Published online: 7-28-2023

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Abstract

Purpose: This study investigated changes in salivary α-amylase (sAA), cortisol, and interleukin-1β (IL-1β) concentrations following completion of an ultra-marathon trail run (UMT) to better understand the physiological stressors imposed by this extreme type of race. **Methods:** Eight subjects participated in this study. Each subject completed a 50 km UMT. Two-minute oral salivary swabs were taken 10 minutes prior to race start and again within 1 minute of race finish. Samples were analyzed for sAA, cortisol, and IL-1β using ELISA kits. **Results:** Our results demonstrated a significant increase in both sAA ($p = 0.002$) and cortisol ($p < 0.001$) from baseline. No significant differences were observed for IL-1β. There was no significant relationship between the observed sAA increase and race speed. The observed increase in cortisol was significantly correlated with race speed ($R^2 = 0.582$, $p = 0.028$). **Conclusion:** Participation in UMTs is associated with activation of the sympathoadrenal and hypothalamic–pituitary–adrenal axes, but not an increase in IL-1β. Better understanding of physiological stress associated with ultra-distance events could lead to improvements in training and performance for individuals engaging in long-distance aerobic events.

Keywords: α-amylase, cortisol, interleukin-1β, endurance exercise

Introduction

Extremely prolonged aerobic exercise elicits increased oxidative (Nieman et al., 2004) and metabolic stress, as well as an immune response (Stelzer et al., 2015). One example of this type of exercise is ultra-marathon trail races (UMTs), which are increasing in popularity. UMTs are competitions that are over 43 km in length and are mainly off road (Suter et al., 2020). These events typically elicit drastic physiological adaptations and result in altered levels of stress biomarkers (Deneen & Jones, 2017; Kupchak et al., 2014). UMTs are also generally characterized by a high percentage of eccentric work, which is exacerbated by the constant elevation shifts along the trail (Farber et al., 1991; Nicol et al., 2006). Additionally, ultra-endurance races require significant energy demands (Kimber et al., 2002) and impose thermal stress which can lead to dehydration of the racer (Sharwood et al., 2004). However, a better understanding of what is occurring in the neuroendocrine system during these events could help athletes perform better and recover faster.

Physiological stress occurs during all types of exercise and intensities; however, stress is amplified by the extreme duration of ultra-distance competitions (Hackney et al., 2016). Of specific interest during these prolonged bouts of exercise is the balance between the hypothalamic–pituitary–adrenal (HPA) axis and the sympathoadrenal medullary (SAM) system (Armstrong & VanHeest, 2002). These two neuroendocrine pathways combine to control the release of cortisol and catecholamines such as epinephrine (EPI) and norepinephrine (NE) (Negrão et al., 2000). Despite lacking a complete understanding of the mechanistic interaction between the HPA and SAM systems, it appears that the stress of ultra-endurance competition affects both systems (Deneen & Jones, 2017). The stress-related component of physical activity is linked with the HPA axis, as measured by individual differences in secretion of salivary cortisol (Gonzalez-Bono et al., 1999). Changes in salivary cortisol are typically observed within 15 minutes from the onset of exercise and peak within 20–30 minutes following exercise (Budde et al., 2015). On the other hand, a more rapid stress response (i.e., within minutes) results from the sympathetic nervous system (SNS) portion of the autonomic nervous system (ANS) (Takai et al., 2004). Catecholamines are released directly into the bloodstream (Chrousos & Gold, 1992), causing the downstream release of α-amylase in saliva (sAA), which acts as an indirect marker of SNS activation (Nater & Rohleder, 2009). Overall, both cortisol and α-amylase tend to increase following prolonged endurance exercise (Silva et al., 2019).

Prolonged exercise, such as UMTs, also induces an inflammatory response (Kasprowicz et al., 2013). Interleukin-1β (IL-1β), a proinflammatory macrophage-derived cytokine, has been shown to be involved in both the regulation and integration of immune system and endocrine function (Fan et al., 1996). During periods of high physical stress, IL-1β is released and is found to be elevated three to nine hours post-exercise (Cannon et al., 1989). However, elevated cortisol can affect IL-1β levels (Ding et al., 2020). Furthermore, IL-1β release is significantly increased as body temperature rises (Capó et al., 2016) which occurs during high-temperature performance and/or longer duration events, such as a UMT.

Our study used a noninvasive salivary collection method to measure α-amylase, cortisol, and IL-1β biomarkers of stress related to activity of the SNS and HPA axis in response to a UMT. Thus, the primary purpose of this project was to examine the changes in sAA, cortisol, and IL-1β levels, from pre- (PRE) to post-ultramarathon (POST). Our hypothesis was that the UMT would cause an increase from PRE to POST for α-amylase, cortisol, and IL-1β. Moreover, we hypothesized that there would be a correlation between elevated α-amylase and cortisol, and faster race completion times.

Methods

Subjects

Eighteen subjects competing in the Wyandotte Psycho-Psummer 50 km trail race (Wyandotte County Lake, Kansas City, KS) volunteered to participate in this study. All subjects were recruited before the race on site at the event where the research team had set up a table with flyers and information regarding the study. Subjects completed their registration for the event, informed consent, and a health history questionnaire prior to the race start. Approval for this study was obtained from the Institutional Review Board at the University of Kansas. The only inclusion criterion was males and females that were healthy and free of any metabolic or cardiovascular disease. The exclusion criterion was the use of any anti-inflammatory drug, including nonsteroidal anti-inflammatory drugs, within 24 hours of race start. Due to attrition during ultra-distance events from the extreme environmental factors to include heat and humidity, eleven subjects completed the study (age: 34.9 ± 9.5 years; height: 174.5 ± 9.1; weight: 69.6 ± 8.3 kg; longest run prior to this competition: 73.2 ± 44.5 km). Of the 11 finishers, 10 subjects provided enough sample to analyze all three markers. Race times for the 10 finishers were $6:57:26 \pm 1:08:59$ (hours:minutes:seconds). Two of the participants were eliminated from subsequent analysis due to race times two hours outside of the standard deviation of the group. Thus, only eight (3 F/5 M) racers' data were used for subsequent analysis.

Environment

The 50 km trail race took place on July 12, 2014. Race conditions during the nine hours were as follows: mostly sunny throughout the race, temperature ranged from 26.0 to 32.2℃, humidity ranged from 55 to 68%, wind speed ranged from 20.5 to 22.2 5 km/h from the south-southwest, estimated indirect sunlight wet bulb globe temperature stayed consistently near 21℃, the heat index ranged from 23 to 27℃, and barometric pressure ranged from 761 to 762 mmHg. The elevation gain of the race was 1189 m. Food and drink were provided at nine aid stations throughout the race, which were staffed by race workers. The caloric and fluid intake was not monitored for this study.

Sample Collection

Approximately 10 minutes prior to the beginning of the race, participants placed a SalivaBio Oral Swab (SOS; Salimetrics, State College, PA) under the tongue for 2 minutes. To minimize contamination, subjects were requested to refrain from consuming any food or drink 10 minutes prior to collection; however, during the race there was no way to enforce this. The SOS was then placed in specific containers provided by the manufacturer prior to and immediately after the race and stored on dry ice for 12 hours until frozen at −65℃ until analysis. Saliva samples from racers that did not complete the race were not analyzed.

Biochemical Analysis of Saliva

At the time of analysis, samples were thawed, centrifuged at 4℃ at $3000g$ for 5 minutes, measured to the nearest 1 ml, and aliquoted into appropriate sample tubes. Excess saliva and samples not being immediately analyzed were refrozen at −65℃. All samples were run in triplicate, using saliva-specific enzyme-linked immunosorbent assay (ELISA) kits for α-amylase, cortisol, and IL-1β (Salimetrics, State College, PA). The α-amylase samples were measured using the differential change time of the enzymatic reaction from 1 and 3 minutes using a filter set at 405 nm with a microplate reader (BioTek Synergy, Winooski, VT). The α-amylase absorbent collection material values were corrected based on drool rates and samples were controlled for salivary flow rate, per recommendation from manufacturer (Salimetrics, State College, PA) by spinning down the SOS and measuring the volume remaining, noting the length of time the swab is in the mouth (2 minutes). The difference in grams is approximately equal to the volume in milliliters and then multiplying the assay results by the saliva flow rate using the following formula:

Units sAA activity/ml × ml/min = Units sAA activity/min

The samples for cortisol and IL-1β were analyzed using a microplate reader (BioTek Synergy, Winooski, VT) set at

an absorbance of 450 nm. The intra-assay CV was 1.778 for cortisol and 3.769 for IL-1β for all samples.

Statistical Analysis

Statistical analyses were performed using IBM SPSS software (22.0, Chicago, IL). Statistical significance was set at the $p < 0.05$ level, and values expressed as mean $\pm$ SD. The mean change in salivary biomarkers α-amylase, cortisol, and IL-1β from pre- to post-race values was compared using a paired-samples t-test. Linear regression was used to determine relationships between race speeds and pre- to post-race analyte levels. Additionally, within-group Hedges' g effect sizes (g) were calculated for all dependent variables.

Results

Saliva

There were no significant changes in saliva flow rate ($p = 0.89$; $g = 0.04$) from PRE (224.5 ± 136.45 ml/min) to POST (216.14 ± 206.76 ml/min).

α-Amylase

There was a 1.61-fold increase ($p = 0.002$; $g = 1.26$) in sAA from PRE (62.92 ± 63.74 U/ml) to POST (164.38 ± 93.07 U/ml) (Figure 1a). There was no relationship between the change in sAA from PRE to POST and speed ($R^2 = 0.028$, $p = 0.692$) (Figure 2a).

Cortisol

There was a 1.71-fold increase ($p < 0.001$; $g = 4.89$) in salivary cortisol from PRE (0.071 ± 0.005 μg/dl) to POST (0.0968 ± 0.004 μg/dl) (Figure 1b). There was a significant relationship between the change in cortisol from PRE to POST and speed ($R^2 = 0.582$, $p = 0.028$) (Figure 2b).

IL-1β

There was no change ($p = 0.32$; $g = 0.336$) in IL-1β from PRE (16.63 ± 0.15 pg/ml) to POST (16.71 ± 0.13 pg/ml) (Figure 1c). Correlations between race time and IL-1β were not examined due to there not being a significant difference between PRE and POST values.

Discussion

The current investigation found that sAA and cortisol significantly increased after completion of a UMT. However, there was no significant change in IL-1β. There was also no relationship between the magnitude of

increase in sAA and race speed. There was a significant correlation between the magnitude of the cortisol increase and race speed with the former accounting for 62% of the variability in the latter. We partially confirmed our hypotheses by showing increased sAA and cortisol from pre- to post-race; however, we did not see a change following the race in IL-1β. Additionally, we hypothesized that sAA and cortisol would be higher with faster race completion times, although we only found a significant association between cortisol and race speed.

α-Amylase

α-Amylase is a digestive enzyme that is secreted from the salivary glands in response to stimulation by the ANS and has been widely used as a less invasive biomarker of SNS activation (Ali & Nater, 2020). Both psychological and physical stresses cause increases in α-amylase and the intensity of the stimulus is directly correlated with the degree of response (Chatterton Jr et al., 1996). Previous literature is conflicting regarding the relationship between α-amylase, EPI, and NE. Chatterton et al. (1996) found significant correlations between α-amylase, EPI, and NE concentrations during exercise, while other research has found only a relationship between α-amylase and NE (Thoma et al., 2012). However, other studies did not have similar findings, showing no relationship between plasma catecholamines and sAA levels (Ehlert et al., 2006; Nater et al., 2006; Nater & Rohleder, 2009). Despite this, sAA is widely viewed as representing noradrenergic activation (Segal et al., 2012). Additionally, sAA is advantageous due to is resistance to the anticipatory response (Kivlighan & Granger, 2006), which otherwise may lead to an increase in sAA due to pre-race anxiety. Conversely, the sAA response can be influenced by the environment; specifically exercise in elevated temperatures exacerbates the response of the SNS (Silva et al., 2019). This may have influenced the observed sAA elevation seen with completion of a UMT beyond the stressors imposed by the UMT alone.

The SNS is involved in many important functions during exercise including hemodynamic modulation and substrate mobilization to ensure metabolic demands of the body are met (Christensen & Galbo, 1983; Katayama & Saito, 2019). Additionally, the magnitude of the SNS response to is directly related to the magnitude of the stressor (i.e., exercise intensity) (Michael et al., 2017). The results of the current investigation showed a significant increase in sAA from PRE to POST, which is consistent with other research looking at sAA response to endurance-type events (Deneen & Jones, 2017; Gill et al., 2014; Piacentini et al., 2015).

Due to the critical nature of the SNS during prolonged exercise, several studies have attempted to demonstrate a link between α-amylase levels and performance across a wide variety of activities both physical (Kivlighan & Granger, 2006; Lim, 2018; Lippi et al., 2015; Nater &
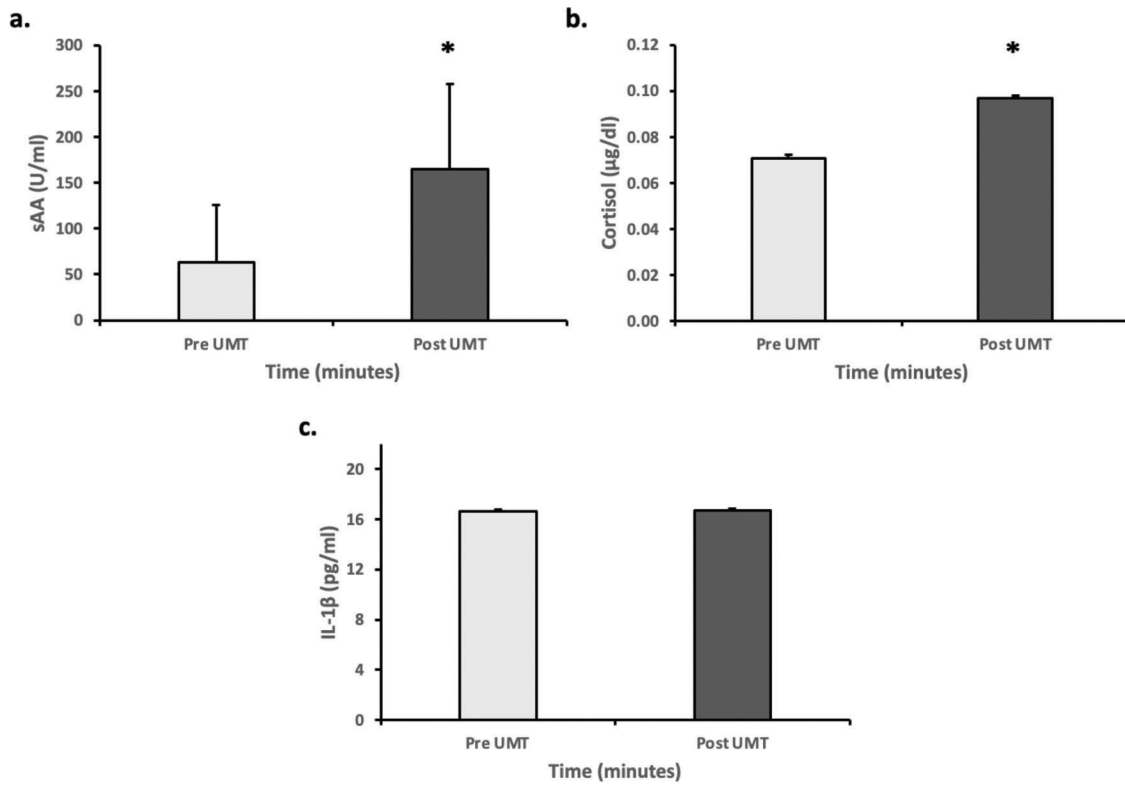
Figure 1. (a) Changes in sAA levels from PRE to POST. (b) Changes in salivary cortisol levels from PRE to POST. (c) Changes in salivary levels of IL-1β from PRE to POST. *Significant difference ($p < 0.05$).
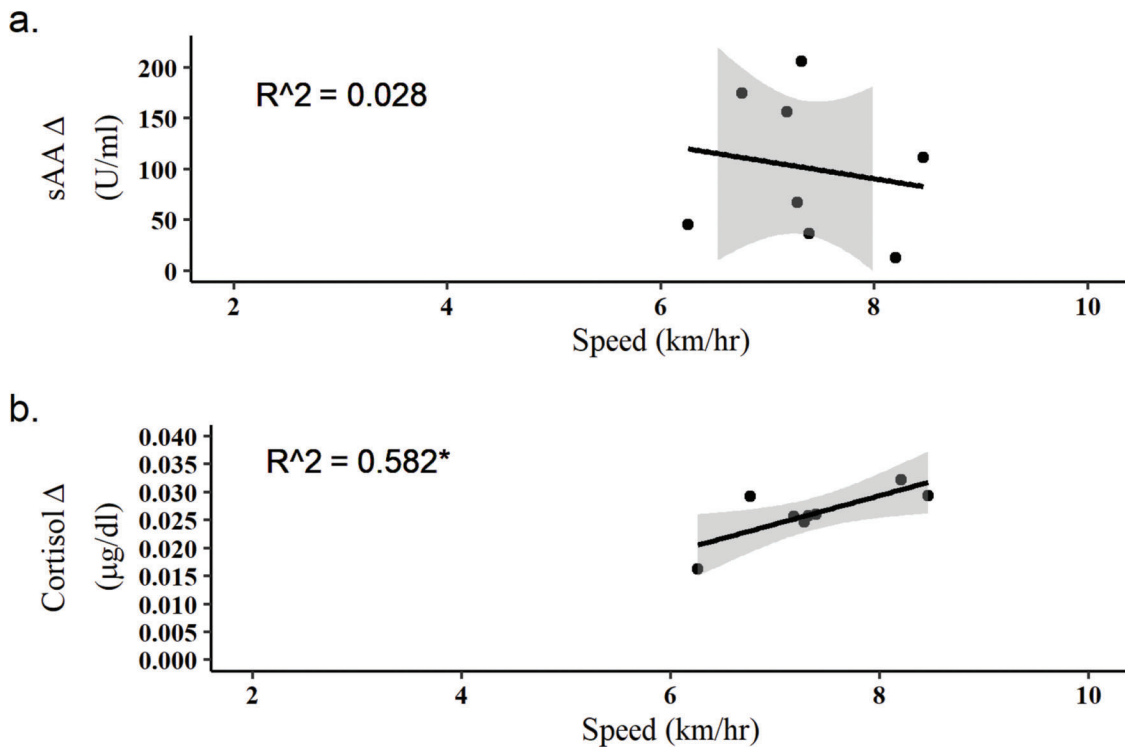

Figure 2. (a) Relationship between the increase in sAA from PRE to POST and race completion time. (b) Relationship between the increase in salivary cortisol from PRE to POST and race completion time. The shaded area represents the 95% confidence interval. *Significant R^2 value ($p < 0.05$).

Rohleder, 2009) and psychological (i.e., academic performance) (Keller et al., 2012), although limited data exist for endurance-type activities such as a UMT. For example, Lippi et al. (2015) showed no correlation between the change in pre- and post-race sAA levels and finish times in amateur runners completing a half marathon; however, they did demonstrate that higher baseline sAA values were positively correlated with completion times (Lippi et al., 2015). Further, Kivlighan and Granger (2006) demonstrated that elite rowers exhibited higher post-race levels of α-amylase with faster race times. This is in contrast to the current investigation which found no correlation between increases in sAA and UMT completion times although caution should be taken comparing results across studies due to the disparate distances and exercise modalities.

Cortisol

Cortisol represents activation of the HPA axis in response to both physical and mental stress and is critical to regulating energy metabolism and availability during exercise (Hackney & Walz, 2013). Numerous studies have shown an increase in cortisol levels with prolonged endurance exercise (Cook et al., 1987; Deneen & Jones, 2017; Kupchak et al., 2014; Piacentini et al., 2015). The current investigation also showed significant increases in salivary levels of cortisol following a UMT. Thus, despite the relatively low to moderate intensity, ultra-endurance exercise imposes significant physiological stress on the body (Peters, 2003). Furthermore, the HPA axis is linked with the SAM axis through the actions of cortisol. Specifically, cortisol is responsible for induction of the enzyme (phenylethanolamine-N-methyltransferase) that catalyzes the conversion of EPI from NE (Wurtman, 2002).

With the critical role that cortisol plays in bioenergetics during exercise, particularly prolonged exercise, it may be reasonable to assume there is a relationship between the magnitude of the cortisol response to exercise and performance (Vaamonde et al., 2022). However, studies have attempted to relate cortisol measurements with performance in endurance-type events, but no consensus has been reached (Bae et al., 2019; Bosco et al., 1996; Hloogeveen & Zonderland, 1996; Syed-Abdul, 2020). For instance, Bae et al (2019) showed no correlation between marathon performance and cortisol increases in male runners. Similarly, a study of endurance-trained cyclists showed no correlation between cortisol increases and performance during completion of a cycling protocol in professional cyclists (Hloogeveen & Zonderland, 1996). Conversely, Bosco et al (1996) showed that lower basal cortisol levels were associated with shorter distances completed during the Cooper's 12-minute running test, a measure of cardiorespiratory fitness, in professional soccer players.

To the best of our knowledge this is one of the first studies to demonstrate a significant relationship between cortisol increases and increased performance in an extreme endurance-type event. This may be due to greater increases in cortisol during the UMT providing greater substrate availability, thus increasing exercise performance capacity. Similarly, greater cortisol increases may result in increased conversion of EPI from NE which may enhance the SNS response to exercise. However, caution should be taken in extrapolating these results beyond this context as several factors can modulate the cortisol response beyond exercise, including psychological stressors (e.g., competition anxiety) and environmental factors (e.g., temperature) and they may not be broadly applicable. More research is needed in this area to determine if this relationship between cortisol and endurance performance is replicable.

Generally, a decrease in cortisol concentration is observed in the afternoon (Lac, 2001), which was when many of the racers were finishing. We did not filter or adjust for this decrease, and this may have contributed to our results. However, all samples were collected from subjects within ten minutes of each other pre-race, and within two minutes of their post-race completion. This is due to the fact that the participants did not finish at the same time which may have also impacted cortisol results.

IL-1β

The immune response of IL-1β, a mediator of inflammation, is stimulated by glucocorticoids and can signal both corticotropin-releasing hormone and adrenocorticotropic hormone, furthering the stress response (Katsuura et al., 1990). Chronic aerobic exercise has been shown to potentiate the IL-1β secretion response by macrophages (Baum et al., 1999). Thus, we hypothesized that IL-1β would increase during the UMT; however, no significant changes in salivary IL-1β concentrations were observed after completing the race. These results are consistent with those of Suzuki et al. (2006) who found no significant changes in plasma IL-1β following an ironman triathlon race. Shorter durations of both moderate- and high-intensity aerobic exercise also produced no change in serum IL-1β concentrations (Zar et al., 2022). As previously noted, serum IL-1β levels peak at 3 to 9 hours post-exercise, so the lack of later collection time points may have failed to capture the anticipated increase in IL-1β. Additionally, the intensity of the UMT may not be intense enough to stimulate an IL-1β response as the subjects may be more accustomed to this style of race.

Limitations and Future Considerations

There were numerous limitations to this field study. Due to the race committee not allowing additional collection

times during the competition, additional collection times were not possible, however, mid-race, 3- and 72-hour post-race collections could help fill in the gaps in the biomarker curves. Future research could benefit from a 3- and 72-hour post-race salivary sample to observe the adaptations in cortisol and IL-1β.

Runners' fuel and water consumption near the end of competition was not controlled; however, the last aid station was outside the 10-minute window. Control of food and drink intake is needed for future studies. Additionally, measures of urine specific gravity would have helped to determine hydration status of the participants.

As previously noted, temperature impacts the response of both sAA and salivary cortisol, although it is unclear if that is modified by an individual's heat acclimation status (Silva et al., 2019). Although endurance-trained athletes require less adaptation for hot environments (Taylor, 2000), future studies should attempt to quantify the degree of heat acclimation for events in hot environments as this may impact biomarker results as well as race performance. It is impossible to uncouple the stress of a UMT from environmental factors, but future studies should also investigate competitions taking place at varying climates.

With a larger sample size, it would have been preferable to group participants by age, sex, and training status as those may also impact results. Previous research has shown that females demonstrate a larger cortisol response than males (Larsson et al., 2009). Similarly, aging increases the cortisol response to psychological stressors (Otte et al., 2005). Moreover, to account for variations in training status, a VO$_2$max assessment could have been performed two weeks prior to collection, although for this study it was not feasible.

Of future interest is the difference in magnitude of stress imposed by different running events such as marathon, Ironman style, and ultra-trail running as it is difficult to generalize results across disparate distances, intensities, and modalities. Furthermore, research could compare the anticipatory response of these markers by collecting 24 hours pre-race and immediately pre-race.

Conclusion

The current investigation showed that a UMT caused significant increases in both sAA and salivary cortisol levels demonstrating that the UMT resulted in significant activation of both the HPA and SAM pathways. Conversely, we did not observe any increases in IL-1β levels as a result of completing the UMT, and no correlation was observed between sAA and cortisol increases and UMT completion time. These results aid in our understanding of the physiological stressors imposed during ultra-distance-type events and how the response to those stressors may alter endurance performance.

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