Salivary Anti-Microbial Protein Responses During Multi-Stage Ultra-Marathon Competition Conducted in Hot Environmental Conditions: Does Hydration Play a Role?

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Introduction

Upper respiratory symptoms (URS) commonly reported after endurance exercise, in highly trained individuals1-3,4, are often attributed to the transient decreases observed in salivary immunoglobulin A (S-IgA)5,6,7. More recently, other salivary anti-microbial proteins (AMP) such as α-amylase and lysozyme have become recognised as having a substantial role in salivary immune defence, and when collectively assessed with S-IgA may provide a more comprehensive assessment of oral-respiratory mucosal status7,8.

Exercise-induced dehydration is a common feature in endurance events conducted in hot ambient environments9. Hypohydration depresses saliva flow rate, and the degree of depression is associated with the severity of hypohydration10. Thus, sub-optimal hydration status may perturb S-AMP responses, potentially leading to increased prevalence of URS.

Acute periods of physiological stress are associated with increases in plasma cortisol release11,12,13 that have been related to the immunodepressive alterations in S-AMP responses14,15, due to the potential alterations in saliva flow rate and mucosal secretions16,17, thus increasing risk of URS.

Aim: To determine S-AMP responses and monitor hydration status of UER during a semi self-sufficient multi-stage ultra-marathon (MSUM) conducted in hot environmental conditions, and record URS during and the month following competition cessation.

Methods

With ethical approval and informed consent salivary AMP (IgA, α-amylase, lysozyme) and cortisol responses, and hydration status of thirty-seven UER (n = 37; M = 23, F = 14; Mean ± SD: age 40 ± 9 y, height 1.66 ± 0.33 m, BM 69.6 ± 9.9 kg, estimated body mass fat (BFM) 17 ± 5%; competing in a 230 km MSUM (28h17m; 41.15m; 7.9 ± 1.3km h⁻¹)), conducted over five consecutive days in hot ambient conditions (Figure 1) were determined. Additionally, 12 matched individuals who accompanied the UER along the MSUM course, but did not compete, volunteered to participate in the study as part of the control group (CON: age 35 ± 13 y, height 1.67 ± 0.09 m, BM 69.6 ± 16.2 kg, BFM 21 ± 6%).

Fluid intakes habits of UER were determined through interview (24h recall technique), and analysed on Dietplan dietary assessment software (v6.60, Forestfield Software, Horsham, West Sussex, UK), which was blindly analysed by a 2nd researcher. The mean coefficient of variation for fluid variables analysed was 0.7%.

Body Mass (BM) was determined pre and post-Stages 1 to 5 using calibrated electronic scales (BF510, Omron Healthcare, Ukyo-ku, Kyoto, Japan) placed on a hard levelled surface. Participants were weighed with dry racing clothes and shoes, but with no additional accessories. Saliva (two-minute unstimulated) and blood (venepuncture without venostasis) samples were taken pre and post-Stages 1 to 5. An additional saliva sample was collected during the evening recovery period (20.00 to 22.00 h).

Plasma osmolality was determined by freezeozepoint osmometry in duplicate (Osmomat 030, Goerz, Germany). Salivary IgA, lysozyme, and cortisol were determined by ELISA, while salivary α-amylase was determined by enzyme reaction assay kit.

Data was analysed using a one-way (between stages) and two-way (between groups, and pre to post) ANOVA (SPSS v17.0.2, Illinois, US). Significant main effects were analysed using post hoc Tukey’s HSD test. Significance was accepted at p < 0.05.

Results

Average total daily fluid intake of UER was 7334 ± 1687 ml-day⁻¹, whilst average fluid intake during running was 734 ± 200 ml h⁻¹. Average exercise-induced BM loss in UER was 2.0% with Stage 1 inducing a significantly higher BM loss (2.5%). Mean BM loss ranged from 0.1% to 3.5%.

Discussion and Conclusion

• Fluid intakes of UER appear to be sufficient to maintain euhydration, which may have contributed to the maintenance of salivary flow rate, and subsequently, permanent presence of S-AMPs.

• Transient fluctuations were observed in all S-AMPs throughout MSUM competition. Results indicate that a counteractive effect appears to be present since a decrease in one immune marker generally results in a subsequent increase of another; thus contributing to the overall maintenance of oral-respiratory mucosal immunity, as reflected by the low prevalence of URS reported during and the month following MSUM competition (n = 1).

• Results are not consistent with previous studies reporting substantial decreases in S-IgA and subsequent high levels of URS post-exercise; possibly due to the accountability of hydration status in this study.

Findings highlight the importance of collectively assessing multiple mucosal protective factors, which may provide a more accurate indicator of overall immune protection. Additionally, hydration status may have a substantial role in protecting the upper respiratory tract against pathogen invasion through maintenance of salivary flow rate.

References

1. Peters, E.M. (1997). Salivary IgA, lysozyme, and cortisol were determined by ELISA, while salivary anti-microbial proteins (AMP) such as α-amylase and lysozyme have become recognised as having a substantial role in salivary immune defence, and when collectively assessed with S-IgA may provide a more comprehensive assessment of oral-respiratory mucosal status7,8.

2. Exercise-induced dehydration is a common feature in endurance events conducted in hot ambient environments9. Hypohydration depresses saliva flow rate, and the degree of depression is associated with the severity of hypohydration10. Thus, sub-optimal hydration status may perturb S-AMP responses, potentially leading to increased prevalence of URS.

3. Body Mass (BM) was determined pre and post-Stages 1 to 5 using calibrated electronic scales (BF510, Omron Healthcare, Ukyo-ku, Kyoto, Japan) placed on a hard levelled surface. Participants were weighed with dry racing clothes and shoes, but with no additional accessories. Saliva (two-minute unstimulated) and blood (venepuncture without venostasis) samples were taken pre and post-Stages 1 to 5. An additional saliva sample was collected during the evening recovery period (20.00 to 22.00 h).

4. Plasma osmolality was determined by freezeozepoint osmometry in duplicate (Osmomat 030, Goerz, Germany). Salivary IgA, lysozyme, and cortisol were determined by ELISA, while salivary α-amylase was determined by enzyme reaction assay kit.

5. Data was analysed using a one-way (between stages) and two-way (between groups, and pre to post) ANOVA (SPSS v17.0.2, Illinois, US). Significant main effects were analysed using post hoc Tukey’s HSD test. Significance was accepted at p < 0.05.

6. Average total daily fluid intake of UER was 7334 ± 1687 ml-day⁻¹, whilst average fluid intake during running was 734 ± 200 ml h⁻¹. Average exercise-induced BM loss in UER was 2.0% with Stage 1 inducing a significantly higher BM loss (2.5%). Mean BM loss ranged from 0.1% to 3.5%.

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Table 1: Changes in plasma osmolality of UER (n = 37) participating in a 230 km MSUM competition conducted in a hot ambient environment and CON (n = 12).

Table 2: Salivary rate flow of UER (n = 37) participating in a 230 km MSUM competition conducted in a hot ambient environment and CON (n = 12).

Table 3: S-cortisol concentration of UER (n = 37) participating in a 230 km MSUM competition conducted in a hot ambient environment and CON (n = 12).

Figure 1.

Figure 2.

Figure 3.