Influence of a fed or fasted state on the s-IgA response to prolonged cycling in active men and women

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Abstract

This study investigated the effect of a fed or fasted state on the s-IgA response to prolonged cycling. Using a randomised, cross-over design, 16 active adults (8 males, 8 females) performed 2 h cycling on a stationary ergometer at 65% maximal oxygen uptake on one occasion following an overnight fast (FAST) and on another occasion following the consumption of a 2.2 MJ high carbohydrate (CHO) meal (FED) 2 h before. Timed, unstimulated whole saliva samples were collected immediately before ingestion of the meal, immediately pre-exercise, 5 min before cessation of exercise, immediately post-exercise and 1 h post-exercise. The samples were analysed for s-IgA concentration, osmolality and cortisol and saliva flow rates were determined to calculate the s-IgA secretion rate. Saliva flow rate decreased by 50% during exercise ($P<0.05$), s-IgA concentration increased by 42% ($P<0.05$), but s-IgA secretion rate remained unchanged. There was a 37% reduction in s-IgA:osmolality post-exercise ($P<0.05$) and salivary cortisol increased by 68% ($P<0.05$). There was no effect of FED versus FAST on these salivary responses. The s-IgA concentration, secretion rate and osmolality were found to be significantly lower in females than in males throughout the exercise protocol ($P<0.05$); however, there was no difference between genders in saliva flow rate, s-IgA:osmolality ratio or cortisol. These data demonstrate that a fed or fasted state 2 h prior to exercise does not influence resting s-IgA or the response to prolonged cycling. Furthermore, these results show lower levels of s-IgA and osmolality in females compared with males at rest.

Keywords

Salivary immunoglobulin A, nutritional status, exercise, gender

Introduction

It is now widely recognised that exercise can have deleterious effects on the immune system (Gleeson, 2007). Prolonged, strenuous exercise can result in a temporary depression of certain aspects of immune function (Mackinnon *et al*., 2000) and there is increasing evidence to suggest that athletes engaged in heavy training and competition tend to suffer from a higher incidence of upper respiratory illness than their sedentary counterparts (Fahlman & Engels, 2005; Nieman *et al*., 1990; Peters & Bateman, 1983; Spence *et al*., 2007).

Immunoglobulin A is the predominant antibody present in mucosal fluids and can be measured non-invasively in saliva (s-IgA). S-IgA plays an important role in immunity as the first line of defence against potential pathogens invading the oral and nasal cavities and functions by inhibiting the colonisation of pathogens, binding antigens for transport across the epithelial barrier and neutralising viruses (Lamm, 1998). A reduction in the concentration of s-IgA has been implicated as a causal factor for the reported increased incidence of upper respiratory illness during heavy training in athletes (Fahlman & Engels, 2005; Gleeson *et al*., 1999; Neville *et al*., 2008). Indeed, several studies have reported significant reductions in s-IgA following an acute bout of intense exercise (Gleeson *et al*., 1999, Nieman *et al*., 2002; Steerenberg *et al*., 1997; Tomasi *et al*., 1982). However, others have reported significant increases in s-IgA (Allgrove *et al*., 2008; Blannin *et al*., 1998; Sarri-Sarraf *et al*., 2007) or no change (Li & Gleeson, 2004; Mackinnon and Hooper, 1994; McDowell *et al*., 1991; Walsh *et al*., 1999). These discrepant findings may be related to several factors including the method of expressing s-IgA, nutritional status of the individual and the exercise protocol employed.

The saliva flow rate is suggested to play a critical role in oral health via a mechanical washing effect and through the supply of both immune and non-immune elements into the oral cavity (Li & Gleeson, 2004; Ranoten & Meurman, 2000; Tenuvuo, 1998). A decrease in the saliva flow rate has been repeatedly shown following prolonged exercise via mechanisms such as dehydration, autonomic imbalance and evaporation of saliva through increased breathing (Blannin *et al*., 1998; Laing *et al*., 2005; Steerenberg *et al*., 1997; Walsh *et al*., 2002). However, a reduced saliva flow rate may artificially increase s-IgA concentration (Li & Gleeson, 2004; Walsh *et al*., 2002). Consequently, expressing s-IgA as a secretion rate (flow rate x concentration) or as a ratio to solute concentration (osmolality) may be more valid, since solute secretion rate appears to be unaffected by exercise (Blannin *et al*., 1998). Indeed, previous studies have shown transient reductions in both the s-IgA secretion rate and s-IgA:osmolality ratio following prolonged cycling (Walsh *et al*., 2002; Laing *et al*., 2005).

The responses of s-IgA to exercise are thought to be mediated by increases in circulating stress hormones such as catecholamines and cortisol. Cortisol has been shown to inhibit transepithelial transport of s-IgA in rodents (Sabbadini & Berczi, 1995), and may inhibit IgA synthesis by B cells in the submucosa (Saxon *et al*., 1978). Salivary cortisol has been shown to correlate highly with circulating levels (Chicharro *et al*., 1998), making it a reliable marker of the stress hormone response to exercise. Nutritional interventions are commonly used by athletes as a method to offset the exercise-induced immune response (Gleeson & Bishop, 2000). Carbohydrate (CHO) ingestion during exercise has been shown to attenuate the rise in

stress hormones, whereas an athlete exercising in a CHO-depleted state experiences larger increases in circulating stress hormones (Gleeson *et al*., 2004b). However, previous investigations have shown little effect of CHO ingestion on s-IgA concentration or secretion rate during prolonged exercise compared with placebo (Bishop *et al*., 2000; Nehlsen-Cannarella *et al*., 2000; Nieman *et al*., 2002). In one previous study it was shown that the prior nutritional status (i.e. fed or fasted) of an individual significantly influenced resting s-IgA, where higher and more variable levels in s-IgA concentration were observed in fasting saliva compared with non-fasting saliva (Gleeson *et al*., 1990). Moreover, Oliver *et al*. (2007) reported that 48 h of energy and fluid restriction resulted in a fall in resting s-IgA secretion rate. However, the precise mechanism(s) of action to explain these differences is currently unclear, whether they are directly related to the meal ingested and/or hydration status remains to be determined.

The effects of nutritional status (fed versus fasted) on the s-IgA response to exercise remain unknown. Given the apparent link between s-IgA and nutritional status at rest (Gleeson *et al*., 1990; Oliver *et al*. 2007), understanding the impact of this factor on the s-IgA response to exercise could have implications for future research designs, as well as consequences on the oral health of individuals performing strenuous exercise. Therefore, the present study investigated the influence of a fed or fasted state 2 h prior to exercise on the s-IgA responses to prolonged cycling. It was hypothesised that a fasted state would increase the immunoendocrine response to exercise resulting in a greater reduction in s-IgA post-exercise. Both males and females were recruited to participate in the study; therefore an additional aim was to assess whether gender would influence the salivary measures.

Participants

After approval from the University Ethical Committee, 16 active, healthy adults (8 males, 8 females), who were participating in regular physical activity at least three times per week, for more than two hours per day, volunteered to take part in the study. Their physical characteristics are outlined in Table 1. Participants were informed of the aims and procedures of the study before providing written informed consent and completing a comprehensive health questionnaire.

****Table 1 near here****

Preliminary measurements

Approximately 1 week before the main trials participants completed a continuous incremental test on an electromagnetically braked cycle ergometer (Lode Excalibur, Groningen, Netherlands) to volitional exhaustion in order to determine their maximal oxygen uptake ($\dot{V}O_{2max}$). Following a 3-min warm-up participants began cycling at 70 W with increments of 35 W occurring every 3 min. Verbal encouragement was provided to each participant to ensure maximal effort. Expired gas samples were collected in Douglas bags (Harvard Apparatus, Edenbridge, UK) during the third minute of each work rate increment 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 $\dot{V}_E$, $\dot{V}O_2$ and $\dot{V}CO_2$. From the $\dot{V}O_2$ - work rate relationship the work rate equivalent to 65% $\dot{V}O_{2max}$ was interpolated. On a second occasion

participants completed a familiarisation trial. The purpose of this was to familiarise them with the exercise protocol and to check that the correct relative exercise intensity was being performed. Participants cycled at a constant work rate corresponding to 65% $\dot{V}O_{2max}$ for 2 h. Expired gas samples were collected over a 1-min period into Douglas bags after 10 min and 30 min of exercise and then every 30 min thereafter. Heart rate and RPE were also measured every 15 min. During this trial the participants were familiarised with the saliva collection procedure. This enabled them to feel comfortable with the collection method and to establish individual flow rates and an appropriate collection time to ensure that an adequate volume (~ 1 mL) of saliva was collected for analysis.

Experimental procedures

Using a randomised cross-over design, participants performed two exercise trials separated by at least one week. They reported to the laboratory at 09:00 h and following an overnight fast (10-12 h). A resting fingertip blood sample was obtained using an autoclick lancet, and the blood glucose concentration was analysed in singular using an Accutrend GC (Roche, Germany) glucose analyser (reported coefficient of variability 3%). This was to assess whether the participants were in a fasted state before beginning the trial. Participants then consumed either: 3 cereal bars and 500 mL of a commercially available sports drink (FED; total energy content: 2210 kJ) or 500 mL of a dilute low calorie cordial drink (FAST; total energy content: 60 kJ) 2 h prior to exercise, as this timing of food and fluid intake is typically recommended for individuals engaging in strenuous physical activity (Williams and Serratosa, 2006). The nutritional content of the meal is outlined in Table 2. The participants were required to consume the meal within a 15-min time period. They

then rested for 1 h 45 min before performing 2 h cycling at 65% $\dot{V}O_{2max}$ on a stationary cycle ergometer. The order of the trials was randomised. Body mass was measured pre- and post-exercise. Heart rate and RPE were obtained at 15-min intervals during and expired gas samples were collected every 30 min of exercise using a Douglas bag. Saliva samples were obtained upon arrival at the laboratory (baseline), immediately pre-exercise, 5 min before cessation of exercise, immediately post-exercise and 1 h post-exercise. Water ingestion was permitted *ad libitum* before, during and after exercise with the exception of the 10-min period prior to each saliva sample collection. During both experimental trials, the participants were instructed to remain fasted until the 1 h post-exercise sample had been collected. For the 24 h preceding each trial, the participants were requested to follow the same (pre-trial 1) diet and eating schedule before the subsequent trial. They were also requested to abstain from alcohol, caffeine and heavy exercise for 48 h prior to each trial. The environmental conditions of the laboratory were 25.4 ± 0.2°C and relative humidity of 41 ± 2%.

****Table 2 near here****

Saliva collection

Saliva collections were made with the participants seated, leaning forward and with their heads tilted down. Participants were instructed to swallow in order to empty the mouth before an unstimulated whole saliva sample was collected over a pre-determined time period into a pre-weighed, sterile vial. Care was taken to allow saliva

to dribble into the collecting tubes making minimal orofacial movement. Samples were then stored at -80°C until 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.

Saliva analysis

After thawing s-IgA concentration was determined by an enzyme-linked immunosorbant assay (ELISA) as described previously by Li & Gleeson (2004). Salivary cortisol was analysed using a commercially available ELISA kit (DX-SLV-2930, DRG Instruments, Marburg, Germany). Osmolality was determined using a cryoscopic (freezing point depression) osmometer (Osmomat 030, Gonotec, GbBH, Berlin, Germany) calibrated with 300 $mOsmol.kg^{-1}$ NaCl solution. The s-IgA secretion rate was calculated by multiplying the saliva flow rate by the s-IgA concentration. The intra-assay coefficient of variation for the analytical methods was 7.0% and 3.9% for s-IgA and cortisol assays, respectively.

Statistical analysis

Results are presented as mean values and standard error of the mean (± SEM). Data were checked for normality, homogeneity of variance and sphericity before statistical analysis. The salivary data were examined using a 3-factor (trial x gender x time) repeated measures ANOVA. Significant differences were assessed using Student's paired *t*-test with Holm-Bonferroni adjustments for multiple comparisons.

Physiological variables and RPE were examined using Student's paired *t*-tests. Statistical significance was accepted at $P < 0.05$.

Results

Physiological variables and RPE

Mean blood glucose values recorded at baseline were 4.4 ± 0.3 mmol.L^{-1} on the treatment days. Results for exercise intensity (% $\dot{V}O_{2max}$), HR, RPE, body mass changes and RER are presented in Table 3. RER was significantly lower in FAST compared with FED, but there were no differences between trials in any of the other physiological variables.

****Table 3 near here****

Salivary variables

Gender differences

Gender did not influence the salivary responses to exercise. However, a significant main effect was observed where s-IgA concentration, s-IgA secretion rate and osmolality were significantly lower in females compared with males ($P < 0.01$; Table 4), but there were no differences between genders in saliva flow rate, s-IgA:osmolality ratio and cortisol.

****Table 4 near here****

Saliva Flow rate

Saliva flow rate showed an initial increase from baseline to pre-exercise. Saliva flow rate then decreased significantly during exercise and returned to baseline at 1 h post-exercise (main effect of time: $F_{3, 42} = 14.4$, $P < 0.001$). There was no effect of FED or FAST on this response (Figure 1A).

Salivary IgA concentration

Salivary IgA concentration increased with exercise (main effect of time: $F_{3, 34} = 8.4$, $P < 0.01$), but there was no effect of FED or FAST on this response (Figure 1B).

Salivary IgA secretion rate

The s-IgA secretion rate did not change significantly throughout the exercise protocol (Figure 1C).

****Figure 1 near here****

Salivary osmolality

Salivary osmolality increased with exercise (main effect of time: $F_{3, 22} = 26.9$, $P < 0.001$). Salivary IgA:osmolality ratio decreased post-exercise (main effect of time: F $F_{3, 40} = 14.0$, $P < 0.001$). There was no effect of FED or FAST on these responses (Table 5).

****Table 5 near here****

Salivary cortisol

Salivary cortisol increased post-exercise compared with pre-exercise (main effect of time: $F_{1, 13} = 7.7$, $P = 0.016$), but there was no effect of FED or FAST on this response: pre exercise 10.4 ± 2.2 versus 9.4 ± 1.4 nmol.L^{-1}, post exercise 16.6 ± 3.7 versus 16.6 ± 3.8 nmol.L^{-1} for FED and FAST trial, respectively.

Discussion

The main findings of the study were that prior nutritional status had no influence on the s-IgA response to prolonged cycling. In addition, lower values of s-IgA concentration, s-IgA secretion rate and salivary osmolality were observed in females compared with males at rest, with no effect on the acute responses to exercise.

It has been previously reported that fasting saliva samples yield higher and more variable s-IgA concentrations than non-fasting samples at rest (Gleeson *et al*., 1990; Gleeson *et al*., 2004b). However, the present results do not support these findings since s-IgA was not significantly affected by the pre-exercise nutritional status, and in fact, s-IgA concentration in the fed state tended to be higher. Furthermore, the spread of the data – highlighted by the SEM - for this group of individuals under the two conditions were very similar. It is possible that the discrepant findings may be partly explained by hydration status, since dehydration can reduce the saliva flow rate which may artificially increase s-IgA concentration (Bishop *et al*., 2000; Walsh *et al*., 2002; Walsh *et al*., 2004). Gleeson *et al*. (1990) did not report the saliva flow rate or the amount of fluid ingested between conditions; therefore, it is not known if the higher and more variable levels in s-IgA concentration in fasting saliva had been influenced

by this factor. Furthermore, the timing of the meal may be important since previous studies have shown that mastication (chewing) can stimulate the saliva flow rate by up to 3-fold (Hector and Linden, 1999), and result in a lower s-IgA concentration recorded immediately after (Proctor and Carpenter, 2001). Since the timing of meal was not reported in the study by Gleeson *et al*. (1990), a direct comparison cannot be made, but the present results show that the ingestion of a high CHO meal 2 h before compared with fasting has no significant influence on the saliva flow rate or composition at rest or following exercise.

Carbohydrate (CHO) is known to attenuate the cortisol response compared with placebo during prolonged exercise (Gleeson *et al*., 2004a). However, several studies have shown no effect of CHO feeding during exercise on s-IgA concentration or secretion rate (Bishop *et al*., 2000; Nehlsen-Cannarella *et al*., 2000; Nieman *et al*., 2002). In the present study salivary cortisol increased post-exercise; but there was no effect of nutritional status on this response. Since the inhibition of s-IgA transport (and synthesis) has been previously attributed to increased levels of circulating stress hormones including cortisol (Hucklebridge *et al*., 1998; Sabbadini & Berczi, 1995), this may explain in part why no differences in the s-IgA response were observed between the two conditions.

The significant reduction in saliva flow rate with exercise observed in this study is consistent with many previous studies (Blannin *et al*., 1998; Steerenberg *et al*., 1997; Mackinnon and Jenkins, 1993; Walsh *et al*., 2002). Salivary secretion is predominantly under the control of the autonomic nervous system (Chicharro *et al*., 1998), and is dependent on both parasympathetic cholinergic nerves and sympathetic

adrenergic nerves. It is now accepted that the reduction in saliva flow rate associated with exercise results in part to a removal of parasympathic vasodilatory influences rather than sympathetic vasoconstriction since only sympathetic secretomotor rather than vasoactive nerve fibres are activated under reflex conditions (Proctor and Carpenter, 2007). Indeed, the saliva flow rate was lowest at the sample point during exercise when parasympathetic activity would be largely inhibited and began to recover upon cessation of exercise (5 min after). It has been reported that dehydration has the greatest negative impact on saliva flow rate during exercise (Walsh *et al*., 2004), which tends to occur following losses of ~3% body mass. In the present investigation only small changes in body mass occurred post-exercise (~0.5%) and despite this, a decrease in saliva flow rate and an increase in osmolality were still observed. These findings contrast Bishop *et al*. (2000) and Walsh *et al*. (2004), where fluid intake (to match pre-determined sweat losses in the latter study) prevented the decrease in flow rate and increase in osmolality, which may suggest a greater effect of neural activation on saliva flow rate and electrolyte secretion during exercise rather than dehydration *per se*. This may question their sensitivity as indicators of hydration status following exercise. It is noteworthy that an initial increase in the saliva flow rate from baseline to pre-exercise 2 h after was observed which may be due to a diurnal variation, since saliva flow rate tends to increase throughout the day (Dawes, 1974). On the other hand, it may relate to an anticipatory increase in sympathetic activity and inhibition of parasympathetic activity, thereby reducing the saliva flow rate (Blannin *et al*., 1998).

Salivary IgA concentration increased significantly with exercise which is in accordance with many previous studies involving prolonged cycling (Blannin *et al*.,

1998; Walsh *et al*., 2002; Li and Gleeson, 2004). This is likely due to the reduction in saliva flow rate and slight dehydration, rather than genuine alterations in the mucosal immune response (Walsh *et al*., 2002), since s-IgA secretion rate did not change significantly throughout the exercise protocol. Many previous studies have reported significant reductions in s-IgA secretion rate following prolonged cycling (Laing *et al*., 2005; Mackinnon *et al*., 1989; Walsh *et al*., 2002). Although s-IgA secretion rate did not change significantly with exercise in the present study, a 30% reduction was observed during exercise compared with pre-exercise, but whether this trend has any physiological significance in terms of risk of respiratory illness is unknown. Despite no significant change in the s-IgA secretion rate with exercise, a clear reduction in the s-IgA:osmolality ratio was observed, which returned to baseline levels at 1 h post-exercise and is in accordance with findings by Laing *et al*. (2005). A reduction in the s-IgA:osmolality ratio post-exercise lends some support to a transient depression of mucosal immunity post-exercise, which returns to baseline levels following 1 h of recovery. However, since significant reductions in the other measures of s-IgA were not demonstrated, this highlights the continued uncertainty of which measure to use when investigating the effects of exercise on mucosal immune function.

Striking genders differences were observed in that resting s-IgA concentration, s-IgA secretion rate and osmolality in females were almost half that of males. Previous studies have reported a lower s-IgA concentration in females at rest (Evans *et al*., 2000; Gleeson *et al*., 1999), although Nieman *et al*. (2002) found no gender differences in s-IgA concentration or secretion rate following a marathon race. Although there was no effect of gender on the acute response to exercise, a significant difference in resting saliva composition may have practical importance if these

parameters are to be used as predictors of infection risk or as a marker of hydration status. If laboratories are to establish clinically relevant ranges, such differences would need to be considered. The physiological significance of these differences is yet to be elucidated. Sex hormones are known to play a role in the immune system at rest (Timmons *et al*., 2005). However, Burrows *et al*. (2002) found no differences in s-IgA in a group of highly trained female endurance athletes over the menstrual cycle. Moreover, there was no relationship between s-IgA concentration and progesterone. It is possible that absolute training status (weight-specific $\dot{V}O_{2max}$ was 20% lower in females) may have influenced these values, as a higher s-IgA concentration was previously reported in elite athletes compared with both active and sedentary individuals (Francis *et al*., 2005). Furthermore, as a dietary analysis of the participants was not conducted; it is not known whether the differences were related to deficiencies in certain nutrients such as protein (Chandra, 1997; Gleeson *et al*., 2004b). The finding of lower levels of mucosal immunity in females compared with males in this study contrast reports that females appear to be more resistant to viral infections in the general population (Beery, 2003). Thus, further research is warranted to investigate s-IgA between genders in different athletic populations to determine the importance of such differences in infection risk.

In summary, the results from the present study show that exercise performed in either a fed or short-term (~10-h) fasted state appears to have little influence on the response pattern of s-IgA and salivary cortisol during prolonged, strenuous exercise. Additionally, these results demonstrate lower levels of resting s-IgA and osmolality in females compared with males, with no effect on the acute response to exercise.

References

Allgrove J.E., Gomes, E., Hough, J. & Gleeson, M. (2008). Effect of exercise intensity on salivary antimicrobial proteins and markers of stress in active men. *Journal of Sports Sciences*, **26**, 653-661.

Beery, T. A. (2003). Sex differences in infection and sepsis. *Critical care nursing clinics of North America*, **15**, 55-62.

Bishop, N. C., Blannin, A. K., Armstrong, E., Rickman, M. & Gleeson, M. (2000). Carbohydrate and fluid intake affect the saliva flow rate and IgA response to cycling. *Medicine and Science in Sports and Exercise*, **32**, 2046-2051.

Bishop, N. C., Walker, G. J., Scanlon, G. A., Richards, S. & Rogers, E. (2006). Salivary IgA responses to prolonged intensive exercise following caffeine ingestion. *Medicine and Science in Sports and Exercise*, **38**, 513-519.

Blannin, A. K., Robson, P. J., Walsh, N. P., Clark, A.M., Glennon, L. & Gleeson, M. (1998). The effect of exercising to exhaustion at difficult intensities on saliva immunoglobulin A, protein and electrolyte secretion. *International Journal of Sports Medicine*, **19**, 547-552.

Burrows M., Bird S. R. & Bishop N. (2002). The menstrual cycle and its effect on the immune status of female endurance runners. *Journal of Sports Sciences*, **20**, 339-344.

Chandra R. K. (1997). Nutrition and the immune system: an introduction. *The American Journal of Clinical Nutrition*, **66**, 460S-463S.

Chicharro, J. L., Lucia, A., Perez, M., Vaquero, A. & Urena, R. (1998). Saliva composition and Exercise. *Sports Medicine*, **26,** 17-27.

Cole, A. S. & Eastoe, J. E. (1988). *Biochemistry and Oral Biology*. 2nd edition, pp. 476-477. London: Wright.

Dawes, C. (1974). Rhythms in salivary flow rate and composition. *International Journal of Chronobiology,* **2**, 253-79.

Evans, P., Derm G., Ford, G., Hucklebrideg, F., Hunt, K & Lambert, S. (2000). Social Class, Sex, Age Differences in Mucosal Immunity in a Large Community Sample. *Brain Behaviour and Immunity*, **14**, 41-48.

Fahlman, M. M. & Engels, H. J. (2005). Mucosal IgA and URTI in American Collage Football Players: A Year Longitudinal Study. *Medicine and Science in Sports and Exercise*, **37**, 374-380.

Francis, J. L., Gleeson, M., Pyne, D. B., Callister, R. & Clancy, R. (2005). *Medicine and Science in Sport and Exercise*, **37**, 571-578.

Gleeson, M. (2007). Immune function in sport and exercise. *Journal of Applied Physiology*, **103**, 693-699.

Gleeson, M. & Bishop, N. C. (2000). Elite Athlete Immunology: Importance of Nutrition. *International Journal of Sports Medicine*, **21**, S44-S50.

Gleeson, M., Cripps, A. W., Clancy, R. L. & Geraghty, S. B. (1990). The variability of immunoglobulins and albumin in saliva of normal and IgA-deficient adults. In: Advances in Mucosal Immunology. T. T. Mcdonald (Ed.) Lancaster UK: Kluwer Academic Publishers, pp. 500-501.

Gleeson, M., W. A. McDonald, D. B. Pyne, A. W. Cripps, J. L. Francis, P. A. Fricker, & R. L. Clancy. (1999). Salivary IgA levels and infection risk in elite swimmers. *Medicine and Science in Sports and exercise*, **31**, 67-73.

Gleeson, M., Nieman, D. C. & Pedersen, B. K. (2004a). Exercise, nutrition and immune function. *Journal of Sports Sciences*, **22**, 115-125.

Gleeson, M. & Pyne, D. B. (2000). Exercise effects on mucosal immunity. *Immunology and Cell Biology*, **78**, 536-544.

Gleeson, M., Pyne, D. B. & Callister, R (2004b). The missing links in exercise effects on mucosal immunity. *Exercise Immunology Review*, **10,** 107-128.

Hanson, L. A., Bjorkander, J. & Oxelius, V. A. (1983). Selective IgA deficiency. In *Primary and Secondary Immunodeficiency Disorders*. (edited by R. K. Chandra), pp. 62-64. Edinburgh: Churchill Livingstone.

Hector, M. P. & Linden, R. W. A. (1999). Reflexes of salivary secretion. In "Neural Mechanisms of Salivary Gland Secretion" (J. R. Garrett, J. Ekstrom and L. C. Anderson, eds.). Frontiers in Oral Biology, Volume 11, Chapter 12 pp. 196-218. Karger, Basel.

Hucklebridge, F., Clow, A. & Evans, P. (1998). The relationship between salivary secretory immunoglobulin A and cortisol: neuroendocrine response to awakening and the diurnal cycle. *International Journal of Psychophysiology*, **31**, 69-76.

Laing, S. J., Gwynne, D., Blackwell, J., Williams, M., Walters, R. and Walsh, N. P. (2005). Salivary IgA response to prolonged exercise in a hot environment in trained cyclists. *European Journal of Applied Physiology*, **93**, 665-671.

Lamm, M. E. (1998). Current concepts in mucosal immunity IV. How epithelial transport of IgA antibodies relates to host defense. *American Journal of Physiology*, **274**, 614-617.

Li, T. L. & Gleeson, M. (2004). The effect of single and repeated bouts of prolonged cycling and circadian variation on saliva flow rate, immunoglobulin A and α-amylase responses. *Journal of Sports Sciences*, **22**, 1015-1024.

Mackinnon, L. T. (2000). Chronic exercise training effects on immune function. *Medicine and Science in Sports and Exercise*, **32**, S369-S376.

Mackinnon, L. T., Chick, T. W., Van, A. S., and Tomasi, T. B. (1989). Decreased secretory immunoglobulins following intense endurance exercise. *Sports Training and Medical Rehabilitation*, **1**, 209-218.

Mackinnon, L. T. & Hooper, S. (1994). Mucosal (secretory) immune system responses to exercise of varying intensity and during overtraining. *International Journal of Sports Medicine*, **15**, S179-S183.

Mackinnon, L. T. & Jenkins, D. G. (1993). Decreased salivary IgA after intense interval exercise before and after training. *Medicine and Science in Sports and Exercise*, **25**, 678-683.

McDowell S. L., Chaloa, K., Housh, T. J., Tharp, G. D. & Johnson, G. O. (1991). The effect of exercise intensity and duration on salivary immunoglobulin A. *European Journal of Applied Physiology*, **63**, 108-111.

Neville, V., Gleeson, M. & Folland, J.P. (2008). Salivary IgA as a risk factor for upper respiratory infections in elite professional athletes. *Medicine and Science in Sports and Exercise,* **40**, 1228-1236.

Nieman, D., Henson, D. A. , Fagoaga, O. R., Utter, A. C., Vinci, D. M., Davis, J. M. & Nehlsen-Cannarella, S.L. (2002). Change in salivary IgA following a competitive marathon race**.** *International Journal of Sports Medicine*, **23**, 69-75.

Nieman, D. C., Johanssen, L. M., Lee, J. W. & Arabatzis, K. (1990). Infectious episodes in runners before and after the Los Angeles Marathon. *Journal of Sports Medicine and Physical Fitness*, **30**, 316-328.

Nehlsen-Cannaralla S. L., Nieman, D. C., Fagoaga, O. R., Kelln, W. J., Shannon, M. & Davis, J. M. (2000). Saliva immunoglobulins in elite women rowers. *European Journal of Applied Physiology*, **81**, 222-228.

Oliver S. J., Laing S. J., Wilson S., Bilzon J. L., Walters R., & Walsh N. P. (2007). Salivary immunoglobulin A response at rest and after exercise following a 48 h period of fluid and/or energy restriction. *British Journal of Nutrition*, **97**, 1109-1116.

Peters, E. M. & Bateman, E. D. (1983). Ultramarathon running and upper respiratory tract infections. *South African Medical Journal*, **64**, 582-584.

Proctor, G. B. & Carpenter, G. H. (2001). Chewing stimulates secretion of human salivary secretory immunoglobulin A. *Journal of Dental Research*, **80**, 909-913.

Proctor, G. B. & Carpenter, G. H. (2007). Regulation of salivary gland function by autonomic nerves. *Autonomic Neuroscience : basic & clinical*, **30**, 133: 3-18.

Sabbadini, E. & Berczi, I. (1995). The submandibular gland: a key organ in the neuro-immuno-regulatory network? *Neuroimmonomodulation*, **2**, 184-202.

Sari-Sarraf V., Reilly T., Doran D. & Atkinson G. (2007). The effects of single and repeated bouts of soccer-specific exercise on salivary IgA. *Archives of Oral Biology*, **52**, 526-532.

Saxon, A., Stevens, R. H., Ramer, S. J. & Clements, P. J (1978). Glucocorticoids Administered In Vivo Inhibit Suppressor T Lymphocyte Function and Diminish B Lymphocyte Responsiveness in In Vitro Immunoglobulin Synthesis. *The Journal of Clinical Investigation*, **61**, 922-930.

Steerenberg, P. A., Van Asperen, I. A., Van Nieuw Amergongen, A., Biewenga, J., Mol, D. & Medema, G. (1997). Salivary levels of immunoglobulin A in triathletes. *European Journal of Oral Science*, **105**, 305-309.

Spence, L., Brown, W. J., Pyne, D. B., Nissen, M. D., Sloots, T. P., McCormack, J. G., Locke, A. S. & Fricker, P. A. (2007). Incidence, etiology, and symptomatology of upper respiratory illness in elite athletes. *Medicine and Science in Sports and Exercise*, **29**, 577-586.

Timmons, B. W., Hamadeh, M. J., Devries, M. C. & Tarnopolsky, M. A. (2005). Influence of gender, menstrual phase, and oral contraceptive use on immunological changes in response to prolonged cycling. *Journal of Applied Physiology*, **99**, 979-985.

Tomasi, T. B., Trudeau, F. B., Czerwinski, D. & Erredge, S. (1982). Immune parameters in athletes before and after strenuous exercise. *Journal of Clinical Immunology*, **2**, 173-178.

Walsh, N. P., Bishop, N. C., Blackwell, J. B., Wierzbicki, S. G. & Montague, J. C. (2002). Salivary IgA response to prolonged exercise in a cold environment in trained cyclists. *Medicine and Science in Sports and Exercise*, **34**, 1632-1637.

Walsh, N. P., Blannin, A. K., Clark, A. M., Cook, L., Robson, P. J. & Gleeson, M. (1999). The effects of high intensity intermittent exercise on saliva IgA, total protein and [alpha]-amylase. *Journal of Sports Sciences*, **17**, 129-134.

Walsh, N. P., Laing, S. J., Oliver, S. O., Montague, J. C., Walters, R. & Bilzon, J. L. (2004). Saliva parameters as potential indices of hydration status during acute dehydration. *Medicine and Science in Sports and Exercise*, **36,** 1535-1542.

Williams, C. & Serratosa, L (2006). Nutrition on match day. *Journal of Sports Sciences*, **24**, 687-697.

Table 1. Participant Characteristics (n = 16). Values are mean (± SEM)

Variable	Males (n=8)	Females (n=8)
Age (yr)	22 (1)	22 (1)
Body Mass (kg)	80.6 (5.8)	60.0 (0.9)*
Height (cm)	182 (1)	165 (1)*
$\dot{V}O_{2max}$ ($mL.kg^{-1}.min^{-1}$)	51.0 (3.6)	40.1 (0.7) *

* Significantly different to Males ($P < 0.05$).

Table 2. Nutritional Information for the Sports Drink and Cereal bar

Variable	Sports Drink (500 mL)	Cereal bar (37 g)
Energy (kJ)	590	540
Protein (g)	-	4
Carbohydrate (g)	32	67
Fat (g)	-	8
Sodium (mg)	250	250

Table 3. Mean (± SEM) physiological variables and RPE obtained during each trial (n = 16).

	FED	FAST
% $\dot{V}O_{2max}$	65.8 (0.7)	65.1 (0.9)
HR (beats.min^{-1})	154 (3)	151 (3)
RPE	12 (1)	12 (1)
Body Mass Change (kg)	-0.2 (0.2)	-0.2 (0.2)
Fluid Intake (L)	1.20 (0.12)	1.27 (0.15)
Fluid Loss (L)	1.41 (0.16)	1.49 (0.18)
RER	0.94 (0.01)	0.91 (0.01)*

* Significantly different to FED ($P < 0.01$).

Table 4. Effect of gender on mean (± SEM) salivary variables at baseline. Males n = 8, Females n = 8.

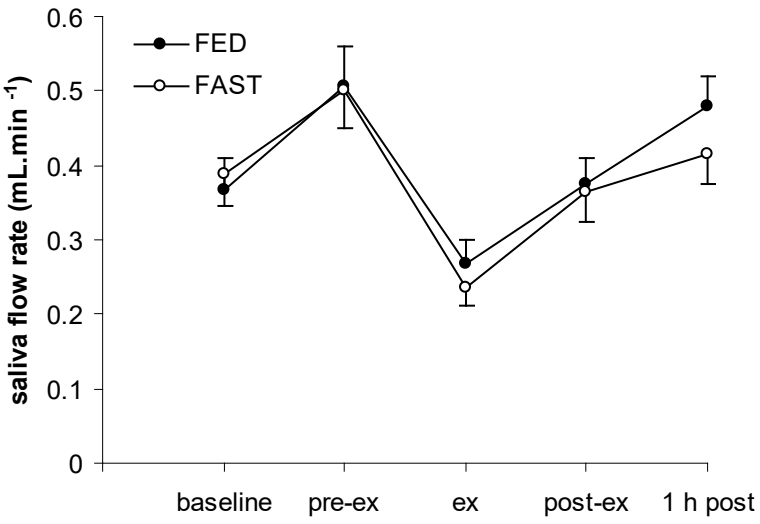
	Males	Females
Saliva flow rate (mL.min^{-1})	0.39 (0.45)	0.36 (0.71)
S-IgA concentration (mg.L^{-1})	224 (38)	116 (20)*
S-IgA secretion rate (μg.min^{-1})	85 (14)	41 (12)*
Osmolality (mOsmol.kg^{-1})	94 (11)	47 (5)*
S-IgA:osmolality	2.4 (0.3)	2.5 (0.4)
Cortisol (nmol.L^{-1})	8.0 (1.8)	12.05 (2.7)

* Significantly different to Males ($P < 0.01$).

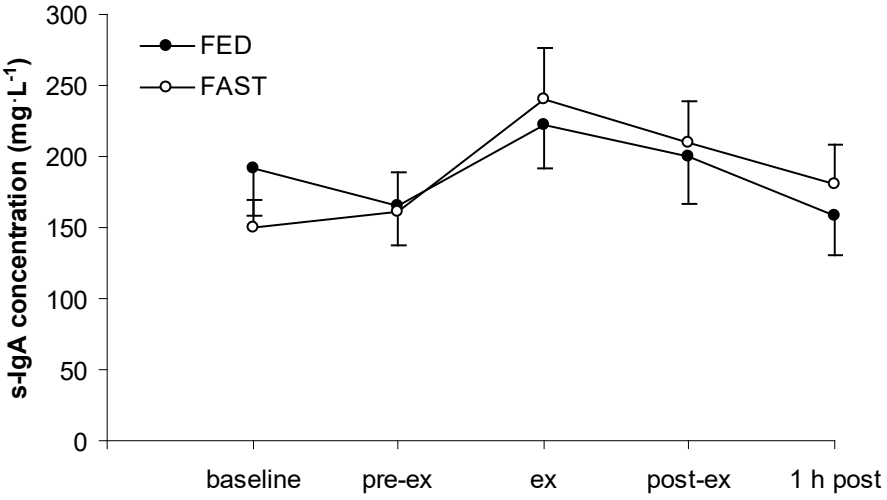
Table 5. Effect of fed (FED) or fasted (FAST) on salivary osmolality and s-IgA:osmolality. Values are mean (± SEM). (n = 16).

	baseline	pre-ex	ex	post-ex	1 h post-ex
Osmolality (mOsmol.kg^{-1})					
FED	77 (12)	71 (8)	144 (21)	148 (23)	69 (9)
FAST	64 (6)	66 (8)	152 (25)	152 (24)	73 (11)
s-IgA:osmolality ratio					
FED	2.6 (0.3)	2.2 (0.2)	1.7 (0.2)	1.5 (0.2)	2.4 (0.4)
FAST	2.4 (0.3)	2.4 (0.3)	1.7 (0.2)	1.5 (0.2)	2.7 (0.3)

(A)



(B)



(C)

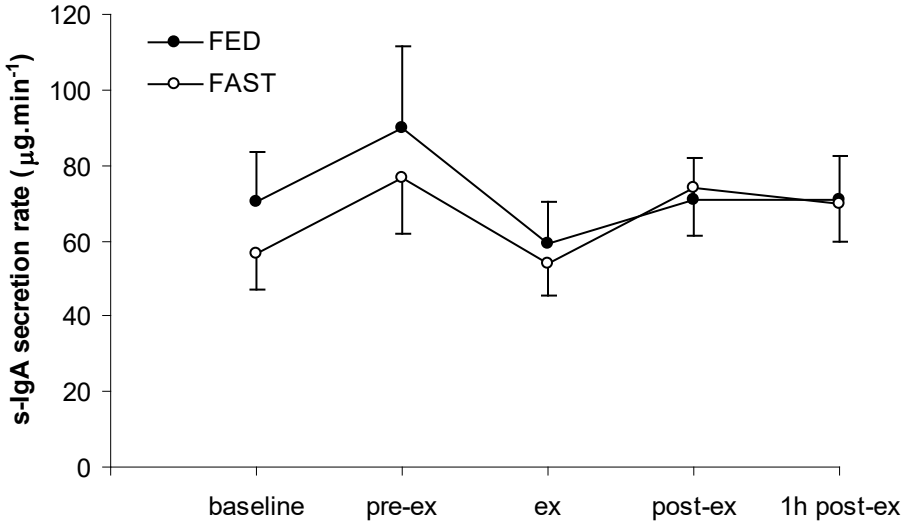


Figure 1. The effect of a fed (FED) or fasted (FAST) state on (A) saliva flow rate (B) s-IgA concentration and (C) s-IgA secretion responses to exercise. Values are mean ± SEM (*n* = 16).