Stimulating whole saliva affects the response of antimicrobial proteins to exercise

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Running head: stimulated saliva flow, exercise and AMPs

Abstract

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

Keywords: cycling, lysozyme, α-amylase, immunogloblulin A, chewing

Introduction

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

Saliva secretion is under strong autonomic neuronal control and thus regulated by parasympathetic and sympathetic nerve fibres that are the effector arms of reflexes activated predominantly by taste and chewing (Humphrey and Williamson, 2001). Generally, when sympathetic stimulation dominates (via noradrenaline) the secretions are high in protein content (e.g. α-amylase), whereas secretions with a high fluid output occur in response to parasympathetic stimulation (Chicharro et al., 1998). However, parasympathetic stimulation can also affect salivary protein secretion, and protein secretion of some glands such as the sublingual and some of the minor glands, may even be entirely under parasympathetic control (Teeuw et al., 2004). Furthermore, sympathetic stimulation also causes some stimulation of the saliva flow rate (Garrett, 1987), thus, rather than acting antagonistically, it could be argued that the two branches of the autonomic nervous system may exert relatively independent effects in which the activity of one branch may synergistically augment the other (Bosch et al., 2002). Other factors known to influence saliva secretion include psychic factors, nutrition, hydration status, medication, local or systemic diseases and physical stress (Humphrey and Williamson, 2001), where significant reductions in the flow rate and changes in the antimicrobial proteins have been observed. For example, reductions in salivary immunoglobulin A (s-IgA) levels have often been reported following strenuous exercise (Tomasi et al., 1982; Mackinnon et al., 1989; Nehlsen-Cannaralla et al., 2000; Nieman et al., 2002). However, other studies report increases (Blannin et al., 1998; Walsh et al., 2004; Li and Gleeson, 2004; Sari-Sarraf et al., 2007; Allgrove et al., 2008; Allgrove et al., 2009; Costa et al., 2012) or no change (McDowell et al., 1991; Walsh et al., 1999). α-amylase (Li and Gleeson, 2004; Allgrove et al., 2008) and lysozyme (Allgrove et al., 2008; West et al., 2010; Costa et al., 2012) typically increase with exercise, although one study reported a significant reduction in lysozyme levels post-exercise (Davison & Diment, 2010) and in another reductions were observed following a dehydration protocol (Fortes et al., 2012). Changes in salivary antimicrobial proteins have been linked to the susceptibility of upper respiratory symptoms in a variety of athletic/exercising populations (Gleeson et al., 1999; Klentrou et al., 2002; Neville et al., 2008; Nieman et al., 2002; Fahlman & Engels, 2005; Cunniffe et al., 2011).

Athletes often consume both food items and beverages during exercise. It has been shown that chewing can increase the flow rate by 3-fold compared with unstimulated saliva secretion (Hector and Linden, 1987), and this has also been shown to increase the secretion of certain salivary proteins, including s-IgA probably via increased epithelial cell trancytosis (Proctor and Carpenter, 2001). Similar increases were also found in the secretion rates of total protein and α-amylase (Proctor and Carpenter, 2001). Furthermore, saliva flow rate and protein concentration is increased in response to gustatory stimulation (Proctor and Carpenter, 2007), with acid and sweet taste stimuli providing the greatest response (Humphrey and Williamson, 2001). Given that exercise and stimulated salivary flow can independently affect antimicrobial proteins, it is possible that stimulating salivary flow during exercise may potentially affect the salivary antimicrobial response, and represent mechanisms by which resistance to oral infection may be altered. Therefore, the aims of the study were to investigate the influence of stimulated saliva flow on salivary antimicrobial proteins by chewing flavoured gum during prolonged exhaustive cycling. It was hypothesised that stimulating saliva flow by chewing would acutely enhance antimicrobial protein secretion during exercise.

Materials and methods

Participants

Following university ethical approval 24 trained male volunteers (mean ± SD: age 23 ± 5 yr; height 1.79 ± 0.07 m; body mass 73.8 ± 8.1 kg; $\dot{V}O_{2max}$ 56.6 ± 4.7 $mL.kg^{-1}.min^{-1}$) with cycling as one of their main sports, volunteered to participate in the study. Participants completed a health questionnaire to report any symptoms of infection or illness in the 12 weeks prior to commencing the study and were informed of the aims and procedures before providing written informed consent. Participants were included if they were healthy endurance-trained male volunteers between the ages of 18-35 yr of age. Participants representing one or more of the following criteria were excluded from the study: smoking or use of any medication or dietary supplements or suffering from any known chronic disease.

Preliminary measurements

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

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

Experimental procedures

Participants were requested to abstain from alcohol, caffeine and strenuous exercise 2 days prior to the trial. Participants arrived at the laboratory at 8:30 h for the main trial following an overnight fast (10 – 12 h) and were required to sit quietly for 5 min before providing a saliva sample. The participants were then asked to empty their bladders before body mass was measured wearing their shorts only. They then performed 2.5 h cycling at 60% $\dot{V}O_{2max}$ on a stationary cycle ergometer. Heart rate and RPE were measured at 20 min intervals and expired gas was collected at 30 min, 90 min and 114 min of exercise for the analysis of $\dot{V}O_2$ using a Douglas bag. Participants were given 200 mL of water every 20 min during exercise; in addition they ingested 300 ml of flavoured water immediately before the exercise began, and again after 50 min and 110 min of exercise. Further saliva samples were collected 70 min and 130 min of exercise. It was ensured that no fluid was consumed in the 10 min prior to each saliva collection. Following completion of the 2.5 h cycling, the participants were allowed a 5 min rest before commencing the ride to exhaustion at 75% $\dot{V}O_{2max}$ with no verbal encouragement and no information on time elapsed. The time to exhaustion was 897 ± 122 s. A final saliva sample was obtained immediately after completing the ride to exhaustion and body mass was measured. Mean temperature and humidity in the laboratory during the trial were 23 ± 0.5 ^{o}C and 32 ± 4 % respectively.

Saliva collection

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

Saliva analysis

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

Statistical analysis

Data were checked for normality, homogeneity of variance and sphericity before statistical analysis. A two-way ANOVA (2 treatments x 4 sample times) with repeated measures design was used to examine the salivary data. Data that were not normally distributed were normalised with log transformation. Significant differences were assessed using Student's paired *t*-test with Holm-Bonferroni adjustments for multiple comparisons. Differences in HR between the steady state exercise and time to exhaustion ride were assessed using Student's paired *t*-tests. Data in text and tables are presented as mean ± SD. For clarity, data in figures are presented as mean ± SEM. Statistical significance was accepted at $P < 0.05$.

Results

Physiological variables and RPE

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

Salivary variables

Saliva flow rate

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

Insert Table 1 near here

Salivary lysozyme concentration

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

Salivary lysozyme secretion rate

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

Insert Figure 1 near here

Salivary α-amylase activity

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

Salivary α-amylase secretion rate

Salivary α-amylase secretion rate increased with exercise (main effect of time: $F_{3, 49}$ = 45.99; $P < 0.001$) and was significantly higher in STIM compared with UNSTIM throughout the exercise protocol (main effect of treatment: $F_{1, 23} = 166.85$; $P < 0.001$; Figure 2).

Insert Figure 2 near here

Salivary IgA concentration

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

Salivary IgA secretion rate

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

Insert Figure 3 near here

Discussion

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

The results show a significant effect of stimulating saliva flow on saliva flow rate, where it was 3-fold higher in STIM compared with UNSTIM and corroborates previous findings (Hector and Linden, 1987). Saliva flow rate decreased following exercise in the STIM trial only. Previous studies have attributed a reduction in flow rate during exercise to dehydration, although the small change in net mean body loss of (0.53 ± 0.11 kg; 0.7 ± 0.1%), and lack of difference in the unstimulated trial suggests that this had little impact (Walsh et al., 1999). It is possible that the decreased parasympathetic nervous system activity during exercise and a removal of vasodilatory influences (Proctor and Carpenter, 2007) may have limited the increase in flow rate that occurs with chewing. Alternatively, the decline may be a result of repetitive periods of chewing whereby the production of saliva may have become temporarily exhausted over time (Proctor and Carpenter, 2001). Although saliva flow rate in STIM was consistently higher than UNSTIM during the protocol, these findings show that the saliva collection method employed during exercise can differently affect the salivary flow response.

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

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

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

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

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

Perspective

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

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

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References

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

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

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

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

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

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

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

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

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

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

Costa, RJ, Fortes, MB, Richardson, K, Blizon, JL and Walsh, NP. The effects of postexercise feeding on saliva antimicrobial proteins. Int J Sport Nutr Exerc Metab. 2012: 22: 184-91

Crawford, JM, Taubman, MA Smith, DJ. Minor salivary glands as a major source of secretory immunoglobulin A in the human oral cavity. Science 1975: 190: 1206-9.

Cunniffe B, Griffiths H, Proctor W, Davies B, Baker JS, Jones KP. Mucosal immunity and illness incidence in elite rugby union players across a season. Med Sci Sports Exerc 2011: 43: 388-97.

Davison G, Diment BC. Bovine colostrum supplementation attenuates the decrease of salivary lysozyme and enhances the recovery of neutrophil function after prolonged exercise. Br J Nutr 2010: 103: 1425-32.

Fahlman MM, Engels HJ. Mucosal IgA and URTI in American Collage Football Players: A Year Longitudinal Study. Med Sci Sports Exerc 2005: 37: 374-380.

Fortes MB, Diment BC, Di Felice U, Walsh NP. Dehydration decreases saliva antimicrobial proteins important for mucosal immunity. Appl Phys Nutri Metab 2012: 37: 850-9

Garrett JR. The proper role of nerves in salivary secretion: A review. J Dent Res 1987: 66: 387-97.

Gleeson MW, McDonald A, Pyne DB, Cripps AW, Francis JL, Fricker PA, Clancy RL. Salivary IgA levels and infection risk in elite swimmers. Med Sci Sports Exerc 1999: 31: 67-73.

Hector MP, Linden RW. The possible role of periodontal mechanoreceptors in the control of parotid secretion in man. Q J Exp Physiol 1987: 72: 285-301.

Humphrey SP, Williamson RP. A review of saliva: normal composition, flow, and function. J Prosthet Dent 2001: 85: 162–9.

Klentrou K, Cieslak, T, MacNeil, M, Vintinner, A, and Plyley, M. Effect of moderate exercise on salivary immunoglobulin A and infection risk in humans. Eur J of App Phys 2002: 87: 153-158.

Li TL, Gleeson M. The effect of single and repeated bouts of prolonged cycling and circadian variation on saliva flow rate, immunoglobulin A and α-amylase responses. J Sports Sci 2004: 22: 1015-24.

Mackinnon LT, Chick TW, Van AS, Tomasi, TB. Decreased secretory immunoglobulins following intense endurance exercise. Sports Training Med Rehab 1989: 1: 209-18.

McDowell SL, Chaloa K, Housh TJ, Tharp GD Johnson GO. The effect of exercise intensity and duration on salivary immunoglobulin A. Eur J of Appl Physiol Occup Physiol 1991: 63: 108-11.

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.

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

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

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

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

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

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

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

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

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

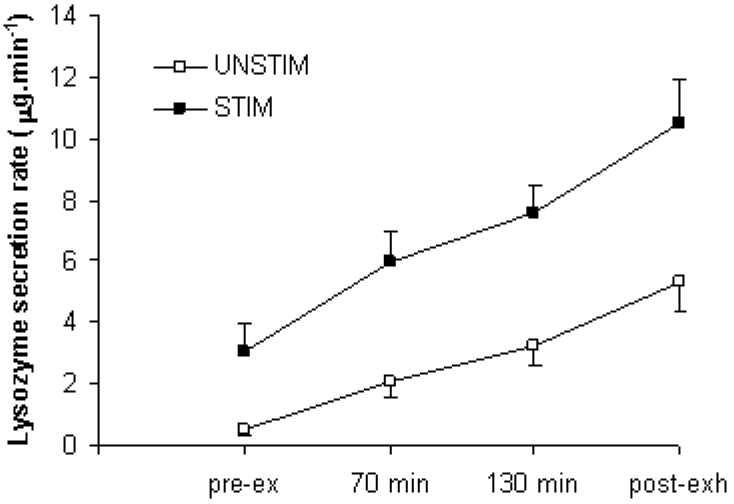

Figure 1. Changes in lysozyme secretion rate during exercise in unstimulated (UNSTIM) and stimulated (STIM) flow conditions. Values are mean ±SE

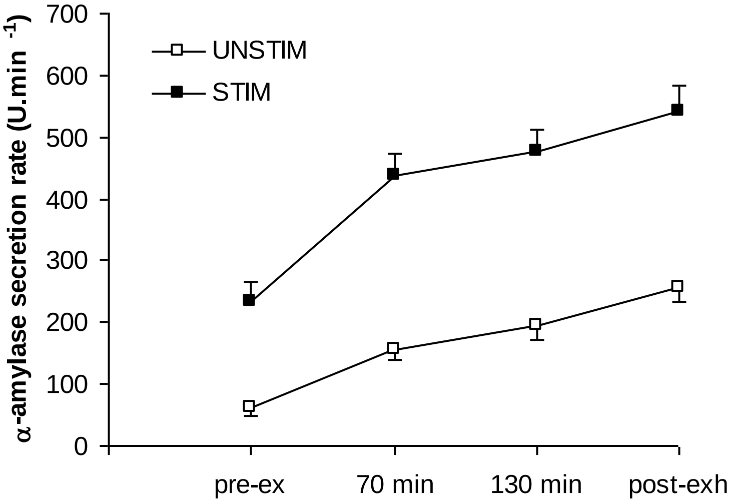

Figure 2. Changes in α-amylase secretion during exercise in unstimulated (UNSTIM) and stimulated (STIM) flow conditions. Values are mean ±SE

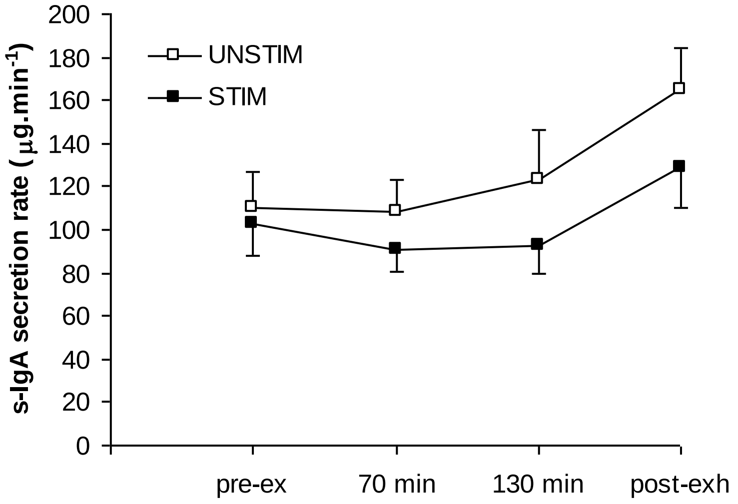

Figure 3. Changes in s-IgA secretion rate during exercise in unstimulated (UNSTIM) and stimulated (STIM) flow conditions. Values are mean ±SE

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

	Pre-ex	70 min	130 min	Post-exh	Main effects *p* 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)	
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)	

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