The influence of hydration status during prolonged endurance exercise on salivary antimicrobial proteins

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Abstract

Purpose: Antimicrobial proteins (AMPs) in saliva including secretory immunoglobulin A (SIgA), lactoferrin (SLac) and lysozyme (SLys) are important in host defence against oral and respiratory infections. This study investigated the effects of hydration status on saliva AMP responses to endurance exercise. Methods: Using a randomized design, 10 healthy male participants (age 23±4y, $\dot{V}O_{2max}$ 56.8±6.5ml/kg/min) completed 2h cycling at 60% $\dot{V}O_{2max}$ in states of euhydration (EH) or dehydration (DH) induced by 24h fluid restriction. Unstimulated saliva samples were collected before, during, immediately post-exercise and each hour for 3h recovery. Results: Fluid restriction resulted in a 1.5±0.5% loss of body mass from baseline and a 4.3±0.7% loss immediately post-exercise. Pre-exercise urine osmolality was higher in DH than EH and overall, saliva flow rate was reduced in DH compared with EH ($p<0.05$). Baseline SIgA secretion rates were not different between conditions; however, exercise induced a significant increase in SIgA concentration in DH (161±134 to 309±271mg/L) which remained elevated throughout 3h recovery. SLac secretion rates increased from pre- to post-exercise in both conditions which remained elevated in DH only. Overall, SLac concentrations were higher in DH than EH. Pre-exercise SLys concentrations were lower in DH compared with EH (1.6±2.0 vs. 5.5±6.7mg/L). Post-exercise SLys concentrations remained elevated in DH but returned to pre-exercise levels by 1h post-exercise in EH. Conclusions: Exercise in DH caused a reduction in saliva flow rate yet induced greater secretion rates of SLac and higher concentrations of SIgA and SLys. Thus, DH does not impair saliva AMP responses to endurance exercise.

Key Words: Hydration Status, Mucosal Immunity, Endurance Exercise, Salivary Antimicrobial Proteins

Abbreviations

Abbreviation	**Meaning**
ACTH	Adrenocorticotrophic hormone
AMPs	Antimicrobial proteins
ANOVA	Analysis of variance
BM	Body mass
DH	Dehydration
EH	Euhydration
FR	Salivary flow rate
HPA axis	Hypothalamic-pituitary-adrenal axis
HR	Heart Rate
RPE	Perceived exertion
SLac	Salivary lactoferrin
SIgA	Salivary secretory IgA
SLys	Salivary lysozyme

Introduction

Mucosal secretions play an important role in immunity as the first line of defence against potential pathogens invading the oral cavity and upper respiratory tract (Gleeson and Pyne 2000). Salivary secretory IgA (SIgA) has been the most commonly studied marker of mucosal immunity and its deficiency has been associated with a higher incidence of infections (Fahlman and Engels 2005, Neville et al. 2008, Gleeson 2000, Gleeson and Bishop 2013). More recently however, the importance of other antimicrobial proteins (AMPs) in saliva has gained greater recognition. There are a diverse range of AMPs in saliva, many of which act to form a protective barrier against bacteria and other pathogens. Two of the most abundant AMPs present in the mucosal secretions of the upper respiratory tract are salivary lysozyme (SLys) and salivary lactoferrin (SLac). The presence of AMPs such as SLys and SLac in salivary secretions without prior exposure to infectious agents is indicative of their integral role in the innate immune system (West et al. 2006a).

Immune resilience is an important factor in the success of an elite athlete (Malm 2004). It is known that prolonged and intense periods of exercise can cause transient perturbations in many cellular and hormonal immune factors (Gleeson et al. 2013). A small collection of studies have investigated the effects of endurance exercise on salivary AMPs. Koutedakis and colleagues (Koutedakis et al. 1996) were the first to report significant reductions in salivary flow rate (FR) and SLys concentrations following exercise in elite swimmers. Other studies have reported equivocal results; short-duration, high intensity exercise led to increased secretion rates of AMPs such as SIgA and SLys in active men (Allgrove et al. 2008) and prolonged endurance exercise either decreased SIgA with no change in SLys secretion rates (Gill et al. 2013, Gillum et al. 2013) or resulted in no change in SIgA concentrations (Davison et al. 2009, Blannin et al. 1998, Li and Gleeson 2005). The variation in the findings of these studies may be due to the differences in exercise intensity, duration and relative stress on the participant. Furthermore, there are numerous possible mechanisms by which exercise could affect the concentration and secretion of AMPs both acutely and chronically including; increased secretion of neuropeptides, secretion of AMPs induced by proinflammatory cytokines, secretion of AMPs from neutrophils or damaged epithelial cells or simply hyperventilation during exercise resulting in drying of the respiratory tract (for detailed review see (West et al. 2006b)). In addition, circulating stress hormones such as cortisol have been implicated in the inhibition of salivary IgA (Hucklebridge et al. 1998) and Lys (Perera et al. 1997) production, mobilization and secretion.

Saliva secretion is regulated by the autonomic nervous system and humans typically produce ~1500 mL saliva per day (Gleeson et al. 2013). However, fluid balance studies have observed significant reductions in salivary FR when in a state of hypohydration (Gill et al. 2013, Fortes et al. 2012). Fortes and colleagues reported significant reductions in salivary FR and secretion of AMPs at a modest dehydration of -3% body mass (BM) loss induced by fluid restriction (Fortes et al. 2012). Dehydration of 1.3-2.4% BM loss resulting from prolonged endurance exercise has also been shown to reduce salivary FR (Gill et al. 2013). In addition to a reduction in salivary FR the authors reported a significant decrease in SIgA secretion rates but no change in SLys. During exercise, the concentration of the saliva, and its FR, are thought to be influenced by sympathetic nervous activity and the hypothalamic-pituitary-axis (HPA axis) (Allgrove et al. 2008). It has been proposed that increases in protein content are influenced by noradrenalin release via sympathetic nervous stimulation, whereas FR is considered to respond primarily to parasympathetic stimulation (Chicharro et al. 1998).

In line with the well-established guidelines, athletes are regularly advised to drink sufficient fluids before, during and after exercise to limit dehydration to <2% loss in BM (American Dietetics Association, Dietitians of Canada, 2009, Maughan and Shirreffs, 2011). However studies suggest that commencing exercise in a mild state of hypohydration is not uncommon across athletic populations (Garth and Burke 2013, Volpe et al. 2009, Maughan et al. 2005). Furthermore, it has been shown that many athletes fail to consume sufficient fluids during exercise to offset fluid loses, resulting in levels of dehydration in excess of 2% BM loss (Gore et al. 1993, Kurdak et al. 2010). Exercising in a state of hypohydration has been shown to increase cardiovascular strain (increased heart rate (HR)) (Adams et al. 2014) and to significantly increase plasma cortisol levels (Maresh et al. 2006) compared to euhydration. Thus the potential for a disturbed immune response when exercising in a state of hypohydration is significantly augmented.

It is apparent that prolonged exercise can effect secretion of salivary AMPs however it is not known how the hydration status of an athlete may affect these changes. The aim of this study was to investigate the effects of moderate dehydration during prolonged endurance cycling on exercise performance and salivary markers of immune function.

Materials and Methods

Participants

Ten recreational male cyclists were recruited to participate in this study. Participant characteristics are presented in Table 1. Inclusion criteria required participants to be weight stable, involved in regular exercise, complete a general health questionnaire without reporting any chronic or underlying illness or disease, free from medications that may disrupt fluid-electrolyte balance and to have not suffered any symptoms of an upper respiratory tract infection during the four weeks prior to the study. Females were not included in the study due to potential disruptions in fluid balance resulting from the menstrual cycle. All participants were informed of the purposes of the study and the risks associated with the procedures. Written informed consent was obtained from each participant and a health questionnaire was completed before the study commenced. The study was approved by the Loughborough University ethical advisory committee.

Table 1: Participant Characteristics

	Age (y)	**Height (cm)**	**Body Mass (kg)**	**$\dot{V}O_{2max}$ (ml/kg/min)**
Mean ± SD	21 ± 1	178.0 ± 7.9	72.4 ± 8.0	58.2 ± 6.2

Experimental Design

Participants underwent two experimental trials in a randomised, counterbalanced-cross over design, consisting of 2 h cycling on an electronically braked ergometer at 60% $\dot{V}O_{2max}$ in either a euhydrated (EH) or a moderately dehydrated state (DH). Saliva samples were collected pre-exercise, throughout the exercise session and each

hour for 3 h post-exercise. Urine samples were collected pre- and immediately post-exercise and again 2 h (blood) and 3 h (urine) post-exercise. Trials were separated by a minimum of 6 days.

Pre-trial Tests & Familiarisation

Prior to the exercise trials, participants completed an incremental cycle exercise test to volitional exhaustion on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) in order to determine $\dot{V}O_{2max}$. Briefly, following a 10 min warm up at 70 Watts (W), participants began the exercise test at 95 W, undergoing 35 W increments every 3 min. Breath samples were collected from participants into Douglas bags during the final minute of each stage and rating of perceived exertion (RPE) was recorded (Borg, 1998). Heart rate (HR) was measured throughout the test using short-range telemetry (Polar RS800CX, Kempele, Finland). A paramagnetic oxygen analyser (Servomex 1420B, Crowborough, UK) and infrared carbon dioxide analyser (Servomex 1415B) were used in combination with a dry gas meter (Harvard Apparatus, Edenbridge, UK) for determination of $\dot{V}_E$, $\dot{V}O_2$ and $\dot{V}CO_2$. The work rate in Watts corresponding to 60% $\dot{V}O_{2max}$ was then calculated from the $\dot{V}O_2$–work rate relationship using a linear equation. Following a 15 min rest period, during which time participants were familiarised with the saliva collection protocols, participants completed a 20 min familiarisation at 60% $\dot{V}O_{2max}$ representing the workload of the subsequent trials. Expired gas samples were collected during each trial at 5, 15 and 20 min to ensure the calculated work rate elicited the desired relative intensity. In addition to the pre-trial $\dot{V}O_{2max}$ test, participants were required to visit the laboratory on the final three mornings prior to their first trial to establish fasted baseline body mass. On each occasion nude body mass was recorded, following a urine void, and the average of these data was used to estimate baseline BM at euhydration (Seca, GmbH, Germany, accurate to 1 d.p.).

Exercise Trials

Participants reported to the laboratory between 07:00-09:00 for each exercise trial having completed an overnight fast (≥10 h). Each participant performed both trials at the same time of day to reduce inter-trial effects of diurnal variations in cortisol (Petrovsky et al. 1998, Kanaley et al. 2001). Participants were requested to complete a 24 h weighed food diary prior to their first trial and to avoid consumption of foods with a high fluid content. Participants were then instructed to replicate their nutritional intake during the 24 h prior to their second trial. Participants were also requested to abstain from any physical activity and alcohol consumption for 48 h prior to each trial to help prevent disturbances in fluid balance. Prior to commencing exercise trials, participants provided a urine void, nude BM measurement and a saliva and blood sample for analysis. Participants cycled for 120 min at 60% $\dot{V}O_{2max}$ on a stationary cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) in a laboratory environment maintained at 21.0 ± 1.0 ˚C. Saliva samples and expired gas were collected at 15, 45, 75 and 105 min of exercise. Heart rate was recorded continually during each exercise trial with short range telemetry (Polar RS800CX, Kempele, Finland). Participant's RPE scores were recorded at 15, 45, 75 and 105 min (Borg, 1998). Post-exercise saliva, blood and urine samples were collected following exercise cessation and BM was measured before participants were provided with 500 mL water. Participants remained in the laboratory for 180 min, sitting quietly, to enable hourly saliva sampling and a final blood (120 min) and urine sample to be collected (180 min).

Treatment

To induce DH, participant's fluid intakes were restricted to 500 mL water during the 24 h period pre- trial. Furthermore, no water was provided prior to or during the 2 h cycling protocol. On completion of the exercise trial, following saliva and urine sampling, participants were provided with 500 mL water. During EH, participants were encouraged to consume their normal fluid intakes during the 24 h leading into the trial. In addition, participants were provided with 500 mL water before commencing the exercise trial and given an additional 250 mL water every 20 min during exercise. The exercise protocol was identical for both trials.

Measures and Analysis

Unstimulated saliva samples were collected at each time point; pre-exercise (baseline), post-exercise (immediately following exercise cessation) and again at 1 h, 2 h and 3 h post-exercise. Participants were instructed to be the seated position, leaning forward with their head tilted forward and asked to swallow to empty their mouth of any residual saliva before the timed sample collection began. Saliva collections lasted 3 min, during which time participants were requested to minimise orofacial movement and passively dribble into a pre-weighed vial. Samples were weighed to estimate saliva volume and micro-centrifuged to remove cells and insoluble matter before storing at -20°C. The saliva flow rate (ml/min) was determined by dividing the volume of saliva by the collection time. Subsequently, saliva samples were analysed for SIgA using an ELISA kit (Salimetrics, Philadelphia, USA) and both lactoferrin and lysozyme were analysed using commercially available ELISA kits (Calbiochem, USA and Biomedical Technologies, USA, respectively). Secretion rates for each of the salivary AMPs were calculated as the multiple of the saliva FR and the antimicrobial protein concentration. All saliva assays were carried out in duplicate. The intra-assay CV for SIgA, lactoferrin and lysozyme were 1.8%, 8.1% and 5.3%, respectively. Urine osmolality was analysed via freezing-point depression using a single sample osmometer (Osomamat 030, Gonotec, Berlin, Germany). Venous blood samples were collected from an antecubital vein immediately before commencing exercise trials, immediately post-exercise and at 120 min post-exercise. Blood samples were collected into a K3EDTA vacutainers and analysed for haematocrit via an automated cell-counter (A^c.T 5diff haematology analyser, Beckman Coulter, High Wycombe, UK). Remaining blood was centrifuged and stored at -20˚C for later analysis of cortisol. Plasma cortisol concentrations were determined using a commercially available solid phase enzyme-linked immunosorbent assay (IBL International, Hamburg, Germany).

Statistical Analysis

All data were analysed using statistical software (IBM SPSS Statistics 21) and are presented as Mean ± Standard Deviation. Changes in hydration and saliva markers and plasma cortisol were analysed using a 2-way repeated measures analysis of variance (ANOVA). A Bonferroni adjustment was included into the analysis to correct for multiple comparisons. Data sets that were found to be significantly non-normal (SLys and SLac) were log transformed prior to analysis. Between trial differences in performance parameters (mean HR, $\dot{V}O_2$and RPE) were determined using an independent samples t-test. The level of significance was set at $p<0.05$.

Results

Hydration Variables

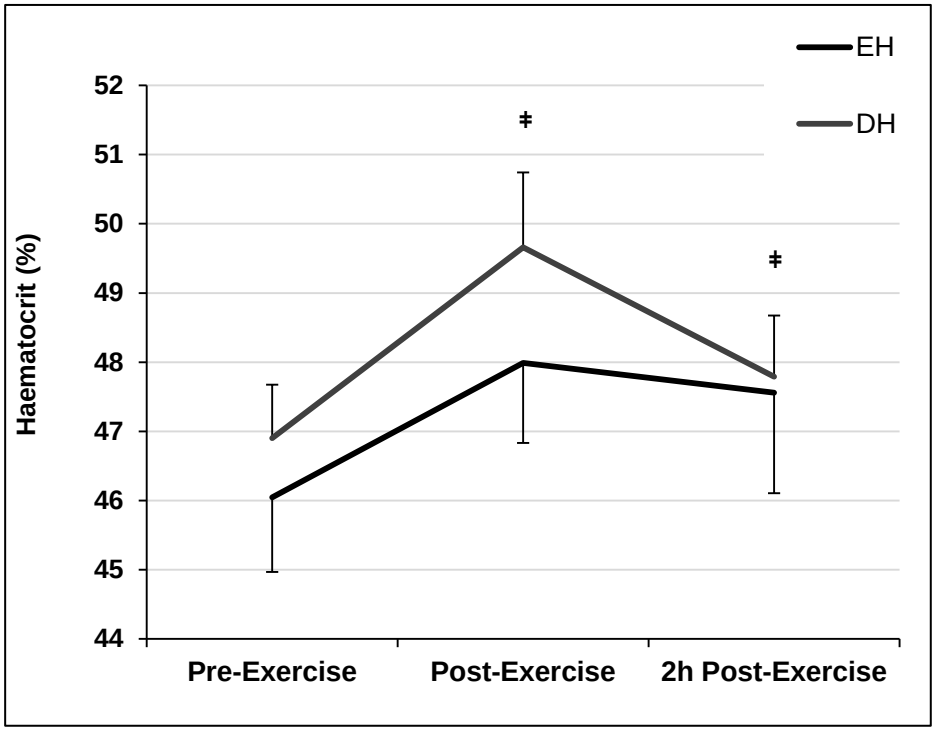
Table 2 provides an overview of the hydration variables during each trial. Twenty-four hour fluid restriction resulted in a 1.5 ± 0.5% loss of body mass from baseline and a 4.3 ± 0.7% loss immediately post-exercise. No differences in BM were reported between pre-exercise and baseline BM (72.5 kg ± 8.0 vs. 72.4 kg ± 8.0, respectively) in EH. Urine osmolality was significantly higher at pre-and post-exercise in DH compared to EH ($p<0.05$). Pre-exercise haematocrit was similar between conditions (46.9 ± 3.4 vs 46.0 ± 3.4%, for DH and EH, respectively) and was significantly elevated by exercise in both conditions (46.5 ± 0.9 to 48.8 ± 1.0%; $p<0.01$), remaining elevated at 2 h post-exercise (47.7 ± 1.0%; $p<0.01$; Fig 1). No difference in pre-exercise salivary FR was observed between conditions. However, immediately post-exercise and for the remainder of the trial, FR was significantly higher in EH than DH ($p<0.05$).

Table 2: Overview of Hydration Variables

	Body Mass (kg)		**Urine Osmolality (mOsm/kg)**		**Haematocrit (%)**	
	EH	DH	EH	DH	EH	DH
Pre-Exercise	72.5 ± 8.0	71.3 ± 8.0	721 ± 237	958 ± 134*	46.0 ± 3.4	46.9 ± 2.4
Post- Exercise (0 min)	71.6 ±7.7	69.4 ± 7.8	584 ± 252	918 ± 137*	48.0 ±3.7ǂ	49.7 ± 3.4ǂ
Post-Exercise (120 min)	72.0 ± 8.4	70.9 ± 6.7	NA	NA	47.6 ± 4.6	47.8 ± 2.8

Data are means ± SD. * Indicates significant condition effect. ǂ Indicates significant change from pre-exercise.

Figure 1: Haematocrit levels at pre-exercise, immediately post-exercise and at 2 h post exercise for Euhydrated (EH) and Dehydrated (DH) trials. Data are means ± SE. ǂ Indicates significant change from pre-exercise.



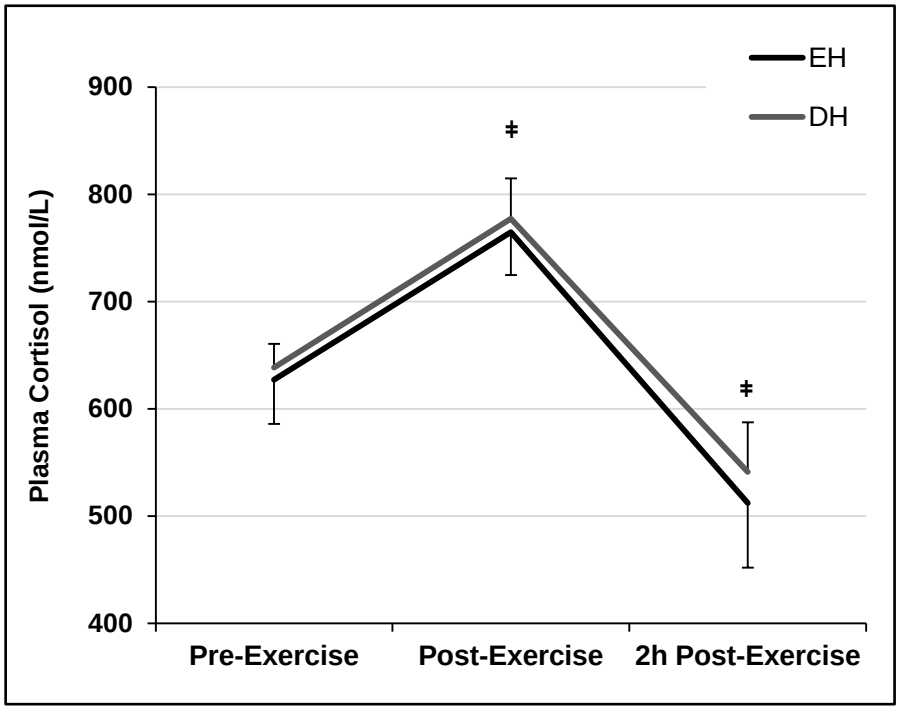
Performance Data

Average power output during trials was 175 ± 22 W, resulting in an exercise VO_2 of 60 ± 4% $\dot{V}O_{2max}$ with no difference between trials. Mean HR and RPE were significantly higher throughout exercise in DH compared to EH (157 ± 13 vs. 151 ± 11 bpm; $p<0.01$, and 14.1 ± 2.2 vs. 13.0 ± 1.8 RPE; $p<0.05$).

Haematology

Figure 2 illustrates plasma cortisol concentrations pre-exercise, immediately post- and 2 h post-exercise. Exercise elicited a significant rise in plasma cortisol levels in both conditions (632 ± 26 to 771 ± 33 nmol/L; $p=0.01$). At 2 h post-exercise, cortisol concentrations had fallen and were significantly lower than pre-exercise levels ($p=0.03$).

Figure 2: Plasma cortisol concentrations at pre-exercise, immediately post-exercise and at 2 h post exercise for Euhydrated (EH) and Dehydrated (DH) trials. Data are means ± SE. ǂ Indicates significant change from pre-exercise.



Salivary Analysis

Salivary AMP concentrations are presented in Table 3. There was a significant increase in SLac concentrations with exercise ($p<0.01$) which returned to pre-exercise values by 1 h post-exercise in EH, but remained elevated in DH. Overall, SLac concentrations were higher in DH than EH ($p=0.02$). Pre-exercise SLys concentrations were significantly lower in DH compared to EH ($p<0.05$). Exercise resulted in an increase in SLys concentration ($p<0.01$), which remained elevated above pre-exercise levels during DH, but returned to pre-exercise concentrations in EH by 1 h post-exercise. Pre-exercise SIgA and SLac concentrations were not difference between conditions. Following exercise SIgA concentrations were higher in DH than EH; overall SIgA concentrations were significantly higher in DH than EH ($p<0.01$).

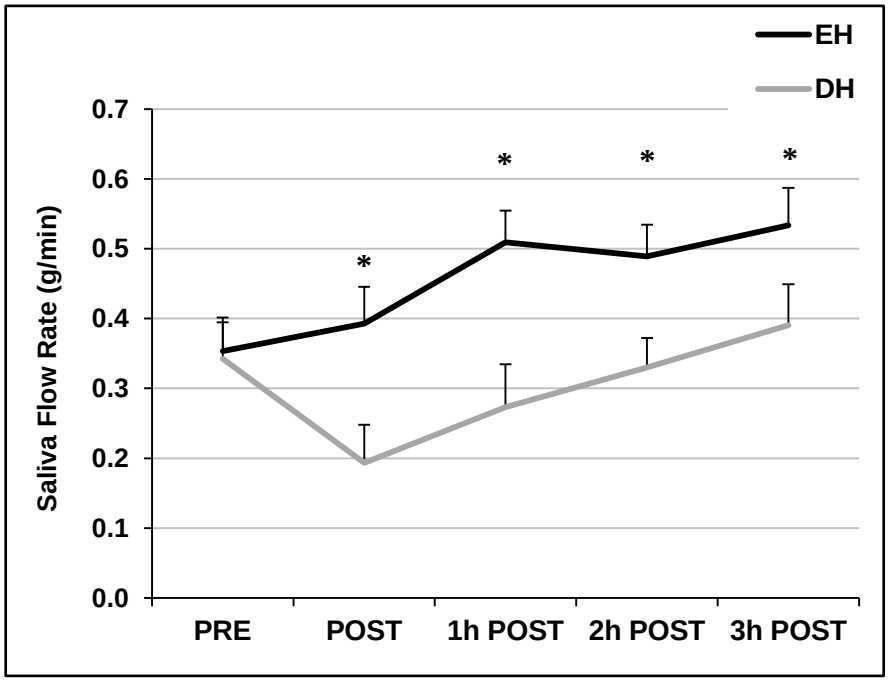
Table 3: Salivary AMP concentrations pre- and post-exercise for Euhydrated (EH) and Dehydrated (DH) trials.

	SLac (mg/L)		SLys (mg/L)		SIgA (mg/L)	
Mean ± SD	EH	DH	EH	DH	EH	DH
Baseline (Pre-Exercise)	5.0 ± 5.5	3.5 ± 2.8	5.5 ± 6.7	1.6 ± 2.0*	104.9 ± 84.4	160.6 ± 134.3
Post-Exercise	11.9 ± 7.0ǂ	15.0 ± 12.7ǂ	11.1 ± 4.8ǂ	9.5 ± 9.1ǂ	136.9 ± 98.3	309.3 ± 271.2*
1 h Post	4.8 ± 3.1	13.6 ± 9.3*ǂ	2.4 ± 1.9	9.1 ± 5.3*	73.9 ± 32.5	241.6 ± 214.7*
2 h Post	7.9 ± 8.6	18.7 ± 21.0ǂ	2.1 ± 2.8	9.5 ± 7.4*	87.9 ± 56.2	239.9 ± 140.7*
3 h Post	5.4 ± 4.5	15.3 ± 12.3*ǂ	3.1 ± 3.5	8.9 ± 6.7	74.6 ± 41.6	246.6 ± 146.6*

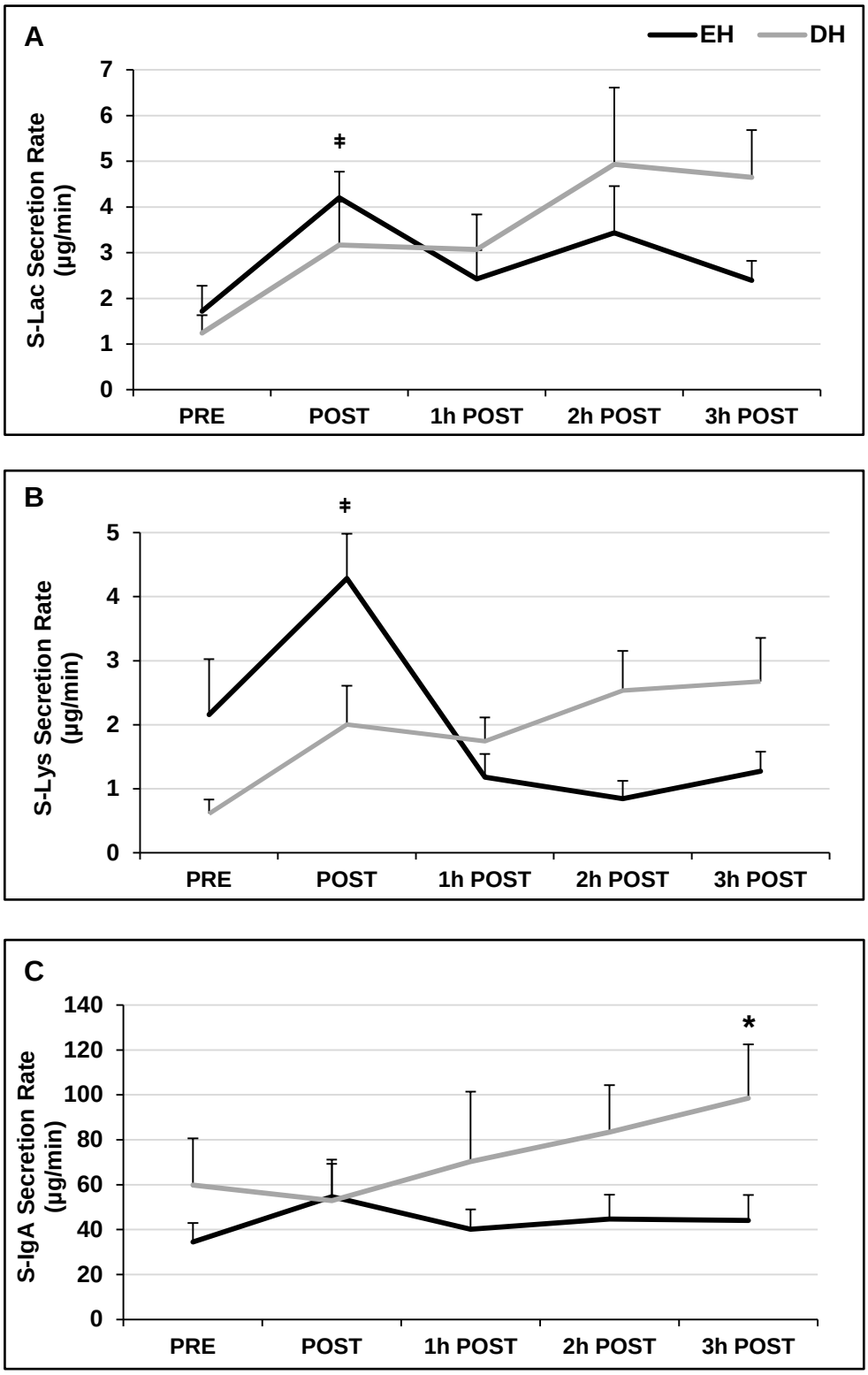
Data are means ± SD. * Indicates significant difference between conditions. ǂ Indicates significant change from pre-exercise.

No differences were observed between pre-exercise salivary FR, however FR was significantly lower during DH than EH at all time points during and post-exercise ($p<0.05$; Fig 3). SLac secretion rate increased significantly following exercise ($p=0.01$; Fig 4A). No differences between EH and DH were observed. Exercise elicited a significant increase in SLys secretion rate in both conditions; with a tendency to be higher in EH than DH ($p=0.05$; figure 4B). SLys secretion rate fell significantly after exercise in EH ($p=0.02$) but remained elevated in DH at the end of the trial. SIgA secretion rates were not acutely effected by exercise; however, at 3 h post-exercise SIgA secretion rates were significantly higher in DH than EH ($p<0.01$; Fig 4C).

Figure 3: Salivary flow rate pre- and post-exercise for Euhydrated (EH) and Dehydrated (DH) trials. Data are means ± SE. * Significant difference between conditions.



Figures 4A-C: AMP secretion rates pre- and post-exercise for Euhydrated (EH) and Dehydrated (DH) trials. Data are means ± SE. * Significant difference between conditions. ǂ Significant change from pre-exercise.



Discussion

The aims of the present study were to investigate the effects of moderate dehydration during prolonged endurance cycling on exercise performance and salivary markers of immune function. In the knowledge that many athletes commence exercise with pre-existing fluid imbalances (American College of Sports Medicine 2007), we were interested in the effects of prior dehydration (with fluid restriction) on exercise performance and immune function during exercise and short term recovery. The main findings of this study were that exercise in a mildly dehydrated state caused a chronic reduction in salivary FR and resulted in transient changes in salivary AMPs during and immediately post-exercise that had mostly returned to pre-exercise levels by 3 h of recovery.

A small collection of studies have reported the effects of a single bout of exercise on AMPs involved in host defence. Allgrove and colleagues investigated salivary AMPs in acute exercise at 50%,70%$\dot{V}O_{2max}$ and during an incremental test to exhaustion and observed temporal increases in secretion rates of SIgA and SLac at exhaustion, post-exercise increases in SLys secretion rate at 70% $\dot{V}O_{2max}$ and no effects at 50% $\dot{V}O_{2max}$(Allgrove et al. 2008). The authors concluded that sympathetic stimulation during high intensity exercise was sufficient to increase SIgA and SLys transport, despite the short-duration of the exercise period. No studies have investigated the effects of endurance exercise (>90 min) on AMPs, with the exception of two field studies which analysed SIgA and SLys (and SLac (Gillum et al. 2013)) during ultra-marathon racing and reported equivocal results. The authors of the multi-stage ultra-marathon observed exercise-induced body mass losses over the duration of the race and postulate that hydration status may play a role in protecting the upper respiratory trace when exercising (Gill et al. 2013).

Current hydration guidelines advise athletes to limit body mass losses during exercise to no greater than 2% in order to prevent deleterious effects of dehydration on exercise performance and health (American Dietetics Association, Dietitians of Canada 2009, Maughan and Shirreffs 2011). Dehydration has been shown to increase cardiovascular strain, core temperature, heart rate, perceived exertion response (Sawka and Coyle 1999) and a mean change in HR of 3 bmp for every 1% change in BM loss has recently been reported (Adams et al. 2014), ultimately inhibiting exercise performance. (Institute of Medicine 2005). In line with these findings, we observed significantly higher mean HR and RPE at the same work rate in DH compared with EH. Twenty-four hour fluid restriction in this study resulted in a mean body mass loss of 1.5%, (0.3-1.9 kg loss). Following exercise in DH, mean body mass loss was 3.9% (2.1-4.2 kg loss). Pre-exercise urine osmolality was 972 mOsmol/kg (777-1113 mOsmol/kg) in DH compared to 687 (353-1092 mOsmol/kg) in EH and haematocrit was 47 % (43-50 %) and 46 % (43-50 %) for DH and EH, respectively. These data highlight the large individual variability in sweat rates and fluid homeostasis between individuals undergoing the same level of fluid restriction.

Given the known effects of fluid intake (Bishop et al. 2006) and sweat rates (Oliver et al. 2007, Walsh et al. 2004) on salivary FR, the potential for exercising in a dehydrated state to interfere with salivary AMP activity is high. Despite this, the effects of hypohydration on salivary AMPs during exercise are relatively understudied. To the author's knowledge, only one study has investigated the effects dehydration and exercise on salivary AMPs. Fortes and colleagues investigated the effects of dehydration caused by exercise in the heat, with subsequent overnight fluid restriction on immune function (Fortes et al. 2012). The authors reported a significant reduction in salivary FR immediately following exercise in the dehydration trial, which remained

suppressed until rehydration was permitted the following morning. In addition, they observed an increase in SIgA concentration, with no change in secretion rate and a decrease in SLys secretion rates with no change in concentration. Other studies that have investigated the effects of exercise on SLys, independent of hydration status, have reported decreased SLys concentrations (Koutedakis et al. 1996), increased secretion rates (Allgrove et al. 2008) and no change in secretion rates (Gill et al. 2013, Gillum et al. 2013) following a range of exercise sessions.

We did not observe any differences in pre-exercise salivary FR between EH and DH, despite 24 h fluid restriction. However, during exercise, salivary FR decreased significantly in DH, with no change in EH, and remained significantly lower than pre-exercise levels until 2 h post-exercise. SIgA secretion rates were not acutely affected by exercise; however by 3 h post-exercise SIgA secretion rates were significantly higher in DH than EH. We also observed significantly higher concentrations of SIgA and SLac in DH than EH throughout the trial, likely the result of a concentrating effect from the attenuated salivary FR caused by dehydration. SLac secretion rates increased with exercise, with no differences between conditions. Participants in both trials presented a significant increase in SLys concentrations which returned to pre-exercise values within 1 h post-exercise in EH but remained elevated for the duration of the trial in DH. Furthermore, we observed a transient increase in SLys secretion rate despite no change in salivary FR during exercise in EH. Despite a reduction in salivary FR in DH, we observed an increase in SLys secretion rates that remained elevated for the duration of the trial.

Due to the differences in salivary FR between conditions, it is important to consider secretion rates when looking at AMP immune responses, and not absolute concentrations. Increases in SIgA secretion rates in DH (above that reported in EH) and elevated SLys secretion rates above pre-exercise values at 3 h post exercise in DH suggest that hypohydration exacerbated the immune response to endurance exercise. Whilst we did not observe any significant differences in cortisol concentrations between conditions, it may be possible that a heightened stress response in DH (evidenced by increased HR and RPE during exercise) resulted in a great immune response. Furthermore, it has been suggested that high-intensity exercise may increase the mobilization of SIgA into the saliva via sympathetic nervous activity (Allgrove et al. 2008), therefore it could be speculated that the increased exercise strain in DH effectively increased the relative intensity of the exercise and caused a greater mobilization of SIgA.

Exercising in a state of severe hypohydration (5% and 7% BM loss) has previously been shown to increase resting and post-exercise cortisol concentrations compared to euhydration (Maresh et al. 2006, Francesconi et al. 1985). However, moderate dehydration in this study did not influence resting cortisol, or differentially effect post-exercise response compared to euhydration in this study. These data support the findings of Mitchell and colleagues, who reported no effect of hydration status (~1 % BM loss versus EH) on cortisol concentrations when exercising in an ambient environment (Mitchell et al. 2002). It is interesting that we observed higher RPE and HR in DH compared to EU without seeing differences in cortisol. This may be explained by relatively high pre-exercise concentrations due to the diurnal pattern of cortisol and the fact that testing was carried out early in the morning which may have somewhat masked an influence of hydration status. Alternatively, the level of dehydration may not have been sufficiently severe to trigger an increased stress response. In conclusion, we observed transient changes in salivary AMPs during and immediately post-exercise that had mostly returned to pre-exercise levels by 3 h of recovery, with little influence of pre-exercise hydration status on these responses.

The responses may have been greater if participants were exercising in the heat and/ or if the level of dehydration was greater; however, exercising in thermoneutral temperature at low-moderate levels of dehydration is a more common occurrence in most athletic populations and therefore this was chosen as the subject of the present investigation. It may have been beneficial to collect a saliva sample prior to the 24 h fluid restriction to provide additional insight into the impact of mild dehydration on mucosal immunity and therefore future studies should include this measurement. It would appear that the moderate levels of dehydration achieved in this study do not impair salivary AMP responses to endurance exercise and are therefore unlikely to be of clinical relevance.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Ethical Standards

Experiments carried out within this study comply with current UK laws.

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