



Original Research

Cortisol and Alpha-amylase changes during an Ultra-Running Event

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ABSTRACT

International Journal of Exercise Science 10(4): 531-540, 2017. Elevated stress hormone concentrations can positively affect an athlete's overall performance during a competition, and in many cases, are necessary to be able to perform exercise. During extreme exercise, the body's ability to utilize energy efficiently can affect an athlete's performance. Elevated hormonal concentrations can have many benefits in regards to an athlete's overall performance during a competition. The purpose of this study was to examine the effects of long distance running, such as seen during an ultra-running event (distances beyond 26.2 miles), on the activity of the hypothalamic-pituitary-adrenocortical (HPA) axis production of cortisol (CORT) as compared to autonomic nervous system production of salivary alpha-amylase (AA). Despite the well-known effects of exercise on CORT and AA response, it is unclear what effect running beyond the marathon distance has on these levels. This study investigates what effect long duration cardio exercise, such as running up to 100K (kilometers) distance, has on the neuroendocrine system, by means of saliva samples provided by participants signed up for an ultra-marathon event. The findings of this study show that the autonomic nervous system may present a response signal during physical stress that is independent of the HPA axis response. At distances beyond the marathon length, the production of CORT and AA was found to be suppressed for athletes, which could help them in their continued performance. Furthermore, this study recognizes a difference in the overall male and female response to stress in regards to CORT and AA production.

KEY WORDS: Autonomic nervous system and exercise, cortisol and running, physiological stress of running, elevated levels of cortisol

INTRODUCTION

Ultra-marathon events have gained rapid popularity within the last few years among avid runners. The Western States 100-mile endurance run is one of the nation's most popular ultra-running events and has consistently increased in numbers of applications each year. In 2000,

there were 583 applicants and this year there were 3510 applicants with only 270 being chosen during the final lottery (<http://www.wser.org/lottery>).

Running a couple of miles or longer, such as a half marathon can be physically and mentally exhausting. Activation of the neuroendocrine system is one of the many systems activated during such a stressful event. Currently, limited information exists on the effects of the neuroendocrine system during an ultra-marathon (distances beyond 26.2 miles/42 kilometers (km) event (4, 5, 19). Much of the current research involves the evaluation of the neuroendocrine system pre and post exercise or in a closely monitored laboratory setting (4, 14, 5, 16). This study is unique, as it is the first attempt to evaluate the stress response system *during* an event with runners competing beyond the marathon distance under natural conditions.

The first goal of this study was to investigate the possible effects varying running distances has on the sympathetic nervous system with the release of cortisol (CORT) and alpha-amylase (AA). This study analyzed CORT and AA at varying intervals throughout an ultra-running event called the Pumpkin Holler Hunnerd, which takes place annually in Tahlequah, Oklahoma. It is important to study these changes during a real time event, as it provides a more accurate assessment of what changes are occurring within the stress-response systems.

Physiological and psychological stressors can contribute to the activation of the Hypothalamic-Pituitary-Adrenal axis (HPA) which is responsible for the release of the stress hormone, CORT (9). CORT is the primary glucocorticoid found throughout the body and when released from the renal cortex, it can have a direct impact on the physiological functioning of the body (8). Elevated hormonal concentrations, such as CORT can positively affect an athlete's overall performance during a competition (18, 2).

To evaluate the sympathetic nervous system this study analyzed salivary AA. Salivary AA correlates to the autonomic nervous system during physiological stressors. The autonomic nervous system releases AA in response to physiological stressors (20, 3). Research shows that AA has been identified as valid alternative to catecholamine measurements of the sympathetic nervous system (13). Despite the well-known effects of CORT and AA response on intense physiological stress, it is unclear what effect running beyond the marathon distance has on these analyses.

This study analyzes CORT and AA before, during and after an official competition in an effort to understand what physiological changes are occurring during high intensity exercise such as seen during an ultra-running event called the Pumpkin Holler Hunnerd.

The second goal of this study was to investigate the relationship of male to female response of the CORT and AA. To date there is limited information on the stress response systems of women participating in ultra-marathon running events. It has only been since 1972, that women were allowed to run in marathons like the Boston Marathon (10). For example, one

study, which evaluates changes in salivary hormones, the population, consisted of 66 men and 4 women (19).

This study was designed to 1) investigate the effects of long distances running on the activity of the HPA axis with the release of CORT compared to the autonomic nervous system production of salivary AA & 2) to investigate the stress response system in relationship to male or female response.

METHODS

Participants

Individuals who signed up to participate in the Pumpkin Holler Hunnerd were asked to participate in this study. Thirty-five participants were involved in this study, 25 women and 15 men. All participants provided an informed consent prior to any sample collection. All experimental procedures were approved by the Northeastern State University Institutional Review Board prior to any data collection.

Hormonal replacement therapies such as birth control, estrogen and progesterone have been shown to have an impact on CORT levels (1, 15). Participants were asked to disclose if they were taking any hormonal therapies (example: birth control, estrogen patches, testosterone) on the initial informed consent.

Protocol

The Pumpkin Holler Hunnerd event offered distances of 10KM, 25KM, 50KM and 100KM, which allowed sampling of runners at a variety of distances in order to compare changes that occur with running shorter versus longer distances. Saliva samples were obtained prior to any exercise the day of the event and throughout the race at the following markers as seen in Table 1.

Table 1. Saliva collection distances for each running distance.

Running Distance					
10km	Start	Finish line			
25km	Start	10km	Finish line		
50km	Start	10km	25km	Finish line	
100km	Start	10km	25km	50km	Finish line

Samples for all participants were collected by means of passive drool and obtained by using Salimetrics Salivary Collection System (Salimetrics, LLC, State College, PA). Each participant was asked to submit 1 mL of saliva at each collection station.

Research has shown that hydration status and fluid intake during the course of an event can alter these analytes (11). To help eliminate the intake of fluids that could alter findings, collection stations were strategically placed approximately 50 meters prior to an aid station. While we could not eliminate all fluid ingestion by participants as that could be detrimental to a runners' performance, we attempted to reduce fluid ingestion before sample collection by

locating saliva collection points just prior to aid stations so that runners would be anticipating fluids there and might not have much left in their bottles. An additional step was also to place signs just ahead of the saliva collection points so that runners would know that it was coming up. They were advised not to drink anything immediately prior to saliva collection during the pre-race briefing.

Upon collection, saliva samples were placed upon dry ice until all samples were collected and transported to a freezer where they were stored at -20°C until plate preparation.

A competitive 96 well salivary CORT Enzyme Immunoassay (Salimetrics, LLC, State College, PA) was used in this study to measure CORT levels in saliva. A salivary AA kinetic enzyme assay (Salimetrics, LLC, State College, PA) was used to measure the AA activity in the saliva. Cortisol EIA's were read at 450nm; AA read at 415nm using the iMark Microplate Absorbance Reader (Bio-Rad, Hercules, CA). All samples were assayed in duplicate.

Raw data was analyzed utilizing assay data analysis software (Myassays.com, 2015) to calculate the final CORT concentration in micrograms per deciliter ($\mu\text{g}/\text{dL}$). The final AA concentration was calculated by subtracting the one-minute reading from the three minute reading and then utilizing the formulas found in the table below.

Table 2 Formula's used to calculate the final AA concentration.

$\Delta\text{Abs.}/\text{min} \times \text{TV} \times \text{DF}$	U/mL of AA
$\text{MMA} \times \text{SV} \times \text{LP}$	activity in sample
Where: $\Delta\text{Abs.}/\text{min}$	Absorbance difference per minute
TV	Total assay volume (0.328 mL)
DF	Dilution factor
MMA	Millimolar absorptivity of 2-chloro-p-nitrophenol (12.9)
SV	Sample volume (0.008 mL)
LP	Light path = 0.97 (specific to plate received with kit)

Results for AA are expressed in U/mL.

Statistical Analysis

Salivary CORT sensitivity range for adults is 0.007-3.0 $\mu\text{g}/\text{dL}$ (Salimetrics). Individuals with levels outside of the kit sensitivity ranges were excluded in the final numbers. The following list the number of female or male participants that AA ranges were out of range and therefore not considered in the final data: 10k = 3 females/1 male; 25K = 5 females/3 males; 50K = 4 females/2 males; 100K = 2 males.

AA kit sensitivity ranges for adults are 2.0-400.0 U/mL (Salimetrics), therefore individual samples were excluded if the result was outside the range. Higher AA levels can be associated with the use of caffeine and/or other confounding factors such as eating or drinking, which could lead to an increase of salivary AA (7).

Some participant's data were excluded based on AA ranges being out of range.

Data are presented as group means +/- standard error (SE). Statistical significance was set at $p < 0.05$. A two-way analysis of variance (ANOVA) was used to compare males and females for CORT and AA. Comparison between pre-exercise, and varying distances throughout the event were performed with a one way ANOVA. A Fisher's post hoc followed upon significant findings.

RESULTS

Before the race began, 35 individual runners had signed up to participate in this study, 25 of which were women and 15 men. Figure 1 shows the mean salivary CORT levels that were collected throughout the Pumpkin Holler Hunderd among the individual distances. The greatest increase in CORT is among the 100km at the 25km collection site. An increase in CORT levels from start to finish is reflected in all distances. Subtle increases can be seen throughout the event; however the 100km runners CORT concentrations vary the greatest. The smallest percent increase can be seen among the 100km runners at 17% as seen in Table 2.

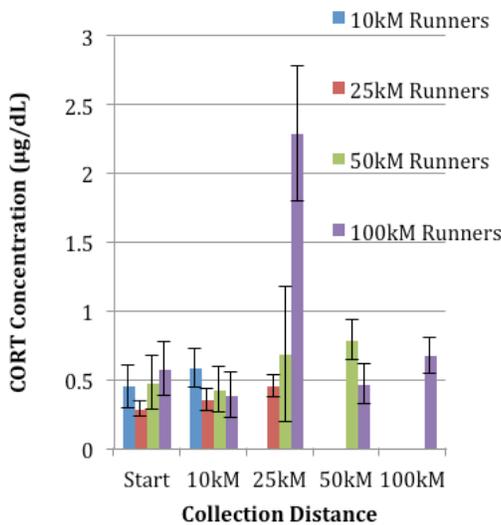


Figure 1. Mean salivary CORT levels in male and female participants.

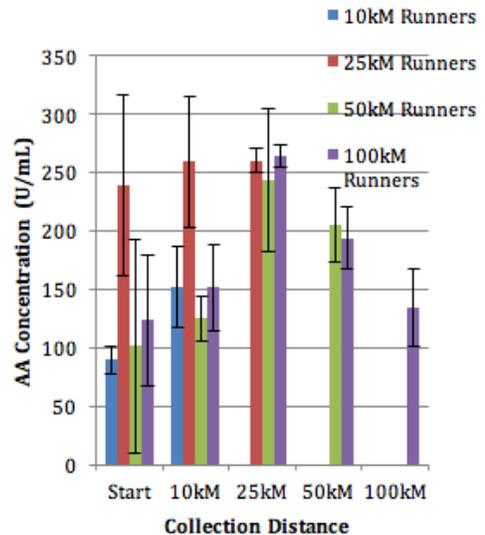


Figure 2. Mean salivary AA levels in male and female participants.

Table 2. Percent change of CORT and AA from start to finish in male/female runners.

Distance Ran	Cortisol	Alpha-Amylase
10 km	29% ↑	70% ↑
25 km	29% ↑	8% ↑
50 km	56% ↑	101% ↑
100 km	17% ↑	8% ↑

Table 2 summarizes the percent change in salivary CORT and AA in each of the distances from start to finish. The greatest percent change can be seen in the AA among the 50km runners.

This increase parallels the overall highest percent increase as seen in CORT among 50km runners. The data reflects there is a similar stress response to those individuals running the 50km distance in CORT and AA production. Figure 2 shows the mean salivary CORT levels that were collected throughout the Pumpkin Holler Hunnerd among the individual distances. The overall greatest response of the sympathetic nervous system is reflected among the 100km runners at the 25km collection site. These results parallel the results we see in the CORT production.

Figure 3 shows the mean CORT levels for females increase from start to finish in every event. Those individuals running the 25km show the least response to stress in terms of CORT production. A 1-way ANOVA for females for CORT shows a significant effect ($p < 0.05$), and Fishers LSD post hoc shows that values at 25K are significantly greater than the start ($p < 0.01$).

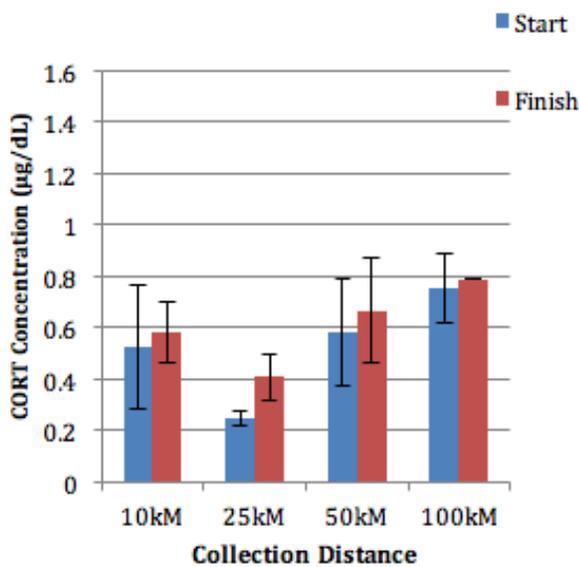


Figure 3. Mean salivary CORT levels for females, demonstrating an increase in CORT from start to finish at each distance.

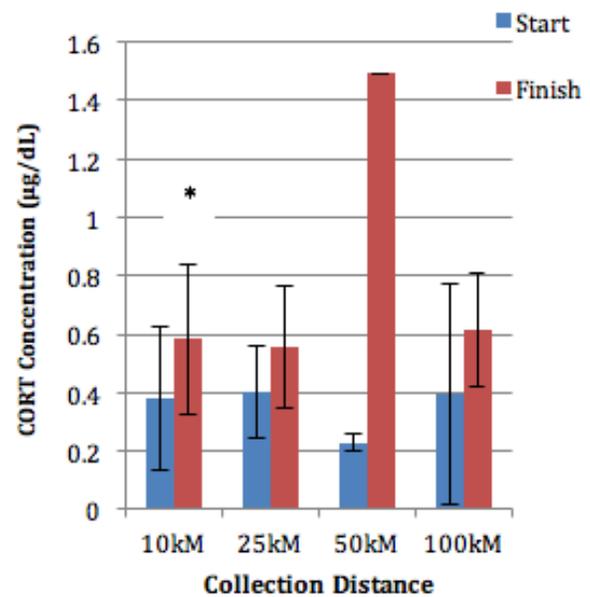


Figure 4. Mean salivary CORT levels for males. * $P < 0.01$. Mean salivary CORT levels for males, showing an increased CORT in each distance at the finish compared to the start level.

The data in figure 4 summarizes the salivary CORT levels in male runners from start to finish in each of the distances. An increase in CORT is seen in each of these events. A Fisher's LSD post hoc indicates that the male 10km is significantly greater than the male start ($p < 0.01$). The data indicates that the males participating in the 50km distance had the greatest CORT levels at the finish line. This could be attributed to having one viable sample among the 50km male finishers.

Figure 5 summarizes the sympathetic nervous response with the release of AA in female runners. The figure shows the mean AA production in female runners at the starting line and the finish line in the various running distances. The data reflects an increase in each of the distances with the exception of the female runners competing in the 100km. 25km female AA

was significantly greater at the start. A 1-way ANOVA of distances (10km, 25km, 50km & 100km) in female AA levels reveals a main effect of exercise [$F(3,33) = 4.06, p < 0.05$], and a Fisher's LSD post hoc showed the 25km were significantly greater than start ($p < 0.05$). On average the participants running the 100km event, show a decrease (18%) in AA production from start to finish.

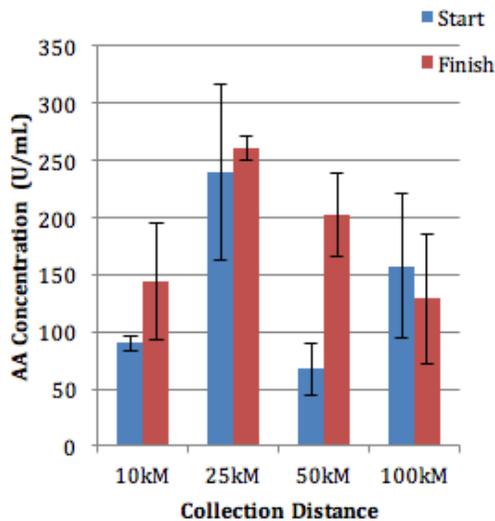


Figure 5. Mean salivary AA in females. These data reflect an increase in AA in each of the distances, except female runners in the 100km distance.

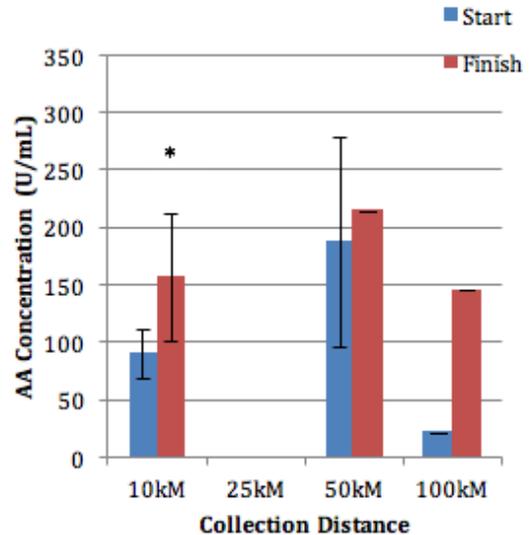


Figure 6. Mean salivary AA for males. * $P < 0.01$ Fisher's LSD post hoc. 10km Pre vs. post exercise. Mean salivary AA for males. 100km runners display the greatest in AA production over the course of the race.

Figure 6 summarizes the mean of the sympathetic nervous system response with the release of AA in male runners. It should be noted there were no viable samples for male participants running the 25km event as each sample was discarded due to being outside the sensitivity ranges. The greatest increase from start to finish is among the 100km runners with a 597% increase in AA production.

DISCUSSION

This study is the first attempt to focus on neuroendocrine changes throughout an ultra-running event while performed under natural means to evaluate the physiological changes. Based on our results it is apparent that the physiological stress of ultra-running does play a significant role in the CORT production of the HPA axis along with the AA production from the sympathetic nervous division.

In previous studies, CORT has been shown to increase as the intensity of exercise increases (12, 19). An increase in CORT and AA during a running event can have an overall positive impact on the athletes' performance (16). These levels could ultimately be used to predict an athletes overall performance. According to some studies a pre-competition CORT and AA can benefit athletes to help them meet the physical demands in competing during an ultra-running event (6).

Considering that these levels can be utilized to help predict an athlete's competitive performance, athletic trainers and coaches could customize training programs based on an athlete's neuroendocrine response to maximize training benefits.

The data in this study indicates that at distances below the ultra-marathon, the HPA axis and the autonomic nervous system respond in a parallel pattern; however, at ultra-marathon distances (50km & 100km), the responses of these two systems vary. This would suggest that the autonomic nervous system might present a response signal during physical stress that is independent of the HPA axis response.

Interestingly, the 100km runners exhibited the greatest response to CORT and AA production at the 25km distance and then began to drop until they reached the finish line. The course was similar for all distances; 25K runners completed an "out and back" type on the trails, 50K runners did one loop, and 100K repeated the 50K loop twice. A reduction of the HPA and sympathetic nervous system could benefit an athlete's performance, as continual activation of these stress response systems could be detrimental to their performance.

Additionally, these pathways differ in their response between male and females, with females showing an overall higher production of CORT in response to stress, while males show an overall higher production of AA in response to stress.

The extensive length of the 100km event had runners crossing the finish line well into the early morning hours. Many factors can cause the production of CORT and AA to fluctuate. CORT production has a circadian rhythm with levels peaking in the morning and showing the lowest values at night (17). Hydration status can play a role in these levels of analytes. Each of the athletes were encouraged to stay hydrated throughout the event. Collection stations were placed approximately 50 meters prior to any aid station to help eliminate the intake of fluids that could alter findings.

Additional studies are recommended which repeats this study over the course of 3 consecutive years to analyze individual subjects' catecholamines and how the level of conditioning plays a role in these stress response systems.

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