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ORIGINAL ARTICLE
EXERCISE PHYSIOLOGY AND BIOMECHANICS

Cortisol and insulin behaviors during an ultramarathon event: are they real markers of extreme exertion?

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ABSTRACT

BACKGROUND: The current work aimed to describe and compare the cortisol and insulin concentrations behavior and rate of perceived exertion (RPE) during a 115 km ultramarathon race.
METHODS: Nine ultrarunners (eight males) were evaluated six times (0, 37, 60, 76, 89 and 115 km). At each moment, saliva samples (for cortisol and insulin assessment) and RPE (CR10 scale) were collected. Statistical analysis included correlation, one-way repeated measure ANOVA, and Statistical Parametric Mapping to define discrete and continues changes and compare cortisol, insulin and RPE profiles.
RESULTS: Our main findings revealed an early peak in cortisol and RPE, accompanied by a decline in insulin responses (402±49 min of the race, $P<0.05$). Cortisol and insulin only showed magnitude differences with inverse behaviors until ~6% (7 km) of the ultramarathon duration. Cortisol and RPE presented similar behaviors, rising from the beginning of the race and remaining elevated throughout the race ($\eta^2=0.91$ and $\eta^2=1.0$, $P<0.001$). Insulin levels decreased when the race started, remaining below 60% of baseline values from the midpoint to the end of the race ($P=0.04$).
CONCLUSIONS: The study showed an imbalance in the catabolic/anabolic hormone profile during an ultramarathon race, with a prominence in catabolic state. It should be considered in the ultramarathon races preparation and participation due to its possible detrimental effect on the athlete’s health.

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KEY WORDS: Marathon running; Hormones; Health; Athletic performance.

Ultra-endurance sports, such as ultramarathons (distances exceeding 42.192 km), have gained popularity.[1,2] The increasing number of participants and finishers in these events, which push the human body to its limits, has made ultramarathons increasingly attractive from a scientific perspective,[1] allowing investigation into human performance boundaries.[3,4] Given the wide range of ultramarathon distances, from 50 km to over 1600 km, across varied terrains and race formats, generalizing and comparing results remains challenging.

While the benefits of regular moderate exercise are well-documented,[5] the extreme nature of ultra-endurance raises questions regarding their impact on hormonal balance and overall health.[6, 7] Cortisol is the primary glucocorticoid in the body and is essential for the integrative adaptive response to stress.[8] It represents the physiological reaction to exercise demands,[9] acting as a potent anti-inflammatory and immunomodulator agent.[10] Its endogenous secretion follows a prominent circadian rhythm, peaking around the sleep-wake transition and reaching minimal levels in the evening and early night.[11] Despite this rhythm, cortisol significantly increases during endurance exercises to maintain an appropriate energy supply.[12, 13]

In contrast, insulin, the primary anabolic hormone, promotes the deposition of energy substrates from food into glycogen, playing a central role in muscle growth and protein turnover, promoting muscle recovery.[14] Interactions between insulin and exercise are essential for metabolic regulation and homeostasis. Post-exercise insulin levels rise to enhance glycogen replacement, decrease carbohydrate oxidation, and increase lipid oxidation.[15]

Psychologically, resilience and endurance are essential in these extreme sports to overcome pain and succeed.[16] Fatigue is commonly assessed through the subjective rate of perceived exertion (RPE), a validated indicator of exercise intensity and physical homeostasis disruption.[17] RPE also elucidates pacing strategies, as its progression rate during competition correlates with race duration and end-point confidence.[16, 17]

To ensure that the participants' health is not compromised in events that combine extreme stress, exertion, and high pain tolerance, monitoring and understanding hormonal responses and fatigue perception are essential.[18, 19] Therefore, the study aimed to determine the behavior of cortisol and insulin concentrations and the RPE during an ultramarathon since, as far as we know, no studies have investigated these factors under real competitive conditions. It was hypothesized that 1) cortisol and RPE would increase during an ultra-endurance race while insulin would decrease; and 2) cortisol would have a direct relationship with RPE and an inverse relationship with insulin.

Materials and methods

Nine ultrarunners athletes (eight males and one female) with the following characteristics (age 40.4±4.9 *vs.* 46 years; height 176.6±8.0 *vs.* 174 cm, weight 69.3±7.3 *vs.* 58.0 kg, % body fat 13.3±3.1 *vs.* 15.6%, maximal oxygen uptake 57.5±4.6 *vs.* 63.3 $ml.kg^{-1}.min^{-1}$, for male and female respectively) entered the Madeira Island Ultra-Trail (MIUT) and voluntary participated in this study. Inclusion criteria were having previous experience in this type of events (at least two other participations) and a maximal oxygen uptake above 50.0 $ml.kg^{-1}.min^{-1}$ (previously determined by an incremental protocol until exhaustion).

The MIUT is a continuous ultra-trail running competition, spanning a 114.8 km course over rough terrain at medium altitude (altitude range between 5 and 1740.5 m), with a total positive/negative elevation of + 7060 /- 7070 m. In the MIUT event analysed in the current work, of the 785 starters, 590 (~75%) completed the event. Participants provided written informed consent after being briefed on all experimental procedures and possible risks associated with the experiments before the commencement of the study. Prior to the study, all subjects underwent a medical questionnaire to ensure the absence of any exogenous anabolic-androgenic steroids, drugs, or other supplements that could affect their physical performance or hormonal balance during the study. Ethical approval was obtained by the local Ethics Committee (CEFADE 20_2023), and all procedures complied with the Declaration of Helsinki Ethics Code and the Standards for Ethics in Sports and Exercise Science Research.

Procedures

Athletes were evaluated six times: pre-race (0 km) and at five designated stops (moments 37, 60, 76, 89, and 115 Km, corresponding to 32, 52, 66, 77, and 100% of the race). At each moment, saliva samples were collected before food ingestion for subsequent analysis of cortisol and insulin, and RPE was assessed using the CR10 scale.[20] Saliva samples were collected without stimulation by putting a cotton tube into the mouth until saturated, stored in the plastic tubes and then frozen at -70 °C. Before analysis, saliva samples were clarified by centrifuging at 12 000 g for 5 min at 4 °C. Cortisol and insulin levels were measured using commercially available ELISA kits (Salimetrics, Newmarket Suffolk, UK). Figure 1 presents a schematic diagram of the data collection done during the MIUT.

Race results were obtained from official reports, and running velocity was assessed dividing the distance covered by the time of each section, minus the stop durations. MIUT started at 00:00 am and the beginning running velocity was assessed at the first checkpoint (13.9 km). To allow the comparisons with previous studies in different ultramarathons, we also calculated the equivalent flat distances: positive elevation (m) / 100 + distance (km) and velocities: equivalent flat distances (km) / race time (h), as proposed by Saugy.[4]

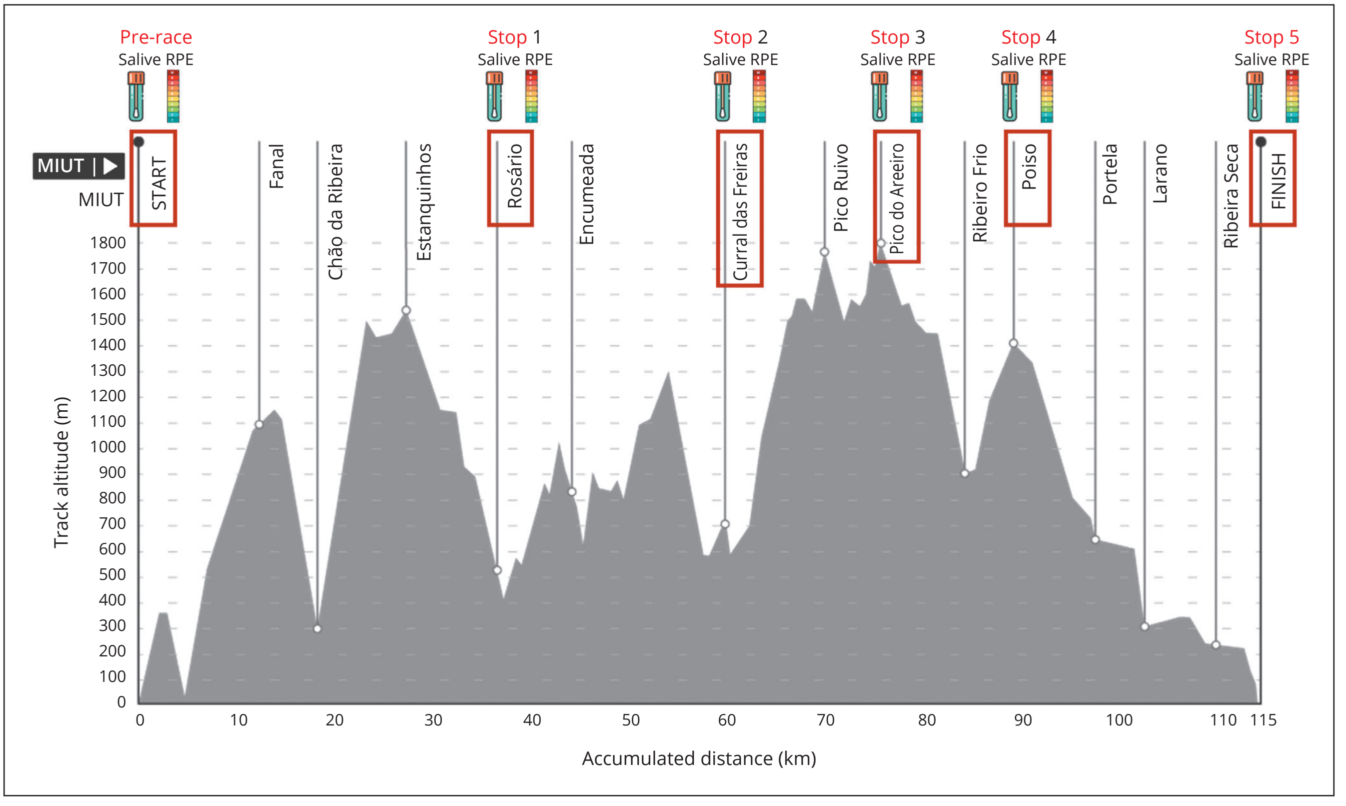

Figure 1.—Schematic representation of the testing timeline during MIUT.
RPE: rate of perceived effort.

Statistical analysis

Statistical analysis was performed with the software SPSS 29.0 (IBM Statistics, USA) for Windows. The normality and homogeneity of distribution were checked using the Shapiro–Wilk test for all the assessed variables, and mean values±SD were calculated. One-way repeated measures ANOVA with pairwise comparisons with Bonferroni correction was used to determine cortisol, insulin, RPE, and running velocity behaviour across the MIUT event, using partial eta-squared (η^2) for effect size calculation: 0.05-0.25 (small), 0.25-0.64 (moderate) and >0.64 (strong).[21] Also, a paired samples Student's *t*-test with effect size estimation[22] was used to assess the differences between the beginning and the end of the MIUT event.

Pearson correlation coefficients were calculated between all the assessed variables at each moment of the collection. The threshold used to determine the correlation values qualitatively was based on the following criteria:[23] <0.1 (trivial), 0.1-0.3 (small), 0.3-0.5 (moderate), 0.5-0.7 (large), 0.7-0.9 (very large) and >0.9 (nearly perfect). Magnitude-based inference and precision of estimation were calculated to assess practical significance with 95% confidence intervals (CI[23]), and correlations are presented as r±95%CI. The Statistical Parametric Mapping (SPM) with one-way repeated measure ANOVA and Bonferroni correction for pairwise comparisons was used to define the continues changes and compare cortisol, insulin, RPE, and running velocity profiles in MATLAB (MATLAB R2019b, The MathWorks Inc., Natick, MA, USA) using the open-source code SPM1d (v.M0.1, www.spm1d.org).[24] All variables were interpolated, transforming the six moments into 100 data points using the spline transformation. Afterward, the data series were normalized based on their maximal value (100%), and the duration of MIUT was transformed to 0-100% of the event. For all statistical procedures, the significance level was set at 5%.

Results

Twelve ultramarathon runners were recruited, however only nine completed the total 115 km (75%, aligning with overall finishers), where three withdrew at Km 70 (two males and one female) due to extreme exertion (self-de-

TABLE I.—*Mean±SD of the time of exercise, stop durations, running velocity, rate of perceived exertion, cortisol, and insulin concentration before and during MIUT.*

Variables (N.=9)	Pre-race/ 14 km	Moment 1 37 km	Moment 2 60 km	Moment 3 76 km	Moment 4 89 km	Moment 5 115 km
Race (% of final distance)	12	32	52	66	77	100
Total time (minutes)	138±14	402±49	666±89	957±131	1152±162	1419±219
Total time (% of final time)	10±1	28±1	47±2	68±2	81±1	100
Stop duration (min)	-	4.3±3.1	12.1±8.5	6.3±5.7	6.1±3.3	-
Running velocity (km/h)	6.1±0.6	5.6±0.6	5.3±0.7	3.8±0.7*	4.5±0.8	5.9±1.1*
Flat running velocity (km/h)	12.8±1.2	10.4±1.2*	8.5±1.2*	7.6±1.3	6.7±1.2	6.2±1.2
Flat running distance (km)	29.4	68.7	105	139.2	158.6	185.4
Total elevation gained (m)	1546	3127	4540	6331	6956	7060
Rate of Perceived exertion (CR10 Borg scale)	0±1	5±1*	6±1	7±1	7±1	7±2
Cortisol (µg/dL)	0.13±0.07	0.88±0.71*	1.39±0.67	1.46±0.77	1.30±0.80	1.15±0.67#
Insulin (µIU/mL)	2.14±1.50	0.62±0.58*	1.52±0.78*	1.11±0.92	0.94±0.79	1.13±0.98#

*Significant differences compared to the previous moment, P<0.05; #significant differences compared to the pre-race moment, P<0.05.

TABLE II.—*Significant partial Pearson correlations between the pairs: running velocity – rate of perceived exertion, cortisol – rate of perceived exertion and insulin – rate of perceived exertion at different race moments.*

Partial Pearson correlations (sex controlled)	r (95% CI)	P
Running velocity - rate of perceived exertion 37 Km	-0.81 (-1.00 to 0.49)	0.016
Total time (minutes)- rate of perceived exertion 37 Km	0.86 (-0.13 to 1.00)	0.006
Cortisol - rate of perceived exertion 60 km	0.94 (0.79 to 0.99)	<0.001
Insulin - rate of perceived exertion 115 km	-0.74 (-0.96 to -0.32)	0.035

termined exhaustion). Finisher's performance data, RPE, and cortisol and insulin levels at each collection point are presented in Table I. Running velocity changed along the event ($F_{5.45}$=68.01, η^2=1.0, P<0.001) but was similar between the initial 14 km and the final 25 km of the race (P=1.00). However, when converted to flat running velocity, it decreased from the start to the end of the race ($F_{5.45}$=211.60, η^2=1.0, P<0.001). Increases in cortisol ($F_{5.45}$=8.10, η^2=0.91, P<0.001) and RPE ($F_{5.45}$=210.64, η^2=1.0, P<0.001), along with a decrease in insulin (t=2.44, ES (d)=0.82, p=0.04), were observed from the race's beginning to the end. Only four correlations were found: two very large positive correlations between total time (minutes) and RPE at 37 km and between cortisol and RPE at the 60 km stop, and two very large negative correlations between running velocity and RPE at 37 km and between insulin and RPE at the 115 km (Table II).

Figure 2 shows the mean behaviors (based on SPM1d) of running velocity, cortisol, insulin, and RPE along the MIUT event. Running velocity slightly decreased until 40% of the race (46 km), then reduced at a higher pace, reaching its minimum value at around 60% (69 km), followed by an increase to a new peak at the race's conclusion. Cortisol presented a crescent behavior until 50% of the race (58 km), stabilized from 50 to 60% (58-69 km), slightly dropped until 80% (92 km), and remained stable again until the end, with maximal values presented between 40 and 60% of the race. Insulin started decreasing up to 20% of the race (23km), then increased and peaked at 40% (46 km) before declining again until 80% (92 km), remaining 60% (69 km) below initial levels until the end. RPE also presented a crescent behavior until approximately 65% (75km) of the race, with peak values between 60-65% (69-75 km), stabilizing thereafter.

Figure 3 shows the ANOVA test based on SPM1d of running velocity, cortisol, insulin, and RPE magnitudes along the MIUT event. The differences were found in the first 38, at 50, and beyond 70% of the race duration (0 to 44, 58 and after 80 km, respectively). Figure 4 shows the pairwise comparisons of the magnitudes and behaviors of cortisol and insulin and both with RPE along the race. From 0 to 6.24%/0 to 7.5 km, cortisol and insulin presented magnitude differences with inverse behavior (cortisol rising and insulin decreasing). After that first moment, cortisol and insulin showed similar concentrations magnitudes. Insulin and RPE also presented magnitude differences with an inverse behavior in beginning and near the race's end (0 to 6.43% and 96.51 to 100%/0 to 7.5 and 111 to 115 km), while cortisol and RPE presented similar magnitudes and behaviors throughout the event (from 0 to 100%/0 to 115 km). Figure 5 shows the pairwise comparisons of the magnitudes and behaviors of cortisol, insulin,

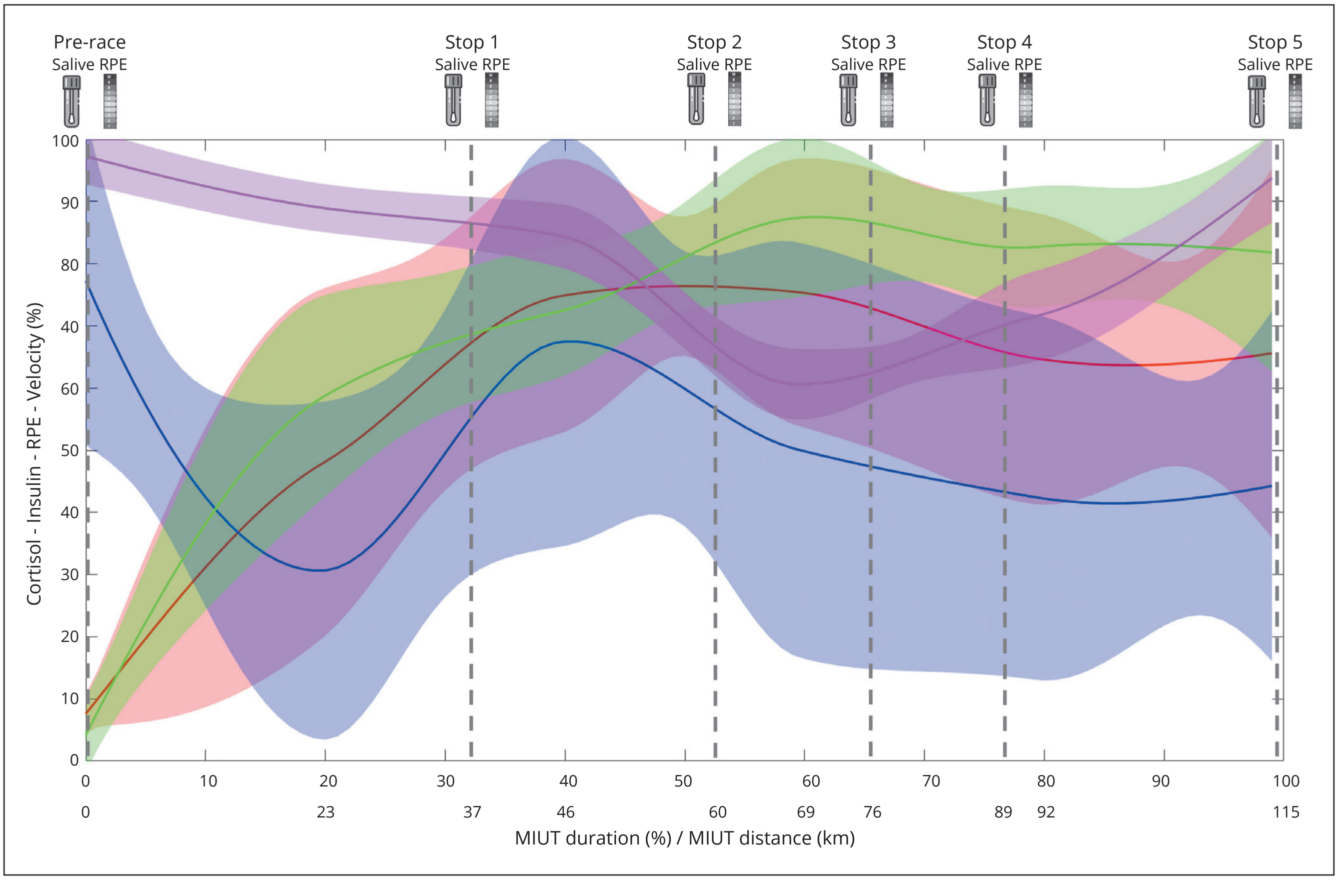

Figure 2.—Mean and standard deviation curves of the behaviors of running velocity (magenta line) and cortisol (red line), insulin (blue line) concentrations and RPE (green line) along the MIUT event (colors in the online version).

and RPE with running velocity along the MIUT event. The running velocity presented a higher magnitude with an inverse behavior compared to cortisol at the beginning of the race (0% to 17.5%/0 to 20 km), decreasing velocity and increasing cortisol. A higher magnitude was also observed compared to insulin (2.8 to 30.3 and 87.3 to 99%/3 to 35 and 100 to 114 km) and to RPE (0 to 31.7 and 53.0 to 73.6% / 0 to 36.5 and 61 to 85 km).

Discussion

Despite the increasing scientific interest and participation in ultramarathons,[1] data on physiological responses and demands during real competitive contexts remain scarce and almost non-existent,[2] despite the known importance of describing them during these extreme duration exercises (close to 24h) as a disease prevention strategy.[7, 25] The current work aimed to describe and compare hormonal behaviors (cortisol and insulin) and the RPE during a 115 km ultramarathon race. Our findings indicated an early peak in catabolic responses and perceived exertion, coupled with a drop in anabolic response. The primary hypothesis was confirmed, as increases in cortisol and RPE and a decrease in insulin were observed from the beginning to the end of the event. The second hypothesis was partially confirmed, with cortisol and RPE displaying similar magnitudes and behaviors throughout the event, while cortisol and insulin exhibited inversely related behaviors until approximately 6% of the race duration.

The cortisol and insulin behavior analysis during the complete event was possible due to statistical parametric mapping method, interpreting the data as a continuous event rather than looking for each moment of stop isolated. Salivary hormonal assessment was chosen because it has already been deemed valid in comparing salivary and serum levels of hormones such as cortisol and insulin both

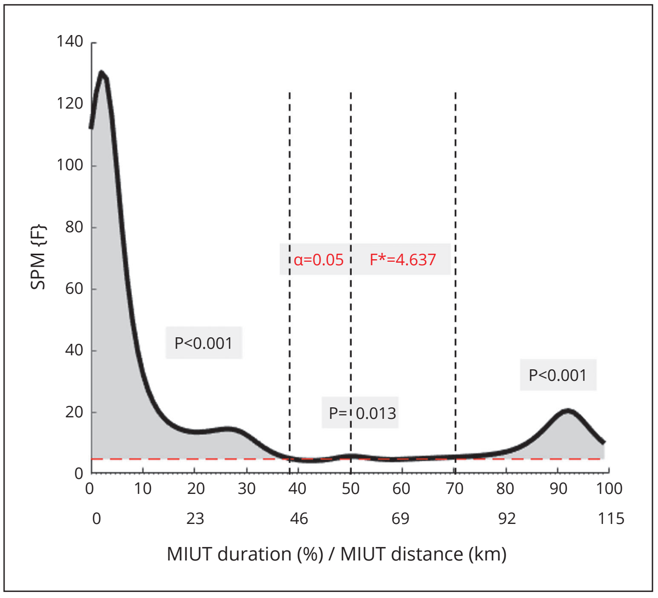

Figure 3.—ANOVA Test based on SPM1d of running velocity, cortisol, insulin and rating of perceived exertion behaviors along the MIUT event. Dashed lines represent the cut for the significant differences.

in basal and under exercise conditions, presenting high correlations, being a reliable and non-invasive method to determine the physiological response to exercise.[26-28]

Ultra-endurance races impose an immense caloric requirement/deficit, necessitating a sufficient provision of metabolic fuels.[29] Given the limited carbohydrate stores in the body, prolonged muscular activity amplifies the demand for carbohydrates. Cortisol, in its broad spectrum of tasks for metabolic processes control,[13] increases significantly during prolonged endurance exercise to elevate blood glucose levels and facilitate fat and protein processing, providing energy to the muscles.[10, 19, 30] Elevated cortisol levels are also expected in conditions of severe caloric deficit, typical of these events.[31] Previous research also indicates that in very long exercises below the anaerobic threshold (around 60% of maximal oxygen uptake), cortisol activation is driven by exercise duration rather than intensity,[12] as in this type of race.

This phenomenon aligns with our results, where cortisol levels rose early, peaked mid-race, and remained elevated throughout the race. Furthermore, as the event started at

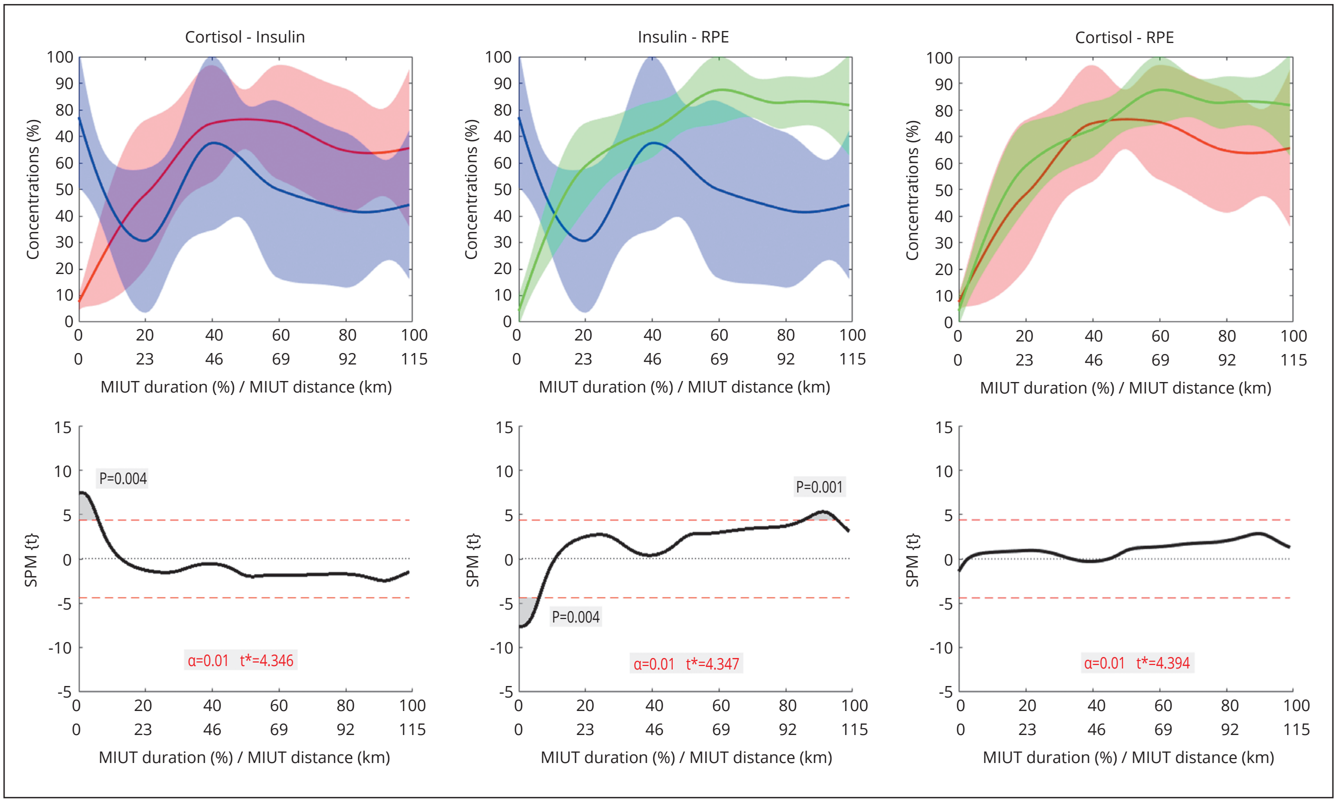

Figure 4.—Comparisons of the behaviors of cortisol (red) and insulin (blue) concentrations (top left) and both with RPE (green: top middle and right panels) along the MIUT event (colors in the online version).

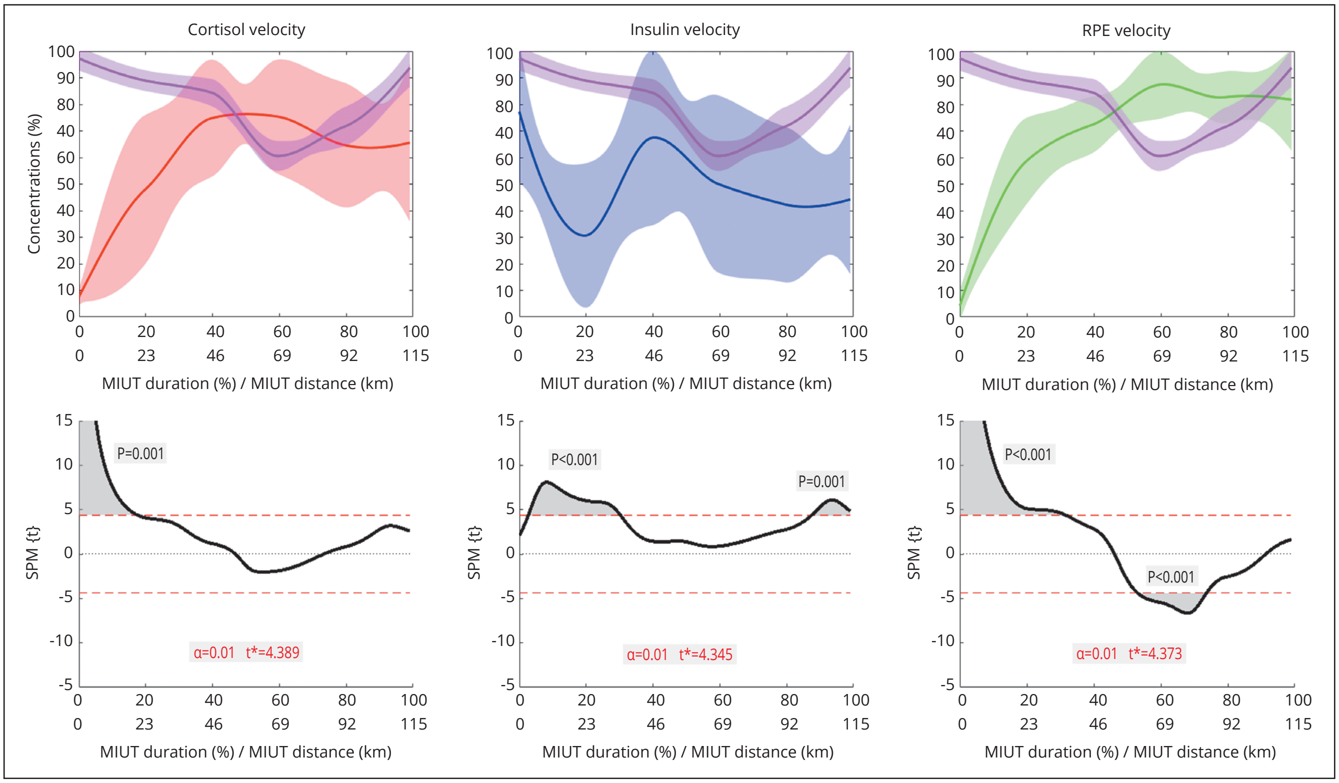

Figure 5.—Comparisons of the behaviors of running velocity (magenta) and cortisol (red), insulin (blue) concentrations (top left and middle panels) and RPE (green: top right panel) along the MIUT event (colors in the online version).

midnight, with the participants' cortisol at the minimum expected levels, the behavior found is justified in response to the sustained stress imposed by the effort, since, based in the circadian rhythm cortisol levels should be kept very low up to around 6-8 am.[11] These findings are corroborated by studies reporting increased cortisol levels following 160 km foot races,[30, 32] and an early surge during a 217 Km mountain ultramarathon, with the highest values observed after 16 hours (similar to our results; see Table I).[19] Other studies showed that cortisol levels increased at the beginning of the race but also remained augmented until the finish line.[6, 19, 29]

Moreover, ultramarathons have been associated with increased muscle damage and inflammation biomarkers (elevated creatine kinase, lactate dehydrogenase, aspartate aminotransferase, and C-reactive protein levels),[19] depletion of the antioxidative capacity and heightened inflammation (elevated neopterin levels),[29] and suppression of anabolic testosterone levels,[25, 32] thus enhancing the catabolic process. In addition, the prolonged hyperproduction of cortisol can also compromise anabolic stimulation in muscles by inhibiting insulin and IGF-1 production and decreasing signaling mediated by amino acids involved in muscle protein synthesis regulation. This anti-anabolic effect is accompanied by the inhibition of the mammalian target of rapamycin (mTOR), thereby compromising protein regenerative synthesis[33] and enhancing the anti-anabolic process.

Although insulin is primarily associated with diabetes research, recent studies have highlighted its relevant role as a biomarker for training, monitoring, and assessing catabolic/anabolic balance. The interaction between insulin and exercise exemplifies the need to modify and adjust opposing metabolic forces under varying conditions.[34] Its decrease during exercise is critical for mobilizing glucose and fatty acids from the liver and adipose tissue.[34, 35] Therefore, in prolonged muscular activities like ultramarathons, with the depletion of glycogen reserves, the demand for carbohydrates from fat and protein processing is amplified, suppressing insulin's effects on fuel storage.[34]

Conversely, after exercise, there is an inverse need to replenish the fuel reserves mobilized during exercise, particularly glycogen in the muscles, reducing carbohydrate oxidation and increasing lipid oxidation.[15] Increased insulin sensitivity in the muscles involved in physical activity facilitates this process. This training-optimized sensitivity is due to adaptations of the vasculature, skeletal muscle, and adipose tissue, justifying lower basal insulin levels in well-trained athletes.[34, 36] Decreased insulin levels have also been reported during prolonged exercise due to increased catecholamines, which inhibit its secretion, thus maintaining glucose homeostasis and ensuring energy availability to the central nervous system,[14] and in energy-deficient athletes.[31]

In our study, insulin decreased up to 20% (23 km) of the race, following cortisol rise. However, it then increased and peaked at 40% (46 km), likely due to the first stop (see Table I), which could be perceived as the end of the exercise bout and a new beginning. Another possible explanation can be the unavoidable food intake during this stage to prevent the energy deficit typical of such races.[29] Even so, this behavior did not repeat in subsequent stops, possibly due to the anti-anabolic cortisol effect or the severe caloric deficit mentioned earlier. These factors may justify the observed insulin decrease after 40% (46 km) of the race, culminating in significant lower levels than the initial (below 60%). It is essential to consider that saliva collections in the programmed stops were performed before any food intake, ensuring that spikes in blood glucose did not influence the analysis.

RPE analysis, a subjective measure of exertion, plays a significant role in understanding athletes' perception of external load during competition. It was reported that the most motivated runners perform more distance in a 24-hour marathon,[16] associated with an abnormally high pain tolerance, which could partially explain ultrarunners' capacity to endure prolonged exertion.[18] Our findings suggest that RPE behavior was influenced by the race's more challenging altitude sections, stabilizing after 65% (75 km) of the event as elevation gains diminished.

Evidence suggests a link between elevations in both cortisol and RPE during exercise.[37] Cortisol, released in response to stress, tends to increase during and immediately after high-intensity exercise.[37, 38] At the same time, RPE follows a similar pattern.[17] One possible explanation for this relationship is that cortisol may contribute to feelings of fatigue and exertion during exercise[38] and also may interact with other stress-response hormones like adrenaline and noradrenaline, amplifying perceived exertion.[9] These latter hormones are involved in the body's stress response, increasing heart rate, blood pressure, and respiration. While the relationship between cortisol levels and RPE during exercise is not fully understood, our results showed a link between the two. Further research is needed to explore the mechanisms underlying this relationship and its implications for exercise performance.

Complementary, along the event, changes in running velocities (both real and flat conversion) were consistent with previous studies on ultramarathons[19, 39] exhibiting similar behaviors among all evaluated participants. The race's demanding phases made it impossible to maintain constant velocities (e.g., altitude changes and rough terrains), explaining the drop from the midsection compared to the beginning and the end. The flat conversion analysis confirmed these results, with the last race section (with a lower altitude gained compared to the rest of the race, +104 m) showing a lower flat running velocity than the midsection. The combined real and flat conversion analysis of distance and velocities showed the impact of terrain difficulty and race intensity, justifying the stabilization of the RPE (one runner even reported a decreased RPE in the last section of the race).

Additionally, studies have characterized ultra-endurance exercises by intensities below the anaerobic threshold, representing 64 to 82% of maximal heart rate (HR_{max}) for efforts between 7 and 14h,[7, 40] or even at lower HR for events of longer duration.[41] Although HR data was not collected, it can be assumed that the average HR in our subjects would be similar to previously reported values, given their maximal oxygen uptake was comparable to other mountain ultramarathon finishers (higher than 50 $ml.kg^{-1}.min^{-1}$) and the event duration was within the mentioned range.[7, 40]

Limitations of the study

The absence of evaluation of this parameter can be assumed as a limitation of this study.

Another limitation was not considering food intake during the event, which may have influenced the interpretation of insulin levels. Furthermore, the varying race durations for different runners (19 to 30 hours) have different impacts on their circadian hormonal concentrations like cortisol.[11] However, evaluations made during a real event help better understand the effects of such extreme efforts on hormonal concentrations and balance, and when interpretated based on their RPE could help to regulate the stopping, food intake and intensity strategies in future events. Future research should consider food intake and break times during ultramarathons and try to find the best relationship between them and the intensity performed. Also, the recovery process of hormonal alterations after race should be analyzed.

Conclusions

The present study found a relationship between cortisol, insulin and RPE responses to an ultramarathon lasting an average of 24 hours, with the increase and maintenance of high cortisol and RPE perceived by the athletes and the decrease and maintenance of low insulin levels during the race. The imbalance observed in the catabolic/anabolic hormonal profile during the ultramarathon and its possible detrimental effect on the athlete's health should be considered in the race preparation and participation.

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Conflicts of interest
The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Authors' contributions
Jaime Milheiro, Paulo Pires, Ana Sousa, André Moreira and José Soares conceived and designed the experiments; Jaime Milheiro and Paulo Pires conducted data collection; Jaime Milheiro, Diogo D. Carvalho and Márcio F. Goethel conducted data analysis; Jaime Milheiro, Diogo D. Carvalho and Márcio F. Goethel interpreted the data; Jaime Milheiro, Diogo D. Carvalho and Márcio F. Goethel drafted the manuscript; Jaime Milheiro, Diogo D. Carvalho, Paulo Pires, Márcio F. Goethel, Ana Sousa, André Moreira and José Soares revised the manuscript critically. All authors read and approved the final version of the manuscript.

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