

1 **Stimulating whole saliva affects the response of antimicrobial proteins to exercise**

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18 Running head: stimulated saliva flow, exercise and AMPs

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23 **Abstract**

1 This study investigated the salivary secretion rates of antimicrobial proteins in
2 response to prolonged, exhaustive exercise in both stimulated and unstimulated saliva
3 flow sample methods. Twenty four trained men cycled for 2.5 h at 60% $\dot{V}O_{2max}$ and
4 then to exhaustion at 75% $\dot{V}O_{2max}$. Timed collections of whole saliva were made
5 before exercise, mid-exercise, at the end of the moderate exercise bout and post
6 exhaustive exercise. After each unstimulated (UNSTIM) collection a stimulated
7 sample (STIM) was collected following chewing flavoured gum for one minute.
8 Saliva was analysed for lysozyme, α -amylase and salivary immunoglobulin A (s-
9 IgA), and secretion rates were calculated. Saliva flow was 156% higher in STIM
10 compared with UNSTIM ($P<0.001$) and decreased with exercise in STIM only
11 ($P<0.001$). Exercise increased lysozyme and α -amylase levels and secretion rates
12 were 144% higher and 152% higher in STIM compared with UNSTIM for lysozyme
13 and α -amylase, respectively (all $P<0.001$). S-IgA concentration ($P<0.05$) and
14 secretion rate ($P<0.001$) increased with exercise but were both lower in STIM
15 compared with UNSTIM ($P<0.001$). In conclusion, a stimulated saliva flow collection
16 during exercise by chewing flavoured gum increased the quantity of saliva and the
17 secretion of lysozyme and α -amylase, but had a limited impact on the secretion of s-
18 IgA.

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20 Keywords: cycling, lysozyme, α -amylase, immunoglobulin A, chewing

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22

1 **Introduction**

2 Saliva is a clear, slightly acidic mucoserous exocrine secretion consisting of inorganic
3 and organic compounds of usually more than 99% water. Saliva secretions play an
4 important role in maintaining the integrity of oral health via a mechanical washing
5 effect and through the secretion of antimicrobial proteins. These proteins constitute
6 the first line of defence against infectious agents and include both innate (lysozyme,
7 α -amylase) and adaptive (immunoglobulin A) immune components (Humphrey and
8 Williamson, 2001). The secretion of saliva into the mouth originates from three pairs
9 of major salivary glands; the submandibular glands contribute ~65% of total
10 unstimulated whole saliva secretion, the parotid glands contribute ~20%, the
11 sublingual glands contribute 7-8% and the numerous minor salivary glands contribute
12 less than 10% (Pedersen et al., 2002). The average daily flow of whole saliva varies
13 between 1 and 1.5 L (Humphrey and Williamson, 2001).

14
15 Saliva secretion is under strong autonomic neuronal control and thus regulated by
16 parasympathetic and sympathetic nerve fibres that are the effector arms of reflexes
17 activated predominantly by taste and chewing (Humphrey and Williamson, 2001).
18 Generally, when sympathetic stimulation dominates (via noradrenaline) the secretions
19 are high in protein content (e.g. α -amylase), whereas secretions with a high fluid
20 output occur in response to parasympathetic stimulation (Chicharro et al., 1998).
21 However, parasympathetic stimulation can also affect salivary protein secretion, and
22 protein secretion of some glands such as the sublingual and some of the minor glands,
23 may even be entirely under parasympathetic control (Teeuw et al., 2004).
24 Furthermore, sympathetic stimulation also causes some stimulation of the saliva flow

1 rate (Garrett, 1987), thus, rather than acting antagonistically, it could be argued that
2 the two branches of the autonomic nervous system may exert relatively independent
3 effects in which the activity of one branch may synergistically augment the other
4 (Bosch et al., 2002). Other factors known to influence saliva secretion include psychic
5 factors, nutrition, hydration status, medication, local or systemic diseases and physical
6 stress (Humphrey and Williamson, 2001), where significant reductions in the flow
7 rate and changes in the antimicrobial proteins have been observed. For example,
8 reductions in salivary immunoglobulin A (s-IgA) levels have often been reported
9 following strenuous exercise (Tomasi et al., 1982; Mackinnon et al., 1989; Nehlsen-
10 Cannaralla et al., 2000; Nieman et al., 2002). However, other studies report increases
11 (Blannin et al., 1998; Walsh et al., 2004; Li and Gleeson, 2004; Sari-Sarraf et al.,
12 2007; Allgrove et al., 2008; Allgrove et al., 2009; Costa et al., 2012) or no change
13 (McDowell et al., 1991; Walsh et al., 1999). α -amylase (Li and Gleeson, 2004;
14 Allgrove et al., 2008) and lysozyme (Allgrove et al., 2008; West et al., 2010; Costa et
15 al., 2012) typically increase with exercise, although one study reported a significant
16 reduction in lysozyme levels post-exercise (Davison & Diment, 2010) and in another
17 reductions were observed following a dehydration protocol (Fortes et al., 2012).
18 Changes in salivary antimicrobial proteins have been linked to the susceptibility of
19 upper respiratory symptoms in a variety of athletic/exercising populations (Gleeson et
20 al., 1999; Klentrou et al., 2002; Neville et al., 2008; Nieman et al., 2002; Fahlman &
21 Engels, 2005; Cunniffe et al., 2011).

22
23 Athletes often consume both food items and beverages during exercise. It has been
24 shown that chewing can increase the flow rate by 3-fold compared with unstimulated
25 saliva secretion (Hector and Linden, 1987), and this has also been shown to increase

1 the secretion of certain salivary proteins, including s-IgA probably via increased
2 epithelial cell transcytosis (Proctor and Carpenter, 2001). Similar increases were also
3 found in the secretion rates of total protein and α -amylase (Proctor and Carpenter,
4 2001). Furthermore, saliva flow rate and protein concentration is increased in
5 response to gustatory stimulation (Proctor and Carpenter, 2007), with acid and sweet
6 taste stimuli providing the greatest response (Humphrey and Williamson, 2001).
7 Given that exercise and stimulated salivary flow can independently affect
8 antimicrobial proteins, it is possible that stimulating salivary flow during exercise
9 may potentially affect the salivary antimicrobial response, and represent mechanisms
10 by which resistance to oral infection may be altered. Therefore, the aims of the study
11 were to investigate the influence of stimulated saliva flow on salivary antimicrobial
12 proteins by chewing flavoured gum during prolonged exhaustive cycling. It was
13 hypothesised that stimulating saliva flow by chewing would acutely enhance
14 antimicrobial protein secretion during exercise.

15

16 **Materials and methods**

17 **Participants**

18 Following university ethical approval 24 trained male volunteers (mean \pm SD: age 23
19 \pm 5 yr; height 1.79 ± 0.07 m; body mass 73.8 ± 8.1 kg; $\dot{V}O_{2\max}$ 56.6 ± 4.7 mL.kg⁻¹.min⁻¹) with cycling as one of their main sports, volunteered to participate in the
20 study. Participants completed a health questionnaire to report any symptoms of
21 infection or illness in the 12 weeks prior to commencing the study and were informed
22 of the aims and procedures before providing written informed consent. Participants
23

1 were included if they were healthy endurance-trained male volunteers between the
2 ages of 18-35 yr of age. Participants representing one or more of the following criteria
3 were excluded from the study: smoking or use of any medication or dietary
4 supplements or suffering from any known chronic disease.

5

6 **Preliminary measurements**

7 At least 2 weeks prior to the main trial participants performed an incremental test to
8 volitional exhaustion on an electronically braked cycle ergometer (Lode Excalibur,
9 Groningen, Netherlands) to determine their maximal oxygen uptake ($\dot{V}O_{2\max}$).
10 Following a 3-min warm-up, participants began cycling at 95 W with increments of
11 35 W every 3 min with verbal encouragement was provided to each participant to
12 ensure maximal effort. Samples of expired gas were collected in Douglas bags
13 (Harvard Apparatus, Edenbridge, UK) during the third minute of each work rate
14 increment and ratings of perceived exertion (RPE) using the Borg scale were recorded
15 and heart rate (HR) was measured continuously using short-range radio telemetry
16 (Polar Beat, Polar Electro Oy, Kempele, Finland). An oxygen/carbon dioxide analyser
17 (Servomex 1400, Crowbridge, UK) was used along with a dry gas meter (Harvard
18 Apparatus, Edenbridge, UK) for the determination of oxygen uptake ($\dot{V}O_2$). Criteria
19 for attaining $\dot{V}O_{2\max}$ included the participants reaching volitional exhaustion and a
20 heart rate within 10 beats/min of HR_{\max} . From the $\dot{V}O_2$ work rate relationship, the
21 work rates equivalent to 60% and 75% $\dot{V}O_{2\max}$ for each participant were interpolated.
22
23 Participants then completed a familiarisation ride. This ensured the subjects were able
24 to cope physically with the demands of the test, and for them to practice the saliva

1 collection procedure. It also allowed saliva flow rates to be determined to ensure there
2 was a large enough volume (~ 1.5 mL) collected for analysis. The familiarisation trial
3 was conducted in the same manner as the main trial. Participants cycled at 60%
4 $\dot{V}O_{2\max}$ for 2.5 h and then after a 5 min rest, completed a ride to fatigue at 75%.
5 $\dot{V}O_{2\max}$. Expired gas samples were collected into Douglas bags at 30, 90 and 114 min
6 to enable adjustments to be made to the work rate so that 60 % of $\dot{V}O_{2\max}$ was
7 achieved throughout the 2.5 hours of cycling. Heart rate and RPE were also measured
8 every 15 min.

9

10 **Experimental procedures**

11 Participants were requested to abstain from alcohol, caffeine and strenuous exercise 2
12 days prior to the trial. Participants arrived at the laboratory at 8:30 h for the main trial
13 following an overnight fast (10 – 12 h) and were required to sit quietly for 5 min
14 before providing a saliva sample. The participants were then asked to empty their
15 bladders before body mass was measured wearing their shorts only. They then
16 performed 2.5 h cycling at 60% $\dot{V}O_{2\max}$ on a stationary cycle ergometer. Heart rate
17 and RPE were measured at 20 min intervals and expired gas was collected at 30 min,
18 90 min and 114 min of exercise for the analysis of $\dot{V}O_2$ using a Douglas bag.
19 Participants were given 200 mL of water every 20 min during exercise; in addition
20 they ingested 300 ml of flavoured water immediately before the exercise began, and
21 again after 50 min and 110 min of exercise. Further saliva samples were collected 70
22 min and 130 min of exercise. It was ensured that no fluid was consumed in the 10 min
23 prior to each saliva collection. Following completion of the 2.5 h cycling, the

1 participants were allowed a 5 min rest before commencing the ride to exhaustion at
2 75% $\dot{V}O_{2max}$ with no verbal encouragement and no information on time elapsed. The
3 time to exhaustion was 897 ± 122 s. A final saliva sample was obtained immediately
4 after completing the ride to exhaustion and body mass was measured. Mean
5 temperature and humidity in the laboratory during the trial were 23 ± 0.5 °C and $32 \pm$
6 4 % respectively.

7

8 **Saliva collection**

9 The saliva collections were made with the participants seated, leaning forward and
10 with their heads tilted down. They were instructed to swallow in order to empty the
11 mouth before a whole saliva sample was collected over a 3-min period into a pre-
12 weighed, 50 mL screw top sterile vial (Fisher Scientific, UK; UNSTIM). Care was
13 taken to allow saliva to dribble into the collecting tubes with making minimal
14 orofacial movement. At the end of the collection period subjects were instructed to
15 collect any saliva remaining in the mouth and expectorate it (Navazesh and
16 Christensen, 1982). The UNSTIM sample was immediately followed by 1 min of
17 chewing a commercially available sugar-free mint flavoured gum (1.8g portion, 11 kJ,
18 1.1 g carbohydrate) where participants were instructed to chew at a regular rate and
19 force (Proctor and Carpenter, 2001). Immediately after removing the gum the
20 participants provided a second saliva sample (STIM) as described above by dribbling
21 into a different tube for a further 1 min. Samples were then stored at -80°C until
22 analysis.

23

1 **Saliva analysis**

2 Saliva volume was estimated by weighing to the nearest mg and the saliva density
3 was assumed to be 1.0 g.mL^{-1} (Cole and Eastoe, 1988). Saliva flow rate (mL.min^{-1})
4 was determined by dividing the volume of saliva by the collection time. Salivary IgA
5 concentration was determined in duplicate by an enzyme-linked immunosorbent assay
6 (ELISA) and alpha-amylase activity was measured in duplicate using a
7 spectrophotometric method as described previously (Li and Gleeson, 2004).
8 Lysozyme (Biomedical Technologies Inc., USA) concentration following a 1000-fold
9 dilution of saliva with phosphate buffered saline (PBS), was analysed in duplicate
10 using a commercially available ELISA kit on a subset of twelve participants selected
11 at random, the number of which was determined upon findings from a previous study
12 (Allgrove et al., 2008). Osmolality was determined using a cryoscopic (freezing point
13 depression) osmometer (Osmomat 030, Gonotec, GbBH, Berlin, Germany) calibrated
14 with $300 \text{ mOsmol.kg}^{-1}$ NaCl solution. Secretion rates for the salivary analytes were
15 calculated by multiplying the concentration by the saliva flow rate. The intra-assay
16 coefficient of variation for the analytical methods used were 2.4%, 7.9% and 8.2% for
17 α -amylase, IgA and lysozyme, respectively.

18

19 **Statistical analysis**

20 Data were checked for normality, homogeneity of variance and sphericity before
21 statistical analysis. A two-way ANOVA (2 treatments x 4 sample times) with repeated
22 measures design was used to examine the salivary data. Data that were not normally
23 distributed were normalised with log transformation. Significant differences were

1 assessed using Student's paired *t*-test with Holm-Bonferroni adjustments for multiple
2 comparisons. Differences in HR between the steady state exercise and time to
3 exhaustion ride were assessed using Student's paired *t*-tests. Data in text and tables
4 are presented as mean \pm SD. For clarity, data in figures are presented as mean \pm SEM.
5 Statistical significance was accepted at $P < 0.05$.

6

7 **Results**

8 **Physiological variables and RPE**

9 Attainment of an average of 60% $\dot{V}O_{2\max}$ was achieved during the steady state
10 exercise; where mean $\dot{V}O_2$ was $60.1 \pm 2.8\%$ $\dot{V}O_{2\max}$. Mean HR was 137 ± 11
11 $\text{beats}\cdot\text{min}^{-1}$ and $174 \pm 9 \text{beats}\cdot\text{min}^{-1}$ during the steady state exercise and the time to
12 exhaustion trial, respectively. Mean RPE measured during the steady state exercise
13 was 12 ± 2 and the post-exercise body mass loss was $0.53 \pm 0.10 \text{ kg}$ ($0.7 \pm 0.1\%$).

14

15 **Salivary variables**

16 **Saliva flow rate**

17 Saliva flow rate was significantly higher in STIM compared with UNSTIM
18 throughout the exercise protocol (main effect of treatment: $F_{1, 23} = 177.10$, $P <$
19 0.001). Saliva flow rate decreased at 130 min of exercise and post-exhaustion in
20 STIM only (interaction: $F_{3, 56} = 10.37$, $P < 0.001$; Table 1).

21

22 ***Insert Table 1 near here***

1

2 **Salivary lysozyme concentration**

3 Salivary lysozyme concentration increased with exercise (main effect of time: $F_{3,33} =$
4 23.97, $P < 0.001$), but there were no differences between methods (Table 1).

5

6 **Salivary lysozyme secretion rate**

7 Salivary lysozyme secretion rate increased with exercise (main effect of time: $F_{3,33} =$
8 18.00, $P < 0.001$), and was significantly higher in STIM compared with UNSTIM
9 throughout the exercise protocol (main effect of treatment: $F_{1,11} = 35.05$, $P < 0.001$;
10 Figure 1).

11

12 *****Insert Figure 1 near here*****

13

14 **Salivary α -amylase activity**

15 Salivary α -amylase activity increased with exercise (main effect of time: $F_{3,69} =$
16 107.77; $P < 0.001$), but there were no differences between methods (Table 1).

17

18 **Salivary α -amylase secretion rate**

19 Salivary α -amylase secretion rate increased with exercise (main effect of time: $F_{3,49}$
20 = 45.99; $P < 0.001$) and was significantly higher in STIM compared with UNSTIM

1 throughout the exercise protocol (main effect of treatment: $F_{1, 23} = 166.85$; $P <$
2 0.001; Figure 2).

3

4 ***Insert Figure 2 near here***

5

6

7 **Salivary IgA concentration**

8 Salivary IgA concentration increased with exercise duration (main effect of time: $F_{3,}$
9 $_{50} = 4.45$; $P < 0.05$), and was significantly higher in UNSTIM compared with STIM
10 throughout the exercise protocol (main effect of treatment: $F_{1, 23} = 44.84$; $P < 0.001$;
11 Table 1).

12

13

1

2 **Salivary IgA secretion rate**

3 Salivary IgA secretion rate increased post-exhaustion compared with baseline levels
4 (main effect of time: $F_{3,46} = 9.81$; $P < 0.001$) and was significantly higher in
5 UNSTIM compared with STIM (main effect of treatment: $F_{1,23} = 5.15$; $P < 0.05$;
6 Figure 3)

7

8 ***Insert Figure 3 near here***

9

10

11

12 **Discussion**

13 The main findings of the study were 1) saliva flow rate decreased with exercise in
14 STIM only and was higher in STIM compared with UNSTIM 2) α -amylase activity
15 and secretion rate and lysozyme concentration and secretion rate all increased with
16 exercise in both STIM and UNSTIM, and secretion rates were higher in STIM
17 compared with UNSTIM 3) s-IgA concentration and s-IgA secretion rate increased
18 post-exercise and were both lower in STIM compared with UNSTIM.

19

20 The results show a significant effect of stimulating saliva flow on saliva flow rate,
21 where it was 3-fold higher in STIM compared with UNSTIM and corroborates
22 previous findings (Hector and Linden, 1987). Saliva flow rate decreased following
23 exercise in the STIM trial only. Previous studies have attributed a reduction in flow

1 rate during exercise to dehydration, although the small change in net mean body loss
2 of (0.53 ± 0.11 kg; $0.7 \pm 0.1\%$), and lack of difference in the unstimulated trial
3 suggests that this had little impact (Walsh et al., 1999). It is possible that the
4 decreased parasympathetic nervous system activity during exercise and a removal of
5 vasodilatory influences (Proctor and Carpenter, 2007) may have limited the increase
6 in flow rate that occurs with chewing. Alternatively, the decline may be a result of
7 repetitive periods of chewing whereby the production of saliva may have become
8 temporarily exhausted over time (Proctor and Carpenter, 2001). Although saliva flow
9 rate in STIM was consistently higher than UNSTIM during the protocol, these
10 findings show that the saliva collection method employed during exercise can
11 differently affect the salivary flow response.

12

13 Stimulating saliva flow did not affect lysozyme concentration or α -amylase activity.
14 Rudney (1989) also reported that salivary lysozyme was unaffected by the flow rate.
15 However, when these proteins were expressed as a secretion rate, significantly higher
16 values in STIM compared with UNSTIM were observed. Similar increases in parotid
17 α -amylase activity secretion rate were reported by Proctor and Carpenter (2001)
18 following chewing, as would be expected given that α -amylase is an enzyme that
19 functions to break down starch and glycogen to maltose in the oral cavity. In contrast
20 to these proteins, stimulating saliva flow resulted in a significantly lower s-IgA
21 concentration compared with UNSTIM throughout the exercise protocol, which has
22 been previously attributed to the increased saliva flow rate from chewing activating
23 the parotid gland functioning to dilute the saliva (Proctor and Carpenter, 2001).
24 However, when expressed as a secretion rate s-IgA levels were slightly lower in

1 STIM compared with UNSTIM. These findings do not support other studies where
2 stimulating saliva flow increased the secretion rate of s-IgA at rest (Proctor and
3 Carpenter, 2001). However, Proctor and Carpenter (2001) stimulated saliva by
4 chewing on a piece of polythene tube whereas the current study administered a
5 commercially available flavoured chewing gum. The resulting differences between
6 masticatory stimulation only, from chewing the polythene tube, and gustatory and
7 masticatory stimulation combined when chewing flavoured gum, may explain some
8 of these differences. Despite a detrimental effect on the rate of s-IgA secretion during
9 exercise observed in STIM, levels remained above resting values, which may be
10 relevant in terms of oral immunity.

11

12 Significant increases in the salivary antimicrobial proteins were observed with
13 exercise which has been reported in previous studies (Blannin et al., 1998; Walsh et
14 al., 2004; Li and Gleeson, 2004; Sari-Sarraf et al., 2007; Allgrove et al., 2008; West et
15 al., 2010, Costa et al., 2012). α -amylase and lysozyme levels increased consistently
16 during the exercise protocol, whereas S-IgA levels were elevated following the ride to
17 exhaustion at 75% $\dot{V}O_{2max}$. These changes are thought to be related to an increase in
18 sympathetic nervous system (SNS) activity enhancing their transport and/or secretion
19 into saliva (Chatterton et al., 1996; Bishop et al., 2000; Walsh et al., 2002) and
20 suggest that there may be a threshold level of SNS activity to increase s-IgA secretion
21 during exercise, a finding that has been previously demonstrated in the rat model
22 (Carpenter et al., 2000).

23

1 Differences in the secretion of the antimicrobial proteins with chewing may be related
2 to the way these proteins are stored and secreted into saliva. Lysozyme and α -amylase
3 are stored in secretory granules which are released spontaneously upon autonomic
4 stimulation (Bosch et al., 2002). However, s-IgA is secreted onto mucosal surfaces
5 across epithelial cells via the polymeric immunoglobulin receptor, (Proctor and
6 Carpenter, 2001) which is activated by neuronal stimuli that may differ to other
7 salivary proteins. These findings show that the combination of masticatory and
8 gustatory stimuli through chewing flavoured gum activate the secretion of stored
9 salivary proteins into saliva (lysozyme and α -amylase) but do not enhance the
10 (transport and subsequent secretion) of the receptor mediated secretion of s-IgA. A
11 further explanation may be related to the relative contributions of the different
12 salivary glands during unstimulated and stimulated saliva flow, since specific salivary
13 glands have been shown to be activated by some stimuli more than others (Noble,
14 2000). For example, mastication predominantly activates the parotid glands, which
15 produce large amounts of α -amylase. In contrast, strong taste stimuli activate the
16 submandibular and sublingual glands (from which lysozyme is mainly produced)
17 more than the parotid gland. Thus, the increase in α -amylase and lysozyme by
18 chewing flavoured gum may be explained by the increase of salivary secretion from
19 these specific glands. The relative contribution of salivary glands for s-IgA secretion
20 is not clear. Crawford et al. (1975) reported s-IgA concentration to be four times
21 higher in the minor salivary glands than parotid glands although other data suggest a
22 low parotid s-IgA secretion rate is associated with high susceptibility to dental caries,
23 suggesting a greater role of the parotid gland in s-IgA secretion (Brandtzaeg, 1976).
24 These uncertainties make it difficult to relate the changes in s-IgA secretion in

1 stimulated and unstimulated saliva flow to the stimulation of specific glands. From a
2 practical standpoint, these findings show that when investigating s-IgA, the saliva
3 flow rate must be considered.

4

5 A limitation of these findings is that it is not possible to distinguish whether the
6 differences between stimulated and unstimulated saliva flow were due to masticatory
7 or gustatory stimuli alone or in combination and future work might seek to address
8 this to determine which may have the most favourable response. In addition, the
9 practice of chewing during exercise may not be recommended in case of a risk of
10 choking, therefore administering a bitter isotonic beverage might be recommended as
11 a potential alternative. Nevertheless, the finding of an increase in the secretion rates of
12 lysozyme and α -amylase with exercise which is further enhanced by stimulating
13 saliva flow suggest mechanisms by which resistance to oral infections might be
14 enhanced. These effects might have further benefits in immunocompromised
15 individuals where significant reductions in salivary antimicrobial proteins (i.e. below
16 basal levels) have been observed. At present, there are limited data to directly relate
17 the levels of these proteins in saliva to a reduced risk of upper respiratory symptoms
18 (Cunniffe et al., 2011) and given the present findings, this may be of interest.

19

20 **Perspective**

21 These findings show that prolonged exhaustive exercise in trained men can result in
22 increases in salivary antimicrobial proteins probably via an increase in SNS activity,
23 which may be regarded as beneficial to oral immune status. Moreover, a stimulated
24 saliva flow collection with exercise through chewing flavoured gum has a further
25 enhancing effect on α -amylase and lysozyme secretion rate but has little effect on s-

1 IgA secretion. The differences in these effects are likely related to the way that these
2 proteins are stored and secreted into saliva and/or by the activation of different
3 salivary glands by masticatory and gustatory stimuli. Understanding the effect of
4 exercise on salivary antimicrobial proteins and mechanisms or interventions that
5 might affect this response can enable us to employ measures to enhance immune
6 function which might reduce the incidence of URI.

7

8 **Acknowledgements**

9 We would like to acknowledge Alice Gallen, Sam Cheffins, and Ben Silver for their
10 help with the data collection. The authors are grateful for the funding support from
11 Wrigley.

12

13 **References**

14

15 Allgrove, JE, Gomes, E, Hough, J, Gleeson, M. Effect of exercise intensity on
16 salivary antimicrobial proteins and markers of stress in active men. *J Sports Sci* 2008:
17 26: 653-61.

18

19 Allgrove JE, Geneen, L, Latif, S, Gleeson, M. Influence of a fed or fasted state on the
20 s-IgA response to prolonged cycling in active men and women. *Int J Sport Nutr Exerc*
21 *Metab* 2009: 19: 209-21.

22

23 Bishop, NC, Blannin, AK, Armstrong, E, Rickman, M, Gleeson, M. Carbohydrate and
24 fluid intake affect the saliva flow rate and IgA response to cycling. *Med Sci Sports*
25 *Exerc* 2000 : 32: 2046-51.

1

2 Blannin, AK, Robson, PJ, Walsh, NP, Clark, AM, Glennon, L, Gleeson, M. The effect
3 of exercising to exhaustion at difficult intensities on saliva immunoglobulin A, protein
4 and electrolyte secretion. *Int J Sports Med* 1998; 19:547-52.

5

6 Bosch, JA, Ring, C, Geus, EJC, Veerman, ECI, Nieuw Amerogen, AV Stress and
7 secretory immunity. *Int Rev Neurobiol* 2002; 52: 213-53.

8

9 Brandtzaeg, P. Synthesis and secretion of secretory immunoglobulins: with special
10 reference to dental diseases. *J Dent Res* 1976; 55: C102-14.

11

12 Carpenter, GH, Proctor, GB, Andersen, LC, Zhang, XS, Garrett, JR. Immunoglobulin
13 A secretion into saliva during dual sympathetic and parasympathetic nerve stimulation
14 of rat submandibular glands. *Exp Physiol* 2000; 85: 281-6.

15

16 Chatterton, RT, Vogelsong, KM, Lu, YC, Ellmen, AB, Hudgens, GA. Salivary alpha-
17 amylase as a measure of endogenous adrenergic activity. *Clin Physiol* 1996; 1: 433-
18 48.

19

20 Chicharro, JL, Lucia, A, Perez, M, Vaquero, A, Urena, R. Saliva composition and
21 Exercise. *Sports Med* 1998; 26: 17-27.

22

23 Cole, AS, Eastoe, JE. *Biochemistry and Oral Biology*. 2nd ed. London: Wright: 1988
24 476-7 p.

25

1 Costa, RJ, Fortes, MB, Richardson, K, Blizon, JL and Walsh, NP. The effects of
2 postexercise feeding on saliva antimicrobial proteins. *Int J Sport Nutr Exerc Metab.*
3 2012; 22: 184-91
4
5 Crawford, JM, Taubman, MA Smith, DJ. Minor salivary glands as a major source of
6 secretory immunoglobulin A in the human oral cavity. *Science* 1975; 190: 1206-9.
7
8 Cunniffe B, Griffiths H, Proctor W, Davies B, Baker JS, Jones KP. Mucosal immunity
9 and illness incidence in elite rugby union players across a season. *Med Sci Sports*
10 *Exerc* 2011; 43: 388-97.
11
12 Davison G, Diment BC. Bovine colostrum supplementation attenuates the decrease of
13 salivary lysozyme and enhances the recovery of neutrophil function after prolonged
14 exercise. *Br J Nutr* 2010; 103: 1425-32.
15
16 Fahlman MM, Engels HJ. Mucosal IgA and URTI in American Collage Football
17 Players: A Year Longitudinal Study. *Med Sci Sports Exerc* 2005; 37: 374-380.
18
19 Fortes MB, Diment BC, Di Felice U, Walsh NP. Dehydration decreases saliva
20 antimicrobial proteins important for mucosal immunity. *Appl Phys Nutri Metab* 2012:
21 37: 850-9
22
23 Garrett JR. The proper role of nerves in salivary secretion: A review. *J Dent Res* 1987:
24 66: 387-97.
25

1 Gleeson MW, McDonald A, Pyne DB, Cripps AW, Francis JL, Fricker PA, Clancy RL.
2 Salivary IgA levels and infection risk in elite swimmers. *Med Sci Sports Exerc* 1999:
3 31: 67-73.
4
5 Hector MP, Linden RW. The possible role of periodontal mechanoreceptors in the
6 control of parotid secretion in man. *Q J Exp Physiol* 1987: 72: 285-301.
7
8 Humphrey SP, Williamson RP. A review of saliva: normal composition, flow, and
9 function. *J Prosthet Dent* 2001: 85: 162-9.
10
11 Klentrou K, Cieslak, T, MacNeil, M, Vintinner, A, and Plyley, M. Effect of moderate
12 exercise on salivary immunoglobulin A and infection risk in humans. *Eur J of App*
13 *Phys* 2002: 87: 153-158.
14
15 Li TL, Gleeson M. The effect of single and repeated bouts of prolonged cycling and
16 circadian variation on saliva flow rate, immunoglobulin A and α -amylase responses. *J*
17 *Sports Sci* 2004: 22: 1015-24.
18
19 Mackinnon LT, Chick TW, Van AS, Tomasi, TB. Decreased secretory
20 immunoglobulins following intense endurance exercise. *Sports Training Med Rehab*
21 1989: 1: 209-18.
22
23 McDowell SL, Chaloa K, Housh TJ, Tharp GD Johnson GO. The effect of exercise
24 intensity and duration on salivary immunoglobulin A. *Eur J of Appl Physiol Occup*
25 *Physiol* 1991: 63: 108-11.

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5
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7
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9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24

Navazesh, M, Christensen, CM. A comparison of whole resting and stimulated salivary measurement procedures. *J Dent Res* 1982: 61: 1158-62

Neville V, Gleeson M, Folland, JP. Salivary IgA as a risk factor for upper respiratory infections in elite professional athletes. *Med Sci Sports Exerc* 2008: 40: 1228-36.

Nieman D, Henson DA, Fagoaga OR, Utter AC, Vinci DM, Davis JM, Nehlsen-Cannarella, SL. Change in salivary IgA following a competitive marathon race. *Int J Sports Med* 2002: 23: 69-75.

Nehlsen-Cannaralla SL, Nieman DC, Fagoaga OR, Kelln WJ, Shannon M, Davis JM. Saliva immunoglobulins in elite women rowers. *Eur J Appl Physiol* 2000: 81: 222-8.

Noble RE. Salivary alpha-amylase and lysozyme levels: a non-invasive technique for measuring parotid vs. submandibular/sublingual gland activity. *J Oral Sci* 2000: 42: 83-6.

Pedersen AM, Bardow A, Jensen SB, Nauntofte B. Saliva and gastrointestinal functions of taste, mastication, swallowing and digestion. *Oral Dis* 2002: 8: 117-29.

Proctor GB Carpenter GH. Chewing stimulates secretion of human salivary secretory immunoglobulin A. *J Dental Res* 2001: 80: 909-13.

1 Proctor GB, Carpenter GH. Regulation of salivary gland function by autonomic
2 nerves. *Auton Neurosci*. 2007; 133: 3-18.

3 Rudney JD. Relationships between human parotid saliva lysozyme, lactoferrin,
4 salivary peroxidase and sIgA in a large sample population. *Arch Oral Biol* 1989; 34:
5 499-506.

6

7 Sari-Sarraf V., Reilly T, Doran DA, Atkinson G. The effects of single and repeated
8 bouts of soccer-specific exercise on salivary IgA. *Arch Oral Biol* 2007; 52: 526-32.

9

10 Teeuw W, Bosch JA, Veerman EC, Amerongen AV. Neuroendocrine regulation of
11 salivary IgA synthesis and secretion: implications for oral health. *Biol Chem* 2004:
12 385: 1137-46.

13

14 Tomasi TB, Trudeau FB, Czerwinski D, Erredge S. Immune parameters in athletes
15 before and after strenuous exercise. *J Clinl Immunol* 1982; 2: 173-78.

16

17 West NP, Pyne DB, Kyd JM, Renshaw GM, Fricker PA, Cripps AW. The effect of
18 exercise on innate mucosal immunity. *Br J Sports Med* 2010; 44: 227-31.

19

20 Walsh NP, Bishop NC, Blackwell JB, Wierzbicki SG, Montague JC. Salivary IgA
21 response to prolonged exercise in a cold environment in trained cyclists. *Med Sci*
22 *Sports Exerc* 2002; 34: 1632-7.

23

1 Walsh NP, Blannin AK, Clark AM, Cook L, Robson P J, Gleeson M. The effects of
2 high intensity intermittent exercise on saliva IgA, total protein and [alpha]-amylase. J
3 Sports Sci 1999: 17: 129-34.

4

5 Walsh NP, Laing SJ, Oliver SO, Montague JC, Walters R, Bilzon, JL. Saliva
6 parameters as potential indices of hydration status during acute dehydration. Med Sci
7 Sports Exerc 2004: 36: 1535-42.

8

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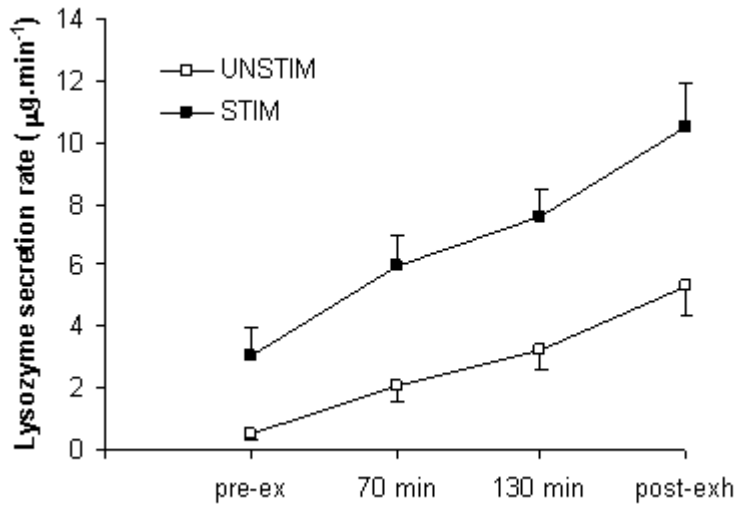
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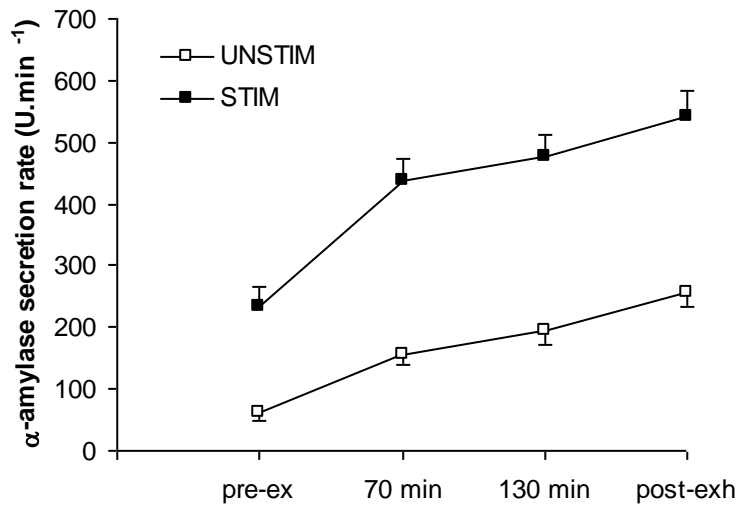
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2 **Figure 1.** Changes in lysozyme secretion rate during exercise in unstimulated (UNSTIM) and
 3 stimulated (STIM) flow conditions. Values are mean \pm SE

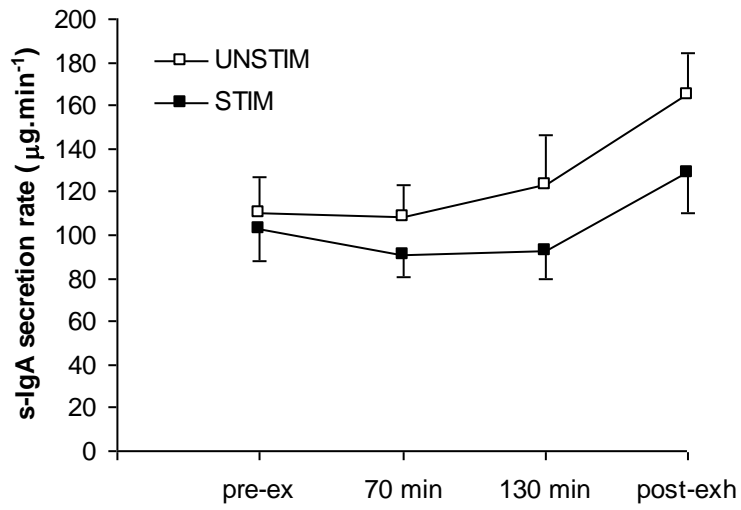
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6 **Figure 2.** Changes in α -amylase secretion during exercise in unstimulated (UNSTIM) and
 7 stimulated (STIM) flow conditions. Values are mean \pm SE

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2 **Figure 3.** Changes in s-IgA secretion rate during exercise in unstimulated (UNSTIM) and
3 stimulated (STIM) flow conditions. Values are mean \pm SE

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- 1 **Table 1.** Changes in saliva flow rate, s-IgA concentration, lysozyme concentration and α -amylase activity during exercise in unstimulated
 2 (UNSTIM) and stimulated (STIM) flow conditions. Values are mean \pm SD

	Pre-ex	70 min	130 min	Post-exh	Main effects <i>p</i> values, trial; time; trial x time
Saliva flow rate					
(ml.min ⁻¹)					< 0.001; 0.027; < 0.001
UNSTIM	0.45 (0.25)	0.48 (0.26)	0.47 (0.47)	0.55 (0.24)	
STIM	1.40 (0.62)	1.27 (0.43)	1.13 (0.38)*	1.20 (0.40)*	
S-IgA concentration					
(mg.l ⁻¹)					< 0.001; 0.015; > 0.1
UNSTIM	291 (250)	242 (123)	285 (194)	326 (186)	
STIM	76 (42)	73 (39)	80 (41)	111 (79)	
Lysozyme concentration					
(mg.l ⁻¹)					> 0.1; < 0.001; > 0.1
UNSTIM	1.15 (1.34)	4.66 (4.72)	7.61 (5.15)	9.73 (6.24)	

STIM	2.08 (2.01)	4.52 (2.80)	6.49 (2.63)	8.50 (3.56)
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α-amylase activity

(U.ml⁻¹)

> 0.1; < 0.001; > 0.1

UNSTIM	143 (23)	352 (26)	418 (28)	463 (22)
STIM	171 (22)	349 (22)	427 (17)	454 (17)

1 ***Significantly different to pre-exercise (P<0.05)**

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