

Article

Physiological, Biomechanical, and Thermographic Responses in Male Athletes during an Ultra-Endurance Race

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Abstract: This study investigates the comprehensive physiological, biomechanical, and thermographic responses of male athletes during an ultra-endurance race, the Santander Four Days (S4D). Involving a 160 km race over four consecutive days with a 10 kg backpack, the study focuses on key aspects such as body mass, cortical arousal, handgrip strength, heart-rate variability, hydration status, blood glucose and lactate concentrations, and thermographic responses. The results indicate changes in heart-rate variability, indicating increased cardiovascular strain, consistent neuromuscular performance, significant body-weight reduction possibly due to dehydration and energy use, stable pH and glucose, but increased protein in urine suggesting renal stress, and varied body temperatures reflecting physical exertion and environmental factors. These findings highlight the body's adaptive mechanisms and the importance of specialized training and recovery strategies in such physically demanding events.

Keywords: ultra-endurance; thermography; heart-rate variability; stress; cortical arousal; strength



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1. Introduction

Ultra-endurance events are athletic competitions that significantly exceed the duration of traditional endurance events, typically lasting six hours or more. These events include activities such as ultramarathons, Ironman triathlons, and long-distance cycling races. They are defined as those lasting more than six hours, often requiring sustained physical exertion and exceptional stamina [1,2]. Participation in these events has increased in the last 25 years. The most popular are ultramarathon races, ultratriathlons, ultradistance swimming, ultracycling, and cross-country skiing [3]. There is also an increasing number of ultra-endurance mountain races, which are demanding events that produce a high impact on the psychophysiological response of participants [4]. Specifically, ultra-endurance athletes report a large rate of perceived exertion (RPE) [4], a decrease in blood glucose values [5], and an increase in sympathetic modulation analyzed by heart-rate variability (HRV), heart rate [6], muscular pain, and a decrease in cortical arousal [7]. Also, these long-duration events produce dehydration and a decrease in leg muscle strength [4]. They are performed at intensities lower than the anaerobic threshold [8] but produce a large muscular breakdown, as shown by increased creatinine kinase values [9].

Previous authors have found that different parameters are related to performance in ultra-endurance races [2,4]. Regarding training programs, traditionally, high-volume training has been the principal paradigm; however, the advantages of implementing high-intensity programs for ultra-endurance athlete performance have been shown [2,4]. In

addition, complementary strength training is also related to increased performance and injury prevention [4]. Specifically, in ultra-endurance mountain races, previous authors have shown how stress levels and general mental health are important performance predictors [10]. Regarding body composition, lower levels of body fat are a key factor for finishing ultra-endurance mountain races [10]. In line with this, hydration and nutritional patterns have also been shown to be crucial in these extreme events. For example, athletes that are more hydrated at the start line presented higher performance in ultra-endurance mountain races [2]. Ultra-endurance events often result in substantial energy deficits, producing a catabolic state [11]. Therefore, it is necessary to adapt the body to use fats with principal substrate energy [2].

Ultra-endurance athletes often face challenges such as hyperthermia that can hinder performance and even jeopardize their health during ultra-endurance events [4]. Heat acclimatization, maintaining proper hydration, and athlete morphology play pivotal roles in preventing hyperthermia [12]. In this context, infrared thermography has emerged as an effective tool for analyzing athletes' thermal responses during movement and for injury-prevention interventions [13,14]. Specifically, in soccer players, an increase of 1.0–1.5 °C is associated with a high injury risk, and when the values exceed 1.5 °C, the risk becomes even more pronounced [15]. Moreover, in endurance sports, a rise in temperature is expected at the end of prolonged exercise due to the heat produced by muscular contraction [16,17]. Additionally, ultra-endurance sports induce an inflammatory response that correlates with the duration of physical exertion [18]. Therefore, the application of thermography is crucial for anticipating and preventing potential injury [19]. However, this has not yet been studied in the context of ultra-endurance events.

Therefore, to better understand the psychophysiological variables related to performance in these events carried out on several consecutive days, as well as the effect of these extreme races on the body, we conducted this research with the primary aim of assessing the physiological, biomechanical, and thermographic responses of male athletes participating in an ultra-endurance race of 4 days.

2. Materials and Methods

2.1. Participants

In this study, the small sample size of three male volunteer athletes can be justified by the difficulty in recruiting participants for such extreme events. The participants had an average age of 38 years, a height of 173.6 cm, a weight of 67 kg, and a body-mass index (BMI) of 22.2 kg/m². To recruit the participants, we contacted the organizers of the S4D event and arranged for the study to be conducted on the day of the event. Participants were given the opportunity to voluntarily participate in the study. The inclusion criteria required participants to be healthy individuals and volunteers willing to participate. The exclusion criteria included any participants who were taking medication, had any known pathologies or did not sign the informed consent form. The participants had over 20 years of experience in aerobic endurance training, with a background in competing in endurance and ultra-endurance events such as triathlons, trail running, mountain races, and cycling races. They typically trained five days per week, averaging 45–90 min per session, with an average weekly training volume of approximately 6 h, including strength training 1–2 times per week. Prior to commencing the research, the experimental procedures were thoroughly explained to the participants, who then provided their voluntary written informed consent in accordance with the Declaration of Helsinki. The methods employed in this study were developed and approved by the University Ethics Committee (CIPI/002/17).

2.2. Ultra-Endurance Event

The ultra-endurance event was held in Santander as part of the Santander Four Days (S4D) civic–military race (noncompetitive event). Participants were required to walk 40 km per day for four consecutive days, carrying a 10 kg backpack. The total distance covered during the event was 160 km.

2.3. Design and Procedure

The probe began at 7:00 a.m., with the participating athlete having his last meal before 9:00 pm the previous evening. In the hour preceding the start and immediately after the conclusion of the ultra-endurance event, we assessed the following parameters, drawing on previous research conducted using ultra-endurance probes [2,4,10].

Body mass was assessed using a SECA model 711 scale (SECA GmbH & Co. KG, Hamburg, Germany), which has a precision of 100 g and a measurement range of 0.1 to 130 kg. The scale was situated on a flat, smooth surface and calibrated to zero. Participants, barefoot and wearing minimal clothing, stood at the center of the platform. They avoided contact with any surrounding objects, ensuring their weight was evenly distributed on both feet while facing forward.

Cortical arousal was evaluated using the Critical Flicker Fusion Threshold (CFFT) within a viewing chamber (Lafayette Instrument Flicker Fusion Control Unit Model 12021), adhering to established procedures from previous studies. An increase in CFFT indicated enhanced cortical arousal and information-processing capabilities, whereas values below the baseline suggested diminished efficiency in information processing and central nervous system fatigue. CFFT is a measure used to evaluate cortical arousal and information-processing capabilities. Variations in CFFT values were interpreted as follows: an increase in CFFT indicates enhanced cortical arousal and improved information-processing capabilities, reflecting central nervous system (CNS) activation. Conversely, a decrease in CFFT suggests diminished efficiency in information processing, which can be associated with CNS fatigue [20,21].

Isometric handgrip strength was measured using a TKK 5402 dynamometer (Takei Scientific Instruments Co., Ltd., Niigata City, Japan). The measurement was taken on the athlete's dominant hand. The athlete was seated with the shoulder at 0° flexion, the elbow at 90° flexion, and the forearm in a neutral position. The highest value obtained from two trials was recorded.

Lower limb strength was assessed using a horizontal jump test. The athlete stood behind a marked line on the ground, with feet shoulder-width apart. Three attempts were made, and the highest result was taken for analysis.

Heart-Rate Variability (HRV) was monitored using a Polar V800 HRV monitor (Kempele, Finland). Measurements commenced a few minutes before the event started and concluded at the event's end.

Following previous protocols [2,4,10], the following parameters were analyzed immediately after the event:

Hydration status was evaluated using a colorimetry procedure with a urine color chart, which identified pH status and the presence of glucose, nitrites, protein, and glucose on the urine strip.

Blood glucose concentration was determined by analyzing 5 µL of capillary blood from the finger using a portable analyzer (One Touch Basic, LifeScan Inc., Madrid, Spain).

Blood lactate concentration was measured by collecting a 5 µL sample of capillary blood from the subject's finger and analyzing it with the Lactate Pro II system (Arkay, Inc., Kyoto, Japan).

Thermography response: Thermal images were captured using a second-generation FLIR One iOS Thermal Camera Smartphone Module (FLIR Systems, Wilsonville, OR, USA) through the Thermal Camera+ for the FLIR One app installed on an Apple iPhone 6. The emissivity setting in the app was adjusted to "matte: 95%" to closely match the human skin emissivity of 98% [22]. Before capturing each thermal image, the camera was recalibrated using the app's built-in function to reduce noise. The thermal images captured with the FLIR One have a resolution of 160 × 120 pixels in a "compressed image format" to reduce storage size, and the accompanying software automatically upscales them to 640 × 480 pixels. The corresponding visible spectrum images are taken at a resolution of 1440 × 1080 pixels. According to the FLIR One specifications, it can measure object temperatures ranging from −20 °C to 400 °C (−4 °F to 752 °F) with an accuracy of ±3 °C (5.4 °F)

or $\pm 5\%$. All thermal images were collected in compliance with the recommendations of the European Association of Thermology (Ring & Ammer, 2012). The thermograms were obtained in a room with a controlled and constant temperature of 20 °C and 40% humidity. Following protocols of previous studies, the thermograms were performed on the face, chest, abdomen, right and left arm, and right and left leg. The analysis of the skin surface temperature was conducted by locating the middle point of each anatomical region and through a circle or rectangle at the center of each region, as shown in Figure 1 [23,24].

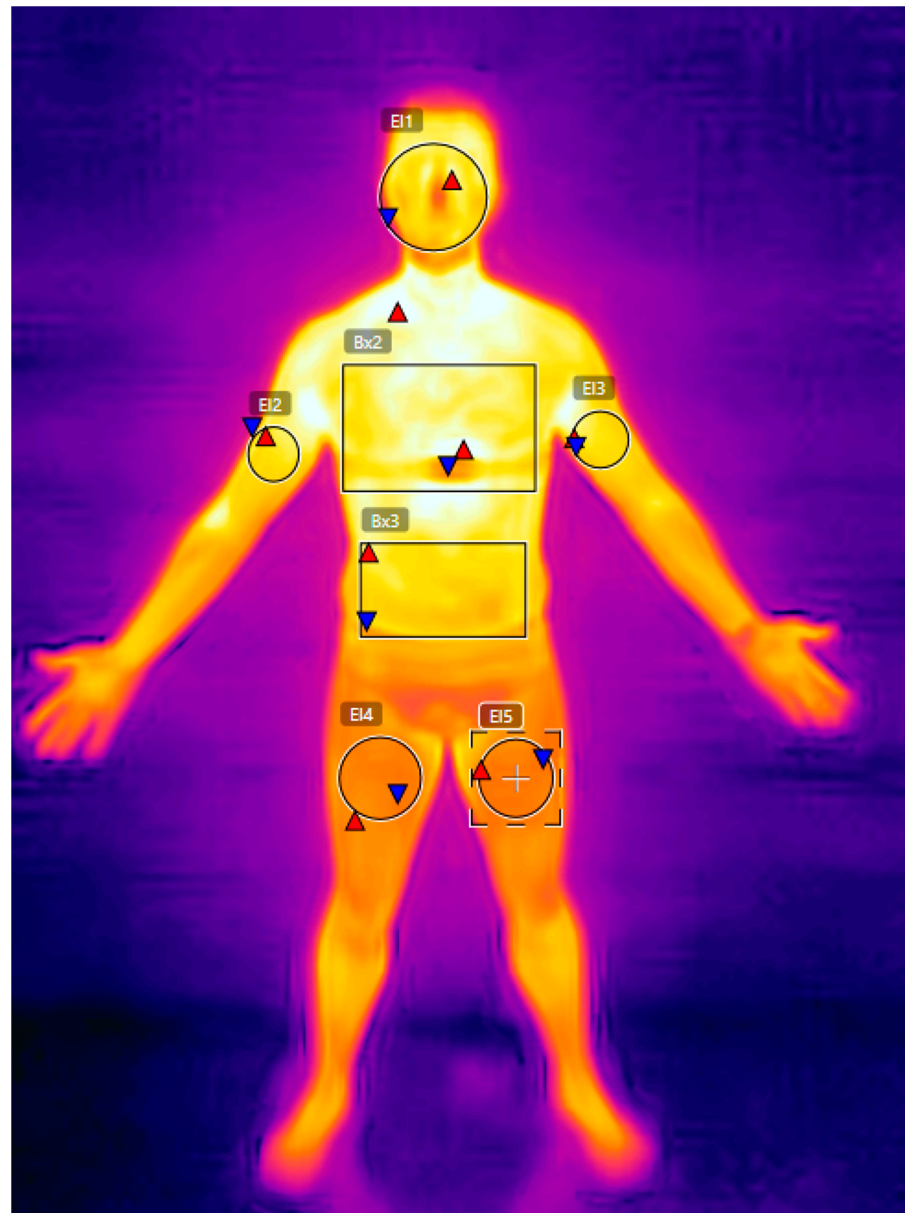


Figure 1. Thermographic analysis model.

2.4. Statistical Analyses

Statistical analyses were performed using SPSS version 21.0. The data were first checked for normality using the Shapiro–Wilk test. Descriptive statistics, including means and standard deviations, were calculated for all variables. A Wilcoxon signed-rank test was used to analyze differences in cortical arousal, handgrip, weight, horizontal jump, glucose, lactate, and urine values. In addition, Cohen’s *d* was calculated for heart-rate variability and thermographic values to determine the effect size of the differences observed. For all tests, a significance level of $p < 0.05$ was used.

3. Results

The heart-rate variability (HRV) metrics exhibited significant alterations during the ultra-endurance race. In the time-domain analysis, the mean heart rate escalated, with marked fluctuations over the four days. Notably, the RMSSD and pNN50 metrics substantially decreased from their pre-event values on Day 1. In the frequency domain, LF Power increased while HF Power showed a decline, resulting in a notable elevation in the LF/HF ratio, especially on Day 2. The nonlinear domain also displayed shifts, with SD1 and SD2 metrics notably decreasing from pre-event values. Overall, the data reflects the physiological challenges and adaptations experienced by participants during the event (Table 1).

Table 1. Heart-rate variability results before and during the event.

	Pre-Event	Day 1	Day 2	Day 3	Day 4	Cohen's d (Pre vs. Day 4)
Time-domain						
Mean HR (bpm)	70.0 ± 15.2	91.9 ± 15.3	95.8 ± 18.3	88.3 ± 10.7	95.1 ± 11.4	1.65
Min HR (bpm)	53.0 ± 14.3	62.9 ± 8.9	64.1 ± 8.5	73.6 ± 10.2	65.2 ± 12.6	0.85
Max HR (bpm)	80.4 ± 9.3	126.6 ± 20.3	145.8 ± 12.2	137.1 ± 18.3	147.6 ± 14.6	7.23
RMSSD (ms)	64.4 ± 20.3	16.5 ± 18.6	18.4 ± 14.3	15.3 ± 12.4	18.5 ± 15.2	−2.26
pNN50 (%)	28.3 ± 9.9	1.3 ± 3.3	1.7 ± 4.3	1.2 ± 5.2	1.3 ± 4.8	−2.73
Frequency domain						
LF Power (n.u)	70.8 ± 6.2	83.2 ± 7.8	92.2 ± 4.1	85.3 ± 6.3	89.1 ± 5.9	2.95
HF Power (n.u)	29.2 ± 6.7	16.7 ± 5.0	7.8 ± 3.2	14.7 ± 6.8	10.9 ± 4.6	−2.73
Ratio LF/HF	3.7 ± 0.9	5.0 ± 1.1	11.8 ± 2.0	5.8 ± 1.8	8.2 ± 2.3	5.00
Nonlinear						
SD1 (ms)	45.6 ± 9.6	11.7 ± 8.9	13.0 ± 11.3	10.8 ± 12.4	13.1 ± 10.8	−3.39
SD2 (ms)	95.2 ± 25.6	29.0 ± 10.5	42.6 ± 21.3	34.2 ± 18.3	34.4 ± 14.5	−2.38
ApEn	1.2 ± 0.2	1.4 ± 0.6	1.2 ± 0.5	1.2 ± 0.4	1.3 ± 0.2	0.50
SampEn	1.3 ± 0.4	1.5 ± 0.3	1.2 ± 0.3	1.3 ± 0.3	1.3 ± 0.2	0.00

HR: Heart Rate; bpm: beats per minute; Mean HR: Average Heart Rate; Min HR: Minimum Heart Rate; Max HR: Maximum Heart Rate; RMSSD: Root Mean Square of the Successive Differences; pNN50: Percentage of successive RR intervals that differ by more than 50 ms; LF: Low Frequency; HF: High Frequency; n.u: normalized units; Ratio LF/HF: Ratio of low-frequency power to high-frequency power; SD1: Standard deviation of instantaneous beat-to-beat variability; SD2: Standard deviation of long-term continuous RR interval variability; ApEn: Approximate Entropy; SampEn: Sample Entropy.

Figure 2 shows the results of (a) flicker fusion, (b) handgrip; (c) weight; and (d) horizontal jump. There were no significant differences in flicker fusion ($p = 0.121$), handgrip ($p = 0.956$), or horizontal jump ($p = 0.090$). However, a significant difference was found in weight ($p = 0.020$), lowering from the first 67.00 ± 6.44 kg to 64.77 ± 5.77 kg in the fourth stage.

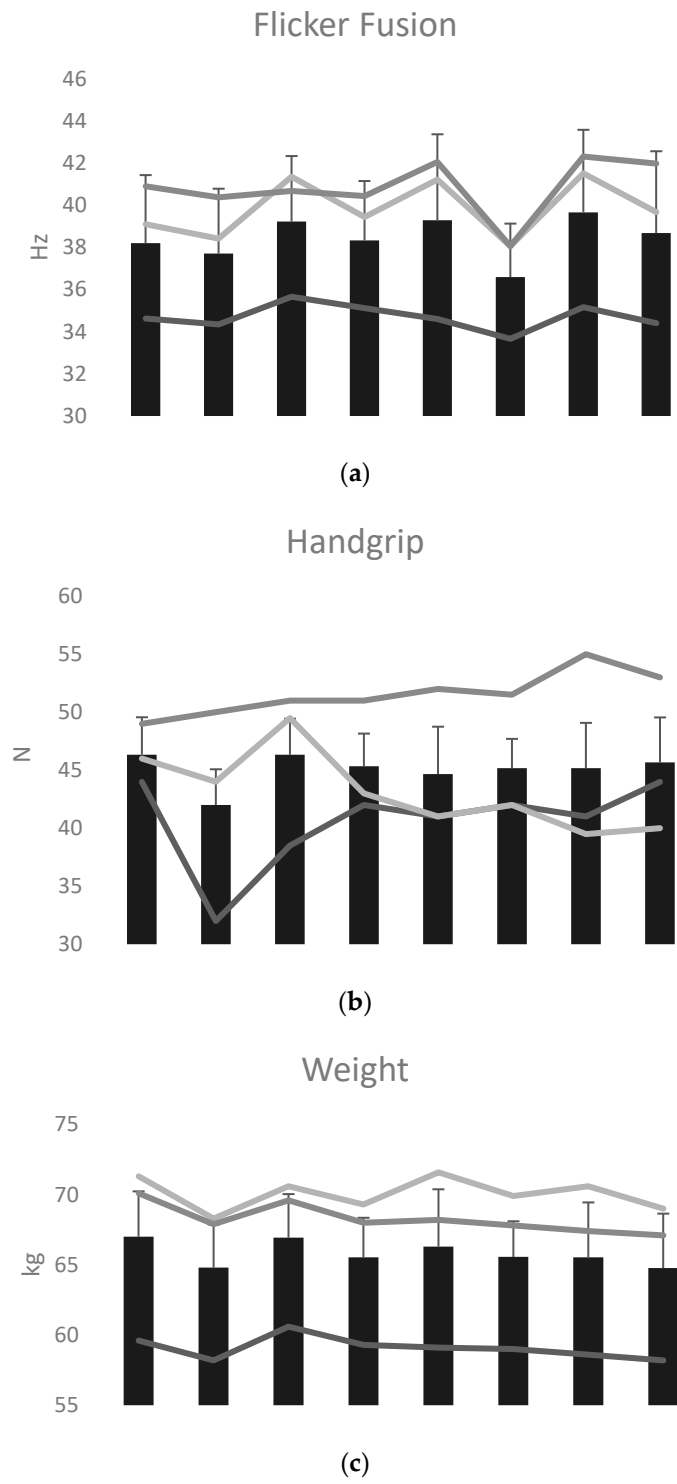
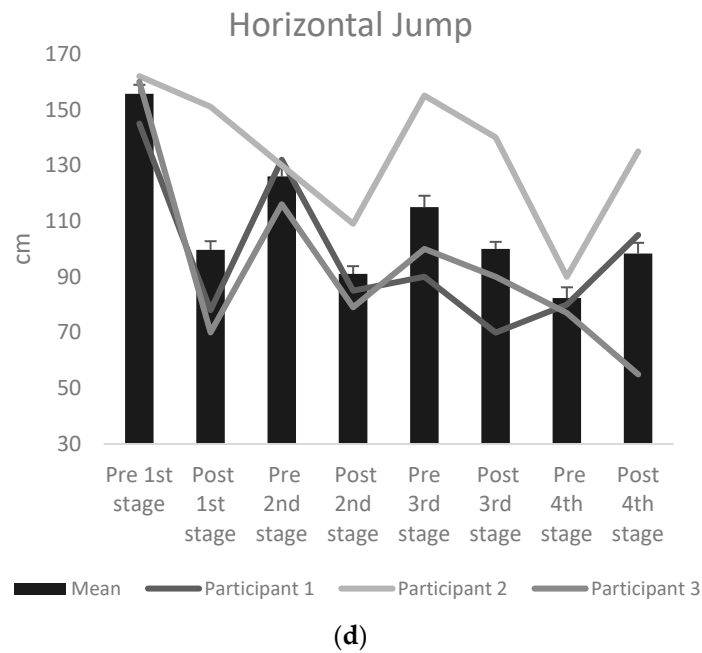


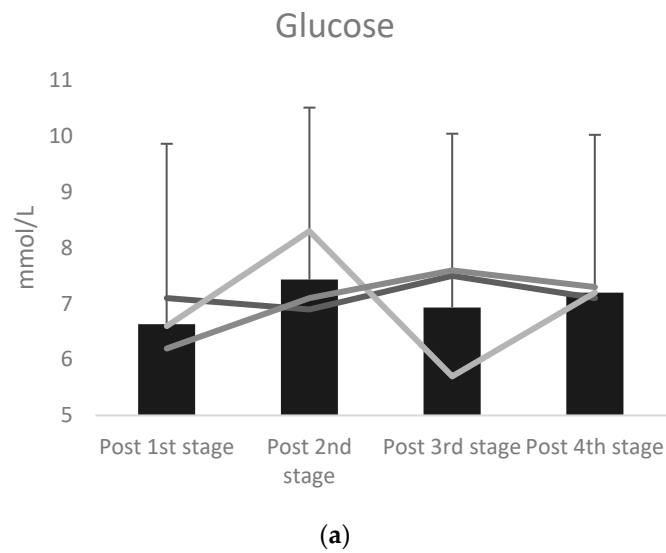
Figure 2. Cont.



(d)

Figure 2. Results for each stage showing (a) flicker fusion; (b) handgrip; (c) weight; and (d) horizontal jump. The black bars represent the average values across all participants, with error bars indicating the standard deviation. Individual results for each of the 3 participants are shown with lines.

On the other hand, no significant differences were found in glucose ($p = 0.670$) and lactate (mmol/L) (Figure 3).



(a)

Figure 3. Cont.

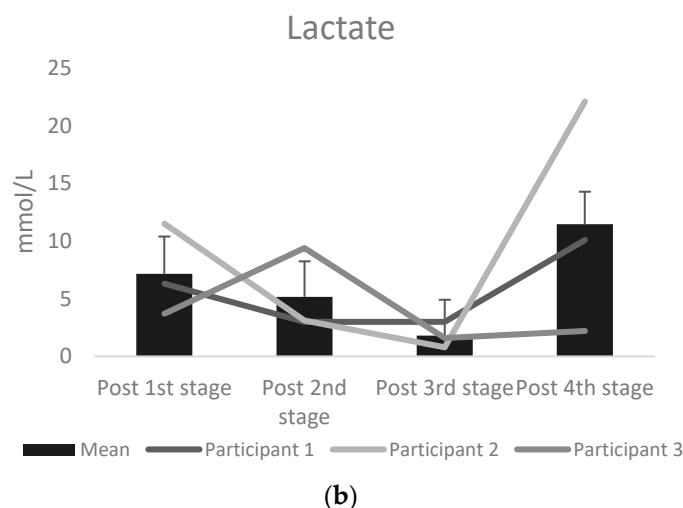


Figure 3. Results in each stage of (a) glucose; (b) Lactate. The black bars represent the average values across all participants, with error bars indicating the standard deviation. Individual results for each of the 3 participants are shown with lines.

Regarding urine parameters, there were no significant differences in pH ($p = 0.112$), colorimetry ($p = 0.142$), glucose ($p = 1.000$), or creatinine kinase ($p = 0.097$) (Table 2). However, a significant difference was found in the protein values in the urine ($p = 0.033$).

Post-event urine analysis over the four stages of the ultra-endurance race revealed changes in various parameters. The pH of the urine remained consistent between the first two stages and then increased to a constant value in the third and fourth stages. Urine colorimetry values, which provide a measure of hydration, demonstrated variability, indicating differences in hydration status over the stages. Interestingly, glucose and protein presence in urine showed stability in the initial stages, but a significant spike in proteinuria was observed post the third stage, which then decreased by the fourth stage. Creatinine kinase, a marker for muscle damage, was only available from the second stage onwards, showing a decreasing trend over the stages (Table 2).

Table 2. Urine values obtained during the 4-day event.

	Units	Post-1st Stage	Post-2nd Stage	Post-3rd Stage	Post-4th Stage	<i>p</i>
pH urine		5.33 ± 0.58	5.33 ± 0.58	6.00 ± 0.00	6.00 ± 0.00	0.112
Colorimetry urine		7.33 ± 0.58	4.67 ± 3.21	4.33 ± 3.1	5.00 ± 2.65	0.142
Glucose urine		0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.000
Creatinine Kinase	μmol/L	n/a	418.33 ± 112.77	300.67 ± 80.41	219.33 ± 63.01	0.097
Protein urine		0 ± 0	0 ± 0	343.34 ± 271.34	53.35 ± 40.40	0.033

Table 3 presents the average body temperature measurements in °C from various body regions of three participants before and after the event. We found an increase in temperature in the face, chest, abdomen, and right and left arm, and a decrease in temperature in the right and left leg.

Table 3. Thermographic result before and after the ultra-endurance event in °C.

Body Part	Pre	Post	% Difference	Cohen's d (Pre vs. Post)
Face	29.4 ± 0.8	29.1 ± 1.1	1.0	−0.38
Chest	30.9 ± 0.6	30.2 ± 1.0	2.4	−1.17
Abdomen	30.5 ± 0.9	30.3 ± 1.2	0.7	−0.22
Right Arm	30.3 ± 0.9	29.9 ± 0.9	1.4	−0.44
Left Arm	30.4 ± 0.9	29.5 ± 1.4	3.2	−1.00
Right Leg	26.3 ± 0.7	26.4 ± 2.4	−0.6	0.14
Left Leg	26.3 ± 0.8	28.3 ± 2.3	−7.9	2.50

4. Discussion

The research aimed to assess the physiological, biomechanical, and thermographic responses of male athletes during an ultra-endurance race. Findings revealed alterations in heart-rate variability metrics, suggesting increased cardiovascular demands and heightened sympathetic activity. While neuromuscular and perceptual metrics like flicker fusion, handgrip, and horizontal jump remained stable, there was a significant weight reduction, indicating potential dehydration and energy expenditure. Urinalysis highlighted consistent pH, colorimetry, and glucose levels but a notable increase in proteinuria after the third stage, hinting at renal stress. Thermographic data showed increased body temperatures in the face, chest, abdomen, and arms but decreased temperatures in the legs.

Ultra-endurance events provide a unique scenario for directly assessing the adaptability of the human body [2]. These extreme physical challenges not only test the limits of human performance but also offer valuable insights into how the body responds and adapts to prolonged, intense stress [1]. This aspect makes ultra-endurance events an invaluable tool for understanding the physiological, biomechanical, and psychological dimensions of human endurance and resilience [4,10].

In the study, heart-rate variability (HRV) analysis during the four stages of the ultra-endurance event revealed a decline in parasympathetic activity and a rise in sympathetic nervous system activation, aligning with similar findings in ultra-endurance running events [8,25]. Notably, autonomic modulation remained unchanged across the stages. This is a unique observation in multi-stage ultra-endurance walking events, as previous research primarily focused on cycling events, where HRV showed significant declines, indicating altered autonomic nervous system function [26]. The methodology of HRV assessment, varying stage lengths, and the distinction between walking and cycling activities could account for these differences. The study's results suggest that each stage imposed a consistent level of stress on the autonomic nervous system, regardless of accumulated fatigue. This could be attributed to the event's low intensity, which might have allowed sufficient recovery for the athletes to maintain a consistent performance level across stages [8,25]. In our multi-stage research, we understand that anticipatory anxiety before the competitive race, which affects parasympathetic activity, would have an exclusive effect on the pre-event measurement, similar to that reported in the literature [27]. Possibly, in our study, as it involves a noncompetitive multi-stage event, the rest of the pre-stage measurements were influenced by an adaptation process.

Specifically, the literature shows that after completing a 64 km ultramarathon event, parasympathetic-related indices require 2 days to return to initial values. However, subjective measures of fatigue and well-being indicate that athletes need 5 days after the event for full recovery [28].

Regarding cortical arousal, a decline was observed at the end of each stage, indicating that while the event induced stress and potential central nervous system fatigue [29,30], it might not have led to significant mental fatigue, which could positively favor the recovery process between stages [31–33].

The metabolic response of participants in the ultra-endurance event was marked by elevated blood lactate concentrations at the conclusion of each stage, suggesting a

higher dependency on glycolysis for ATP supply, consistent with findings in [34]. These elevated glucose levels highlight glycolysis as a primary energy pathway during the event, comparable to intensities observed in different exercises, such as half-marathon races and resistance training [35], and even surpassing levels noted in other ultra-endurance studies [25]. The added challenge of carrying a 10 kg backpack likely contributed to this increased glycolytic activity, as demonstrated by a notable rise in VO_2 and cardiorespiratory response compared to a no-load condition [36]. This aligns with previous research on soldiers performing graded races with equipment weight [37]. The study also observed significant weight loss from the first to the fourth day, underscoring the high energy demands of multi-stage ultra-endurance events, often resulting in an energy deficit [38].

In the study, hand strength remained stable between the first and fourth stages of the ultra-endurance event, likely because hand muscles are not heavily utilized in prolonged walking activities [39]. This stability is also influenced by the consistent sympathetic activity throughout the event [29]. In contrast, leg strength showed a noticeable decline from the first to the fourth day, reflecting the extensive use of lower body musculature in such events [39]. This decline was significant in the initial three days, aligning with the literature on fatigue and muscle damage in endurance sports [40]. Interestingly, a slight increase in leg strength was observed in the post-values of the fourth stage, possibly due to the participants' sense of accomplishment in completing the 160 km challenge, highlighting the interplay between performance and emotions in endurance activities [41]. This research, focused on low-intensity, long-duration exercises, is not related to peripheral fatigue. Peripheral fatigue is more commonly associated with high-intensity activities and the biochemical and metabolic changes that occur at the muscular level to maintain homeostasis [42].

The urinary marker analysis across the event stages revealed an uptrend in urine pH during the third and fourth stages, suggesting possible alkalinization, potentially due to dietary or hydration changes. The urine's colorimetry decreased initially but increased slightly in the fourth stage, possibly indicating fluctuations in urinary concentration or fluid intake. Notably, the consistent absence of glucose in the urine throughout all stages highlighted adherence to effective nutritional guidelines, in line with observations by Belinchón-deMiguel et al. (2019) [4]. Creatinine kinase (CK) levels, indicative of muscle damage, showed a decreasing trend from the second to the fourth stage, suggesting possible muscle recovery or adaptation. A significant increase in urinary proteins during the third stage could indicate renal impairment or muscle breakdown, aligning with Belli et al.'s (2018) findings that link urinary proteins to muscle degradation [43]. The variability of these markers across stages highlights the dynamic physiological response to endurance activities and the necessity for continuous health-monitoring in athletes. This transient spike in urinary proteins particularly underscores the need for careful evaluation of exercise and recovery protocols. [44].

In the observed body temperature data, the post-event decrease in facial temperature could be attributed to the face's high exposure to environmental elements during the race [45]. The reduced chest temperature might reflect heat dissipation due to physical exertion and the body's primary thermoregulatory response of sweating [46]. The abdomen, being a core body region, showed minimal temperature fluctuation, emphasizing the body's prioritization of maintaining core temperature for vital organ functioning [47]. The right arm's temperature decrease highlights the limbs' quicker cooling under exertion, while the right leg's temperature increase may result from its intensive muscular activity during the race [48]. The differential temperature changes in various body regions underscore the body's dynamic response to the physical demands and environmental conditions of the event, and the importance of monitoring for risks like hypothermia or overheating is reinforced [48]. Consequently, we observe that physical exertion and its inherent production of metabolic heat present a challenge for thermoregulation [49]. The ability to sweat, peripheral vasodilation, and the crucial relationship between the skin and the environment determine an individual's capacity to manage the delicate balance

between heat production and avoiding imbalances caused by heat accumulation during sustained physical exercise [50].

The practical implications of our findings suggest specific training and recovery strategies that can be implemented by athletes and coaches. These recommendations include targeted hydration protocols and muscle recovery techniques, which are essential for optimizing performance and ensuring athlete safety during ultra-endurance events.

5. Conclusions

A four-day ultra-endurance race produced changes in heart-rate variability, indicating increased cardiovascular strain, consistent neuromuscular performance, significant body-weight reduction possibly due to dehydration and energy use, and stable pH and glucose but increased protein in urine suggesting renal stress and varied body temperatures reflecting physical exertion and environmental factors. These findings highlight the body's adaptive mechanisms and the importance of specialized training and recovery strategies in such physically demanding events.

5.1. Limitations of the Study

Despite the comprehensive nature of the research, several limitations should be acknowledged. First, the study had only three male athletes, which severely restricts the generalizability of the findings and raises questions about the reliability of the conclusions. The study did not adjust for other influential factors such as age, fitness level, diet, and sleep, potentially introducing bias into the results. Additionally, this was a short-duration study that observed athletes only during and immediately after the event, providing no insight into long-term effects or recovery. The exclusive focus on male athletes further limits the applicability of the findings to a broader demographic, including female athletes. The specific conditions of the ultra-endurance event studied may not be representative of other endurance events, limiting the generalizability of the results. The small sample size ($n = 3$) in this study significantly compromises the generalizability of the results and the robustness of the conclusions. This limitation arises from the difficulty in recruiting participants for such extreme events. Future studies should aim to include a larger and more diverse sample to enhance the validity of the findings. The demands of conducting larger prospective cohort studies, with continuous monitoring and data collection, pose significant challenges in terms of time and resources. Another limitation of our study is the lack of baseline VO_2 max measurements due to logistical constraints arising from participants being from various locations, which could have provided a more comprehensive understanding of the athletes' cardiovascular fitness and its impact on performance. In line with this, the absence of dietary intake data before and during the event was another limitation. Future studies should include comprehensive dietary analysis to better understand the impact of nutrition on performance and recovery. Another limitation of our study is the lack of adjustment for potential confounding variables. Future studies should incorporate statistical methods to control these variables to strengthen the validity of the findings.

5.2. Future Research Lines

Future studies should aim to include a more diverse set of participants, including female athletes, to capture gender-specific responses during ultra-endurance races. The integration of additional physiological and biomechanical metrics, alongside psychological assessments, would provide a more holistic understanding of the demands and effects of such events. Monitoring muscle soreness and injury rates during and after the event could offer valuable insights into the physical toll and help develop better recovery strategies. Additionally, assessing the biomechanics of running or walking gait would provide insights into how the event affects movement efficiency and injury risk. Regular medical assessments to monitor for overtraining syndrome and other medical conditions should be included to identify potential health issues and ensure participant safety. Evaluating the athletes' mental health and stress levels before, during, and after the event would provide

crucial information on the psychological demands of ultra-endurance events. Including nursing care and interventions during the event to manage hydration and nutrition could improve athletes' performance and safety. Research should also investigate the impact of various interventions, such as different hydration and nutritional strategies, to optimize performance and recovery. Furthermore, long-term studies are needed to assess the prolonged effects and recovery processes of ultra-endurance athletes.

5.3. Practical Application

The findings of this study hold practical implications for coaches, athletes, and event organizers. Recognizing the physiological demands and the potential risks associated with ultra-endurance races can guide tailored training regimens and recovery protocols. The observed weight reduction and potential dehydration underline the importance of adequate hydration and nutrition strategies during such events. Furthermore, monitoring urinary markers and body temperature can act as crucial indicators of overall health and potential stress, enabling timely interventions.

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Conflicts of Interest: The authors declare no conflicts of interest.

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