Article

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Mucosal immunity and upper respiratory tract symptoms in recreational endurance runners

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Abstract

Purpose: The present study investigated the effects of a 12-week endurance training intervention on salivary proteins and upper respiratory tract symptoms (URS) in twenty-five young men. **Methods:** Saliva samples of 25 recreational male endurance runners (age 34.6 years, BMI=23.8 $kg \cdot m^{-2}$, VO_{2peak}=47.2 $ml \cdot kg^{-1} \cdot min^{-1}$) were collected before (PRE) and after (POST) the training intervention, in a fasting state, as well as both before and after a maximal incremental treadmill run. The training consisted of both continuous and interval training sessions, 4-6 x per week based on the polarized training approach. Participants filled in Wisconsin Upper Respiratory Symptom Survey-21 and were retrospectively divided into two groups according to whether they reported upper respiratory tract symptoms (URS, n=13) or not (HEALTHY, n=12). **Results:** Basal salivary Immunoglobulin A (sa-sIgA) levels were significantly higher (+70%, $p<0.05$) in HEALTHY both at PRE and POST whereas no significant differences were observed in salivary immunoglobulin M (sa-IgM), salivary immunoglobulin G (sa-IgG), lysozyme or salivary α-amylase activity (sAA). Sa-sIgA concentration at PRE significantly correlated with the number of sick-days ($R=-0.755$, $p<0.001$) in all subjects. The incremental treadmill run acutely increased salivary α-amylase (sAA) significantly ($p<0.05$) at PRE (200%) and POST (166%) in HEALTHY but not in URS. **Conclusions:** This study demonstrated that subjects, who experienced URS during the twelve weeks of progressive endurance training intervention, had significantly lower basal sa-sIgA levels both before and after the experimental endurance training period. In addition to sa-sIgA, acute sAA response to exercise might be a possible determinant of susceptibility to URS in endurance runners.

Keywords: endurance training, exercise immunology, saliva, antimicrobial proteins, health, upper respiratory tract symptoms, performance

Introduction

A high endurance training load has been associated with an increased incidence of upper respiratory tract symptoms (URS) (Gleeson 2000; Gleeson et al. 2011). Nieman (1994) has described the relationship between the risk of contracting an URS and the amount of regular exercise to be J-curved. Thus, a sedentary individual would be at moderate risk, and an individual who is moderately active would exhibit a decreased risk for contracting an URS, while athletes, who are exposed to high training loads on a daily basis are at a risk much above that of the sedentary individual (Nieman 1994). As acute respiratory infection symptoms have previously been associated with decreased performance in endurance athletes (Friman & Wesslén 2000), identifying early markers helping to avoid the onset of URS are crucial for coaching purposes.

The mucosal membranes (e.g. in the oral cavity and respiratory tract) are continuously exposed to pathogens. Thus, assessment of salivary proteins and antimicrobial proteins has been shown to represent the status of mucosal immunity (Gillum et al. 2014; Papacosta&Nassis, 2011) and is conducted in order to investigate the exercise induced changes in immune system functions (Gleeson et al. 2004). While sIgA is the most abundant antimicrobial protein found in mucus secretions including saliva, also α-amylase, lactoferrin and lysozyme provide a first line of defense against pathogens that might be present on mucosal surfaces (Marcotte & Lavoie 1998). In addition, saliva IgM and locally produced IgG are considered to play a smaller role in the protection of mucosal surfaces (Bishop & Gleeson 2009; Walsh et al. 2011).

71 Increases in saliva concentrations of sIgA (sa-sIgA) and other antimicrobial protein
72 concentrations and/or increased secretion rates are associated with acute exercise whereas
73 conversely, decreases have been reported in athletes throughout a training season, leaving the
74 athlete susceptible for upper respiratory tract symptoms (Gleeson et al. 2012; Papacosta &
75 Nassis, 2011). Decreased secretion rates of salivary markers and low concentrations of IgA
76 have typically been implicated as a risk factor for subsequent episodes of URS in endurance-
77 type activities as well as in high-intensity intermittent physical activities and high intensity
78 resistance training (Bishop & Gleeson 2009; He et al. 2014; Papacosta & Nassis, 2013; Walsh
79 et al. 2011). However, numerous studies have not been able to find associations between
80 salivary markers and upper respiratory tract symptoms (Novas et al. 2003; Peters et al.2010;
81 Tiollier et al. 2004). In addition to the changes in resting salivary protein levels also acute
82 response to exercise of salivary proteins have been suggested to be related to susceptibility to
83 upper respiratory symptoms, as the possible transient activation response after exercise would
84 increase protection in the immediate post exercise period (West et al. 2010).

85

86 This study was designed to examine the effects of prolonged progressive endurance training
87 on basal levels of salivary proteins (total proteins, sa-sIgA, sa-IgM, sa-IgG, sa-Lyso, sAA)
88 and susceptibility to URS in recreationally endurance-trained men. In addition, the acute
89 effects of maximal endurance exercise on the salivary proteins during prolonged supervised
90 training were studied. It was hypothesized that twelve weeks of progressive endurance
91 training that includes continuous and interval training with a frequency of 4-6 sessions per
92 week would lead to a suppression of salivary sIgA and lysozyme concentrations. Furthermore,
93 we expected to observe significant differences in salivary biomarkers before the training
94 period between the runners who suffered from respiratory tract symptoms and those who
95 remained healthy throughout the entire training period.

96 **Materials and methods**

97

98 *Subjects*. Twenty-five young men volunteered for this study (age = 34.6 ± 1.3 years, weight =
99 77.4 ± 1.5 kg, height 1.82 ± 0.02 m, fat percentage = 16.2%± 1.2). Subjects were
100 retrospectively assigned to either a symptom free (HEALTHY) or an upper respiratory tract
101 symptom (URS) group, according to self-reported symptoms throughout the training period.
102 Participants were categorized as HEALTHY if they did not report any respiratory symptoms
103 during the entire training period (n=12) or as URS (n=13), if they reported to have at least one
104 episode of URS symptoms. The participants were reminded about the questionnaires during
105 the supervised training sessions to avoid the possibility of forgetting to report the symptoms.
106 All the participants in HEALTHY group returned an empty WURSS-21 questionnaire. All
107 subjects had a minimum of one year endurance training experience with 2-6 sessions and at
108 least 3 h of moderate to high-intensity training per week prior to inclusion in the study.
109 Participants who smoked, used any medication, or had a history of cardiac, hepatic, renal,
110 pulmonary, neurological, gastrointestinal, haematological or psychiatric illness or disease
111 were excluded from the study. Subjects were required to complete a comprehensive health-
112 screening questionnaire and a resting ECG was reviewed by a cardiologist prior to entering
113 the study. Subjects were informed about the potential risks and discomforts associated with
114 the measurements and gave written informed consent prior to participation. The study was
115 conducted according to the declaration of Helsinki, and approved by the Ethical Committee of
116 the University of Jyväskylä, Finland.

117

118 *Design*. The present data stem from a a larger endurance training study (Schumann et al.
119 2014). Following health-screening, the subjects participated in a twelve-week progressive
120 endurance running program conducted during the winter months (average temperature -7 to -

1 °C). If unable to perform training sessions (e.g. due to upper respiratory tract symptoms) subjects were instructed to catch up on missing training sessions so that the total amount of training at POST was similar in all subjects. If the subjects were unable to catch up the training load, the overall durations of the training period was extended. Saliva samples were collected both before (PRE) and after (POST) the training intervention in a fasting state (basal) as well as before and 5 min after an incremental treadmill incremental treadmill run to voluntary exhaustion. In order to control the experimental conditions, subjects received both verbal and written instructions about the measurement preparation in an attempt to minimize physical and mental stress. Subjects were also asked to allow for at least 7-8 h of sleep on the day before each testing as well as to maintain their normal nutritional intake.

Salivary markers. Subjects arrived at the laboratory for the fasting measurement between 7:00 and 9:00 a.m. following an overnight fast of 12 hours. A glass of water (120 ml) was ingested 5 minutes before the saliva collection by Cotton-swab (Salivette, Sersted, Vümbrecht, Germany). The subjects were asked to rest in a seated position for 2 minutes, while keeping the cotton-swab collector in their oral cavity. Following collection, samples were centrifuged at 15 000 g for 2 minutes. Thereafter, saliva samples were stored frozen (-20°C) until analysis. All samples were analysed at the same time by immunoturbidimetry (Konelab, 20XTi, Thermo Electron Corporation, Vantaa, Finland) using commercial reagents (sa-sLyso: Instruchemie, Netherlands; others: Thermo Scientific, Vantaa, Finland). The detection limits and inter-assay coefficients of variation, respectively were 1.2 $pg \cdot ml^{-1}$ and 3.1 % for sa-IgG, 0.2 $g \cdot l^{-1}$ and 5.9 % for sa-sIgA, 0.1 $pg \cdot ml^{-1}$ and 7 % for sa-IgM, 1.0 $g \cdot l^{-1}$ and 2.2 % for salivary total protein, 4 $U \cdot l^{-1}$ and 3.6 % for sa-sAA 5 $\mu g \cdot mL^{-1}$ and 5.8 % for sa-Lyso.

The peak aerobic capacity (VO_{2peak}) of each subject was assessed by an incremental treadmill run to voluntary exhaustion. The initial velocity for all subjects was 9 $km \cdot h^{-1}$ and increased by 1 $km \cdot h^{-1}$ every 3 min, while the incline was kept constant at 0.5º. The treadmill was stopped

every 3 min for 20 s in order to collect capillary blood samples from the fingertip for the determination of blood lactate concentrations. VO_{2peak} was determined as the highest 60 s oxygen consumption value recorded (Masterscreen CPX, Carefusion, San Diego, USA). Heart rate was measured using a Polar S810 heart rate monitor (Polar Electro, Kempele, Finland). Time to exhaustion was defined as the maximal testing time until voluntary exhaustion.

The amount and severity of upper respiratory tract symptoms was evaluated by a shorter version of the Wisconsin Upper Respiratory Symptom Survey (WURSS-21, Barrett et al. 2005). WURSS-21 is an evaluative illness-specific quality of life instrument, designed to assess the negative impact of the common cold. The construct validity of this questionnaire has been supported by measures of reliability, responsiveness, importance to patients and convergence (Barret et al. 2005). The WURSS-21 includes 10 items assessing symptoms, nine items assessing functional impairments and one item assessing global severity and global change. In the present study, subjects were asked to complete the questionnaire on every day of suffering from URS until complete resolution of the illness episode as indicated by answering “not sick”. To calculate the symptom score, the daily illness severity scores were summed.

The subjects were required to maintain training program and their habitual physical activity throughout the study period. The prescribed training program has been described in detail by Schumann et al. (2014). The prescribed endurance training consisted of both continuous and interval training sessions, 4-6 x per week based on the polarized training approach (Muñoz et al. 2013). Both the training intensity and volume progressively increased throughout the twelve weeks of training. The exercises focused on running but alternative endurance training modes such as cycling and cross country skiing were permitted for all low-intensity continuous training sessions (i.e. the long run as well as the light run) in order to minimize the risk of injuries. The training intensity was based on heart rate zones calculated from maximal

heart rate determined during the incremental treadmill protocol. The training program included one to two incremental (35-45 min, 65-85%), one long (70-120 min, 60-65%), one interval (20-25 min, 80-85%) and one to two light (35-40 min, 60-65%) runs. Training intensity, duration and distance were consistently controlled and recorded by heart rate monitors (RS800cx, Polar Electro Oy, Kempele, Finland), using manually pre-programmed exercise files. Two training sessions per week were supervised and the remaining training sessions were performed individually.

Statistical methods. Data are presented as mean ± SD. Before applying further statistical methods, the data was checked for sphericity, normality and the homogeneity of variances was analyzed via Levene statistics. As expected, salivary markers were non-normally distributed and rank-transformation was used before further analysis. Absolute changes were analyzed via two-way repeated analysis of variance for main (group, time) and interaction (group × time) effects. This was followed by one-way repeated measures ANOVA on both groups (HEALTHY, URS) to examine a main effect of time. If interaction was observed at $p \leq 0.05$, the change from PRE to POST was compared between group and time using paired t-test. Spearman's rank correlation coefficient was used to assess associations between salivary markers, training data and upper respiratory tract symptoms. Data was analyzed using PASW statistic 22.0 (SPSS, Chicago, IL, USA). The level of statistical significance was set at $p<.05$.

189 **Results**

190 Anthropometrics and endurance performance of HEALTHY and URS groups are presented in Table 1.
191 The number of the days that the subjects suffered from upper respiratory tract symptoms in
192 the URS group varied between 4 and 28 with an average of 11 ±7 days (Table 2). The
193 symptom scores in the URS group varied between 62 and 1520 and the average severity score
194 per day was 36 ±31. The duration on the training intervention on average was 12 (±0.3) weeks
195 in HEALTHY and 14 (±0.5) weeks in URS. The subjects trained on average 65 ± 8 training
196 sessions during the entire training period and the total amount of training varied between 50
197 and 68 hours. The subjects ran on average a total of 455 ± 14 kilometers during the study.
198 There were no significant differences between the URS and HEALTHY in the training load.
199 The duration of the incremental run was 25 ± 3.5 min at PRE and 27 ± 2 min at POST in
200 HEALTHY and 25 ± 4 min at PRE and 27 ± 4 min at POST and there were no between-
201 groups difference. After the training VO_{2peak} improved similarly in HEALTHY (+6± 4%,
202 $p<.001$) and URS (+4 ± 4%, $p<.01$), while no between-group differences were observed.

203 ***Table 1 & 2 somewhere here***

204 Significant changes in salivary markers were not observed between PRE and POST in either
205 of the groups. Sa-sIgA levels were significantly higher in HEALTHY compared to URS at
206 PRE (240%, $p<.001$) and POST (130%, $p<.01$, Figure 1). However, no significant between-
207 group differences were observed in total salivary proteins, sa-IgG, sa-IgM, sa-Lyso or sAA.
208 There was a significant correlation between sa-sIgA at PRE and symptom days in all the
209 participants ($R=-0.755$, $p<.01$). In URS, a significant correlation between the reported number
210 of symptom days and sa-sIgA ($R=-.719$, $p<.001$) as well as between the symptoms score and
211 sa-sIgA levels ($R=-.573$, $p<.01$) was observed.

212 ***Figure 1 somewhere here***

213 Maximal endurance exercise acutely increased sAA concentrations in HEALTHY ($p<.05$) but
214 not URS both at PRE and POST. In URS a significant decrease was observed in sAA to total
215 protein ratio ($p<0.05$) (Table 3). Total salivary protein content increased significantly ($p<.01$)
216 in both groups and both measurement points. There was a significant ($p<.001$) group × time
217 interaction in acute sa-sIgA and sa-sIgA to total protein -ratio response at PRE. In HEALTHY
218 a significant decrease ($p<.01$) was observed, whereas in URS significantly increased sa-sIgA
219 levels ($p<.01$) were observed in response to the incremental treadmill run at PRE. A
220 significant response in sa-sIgA concentrations was not observed in either of the groups at
221 POST. Sa-IgM to total protein ratio decreased in URS at PRE ($p<0.05$) and at POST ($p<0.05$).
222 Sa-Lyso concentrations increased significantly in both measurement points ($p<.05$) in
223 HEALTHY and at POST in URS.

224 ***Table 3 somewhere here***

225

226 **Discussion**

227

228 Saliva plays a key role in reducing the accessibility of microbe-susceptible cells in the oral
229 cavity and upper digestive and respiratory tract (Gröschl 2009; Mahanonda et al, 2011). This
230 study demonstrated that subjects, who experienced upper respiratory tract symptoms during
231 the twelve weeks of progressive endurance training that included 4-6 training session per
232 week based on the polarized training approach, had significantly lower basal sa-sIgA levels
233 both before and after the experimental endurance training period. These findings were
234 accompanied by the significant acute increase in sAA after maximal endurance exercise in
235 HEALTHY but not URS both at PRE and POST. In addition, a significant response to
236 maximal incremental run in salivary lysozyme in URS was only observed at POST and the sa-

237 sIgA concentration at PRE correlated significantly with the symptom days (R=-0.755,
238 p<.001).

239 In the present study we did not determine whether the cause of the symptoms were a local
240 inflammation or infections and that is why upper respiratory symptoms were used to cover
241 both causes. Nevertheless, the subjects who reported upper respiratory tract symptoms during
242 the training period had lower sa-sIgA levels at both PRE and POST. This is in line with
243 previous studies performed in elite athletes (Gleeson & Bishop 2013). Neville et al. (2008)
244 showed a significant reduction (28%) in sa-sIgA concentrations, occurring during the 3 weeks
245 prior to URS episodes and a return to baseline two weeks later, which might explain the
246 difference between our groups at POST. Moreover, sa-sIgA concentrations correlated
247 significantly with the number of sickness days, which is in line with the study by Neville et al.
248 (2008). While we are aware that lower sa-sIgA concentrations at POST might be also
249 attributed to the inadequate recovery from an URS episode commenced just before the POST
250 measurements (Neville et al. 2008), none of the participants in the present study had
251 symptoms during the final 10 days prior to the POST measurements. Interestingly, the lower
252 sa-sIgA levels or the missed training days in URS did not seem to affect endurance
253 performance adaptations as both groups increased VO_{2peak} and time to exhaustion to a similar
254 extent. However, it should be kept in mind that the overall training volume was matched
255 between the subjects also in the case of sickness which is also shown by the longer training
256 duration in URS (14±0.5 weeks) than in HEALTHY group (12±0.3 weeks).

257

258 In the present study a total of 19 episodes of URS symptoms in 13 subjects were recorded
259 during the entire training period. Previous studies have shown that athletes utilizing high
260 training loads with high intensities seem to be more susceptible to respiratory tract symptoms

compared to athletes training with a lower training volume and/or lower intensity, especially during endurance training (Spence et al. 2007). In the present study, however, the amount of training did not differ between the groups. Furthermore, despite a progressive increase in training load throughout the twelve weeks of training, there were no significant changes in salivary proteins, which might indicate that the training load was not high enough to have an effect on salivary biomarkers. Previous studies (Francis et al. 2005) have reported large within and between subject variations in sa-sIgA concentrations. It should be noted that in the present study the incidence of upper respiratory tract symptoms was relatively small and the URS group was not homogenous regarding the number of days with reported symptoms. On the other hand, the salivary sIgA concentrations in URS were consistently low among all subjects, while in HEALTHY the deviation was notably higher. This in turn may provide some evidence for low salivary sIgA concentrations as a risk factor for the development of upper respiratory tract symptoms, however, notable inter-individual differences exist. It has been shown that the upper respiratory tract symptoms are most common during winter months (Makinen et al. 2009). In the present study the training was conducted during the winter months where the average temperatures typically vary between -1 and -7 °C. Training in cold environment could have affected salivary proteins and upper respiratory symptoms but this cannot be confirmed based on the present study design.

In the present study a significant acute increase was observed in salivary total protein concentrations in both groups after the incremental treadmill run both before and after the training. In agreement with a study of Chicharro et al. (1998), this transient increase might be attributed to β-sympathetic actions on the salivary glands. However, to the best of our knowledge limited data exists regarding the use of acute exercise-induced changes in salivary immunoglobulins and antimicrobial proteins as predictors of upper respiratory tract

symptoms. The acute effects of exercise on sa-sIgA concentrations are not consistent in the previous literature (Papacosta & Nassis 2011). Papacosta & Nassis (2011) reported that typically high intensity endurance exercise of less than 30 min in duration leads to an increase in sa-IgA concentrations. Fahlman et al. (2001) reported a significant transient reduction in sa-sIgA after high-intensity exercise which was not related to URS incidences. Interestingly, Nieman et al. (2006) reported higher incidence of upper respiratory tract symptoms after ultra-marathon in those subjects who exhibited a larger pre- to post exercise reductions in sa-sIgA secretion but an observed reduction (10 %) in sa-sIgA concentration was not related to URS. The present study observed significantly different response to maximal treadmill run until voluntary exhaustion in sa-sIgA concentration between the URS and HEATHY groups at PRE, in HEALTHY a significant decrease was observed, whereas in URS sa-sIgA concentrations increased. Interestingly, however, at POST the response in sa-sIgA remained statistically unaltered following the incremental treadmill run in both groups, which might indicate that the training affected the exercise response. However, in the present study significant associations between the acute sa-sIgA response and URS during 12 weeks of endurance training were not observed. Typically studies have reported that salivary IgG remains to be unaffected by acute exercise, whereas saliva IgM has previously been shown to respond simultaneously with salivary sIgA concentrations (Bishop & Gleeson 2009). In the present study, however, incremental treadmill running did not have a significant effect on concentrations of sa-IgG or sa-IgM before or after the training. However, IgM to total protein ratio significant decreased in URS group, whereas in HEATHY significant changes were not observed.

Saliva α-amylase is an antimicrobial protein and its secretion is stimulated by the activity of the sympathetic nervous system. It has previously been suggested that sAA might be sensitive

311 to exercise-induced stress as it is locally secreted in the salivary gland by the stimulation of
312 the autonomic nervous system and could be a predictor of plasma noradrenalin under
313 physiological or psychological stress (Allgrove et al. 2008). Interestingly, in the present study
314 the acute response of sAA to the incremental treadmill run was blunted in the URS. Whereas
315 the incremental run increased sAA significantly in HEALTHY at PRE and POST and when
316 sAA to total protein ratio was used a significant decrease in sAA was observed. Importantly,
317 there were no significant differences in the time to exhaustion or maximal oxygen
318 consumption between the two groups, which might cause the different sAA response (Kunz et
319 al. 2015). Previous studies have suggested that high intensity exercise increases sAA, whereas
320 submaximal exercise does not affect sAA (Ali & Pruessner 2012). In addition, it has been
321 suggested that sAA (as a marker of sympathetic nervous activity) might be related to
322 difficulties in the regulation of the exercise-induced stress response (Ali & Pruessner 2012;
323 Rohleder & Nater 2009). The increased saliva alpha-amylase activity after exercise may
324 improve the protective effect of saliva, since the enzyme is known to inhibit bacterial
325 attachment to oral surfaces and a lack of this response might lead to a higher risk of
326 developing URS.
327
328 A significant acute increase in sa-Lyso was observed following the incremental treadmill run
329 before and after the training, both in HEALTHY and at POST in URS. The results of previous
330 studies investigating the effects of exercise on sa-Lyso concentrations are controversial.
331 Allgrove at al. (2008) reported an acute increase in sa-Lyso after an incremental cycling test
332 to exhaustion, while Inoue et al. (2004) showed a significant decrease in salivary lysozyme
333 concentrations following intensive exercise in elite swimmers. Lysozyme is part of the innate
334 immune system that has a wide variety of antimicrobial activities. Allgrove et al. (2008)
335 suggested that an acute elevation in sa-Lyso after an exercise bout might be mediated by the

perturbations in sympathetic nervous system activity and secretion of glucocorticoids. The increase in sa-Lyso is typically considered as a temporary enhancement in immune function that may increase protection in the periods immediately post exercise but the significance of this change needs further investigation (West et al. 2015).

This study confirmed the findings of previous studies that salivary sIgA could be a useful marker to predict upper-respiratory tract symptoms and to screen illness-prone runners before prolonged endurance training. In this study, no significant between-group differences were observed in other salivary markers (sa-IgM, lysozyme, sa-IgG or sAA) in the fasting state, whereas, our findings suggest that the lack in acute response to exercise, especially in sAA might predict a higher incidence of upper respiratory symptoms. Nevertheless, more research is needed on the use of acute responses to exercise in salivary proteins, especially sAA and lysozyme, as a marker of increased susceptibility to URS.

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Conflict of interest statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the manuscript.

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449 **List of Tables**

450 Table 1. Anthropometrics and endurance performance of HEALTHY and URS (**p<0.01, ***p<0.001 difference between PRE and POST).

Group	HEALTHY	URS
Number of subjects	12	13
Age (years)	34.5 ± 7.78	34.7 ± 5.84
Height (m)	1.81 ± 0.07	1.82 ± 0.06
Body weight (kg)	76.9 ± 6.85	77.9 ± 8.29
BMI ($kg \cdot m^{-2}$)	23.7 ± 2.06	23.2 ± 2.31
PRE VO_{2peak} ($ml \cdot kg^{-1} \cdot min^{-1}$)	46.6 ± 6.02	47.9 ± 5.55
POST VO_{2peak} ($ml \cdot kg^{-1} \cdot min^{-1}$)	49.3 ± 5.63***	49.7 ± 5.84**

451

452

453 Table 2. Individual number of sick days, severity scores and average severity score per day in URS group.

Participant	Number of sick days	Symptom score	Averge symptom score per day
1	28	330	11.8
2	10	1040	104
3	6	172	27.8
4	6	71.0	11.8
5	4	159	39.8
6	4	62	15.5
7	9	184	20.4
8	9	300	33.3
9	18	1520	84.4
10	15	351	23.4
11	5	75.0	15.0
12	16	119	7.44
13	10	783	78.3

454

455

456 Table 3. Acute responses in salivary proteins and concentration to total protein ratio after the incremental treadmill run to exhaustion before (PRE) and after
457 (POST) the training intervention.*=significant difference to pre value, #=significant time×group interaction (*p<0.05, **p<0.01, # p<0.05, ## p<0.01, ###
458 p<0.001).

459

	PRE				POST			
	HEALTHY		URS		HEALTHY		URS	
	Before	After	Before	After	Before	After	Before	After
sProtein (mg/l)	710 ± 140	1400± 300*	490 ± 130	1900 ± 510**	630 ± 110	1300 ± 260**	310 ± 56	1400 ± 220**
sIgA(mg/l)	150 ± 45	74 ± 21*###	49 ± 9.3	89 ± 18**###	110 ± 32	120 ± 32	56 ± 11	67 ± 13
sIgA (g/100g protein)	27 ± 7.1	7 ± 1.6*#	16 ± 3.5	10 ± 3.2#	20 ± 4.0	13.2 ± 5.1*	25 ± 6.2	7.6 ± 5.6*
sIgM (mg/l)	14 ± 6.5	18 ± 6.9	15 ± 4.5	14 ± 2.5	14 ± 8.2	19 ± 8.5	14 ± 5.0	13 ± 4.4
sIgM (g/100g protein)	2.6 ± 1.3	1.8 ± 1.0	6.1 ± 2.3	1.4 ± 0.4*	2.4 ± 1.5	2.1 ± 1.1	6.4 ± 3.1	1.4 ± 0.5*
sIgG (mg/l)	21 ± 5.7	15 ± 4.8	15 ± 2.6	23 ± 10	23 ± 6.3	16 ± 4.3	12 ± 2.6	23 ± 10
sIgG (g/100g protein)	2.7 ± 0.5	1.0 ± 0.2	5.7 ± 2.0	1.7 ± 2.6	3.2 ± 0.7	1.2 ± 0.3	6.7 ± 3.5	2.1 ± 1.7
α-amylase (U/ml)	69 ± 15	210 ± 54*	79 ± 28	200 ± 100	71 ± 15	198 ± 46*	61 ± 27	130 ± 36
α-amylase U/100g protein)	14 ± 4.5	13 ± 2.6	18 ± 5.8	10 ± 3.1*	14 ± 3.0	15 ± 3.4	32 ± 22	16 ± 5.9*
Lysozyme (mg/l)	3.7 ± 1.0	15 ± 3.1**	3.9 ± 1.5	9.7 ± 2.8	8.7 ± 5.4	23 ± 13*	2.2 ± 0.8	11 ± 3.8*
Lysozyme (g/100g protein)	0.6 ± 0.2	0.8 ± 0.2	2.8 ± 2.1	0.7 ± 0.2	0.9 ± 0.5	1.4 ± 0.6	1.0 ± 0.6	0.7 ± 0.2

460 **Figure captions**

461 Figure 1. Fasting salivary sIgA, total salivary proteins, IgM, α-amylase, IgG and
462 lysozyme in HEALTHY and URS. #=significant difference between the groups. (##
463 $p<0.01$, # ## $p<0.001$).

464