The missing links in exercise effects on mucosal immunity

Running Title: Exercise Effects on Mucosal Immunity

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Abstract

This review highlights research limitations within the existing exercise immunology literature and summarises unanswered questions to assist researchers and clinicians interested in exploring relationships between exercise, training and mucosal immunity. The primary limitations of the existing literature include: inadequate descriptions of training stimuli, age, gender and physical activity of subjects; failing to account for the influence of these factors and the underlying fitness and health status of subjects; methodological differences in assessments of mucosal immunity; limited understanding of the sources of biological variability in mucosal immunity; limited clinical and laboratory diagnosis of respiratory illness; and neglect of psychological, environmental, nutritional and pharmacological influences. Despite a considerable volume of research on mucosal immunity the unanswered research questions include: whether athletes are really more prone to illness; whether illness impacts on athletic performance; identifying subject or training characteristics that influence the mucosal immune responses to exercise; defining how exercise influences the acute mucosal immune response; assessing whether moderate exercise can enhance mucosal immune status; defining more clearly the treatment and management strategies for the athlete suffering recurrent illness, overtraining or long-term fatigue; and the effectiveness of

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dietary and therapeutic interventions. Answers to these questions should define future research strategies and assist clinicians seeking guidance on the assessment, treatment and management of athletes suffering from respiratory illness, particularly those with recurrent illness, long-term post-viral fatigue or suspected of being overtrained.

Keywords: Age, gender, URTI, overtraining, methods

Introduction

Exercise immunology has emerged as an active area of medical and scientific research in the last 20 years. Although a large number of research findings have been compiled, the search continues for a well defined clinical approach and detailed understanding of the mechanisms accounting for variations in immune regulation observed following exercise. One of the primary areas of interest has been the impact of exercise on mucosal immunity, driven by the perception of a link between exercise-induced immune suppression and common illnesses, particularly upper respiratory tract infections (URTI). Despite two decades of research since the first paper on mucosal immune responses (96) there are still missing links in our understanding of the interactions between exercise, immunity and susceptibility to infections. Although relationships of mucosal immunity and exercise have been reviewed previously, most of this work has been in the form of a traditional review of experimental findings (32, 42). The purpose of this review is two-fold: first to highlight research limitations within the existing literature in exercise immunology and its various subdisciplines; and secondly, to summarise the unanswered questions from both a clinical and experimental perspective. To take exercise immunology research to the next level of comprehension it is time to bring further rigour to the published science.

The current understanding of the impact of exercise on mucosal immune responses has been relatively limited to measures of changes in salivary immunoglobulin A (Sal-IgA), measured as either concentrations or secretion rates. The key findings of the existing literature can be summarized as:

- Sal-IgA concentrations typically decrease in response to high intensity exercise but remain either unaltered or increased in response to moderate-low intensity exercise (21, 42);
- Sal-IgA concentrations decrease over long-term training at high intensity (>6 months) (39) but substantial changes may not necessarily be observed over shorter training periods (<4 months) (40);
- The lower the Sal-IgA concentration the greater the risk of infection or symptoms of infection, such as those associated with viral reactivation (41);
- The variability in Sal-IgA concentrations differs between populations with different fitness levels (27);
- Group studies do not reveal the whole picture as group means or medians do not account for the biological variability in Sal-IgA between subjects and tend to obscure the trends within individuals (29, 35);
- The immunosuppressive effects of exercise on Sal-IgA can be ameliorated by discrete dietary influences (30, 37)

Research Limitations

Inadequate descriptions of training status

Given that the majority of existing investigations have focused on changes in Sal-IgA levels, and that variability differs between subjects with different physical activity characteristics, then clear and unambiguous descriptions of study populations are necessary for comparison of findings between studies. One of the most problematic areas is the training status of the participants. It is important to clearly define the underlying fitness level of subjects, given evidence that athletes with an extensive training background or history (and presumably a higher level of fitness) are more likely to experience illness than moderately trained subjects (79, 92). Studies have also shown transient or long-term immunosuppression in more highly trained athletes that may not be apparent in recreational athletes or sedentary individuals (15). There is a need for standardisation between studies given that global terms such as ‘collegiate athlete’, ‘well-trained’, ‘highly-trained’ or ‘elite level’ are often used loosely to describe the training status. The description of training status can be quantified in physiological terms such as maximal oxygen uptake, in performance terms such as training mileage (km.week-1) or duration (hours), or by the level of sport reached in terms of national or international representation (e.g. world rankings, world championships, international competition).

Inadequate descriptions of training stimuli

The intensity of exercise can be quantified relatively easily in controlled laboratory experiments using the percentage of maximal oxygen uptake, peak heart rate, peak power output, running (treadmill) speed, or time to exhaustion. In longitudinal training studies, particularly where the mucosal responses of athletes have been monitored over several weeks or months, quantifying the demands of training in a uniform manner has proved more difficult. In individual endurance-type sports it should be relatively straightforward to quantify training volumes in terms of kilometres or metres completed, or training time or total loads achieved (25). Most studies have provided only limited details of training loads and typically report mean or prescribed training loads rather than quantifying the actual training activities completed by individual athletes or players. Very few studies have examined team sports (4) and the process of quantifying the diverse range of training activities such as running-based conditioning, resistance training, skill drills and team drills, has proved quite problematic. Although some detailed methods for modelling or quantifying training loads in various sports have been devised (75), application to immunological studies is yet to be fully described.

Age and gender differences in subjects

There is little understanding of the effects of age, if any, on the mucosal immune response to exercise. There have been a few studies on subjects other than young adults and almost no studies comparing the responses of subjects in substantially different age groups. Most studies have been conducted with subjects aged 18-30 years of age. There are three logical reasons for this; it is the age range of most elite athletes, of the most active segment of the adult population,

and of the university populations from which many subjects, particularly control groups, are recruited. The application of these research findings to trained athletes is appropriate, but application of the findings to the rest of the community, particularly with regard to health risks and benefits, requires other age groups to be studied. Akimoto and co-workers investigated the effects of exercise on Sal-IgA in elderly men and women (1). Nieman and colleagues (80, 83) investigated the effects of marathon competition in subjects ranging in age from 21-72 years and 25% of their population was aged over 50 years. Both Krzywkowski *et al*. (23-48 years) (55) and Reid et al. (22-55 years) (89) recruited across wider adult population age ranges. However, these studies have not provided a clear basis on which to determine whether age influences the IgA responses to exercise.

Few studies have been conducted with children (21, 94) or adolescents (81, 94) although a small number of studies have included athletes aged from 16 years (36, 62, 84). Only two studies have directly investigated the effects of age on IgA responses to exercise (83, 94). Miletic et. al. reported differences in Sal-IgA at rest in 20-30 year-olds compared to 60-80 year-olds (72). Thus, well-designed studies investigating the effects of age on mucosal immune responses to exercise are needed, particularly as exercise is promoted to reduce obesity and other health risks across the age spectrum. Most studies have used male subjects only or a mixed gender population. Only a few studies have been conducted on females alone (24, 76, 78). Strikingly, no study has investigated whether gender has an effect on the Sal-IgA response to acute exercise or exercise training, although gender differences in pre-exercise levels have been reported in elite swimmers (41). Whether there are gender differences in pre-exercise Sal-IgA levels for other sports and whether this influences the Sal-IgA response to exercise remains to be investigated.

Physical activity history or status

Most studies of immune function and exercise have examined responses in a homogeneous population, usually a group of athletes from one sport. This has been appropriate for understanding the impact of exercise on immune function on that particular group of athletes, but the lack of studies comparing the responses of subjects with different training backgrounds or fitness levels may be limiting the development of our understanding of influences on mucosal immune function. Variation in the design features among single sport studies makes the lack of comparative studies even more frustrating for resolving inconsistencies between research findings.

There is variety in the physical activity status of the populations studied with a bias towards more studies of highly trained and elite competitor groups in spite of the inconsistencies in describing these groups. Studies of highly trained athletes typically include those competing or aspiring to compete at national and international level. These groups are usually physically homogeneous due to both selection pressures and the high levels of structured training performed, and come from a range of sports including swimming (36, 59, 84), rowing (78), kayaking (60), tennis (81), squash (61), field hockey (61), wrestling (53) and cross-country skiing (96).

A rapidly increasing number of studies have been conducted on groups justifiably described as ‘trained’ but which are less likely to be as homogeneous as the highly trained groups due to the lower levels of competitive pressure and less structured or intensive training. These groups are predominantly endurance trained runners/cyclists/triathletes (16, 46, 55, 80, 97) but also include sprinters/jumpers (71) and games players (4, 94, 98). Many of these groups are composed of only male subjects. There are also groups that may be collectively described as ‘active’ but within which a variety of physical activity levels and types of activity backgrounds are found, as well as a mix of genders (9, 22, 24, 51, 62, 67-69). Interestingly there are very few studies examining participants from team sports (4, 94, 98) and the impact of weekly competition in addition to training on mucosal immune function is unknown, other than for one study of young basketball players who showed variable responses dependent on pubescent status (24).

Only one of the studies of elite athletes (80) provides any quantitative data on fitness levels. Most studies report only broader details of the training squad from which the athletes were recruited. In contrast, some studies of trained and active subjects have reported maximal oxygen uptake (VO_{2max}) values or indirect estimates of aerobic fitness (1, 89). A small number of studies reported other performance measures pertinent to the subject population such as best 10 km time (16), best 400 m swim time (20), best 100 m sprint time (71) or 1 repetition maximum (1RM) bench press and squat (70). The values of VO_{2max} reported support the descriptions of subjects as active or trained with the mean values for male subjects all 50 $ml.kg^{-1}.min^{-1}$ or above. Only a few studies have compared the responses of groups differing in fitness level (41, 62, 96), and these studies have typically used active subjects as controls for studies of elite athlete groups. No studies have directly examined the effects of comparable exercise on Sal-IgA in groups with different levels of fitness.

Body composition has rarely been reported in studies examining immune function and exercise (80, 81). Body fat levels might expected to be in the low to desirable range in active populations, although the almost complete absence of supporting data is surprising. No comparisons have been made of athletes with extremely low levels of body fat compared to those in the healthy body fat range. If the factors that lead to excess body fat influence mucosal immunity it is possible that factors contributing to very low levels of body fat may also exert a negative effect on immunity. Finally only a few studies have compared Sal-IgA at rest and in response to exercise in groups (primarily elite athletes) with or without upper respiratory tract symptoms (41, 61, 80, 84) or healthy compared to stale (overtrained) athletes (59). In particular, the influence of moderate intensity activity on athletes with indications of reduced mucosal immunity is uncertain.

Methodological differences between assays

Despite highlighting the need for analytical standardization of Sal-IgA methods in a previous review (31), studies continue to be published with poorly defined methodology or using inappropriate methods for detection of Sal-IgA. To allow meaningful comparisons between studies it is essential that the assay be calibrated against the International Reference Preparation for proteins (BCR CRM 470) (99).

This standard clearly specify the detection antibodies and ensures the method is capable of detecting both the subclasses of IgA present in saliva (13). It is essential that newer technologies for detecting IgA are validated for saliva samples, as most automated instruments designed to detect IgA in serum underestimate the true value of total Sal-IgA due to the selection of antisera in these systems focused on detection of IgA1 subclass only (M.Gleeson, unpublished data). Manuscripts using new technologies should provide validation data for the detection of both subclasses in saliva samples (13).

The source and collection conditions for the saliva samples are also critical (31) as saliva is composed of secretions from several salivary glands and the concentration of IgA varies between the glands. The use of flow rate stimulants and relationship to food intake must be specified to allow meaningful comparisons. Saliva samples should ideally be collected under conditions that reduce the biological variability of Sal-IgA concentrations in order to maximise the ability to detect meaningful differences between athletic groups, or responses to exercise under specified conditions, including therapeutic or training interventions. The absorbent collection systems used for detection of other analytes in saliva need to be validated for the detection of IgA, as some absorbent material will lead to the underestimation of the total Sal-IgA concentration due to binding of the immunoglobulin molecule. Storage and handling conditions are also critical to maintain the integrity of IgA in saliva, that is susceptible to IgA proteases (11). Some recommendations for researchers establishing experimental approaches and preparing manuscripts are shown in Table 1.

Table 1: Recommendations for the standardization of studies of relationships between exercise, training and mucosal immunity. Researchers could consult this checklist when establishing the experimental design and methodology of studies, and preparing manuscripts.

Classification of subjects	• Selection criteria for subject (experimental) and control groups • Source of subjects • Fitness levels • Age and gender mix
Source of samples	• Saliva: whole mixed; parotid; submandibular • Biopsies: region of small or large intestine; nasal • DNA: extraction source
Collection conditions for samples, particularlysaliva samples	• Fasting or non-fasting • Stimulated or non-stimulated • Time of day, particularly for repeated collections • Time of collection in relation to prior exercise and food intake • Collection apparatus: passive drool, use of suction pumps, use of salivettes or other absorbent devices • Collection, transport and storage temperatures
Analytical methods for immune parameters	• Analytical technique • Analytical instrumentation • Calibration material • Selection of analytical antibodies • Analytical conditions • Reproducibility of assay: within and between assays at relevant concentrations • Quality assurance for assay
Classification of fitness an measurements of physiological parameters	• Performance rankings or classification of subjects • Physiological and anthropometric characteristics • Training loads (frequency, duration or volume, intensity)

One of the limitations of comparisons between studies in the exercise immunology literature is the lack of definition of a baseline level and when this should be measured. Some studies have used fasting saliva sample collections for the baseline measurement of Sal-IgA (3, 97) without consideration that fasting saliva yields higher and more variable concentrations of Sal-IgA than non-fasting samples (32, 34). Reports indicating diurnal differences in Sal-IgA concentrations in exercising populations (97) could be due to differences in fasting and non-fasting status (34) or hydration status of the subjects (44) and not attributable to other biological or exercise effects. Other studies indicate a full day of rest from exercise prior to establishing a baseline level, while other studies specify only 10-30 minutes of quiet rest. Some of the discrepancies between studies may be accounted by the variations in study design, and until knowledge of the complex dynamics of response of the immune system to exercise is more clearly defined, protocols that reduce other sources variability are essential.

Limited understanding of sources of biological variability

Striking variation in Sal-IgA concentrations between subjects is widely observed and reported (16, 70). The sources of this variation are both methodological and biological, but our understanding of the biological sources of the variation is still rudimentary. In any study population there are two components of variation in the IgA levels obtained relating to the subjects: the within-subject variation (i.e. whether the sample obtained now is representative of that individual), and the between-subject variation (i.e. that different individuals in a group have different concentrations of IgA). Recently Francis and co-workers examined the contributions of both components of variations for pre-exercise Sal-IgA levels in three populations: elite swimmers, active adults, and a sedentary group (27). They found that the within-subject and between-subject sources of variability differed substantially in the three populations.

In research with a repeated measures design, the within-subject variability determines the magnitude of response and sample size needed to achieve statistical significance. Francis *et al*. found that the within-subject variability was much greater in the elite swimmers (47%) than the other active (23%) and sedentary (28%) groups studied (27). This means that a single saliva sample from an elite swimmer provides a less reliable estimate of that swimmer's average Sal-IgA concentration than a single sample from an active or sedentary adult. To date there is a lack of published data on the reproducibility of both Sal-IgA levels in populations of different activity levels and the IgA responses to exercise. Until repeatability studies are conducted investigators are likely to continue to underestimate the sample sizes needed to obtain reliable estimates of the effects of exercise on mucosal immunity.

Studies of mucosal immunity and exercise have typically been conducted on highly trained or elite athletes and involving small numbers of subjects. Consequently differences in Sal-IgA levels of 25-30% are often reported as not statistically significant. The possibility of greater within-subject variability in elite athletes has implications for monitoring Sal-IgA levels as an indicator of changing mucosal immune status. Multiple sampling may be required to establish the Sal-IgA range from which deviations would have clinical significance. The differ-

Table 2: Considerations for the statistical analysis and interpretation of experimental and observational data.

Specification of statistical analysis	• Evaluation of normality of measures • Justification for use of mean or median data • Details of statistical analyses performed • Details of statistical programs used for each analysis • Details of commercial statistical packages used: e.g. SPSS • Justification of sample size • Implication of power to detect differences on interpretation of data • Evaluation of reproducibility of measures in each subject group • Justification for appropriateness of each control or comparison groups
Clear interpretation of data	• Acknowledgement and clarification of any limitations in interpretation of data • Clinical (practical) significance of results • Consideration of both mean effects and individual responders • Distinction of real effects from assay and normal biological variability

ences in between-subject variability between the three different exercising populations studied by Francis *et al*. also has implications for the design of research studies (27). If active and sedentary populations have less within-subject but more between-subject variation than elite athletes, then these populations may be inappropriate control groups for studies of elite athletes. There is currently little understanding of the influences on biological variability in the different exercising populations. The explanations for greater variability in IgA levels between elite athletes also requires further investigation. A suggested checklist for the statistical analysis and interpretation of experimental and observational data is presented in Table 2.

Clinical and laboratory diagnosis of respiratory illness is limited

The strong research interests in interactions between exercise, URTI and mucosal immunity have been pursued on the assumptions that elite athletes have a higher incidence of URTI than the general community, and that this association is related to suppression of mucosal immune protection (14) by high intensity or endurance exercise. Assessment of the existing studies is confounded by two major limitations: an accurate diagnosis of the aetiology of the upper respiratory symptoms; and the rare use of physician diagnosis of the episodes. While there is evidence that some elite athletes are susceptible to recurrent URTI (7, 10), particularly in the pre-competition period (34), most studies have failed to find an increased incidence of URTI compared to control groups (34). A 10-year retrospective study of elite swimmers reported an incidence similar to the general population (64). Very few studies have used physician-verified URTI in the study design, with most studies using self-reports of a limited number of common symptoms and signs. This may lead to an over estimate of the incidence of URTI in elite athletes, coupled with the fact that athletes are more likely to seek medical attention for URTI than the general population.

Aetiology of upper respiratory symptoms

Even where there is physician consultation it is rare for laboratory confirmation of the illness to be ascertained due to the high cost of pathology investigations and the vast number of potential pathogens. While it has been assumed that athletes suffer from the same URTI as the general population (90) very few specific studies have been undertaken to identify the aetiology of URTI in athletic populations and it is possible that the symptoms are attributable to other factors. Currently physician diagnosis of URTI is restricted by a lack of effective differential diagnostic criteria for true URTI, as distinguished from reactivated viral illnesses, or inflammatory causes due to airway inflammation or allergy. Until the true aetiology of the symptoms associated with UTRI in athletic populations has been determined it is likely that the incidence will continue to be over reported and the ability of physicians to assist those athletes with recurrent illness will be limited.

There has been only limited examination of the clinical significance of the symptoms associated with URTI as most infections are self-limited viral illnesses (66, 90), and bacterial infections in athletes are rare (39, 90) and usually clinically distinguishable from other causes of URTI symptoms. Examination of Epstein-Barr virus (EBV) as a cause of the symptoms in elite athletes revealed that reactivation of EBV could account for 64% of elite swimmers experiencing URTI (43) but only 7% of episodes of URTI in a similar study of long-distance runners (63). These findings highlight the need for sport specific studies and the possibility of other causes of symptoms, such as non-infective airway inflammation (91).

Neglect of psychological, environmental and biological influences

There is a wealth of information from experimental or observational studies that have independently examined relationships between psychological, physiological or environmental influences on mucosal immunity and resistance to illness and infection. There are many studies in the medical and scientific literature examining the physical effects of exercise (31, 32, 42) but relatively few on psychological (47) or environmental effects (51) on mucosal immunity. In practice these influences are likely to act in concert rather isolation with the interactive or cumulative effects possibly leading to immunosuppression in susceptible individuals. A reductionist approach using controlled studies on single influences such as the physical effects of exercise (86), psychological stress and adverse environmental conditions on mucosal immunity (26), may not necessarily account for the combined effects of these stressors. Failure to consider these interactions may confound some of the conclusions of exercise (physical stressor) studies that did not address psychological factors, or the influence of external environmental factors such as seasonal influx of winter ‘flu’ (66), or pathogen exposure through airborne transmission or direct physical contact (19).< Psychological stress in the athletic setting could originate from anxieties associated with training performance, competition or some other lifestyle stress unrelated to sport (47). It is conceivable that some athletes will experience significant stress from the combined

effects of competing in a major international competition after a season of prolonged training in hot and humid ambient conditions (14), such as those experienced for the 1992 (Barcelona), 1996 (Atlanta) and 2004 (Athens) Olympic Games. Other areas worthy of investigation include the effects of high altitude training, hot and cold climates, and circadian disturbances associated with travel and time zone dislocation.

Infections become established when host defence mechanisms are unable to adequately respond to the colonization of invading pathogenic agents such as bacterial or viral challenge (64). In the absence of a pathogenic challenge, infections are unlikely to be experienced even if mucosal immunity has been lowered by an underlying immune disorder, or a combination of physical or psychological stress. While mucosal immune status of the upper airways has been extensively studied by analysis of salivary immunoglobulin levels [6-9], and associations have been established between low levels of Sal-IgA and susceptibility to URTI (41), the importance of gut immunity has largely been overlooked (2). Disturbance of the gut mucosa is a common result of high intensity training or competition, and changes in gut mucosal immunity with exercise have been reported (2). The integrity of gut immunity becomes important in the context of athletes travelling to foreign locations where gut flora may be disturbed by local strains of water borne agents such as *Escherichia Coli*.

The highly interactive nature of the common mucosal immune system with other elements of host defence also needs to be considered (31). Clearly many elements of the human immune system work in concert and exert pleiotropic effects. The level of counter-redundancy between different compartments of the human immune system should be considered in the design of experiments. An example can be seen when suppression of IgA is counterbalanced by an elevation in IgM or IgG. Individuals who cannot sufficiently counter-compensate for IgA suppression with IgM or IgG antibody producing cells experience significant clinical consequences, such as recurrent URTI and gastrointestinal infections, in those individuals with total IgA-deficiency (13).

Neglect of nutritional and pharmacological influences

The impact of diet on immune status has been well studied and a balance between the major food groups, protein, carbohydrates and fat and several essential minerals and vitamins is required for immune competence. The impact of diet on immune function in relation to exercise has been extensively reviewed (30, 37). These reviews highlight the need for a balance between the nutritional requirements of highly trained athletes and maintenance of effective immune function. Very few exercise immunology studies provide any details of the nutritional status of the subjects and therefore the likelihood of any confounding effect of nutritional influences on immune suppression cannot be readily determined. No study has yet assessed the habitual dietary status of subjects and whether this affects Sal-IgA responses to exercise. In particular, the effect of energy balanced versus energy restricted or a low calorie diet on mucosal immune function has not been examined (22). It is possible that the decreases in IgA observed by Boyum and co-workers were at least in part a consequence of dietary energy deficiency (10).

A number of studies have examined the influence of carbohydrate ingestion in ameliorating the immunosuppression associated with exhaustive exercise (3, 6, 78). A few studies have examined the effects of a range of nutritional supplements on Sal-IgA responses to exercise. These supplements have included bovine colostrum (71), protein (55), and ginseng. The influence of Vitamins C and E on the incidence of URTI have been studied in exercising populations but there have been no studies on the effect of vitamin status or supplementation on the mucosal immune responses to exercise.

Several pharmacological agents, such as epileptic medications and immunosuppressive agents, are known to cause reduced Sal-IgA concentrations but it is unlikely that these would be used by many athletes, other than the limited use of corticosteroids. A recent clinical trial examined the effectiveness of the prophylactic administration of the anti-viral agent Valtrex in controlling EBV reactivation and upper respiratory symptoms in elite distance runners (17). No changes in mucosal immunity or upper respiratory tract symptoms were observed over the 4-month study period although EBV reactivation was reduced. Future studies will address the precise causes of upper respiratory symptoms in elite athletes and identify pharmaceutical agents effective against viruses.

Unanswered Questions

Are athletes really more prone to illness and does illness really impact on performance?

A commonly held view in the general and sporting communities is that athletes are more prone to illness. This view is supported in part by several cross-sectional and longitudinal studies showing various groups of athletes have a higher incidence of illness than recreational athletes or sedentary individuals (15). However not all studies are in agreement with these observations (15, 86, 92) and a more appropriate conclusion is that some athletes, particularly those undertaking prolonged intensive training coupled with inadequate recovery, may be more at risk (86). A retrospective study of illness in competitive swimmers over an 11-year period showed that 15% of swimmers experienced more than four episodes of illness per year, a rate considered abnormal high in the general population (28). Athletes with a prior history of illness interfering with training or competition warrant more specific medical attention and training management (89). Future studies need to address a broader range of individual and team sport athletes to ensure that conclusions and recommendations are transferable between sports. Athletes who are illness prone, coaches, other participants and officials in all sports are seeking guidance to avoid illness and the cross-infection of athletes.

The relationship between suppression of mucosal immune parameters, the presence of illness, and the resulting impact on competitive performance is still not well understood. While it is clear that suppression of mucosal immune parameters is associated with an increased risk of illness, few studies have attempted to directly quantify the effect of illness on competitive performance. Athletes generally experience mild to moderate, rather than severe, symptoms of illness or infection, and the key question is whether the presence of mild self-reported symptoms actually influences exercise performance. A study of international swimmers reported that athletes experiencing illness just prior to competition

showed a marginal decline in competitive performance but this failed to reach statistical significance (84). In competitive sports, it is more appropriate to consider the practical and clinical significance of illness on performance, rather than strict statistical significance (49, 50). Future studies need to address the question of the quantitative impact of illness on performance particularly in individual sports, such as swimming and running, where it is possible to reliably quantify and compare performance between competitions.

The underlying genetic predisposition of athletes to illness or infections should prove a fruitful area of research over the next decade as new technologies developed for clinical medicine are transferred to sports medicine research. Recent studies have examined the cytokine gene polymorphisms and identified significant differences in the balance of pro-inflammatory and ant-inflammatory cytokines that control immune responses to infections in different ethics groups (73, 74). These studies have identified a combination of cytokine polymorphisms that result in a predisposition to pro-inflammatory responses to infection (8). It may be possible to identify those athletes who have a high susceptibility to infections with future genetic testing.

Is mucosal immune status representative of overall immune status, anti-viral or anti-bacterial protection?

While mucosal immunity provide the first-line-of-defence at external body surfaces it is only one arm of the immune system and changes in mucosal protection may not reflect competencies in other aspects of immune defence. Changes in Sal-IgA provide an easy marker for monitoring alterations in mucosal immune status and reduced concentrations are an indicator for susceptibility to respiratory infections in elite athletes (7, 27). Given that low levels of Sal-IgA *per se* do not equate to infection, any interpretation of changes in Sal-IgA should view low levels as a risk factor for infections only. It should also be remembered that the measurement of total Sal-IgA does not give any information about the level of specific antibodies in saliva and therefore no indication of specific immune protection.

Whether changes in total Sal-IgA also reflect changes at other mucosal surfaces is uncertain, but as part of the common mucosal immune system it is recognised that specific antibody responses at one site are reflected at other mucosal surfaces (12, 56). Studies examining whether exercise induces changes at mucosal surfaces other than in saliva have been limited. Slight increases in the percentage of IgA and IgM immunocytes in gastrointestinal biopsies have been reported after a marathon run (82). Another study reported substantial reductions in breast milk IgA after a treadmill test by lactating women (45). The only study to examine two mucosal sites reported an increase in Sal-IgA but not nasopharyngeal IgA levels following application of a topical nasal-IgA spray prior to exercise (56).

It is possible that changes in Sal-IgA concentrations provide a maker for monitoring T-cell cytokine regulation at mucosal surfaces, not only for the humoral antibody responses but also for cellular control. While the anti-bacterial activities of secretory IgA are well recognised, IgA also plays a significant anti-viral role at mucosal surfaces (65, 100). Changes in Sal-IgA mirrored the pattern of EBV reactivation in saliva, with low levels of Sal-IgA preceding the appearance of viral EBV-DNA and high levels appearing in response to the EBV reactivation (43). One interpretation of this data is that exercise-induced immune suppression,

reflected by the low levels of Sal-IgA, also results in diminished control of the EBV-specific cytotoxic T-cells resulting in EBV reactivation, which in turns induces an increase in Sal-IgA to control the reactivated virus. The unanswered questions are how does exercise exert this influence on both the humoral and cellular arms of immune regulation, and can Sal-IgA or some other immune parameter be used to predict when an athlete is at increased risk of illness?

Does the type of exercise influence the acute mucosal immune response?

There are three broad categories of studies that have been used to investigate Sal-IgA responses to acute exercise: training sessions; competition performance; and laboratory-based exercise testing. Training sessions have been widely used to investigate the acute responses to exercise include swimming (41), athletics (17), rowing (78), kayaking (35), soccer (4), basketball (94), squash (61), tennis (81), and strength training (70). A variation on this approach used by Dimitriou *et al*. was a 5 x 400 m swim at 85% of season-best performance (20). The acute mucosal immune response to training has been variable in physically active subjects but generally indicate that Sal-IgA decreases after intense training, particularly in elite athletes (2). The responses in sedentary individuals who commence training programs have also been variable, but in general there has been an improvement in Sal-IgA levels, consistent with the responses observed to moderate intensities of exercise (4).

Responses to competition have not been well studied and usually only as part of a larger study investigating acute responses to prior training sessions. Cross-country skiing (96), basketball (94) and hockey (58) games, and marathon running (80, 83) competition performances have been examined. It is not possible to draw a consensus from these studies as they are a mix of different ages, subject fitness and training levels, all of which have independent influences on mucosal immune responses. There are no studies assessing the impact of a season of competition performances on mucosal immunity. This is particularly warranted for team sports where athletes are required to compete on a weekly basis. Further studies are required to determine whether mucosal immune responses to competitive events differ from responses to routine training.

Laboratory-based tests have used a variety of protocols to investigate the acute effects of exercise on immune function (51). These have included: incremental tests to exhaustion (21, 46); short duration (15-45 min) submaximal tests (52, 76, 88) which have varied markedly in intensity from 30-80% VO_{2max}; longer duration (1.5-3 hours) submaximal tests (52, 76, 88), or submaximal tests to exhaustion (9). A small number of studies have compared responses to different intensities or durations of exercise (9, 62, 67). There have also been studies using a very high intensity short duration tests involving single bouts (21, 53) or repeated bouts (22, 24, 98) of cycle exercise. The widely varying study protocols and poor description of the subjects' fitness levels have made it difficult to draw definitive conclusions on the Sal-IgA responses to the laboratory-based tests, but in general there is a reduction in Sal-IgA in response to an acute bout of intense exercise and an increase in response to moderate exercise (4, 51).

What factors influence responses to long-term exercise training?

These studies fall into two main groups: those investigating responses in highly

trained athletes and those investigating responses to a conditioning program in untrained subjects. The most commonly used design has been to follow elite athletes over different phases of training. Such studies are usually weeks to months in duration and have been conducted with swimmers (41, 62, 84), cyclists (46), and runners (17). There is an indication from these studies that the training phase may influence the degree of suppression of Sal-IgA, with the endurance phases having the greatest impact (51,) but this observation requires further rigorous scientific evaluation. Shorter duration studies have also been conducted with elite athletes, usually examining the impact of an intensive training period (53). The conclusions from these studies are that substantial changes in Sal-IgA may not be observed over short periods, but in elite athletes undertaking long-term high intensity training there is often a reduction in Sal-IgA over the longer training season (38). In most cases the degree of Sal-IgA suppression is relative to the intensity of the training (10, 36, 37) and the decline is reversed when training is ceased or reduced (29, 35).

Only a few studies have examined the effect of exercise training on Sal-IgA in previously untrained subjects. These studies vary widely in terms of the subjects recruited and exercise modalities used, and include strength training (70), endurance running (68), intermittent supra-maximal cycling (63), and combination resistance and aerobic conditioning in elderly subjects (1). The responses have been variable and again most likely reflect the moderate low intensity training undertaken in these studies. Until there is better understanding of the effects of intensity and duration on mucosal responses, the influence of the underlying fitness of the subjects can not be assessed independently.

There are no studies addressing the true long-term nature of competitive sport. Future studies need to follow athletes over several years of training, particularly as athletes move into the elite level of training, and then after retirement from competitive sports. An area of particular neglect is the impact of children and youth commencing a high intensity training required to attain elite athlete status. Epidemiological studies addressing these long-term issues have the potential to answer the underlying question of why some athletes are more susceptible to illness and infections than others.

What prescription can be offered to the athlete suffering recurrent infections and long-term fatigue?

A recent investigation found that reversible underlying medical conditions were present in 68% of athletes presenting with persistent fatigue and/or recurrent infections (89). The initial strategy for athletes suffering fatigue should be to reduce or cease training for a short period of time to allow recovery. It is important during this reduction in training to ensure appropriate nutrition, and avoid energy, carbohydrate or protein deficits. If fatigue persists or infections recur despite rest and nutrition then further medical investigation is warranted. Such clinical investigations should systematically screen for common causes of fatigue and the presence of unresolved viral infections. Reid *et al*. (89) found that over 40% of the athletes had more than one identifiable medical condition, and the most common finding was IgG3 deficiency. Further studies investigating the medical causes of fatigue and recurrent infections in athletes will provide a greater understanding of the most likely causes of these conditions in athletes.

Does moderate exercise enhance mucosal immune status?

There are two components to this question. First, does an acute bout of moderate intensity exercise enhance mucosal immune status? Second, does moderate intensity exercise training result in enhanced mucosal immune status? There are indications that an acute bout of moderate intensity exercise increases IgA concentrations and possibly IgA secretion (21, 76). The changes in some of the relevant studies are not statistically significant, probably due to the small numbers of subjects. The common feature of these studies is that the duration of activity was 30 minutes or less, and that the exercise was at an intensity less than 60% of VO_{2max} or comparable heart rate or blood lactate level. More prolonged exercise at workloads that are initially submaximal may not produce this increase in IgA secretion (3, 10, 97). What is not known is how variations in the type, intensity and duration of exercise in this more moderate range influence mucosal immunity, and whether subject characteristics influence the response. Interestingly, none of these investigations have been performed in elite athletes.

The effects of moderate intensity training on mucosal immune status indicate a trend for enhancement of IgA concentration or secretion. In elite athletes, pre-exercise IgA levels are usually higher following less intense training (39, 41) but whether this observation is a consequence of moderate intensity exercise or simply a response to the reduction in higher intensity training is unclear. In less elite exercisers, a small increase in IgA concentration in children training and playing basketball over a season was observed (95), and aged men and women had higher resting IgA concentrations after a year of moderate exercise training (1). Increases in IgA levels with brisk walking training in mildly obese women (77), while moderate exercise training increased IgA concentration and secretion rate as well as reducing URTI risk (54). There is a clear need for more studies examining the effects of different types of moderate exercise training programs on mucosal immune status in all populations. No studies have investigated the effects of moderate intensity training in elite athletes; therefore, the efficacy of moderate intensity training as a therapeutic intervention for athletes with illness or fatigue is unknown.

What are the practical guidelines for monitoring and managing mucosal immunity?

A number of researchers have described practical guidelines for monitoring and managing the immune status of athletes in training (33, 87). However these are relatively broad guidelines that have not been directly tested under controlled or field conditions. Three key recommendations are: the assessment of mucosal immune status, guidelines for nutritional support, and management of training loads to limit physical stress on the immune system and the risk of respiratory and gastrointestinal illness. Monitoring of mucosal immunity has traditionally involved assessment of a panel of salivary immunoglobulins: Sal-IgA, Sal-IgM, Sal-IgG and often albumin levels (42) or osmolality (98) to correct for flow rate and the effects of dehydration. Issues of the most appropriate means of reporting Sal-IgA in terms of absolute concentration or as a function of salivary flow rate are well understood (57). Given the degree of within- and between-subject biological variability in immunological parameters (27), and the inherent counter redundancy of the human immune system, it appears that a multi-factorial

approach involving a small but targeted group of immunological markers offers more promise than attempts to identify a single 'gold standard' marker (93). Multi-factorial models have been developed for diverse conditions such as cardiovascular disease and physiological aspects of overtraining (48), and a combination of cellular and soluble immune markers is likely to form the basis of immune assessment of athletes (85). Emerging diagnostic technologies in the form of portable hand-held analysers enabling rapid point-of-care monitoring in the field will facilitate more effective clinical management of athletes and generate new research opportunities.

From a physical training perspective it appears that modification of either training volume (duration), intensity or a derived overall training load (volume x intensity), offers some promise in maintaining immune competence in those sports where aerobic fitness is a key requirement (25). This approach involves daily and weekly recording of exercise and training activities using pre-determined training descriptors. Weekly and monthly tallies of training loads may be useful in identifying thresholds of training beyond which resistance to fatigue and illness is compromised (25). These concepts, developed with highly trained speed skaters (25) and kayakers (60), raise several questions for future investigators including: how to set thresholds for individual athletes in a prospective manner; the degree of within- and between-athlete variability in the thresholds; differences between indoor and outdoor sports; and whether the paradigm translates directly to both team and individual sports.

Does mucosal immune status have any role to play in assessing overtraining?
The definition of overtraining remains problematic and one for which there is no gold standard. While low levels of Sal-IgA has been reported in athletes who are under performing (9, 10, 23, 28) there have been no formal evaluation of the utility of Sal-IgA concentrations to predict overtraining in athletes. The only study to follow the recovery phase of these athletes reported that the Sal-IgA level returned to normal level with rest (9). The suppressed Sal-IgA levels in these studies have all been associated with increased incidences of URTI or persistent fatigue, which adds further complexity to the picture of what role Sal-IgA has in overtraining.

What are the possibilities with therapeutic and dietary interventions?
The area of dietary practices and use of nutritional supplements has broad appeal in both the general and sporting communities (37). With respect to mucosal immunity, there is evidence that use of probiotics such as *Lactobacillus acidopholous* can have a positive effect of Sal-IgA concentration under controlled conditions, with benefits accruing to both the respiratory and gastrointestinal tracts (18). There is emerging evidence of the possibility of the humoral and cellular immune systems with probiotic enhancement (23), although the effects of probiotics on immune function in highly trained athletes is unknown. Several studies have pointed to the benefits of carbohydrate ingestion before and during exercise for maintaining immune competence (3, 5, 7). Bishop and co-workers showed that ingestion of carbohydrate-rich sports drink by cyclists undertaking 2 h of moderate intensity exercise increased the saliva flow rate, but decreased the Sal-IgA concentration, with no overall effect on salivary IgA secretion rate (3).

The authors concluded that endurance athletes should adopt strategies that maintain adequate carbohydrate and fluid intake during heavy schedules of training and competition (3). The clinical consequences of these practices remain unclear. Future studies are required to clarify issues such as the appropriate dosage, timing and ingestion patterns for prophylactic use of dietary supplements; specific guidelines for those athletes who experience chronic respiratory or gastrointestinal illness; and detailed instructions for supplementation during international travel.

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