

The effects of exercise, sex, and menstrual phase on salivary antimicrobial proteins

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ABSTRACT

*Salivary antimicrobial protein (AMP) expression is a primary determinant of mucosal immunity. This expression can be altered by exercise. While salivary IgA has been extensively studied, less is known about Lysozyme (Lys) and Lactoferrin (Lac). Knowledge on how sex and menstrual phase affect mucosal immunity is also limited. The purpose of this study was to examine how sex, menstrual phase, and exercise impact IgA, Lys, and Lac expression. Men (n=9) and women (n=9) ran for 45 min at 75% VO_{2peak} . Women were tested in the follicular and luteal phase. Saliva was collected pre-exercise, immediately post-exercise and 1 h post-exercise. Pre-exercise, women had higher secretion rates of IgA compared to men (154 ± 106 vs 85 ± 44 $\mu\text{g}/\text{min}$) ($p < 0.05$). Lac secretion rate increased with exercise in both sexes and remained above baseline 1 h after exercise in men (7460 ± 4839 ng/min), but had returned to pre-exercise levels at 1 h post-exercise in women (5720 ± 4661 ng/min) (time*sex interaction, $p < 0.05$). Men had higher secretion rates of Lys ($p < 0.05$) at each time point compared to women (Men pre-exercise: 31042 ± 23132 , post-exercise: 29521 ± 13205 , 1 h post-exercise: 41229 ± 31270 ng/min vs Women pre-exercise: 11585 ± 10367 , post-exercise: 22719 ± 19452 , 1 h post-exercise: 17303 ± 11419 ng/min). Both sexes increased the secretion rate of Lys and Lac with exercise, whereas IgA was unchanged. Menstrual phase did not affect IgA, Lys, or Lac and men and women did not differ in saliva flow rates. In conclusion, regularly menstruating women who are not taking hormonal contraceptives differently express AMPs compared to men.*

Keywords: Lactoferrin; Lysozyme; IgA; upper respiratory symptoms; oestrogen

Conflict of Interest

The authors report no conflict of interest with the present study. The authors alone are responsible for the writing and content of this paper.

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INTRODUCTION

The mucosal immune system, comprised primarily of salivary IgA, lactoferrin (Lac), and lysozyme (Lys), serves as the first line of defence against invading pathogens at the mucosal surface (21, 42). IgA is the most abundant antibody at the mucosal surface and is the most commonly researched biomarker for innate mucosal immunity during exercise (29). Although IgA has been well studied, it is necessary to assess other constituents of the mucosal immune system that are important to immune protection. The two most abundant antimicrobial proteins (AMPs) are Lys and Lac. These AMPs are produced by salivary glands and epithelial cells, and are located in neutrophils (14). Lys provides protection against gram-positive bacteria (28) while Lac confers protection by inhibiting iron uptake to reduce bacterial growth (43). Lys and Lac function synergistically to augment immunity (12) and Lac may improve Lys ability to remove gram-positive bacteria (28). In short, Lac and Lys play an important role in host defence at the mucosal barrier.

The potential for IgA, Lys, and Lac to be influenced by sex is appropriate because they arise from mucous membranes and mononucleated cells (25, 35), both of which have oestrogen receptors (13). In addition, salivary glands and the oral epithelium express both oestrogen receptor β (41) and progesterone receptors (34), which have been shown to affect saliva composition and flow rate.

Epidemiologic data suggests that active (30) or athletic (24, 27) women experience more upper respiratory symptoms (URS) than men. URS are partially linked to mucosal immunity (16, 23, 26, 31, 36). Salivary IgA concentrations and secretion rates are higher in athletic men compared to athletic women at rest (20) and immediately prior to both prolonged cycling (2) and maximal exercise (38).

Importantly, previous work to quantify sex differences in IgA after exercise has failed to control for both hormonal contraceptive use and menstrual phase and/or status. This lack of control may confound the prior work that has been done in this area. For example, eumenorrhoeic runners demonstrated significantly higher salivary IgA secretion rates and fewer URS than amenorrhoeic runners (39). Similarly, higher salivary IgA concentration and relative IgA (IgA:100 mg protein) was found in resting women compared to men when menstrual phase and contraceptive use was controlled (22). Leukocytes, neutrophils, and monocytes were higher in women who took oral contraceptives compared to both men and women who were not taking oral contraceptives after cycling for 90 min at 65% $\text{VO}_{2\text{pk}}$ (40). Furthermore, the mRNA of both pro- and anti-inflammatory cytokines were different between sexes and menstrual phase after running for 60 min at a moderate intensity (33). After prolonged, exhaustive running, IL-1ra, IL-6, and IL-10 were different between men and regularly menstruating women not taking hormonal contraceptives (1). When taken together, these studies imply that circulating concentrations of ovarian hormones differ between phases (or occurrence) of the menstrual cycle and according to hormonal contraceptive use. These fluctuations may alter immune parameters. Therefore, in order to accurately assess the impact of sex, both menstrual phase and hormonal contraceptive use need to be controlled.

There are limited data detailing how physiologic fluctuations of ovarian hormones across the menstrual cycle impact IgA, Lac, or Lys expression in response to exercise. Furthermore, potential sex differences in these saliva parameters are needed if relevant clinical or practical recommendations are to be made. Given that menstrual phase and hormonal contraceptive use have the potential to impact immune parameters after exercise, our purpose was to determine sex and menstrual phase differences in IgA, Lac, and Lys in response to acute treadmill running.

METHODS

Participants. 9 men and 9 women volunteered to participate in this study. All procedures were approved by the Institutional Review Board at California Baptist University and subjects gave their informed, written consent prior to participation. All subjects were negative for cardiovascular, pulmonary, and metabolic disease. For at least 3 months prior, subjects had not been ill and were recreationally active, meeting the American College of Sports Medicine's weekly physical activity recommendations (18). Female subjects were eumenorrhoeic. They had maintained a regular menstrual cycle (28-32 days) and had not used hormonal contraceptives for the previous 6 months.

Preliminary Assessment. Male and female subjects were matched according to age and aerobic capacity. Three site skinfold (Lange, Beta Technology, Santa Cruz, CA) measurements (Men: chest, abdomen, thigh; Women: triceps, suprailiac, thigh) were used to determine percent body fat. Each site was measured in duplicate and the mean value was used to calculate percent body fat (5). A continuous graded treadmill test was used to determine VO_{2peak} . This treadmill test started at 6.4 km/h for women and 8 km/h for men with 1% grade. The speed increased 1.6 km/h every minute while the grade remained constant. Subjects ran until volitional fatigue. VO_{2peak} was assessed through open circuit spirometry (Viasys, San Diego CA) and defined as the highest 30-s value when 2 of the following criteria were met: 1) a plateau in VO_2 (change in $VO_2 < 150$ mL/min) with increased workload, 2) a maximal respiratory exchange ratio greater than 1.1, and 3) heart rate greater than 90% of the age predicted maximum (220-age). From the VO_{2peak} testing procedure, a speed that would elicit 75% VO_{2peak} was selected and used for the experimental trial. This workload was shown to optimally increase AMP expression (3). VO_{2peak} was expressed per mL of fat free mass (FFM) so that the aerobic fitness of sexes could be compared. Sex based variations in salivary immune markers may reflect differences in fitness level as elite athletes exhibit higher IgA concentration compared to active or sedentary individuals (17). However, subjects in the current study had similar VO_{2peak} values (when expressed as mL/kg FFM/min) in an effort to minimize the impact of fitness on mucosal markers. Subject characteristics are listed in Table 1.

Experimental Design. Subjects were asked to refrain from exercise and alcohol for 24 h and from caffeine for 12 h prior to testing. They were also instructed to perform an overnight fast (10 h), after which they arrived at our laboratory at

Table 1. Baseline Subject Characteristics.

	Height (cm)	Weight (kg)	Age	% BF	VO _{2pk} (mL/kg FFM/min)
Men (n = 9)	182.1 ± 7.9*	79.7 ± 5.7*	21.1 ± 1.1	11.5 ± 4.2*	63.0 ± 5.4
Women (n = 9)	167.7 ± 7.1	61.4 ± 8.7	22.4 ± 2.4	18.7 ± 3.8	62.6 ± 9.4

Data are mean ± SD. *Between group difference $p < 0.05$

08:00. Subjects were instructed to consume 500 mL of water 1 h before testing to control for hydration. They voided their bowel and bladder before nude body weight was assessed. Subjects ran on a motorized treadmill (Trackmaster, Newton, KS) in a 20°C, 10-15% relative humidity environment for 45 min at a pre-determined speed that elicited 75% of VO_{2peak}. Expired gases were collected every 15 min during exercise and heart rate was recorded every 5 min to ensure appropriate exercise intensity. Nude body weight was assessed after the post exercise saliva collection.

All subjects completed 2 exercise trials. Females completed trials in the follicular (Fol) and luteal (Lut) phase using a counterbalanced design (Fol trial 1: $n = 4$; Lut trial 1: $n = 5$). Men were matched with women to ensure equal time between trials (14.9 ± 0.9 days for women; 17.0 ± 1.7 days for men). Exercise trials for an individual subject were identical in speed, grade, duration, and time of day. Subjects were asked to continue their habitual exercise between trials.

Saliva Collection. During each collection, subjects were seated and asked to swallow to cleanse their mouth prior to un-stimulated collection via passive drool into pre-weighed tubes. Subjects sat with their head tilted forward and were asked to maintain minimal oro-facial movement during collection. Saliva volumes were estimated by weighing to the nearest mg. Density of saliva was assumed to be 1.00 g/mL (8). Flow rate was calculated as the volume of saliva collected divided by the collection time. Secretion rate was calculated as the product of the flow rate and concentration of salivary protein. Saliva was collected at 3 time points: pre-exercise, immediately post-exercise, and 1 h after exercise, as done previously (3). Participants were given 250 mL of water after the post exercise saliva collection in accordance with previous work (3). No other food or drink was consumed until after the 1 h post-exercise saliva collection.

Saliva Analysis. After collection and before storage, saliva was mixed and osmolality was assessed using a freeze point depression osmometer (Advanced Instruments, Norwood, MA, USA) that had been calibrated using 50 mOsm/kg NaCl and 850 mOsm/kg NaCl controls and checked with 290 mOsm/kg NaCl solution according to manufacturer's instruction. Remaining saliva was stored at -80°C for subsequent batch analysis of salivary AMP, with minimal freeze-thaw cycles. These samples were later thawed and analyzed with ELISA according to manufacturer's instruction. IgA (Salimetrics, State College, PA, USA) was detectable at 2.5 µg/mL with an intra-assay coefficient of 4.5% and an inter-assay coefficient of 8.7%. Lac (AssayPro, St. Charles, MO, USA) was detectable at 0.1 ng/mL with an intra-assay coefficient of 4.1% and an inter-assay coefficient of 7.1%. Lys (AssayPro, St. Charles, MO, USA) was detectable at 0.3 ng/mL with

an intra-assay coefficient of 4.3% and an inter-assay coefficient of 7.3%. Data were generated using Gen5 software (BioTek Instruments, Inc, Winooski, VT, USA). All samples for individual subjects were analyzed on the same plate.

Menstrual Phase Analysis. With day 1 as the onset of bleeding, female subjects were tested in the Fol (day 4.1 ± 0.3) and Lut (day 20.4 ± 0.2) phase of their menstrual cycle. Progesterone concentration was used to validate the presence of the Lut phase. 17- β estradiol (Salimetrics, State College, PA) was detectable at 0.1 pg/mL with an intra-assay coefficient of 8.1% and an inter-assay coefficient of 8.9%. Progesterone (Salimetrics, State College, PA) was detectable at 5 pg/mL with an intra-assay coefficient of 8.4% and an inter-assay coefficient of 9.6%. Ovarian hormone concentrations are listed in Table 2.

Table 2. Baseline Ovarian Hormones

	Oestradiol (pg/mL)	Progesterone (pg/mL)
Follicular (n=9)	5.4 \pm 1.2*	98.1 \pm 58.6*
Luteal (n=9)	6.6 \pm 1.1	207.2 \pm 146.9

Data are mean \pm SD. *Between group difference $p < 0.05$

Statistical Analysis. Data in text and in tables are expressed as mean \pm SD. For clarity, data on Figures are expressed as mean \pm SEM. Trial 1 was statistically similar to trial 2 for both men and women. Thus, for simplicity, Figures and Tables that compare sexes combined both trials. A 3 way mixed-design ANOVA (exercise time x trial x sex), using Statistica version 8 (Tulsa, OK, USA), was used to determine the effect of exercise on dependent variables. A 2-way repeated measures ANOVA (exercise time x trial) was used to assess differences in women across the menstrual cycle. Significant differences were further evaluated using Student's paired t test with Holm-Bonferroni adjustments for multiple comparisons. Independent t-tests were used to determine differences between sexes in descriptive data. Pearson's product moment correlation was used to find the association between oestrogen, progesterone, flow rate, IgA, Lac, and Lys. Statistical significance was set at $p < 0.05$. All salivary analytes were log transformed to correct for violations of normality. When appropriate, adjustments were made using Greenhouse-Geisser to account for violations against sphericity.

While we could not find published work regarding sex differences in mucosal immunity (specifically Lac and Lys) after exercise, the estimated sample size was 9 subjects, using an alpha level of 0.05 and a beta level of 0.80, to find differences in mucosal immunity after exercise (3).

RESULTS

This study was designed to answer 2 specific questions. First, does sex affect IgA, Lac, Lys at rest or after 45 min of treadmill running? Second, does menstrual phase affect IgA, Lac, Lys at rest or after 45 min of treadmill running? To aid in reader interpretation, the answers for these two questions will each be discussed

for individual dependent variables starting with sex differences before discussing the effect of menstrual phase.

Sex Differences

Exercise Challenge. Metabolic work was not different between men ($72.5 \pm 3.5\%$ VO_{2peak}) and women ($73.6 \pm 4.7\%$ VO_{2peak}). Dehydration was similar between sexes as body weight reductions of men ($0.8 \pm 0.3\%$) and women ($0.6 \pm 0.3\%$) were not significantly different.

Saliva Analysis. Saliva flow rate and osmolality did not differ between sexes. Saliva flow rate increased from pre-exercise to 1 h after exercise ($p < .00$), while saliva osmolality increased from pre-exercise to immediately post-exercise ($p < 0.05$) (Table 3).

Table 3. Saliva Analysis

	Pre-Exercise		Immediately Post-Exercise		1 Hour Post-Exercise	
	Osm (mOsm/kg)	Flow Rate (mL/min)	Osm (mOsm/kg)	Flow Rate (mL/min)	Osm (mOsm/kg)	Flow Rate (mL/min)
Men	69.4 \pm 15.3	0.53 \pm 0.2	96.6 \pm 30.4*	0.48 \pm 0.2	70.6 \pm 15.6	0.71 \pm 0.4*
Follicular	63.2 \pm 18.3	0.67 \pm 0.5	85.3 \pm 10.1*	0.67 \pm 0.59	68.2 \pm 16.1	0.87 \pm 0.63*
Luteal	67.3 \pm 18.4	0.90 \pm 0.6	91.9 \pm 13.3*	0.73 \pm 0.56	77.7 \pm 14.3	0.93 \pm 0.61*

Changes in saliva pre-exercise, post-exercise, and 1 h after 45 min of treadmill running at 75% VO_{2pk} . Data are mean \pm SD. Data from exercise Trial 1 and exercise Trial 2 were combined for men. * main effect of time, $p < 0.05$ compared to pre-exercise.

IgA Analysis. Women had higher secretion rates ($p < 0.05$) and g:Osm ($p < 0.05$) of IgA than men pre-exercise, but IgA secretion rates were similar between men and women immediately after and 1 h after exercise. There was a main effect of exercise time for IgA secretion rate ($p < 0.05$) and g:Osm ($p < 0.001$). The secretion rate was higher 1 h after exercise than it was before or immediately post-exercise, while post-exercise g:Osm values were lower than pre-exercise and 1 h after exercise (Figure 1 A-C).

Lac Analysis. There was an exercise time x sex interaction ($p < 0.05$) for Lac secretion rate and μ g:mOsm ($p < 0.05$). Both sexes' post-exercise value increased from pre-exercise. However, the Lac secretion rate and μ g:mOsm in women had returned to pre-exercise values 1 h after exercise while men remained elevated at this time point. There was a main effect of exercise time as Lac concentration increased from pre-exercise to post-exercise ($p < 0.05$) (Figure 2 A-C).

Lys Analysis. Men expressed higher Lys concentration ($p < 0.001$), secretion rates ($p < 0.001$), and μ g:mOsm values ($p < 0.001$) than women at all time points. There was a main effect of exercise time for Lys concentration ($p < 0.05$) and secretion rate ($p < 0.05$) as both values increased from pre-exercise to post-exercise. Lys concentration returned to baseline 1 h after exercise while Lys secretion rate remained elevated 1 h after exercise compared to pre-exercise (Figure 3 A-C).

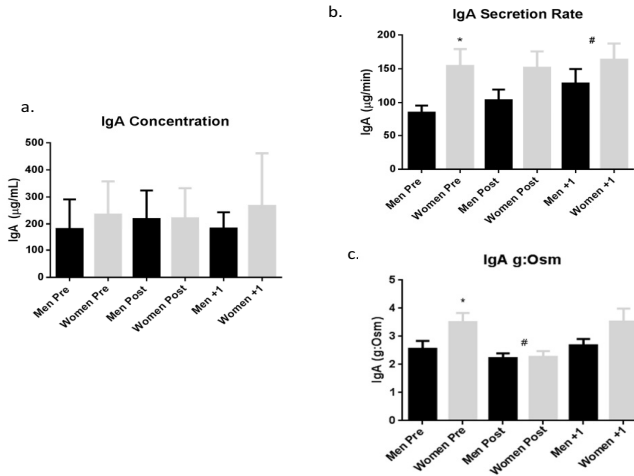


Fig 1. Saliva IgA analysis after 45 min of treadmill running at 75% $\text{VO}_{2\text{pk}}$. Trials 1 and 2 were combined. Data represented as mean \pm SEM. A) IgA concentration. B) IgA secretion rate: * $p < 0.05$ Pre-exercise women compared to pre-exercise men. # $p < 0.05$ main effect of time, 1 h post-exercise greater than pre-exercise and post-exercise samples. C) IgA g:Osm: * $p < 0.05$ pre-exercise women compared to pre-exercise men, # $p < 0.05$ main effect of time, post-exercise compared to pre-exercise and 1 h post-exercise samples.

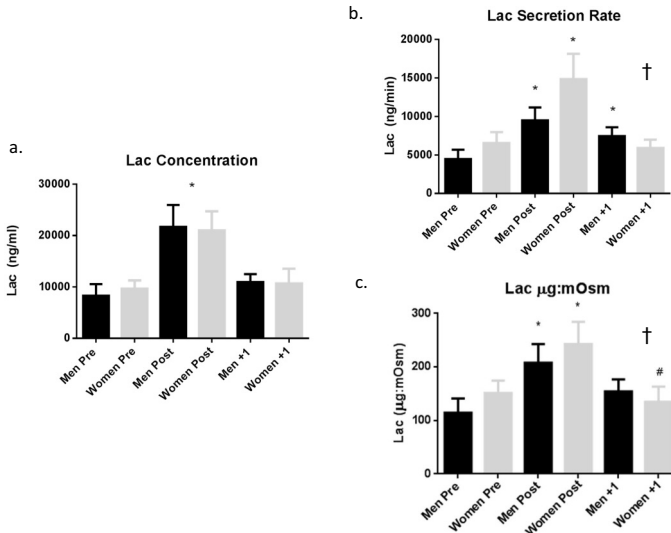


Fig 2. Saliva Lac analysis after 45 min of treadmill running at 75% $\text{VO}_{2\text{pk}}$. Trials 1 and 2 were combined. Data represented as mean \pm SEM. A) Lac concentration: * $p < 0.05$ main effect of time, post-exercise compared to pre-exercise and 1 h post-exercise. B) Lac secretion rate: * $p < 0.05$ from pre-exercise, † time*sex interaction. C) Lac $\mu\text{g:mOsm}$: * $p < 0.05$ from pre-exercise. # $p < 0.05$ from post-exercise, † time*sex interaction.

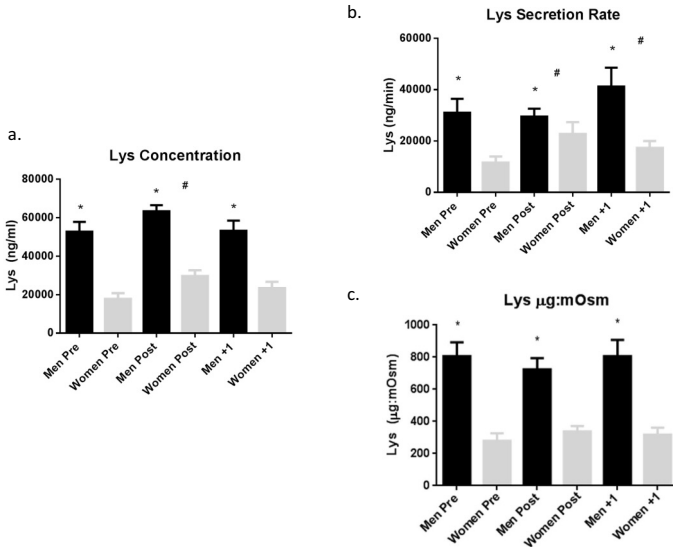


Fig 3. Saliva Lys analysis after 45 min of treadmill running at 75% VO_{2pk} . Trials 1 and 2 were combined. Data represented as mean \pm SEM. A) Lys concentration: * $p < 0.05$ men compared to women, # $p < 0.05$ main effect of time compared to pre-exercise and 1 h after exercise. B) Lys secretion rate: * $p < 0.05$ men compared to women, # $p < 0.05$ main effect of time from pre-exercise. C) Lys $\mu g:mOsm$: * $p < 0.05$ men compared to women.

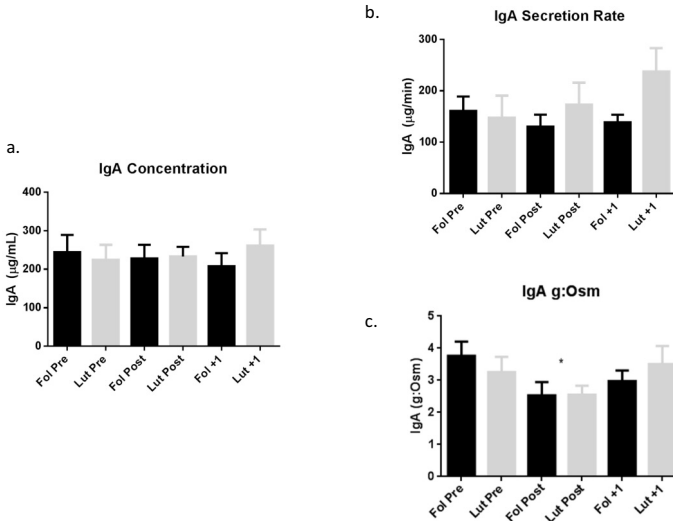


Fig 4. Menstrual phase analysis of salivary IgA after 45 min of treadmill running at 75% VO_{2pk} . Data represented as mean \pm SEM. A) IgA concentration. B) IgA secretion rate. C) IgA $g:Osm$: * $p < 0.05$ compared to pre-exercise.

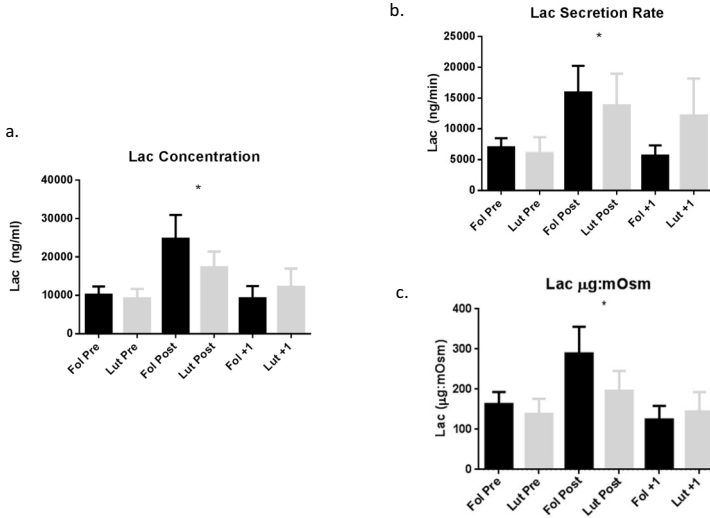


Fig 5. Menstrual phase analysis of salivary Lac after 45 min of treadmill running at 75% VO_{2pk} . Data represented as mean \pm SEM. A) Lac concentration: * $p < 0.05$ compared to pre-exercise. B) Lac secretion rate: * $p < 0.05$ compared to pre-exercise. C) Lac $\mu g:mOsm$: * $p < 0.05$ compared to 1 h after exercise.

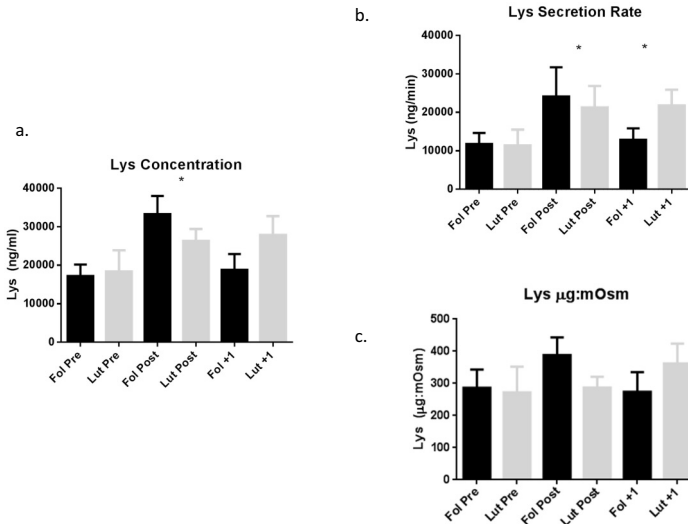


Fig 6. Menstrual phase analysis of salivary Lys after 45 min of treadmill running at 75% VO_{2pk} . Data represented as mean \pm SEM. A) Lys concentration: * $p < 0.05$ compared to pre-exercise. B) Lys secretion rate: * $p < 0.05$ compared to pre-exercise. C) Lys $\mu g:mOsm$.

Menstrual Phase

Ovarian Hormones. Salivary 17- β estradiol concentration was lower in Fol compared to Lut phase ($p < 0.01$), while progesterone values were higher ($p < 0.05$) in the Lut phase (Table 2). Ovarian concentrations were similar to prior work (4) and confirmed that women were tested in the appropriate menstrual phase.

Exercise Challenge. Metabolic work was not different between Fol ($74.2 \pm 4.4\%$ $\text{VO}_{2\text{peak}}$) and Lut ($73.0 \pm 5.2\%$ $\text{VO}_{2\text{peak}}$). Body weight reduction was also not different between Fol ($0.6 \pm 0.3\%$) compared to Lut ($0.7 \pm 0.3\%$).

Saliva Analysis. Saliva flow rate and osmolality were unaffected by menstrual phase. Exercise increased osmolality from pre-exercise to immediately post-exercise ($p < 0.05$) and flow rate increased from pre-exercise to 1 h after exercise ($p < 0.05$) (Table 3).

IgA Analysis. IgA concentration, secretion rate, and $\text{Osm}:\mu\text{g}$ were unaffected by menstrual phase. There was a main effect of exercise time on $\text{g}:\text{Osm}$ ($p < 0.05$). More specifically, pre-exercise values were higher than post-exercise values (Figure 4 A-C).

Lac Analysis. Menstrual phase did not affect Lac concentration, secretion rate, or $\text{Osm}:\text{ng}$. Exercise increased both Lac concentration ($p < 0.001$) and secretion rate ($p < 0.05$) from pre-exercise to post-exercise. Post-exercise $\mu\text{g}:\text{mOsm}$ values were higher than those measured 1 h after exercise (Figure 5 A-C).

Lys Analysis. Lys concentration, secretion rate, and $\mu\text{g}:\text{mOsm}$ was not affected by menstrual phase. Exercise increased Lys concentration ($p < 0.05$) and secretion rate ($p < 0.05$) from pre-exercise to post-exercise and the secretion rate remained elevated 1 h after exercise (Figure 6 A-C).

Correlation. Neither progesterone nor oestrogen was correlated with IgA, Lac, or Lys. Further, neither oestrogen or progesterone was associated with flow rate.

DISCUSSION

The main finding of this study was that men and women differ in AMP expression. Specifically, IgA was higher pre-exercise in women while men's Lys values were higher than women's at all time points. Lac values increased after exercise in both sexes, but remained elevated in men. These changes occurred despite men and women having similar levels of exercise-induced dehydration and similar saliva flow rates. We did not detect any changes in AMP expression in Fol compared to Lut phase. Finally, treadmill running at 75% $\text{VO}_{2\text{peak}}$ increased the secretion rate of Lac and Lys for both sexes.

Sex Differences

Previous work suggests that men have higher IgA levels than women at baseline (2,38) and higher resting IgA secretion rates than women who trained a similar number of hours per week (20). However, this prior work was confounded by the fact that neither hormonal contraceptive use nor menstrual phase was controlled. This is a concern because mucosal immunity in athletic populations has been shown to vary according to the ovarian hormone profile. Indeed, eumenorrhoeic runners demonstrated significantly higher salivary IgA secretion rates than amenorrhoeic runners (26). The authors were only able to find one published report that examined sex differences in salivary IgA and controlled for menstrual phase and hormonal contraceptive use (22). That study reported higher IgA concentration and relative IgA (IgA:100 mg protein) in women compared to men and is consistent with the present analysis. More specifically, our data reflect higher secretion rates and g:Osm of IgA prior to, but not after, treadmill running. As such, these data support the prior observations that sex differences of IgA are only evident at rest and that exercise, *per se*, does not distinctly alter IgA expression between sexes (2, 32).

Since the presence of a menstrual cycle (or ovarian hormone levels) were not accounted for in previous studies that suggest an increase in IgA in men compared to women, the inclusion of amenorrhoeic subjects may partially account for the finding of men possessing higher IgA levels than women. Similarly, higher IgA in men could be due to a blunting of adrenaline (epinephrine) that is seen both at rest (44) and during exercise (37) in the presence of high oestrogen that results during exogenous ovarian hormone intake. Indeed, sympathetic nerve stimulation and adrenaline increase the transport of IgA into the saliva in an animal model (7). Taken together, women consuming exogenous oestrogen may have a blunted adrenaline profile, both at rest and during exercise, and this could lead to lower IgA levels compared to men. The present study suggests that regularly menstruating women who are not taking hormonal contraceptives exhibit higher values compared to men, yet the mechanism responsible for the potential sex difference is unknown. It should be noted that there is high intra- and inter-subject variability in IgA secretion rates and further research is warranted before clinical applications are made.

We are not aware of previously published reports detailing the impact of sex on Lac or Lys in response to exercise. Unlike Lys and IgA, our data suggests that Lac secretion rate and $\mu\text{g}:\text{mOsm}$ between sexes is similar at rest. This is supported by previous epidemiologic work (15). However, also unlike Lys and IgA, exercise affected men and women differently. Both groups increased Lac post-exercise, but men were still above baseline values 1 h after exercise while women had returned to pre-exercise values. Lys, however, was higher in men both at rest and in response to exercise. Epidemiologic data regarding resting Lys concentration and secretion rate are equivocal as stimulated submandibular saliva contained greater concentration in men compared to women (46), while stimulated parotid saliva contained a higher secretion rate of Lys in women compared to men (15). The secretion rate of Lys from unstimulated parotid saliva was congruent

for men and women, and across ages (15). While the clinical significance of sex differences is unknown, our data may have practical applications, and as such, should be the focus of further study.

Menstrual Phase

The present data suggest that physiologic variations of ovarian hormones across the menstrual cycle have a limited effect on AMP expression. Similar data were found for non-trained (22) and trained endurance athletes (6). Specifically, there was no difference in saliva flow rate, IgA concentration, or IgA secretion rate in Fol versus the Lut phase (6, 22). Furthermore, the present data show no correlation between oestrogen or progesterone and IgA or flow rate, which is consistent with others (6). Also, Lys is reported to be consistent throughout the menstrual cycle (1) with no published reports for the menstrual phase effect of Lac. Taken together, while ovarian hormones may be partially responsible for sex differences, the physiologic variation across the menstrual cycle did not significantly alter AMP expression.

Exercise Stress

There was a statistically significant increase in the secretion rate of IgA from pre-exercise to 1 h after treadmill running at 75% $\text{VO}_{2\text{peak}}$. However, IgA concentration and g:Osm did not increase from pre-exercise values. The increase in the secretion rate likely resulted from the increased flow rate. The flow rate was highest 1 h post-exercise. In support of the current data, post-exercise IgA secretion rates have been similar to pre exercise values after 2 h of running at 75% $\text{VO}_{2\text{peak}}$ (9), 2 h of cycling at 65% $\text{VO}_{2\text{peak}}$ (2), and cycling at 75% $\text{VO}_{2\text{peak}}$ for 22 min (3).

Exercise increased the secretion rate of Lac and Lys for both sexes. This is consistent with previous reports of intense, but not sub-maximal, cycling increasing Lys secretion rate and concentration (3). Similarly, vigorous rowing (45) and sprinting (10) increased Lac and Lys concentration immediately post-exercise. Moderate cycling for 2 h, however, decreased Lys secretion rate and concentration immediately post-exercise, but returned to pre-exercise levels within 1 h post-exercise (11). Prolonged, low intensity running did not alter Lac or Lys secretion rates immediately after or 1.5 h post-exercise (19). Thus, Lys and Lac expression may be altered by exercise; however, this expression is dependent on exercise duration and intensity.

Taken together, the maintenance of IgA in combination with an increase Lac and Lys suggest that exercise of this intensity and duration may serve to enhance the immune system and increase protection against URS in both sexes. While clinical markers were not addressed, our data along with Allgrove and colleagues (3) provides guidelines for the intensity of exercise necessary to increase AMP expression.

Limitations

A limitation to the current study, which analyzed the salivary antimicrobial responses of nine men and nine women at rest and during exercise, is the small sample size. This likely contributed to the large variability we reported with salivary AMPs, particularly in women. While this potential limitation is noteworthy, such variability has also been shown to persist in studies that have used much larger n sizes. For example, Gleeson and colleagues examined sex differences in IgA in an athletic population over four months of training. In that study, the coefficient of variation (CV) for resting IgA concentration and secretion rate for 46 men was 64% and 67%, respectively. The CV for resting IgA concentration and secretion rate for 34 women was 43% and 67%, respectively (20). Similar variation was reported for IgA concentration and secretion rate for 50 men and women runners prior to a marathon. In that study, the CV for IgA concentration and secretion rate was 36% and 50%, respectively (data were not analyzed according to sex) (32). As such, while the CVs for pre-exercise IgA concentration (men = 59% and women = 52%) and secretion rate (men = 52% and women = 68%) are certainly higher than desired, they appear to be in line with prior research.

CONCLUSION

AMP expression differs between sexes. Specifically, regularly menstruating women who are not taking hormonal contraceptives express greater IgA while men demonstrate an increase in Lys. Both sexes increased Lac, but this elevation remained longer in men compared to women. Physiologic variations of ovarian hormones across the menstrual cycle do not affect AMP expression. Both men and women experience an increase in Lac and Lys expression in response to acute treadmill running at 75% $\text{VO}_{2\text{peak}}$. Finally, these data highlight the need for greater control in exercise immunology studies that assess the impact of sex.

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