100 stimuli ( 5 columns by 20 rows) printed on a $29.7 \times 21$ - cm sheet of paper, and the participant has to name the color of the ink in which the words are written, ignoring the automatic reading of the word's incongruent meaning (i.e., the word "blue" written in red ink). The number of correct items named within 45 seconds was used to measure the performance (15).

The BM measurements were made with calibrated electronic scales (Seca 813; Vogel and Halke, Hamburg, Germany) that were on firm surfaces. Before the event, the scales were examined for consistency. Following a previous study (20), both prerace and postrace measurements were made with the runner clothed in running wear and shoes, but other items such as waist packs and hydration vests were
removed and nothing was permitted in the runner's hands. Based on USG, participants were categorized as adequately hydrated $\left(<1.020 \mathrm{~g} \cdot \mathrm{ml}^{-1}\right)$, as mildly dehydrated ( $1.020-$ $1.030 \mathrm{~g} \cdot \mathrm{ml}^{-1}$ ), or as severely dehydrated ( $>1.030 \mathrm{~g} \cdot \mathrm{ml}^{-1}$ ) $(5,25)$. Considering BM change, a loss $>4 \%$ was classified as dehydration, a loss $\leq 1 \%$ as overhydration, and a loss between 1 and $4 \%$ as euhydration $(20,34)$.

## Statistical Analyses

Statistical analyses were carried out using the Statistical Package for the Social Sciences software (IBM SPSS Statistics for Windows; version 22.0; IBM Corp., Armonk, NY, USA). After testing for normal distribution (Kolmogorov-Smirnov test, with Lilliefor's correction), SDNN and RMSSD were logarithmically transformed to allow parametric comparisons.

A repeated measures multivariate analysis of variance (ANOVA) was used to assess the effects of race and posture (supine vs. standing) and their interaction on BP and HR dynamic indices. For each ANOVA, if a significant main effect or interaction was identified, pairwise comparisons were adjusted using Bonferroni's correction. Additionally, relative changes from supine to standing position (orthostatic changes) in BP and HR dynamic indices were compared between prerace and postrace using a paired samples Student's $t$-tests. The USG, BM, and Stroop performance were compared before and after the race using paired samples Student's $t$-tests.

Pearson correlation and partial correlation analyses were conducted among selected variables. First, we analyzed whether performance was associated with BM, USG, and Stroop performance. Second, we assessed possible relationships among Stroop performance, hydration status (i.e., BM change and postrace USG), and postrace orthostatic change in HR dynamic indices, SBP, and DBP. Stroop performance analyses were adjusted by age. The meaningfulness of the outcomes was estimated through the effect size (ES, means divided by the $S D$ ): an ES $<0.5$ was considered small; between 0.5 and 0.8 , moderate; and greater than 0.8 , large (50). Likewise, correlations greater than 0.5 were considered large; between 0.3 and 0.5 , moderate; and smaller than 0.3 , small (50). The significance level was set at $p$ value $<0.05$, and data are presented as means and $S D$ s $( \pm S D)$.

## Results

Thirty-three athletes ( 29 men and 4 women) successfully completed the race (finishers to starters ratio: 68\%) with an average finish time of 22 hours 29 minutes $\pm 3$ hours $43 \mathrm{mi}-$ nutes. Both the average finish time and the finishers to starters ratio for the subjects of the present study were similar when all race participants were considered ( 22 hours $37 \mathrm{mi}-$ nutes $\pm 3$ hours 47 minutes and $63.5 \%$, respectively). Furthermore, all levels of performance were represented in our sample as shown by their rank ranging from 3rd to 286th place (of 291 finishers). Temperature at the start was $23.2^{\circ} \mathrm{C}$, and it ranged between 21.7 and $23.8^{\circ} \mathrm{C}$ (first midpoint), 13.5
and $19.4^{\circ} \mathrm{C}$ (second midpoint), and 9.9 and $15^{\circ} \mathrm{C}$ (finish line). Humidity at the start was $48 \%$, and it ranged between 41 and $47 \%$ (first midpoint), 50 and $67 \%$ (second midpoint), and 55 and $68 \%$ (finish line).

## Orthostatic Challenge

Nine participants resigned to undergo either prerace or postrace orthostatic test because of time constraints. Three participants were excluded from HR dynamic analyses because of an excessive number of artifacts $(>1 \%)$ in their HR recording. Postrace, 6 participants could not assume the standing position because of sickness or dizziness and 4 participants showed OH. Prerace, all the subjects completed the orthostatic challenge and none of them exhibited OH. Sixteen participants were eventually included in HR dynamic analysis and 19 in BP analysis.

Univariate contrast analysis revealed a significant effect for "race" on $\operatorname{HR}\left(F=12.75, p<0.01, \eta^{2}\right.$ partial $\left.=0.46\right)$, $\ln S D N N\left(F=9.72, p<0.01, \eta^{2}\right.$ partial $\left.=0.39\right), \ln$ RMSSD $\left(F=8.19, p \leq 0.05, \eta^{2}\right.$ partial $\left.=0.35\right)$, and $\operatorname{SampEn}(F=8.39$, $p \leq 0.05, \eta^{2}$ partial $\left.=0.36\right)$. "Posture" factor significantly affected HR $\left(F=75.39, p<0.01, \eta^{2}\right.$ partial $\left.=0.83\right)$, $\ln$ RMSSD $\left(F=11.86, p<0.01, \eta^{2}\right.$ partial $\left.=0.44\right), \alpha 1(F=$ 55.28, $p<0.01, \eta^{2}$ partial $\left.=0.79\right)$, and SampEn $(F=52.11$, $p \leq 0.05, \eta^{2}$ partial $=0.78$ ). However, no significant effects were found for "race $\times$ posture" interaction. Pairwise comparisons showed that HR was significantly lower in prerace compared with postrace recording $(p<0.01)$, whereas $\ln S D N N, \ln R M S S D$, and SampEn were significantly higher in prerace recording ( $p \leq 0.05$ ). Meanwhile, HR and $\alpha 1$ were significantly lower in supine posture compared with standing, whereas $\ln S D N N$ and SaEn were significantly higher in supine posture ( $p<0.01$ in all cases). In addition, orthostatic changes in HR and RMSSD were significantly and largely attenuated after the race ( $15.05 \pm 12.09 \%$ vs. $21.78 \pm 9.89 \%, \mathrm{ES}=-0.63, p \leq 0.05 ;-4.24 \pm 52.96 \%$ vs.
$-31.87 \pm 12.12 \%, \mathrm{ES}=0.72, p \leq 0.05$, respectively). Table 2 and Figure 1 show the time course of HR dynamic indices during orthostatic challenge before and after the race.

Regarding BP analysis, univariate contrast analysis showed a significant effect for race on SBP $(F=23.03$, $p<0.01, \eta^{2}$ partial $=0.56$ ) and a significant effect for posture on DBP $\left(F=51.52, p<0.01, \eta^{2}\right.$ partial $\left.=0.74\right)$. In addition, race $\times$ posture interaction significantly affected both SBP $\left(F=10.50, p<0.01, \eta^{2}\right.$ partial $\left.=0.37\right)$ and $\operatorname{DBP}(F=$ $6.94, p \leq 0.05, \eta^{2}$ partial $=0.28$ ). Further pairwise comparisons revealed that in prerace condition, SBP and DBP significantly and largely increased from supine to standing $(125.10 \pm 11.10$ vs. $131.58 \pm 13.82 \mathrm{~mm} \mathrm{Hg}, \mathrm{ES}=0.53$, $p<0.01 ; 74.79 \pm 7.07$ vs. $85.16 \pm 7.82 \mathrm{~mm} \mathrm{Hg}, \mathrm{ES}=$ $1.43, p<0.01$, respectively), whereas after the race, no significant changes were observed ( $118.84 \pm 13.75$ vs. $113.58 \pm$ 12.53 mm Hg and $75.26 \pm 8.42$ vs. $78.42 \pm 9.62 \mathrm{~mm} \mathrm{Hg})$. Additionally, orthostatic changes in both SBP and DBP were significantly and largely diminished after the race $(-3.84 \pm$ $10.17 \%$ vs. $5.18 \pm 5.91 \%$, $\mathrm{ES}=-1.11, p<0.01 ; 4.71 \pm$ $11.18 \%$ vs. $14.08 \pm 6.53 \%$, $\mathrm{ES}=-1.05, p \leq 0.05$, respectively). Figure 2 shows the time course of SBP and DBP during orthostatic challenge before and after the race.

## Executive Function

Stroop performance did not change from prerace to postrace condition ( $47.32 \pm 8.27$ vs. $46.29 \pm 7.52$ correct items; $p=$ 0.30 ). Correlation analyses showed that both prerace (using a partial correlation controlling for age differences) and $\Delta$ Stroop performance were unrelated to finishing time.

## Hydration Status

All the finishers were assessed on BM, but unfortunately only 23 postrace urine samples could be collected. The BM showed a significant but small decrease after the race $(68.12 \pm 8.77$ vs. $70.63 \pm 9.20 \mathrm{~kg}, \mathrm{ES}=-0.28, p<0.01)$,

Table 2. Linear and nonlinear HR dynamics during supine and standing positions before and after the race.*||

|  | Prerace |  | Postrace |  | Significant main or interaction effects |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  | Supine | Standing | Supine | Standing |  |
| HR (b $\cdot \mathrm{min}^{-1}$ ) | $63.73 \pm 7.39$ | $77.29 \pm 8.57 \dagger$ | $74.07 \pm 9.35 \ddagger$ | $84.60 \pm 8.84 \dagger$ § | Race, posture |
| InSDNN (ms) | $3.53 \pm 0.50$ | $3.65 \pm 0.39$ | $2.98 \pm 0.54 \ddagger$ | $3.14 \pm 0.57 \ddagger$ | Race |
| InRMSSD (ms) | $3.49 \pm 0.49$ | $3.07 \pm 0.40 \dagger$ | $2.73 \pm 0.81 \ddagger$ | $2.54 \pm 0.62 §$ | Race, posture |
| $\alpha_{1}$ | $1.11 \pm 0.24$ | $1.60 \pm 0.18 \dagger$ | $1.31 \pm 0.40$ | $1.63 \pm 0.20 \dagger$ | Posture |
| SampEn | $1.74 \pm 0.23$ | $1.14 \pm 0.27 \dagger$ | $1.56 \pm 0.24 \S$ | $1.02 \pm 0.26+\S$ | Race, posture |

*HR = heart rate; $\ln S D N N=$ log-transformed $S D$ of normal RR intervals; $\ln R M S S D=$ log-transformed root-mean-square difference of successive normal RR intervals; $\alpha 1=$ short-term fractal scaling exponent; SampEn = sample entropy.
$\dagger$ Significantly different from supine position ( $p<0.01$ ).
$\ddagger$ Significantly different from prerace ( $p<0.01$ ).
$\S$ Significantly different from prerace ( $p \leq 0.05$ ).
$\|$ Significantly different from supine position ( $p \leq 0.05$ ).


Figure 1. Relative change (\%) of HR dynamic indices from supine rest to active standing before (black bars) and after the race (gray bars). $\mathrm{HR}=$ heart rate; $S D N N=S D$ of normal RR intervals; RMSSD = root-mean-square difference of successive normal RR intervals; $\alpha_{1}=$ short-term fractal scaling exponent; SampEn $=$ sample entropy. *Significantly different from prerace ( $p \leq 0.05$ ); **significantly different from prerace ( $p<0.01$ ).
with a mean percent BM loss of $3.51 \pm 2.03 \%$. On the contrary, USG showed no significant changes from prerace to postrace $\left(1.020 \pm 0.005\right.$ vs. $1.021 \pm 0.005 \mathrm{~g} \cdot \mathrm{ml}^{-1}, p=$ 0.36 ). Thirteen participants ( $38.2 \%$ ) were identified as dehydrated and 3 participants ( $8.8 \%$ ) as overhydrated according to their BM change. Meanwhile, considering USG values, mild dehydration was identified in 9 athletes (26.5\%) before the race and 11 athletes after the race (47.8\%), whereas no signif-
icant dehydration was found either prerace or postrace. $\Delta \mathrm{BM}$ and postrace USG displayed a nearly significant correlation $(r=0.39, p=0.06)$. However, relative change in BM from prerace to postrace was inversely and moderately associated with finishing time ( $r=-0.34, p \leq 0.05$ ), whereas no significant relationship was identified between finishing time and postrace USG. Eventually, prerace BM and USG were also unrelated to finishing time.


Figure 2. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) during supine (black bars) and standing (gray bars) positions prerace and postrace.*Significantly different from prerace ( $p \leq 0.05$ ); **significantly different from prerace ( $p<0.01$ ); \#significantly different from supine position ( $p \leq 0.05$ ); \#\#significantly different from supine position ( $p<0.01$ ).


Figure 3. Relationship between $\Delta$ Stroop and postrace orthostatic change in HR.

## Relationship Between Orthostatic Challenge, Executive Function, and Hydration Status

$\Delta$ Stroop was unrelated to postrace USG and $\Delta \mathrm{BM}$, but it was largely correlated with postrace orthostatic change in HR ( $r=0.60, p<0.01$; Figure 3). No relationship was found between either postrace USG or $\triangle B M$ and postrace SBP, DBP, and HR dynamic orthostatic change.

## Discussion

In this study, we analyzed 50 participants of a $118-\mathrm{km}$ mountain race providing a joint assessment of BP and HRV responsiveness to orthostatism, EF, and hydration status before and after the race. According to our results, BP and HRV responsiveness to orthostatism is altered after an ultradistance mountain competition. However, contrary to our hypothesis, EF did not decline after the race. Regarding hydration status, USG did not change from prerace to postrace, whereas a significant BM loss of $3.51 \pm 2.03 \%$ was recorded. Moreover, BM loss was inversely correlated with finishing time, whereas no relationship was found between USG and performance. Eventually, orthostatic HR response after the race showed a large relationship with executive performance.

The significant decrease in BM after the race is in line with several previous studies (19-21,24,30,38,41,45,46,56). Likewise, our mean percent BM loss (3.51\%) falls within formerly reported after other ultra-endurance competitions: greater than that measured after shorter races (i.e., $80.5-\mathrm{km}$ mountain race, $85-\mathrm{km}$ mountain race, or $100-\mathrm{km}$ flat race) $(30,38,41)$ but smaller than that recorded after longer or multistage races (i.e., Ironman triathlon, 24 -hour ultramarathon, or marathon of sands) $(24,45,56)$. According to BM
loss, our percentage of dehydrated athletes (38.2\%) is also within the range reported by Hoffman et al. (19) after analyzing 887 athletes at northern California 161-km ultramarathons during 5 consecutive years (7.3-48.9\%), whereas our percentage of overhydrated runners $(8.8 \%)$ is close to the lower limit of this range (6.7-47.5\%).

Concerning USG, prerace values indicated that $26.5 \%$ of runners were not adequately hydrated before the start. This outcome could be surprising at first sight; but considering that prerace USG was determined from morning first void, it is possible that athletes improved their hydration status in the time lapse before the start, as previously suggested (13). Regarding postrace values, previous studies carried out in other ultra-endurance events have shown divergent results. Our absence of a significant prerace to postrace difference has been formerly reported after a mountain marathon, a 24 -hour ultramarathon or a $1,230-\mathrm{km}$ cycling event $(9,13,33)$, whereas a significant increase has been observed after a $80.5-\mathrm{km}$ mountain race, a $100-\mathrm{km}$ flat race, a 24 -hour mountain bike race, or an Ironman triathlon $(9,27,30,31,38)$. According to USG values, our percentage of postrace mildly dehydrated participants $(47.8 \%)$ is similar to that reported by Geesman et al. (13) after a $1,230-\mathrm{km}$ cycling event ( $50 \%$ ), whereas our absence of severely dehydrated athletes after the race also coincides with the abovementioned study but differs from Mahon et al. (30), where an incidence of $22 \%$ was described after an $80.5-\mathrm{km}$ mountain race.

Therefore, current outcomes further corroborate that USG and BM losses provide different insights into hydration status after an ultra-endurance exercise. Actually, Rogers et al. (37) postulated almost 20 years ago that approximately $60 \%$ of BM loss after a long-distance triathlon was because of factors other than pure fluid loss. And more recently, Mueller et al. (31) have demonstrated using dual-energy X-ray absorptiometry measurements that BM loss after an Ironman triathlon was because of a 28 and $72 \%$ loss in fat and lean mass, respectively, being the latter attributable to a loss of glycogen, as fuel for energy production, and the corresponding loss of body water.

Regarding the association between performance and hydration status, the absence of a significant relationship between postrace USG and finishing time is consistent with a previous investigation (30). At the same time, the inverse relationship between BM change and finishing time reinforces previous studies conducted in endurance and ultra-endurance events, such as road marathons (55), $100-\mathrm{km}$ flat ultramarathons (38), 161-km mountain ultramarathons (19), Ironman triathlons (46,54), 24 -hour ultramarathons (24), and even a multistage trail race in tropical conditions (21). This plethora of results, however, takes issue with current guidelines advising that BM loss $>2 \%$ should be avoided during endurance exercise $(36,39)$. Those guidelines, which state that such weight losses involve a level of dehydration that impairs aerobic exercise performance, are based on laboratory studies using shorter and fixed-intensity exercise protocols (17,32).

Therefore, considering the abovementioned results from Mu eller et al. (31) and the fact that none of our participants showed a severe dehydration according to USG results, it is arguable that greater weight losses among best performers during self-paced ultra-endurance events could be mainly a reflection of their greater energy expenditure.

The incidence of either sickness or dizziness (6 out of 31) in assuming the upright posture after the race was smaller than previously reported after either a mountain marathon ( 6 out of $7 ; 33$ ) or an Ironman triathlon ( 7 out of $23 ; 18$ ). Our results showed that cardiac autonomic modulation during supine rest became less complex and more predictable after the race (i.e., lower SampEn and higher $\alpha 1$ ), although the increase in $\alpha 1$ did not reach the significance level $(p=0.11)$. Concomitantly, both overall and vagally mediated HRV (i.e., $\ln S D N N$ and $\ln R M S S D)$ were significantly reduced in postrace assessment (Table 2). This is in agreement with previous studies involving mountain marathon races $(3,33)$ and ultraendurance events (i.e., Ironman triathlon and 120- and 190km mountain races) $(11,18)$.

However, orthostatic response varied across linear and nonlinear indices and also between prerace and postrace evaluations (Figure 2). Before the race, upright posture induced a significant decrease in $\ln$ RMSSD and SampEn jointly with a significant increase in $\alpha 1$. This could be considered the likely HR dynamic response to an orthostatic challenge (51). After the race, SampEn and $\alpha 1$ kept a similar response to the orthostatic challenge, whereas $\ln$ RMSSD did not change from supine to standing position. This blunted vagal reactivity has been previously reported after an Ironman triathlon (18); conversely, former studies conducted on mountain marathon races have shown a maintained vagal reactivity to orthostatic challenge $(3,33)$. Therefore, it seems that vagal responsiveness is greatly affected after an ultra-endurance event (i.e., Penyagolosa Trails CSP115 and Ironman triathlon) compared with shorter races. Meanwhile, complexity and fractal properties of HR dynamics appear to be more resilient to exercise stress than linear HRV.

Despite increased sympathetic and reduced vagal modulation (i.e., augmented HR and $\alpha 1$ coupled with reduced $\ln S D N N$ and $\ln R M S S D$ ), SBP during supine rest was reduced after the race, in line with previous studies $(3,18,33)$. Furthermore, after the race, BP did not increase as a result of orthostatic challenge, whereas before the race, SBP and DBP significantly increased from supine to standing position (Figure 1). Gratze et al. (18) also found that participants were unable to raise their SBP as a response to active standing after an Ironman triathlon, whereas Murrell et al. (33) even reported a significant decrease in orthostatic SBP and DBP after a mountain marathon race. Notwithstanding, in this latter study, participants failed to show a significant increase in either SBP or DBP during baseline orthostatic test, unlike the study by Gratze et al. (18) and ours.

Eventually, the absence of significant correlations between postrace BP and HR dynamic orthostatic response, on one
hand, and hydration status (either measured by USG or BM change), on the other hand, corroborates that diminished orthostatic tolerance after a long-distance mountain race is unrelated to hydration status (33). Interestingly, whereas a previous laboratory study found that orthostatic HR significantly increased in response to an induced dehydration (8), our results show that postrace orthostatic HR was uncorrelated to BM change. Exercise-related effects on autonomic control of HR (i.e., reduced orthostatic responsiveness) might explain this contradictory results.

The absence of a significant difference between prerace and postrace executive performance coincides with a former study carried out in a 100 -hour adventure race (28). However, other cognitive abilities such as psychomotor vigilance and choice reaction time have been shown to be diminished after ultra-endurance events (i.e., $166-\mathrm{km}$ Ultra Trail du Mont Blanc, a 36 -hour ultra-endurance event, $80.5-\mathrm{km}$ mountain race) $(10,22,30)$. Therefore, it may be arguable that EF shows a greater resiliency than psychomotor vigilance and choice reaction time performance after an ultraendurance event, as previously suggested (49). Further studies including a broader cognitive assessment are nevertheless required to verify this postulate.

Meanwhile, the lack of a significant relationship between hydration status (either measured with USG or BM change) and Stroop performance after ultra-endurance events endorses previous research in the field (30). The reason why we observed no negative effects of dehydration on cognitive function is probably the absence of significant changes in Stroop performance after the race, on one hand, and the fact that dehydration was not severe enough among our participants to affect EF, on the other hand. Notwithstanding, Kempton et al. (26) demonstrated that acute dehydration provoked an increased neural activation during an EF task (i.e., compared with euhydration condition). Accordingly, they concluded that dehydrated participants exerted a higher level of neuronal activity to achieve the same performance level. Therefore, we could not discard that exercise-related dehydration in our study could have also led to this detrimental effect.

Besides, Cona et al. (6) have recently observed a significant baseline better cognitive functioning (i.e., inhibitory control and dual tasking) in fast vs. slow runners of an ultradistance mountain race (i.e., $80-\mathrm{km}$ Trans d'Havet race). Our results, on the contrary, did not show a significant relationship between prerace Stroop performance and finishing time. $\Delta$ Stroop performance was also unrelated to finishing time, as previously observed for psychomotor vigilance performance (22). Conversely, the large relationship found between $\Delta$ Stroop performance and postrace orthostatic HR implies that athletes who showed lesser HR responsiveness displayed greater EF worsening (Figure 3). Actually, Temesi et al. (49) concluded that sympathetic nervous activation could buffer the drop in EF provoked by sleep deprivation and central fatigue after an ultra-endurance event.

Moreover, a controlled laboratory study showed that decreased performance in the Stroop test and lower cardiac autonomic reactivity were connected and also constituted descriptive features of overtrained athletes (23).

Similar to other related studies $(3,18,33)$, we decided to use the stand test because of its practical and physiological generalizability to the realistic problems that occur after exhaustive and prolonged exercise (i.e., the difficulty to maintain an upright posture after a supine rest period). Although it is unclear how the hemodynamic changes during postural change may translate to those induced during a more severe orthostatic stress test (i.e., lower body negative pressure, tilt), both active standing and passive head-up tilt have been reported to provoke comparable changes in spontaneous baroreflex and related hemodynamic variables (2).

## Practical Applications

Our results endorse previous field studies that challenge the well-established belief that euhydration is necessary to obtain the best performance during ultra-endurance races. Therefore, it is advisory for coaches to take into consideration athletes' performance level when interpreting their BM changes during an ultra-endurance competition. Contradictory results obtained from USG and BM measures lead us to suggest that greater weight losses among best performers during self-paced ultra-endurance events could be mainly a reflection of their greater energy expenditure. On the other hand, coaches should be aware that increased vulnerability to orthostatism is a common phenomenon after an ultra-endurance event; so, sudden posture changes (i.e., from sitting to standing in an aid station) are advised against in the final stages of such a race. Eventually, diminished HR responsiveness to orthostatism could constitute a practical and important (in terms of safety) indicator of executive performance worsening during and at the end of ultra-endurance events.

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[^0]:    Address correspondence to Ignacio Martínez-Navarro, ignacio. martinez-navarro@uv.es.
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