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Acute and chronic effects of exercise on markers of mucosal immunity

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1. ABSTRACT

Decreased secretion rate of salivary markers of mucosal immunity, and in particular salivary immunoglobulin A (s-IgA), have been implicated as risk factors for subsequent episodes of respiratory infection in athletes. IgA is the predominant Ig in mucosal secretions and acts with innate mucosal defences to provide the 'first line of defence' against pathogens and antigens presented at the mucosa. As well as summarising the evidence concerning the effects of acute exercise and longer-term intensive training on these markers of mucosal immunity, this review explores the factors that impact upon salivary responses to exercise, such as method of saliva collection, stimulation of saliva collection and the method of reporting s-IgA data. The influence of adequate hydration and nutritional supplementation during exercise as well as exercising in extreme environmental conditions on salivary responses is also explored. Finally, the possible mechanisms underlying the acute and longer-term effects of exercise on salivary responses are examined, with particular emphasis on the potential role of the sympathetic nervous system and the expression and mobilisation of the polymeric Ig receptor.

2. INTRODUCTION

Over the past two decades a growing body of evidence has supported the notion that both acute, strenuous exercise and intensive periods of physical training are associated with immune suppression and this may be linked to the apparent higher incidence of upper respiratory tract infections (URTI) in highly conditioned athletes (1,2). The acute decrease in immune function is transient and is dependent on the exercise intensity and duration. Repeated activity with insufficient recovery, such as during heavy periods of training and competition, appears to exacerbate the situation leading to a chronic depression of several aspects of immune function (3-5). Although the majority of studies have concentrated on continuous endurance-type activities such as distance running, cycling and swimming, evidence to support the concept of an exercise-induced immune depression has also been found following high-intensity intermittent activities such as football and tennis as well as high intensity resistance exercise (6-8).

In contrast to the effects of intensive exercise on immune function, moderate amounts of physical activity in previously sedentary individuals, such as 30 min of brisk walking on most days of the week, are associated with either little effect on (9,10) or elevations (11,12) of markers of immunity and such activity has been associated with a lower incidence of URTI compared with sedentary individuals (1,9,11). The relationship between the amount of exercise and incidence of URTI has been proposed by Nieman (13) as a J-shaped curve and is largely supported by epidemiological data (9,11,14,15). However, identifying a definitive link between prevalence of URTI and any exercise-induced alterations in cellular and mucosal immunity has proved more difficult, not least because of the many confounders that could influence immune function (e.g. age, nutritional status, seasonal variations in immune function, psychological well-being) and pathogen exposure (e.g. shared drinking bottles, close contact with infected individuals). Nevertheless, a decrease in saliva secretion rate of markers of mucosal immunity, and in particular salivary immunoglobulin A (s-IgA), have been implicated as a risk factor for subsequent episodes of URTI in athletes (1,16).

3. MARKERS OF MUCOSAL IMMUNITY

Mucosal surfaces such as those in the gut, uro-genital tract, oral cavity and respiratory system are protected by a network of organised structures known as the Common Mucosal Immune System (17). These structures include Peyers patches and isolated lymphoid follicles in gut-associated lymphoid tissue, nasal-associated lymphoid tissue, bronchial/tracheal-associated lymphoid tissue and salivary glands. The production of IgA is the major effector function of the mucosal immune system and IgA is the predominant Ig in mucosal secretions. As such, secretory IgA, co-operating with innate mucosal defences such as alpha-amylase (ptyalin), lactoferrin and lysozyme, provides the 'first line of defence' against pathogens and antigens presented at mucosal surfaces. In addition, secretory IgM and locally produced IgG play a less significant role in protection of mucosal surfaces (17).

Secretory IgA is a dimeric molecule, with the IgA monomers joined 'tail-to tail' by a small polypeptide structure known as the J-chain and containing epithelial-derived secretory component (Figure 1), which is the cleaved extracellular portion of the polymeric Ig receptor (pIgR) (18). The pIgR is synthesised by mucosal epithelial and glandular cells and expressed on the basolateral membrane, where it is ideally placed to bind to locally produced IgA (and IgM). Transepithelial transport of the resulting pIgR-IgA complex occurs via endocytosis and vesicular transport to the apical cell membrane, where it is proteolytically cleaved between its internal and external domains (19), leaving the external portion, secretory component, bound to IgA (Figure 2). Furthermore, the covalent binding of secretory component makes secretory IgA more resistant to proteases in secretions such as saliva (20). The transport of IgA bound to pIgR across the epithelium affords three potential ways in which secretory IgA provides a first line of defence against microbial pathogens: through prevention of pathogen adherence and penetration of the mucosal epithelium, by neutralising viruses within the epithelial cells during transcytosis and by excretion of locally formed immune complexes across mucosal epithelial cells to the luminal surface (19).

4. IMMUNE MARKERS IN SALIVA

Salivary-IgA (s-IgA) is the most commonly studied marker of the mucosal immune system mainly due to the obvious advantage of being easy to collect in both field and laboratory situations. There are two subclasses of IgA: IgA1 and IgA2, with the former the predominant subclass in saliva (17). s-IgA is produced by plasma cells (differentiated B lymphocytes) adjacent to ducts and acini of salivary glands (21). A high incidence of infections is reported in individuals with selective deficiency of IgA (22) or poor saliva flow rates (23). Moreover, high levels of salivary-IgA are associated with low incidence of URTI (24). Other proteins present in saliva that play an important role in protecting the oral mucosa include salivary alpha-amylase, which inhibits the adherence and growth of specific bacteria (25); lysozyme, which facilitates the destruction of bacteria by cleaving the polysaccharide component of their cell walls (26) and lactoferrin, which has anti-inflammatory and antimicrobial activities, such as competing with bacteria for ferric iron and preventing bacterial growth. Lactoferrin also acts against several viruses including the common respiratory viruses adenovirus and respiratory syncytial virus but does not inhibit the growth of human rhinovirus (27), a major causative agent of the common cold.

Saliva is produced by three pairs of major salivary glands (parotid, submandibular and sublingual) in addition to many smaller glands found in the submucosa under most soft tissue surfaces of the mouth (28) and humans produce around 1500 ml of saliva each day. Whereas IgA is transported into saliva across salivary glandular epithelial cells as described above, alpha-amylase and lactoferrin are synthesised and secreted by acinar cells in the secretory endpiece of salivary glands. The main sources of lysozyme are macrophages in the oral mucosa, although there is some secretion of lysozyme from the basal cells of striated ducts in the parotid glands. Following secretion, both lysozyme and lactoferrin require pepsin digestion for synthesis into an active form for their microbial activity (27). The fluid component of saliva is supplied by a dense network of blood vessels, via the interstitial space (28).

Secretion of saliva and its constituent proteins is regulated by the autonomic nervous system. The salivary glands are innervated by branches of both the parasympathetic and sympathetic nervous systems. Parasympathetic stimulation elicits a high volume of watery saliva that is low in protein content and this secretion is associated with a pronounced vasodilation of the gland, thought to be mediated by local release of vasoactive peptide. In contrast, saliva elicited by sympathetic stimulation is low in volume and high in protein, mainly due to increased exocytosis of salivary proteins from salivary cells (28). Studies in rats have shown that the secretion of IgA can be increased by both parasympathetic and sympathetic nerve stimulation and adrenaline has recently been shown to increase the transport of human IgA into saliva by rat salivary cells via increased mobilisation of the pIgR (29,30). The presence of elevated levels of alpha-amylase in saliva is considered an indicator that saliva secretion was primarily stimulated by increased adrenergic activity (31). Since intensive exercise is associated with enhanced sympathetic nervous system activation it seems logical to assume that strenuous physical activity could modify secretion of saliva and its constituent proteins. Furthermore, given the apparent relationship between prolonged, strenuous exercise and incidence of URTI, any exercise effects on these markers of mucosal immunity is potentially of great importance.

5. SALIVARY IgA AND ACUTE EXERCISE

In response to acute bouts of high intensity exercise, many studies report a decrease in s-IgA concentration following exercise that recovers to resting levels within 1 h of exercise completion (32-35), although some studies have reported either no

change (36,37) or even increases (38,39) in s-IgA concentration. The reason for these inconsistent findings may be at least partly due to the different methods used to express IgA data and this makes it difficult to make direct comparisons between some studies. One of the major sources of variation in s-IgA levels is an alteration in salivary flow rate, which is not always accounted for. Exercise is generally associated with a decrease in saliva volume (38,40,41) and if alterations in flow rate have not been taken into account an apparent increase in s-IgA levels may actually reflect a concentrating effect on absolute s-IgA concentration. Similarly, stimulating saliva flow, for example by chewing, could result in a diluting effect on the secreted s-IgA which would give the (perhaps false) impression of a decrease in s-IgA concentration.

Authors have employed a variety of methods to overcome this problem. One approach has been to assess s-IgA concentration as a ratio to total saliva protein or albumin, with the assumption that the total protein or albumin secretion rates into saliva do not change in response to exercise. For example, in the first published study to look at the relationship between s-IgA and exercise, Tomasi *et al.* (32) reported a 20% decrease in s-IgA concentration following 2-3 h of competition in elite cross-country skiers that became a 40% decrease when expressed relative to total saliva protein concentration. Furthermore, Mackinnon *et al.* (42) reported a 60% decrease in absolute s-IgA concentration and a 65% decrease in s-IgA relative to total protein in trained cyclists following a 2 h cycle at 90% of ventilatory threshold. However, it has been suggested that correcting for total protein is misleading since protein secretion rate itself has been shown to increase during exercise (38,40).

The expression of s-IgA as a secretion rate may be more appropriate as it takes any alterations in saliva volume directly into account and both saliva flow rate and IgA concentration are influential factors in host defence. For example, in trained runners, a 21% decrease in s-IgA concentration and a 25% decrease in s-IgA secretion rate were reported 1.5 h after completing a competitive marathon race (33); this was compared with a 31% decrease when s-IgA was expressed relative to total protein concentration. Furthermore, following a 160 km run s-IgA secretion rate fell by 50% decrease without any concurrent change in s-IgA concentration or s-IgA concentration relative to total protein (43). Moreover, a low s-IgA secretion rate (but not concentration) after 90 km was found to be the best predictor of URTI in the two weeks after the race.

The expression of absolute concentrations of s-IgA as a secretion rate could also be argued to be more important to immune defence as it represents the actual amount of IgA available on the mucosal surfaces (3). On the other hand, it could be argued that changes in s-IgA secretion rate are merely providing an explanation for how the changes in absolute salivary IgA concentration are accomplished and it is therefore the absolute concentration of s-IgA that is more biologically significant. Certainly studies that have investigated the effects of exercise training on salivary IgA measurements at rest (as discussed in section 8) have often used absolute concentrations for comparison, probably with the assumption that saliva flow rate will be similar under resting conditions, in contrast to comparisons made before and after acute physical activity when marked decreases in saliva flow rate are commonly observed. Having said this, a recent study of American Football players found that while both resting s-IgA concentration and secretion rate decreased over a competitive season only secretion rate was found to predict URTI incidence (16).

The effects of exercise on s-IgA concentration and secretion rate appear to be largely dependent on overall exercise intensity. s-IgA concentration increased by 30-45% yet secretion rate remained unchanged in response to 30 min of cycling at 30% and 60% of maximal heart rate in male and females of varying levels of recreational fitness (44). Furthermore s-IgA secretion rate and concentration remained unaffected by a 2 h moderate intensity training session in elite women rowers (45) and in response to intermittent and continuous exercise for a total of 90 min at ~ 60% maximal oxygen uptake ($\dot{V}O_{2max}$) (37).

An alternative method of expressing s-IgA data is relative to saliva osmolality, since osmolality falls in proportion to the fall in saliva flow rate and mainly reflects the inorganic electrolyte concentration, with protein accounting for less than 1% of saliva osmolality (38). Using this measure, s-IgA concentration to osmolality ratio was found to be unaffected by exhaustive exercise at 55% and 80% $\dot{V}O_{2max}$ in males of differing levels of fitness but did increase by 70% after exercise compared with resting values when the data from both trials was combined (38). Likewise, s-IgA concentration increased 3-fold, saliva flow rate decreased by 40% and s-IgA secretion rate increased by 60% immediately after exercise. However, exercise had no effect on s-IgA concentration relative to total protein ratio, most likely because there was an ~80% increase in the protein secretion rate by the end of exercise.

Another factor to consider when comparing studies is whether or not saliva flow has been stimulated, for example by consuming food or drink during exercise trials, since this also been shown to change the composition of the saliva as well as the volume. In humans, stimulated saliva secretion (obtained by chewing a tasteless polythene tube) increased the concentration of s-IgA secreted from the parotid saliva gland, due to increased epithelial cell transcytosis of IgA (46). Alpha-amylase also showed a similar pattern of increased secretion on stimulation of saliva secretion. In agreement with this, although both stimulated (obtained by sucking mints) and unstimulated s-IgA secretion rate decreased in response to cycling at 85% $\dot{V}O_{2max}$ until fatigue, values were higher in stimulated saliva compared with unstimulated saliva (47). We have recently found higher saliva flow rates when saliva was stimulated by chewing mint-flavoured gum for one minute during and after 2.5 h of cycling at 60% $\dot{V}O_{2max}$, followed by a cycle to exhaustion at 75% $\dot{V}O_{2max}$ (Gleeson *et al.*, unpublished data, 2007) and this was associated with a higher secretion rate of both lysozyme and alpha-amylase compared with unstimulated saliva flow. Interestingly, s-IgA concentration

was lower when saliva flow was stimulated, in contrast to previous studies. This does not reflect a diluting effect as a consequence of the increased saliva volume because when flow rate was accounted for s-IgA secretion rate was also lower in the stimulated samples. However, it may reflect the method of stimulation used (taste vs. tasteless, sucking vs. chewing) as these are known to exert differential effects on the major salivary glands (48).

Different methods of saliva collection may also contribute to the discrepancies in the literature. Passive collection into cotton swabs are used to minimise the risk of gingival bleeding that is associated with the expectoration method of saliva collection (49). However, care is needed to ensure that the sample volume is optimal since some concerns regarding the determination of s-IgA from cotton swab collections have been reported on sample volumes of less than 200 μ l and more than 2 ml (50). Furthermore, s-IgA concentrations from cotton swabs are reported to be lower compared with those collected by passive dribbling (51). In addition, the placement of the cotton swab (under the tongue, against the inside of the cheek) could potentially affect the composition of the saliva collected as this would preferentially stimulate different saliva glands, which themselves differ in the composition of saliva that they produce (52). The importance of ensuring that cotton swabs are not saturated is further highlighted in another study that compared saliva collection methods of spitting, draining, suction and swabs (53). All collection methods were capable of providing the same general information on salivary flow rates; however, the swab technique was found to be the least reliable and the spitting and draining methods were found to be the most reproducible. The choice of collection method may also depend on the subject population; studies of children and the elderly may prefer to use swab collections because they are rated positively for both comfort and acceptability compared with passive dribbling (51).

A further consideration is the circadian variations exhibited by both s-IgA and alpha-amylase. Li and Gleeson (54) demonstrated that s-IgA concentration decreased throughout the day from its highest value in the early morning to its lowest value in the evening. Alpha-amylase secretion rate was lowest in the morning and increased to its highest value in the late afternoon. With this in mind, it is possible that reports of decreased s-IgA (and increased alpha-amylase) after prolonged exercise performed in the morning may be at least partly reflecting usual diurnal variations in these markers (42,55). However, Li and Gleeson (54) also investigated the effect of cycling $\sim 70\% \dot{V}O_{2max}$ for 2 hours at different times of day on salivary responses but did not find any influence of circadian variations on the acute salivary responses. This is supported by Dimitriou *et al.* (56), who found higher s-IgA concentrations and lower saliva flow rates and s-IgA secretion rates early in the morning compared with 12 h later but no effect of these on subsequent responses to short-duration (<30 min) intensive intermittent swimming.

6. NUTRITIONAL INFLUENCES ON s-IgA RESPONSES TO ACUTE EXERCISE

Inadequate or inappropriate nutritional practices can compound the negative effects of intensive exercise on immune function. Recent studies have demonstrated that ensuring adequate carbohydrate intake during prolonged strenuous exercise can minimise the effects of exercise on measures of innate and cell-mediated immunity (57-60). However, carbohydrate ingestion during a 2 h cycle at $60\% \dot{V}O_{2max}$ had little effect on saliva flow rate, s-IgA concentration and s-IgA secretion rate responses to the exercise (41). Similar findings have been reported following a competitive marathon race (33), intermittent soccer-specific exercise (61) and repeated bouts of 90 min cycling at $60\% \dot{V}O_{2max}$ separated by 3 h (62). In contrast, consuming a high carbohydrate diet throughout a 6 day period of increased training was associated with post-exercise elevations in s-IgA concentration (63). However, saliva flow rate was not reported in this study, therefore it is unclear whether the increase in s-IgA concentration observed is simply reflecting a post-exercise decrease in saliva volume. Furthermore, it is not clear how much (if any) fluid was consumed during exercise. This is important because restricting fluid intake during prolonged submaximal exercise has been associated with increases in s-IgA concentration (41).

The impact of fluid intake on salivary responses to exercise is further demonstrated by a recent study that investigated the influence of a 48 h period of both fluid and energy restriction on s-IgA responses to exercise (64). Restricting fluid intake to ~ 200 ml/day was associated with a 64% decrease in saliva flow; this was associated with a concurrent increase in s-IgA concentration. Furthermore, the additional effect of restricting energy intake to around 1200 kJ/day resulted in a decrease in s-IgA secretion rate after 24 h. This effect was not exacerbated by performing a 30 min treadmill capacity test after 48 h of treatment and was reversed within 6 h of rehydration and refeeding.

Other nutritional practices that have received much attention in relation to potential effects on the immune response to exercise are vitamin C and glutamine supplementation, although convincing evidence to suggest that these can prevent exercise-induced immune impairment is lacking. Specifically in relation to s-IgA, consuming 1500 mg of vitamin C or placebo 7 days before an ultramarathon had no effect on saliva flow rate, s-IgA concentration, secretion rate and s-IgA:protein ratio, all of which decreased regardless of treatment (65). Similarly, s-IgA concentration, secretion rate and s-IgA:protein ratio fell in response to 2 h cycling at $75\% \dot{V}O_{2max}$ regardless of whether glutamine or placebo had been consumed during and after the exercise (66). Furthermore, taking glutamine supplements 4 times daily for 14 days had no effect on stimulated saliva flow rate, s-IgA concentration and secretion rate in response to 9 days of twice-daily interval training compared with taking placebo (67). The authors did report a significant increase in mean nasal IgA across the study period in those runners who received glutamine; however, this may have been influenced by higher baseline values in the glutamine group.

The use of extracts of Echinacea as prophylactic against URTI is becoming increasingly common among both athletes and the general population. A recent meta-analysis supports the benefits of Echinacea in reducing the incidence and duration of the common cold (68). At present there are few large-scale well controlled studies investigating the effect of this supplement on the immune response to exercise. However, there is some evidence to suggest that 28 days of supplementation with *Echinacea purpurea* is associated with reduced incidence of URTI in a group of triathletes before and after a sprint triathlon (69). Echinacea appears to exert its immunomodulatory effects mainly on innate measures of immunity, particularly macrophage function (70). However, it may also exert effects on mucosal immunity since a recent study reports that 4 weeks of *E. purpurea* supplementation minimised the fall in s-IgA concentration and secretion rate observed in response to repeated Wingate tests (71).

Caffeine is widely used by athletes for its ergogenic properties, with doses as low as 2-3 mg/kg body mass shown to improve performance (72). Caffeine ingestion is associated with enhanced sympathetic nervous system activity (73,74) and therefore it may be speculated that such a practice may affect salivary responses to exercise. A recent study from our laboratory lends support to this hypothesis: ingestion of 6 mg/kg body mass caffeine 1 h before cycling for 90 min at 70% $\dot{V}O_{2max}$ was associated with higher s-IgA concentrations and secretion rate during the exercise but had no effect on saliva flow rate (75). These effects were not apparent 1 h after exercise, suggesting that any influence of caffeine is only short-lived. Caffeine ingestion was also associated with higher saliva alpha-amylase activity and secretion rate and higher plasma adrenaline concentration, suggesting enhanced sympathetic nervous activity with caffeine ingestion.

7. ENVIRONMENTAL INFLUENCES ON s-IgA RESPONSES TO ACUTE EXERCISE

Extremes of cold, heat and altitude may indirectly influence immune function via their influences on central nervous system and specifically on the hypothalamic-pituitary-adrenal and sympathicoadrenal-medullary axes, which control the release of adrenal hormones (76). Given that sympathetic nervous system activation appears to be closely related to the exercise-associated changes in salivary markers of mucosal immunity, any additional stimulation of these pathways through exercise in adverse environmental conditions may be expected to cause greater disturbance to these markers of mucosal immune function.

The decrease in s-IgA concentration observed after cross-country skiing was suggested to be at least partly associated the effect of the cold dry air on the temperature of the mucosal membranes (32). However, Housh *et al.* (77) reported that running for 30 min at 80% $\dot{V}O_{2max}$ at 6°C had no effect on s-IgA concentration compared with running at 19 and 34°C, although this study did not take saliva flow rate into account. Walsh *et al.* (35) found s-IgA concentration was lower following 2 h cycling at 70% $\dot{V}O_{2max}$ at -6°C compared with when the same exercise was performed at 19°C but this was related to higher flow rates since no effect of the cold was found on s-IgA secretion rate. The importance of assessing flow rate is further emphasised by Mylona *et al.* (78) who found 30 min of exercise at ~70% heart rate reserve at 1°C was associated with higher saliva flow rates than when exercise was performed at 24°C. This most likely accounted for the increased s-IgA secretion rate following exercise in the cold since absolute s-IgA concentrations were unaffected. In agreement with the study of Housh *et al.* (77), cycling for 2 h at 60% $\dot{V}O_{2max}$ at 30°C had little effect on saliva flow rate, s-IgA concentration or s-IgA secretion rate compared with exercising at 20°C (79).

There is little research investigating the effect of hypoxia (altitude) on s-IgA responses. One recent study reports a progressive decrease from baseline in resting s-IgA concentrations in a group of elite cross-country skiers who were training at 1200m but living for 11 h/day at simulated altitudes of 2500, 3000 and 3500m (6 days at each). Values did not differ significantly from a control group that trained and lived at 1200m but differences tended to be greater as the simulated living altitude increased (80).

8. SALIVARY IgA AND EXERCISE TRAINING

The effects of exercise training on salivary IgA have been investigated in a number of ways: Cross-sectional studies have compared s-IgA responses in athletes and non-athletes (sedentary people) and longitudinal studies have reported the effect of a training program - typically 4-12 weeks duration - in previously sedentary people. In addition, short-term longitudinal studies have reported the effect of a period - typically 1-3 weeks - of intensified training on s-IgA levels in already well trained athletes and longer term longitudinal studies have monitored immune function in athletes over the course of a competitive season lasting typically 4-10 months.

Levels of s-IgA vary widely between individuals and although some early studies indicated that s-IgA concentrations are lower in endurance athletes compared with sedentary individuals (32), the majority of studies indicate that the levels are generally not different in athletes compared with non-athletes except when athletes are engaged in heavy training (17).

Several studies have examined changes in immune function during intensive periods of military training. However, this often involves not only strenuous physical activity, but also dietary energy deficiency, sleep deprivation and psychological challenges. These multiple stressors are likely to induce a pattern of immunoendocrine responses that amplify the exercise-

induced alterations. Several studies have documented a fall in s-IgA concentration and some, though not all have observed a negative relationship between s-IgA concentration and occurrence of URTI.

s-IgA was evaluated as a marker of the severity of stress during a 19-day Royal Australian Air Force survival course, during which the 29 participants experienced hunger, thirst, boredom, loneliness, and extreme heat and cold combined with demanding physical effort (81). Dietary restriction, consumption of alcohol, body mass loss, occurrence of URTI, and negative emotions were negatively associated with s-IgA or the ratio of s-IgA to albumin and the authors concluded that this ratio is a useful marker of the severity of stresses encountered during stressful training.

A recent study examined the impact of a 3-week period of military training followed by an intensive 5-day combat course in 21 French Commandos on s-IgA levels and incidence of URTI (82). Saliva samples were collected by passive dribbling at 8 a.m. before entry into the Commando training, the morning following the 3-week training, after the 5-day combat course and after one week of recovery. After the 3-week training, s-IgA concentration was not changed, although it was reduced by ~40% after the 5-day course and returned to pre-training levels within a week of recovery. The incidence of URTI increased during the trial but was not related to s-IgA. Among the thirty episodes of URTI reported, there were 12 rhino-pharyngitis, 6 bronchitis, 5 tonsillitis, 4 sinusitis and 3 otitis. This study indicates that sustained stressful situations have an adverse effect on mucosal immunity and incidence of URTI, though a causal relationship between the two could not be established. The large proportion of rhino-pharyngitis indicated that the nasopharyngeal cavity is at a higher risk of infection.

In contrast, a longer-term study examined resting s-IgA concentrations and URTI incidence during the first 19 weeks of UK Parachute Regiment training and found that IgA responses were similar between recruits and controls (83). In addition, s-IgA concentrations were not related to incidence of URTI. Saliva flow rate did decrease in the recruits towards the end of the training however; the authors suggested that this may indicate an ensuing state of hypohydration.

Several longitudinal studies have monitored immune function in high-level athletes over the course of a competitive season. The impact of long-term training on systemic and mucosal immunity was assessed prospectively in a cohort of elite Australian swimmers over a 7-month training season in preparation for national championships (1). Resting levels of s-IgA fell with each additional month of training and both the preseason and the mean pretraining s-IgA concentrations were found to predict the number of infections in the swimmers. Another study of elite male Australian swimmers during a 30 day period of intensive training observed low levels of s-IgA immediately before the onset of upper respiratory symptoms, indicating transient mucosal immune suppression in this cohort (84). More recently it has been reported that periods of competitive and heavy training within a 12 month competitive College football season were associated with a reduction in both resting s-IgA concentration and secretion rate and an increase in incidence of URTI (16). Secretion rate of s-IgA (but not concentration) was found to predict URTI incidence.

Shorter-term observations have not been able to replicate these findings. Saliva samples collected from elite Australian swimmers at 2 week intervals during the final 12 weeks of training before the National Championships demonstrated small, yet significant increases in pre-exercise s-IgA, IgG and IgM concentrations and post-exercise s-IgA concentration but these changes were unrelated to incidence of URTI (85). Furthermore, samples taken from 41 elite Australian swimmers in the May and August before the 1998 Commonwealth Games found no differences in salivary concentrations of IgA, IgG and IgM between the two collection times (86). Importantly, no differences in salivary Ig concentrations were found between swimmers who had experienced URTI in the 6-week period leading up to and including the Commonwealth Games and those who had remained healthy. However, these findings may be influenced by the sporadic saliva sampling protocol used in this study. Nevertheless, such observations do highlight the challenge researchers find themselves presented with when trying to identify relationships between IgA and URTI; individuals with lower levels of IgA will not necessarily present with URTI and those who present with URTI will not necessarily demonstrate low levels of IgA. Furthermore, it is difficult to define a common 'threshold' of s-IgA concentration below which any individual is placed at increased risk of contracting URTIs. Specific laboratory assay techniques may provide standard threshold values for a particular laboratory; for example s-IgA concentrations below 40 mg/ml (1) or s-IgA secretion rates below 40 µg/min (16), but given differences in collection methods and assay techniques these can not necessarily be applied universally. Finally, studies that have attempted to relate measures of s-IgA with incidence of URTI have invariably used regression or correlation analyses, but it should be noted that the absence of a statistical relationship does not necessarily indicate the absence of a biological one. Measures of s-IgA can vary widely between individuals and therefore it might be more meaningful from a practical standpoint to monitor s-IgA on an individual basis of what is 'usual' for that athlete. In this way, a substantial fall in s-IgA concentration or secretion rate from the usual profile for that individual, irrespective of the absolute values, might give a better indication of increased susceptibility for URTI at that time.

Alterations in s-IgA may play a role in the apparent altered susceptibility to URTI associated with moderate exercise. A significant negative relationship between salivary-IgA levels and total sickness days was found in response to a 12-week moderate exercise training programme in 9 previously sedentary men and women (12). However, a significant relationship between salivary IgA concentration and days reporting cold symptoms only was not found, with the authors suggesting that this was due to some ambiguity in the description of the cold-related symptoms. Increases in resting s-IgA concentration and

secretion rate were also observed after older men and women (mean age ~65 years) completed a 12 month training programme that involved performing 60 min of endurance exercise and 60 min of resistance exercise every week (87).

9. EXERCISE EFFECTS ON OTHER SALIVARY MARKERS OF MUCOSAL IMMUNITY

Although the majority of studies investigating changes in mucosal immunity with exercise have focused on s-IgA, there is some evidence that concentrations of s-IgG are unchanged by acute bouts of exercise, whereas absolute concentrations of s-IgM appear to parallel the decrease in s-IgA levels and usually recover within 24 h (17). With regard to exercise training, s-IgM and s-IgG have been shown to be higher in elite swimmers compared with a moderately active control group (5). In addition, lower concentrations of s-IgM can be seen within a few days of intensive training but decreases may be seen following several months of intensive training (17). Comparable with acute exercise, changes in s-IgM in response to longer term training appear to parallel those of s-IgA. This supports the idea (discussed further below) that exercise-induced alterations in s-IgA concentration may reflect altered IgA transcytosis via the pIgR rather than altered rates of IgA synthesis by plasma cells because both s-IgA and the pentameric s-IgM are transported across the glandular cell epithelium bound to this receptor.

Lysozyme and lactoferrin are innate defence mechanisms present at mucosal surfaces throughout the body including saliva and together with alpha-amylase may be considered as components of innate mucosal immunity. Salivary alpha-amylase activity is stimulated by increased activity of the sympathetic nervous system with the majority of this protein produced by the parotid gland (88). In accordance with this, several studies have found that exercise increases the alpha-amylase activity of saliva in a manner that is dependent on exercise intensity (41,54). To date there is little data regarding changes in salivary lysozyme concentration and acute (or chronic) exercise, although recent results from our laboratory suggest that intense and exhaustive exercise of both shorter (~20 min) and longer (~3 h) duration is associated with increases in salivary lysozyme secretion rate (89, Gleeson *et al.*, unpublished data, 2007). These effects also appear to be dependent on exercise intensity since no change was seen following ~20 min of cycling at 50% $\dot{V}O_{2max}$ (89).

The effect of exercise on lactoferrin concentration has received little attention to date. Elevated concentrations of lactoferrin in serum have been reported immediately after ~30 min of intense running and were associated with elevated serum antibacterial activity to live *Micrococcus luteus* (90). Furthermore, increased levels of serum lactoferrin have also been observed following a 2 h submaximal cycle followed by a bout of eccentric resistance exercise (91). These aside, information concerning the effect of exercise on salivary levels of lactoferrin is scarce. Significantly however, a recent pilot investigation conducted at the Australian Institute of Sport has found salivary lactoferrin concentrations decreased during a training season in elite rowers (unpublished data cited in 27).

10. POSSIBLE MECHANISMS

To date, the mechanisms by which exercise influences salivary responses remain to be fully elucidated. However, recent studies of salivary gland control and protein secretion in animal models have provided insights into several potential mechanisms which may be involved during exercise. Underlying these mechanisms are alterations in the stimulation of the autonomic nerves that innervate the salivary glands directly or the blood vessels that supply the glands.

Variations in the volume and/or source of secreted saliva will influence the resultant secretion rate of the constituent proteins. It has been suggested that stimulation of saliva glands by sympathetic nervous activity reduces saliva flow rate via vasoconstriction of the blood vessels supplying the salivary glands (52). While sympathetic stimulation is known to exert some control over glandular blood flow it is important to note that this is not part of the reflex salivary response to stimuli such as anxiety, chewing, taste and sight of food (28). Under reflex conditions, it has been shown that vasoconstriction is not responsible for altered saliva volume because only sympathetic secretomotor nerve fibres and not vasoactive nerve fibres are stimulated (28). This suggests that the decrease in flow rate associated with exercise is more likely related to a removal of parasympathetic vasodilatory influences rather than sympathetically-mediated vasoconstriction, particularly since sensations of 'dry mouth' associated with psychological stress are related to parasympathetic withdrawal rather than sympathetic activation (26). The finding that increased sympathetic activity through caffeine ingestion had no effect on saliva flow rate responses to intensive exercise lends further support to this proposal (75).

The relative contributions of the different salivary glands to saliva secretion will affect the composition of mixed saliva; this itself will vary according to the nature of the neural stimulation. Parasympathetic and sympathetic nerves appear to innervate most cell types in salivary glands (28). Sublingual and minor glands are stimulated mainly by cholinergic (parasympathetic) nerves with parotid and submandibular glands mainly stimulated by the adrenergic (sympathetic) nerves (52). Parotid saliva contains alpha-amylase and minute amounts of lysozyme, whereas sublingual saliva produces higher levels of lysozyme (92). In addition, minor salivary glands are a major source of s-IgA, producing up to 30-35% of the IgA that enters the oral cavity and mean s-IgA concentration in the secretions from these glands is reported to be four times higher than in parotid gland secretions (93). Furthermore, *in vivo* high frequency sympathetic stimulation in the rat, such as would be experienced with high-intensity exercise, resulted in a 2.6 times increase in IgA secretion from the parotid gland compared with the unstimulated rate, yet resulted in increases in IgA secretion of 6-times from the submandibular gland (94,95). Increases in response to

parasympathetic stimulation were similar (2.6 times the unstimulated rate) in both glands, suggesting that increases in s-IgA secretion in response to exercise are more likely related to sympathetic stimulation than parasympathetic withdrawal.

The rate of secretion of s-IgA itself is dependent on the production of s-IgA by the plasma cells and/or the rate of IgA transcytosis across the epithelial cell. The latter is determined by the availability of the pIgR to permit transcytosis across the epithelial cell (26). The time-course (minutes) of the alterations in s-IgA secretion that are observed in response to acute exercise suggest that this is the principal mechanism by which acute intensive exercise influences s-IgA secretion. It has recently been demonstrated that acute stimulation of beta-adrenoreceptors in anaesthetised rats increased s-IgA secretion via elevated transcytosis from the glandular pool in a dose-independent manner above a certain threshold of stimulation (96). This increased IgA transcytosis appears to be associated with increased availability of the pIgR because stimulation with adrenaline has been shown to increase uptake of human IgA by rat salivary cells via increased mobilisation of this receptor (30). Although such a mechanism has not yet been demonstrated in humans, the finding that increases in s-IgA secretion rate are associated with elevations in plasma adrenaline following caffeine ingestion lends some support to this suggestion (75).

While enhanced IgA transcytosis probably accounts for elevations in s-IgA secretion observed by some after exercise, it cannot account for the findings of either no change or decreases in s-IgA secretion rate with intense physical activity. The finding that increased mobilisation of the pIgR only occurred above a certain threshold of frequency of stimulation (96) could account for the finding of little change in IgA levels at more moderate intensities of exercise. However, the finding of decreased concentrations of IgA in response to exercise is harder to explain. Nevertheless, a recent study in rats demonstrated that following a treadmill run to exhaustion (lasting ~60 min) decreases in s-IgA concentration were associated with a decline in pIgR mRNA expression (97). Although highly speculative, this might imply that there is a second critical threshold (or duration) of stimulation, above which pIgR expression becomes downregulated.

An inverse relationship between levels of s-IgA and salivary cortisol has been demonstrated in young adults (98). However, it is unlikely that cortisol plays a major role in the regulation of s-IgA secretion in response to exercise because changes in both salivary IgA concentration and secretion rate have been observed in the absence of any alterations in plasma or salivary cortisol (39,54,89).

Modification of s-IgA synthesis could play a greater role in the changes in s-IgA secretion observed in response to long term intensive training and chronic psychological stress (99-101). In addition, it may be that repeated mobilisation of the pIgR could deplete the available formed IgA pool, leading to decreases in s-IgA output. However, to date there is scant research in either the animal model or humans to support these speculations.

11. SUMMARY AND PERSPECTIVE

Decreased saliva secretion rate of markers of mucosal immunity, and in particular s-IgA, has been implicated as a risk factor for subsequent episodes of respiratory infection in athletes. However, salivary responses to exercise are affected by several factors including saliva collection methods, stimulation of saliva collection, adequate hydration and method of data reporting. To date the majority of exercise studies have assessed s-IgA as a marker of mucosal immunity but more recently the importance of other microbial proteins in saliva including alpha-amylase, lactoferrin and lysozyme as markers of innate mucosal immunity has gained greater recognition. Future studies should aim to elucidate the impact of acute and chronic exercise on all of these markers of mucosal immunity collectively to give a more physiological indicator of salivary defence rather than the isolated effect of one marker alone. Recent *in vivo* and *in vitro* findings from animal studies have offered some insight into potential mechanisms by which exercise affects salivary responses; however the precise manner by which this occurs in humans remains to be conclusively determined. Evidence to date suggests that the mechanisms underlying the alterations in markers of mucosal immunity with exercise are largely related to the activation of the sympathetic nervous system and its associated effects on saliva protein exocytosis and Ig transcytosis. Alterations in saliva flow rate with exercise are now thought to be more likely related to withdrawal of parasympathetic input than increases in sympathetic activity.

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Abbreviations: Ig: immunoglobulin, pIgR: polymeric immunoglobulin receptor, s-IgA: salivary immunoglobulin A, URTI: upper respiratory tract infection, VO^{2max} : maximal oxygen uptake

Key Words: Exercise, Immune, Mucosal, Saliva, s-IgA, Immunoglobulin, Alpha-Amylase, Lactoferrin, Lysozyme, Polymeric Ig Receptor, Review

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Figure 1. Schematic representation of s-IgA. Each IgA monomer comprises two heavy chains and two light chains, joined by disulphide bonds. The majority of s-IgA is dimeric, with two IgA monomers joined by the J-chain and covalently bound to a secretory component. Fab: fragment antibody binding portion, Fc: fragment crystallisable portion.

Figure 1

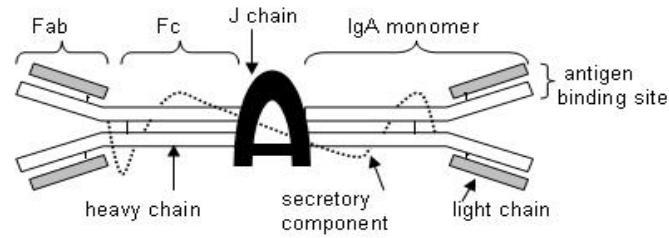


Figure 2. Epithelial transport of IgA into saliva. IgA produced by local plasma cells becomes covalently bound to the polymeric immunoglobulin receptor (pIgR) at the basolateral membrane of the glandular epithelial cell. Transepithelial transport of the resulting pIgR-IgA complex occurs via endocytosis and vesicular transport before the pIgR is proteolytically cleaved at the apical membrane, leaving its external domain, secretory component bound to IgA. This secreted form of IgA is salivary IgA (s-IgA).

Figure 2

