EXERCISE IMMUNOLOGY REVIEW

VOLUME 17 • 2011

CONTENTS

Exercise Immunology Review

Editorial Statement

Exercise Immunology Review, an official publication of the International Society of Exercise Immunology and of the German Society of Sports Medicine and Prevention, is committed to developing and. enriching knowledge in all aspects of immunology that relate to sport, exercise, and regular physical ativity. In recognition of the broad range of disciplines that contribute to the understanding of immune function, the journal has adopted an interdisciplinary focus. This allows dissemination of research findings from such disciplines as exercise science, medicine, immunology, physiology, behavioral science, endocrinology, pharmacology, and psychology.

Exercise Immunology Review publishes review articles that explore: (a) fundamental aspects of immune function and regulation during exercise; (b) interactions of exercise and immunology in the optimization of health and protection against acute infections: (c) deterioration of immune function resulting from competitive stress and overtraining; (d) prevention or modulation of the effects of aging or disease (including HIV infection; cancer; autoimmune, metabolic or transplantation associated disorders) through exercise. (e) instrumental use of exercise or related stress models for basic or applied research in any field of physiology, pathophysiology or medicine with relations to immune function.

Editor: Prof. Dr. Hinnak Northoff
Managing Editor: Dr. Derek Zieker

Send editorial correspondence to:
Secretarial office EIR
Institute of clinical and experimental
Transfusion Medicine (IKET)
University of Tuebingen
Otfried-Mueller-Str. 4/1
72076 Tuebingen, Germany
ZKT.sekretariat@med.uni-tuebingen.de

Exercise Immunology Review (ISSN 1077-5552) is published and sponsored annually by the Association for the Advancement of Sports Medicine (Verein zur Förderung der Sportmedizin) and printed by TOM-Systemdruck GmbH, Hansanring 125.
Subscription rates are $25 in the US and €25 in Europe and other countries. Student rates ($15 or €15) available for up to 3 yrs. Along with payment send name of institution and name of adviser.
Postmaster: Send address changes to Exercise Immunology Review, TOM-Systemdruck GmbH.

Copyright © 2002 by Hinnak Northoff. *Exercise Immunology Review* is indexed in Sport Database, Sport Discus, Sport Search, SciSearch, EMBASE/ Excerpta Medica, Focus on: Sports Science & Medicine, Index Medicus, MEDLINE, Physical Education Index, Research Alert, International Bibliography of Periodical Literature, International Bibliography of Book Reviews, and CINAHL database.

Notice: authorization to photocopy items is granted for internal or personal use only. All other cases contact Hinnak Northoff.

To subscribe or renew subscription phone +49 2571 5 78 89-0,
or write to TOM-Systemdruck, GmbH, Hansaring 125, D-48268 Greven,
e-mail: ribbers@tom-systemdruck.de

From the editors

This year's issue of EIR contains six articles, which is in the usual range, but two of them are quite unusual: For the first time we present a more or less complete consensus summary of the field covered by this journal – in form of a (two-part) "position statement". Although the sheer size of the two papers could suggest it, they are not reviews, but rather a consensus summary of current opinion as a result of a great cooperation between numerous experts world-wide, who have put together what they think to be the essence of today's accepted knowledge and standards. I thank Neil Walsh for initiating and coordinating this immense task and I thank all contributing authors for joining in this common endeavour.

The two parts of the position statement have different sets of authors. Each part begins with a short consensus summary followed by somewhat more detailed explanations of the addressed areas. Part one focuses on the scientific basis of what is known, accepted and deemed to be important about the influence of exercise on immune functions. Part two focuses on applicability – which consequences and recommendations are judged to be reasonable and broadly acceptable on the basis of today's knowledge.

In addition to the position statements EIR 17 holds four more articles. Two of them are classic reviews, one by Gillum et al. about sex differences in the immune reaction to exercise and one by Maltseva et al. who propose a possible role for KIRs in mediating / modulating the effects of exercise on the immune system. There are also two original study reports, one by Ogawa et al. on ATP and extracellular HSP, and another one by (Mike) Gleeson et al., which again probes the influence of that tiny little difference between the two sorts of people who participate in studies on exercise-induced immune responses.

Actually, – in the two papers presented in this issue – that tiny difference is called "sex". In the past, the term "gender" has been used (in EIR and probably elsewhere) in comparable settings. However, native English speakers have convinced me that use of "sex" is probably appropriate in most situations – language wise – as "gender" refers to a social construct, whereas "sex" is biological.

So, if you ever run a search for papers dealing with that wonderful tiny difference - just feed the machine with both terms.

For the editors

Hinnak Northoff

Position Statement
Part one: Immune function and exercise

Neil P. Walsh[1], Michael Gleeson[2], Roy J. Shephard[3], Maree Gleeson[4] Jeffrey A. Woods[5], Nicolette C. Bishop[2], Monika Fleshner[6], Charlotte Green[7], Bente K. Pedersen[7], Laurie Hoffman-Goetz[8], Connie J. Rogers[9], Hinnak Northoff[10], Asghar Abbasi[10], Perikles Simon[11]

[1] School of Sport, Health and Exercise Sciences, Bangor University, UK.
[2] School of Sport, Exercise and Health Sciences, Loughborough University, UK.
[3] Faculty of Physical Education and Health, University of Toronto, Canada.
[4] Hunter Medical Research Institute and Faculty of Health, University of Newcastle, Australia.
[5] Department of Kinesiology and Community Health, University of Illinois at Urbana-Champaign, USA.
[6] Department of Integrative Physiology, University of Colorado, USA.
[7] The Centre of Inflammation and Metabolism at the Department of Infectious Diseases, and Copenhagen Muscle Research Centre, Rigshospitalet, the Faculty of Health Sciences, University of Copenhagen, Denmark.
[8] Department of Health Studies and Gerontology, University of Waterloo, Canada.
[9] Department of Nutritional Sciences, Pennsylvania State University, USA.
[10] Institute of Clinical and Experimental Transfusion Medicine, University of Tuebingen, Germany.
[11] Department of Sports Medicine, Disease Prevention and Rehabilitation, Johannes Gutenberg-University Mainz, Germany.

CONSENSUS STATEMENT

An ever-growing volume of peer-reviewed publications speaks to the recent and rapid growth in both scope and understanding of exercise immunology. Indeed, more than 95% of all peer-reviewed publications in exercise immunology (currently >2, 200 publications using search terms "exercise" and "immune") have been published since the formation of the International Society of Exercise and Immunology (ISEI) in 1989 (ISI Web of Knowledge^{SM}). We recognise the epidemiological distinction between the generic term "physical activity" and the specific category of "exercise", which implies activity for a specific purpose such as improvement of physical condition or competition. Extreme physical activity of any type may have implications for the immune system. However, because of its emotive component, exercise is likely to have a larger effect, and to date the great majority of our knowledge on this subject comes from exercise studies.

In this position statement, a panel of world-leading experts provides a consensus of current knowledge, briefly covering the background, explaining what we think we

Correspondence:
Neil Walsh; email: n.walsh@bangor.ac.uk; telephone: +44 1248 383480

know with some degree of certainty, exploring continued controversies, and pointing to likely directions for future research. Part one of this position statement focuses on 'immune function and exercise' and part two on 'maintaining immune health'. Part one provides a brief introduction and history (Roy Shephard) followed by sections on: respiratory infections and exercise (Maree Gleeson); cellular innate immune function and exercise (Jeffrey Woods); acquired immunity and exercise (Nicolette Bishop); mucosal immunity and exercise (Michael Gleeson and Nicolette Bishop); immunological methods in exercise immunology (Monika Fleshner); anti-inflammatory effects of physical activity (Charlotte Green and Bente Pedersen); exercise and cancer (Laurie Hoffman-Goetz and Connie Rogers) and finally, "omics" in exercise (Hinnak Northoff, Asghar Abbasi and Perikles Simon).

The focus on respiratory infections in exercise has been stimulated by the commonly held beliefs that the frequency of upper respiratory tract infections (URTI) is increased in elite endurance athletes after single bouts of ultra-endurance exercise and during periods of intensive training. The evidence to support these concepts is inconclusive, but supports the idea that exercised-induced immune suppression increases susceptibility to symptoms of infection, particularly around the time of competition, and that upper respiratory symptoms are associated with performance decrements. Conclusions from the debate on whether sore throats are actually caused by infections or are a reflection of other inflammatory stimuli associated with exercise remains unclear.

It is widely accepted that acute and chronic exercise alter the number and function of circulating cells of the innate immune system (e.g. neutrophils, monocytes and natural killer (NK) cells). A limited number of animal studies has helped us determine the extent to which these changes alter susceptibility to herpes simplex and influenza virus infection. Unfortunately, we have only 'scratched the surface' regarding whether exercise-induced changes in innate immune function alter infectious disease susceptibility or outcome and whether the purported anti-inflammatory effect of regular exercise is mediated through exercise-induced effects on innate immune cells. We need to know whether exercise alters migration of innate cells and whether this alters disease susceptibility. Although studies in humans have shed light on monocytes, these cells are relatively immature and may not reflect the effects of exercise on fully differentiated tissue macrophages. Currently, there is very little information on the effects of exercise on dendritic cells, which is unfortunate given the powerful influence of these cells in the initiation of immune responses.

It is agreed that a lymphocytosis is observed during and immediately after exercise, proportional to exercise intensity and duration, with numbers of cells (T cells and to a lesser extent B cells) falling below pre-exercise levels during the early stages of recovery, before returning to resting values normally within 24 h. Mobilization of T and B cell subsets in this way is largely influenced by the actions of catecholamines. Evidence indicates that acute exercise stimulates T cell subset activation *in vivo* and in response to mitogen- and antigen-stimulation. Although numerous studies report decreased mitogen- and antigen-stimulated T cell proliferation following acute exercise, the interpretation of these findings may be confounded by alterations in the relative proportion of cells (e.g. T, B and

NK cells) in the circulation that can respond to stimulation. Longitudinal training studies in previously sedentary people have failed to show marked changes in T and B cell functions provided that blood samples were taken at least 24 h after the last exercise bout. In contrast, T and B cell functions appear to be sensitive to increases in training load in well-trained athletes, with decreases in circulating numbers of Type 1 T cells, reduced T cell proliferative responses and falls in stimulated B cell Ig synthesis. The cause of this apparent depression in acquired immunity appears to be related to elevated circulating stress hormones, and alterations in the pro/anti-inflammatory cytokine balance in response to exercise. The clinical significance of these changes in acquired immunity with acute exercise and training remains unknown.

The production of secretory immunoglobulin A (SIgA) is the major effector function of the mucosal immune system providing the 'first line of defence' against pathogens. To date, the majority of exercise studies have assessed saliva SIgA as a marker of mucosal immunity, but more recently the importance of other antimicrobial proteins in saliva (e.g. α-amylase, lactoferrin and lysozyme) has gained greater recognition. Acute bouts of moderate exercise have little impact on mucosal immunity but prolonged exercise and intensified training can evoke decreases in saliva secretion of SIgA. Mechanisms underlying the alterations in mucosal immunity with acute exercise are probably largely related to the activation of the sympathetic nervous system and its associated effects on salivary protein exocytosis and IgA transcytosis. Depressed secretion of SIgA into saliva during periods of intensified training and chronic stress are likely linked to altered activity of the hypothalamic-pituitary-adrenal axis, with inhibitory effects on IgA synthesis and/or transcytosis. Consensus exists that reduced levels of saliva SIgA are associated with increased risk of URTI during heavy training.

An important question for exercise immunologists remains: how does one measure immune function in a meaningful way? One approach to assessing immune function that extends beyond blood or salivary measures involves challenging study participants with antigenic stimuli and assessing relevant antigen-driven responses including antigen specific cell-mediated delayed type hypersensitivity responses, or circulating antibody responses. Investigators can inject novel antigens such as keyhole limpet haemocyanin (KLH) to assess development of a primary antibody response (albeit only once) or previously seen antigens such as influenza, where the subsequent antibody response reflects a somewhat more variable mixture of primary, secondary and tertiary responses. Using a novel antigen has the advantage that the investigator can identify the effects of exercise stress on the unique cellular events required for a primary response that using a previously seen antigen (e.g. influenza) does not permit. The results of exercise studies using these approaches indicate that an acute bout of intense exercise suppresses antibody production (e.g. anti-KLH Ig) whereas moderate exercise training can restore optimal antibody responses in the face of stressors and ageing. Because immune function is critical to host survival, the system has evolved a large safety net and redundancy such that it is difficult to determine how much immune function must be lost or gained to reveal changes in host disease susceptibility. There are numerous examples where exercise alters measures of immunity by 15-25%. Whether changes of this magnitude are sufficient to alter host defence, disease susceptibility or severity remains debatable.

Chronic inflammation is involved in the pathogenesis of insulin resistance, atherosclerosis, neurodegeneration, and tumour growth. Evidence suggests that the prophylactic effect of exercise may, to some extent, be ascribed to the anti-inflammatory effect of regular exercise mediated via a reduction in visceral fat mass and/or by induction of an anti-inflammatory environment with each bout of exercise (e.g. via increases in circulating anti-inflammatory cytokines including interleukin (IL)-1 receptor antagonist and IL-10). To understand the mechanism(s) of the protective, anti-inflammatory effect of exercise fully, we need to focus on the nature of exercise that is most efficient at allieviating the effects of chronic inflammation in disease. The beneficial effects of endurance exercise are well known; however, the anti-inflammatory role of strength training exercises are poorly defined. In addition, the independent contribution of an exercise-induced reduction in visceral fat versus other exercise-induced anti-inflammatory mechanisms needs to be understood better. There is consensus that exercise training protects against some types of cancers. Training also enhances aspects of anti-tumour immunity and reduces inflammatory mediators. However, the evidence linking immunological and inflammatory mechanisms, physical activity, and cancer risk reduction remains tentative.

In the very near future, genomics, proteomics, and metabolomics may help exercise immunologists to better understand mechanisms related to exercise-induced modulation of the immune system and prevention (or reduced risk) of diseases by exercise training. In addition, these technologies might be used as a tool for optimizing individual training programmes. However, more rigorous standardization of procedures and further technological advances are required before practical application of these technologies becomes possible.

Key Words: exercise; sport; training; immune; pathogen; infection; innate; acquired; mucosal; saliva; leukocyte; monocyte; neutrophil; granulocyte; lymphocyte; immunoglobulin; method; cytokine; interleukin; inflammation; cancer; genomics; proteomics; metabolomics

INTRODUCTION AND HISTORY

Two recent papers have summarized the scientific history of exercise immunology (263) and its development as a specific discipline (264) with its own international society and a dedicated journal. Exercise immunology has quite a short history relative to many branches of the exercise sciences, the modern era of careful epidemiological investigations and precise laboratory studies beginning in the mid 1980s. However, an ever-growing volume of peer-reviewed publications speaks to a rapid growth in both scope and understanding of the topic since that date. In addition to enquiries into many areas of intrinsic scientific interest, exercise immunologists have found diverse applications for their talents in augmenting population health and maintaining high performance athletes in peak physical condition.

From early during the 20th century, clinicians had pointed to what seemed adverse effects of prolonged heavy exercise upon both resistance to and the course of various viral and bacterial diseases (25, 261). These concerns were seemingly sub-

stantiated by a 2-6 fold increase in the reported symptoms of upper respiratory infection (URTI) for several weeks following participation in marathon or ultra-marathon events (200, 224). The influence of exercise on the risks of URTI is discussed in the following section. A transient fall in the circulating natural killer (NK) cell count following a sustained bout of vigorous exercise (270) seemed to offer a mechanism explaining the increase in risk; the temporary lack of NK cells and killer cell activity offered an "open window," a period when a reduced resistance to viral infections allowed easier access to infecting micro-organisms. Innate immunity is discussed in detail later in this part of the position statement. In one report, the reduction in NK cell count persisted for seven days following exercise (259), but in most studies, circulating NK cell numbers and activity have been described as being depressed for only a few hours, raising doubts as to whether the "window" was open long enough to account for the increased vulnerability to infection. Moreover, technical advances (particularly in automated cell counting and identification) (85) have underlined that exercise does not destroy NK cells; rather, they are temporarily relocated to reservoir sites such as the walls of peripheral veins in response to the exercise-induced secretion of catecholamines and activation of adhesion molecules (266). A more plausible explanation for the reported increase in URTI during heavy training and following participation in long-distance events appeared as attention shifted to immunoglobulins in general, and in particular to a depression of front-line defences through a decrease in the mucosal secretory functions of the nose and salivary glands (152, 298). The influence of exercise on mucosal immunity is discussed in more detail later in this part of the position statement.

The hypothesis of a U-shaped relationship between physical activity and resistance to disease, although based on a relatively limited amount of laboratory and epidemiological data (202, 267), has made intuitive sense, jibing with the more general belief that although regular moderate doses of physical activity have beneficial effects on health, excessive amounts or intensities of physical activity have negative consequences. In the case of the immune system, one suggestion has been that an excess of physical activity provokes something analogous to clinical sepsis, with tissue destruction from an excessive inflammatory reaction (260). Although initially conceived simply in the context of URTI (201), the concept of a U-shaped response has now been extended to cover the effects of physical activity upon a variety of clinical disturbances of immune function. In terms of cancer prevention and therapy (268), regular moderate physical activity has been shown to reduce the risk of developing certain forms of the disease (265); it also limits the risk of metastasis, at least in experimental animals (156). Exercise and cancer is discussed in more detail in this part of the position statement. On the other hand, excessive exercise has been shown to cause DNA damage and apoptosis (176, 186). Ageing is increasingly considered in part as an expression of disturbed immune function; high concentrations of pro-inflammatory cytokines are seen in the elderly, and seemingly contribute to such problems of ageing as sarcopenia, neural degeneration and Alzheimer's Disease. Moreover, appropriate amounts of physical activity can control levels of pro-inflammatory cytokines, and appear to have a beneficial effect on these manifestations of ageing (188). Certain autoimmune conditions also respond to carefully regulated physical activity programmes, although it has yet to be established clearly whether benefit occurs

through some direct modulation of cell counts and cytokines, or through changes in the activity of transcription factors for pro-inflammatory cytokines (9).

Developments in fluorescent antibodies have allowed exercise immunologists to identify an ever-growing number of cell sub-types and receptors. At the same time, new cytokine identification kits and methods in molecular technology (173) have allowed the examination of humoral factors that are present in the body for very short periods and in extremely low concentrations; an increasingly complex range of pro- and anti-inflammatory cytokines has been revealed. The exercise immunologist seems drawn into the main streams of sports medicine, physiology and even psychology. A fascinating cascade of cytokines is now thought to have an important role not only in controlling exercise-induced inflammation, but also in regulating the release and necessary flow of metabolites (221). Development of the sub-discipline of psycho-neuroimmunology (141) has emphasized that vigorous exercise should be considered as but one example of the body's reaction to a variety of stressors (221), with an important two-way communication between peripheral immunocytes and hypophyseal centres, involving a wide variety of hormones and autonomic pathways (157). A section in the second part of the position statement deals with stress and immune function.

On the sports field, exercise immunologists are increasingly asked to develop procedures to detect such abuses as blood doping (185) and gene transfer (11) (see "Omics" section in this part of the position statement). However, attempts to pinpoint immunological markers of over-training have as yet proved inferior to traditional indices such as mood state and physical performance (as discussed in the second part of this position statement). A variety of nutritional supplements to date seem to have had only limited success in blunting the immune impairment associated with heavy exercise (as discussed in the second part of this position statement).

These are a few of the important topics on which a panel of world experts provide a succinct consensus of current knowledge, briefly covering the relevant background, exploring continued controversies, and pointing to likely directions of future research.

RESPIRATORY INFECTIONS AND EXERCISE

Background

There are more uncertainties than evidence based facts on the nature of upper respiratory tract infections (URTI) associated with exercise, particularly in high performance athletes. Although URTI or 'sore throats' are the most common reason for presentation of elite athletes to a sports medicine clinic (62, 77, 80), the debate on whether sore throats are actually caused by infections, or are a reflection of other inflammatory stimuli associated with exercise remains unclear (48, 106, 242).

The costs associated with identification of the underlying causes of upper respiratory symptoms (URS) and the delay in obtaining results of investigative tests

means that infections are not usually verified by pathology examinations. Physician confirmation of an infective cause of the symptoms, based on clinical signs and symptoms, has until recently been considered the 'gold standard' for exercise studies, but the involvement of physicians in assessments and diagnosis is not common in research settings. Recently, the 'gold standard' of physician verified diagnosis of URTI has also come under scrutiny, and been found less than ideal (48). Very few studies have examined the underlying causes of URS and extensive clinical investigations of athletes are rare (48, 242).

The focus on respiratory infections in exercise has been stimulated by the commonly held beliefs that the frequency of URTI is increased in elite endurance athletes and that their incidence is associated with more intensive training (201). The evidence to support these concepts is inconclusive, but does, support the idea that exercised-induced immune suppression increases susceptibility to symptoms of infection and that URS are associated with performance decrements.

Evidence based consensus and uncertainties

Over the past thirty years, there have been numerous investigations examining the association between changes in immune parameters and the risk of URTI in athletic and non-exercising populations. The only immune measures to date to show consistent relationships with URS in exercising populations have been changes in salivary IgA concentrations and secretion rates (19, 89, 263). A section focusing on exercise and mucosal immunity appears later in this part of the position statement.

Altered mucosal immunity and risk of symptoms of URTI

The inverse relationship between salivary IgA concentrations and risk of URTI in exercising and non-exercising populations has demonstrated differences between these two populations (76, 89, 98, 232). The different population risk profiles are predominantly due to differences in the levels of intensity and quantum of exercise undertaken by very fit elite athletes and non-elite exercising or sedentary populations. The impact of exercise intensity on salivary IgA concentrations and secretion rates has demonstrated greater decreases in salivary IgA associated with prolonged high intensity exercise, whereas moderate increases in salivary IgA occur in response to short duration moderate intensity exercise (6, 19, 23, 98, 129, 148, 163, 232).

Although study populations vary, the association of an increased risk of URS and/or URTI with lower concentrations of salivary IgA and secretion rates has been consistent for high-performance endurance athletes undertaking intensive training (64, 91, 92, 95, 97, 148, 187, 195-198, 201, 320). Similarly, the increases in salivary IgA observed after moderate exercise training may contribute to the reduced susceptibility to URTI associated with regular moderate exercise (3, 129).

Symptoms and frequency

Although there are many anecdotal reports that URTIs are more common in elite athletes, there is very little reported evidence to support this commonly held belief. This uncertainty is compounded by the current uncertainty around whether the URS are due to infections or other inflammatory stimuli mimicking URTI (48, 242).

Retrospective and prospective longitudinal studies have identified that the majority of elite athletes experience symptoms of URTI at a rate similar to the general population (48, 78, 234). However, the episodes of URS in elite athletes do not follow the usual seasonal patterns of URTI observed in the general population, but rather occur during or around competitions (97, 160, 198, 224). Symptoms occur more frequently during the high intensity training and taper period prior to competitions in some sports, such as swimming (79, 89, 91), but in other endurance sports, such as long distance running, URS appear more frequently after a competition (49, 198, 224). Illness-prone athletes may also be susceptible to URS during regular training periods or following increases in training load (80). The commonly reported short-term duration of URS (1-3 days) in most studies suggests that in most instances a primary infection is unlikely and the symptoms may be due to viral reactivation (97, 242) or other causes of exercise-induced inflamma-

Table 1. Pathogens identified and the number of cases in comprehensive prospective studies of athletes presenting with symptoms of upper respiratory infections in 1) a cohort of high performance triathletes during training and competitions (282); 2) a study of elite athletes from a variety of sports undertaking routine training presenting to a sports clinic with URS (48); and 3) a cohort of elite athletes experiencing recurrent episodes of URS associated with fatigue and performance decrements (242). Where investigations were not performed this is recorded as (-).

Pathogen identified by microbial and viral investigation	Triathletes (n=63) undertaking routine training and competitions Spence et al. (282)	Elite athletes (n=70) presenting to a sports clinic Cox et al. (48)	Elite athletes (n=41) with persistent fatigue and poor performance Reid et al. (242)
Rhinovirus	7	6	-
Influenzae (A & B)	7	1	-
Parainfluenzae (1, 2 & 3)	4	3	-
Adenovirus	0	2	-
Coronavirus	2	0	-
Metapneumovirus	1	0	-
Epstein Barr virus (primary infection)	1	1	3
EBV reactivation	-	1	8
Cytomegalovirus	0	0	5
Herpes simplex virus (types 1 & 2)	0	-	-
Ross River virus	-	-	1
Toxoplasmosis	-	-	1
Mycoplasma pneumoniae	0	1	1
Streptococcus pneumonia	2	1	-
Staphylococcus pyogenes	0	1	-
Haemophilus influenzae	0	0	-
Moraxella catarrhalis	0	0	-
Enterococcus spp	0	0	-

tion. The evidence that URS are associated with poor performance is also limited. In the month prior to an international competition URS have been associated with decrements in performance in elite swimmers (235), suggesting that regardless of whether the URS are due to infections or other inflammatory stimuli, they can impact on performance at an elite level. However, a small proportion of high-performance endurance athletes experience recurrent episodes of URS at significantly higher rates than the incidence in the general population (92, 234), and in these athletes the URS are associated with significant persisting fatigue and poor performance (79, 91, 93, 242).

Infections versus inflammation

The few studies that have undertaken pathology testing to identify infectious from non-infectious causes of the episodes of URS in high-performance athletes have revealed that bacterial infections account for about 5% of the episodes (48, 94, 242, 282). Most episodes of URS with an identified infectious cause are of viral origin, but these account for only about 30-40% of the episodes in each study (48, 282). The bacterial and viral pathogens identified in these comprehensive studies indicate that the infections are caused by the usual respiratory pathogens associated with URTI (246) in the general population (Table 1).

However, the profile of infections in a study of elite athletes experiencing recurrent URS associated with long-term fatigue and poor performance identified a high percentage as having herpes group viruses (e.g. cytomegalovirus) or evidence of Epstein Barr Virus (EBV) reactivation (242) (Table 1). Epstein Barr viral reactivation has also been demonstrated in association with URS in some endurance sports (97, 242), which may account for the short duration of the symptoms reported in most studies, resulting from viral reactivation rather than primary infection. However, in a study examining the prophylactic use of an antiviral treatment in elite runners, it was shown that not all episodes of URS were associated with EBV expression (50) and that the frequency of EBV expression differed between sports (50, 97). Although an anti-herpes virus treatment was effective in reducing EBV expression in elite long-distance runners, it was not effective in reducing the frequency of episodes of URS, once again suggesting other non-infective causes for the URS in elite athletes (50).

Physician diagnosis of infections as the cause of the URS has recently come under scrutiny (48) and in conjunction with a previous study by Reid et al. (242) has identified that elite athletes suffering recurrent episodes of URS need more exhaustive clinical assessments to exclude non-infectious yet treatable causes of the symptoms, such as asthma, allergy, autoimmune disorders, vocal cord dysfunction and unresolved non-respiratory infections. In these studies, other diseases with an inflammatory basis accounted for 30-40% of episodes of URS in elite athletes. These studies identified that URS were divided into approximately one-third proportions as having an infectious cause, non- infectious medical cause and an unknown aetiology. The speculative causes of the ‘unknown-aetiology’ group could include physical or mechanical damage such as drying of the airways (16); asthma and allergic airway inflammation (106); psychological impacts of exercise on membrane integrity and immunity (22); and the migration

to the airways of inflammatory cytokines generated during damage to muscles susained in eccentric exercise (214, 222). Multiple stressors experienced by athletes, biological, physical and psychological, are likely to induce neurological and endocrine responses in addition to alterations in immune parameters; these share common exercise-induced pathways (207) that may result in URS. However, there is currently little direct evidence to support any of these mechanisms being associated with URS, respiratory infections or susceptibility to infections in athletes.

Cytokine regulation

Cytokine responses to exercise (particularly those associated with micro-trauma and or glycogen depletion of muscle tissue (27, 214, 222, 294)) are reasonably well characterised (as discussed in the section on anti-inflammatory effects of physical activity later in this part of the position statement). They are likely to play an important role in modulating post-exercise changes in immune function that increase the risk of infection or the appearance of inflammatory symptoms (294). The pro-inflammatory responses to exercise have the potential to be involved in expression of URS that mimic URTI. A study comparing cytokine responses to exercise in illness-prone distance runners demonstrated impaired anti-inflammatory cytokine regulation compared to runners who did not suffer frequent episodes of URS (51). A recent cytokine gene polymorphism study by Cox et al. (47) identified an underlying genetic predisposition to high expression of the pro-inflammatory interleukin-6 in athletes prone to frequent URS. These studies add further weight to the evidence that suggests infections are not the only cause of the symptoms of 'sore throat'. They are supported by studies examining the prophylactic use of topical anti-inflammatory sprays to prevent URS in long-distance runners which demonstrate a reduction in the severity of the symptoms but not the frequency of episodes following marathon races (49, 257).

Conclusions and future directions

Interpreting the findings of studies on the role of respiratory infections in exercise is often limited by the lack of pathogen identification. Regardless of the underlying stimulus for the inflammatory symptoms the implications of the upper airway symptoms for athletes may be the same. However, unless the symptoms are confirmed as infections, reference to symptoms as URS rather than as infections or URTI should become the accepted reporting standard, particularly when there is no physician assessment.

The current consensus is that the cause of URS in athletic populations is uncertain. Physician identification can no longer be considered the gold standard and symptoms should only be referred to as infection if a pathogen has been identified. Although diagnostic pathology is rarely performed, in the few studies that have examined pathology, the infections identified in most athletes have been the common respiratory pathogens observed in the general population.

Inflammation from non-infective causes is common among athletes and many will have underlying treatable conditions. As differentiation between the inflammatory causes of URS is currently not feasible in most research settings, appro-

priate treatments are difficult to prescribe universally. Athletes with recurrent URS associated with long-term fatigue and poor performance do, however, warrant more exhaustive clinical investigations, including assessment for possible involvement of the herpes group viruses. Identifying athletes with an underlying genetic predisposition to pro-inflammatory responses to exercise may be useful in managing the training regimens of elite athletes, particularly those who suffer recurrent episodes of URS associated with fatigue and poor performance.

The two main questions to be resolved about the relationship between respiratory infections and exercise are: 1) whether the upper respiratory tract symptoms are actually infections and if so whether they can be prevented or treated; and 2) if the symptoms are not due to infections can the different causes of the inflammation be segregated in the complex paradigm of elite training to optimise the illness-prone athlete's training and performance.

CELLULAR INNATE IMMUNE FUNCTION AND EXERCISE

Background

Innate immunity is our first line of defence against infectious pathogens and is intimately involved in tissue damage, repair and remodeling. The major difference between innate immune responses and adaptive responses is that innate responses do not strengthen upon repeated exposure (there is no memory function). In addition, innate responses are less specific in terms of pathogen recognition. So, whereas innate responses recognize classes of pathogens (e.g. gram-negative bacteria) through toll-like receptors (TLRs), lymphocytes exhibit exquisite specificity for epitopes of individual pathogens (e.g. influenza virus). The innate branch of the immune system includes both soluble factors and cells. Soluble factors include complement proteins which mediate phagocytosis, control inflammation and interact with antibodies, interferon α/β which limits viral infection, and anti-microbial peptides like defensins which limit bacterial growth. Major cells of the innate immune system include neutrophils which are first line defenders against bacterial infection, dendritic cells (DCs) which serve to orchestrate immune responses, macrophages (Mϕ's) that perform important phagocytic, regulatory and antigen presentation functions, and natural killer (NK) cells which recognize altered host cells (e.g. virally infected or transformed). However, many host cells, not just those classified as innate immune cells, can initiate responses to pathogenic infection. Although partitioning the immune system into innate and adaptive systems makes the system easier to understand, in fact, these branches are inextricably linked with each other. For example, the innate immune system helps to develop specific immune responses through antigen presentation, whereas cells of the adaptive system secrete cytokines that regulate innate immune cell function. This section will focus on the influence of acute and chronic exercise on cellular components of innate immunity (Figure 1). A later section in this part of the position statement will focus on exercise and inflammatory cytokines which constitute the products of innate immune and other cells.

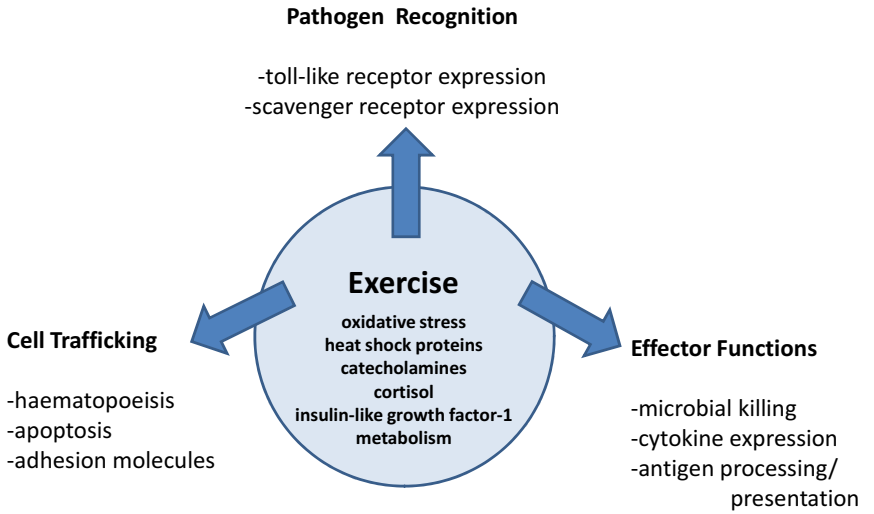

Figure 1. Potential mechanisms whereby acute/chronic exercise affects innate immunity. Exercise-induced factors such as oxidative stress, increased metabolic rate, heat shock proteins, catecholamines, cortisol and insulin-like growth factor can influence: pathogen recognition by altering expression of recognition molecules such as toll-like or scavenger receptors; cell trafficking by altering haematopoieisis, cell death and adhesion molecule expression; and effector functions like oxidative burst, cytokine expression and antigen processing and presentation. This list of potential mechanisms is not all-inclusive and very few have been definitively tested.

Consensus

Acute exercise and cellular innate immune function

Neutrophils

Acute exercise results in a first, rapid and profound neutrophilia (increase in blood neutrophil number) followed by a second, delayed increase in blood neutrophil count a few hours later, the magnitude of which is related to both the intensity and duration of exercise (216, 247). The initial increase is likely due to demargination caused by shear stress and catecholamines, whereas the later increase may be due to cortisol-induced release of neutrophils from the bone marrow (162). Unstimulated neutrophil degranulation, phagocytosis and oxidative burst activity are increased by an acute bout of exercise but there is a reduced degranulation and oxidative burst in response to bacterial stimulation that can last for many hours (215, 216, 247). This indicates that although exercise may mobilize highly functional neutrophils into the circulation, in recovery, their ability to respond to exogenous stimuli may be diminished. Marginated neutrophils are more mature than recently released neutrophils and this likely has implications for the study of exercise on neutrophil function, although this does not appear to influence respiratory burst activity (276).

Monocytes/Macrophages

Many studies have examined the influence of acute exercise on human $CD14^+$ blood monocytes (Mo's) which are relatively immature cells destined to become

tissue Mϕ's. Acute exercise results in a transient (~2 h) monocytosis and most likely represents a shifting of Mo's from the marginated to the circulating pool (206). This could occur as a result of haemodynamic and/or cortisol or catecholamine-induced release from the vascular endothelium (136). Indeed, administration of the beta-blocker propranolol can reduce exercise-induced monocytosis (2) and adrenaline (epinephrine) administration causes monocytosis (307). There are also reports that exercise can affect Mo phenotype, cell surface protein, and cytokine expression. For example, in response to acute exercise, there is a preferential mobilization of $CD14^+/CD16^+$ expressing Mo's (115, 289) that exhibit a pro-inflammatory phenotype relative to $CD14^+/CD16^-$ classical Mo's. It may be that these marginated cells have a more mature inflammatory function for entry into tissues and are knocked off the endothelium in response to exercise. Interestingly, the percentage of these $CD14^+/CD16^+$ cells is reduced in recovery, perhaps indicating remarginalization or tissue recruitment (272). Acute exercise also reduces expression of TLRs 1, 2 and 4 on $CD14^+$ Mo's (140). However, the extent to which these changes reflect a true decrease versus Mo population shifts is unclear. In an attempt to reconcile this, Simpson et al. (272) examined cell surface proteins on Mo subpopulations in response to acute exercise. They found that TLR4 and HLA.DR (major histocompatibility molecule II important in antigen presentation) expression were altered on total $CD14^+$ Mo's but also on individual Mo populations, indicating that changes in cell surface expression are not influenced solely by exercise-induced changes in Mo subpopulations in blood. Several studies have examined Mo cytokine production after acute exercise, finding that, although spontaneous cytokine levels in $CD14^+$ cells change little (245, 285), acute exercise reduces TLR ligand-stimulated interleukin (IL)-6, IL1-α, and tumour necrosis factor-alpha (TNF-α production (140, 286), perhaps as a consequence of reduced TLR expression. Further studies regarding the effects of acute exercise on Mo TLR signaling may clarify these observations.

Because Mo's are relatively immature, exercise-induced changes in their function may not reflect actual tissue Mϕ function which is central to inflammation and immune responses. For this reason, animal studies have examined the influence of exercise on tissue Mϕ number and function. Both moderate and intense acute exercise have potent stimulatory effects on phagocytosis (210), anti-tumour activity (52, 327, 328), reactive oxygen and nitrogen metabolism (327, 328), and chemotaxis (206, 209). However, not all functions are enhanced by exercise. We have documented prolonged exercise-induced reductions in Mϕ MHC II expression (325) and antigen presentation capacity (35, 36). Some effects may be dose-dependent as exhaustive exercise was shown to decrease alveolar Mϕ anti-viral function; this effect was correlated with increased susceptibility to Herpes simplex virus (HSV)-1 infection (133, 134) and related to increased release of adrenal catecholamines, but not corticosterone (133). Thus, it appears that exercise, perhaps dependent on dose with respect to some functions, can affect tissue Mϕ's and, in some studies, disease outcomes in animals. Whether these same effects can be generalized to humans is unknown.

Dendritic cells

The effect of acute exercise on DCs has received little attention despite the important emergent role of these cells in the initiation of immune responses. There are

only two studies reporting that exercise can increase circulating numbers of DCs (59, 109) and, to our knowledge, nothing is known about acute effects of exercise on DC function.

Natural killer (NK) cells

There is a vast literature on the acute effects of exercise on circulating NK ($CD3^{-}CD16^{+}CD56^{+}$) cells, perhaps because of their ease of study and large magnitude change in response to exercise. Like other blood leukocytes, NK cells are rapidly mobilized into the circulation in response to acute exercise, most likely by increased shear stress and catecholamine-induced down-regulation of adhesion molecule expression (15, 122, 301). There appears to be a differential mobilization such that $CD56^{bright}$ NK cells are less responsive than $CD56^{dim}$. Perhaps this indicates a reduced ability to defend against pathogens during acute exercise, as $CD56^{bright}$ cells are more cytotoxic. However, the health significance of exercise-induced changes in circulating NK cells, like other leukocytes, remains unknown. After prolonged exercise, the numbers of circulating NK cells are reduced in blood (87), perhaps as a consequence of remarginalization or tissue migration, but there is a relative increase in the $CD56^{bright}$ subset (302).

NK cell cytotoxicity (NKCC) is a major functional measure of NK activity. Early studies demonstrated that unstimulated NKCC was dependent upon the intensity and duration of the exercise bout (87). Immediately after a single bout of moderate or exhaustive exercise there is a 50-100% increase in human peripheral blood NKCC (87, 329). The exercise-induced increase in NKCC is largely due to an increase in the absolute number and percentage of blood NK cells (87). NKCC expressed on a per cell basis does not appear to change much after acute exercise unless the bout is intense and prolonged, in which case NKCC can be depressed for several hours, possibly indicating an enhanced period of susceptibility to infection (90). Only a few studies have examined whether NK cells mobilized into the circulation in response to exercise have altered sensitivity to stimulating agents like interferon-α or IL-2 (68, 329); however, like unstimulated NKCC, these effects are likely mediated by distributional shifts in NK cell subsets and should not necessarily be interpreted as altered NK cell function on a per cell basis.

Exercise training and cellular innate immune function

Neutrophils

Regular exercise training does not appear to alter blood leukocyte counts, including neutrophils appreciably (90). However, there are a few reports that exercise training reduces blood neutrophil counts in those with chronic inflammatory conditions or neutrophils in sites of chronic inflammation (171) raising the possibility that such exercise acts in an anti-inflammatory fashion in those with inflammation. This effect could be beneficial or deleterious, dependent upon the context. Although there is little known about the influence of exercise training on neutrophil function, regular exercise, especially heavy, intense training, may attenuate neutrophil respiratory burst (103, 233). This could reflect a sustained effect of previous acute exercise, as attenuation of respiratory burst has been documented to last several days post-exercise (295).

Monocytes/Macrophages
Both longitudinal exercise training and cross-sectional studies have shown that physically active people exhibit reduced blood Mo inflammatory responses to lipopolysaccharide, lower TLR4 expression, and a lower percentage of $CD14^+/CD16^+$ 'inflammatory' Mo's (73, 165, 166, 273, 290, 300). The extent to which these effects on the relatively small blood Mo pool contribute to the anti-inflammatory effect of exercise training is unknown. In contrast, animal studies have demonstrated that exercise training can increase induced inflammatory responses of peritoneal Mϕ's (128, 151, 292), indicating a possible difference between the effects of training on blood Mo's when compared with differentiated tissue Mϕ's. Animal studies have the potential to shed additional light on the source of the anti-inflammatory effect of regular exercise, especially in populations that exhibit inflammation. Indeed, in two recent studies, we have shown that exercise training, with or without a low fat diet, reduces visceral adipose tissue (e.g. Mϕ infiltration and pro-inflammatory cytokine gene expression) and systemic inflammation in high fat diet-fed mice (309, 310). Regular exercise may also reduce Mϕ infiltration into other sites of chronic inflammation, including growing tumours (336), and this could be interpreted as a benefit given the tumour supporting role of these cells. In contrast, reduced infiltration of Mϕ's into sites of chronic infection could lead to morbidity, although this has not been demonstrated. In fact, Mϕ's appear to play a definitive role in mediating the beneficial effects of regular moderate exercise as it relates to intranasal infection with HSV-1 in mice (181).

Dendritic cells
There are two reports from the same group demonstrating an effect of exercise training on rat dendritic cells. Liao et al. (147) reported that dendritic cell number increased after training, with no difference in costimulatory molecule (CD80 or CD86) expression, while Chiang et al. (40) found that MHC II expression, mixed leukocyte reaction and IL-12 production were increased in DCs from exercise trained rats. Clearly, given the importance of DCs in early immune regulation, this is an area ripe for investigation.

Natural killer (NK) cells
Despite much research regarding the effects of exercise training on NK cell number and function, there appears to be much controversy regarding its effect. Early cross-sectional or intervention studies with limited subject numbers reported modest increases in NKCC after moderate exercise training in previously sedentary subjects (167, 194, 202, 223, 269, 326). In larger trials, one study (65) found that 15 weeks of moderate exercise training increased NKCC compared with sedentary controls, while another 12-month trial found no change in NKCC in 115 post-menopausal women (31). However, intense training has been shown to alter NK cell subsets and reduce NKCC (93, 293). Studies in animals have demonstrated that regular exercise can increase *in vivo* cytotoxicity (119, 120, 155); however, the specific contribution of NK cells in mediating this exercise effect is unclear (119).

Controversies

Based upon the body of literature, it appears that both acute and chronic exercise have the potential to alter both the number and function of cells of the innate immune system (Figure 1). A limited number of animal studies have helped us determine the extent to which these changes alter susceptibility to herpes simplex (181) and influenza virus (149, 150, 271) infection. Unfortunately, we have only 'scratched the surface' regarding whether exercise-induced changes in immune function alter infectious disease susceptibility or outcome. In addition, although some progress has been made, we know relatively little about how acute and chronic exercise affect innate immune cell trafficking. We need to determine whether exercise alters migration of these cells and whether this alters disease susceptibility. Given the important role of innate immune cells in inflammatory states and the relationship between inflammation and chronic disease, we need to clarify whether the purported anti-inflammatory effect of regular exercise is mediated through exercise-induced effects on innate immune cells. In this regard, it is of interest to know whether exercise affects Mϕ phenotype (e.g. classical versus alternative). Although studies in humans shed light on Mo's, these cells are relatively immature and may not reflect the effects of exercise on fully differentiated tissue Mϕ's. Lastly, there is very little information on the effects of exercise on DCs, which is unfortunate given the powerful influence of these cells early in immune responses.

ACQUIRED IMMUNITY AND EXERCISE

Background

Acquired immunity (also known as adaptive or specific immunity) is designed to combat infections by preventing colonisation of pathogens and destroying invading micro-organisms. With only a few exceptions, it is initiated by the presentation of antigen to T helper ($CD4^+$) lymphocytes within the peptide binding groove of major histocompatibility complex class II molecules on antigen presenting cells $CD4^+$ T cells form a key part of the cell-mediated immune response, since they orchestrate and direct the subsequent response. Helper T cell clones can be divided into two main phenotypes, type 1 (Th1) and type 2 (Th2) cells, according to the cytokines that they produce and release. Th1 cells play an important role in defence against intracellular pathogens, e.g. viruses, the release of the cytokines interferon-γ (IFN- γ) and interleukin-2 (IL-2) stimulating T cell activation and proliferation of clones of effector cells. Memory T cells are also generated, allowing a rapid secondary response upon subsequent exposure to the same antigen. Th2 cells release IL-4, IL-5, IL-6 and IL-13 and appear to be involved in protection against extracellular parasites and stimulation of humoral immunity (production of antibody and other soluble factors that circulate in the blood and other body fluids). Therefore, cytokines released from Th2 cells can activate B lymphocytes, leading to proliferation and differentiation into memory cells and plasma cells (although some antigens can activate B cells independently of $CD4^+$ cells). Plasma cells are capable of secreting vast amounts of immunoglobulin (Ig) or antibody specific to the antigen that initiated the response. The binding of Ig to its target antigen forms an antibody-antigen complex and both free Igs and anti-

body-complexes circulate in the body fluids. $CD8^+$ cells can also be classified into type 1 (Tc1) and type 2 (Tc2) cells according to their cytokine profiles, as described above, but the functional significance of these cells is at yet unclear. A further set of T-cells, the naturally-occurring regulatory T-cells (Tregs) express the phenotype CD4+CD25+ and can suppress the functional activity of lymphocytes by mechanisms that most likely involve secretion of cytokines including IL-10 and TGF-β1.

Consensus: acute exercise and acquired immune function

T and B cell number

Acute exercise elicits characteristic transient biphasic changes in the numbers of circulating lymphocytes. Typically, a lymphocytosis is observed during and immediately after exercise, with numbers of cells falling below pre-exercise levels during the early stages of recovery, before steadily returning to resting values. This pattern of mobilisation is observed for T cells (and T cell subpopulations) and to a lesser extent, B cells. Changes are proportional to exercise intensity and duration, although the effect of intensity is more marked (161, 258). Insufficient recovery between prolonged exercise bouts appears to exaggerate the biphasic response (251). Mobilization of T and B cell subsets in this way is largely influenced by the actions of adrenaline (epinephrine) both directly on the expression of cell adhesion molecules particularly those of the integrin and selectin families, and indirectly via sympathetically mediated influences on cardiac output and the

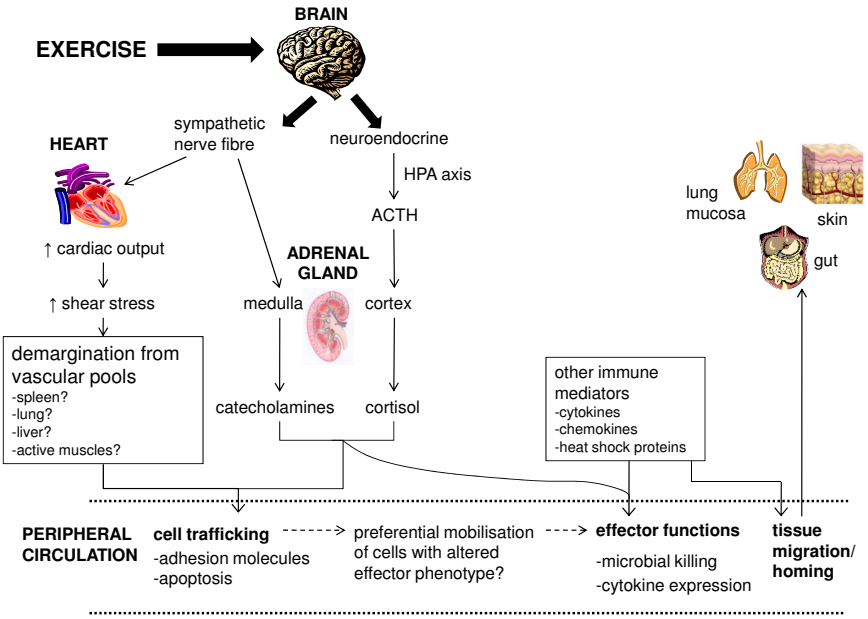

Figure 2. Potential mechanisms by which acute and chronic exercise affects acquired/adaptive immunity. HPA = hypothalamic pituitary adrenal; ACTH = adrenocorticotropic hormone.

subsequent increase in shear stress associated with enhanced blood flow (262) (Figure 2). Lymphocytes express a high density of β_2-adrenergic receptors and the density of these receptors increases with both exercise and exposure to catecholamines (262). The greatest expression of these receptors is found on the surface of NK cells, with fewer on $CD8^+$ and B cells and least of all on $CD4^+$ cells; the differing effects of intense exercise on the relative magnitude of mobilization of the lymphocyte subsets reflects this differential density of adrenergic receptor expression. The decrease in T cell number following exercise is largely due to a decrease in type 1 T cells, since intensive physical activity decreases the percentage of circulating Type 1 T cells but has little effect on the percentage of circulating Type 2 T cells (118, 287). It is unclear whether these changes are due to apoptosis or, as seems more likely, a redistribution of cells to other compartments. A decrease in the percentage of type 1 $CD4^+$ and $CD8^+$ T cells alone does not necessarily indicate that defence against intracellular pathogens such as viruses is suppressed; cytokine production is just one step of the multi-stage process that ultimately leads to lymphocyte proliferation or cytotoxicity. It is possible that any increase or decrease in cell number is countered by a diminished or enhanced response of other aspects of immune cell function. Moreover, the addition of a subpopulation of cells from the marginated pool into the circulation in response to exercise may influence lymphocyte function simply because the mobilized cells may have different functional abilities to those already in the circulation (Figure 2).

T and B cell function

T cells play a fundamental role in the orchestration and regulation of the cell-mediated immune response to pathogens. One important consequence of a defect in T cell function is an increased incidence of viral infections (63). With this in mind, it has been speculated that the apparent increased susceptibility of sportsmen and women to upper respiratory tract infections may be due to exercise-induced decreases in T cell function.

There is evidence that acute exercise stimulates T cell subset activation *in vivo* and in response to mitogen- and antigen-stimulation, as assessed by expression of cell surface markers of T cell activation, including CD69, CD25, the HLA-DR antigen, CD45RO and CD45RA (84, 86, 100). It is not clear whether such increases in activation are due to the recruitment of activated cells into the circulation, or are an effect on the state of activation of individual cells themselves. Most likely it is a combination of both. Numerous studies report decreased mitogen- and antigen-stimulated T cell proliferation following acute exercise, but interpretation of these findings may be confounded by the presence of NK cells and B cells within the cell cultures; alterations in relative numbers of T, B and NK cells in blood samples obtained before and after exercise may affect the proportion of cells that can respond to stimulation in a given volume of blood or number of peripheral blood mononuclear cells (102). Furthermore, *in vitro* stimulation with mitogen does not necessarily reflect the more subtle responses of cells following a specific antigen encounter within the body (20). Moreover, exercise may alter T cell function *in vitro* through an increase in the rate of apoptosis in cell culture rather than a decrease in T cell proliferation rate (101).

Upon stimulation, B cells proliferate and differentiate into memory cells and plasma cells, with plasma cells localised primarily in lymphoid or mucosal tissue and able to produce and secrete vast amounts of Ig (or antibody) specific to the antigen that initiated the response. The binding of Ig to its target antigen forms antibody-antigen complexes; Ig and antibody-antigen complexes circulate in the body fluids. The effect of exercise on humoral immune function has been assessed through measurements of serum and mucosal Ig concentration *in vivo* and serum Ig synthesis following *in vitro* mitogen-stimulation. Serum Ig concentration appears to remain either unchanged, or slightly increased, in response to either brief or prolonged exercise (184, 203, 229). Mitogen-stimulated IgM concentration appears to increase in response to exercise independently of changes in T or B cell number, although there are contrasting findings concerning IgA and IgG (258, 306).

Consensus: exercise training and acquired immune function

In the true resting state (i.e. more than 24 h after their last training session) circulating lymphocyte numbers and functions of athletes appear to be broadly similar to those of non-athletes (192). Longitudinal studies in which previously sedentary people undertake weeks or months of exercise training fail to show any marked changes in T and B cell functions, provided that blood samples are taken at least 24 h after their last exercise bout. In contrast, T and B cell functions appear to be sensitive to increases in training load in well-trained athletes undertaking a period of intensified training, with decreases in circulating numbers of Type 1 T cells, reduced T cell proliferative responses and falls in stimulated B cell Ig synthesis reported (7, 139, 308). This suggests that athletes engaging in longer periods of intensified training can exhibit decreases in T cell functionality. The cause of this depression in acquired immunity appears to be related to elevated circulating stress hormones, particularly cortisol, and alterations in the pro/anti-inflammatory cytokine balance in response to exercise (Figure 2). This appears to result in a temporary inhibition of Type 1 T cell cytokine production, with a relative dampening of the Type 1 (cell-mediated) response.

Conclusions

Acute intensive exercise elicits a depression of several aspects of acquired immune function. This depression is transient and cell numbers and functions usually return to pre-exercise values within 24 h. If recovery between exercise sessions is insufficient, as during prolonged periods of intensified training in elite athletes, this temporary decrease in cell function can become a chronic depression of acquired immunity. Although not clinically immune deficient, it is possible that the combined effects of small changes in several aspects of host defence may compromise resistance to minor illnesses, such as respiratory infections. The clinical significance of these alterations requires more detailed investigation.

MUCOSAL IMMUNITY AND EXERCISE

Background
Mucosal surfaces such as those in the gut, urogenital tract, oral cavity and respiratory system are protected by a network of organised structures known as the Common Mucosal Immune System (96). These structures include Peyer's patches and isolated lymphoid follicles in gut-associated, nasal-associated, and bronchial/tracheal-associated lymphoid tissues and salivary glands. The production of immunoglobulin A (IgA), specifically secretory IgA (SIgA), is the major effector function of the mucosal immune system, SIgA together with innate mucosal defences such as α-amylase, lactoferrin and lysozyme, provides the 'first line of defence' against pathogens present at mucosal surfaces. In addition, secretory IgM and locally produced IgG play a less significant role in protection of mucosal surfaces (96). The transepithelial transport of the polymeric Ig receptor (pIgR)-IgA complex into secretions such as saliva affords three potential ways in which IgA provides an effective 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 (138).

Consensus
A high incidence of infections is reported in individuals with selective deficiency of SIgA (105) or very low saliva flow rates (75). Moreover, high levels of saliva SIgA are associated with low incidence of URTI (252) and low levels of saliva SIgA in athletes (64, 95) or substantial transient falls in saliva SIgA (187) are associated with increased risk of URTI.

Levels of saliva SIgA vary widely between individuals. Although some early studies indicated that saliva SIgA concentrations are lower in endurance athletes compared with sedentary individuals (304), the majority of studies indicate that there are no differences between athletes compared with non-athletes except when athletes are engaged in heavy training (19, 96).

Falls in saliva SIgA concentration can occur during intensive periods of training (4, 32, 64, 93, 95, 97, 187, 303, 304) and some studies (32, 64, 93, 95, 187), though not all (4, 303, 320) have observed a negative relationship between saliva SIgA concentration and occurrence of URTI. Several of the above cited studies examined changes in saliva SIgA during intensive periods of military training (32, 303, 320). However, this often involves not only strenuous physical activity, but also dietary energy deficiency (see section on nutritional countermeasures in part two of the position statement), sleep deprivation (see section on sleep disruption in part two of the position statement) and psychological challenges (see section on the effects of stress on immune function in part two of the position statement). These multiple stressors are likely to induce a pattern of immunoendocrine responses that amplifies the exercise-induced alterations (207).

Increases in saliva SIgA have been observed after a period of regular moderate exercise training in previously sedentary individuals and may, at least in part, con-

tribute to the apparent reduced susceptibility to URTI associated with regular moderate exercise (3, 129).

The saliva SIgA response to acute exercise is variable and may be influenced by exercise mode, intensity and duration as well as the fitness of the subjects, unstimulated versus stimulated saliva collection methods, how saliva SIgA is expressed (e.g. absolute concentration, as a secretion rate or as a ratio to total protein or osmolality) and other factors that may be present such as reduced food intake, dehydration, sleep deprivation, altitude, and psychological stress (19). Levels of saliva SIgA are generally unchanged with resistance exercise sessions (130) and moderate aerobic exercise lasting less than 1 h (19).

The saliva SIgA response to exercise is generally not affected by environmental temperature (116, 137, 312), short periods (<24 h) of fasting (5) or food restriction (207), carbohydrate intake during exercise (18, 146, 199), up to 30 h of sleep deprivation (243), or by time of day (4, 57, 145).

Salivary α-amylase is another antimicrobial protein (317) and its secretion is stimulated by increased activity of the sympathetic nervous system (37), with the majority of this protein produced by the parotid gland (281). In accordance with this, several studies have found that exercise increases the α-amylase activity of saliva in a manner that is dependent on exercise intensity (6, 18, 145, 317).

Controversies

Secretion of saliva and its constituent proteins is regulated by the autonomic nervous system. The secretion of SIgA in rats 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 (33, 34). Since intensive exercise is associated with enhanced sympathetic nervous system activation, it seems surprising that some studies report a decrease in saliva SIgA concentration following a bout of high intensity exercise (>80%$\dot{V}O_2$max) that recovers to resting levels within 1 h of exercise completion (154, 164). Other studies have reported either no change (163, 243, 299) or increases (6, 23, 313) in saliva SIgA concentration after single or repeated bouts of high intensity exercise.

Saliva SIgA concentration (or secretion rate) in response to prolonged (>1.5 h) moderate intensity exercise (50-75%$\dot{V}O_2$max) is more consistently reported to decrease (153, 199, 213, 288, 304) or remain unchanged (23, 116, 163, 195, 255). Different methods of saliva collection and differences in hydration status of subjects may contribute to the discrepancies in the literature (19, 144, 207, 291).

A few small-scale studies have reported that female athletes have lower saliva SIgA concentration (95) and secretion rate (4, 5) compared with their male counterparts, but confirmation of this possible gender difference is required in a larger subject population.

There is little data available regarding changes in salivary lysozyme and lactoferrin concentrations with acute or chronic exercise, although intense and exhaustive

exercise of both short and long duration is associated with increases in salivary lysozyme (6, 316, 317) and lactoferrin secretion (316). These effects also appear to be dependent on exercise intensity, since no change was seen following ~20 min of cycling at 50%$\dot{V}O_2$max (6). Prolonged cycle ergometer exercise at 60%$\dot{V}O_2$max caused a significant increase in salivary α-defensin concentrations and secretion rates (53).

The mechanisms by which exercise influences salivary responses remain to be fully elucidated (Figure 3). The rate of secretion of saliva SIgA is dependent on the production of IgA by the plasma cells in the submucosa and/or the rate of IgA transcytosis across the epithelial cell which is determined by the availability of the pIgR (24). The time-course (minutes) of the alterations in saliva SIgA secretion that are observed in response to acute exercise suggest that this is the princi-

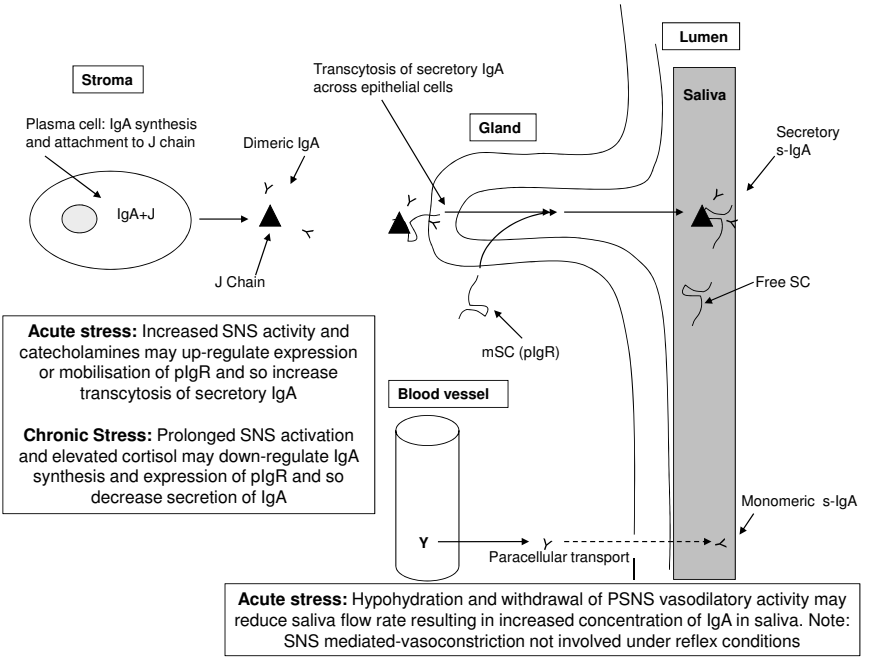

Figure 3. Effects of acute and chronic stress on receptor-mediated transport of locally produced dimeric IgA and paracellular transport of serum derived monomeric IgA into saliva. mSC = membrane secretory component; pIgR = polymeric Ig receptor; SNS = sympathetic nervous system; PSNS = parasympathetic nervous system.

pal mechanism by which acute intensive exercise influences saliva SIgA secretion. In anaesthetised rats, acute stimulation of β-adrenoreceptors above a certain threshold increases saliva SIgA secretion in a dose-independent manner via elevated transcytosis from the glandular pool (230) and this is associated with increased availability of the pIgR (34). Although such a mechanism has not yet

been demonstrated in humans, the finding that increases in saliva SIgA secretion rate are associated with elevations in plasma adrenaline following caffeine ingestion lends some support to this suggestion (21).

Although enhanced IgA transcytosis probably accounts for elevations in saliva SIgA secretion observed after exercise, it cannot account for the findings of either no change or decreases in saliva SIgA secretion rate with intense physical activity. The observation that increased mobilisation of the pIgR only occurred above a certain threshold frequency of stimulation (230) could account for the finding of little change in saliva SIgA levels at more moderate intensities of exercise. However, the finding of decreased concentrations of saliva SIgA in response to acute exercise is harder to explain. Nevertheless, a study in rats demonstrated that following a prolonged treadmill run to exhaustion, decreases in saliva SIgA concentration were associated with a decline in pIgR mRNA expression (127). Although highly speculative, this might imply that there is a second critical threshold (or duration) of stimulation, above which pIgR expression becomes downregulated.

It is unlikely that cortisol plays a major role in the regulation of saliva SIgA secretion in response to acute exercise, because changes in both saliva SIgA concentration and secretion rate have been observed in the absence of any alterations in plasma or salivary cortisol (6, 145, 146, 256, 299) and there appears to be no correlation between saliva SIgA and cortisol responses to exercise (164).

Modification of IgA synthesis could play a major role in the changes in saliva SIgA secretion observed in response to long term intensive training and chronic psychological stress (19, 24, 226). In addition, it may be that repeated mobilisation of the pIgR could deplete the available formed IgA pool, leading to decreases in saliva SIgA output. However, to date there is scant research in either animals or humans to support these speculations.

Conclusions

To date the majority of exercise studies have assessed saliva SIgA as a marker of mucosal immunity but more recently the importance of other antimicrobial proteins in saliva including α-amylase, lactoferrin and lysozyme has gained greater recognition. Acute bouts of moderate exercise have little impact on mucosal immunity, but very prolonged exercise and periods of intensified training can result in decreased saliva secretion of SIgA. Mechanisms underlying the alterations in markers of mucosal immunity with acute exercise are probably largely related to the activation of the sympathetic nervous system and its associated effects on salivary protein exocytosis and IgA transcytosis. Depressed secretion of SIgA into saliva during periods of intensified training and chronic stress are likely linked to altered activity of the hypothalamic-pituitary-adrenal axis, with inhibitory effects on IgA synthesis and/or transcytosis. There is reasonable evidence to indicate that reduced levels of saliva SIgA are associated with increased risk of URTI.

IMMUNOLOGICAL METHODS IN EXERCISE IMMUNOLOGY

Background

There are many examples in the literature and reviewed in this consensus paper that acute exercise and exercise training can alter host defence, leading to changes in disease susceptibility and severity. One important mechanism for such changes is alterations in *immune function*. Herein lies a primary challenge for exercise immunologists; how does one measure immune function in a meaningful way? The immune system is comprised of a large variety of cells, occurs in diverse tissues (i.e., lymph node, Peyer's patches, spleen and liver), and involves the orchestration of hundreds of soluble and cell membrane associated proteins. Successful host defence is the end product of these responses.

Consensus

Exercise immunology experiments test the impact of acute exercise and/or regular exercise training on a number of measures of the immune system. The types of immunological assessments most commonly reported, especially in the human exercise studies involve analyses of blood borne circulating immune proteins (e.g., interleukin (IL)-6, IL-1β, C-reactive protein, IL-8, tumour necrosis factor alpha (TNFα) chemokines), circulating blood leukocytes (e.g., CD4+ T cells, CD8+ T cells, Th1, Th2, Th17, Treg, B cells, neutrophils, monocytes), and salivary/plasma antibody or immunoglobulin (Ig) concentrations. Some studies document dynamic changes in the composition of blood leukocyte populations (e.g., decreases in peripheral blood CD4+ T cells and increases in neutrophils), and some studies isolate the peripheral blood leukocytes and put them in culture with various exogenous stimuli, such as mitogens, that stimulate large populations of immune cells to produce immune products. Using these types of measures, there are many reported examples of robust dynamic changes produced both with acute exercise and after exercise training. As discussed in other sections of this position statement, the nature of the reported changes measured depends on a number of variables that include the training status of the individual, the intensity of the exercise bout, the nutritional status of the individual, the timing of the blood/saliva sample collection and the nature of the specific immunological measure. Due to the reported dynamic changes in such blood borne and salivary measures, it is essential that multiple samples are taken, including pre-, during-, and post- exercise timepoints. Non-exercised, time-matched controls must also be sampled to control for circadian, seasonal, and environmental changes in these dynamic measures. The majority of studies in exercise immunology are sensitive to these aspects of experimental design, making these methodological features strengths of the field.

Another approach to assessing immune function extends beyond blood or salivary soluble proteins, circulating cells, total Ig or *in vitro* stimulated responses. It involves challenging experimental subjects with antigenic (immune stimulating, not disease capable) or pathogenic (immune stimulating, possible disease producing) stimuli and assessing relevant antigen-driven responses including antigen specific cell-mediated delayed type hypersensitivity (DTH) responses or antibody responses and in some instances, changes in disease susceptibility, duration, and

severity. This approach allows assessment of *in vivo* immune function and has several advantages over the previously described measures. Firstly, the generation an antigen specific Ig response reflects a functionally important end product of a multicellular *in vivo* immunological response. For example, the generation of a primary antibody response to a novel antigen like keyhole limpet haemocyanin (KLH) requires antigen presentation (likely by a B cell given KLH is a low dose soluble protein) to CD4+ T cells. KLH specific T cells then provide T cell help in the form of both co-stimulation and cytokines to KLH specific B cells to stimulate the production of anti-KLH IgM and promote isotype switching to anti-KLH IgG1 (driven by Th2 cytokines) and IgG2a (driven by Th1 cytokines). If an acute exercise bout or exercise training impacts *in vivo* immune function, then changes in the generation of KLH specific Ig will be detected. In addition, if there are selective changes in isotype switching, for example an impact on anti-KLH IgG1 and not on anti-KLH IgG2a, or *vice versa*, this suggests selective effects on Th1 and Th2 responses (70, 88, 159, 177). This approach has been successfully used in both humans (274, 275, 278) and animals (55, 69, 71, 82, 179, 311).

The results of the exercise immunology studies that measure *in vivo* anti-KLH Ig responses support the general conclusion that an acute bout of intense exercise suppresses anti-KLH Ig production (178), however, moderate exercise training can restore optimal antibody in the face of stressors (69, 72) and ageing (99, 277). Interestingly, the majority of studies using this measure rarely demonstrate an *increase* in the anti-KLH Ig response with exercise training in **young healthy adults.** This is likely due to the fact that young healthy sedentary and physically active organisms already possess excellent immune responses, and elevating that response further is not necessarily a good thing. Too much immunity is just as detrimental as too little (Figure 4). In other words, the positive effects of exercise training on immune function and host defence may be most readily revealed when *in vivo* immune function is sub-optimal consequent to ageing, stress, or other factors. In fact there are several papers that demonstrate that regular physical activity reduces incidence of illness only if people report high levels of stress (26, 74).

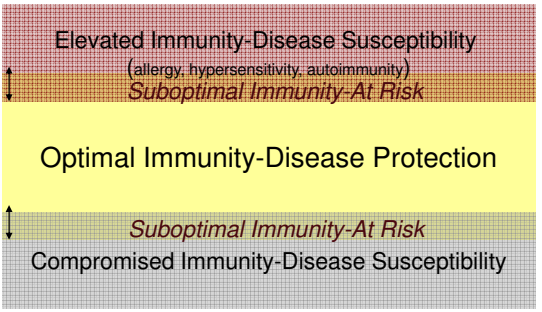

Figure 4. Exercise associated changes in immune function have greatest effects on host defence and disease susceptibility/severity, if the individual has suboptimal immune function due to ageing, stress or other factors.

A related approach that also measures *in vivo* immune function, and is reported in the exercise immunology literature is to inject **not a novel** antigen, such as KLH, but rather a mixture of antigens using influenza vaccine or tetanus vaccine that usually contain a subset of repeated antigens that have been "seen" by people before (30, 60, 61). The advantage of this approach, especially when studying humans, is that people are

willing to receive such injections because they produce useful immunity against influenza and/or tetanus. The disadvantage of this approach is that the subsequent antibody response is a mixture of primary, secondary and tertiary responses. This makes it difficult to accomplish the following: 1) measure group changes in isotypes (very little IgM is detectable in secondary and tertiary versus primary responses); 2) compare concentrations of antigen specific antibody (secondary and tertiary responses characteristically produce higher levels of IgG than primary responses); and 3) make inferences about cellular mechanisms for any detected changes (unique cellular and co-stimulatory signals are required for primary versus secondary and tertiary responses)(70). Thus the assessment of an antigen-specific immune response following vaccination yields important information about *in vivo* immune responses that are superior to measuring dynamic circulating protein or cell changes, but suffers some interpretive limitations not found after primary antigenic challenge.

An additional methodological and interpretation challenge when studying exercise-induced changes in immune responses is to determine if the measured changes in immunity are sufficient to alter host defence or disease susceptibility/severity. This is a complex challenge. It involves issues associated with immune safety net and redundancy (Figure 4) and immune response specificity relative to host disease defence. Because immune function is critical to host survival, the system has evolved a large safety net and redundancy such that it is difficult to determine how much immune function must be lost or gained to incur changes in host disease susceptibility. Studies on human immunodeficiency (HIV) patients offer insight into the issue. It is commonly reported that patients with HIV must lose at least ~50-60% of their total circulating CD4+ T cells before an increase in the incidence of opportunistic infection occurs (182). There are numerous examples of exercise altering circulating cell numbers and other measures of immunity, often by 15-25%. Whether changes of this magnitude are sufficient to alter disease susceptibility or severity likely depends on the state of the host. If, for example, immune function was optimal or functioning at 100% then ± 15-25% change may not impact host defence in a clinically significant way, because the safety net for immune function is great. If instead immune function was suboptimal due to ageing, stress or other factors placing host immunity in the "at risk zone", then a 15-25% change in immune function could have significant consequences for host defence (Figure 4). A second issue to consider when interpreting the functional significance of changes in immune measures for host defence is response specificity. That is, what specific types of pathogens or disease states could be impacted by changes in the aspects of immunity measured? For example, how would transient changes in circulating T cell numbers influence anti-viral host defence? This issue is especially challenging for human research. There are, however, several rodent disease models that establish clear links between changes in specific immune responses and corresponding changes in host defence and disease severity. Work by Shamgar Ben-Eliyahu is one example (12). Although he is not specifically testing the impact of exercise, he is exploring the impact that other stressors (i.e., surgery, drugs etc.) have on immune function and host defence. A strength of his model is that he both demonstrates stress-associated suppression in NK cell tumour killing *ex vivo* and stress-associ-

ated increases in tumour load *in vivo* (14). Furthermore he has verified that the tumour tested in these studies is primarily killed by NK cells and **not** CD8+ T cells (13). Thus using this type of approach one can measure immune function and verify relevance for host defence and disease susceptibility/severity.

A second approach used in immunology research involves challenging animals with pathogens that require specific and well-characterized immunological responses for survival. *Leishmania major*, for example, requires a Th1 dominant response for effective host defence (43). If one blocks the development of Th1 responses, the animal will die. This is a useful experimental model, because one can link changes in specifically Th1 responses (cytokines, clonal expansion, Th1 differentiation or activation, etc.) with corresponding changes in *Leishmania* disease susceptibility, severity and host survival. This type of model could be implemented in exercise immunology studies.

Controversies and future directions

Most studies in exercise immunology are conducted in humans and are usually limited to immune measures derived from the blood, such as soluble immune proteins, cell numbers, *in vitro* cellular responses to mitogen and total Ig concentrations. As previously discussed, it is difficult to determine how such changes could impact host defence, disease susceptibility or severity. Although persistent or chronic elevations in blood concentrations of inflammatory proteins may be reflective of changes in inflammatory processes, it is possible that dynamic, short-lived changes in blood borne immune factors offer little insight into how the *in vivo* immune function and/or host defence is altered. In addition, increases in concentrations of blood borne soluble proteins such as IL1β, IL8, and TNF-α that classically play a role during local tissue inflammation, likely are not related to tissue inflammation. There is no evidence that the acute increases in circulating concentrations of these proteins produced by stressors or exercise function to modulate any inflammatory process, especially in an otherwise healthy host. More likely, the acute elevations in IL-6 and IL1-β found after exercise may be more important for the *metabolic* rather than the *immunological*, responses to exercise.

Given the pleiotropic and context dependent nature of cytokines/chemokines, perhaps we should revise our thinking when trying to interpret acute and dynamic effects of exercise. Firstly, we need to consider any change in cytokine concentration within the context of the cytokine network (180). In other words, the contextual dependence of cytokines cannot be ignored. A nice immunological example of contextual dependence is the effect of transforming growth factor (TGF)-β on CD4+ T cell differentiation. Based on the 3-signal model of T cell activation and differentiation (45), cytokines play a pivotal role in CD4+ T cell differentiation after activation from Th0 (non-polarized) to Th1, Th2, Treg etc. TGF-β plus IL6, for example, drives the differentiation of the Th0 toward a Th17 cell. In contrast, TGF-β in the absence of IL-6 drives the differentiation of the Th0 toward a Treg cell. A second example of cytokine networks and context dependence can be found in the exercise immunology literature, where increases in circulating IL-6 in the presence of TNF-α is indicative of inflammation, whereas increases in cir-

culating IL-6 in the absence of TNF-α may be indicative of increased energy demand (217, 219)(Figure 6).

In conclusion, there are clear effects of both acute exercise and exercise training on measures of immune products and function. Exercise training effects on immune function and host defence are especially demonstrable when immune function is not optimal due to ageing, stress or other factors. Exercise immunology researchers are faced with challenges associated with both the immune measures and the interpretation of changes in such measures. *In vivo* antigen specific immune function can be measured by injecting subjects (both people and animals) with novel antigens and vaccination antigens; assessment of antigen specific immunoglobulin and T cell (by DTH tests) responses is a strong approach. The ability to predict if any change in antibody titre or T cell function is sufficient to alter host defence, specific disease susceptibility or disease severity however, remains debatable.

ANTI-INFLAMMATORY EFFECTS OF PHYSICAL ACTIVITY

Chronic inflammation is involved in the pathogenesis of insulin resistance, atherosclerosis, neurodegeneration, and tumour growth. Evidence suggests that the protective effect of exercise may, to some extent, be ascribed to the anti-inflam-

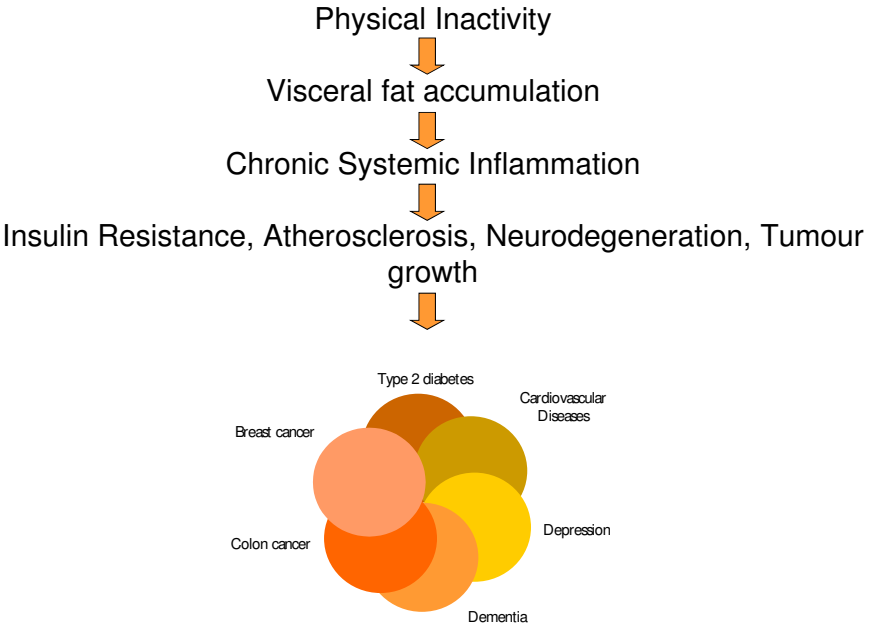

Figure 5. Hypothesis: Physical inactivity leads to accumulation of visceral fat and consequently to the activation of a network of inflammatory pathways, which promotes development of insulin resistance, atherosclerosis, neurodegeneration, and tumour growth, leading to the development of "the diseasome of physical inactivity".

matory effect of regular exercise, mediated via a reduction in visceral fat mass and/or by induction of an anti-inflammatory environment with each bout of exercise.

Background

It is well-established that physical inactivity increases the risk of type 2 diabetes (305), cardiovascular diseases (204), colon cancer (322), breast cancer (175), dementia (253) and depression (211). Physical inactivity leads to the accumulation of visceral fat and consequently the activation of a network of inflammatory pathways. Chronic inflammation promotes the development of insulin resistance, atherosclerosis, neurodegeneration, and tumour growth (104), and subsequently the development of a number of diseases associated with physical inactivity (218) (Figure 5).

The protective effect of exercise against chronic inflammation associated diseases may, to some extent, be ascribed to an anti-inflammatory effect of regular exercise. Several studies show that markers of inflammation are reduced following longer-term behavioural changes involving reduced energy intake and increased physical activity (reviewed in (225)). We suggest that the long-term anti-inflammatory effects of exercise may be mediated both via a reduction in visceral fat mass and the establishment of an anti-inflammatory environment with each bout of exercise.

Consensus

We have suggested that cytokines and other peptides that are produced, expressed, and released by muscle fibres and exert paracrine or endocrine effects should be classified as "myokines" (218). Such myokines may exert a direct effect on fat metabolism and thereby result in indirect anti-inflammatory effects. Moreover, myokines may exert direct anti-inflammatory effects or stimulate the production of anti-inflammatory components.

It is suggested that contracting skeletal muscles release myokines, which work in a hormone-like fashion, exerting specific endocrine effects on visceral fat and other ectopic fat deposits. Other myokines work locally within the muscle via paracrine mechanisms, exerting their effects on signalling pathways involved in fat oxidation.

The first identified and most studied myokine is the gp130 receptor cytokine, interleukin (IL)-6. A number of studies during the past decade have revealed that both type I and type II muscle fibres express the myokine IL-6 in response to muscle contractions. Subsequently IL-6 exerts its effects both locally within the muscle (e.g. through activation of 5' adenosine monophosphate activated protein kinase, AMPK) and, when released into the circulation, in a hormone-like fashion in a number of organs. Within skeletal muscle, IL-6 acts locally to signal through a gp130Rβ/IL-6Rα homodimer resulting in activation of AMPK and/or phosphatidylinositol-3-kinase (PI3K) to increase fat oxidation and glucose uptake (219). Although it has not been demonstrated that IL-6 has specific effects on visceral fat mass, it does appear to play an important role in lipid metabolism. IL-15 is expressed in human skeletal muscle and has been identified as an anabol-

ic factor in muscle growth. In addition to its anabolic effects on skeletal muscle *in vitro* and *in vivo*, IL-15 appears to play a role in lipid metabolism (191). Therefore, IL-15 has been suggested to be involved in muscle – fat cross talk. IL-15 mRNA levels are upregulated in human skeletal muscle following a bout of strength training (190), suggesting that regular training may lead to IL-15 accumulation within muscle. Interestingly, we demonstrated a decrease in visceral fat mass, but not subcutaneous fat mass, when IL-15 was overexpressed in murine muscle (189).

The cytokine response to exercise differs from that elicited by severe infections (Figure 6). Classical pro-inflammatory cytokines, tumour necrosis factor alpha (TNF-α) and IL-1β, in general do not increase with exercise, indicating that the cytokine cascade induced by exercise is markedly different from the cytokine cascade induced by infections, (reviewed in (219)).

To study whether acute exercise induces an acute anti-inflammatory response, a model of "low grade inflammation" was established in which a low dose of *E. coli* endotoxin was administered to healthy volunteers, randomised to either rest or exercise prior to endotoxin administration. In resting subjects, endotoxin induced a 2 to 3 fold increase in circulating levels of TNF-α. In contrast, when the subjects performed 3 h of ergometer cycling and received the endotoxin bolus at 2.5 h, the TNF-α response was totally blunted (284). This study provides some evidence that acute exercise may inhibit TNF-α production.

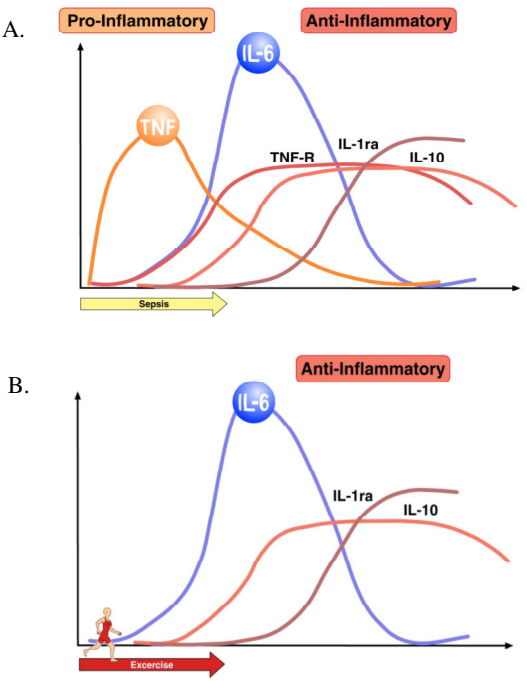

Figure 6. Comparison of sepsis-induced (A) versus exercise-induced (B) increases in circulating cytokines. During sepsis, there is a marked and rapid increase in circulating TNF-α, which is followed by an increase in IL-6. In contrast, during exercise the marked increase in IL-6 is not preceded by elevated TNF-α (220).

Typically, IL-6 is the first cytokine released into the circulation during exercise. The level of circulating IL-6 increases in an exponential fashion (up to 100 fold) in response to exercise and declines in the post-exercise period. The circulating levels of well-known anti-inflammatory cytokines such as, IL-1ra and IL-10, also increase after exercise. However, the

appearance of IL-6 in the circulation is by far the most marked and its appearance precedes that of the other cytokines. A number of studies have demonstrated that contracting skeletal muscle fibres per se produce and release IL-6. Of note, IL-6 infusion totally mimics the acute anti-inflammatory effects of a bout of exercise both with regard to induction of IL-1ra and IL-10 and with regard to suppression of endotoxin-stimulated increases in TNF-α levels. During acute exercise there is also a marked increase in adrenaline (epinephrine), cortisol, growth hormone, prolactin, and other factors that have immunomodulatory effects (104, 193). Taken together, it appears that each bout of exercise induces an anti-inflammatory environment.

Controversies
Patients with chronic inflammatory diseases such as type 2 diabetes are often prescribed exercise to improve quality of life; however, the use of exercise as a treatment for these diseases remains controversial. A systemic review has highlighted that acute and chronic exercise may elicit different responses in patients with chronic inflammatory disease when compared with healthy controls (227). For example, it has been reported that in patients with chronic obstructive pulmonary disease plasma TNF-α levels were abnormally increased compared with healthy controls following moderate-intensity exercise (236). Therefore, more needs to be understood about the nature of exercise that has anti-inflammatory effects in patients with chronic inflammatory diseases without increasing the underlying inflammatory pathology of the disease.

Future directions
To understand the mechanism of the protective, anti-inflammatory effect of exercise fully, we need to focus on the nature of exercise that is most effective at allieviating the effects of chronic inflammation in disease. The beneficial effects of endurance exercise are well known; however, the anti-inflammatory role of strength training exercises is poorly defined and remains an area for future investigation. In addition, the independent contribution of an exercise-induced reduction in visceral fat versus other exercise-induced anti-inflammatory mechanisms needs to be better understood.

EXERCISE AND CANCER

Background
Exercise can have a beneficial role in cancer prevention and therapy. Determining if regular physical activity reduces cancer risk through immunological mechanisms is of public health relevance and could lead to tailored and novel exercise prescriptions.

Consensus
The incidence of several types of cancer is reduced by regular physical activity. Comprehensive reviews by the International Agency for Research on Cancer (17) and the World Cancer Research Fund (330) identified an independent protective effect of physical activity on colon and postmenopausal breast cancer risk. Evi-

dence is also mounting that physical activity reduces risks of endometrial, lung, and pancreatic cancers.

Physical activity has a therapeutic effect in cancer patients by reducing cancer recurrence, enhancing health outcomes, and increasing survival. Women who exercised moderately prior to (81), and after a breast cancer diagnosis, had significant improvements in overall and disease-specific survival and quality of life compared to sedentary counterparts (280, 318). Protective effects of physical activity have also been observed for colorectal cancer patients (169).

There are fewer reports on exercise and neoplasia in animals with chemically-induced, transplantable, or spontaneous tumours (111). These studies describe exercise protecting against intestinal tumour incidence or number, although results with Apc^{min} mice, which develop intestinal tumours spontaneously, have been less consistent (10). A beneficial effect of exercise on mammary tumour incidence, multiplicity, growth rate and/or survival has also been reported (249).

Controversies

The biological mechanisms relating exercise and cancer are not well understood. Potential mediators include reductions in body mass and/or adiposity, decreases in reproductive hormone levels, altered growth factor milieu, enhanced antioxidant defence mechanisms, and changes in immune function, including reduced inflammation and enhanced anti-tumour immunity. Mechanisms studied in detail in humans have not been studied in animal models, and vice versa. Therefore, the relative contribution of these mechanisms in specific cancer types remains unknown. With respect to the hypothesis that exercise induces alterations in immune mediators, more is known about exercise-induced changes in inflammatory mediators than about changes in specific anti-tumour mechanisms; however, controversies exist for both hypotheses.

The association between chronic inflammation and cancer is well established (46). Human cross-sectional studies demonstrate an inverse relationship between regular physical activity and inflammatory biomarkers, including C-reactive protein (CRP), tumour necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) (123, 225). Reductions in CRP levels with exercise training have also been reported (123). Although exercise may reduce inflammatory biomarkers, clinical trials indicate variable outcomes, with an effect of exercise on CRP in some but not all studies (231). Less work has been done with IL-6 in humans, but again there are conflicting results (319). Finally, a recent randomized trial on markers of inflammation following a 12-month exercise intervention reported no change in participant colonic prostaglandin levels (1).

Animal studies demonstrate an anti-inflammatory role of exercise via multiple pathways. Exercise normalized the elevated levels of TNF-α in soluble TNF-receptor knock-out mice (126). Freewheel training lowered TNF-α expression and increased expression of antioxidant enzymes in mouse intestinal T lymphocytes (112, 113) and decreased prostaglandin E_2 level in the serum and polyps from Apc^{min} mice (121). Treadmill exercise decreased the number of

macrophages in polyps from Apc^{min} mice (8), and swimming exercise in rats reduced COX-2 positive cells in colonocytes (54). Taken together, several inflammatory pathways may be altered by exercise, but it is unclear to what extent and under what physiological conditions these changes occur.

Macrophages and natural killer (NK) cells have been studied in both tumour-bearing and healthy subjects following exercise. Collectively, animal model data show a positive effect of exercise on macrophage function, with enhanced clearance of lung metastases (324). Additionally, training results in greater *in vitro* NK cell cytotoxicity (221, 248), enhanced *in vivo* mechanisms of natural immunity and reduced pulmonary tumour metastases in mice (155, 221); however, these effects are small and modified by exercise intensity and timing. No change in NK cell cytotoxicity was observed following a 12-month walking intervention in healthy postmenopausal women (31). There are fewer studies on exercise and antigen-specific T cell functions. Moderately active older adults have higher influenza-specific *in vitro* peripheral blood mononuclear cell proliferation (132) and greater *in vivo* delayed type hypersensitivity (DTH) responses (277) compared with sedentary individuals. Moderate exercise also enhances antigen-specific T-cell mediated cytokine production and proliferation following vaccination (131, 250). Exercise improves antigen-specific T cell function, which may translate into better protection from infectious agents and greater immunosurveillance. Clinical and epidemiological studies show that the incidence of upper respiratory tract infections is lower in moderately active individuals compared with their sedentary counterparts (42). Although no T cell responses were measured, adequate adaptive immune responses play a critical role in the clearance of viral infections of the respiratory tract (323). The potential importance of adaptive immune responses in relation to exercise and virally-induced cancers cannot be overstated. For example, cervical cancer of which nearly all cases are due to human papillomavirus (HPV) is one of the leading causes of cancer death among women worldwide. However, no studies have examined the effect of exercise on the generation of HPV-specific T cells or the role of exercise in minimizing the immunosuppressive environment created by the presence of the tumour.

If an exercise-induced enhancement of anti-tumour mechanisms occurs, protection should be evident for lymphomas, due to the greater role of immune mediation. Only three studies have examined the relationship between physical activity and Hodgkin's and non-Hodgkin's lymphomas (HL, NHL, respectively). Participation in collegiate sports was associated with a trend to reduced risk of HL, although this did not reach statistical significance (212). Women who participated in strenuous physical activity at various time points in adult life had a lower risk of HL (125). Yet, a case-control study on NHL and occupational physical activity (measured as energy expenditure or sitting time) found no significant association (333).

The hypothesis that exercise-mediated changes in immunity contribute to a reduction in cancer risk is prevalent. For example, women participating in a US national sample believed the causes of breast and colon cancers were due to changes in one's immune system (60% of the sample) and lack of exercise (35-45% of the

sample) (314). Nevertheless this hypothesis is based on limited evidence (168) and many studies have significant methodological limitations (283).

Future directions

Physical activity is beneficial in preventing some cancers, and in decreasing recurrence, increasing survival, and improving quality of life for cancer patients. Multiple biological pathways may be involved, including a reduction in inflammation and an enhancement of anti-tumour immunity. Neither of the aforementioned mechanisms has been studied in adequate detail to gain a full understanding of their role in cancer prevention and therapy with respect to exercise. Inflammatory mediators have many physiological, metabolic and immunological roles and are produced in many tissues. Numerous cell types of the innate and adaptive immune system work in partnership to generate anti-tumour host responses. Additional studies will be needed to determine a) which inflammatory mediators and anti-tumour immune mechanisms are most sensitive to exercise, b) the dose, duration and frequency of exercise needed to achieve anti-inflammatory or anti-tumour effects, and c) the timing of sample collection with respect to the exercise bout to adequately capture appropriate levels of anti-inflammatory mediators and anti-tumour immune mechanisms.

Several technical limitations also need to be addressed. We suggest that the development of more sophisticated animal models is required. Although carcinogen-induced tumours have provided valuable insights, they are limited in that these carcinogens induce mutations at multiple genetic loci (117) and trigger both inflammation and immunosuppression (296). In contrast, spontaneous tumour models which 'mimic' human cancers are often limited to single mutations/pathways (i.e., ras, p53, APC, Wnt) and do not reflect complex multi-gene-environment (exercise) interactions. Additionally, many functional immunoassays require fresh cells and hours of assay preparation. Such immune readouts are difficult in epidemiological studies; while cryoprotectants allow freezing of immune cells for later analysis, viability comparisons to fresh cells are often not performed. Functional immunoassays could be conducted using lymphoid tissue harvested from animals, but relevant preclinical immunogenic tumour models would be required.

Concluding position

There is consensus that exercise training protects against some types of cancers. Training also enhances aspects of anti-tumour immunity and reduces inflammatory mediators. However, the data linking immunological and inflammatory mechanisms, physical activity, and cancer risk reduction remains tentative.

"OMICS" IN EXERCISE

Background and consensus

"Omics" is the circumspanning word for technologies which try to analyze an entire biologic field or large parts of it, using high throughput laboratory methods and correspondingly complex, high end- statistics. Accordingly, analysis by the "Omics approach" is often hypothesis free (non-targeted), and provides extremely

detailed and dense information, with a good chance of detecting unexpected responses or biological pathways. Exercise immunologists hope that "omics" will help them to gain a better understanding of mechanisms related to talent identification, exercise-induced disorders, modulation of the immune system by exercise, and prevention of diseases by exercise training. They also hope that "omics" can be used as a tool for optimizing individual training programmes.

Genomics, proteomics, and metabolomics, the classical three, appeared in this order according to the availability of high-throughput/ high-sensitivity methods. There is also diversification and refocusing into transcriptomics, spliceomics, lipoproteomics, pharmacoproteomics, interactomics, and, notably, exerciseomics. Targets of analysis are the genome itself (alleles, single nucleotide polymorphisms, methylations), gene expression (transcription), post-transcriptional regulation (microRNAs), abundance of proteins or metabolites and isomeric shifts and post-translational modifications.

Results on genome-wide screening for allotypes and single nucleotide polymorphisms associated with performance, fitness, or proneness to disease cannot be considered extensively here. Of special interest for exercise immunology are results on diabetes type-2, where at least 11 genes have been associated with the condition, including peroxisome proliferator-activated receptor delta, which is responsive to types/levels of lipids, and the fat mass and obesity associated (FTO) risk allele, which may not be responsible for reduced physical activity, but effects of which can be attenuated by exercise (see reviews (67, 241)).

To our knowledge, gene expression profiling was applied to exercise first in 2002, with work on rat muscle (39), hippocampus (174), and heart (56). A number of genes related to cell growth, signal transduction, calcium-flux, synaptic trafficking, or myosin light chains were found to be altered, some were new, some corresponding to previous findings, some were contradictory.

In humans, Mahoney et al. (158) defined a row of genes associated with muscle growth, remodeling and stress management following eccentric exercise (sterol and lipid metabolism, insulin and calcineurin pathways, c-myc and jun-D). Thalacker-Mercer et al. (297) exposed young and old adults to moderate exercise-induced muscle damage, and found vast differences in transcript activation, alluding to an undue inflammatory response in older subjects.

As first proposed by Fehrenbach et al. (66), many studies have now used peripheral blood gene expression fingerprinting/clustering for analysis of the effects of exercise. Types of exercise ranged from 30 min at 80% $\dot{V}O_2$max (44) to a half-marathon (334, 335) and heat injury in exercising military recruits (279). Time points chosen and platforms used for analysis also varied widely.

Special questions addressed by intervention or design were the effects of different workloads (29, 124), cell fractionation (183, 239), gender and age (205, 237, 238), as well as comparisons of immune suppressed patients versus healthy individuals (135), with every paper using different challenges and time kinetics.

Genes that were activated or suppressed showed remarkably little overlap between studies and between different times. Nevertheless, a number of pathways involved were identified albeit in different composition. They were related to stress genes and heat shock proteins (29, 44, 205, 279, 335), interferon (279), signal transduction (279, 334, 335), pro- and anti-inflammation (29, 44, 110, 135, 205, 237, 239, 279, 297, 334, 335), anti-oxidative system (334, 335), cell growth and wound healing (44, 237, 239, 297), apoptosis (29, 135, 237-239) and necrosis (297), neurotransmitters (124), immunity with natural killer cell activity (183, 237, 238), antigen processing and receptor signaling (239), asthma (107, 205, 237, 239) and arthritis (239).

MicroRNAs (miRNAs) are a large family of 21-22 nucleotide non-coding RNAs with presumed post-transcriptional regulatory activity. miRNA genes were formerly misperceived as junk-DNA, but are now recognized as important regulators of translation. Drummond et al. (58), Safdar et al. (254), and Radom-Aizik et al. (240) all found a number of miRNAs were increased following exercise and linked to adjustment of inflammation (240, 254). They also found dysregulation of exercise reactive miRNA (primary miRNA up, mature down) in aged subjects (58). An overview is given in Exercise Immunology Review, volume 16 (315).

Proteomics were applied to analyze the effects of exercise on rat heart (28), rat infarcted cerebellum (172), human muscle (108, 114), human plasma (332) and pig lipoproteins (244). Changes in expression of myofibrillar proteins, fatty acid metabolism, novel phosphorylation sites (28), and isoelectric species (114) were identified, shedding new light on the role of post-translational modification of proteins. Anti-inflammatory modification of serum complement through moderate exercise was shown (332), and a novel theory of lipoprotein structure including novel markers for vascular disease was proposed (244).

A rapidly increasing number of studies have analyzed the metabolome in relation to exercise - with circumstantial and limited relations to exercise immunology. Potential biomarkers of strenuous exercise and a strategy for analysis of complex data sets were proposed by Pohjanen et al. (228). Evaluating the effects of nutritive interventions in relation to exercise, subjects could be separated according to type of beverage, training, fitness stage and signs of insulin resistance (41, 142, 170, 331). Dampening of exercise-induced oxidative stress in human erythrocytes by administration of N-acetyl cysteine was shown (142). Finally, a role for endogenous medium chain acylcarnitines in lipid oxidation was proposed (143).

Consensus: "omics" in exercise

- There is a rapid activation and deactivation of genes in peripheral blood even after a short bout of exercise (44).
- Clustering is possible and cellular shifts due to exercise are reflected by the changes in the gene expression profile when using whole blood or peripheral blood mononuclear cells (66, 135, 183, 334).
- Gene expression is workload dependent; a secondary response by different genes is detected up to 24 h following exhaustive exercise only (29, 124, 208).
- Expression is influenced by age, and menstrual cycle (205, 237, 238, 297).

- Gene expression profile differences are in line with pathophysiological findings that could explain exercise-induced asthma (107).
- Immuno-suppressed (renal transplant recipient) patients can perform extensive, exhaustive exercise, showing very restricted gene expression changes (metabolism only), at the same time (135).
- Although gene expression profiling gives valuable information, the effects of miRNAs need to be evaluated (58, 315).
- Proteomics and metabolomics have started to shed new light on the role of isomeric forms and post-translational modification of proteins.
- Metabolomics can identify individuals at risk for diabetes, effects of nutrition and effects of exercise (38, 244, 331).

Controversies and future directions

The "omics" approach so far has had a major impact on knowledge about physiological and pathological processes associated with exercise. An enormous amount of new data has been generated, many pathways involved have been identified, new isoforms detected, and multiple candidates for biomarkers found.

Considering the vast amount of data and the high complexity of analysis applied, it is astonishing and potentially disappointing how little- if any- practical application of "omics" technology exists. There is no doubt that "omics" is generating huge steps in scientific advancement (for example detection of new proteins and metabolites, including isoforms related to lipid metabolism, diabetes type-2, and lipoprotein structure, as well as new biological pathways and gender/menstrual phase dependent gene expression). Practical applications will arise from this, but direct application of "omics" technologies for routine practical purposes (e.g., optimization of individual training/treatment programmes) will require one or more further quantum leaps of technology and yet further increased complexity of analysis. These advances need to be such that they re-simplify proceedings, and analysis will have to integrate knowledge from different levels.

In terms of genome screening for talent and for susceptibility to injury, advances may result from technological developments that will allow easier methods of purification or whole genome sequencing. These technological advances will facilitate access to instructive and sensitive personal data. It is unclear so far how the enormous danger of misuse will be handled. Determination of single factors like alpha actinin (ACTN3) variants – even if used commercially – is largely inefficient. Interaction of many different genes in optimal composition is probably required to make an athletic talent, and at this point, research is only starting. So far, it seems highly unlikely that genomics alone will have the predictive power to screen for gifted athletes (321).

At the level of gene expression, an enormous amount of knowledge about new pathways and marker molecules involved in adaption to exercise has been generated – but as yet there is no assay to answer practical questions (concerning type, intensity and duration of activity for adaptation to specific exercise) during training. Although the technology of gene expression profiling is quite advanced and can be handled in many places, practical application of these technologies is not

thinkable without rigorous standardization procedures and further technological advances (e.g. isothermic amplification). The flow of up- and down-regulation of genes in relation to exercise is so dependent on type, intensity, and duration of exercise and nutritional and conditional factors including gender, that it is highly doubtful if any experiment can ever be repeated by a different lab with identical results – even when using the same platform. So, hotspots and time lines have to be identified in order to make reliable predictions from such data, including integration of, and validation by regulatory mechanisms (miRNA) and post-translational modification, thus requiring proteomics and metabolomics.

The latter two technologies, as powerful as they already seem to be, are only just now starting to explore the potential they really have. At present, exceptionally well-equipped laboratories and highly specialized and experienced experts must meet to enable meaningful proteomics and metabolomics studies. But as the power and potential of this approach emerges, advancements of technologies can be expected in the very near future. They will be combined with genomic and gene expression data and resulting networks will then open new levels of meta-analysis for interpretation. First steps are underway (108), although up to now, a handy little tool for talent search or for individually optimized forms of training, using "omics" type analysis, is not available.

Finally, the "omics" approach on all three classical levels will probably be helpful in identifying misuse of substances or genetic interventions for doping purposes, even though direct or specific detection procedures are often preferred in the fight against doping (11). Work paving the way for "dopeomics" is underway (83, 337).

REFERENCES

1. Abrahamson PE, King IB, Ulrich CM, Rudolph RE, Irwin ML, Yasui Y, Surawicz C, Lampe JW, Lampe PD, Morgan A, Sorensen BE, Ayub K, Potter JD and McTiernan A. No effect of exercise on colon mucosal prostaglandin concentrations: a 12-month randomized controlled trial. Cancer Epidemiol Biomarkers Prev 16: 2351-2356, 2007.
2. Ahlborg B and Ahlborg G. Exercise leukocytosis with and without beta-adrenergic blockade. Acta Med Scand 187: 241-246, 1970.
3. Akimoto T, Kumai Y, Akama T, Hayashi E, Murakami H, Soma R, Kuno S and Kono I. Effects of 12 months of exercise training on salivary secretory IgA levels in elderly subjects. Br J Sports Med 37: 76-79, 2003.
4. Allgrove JE. Factors influencing the mucosal immune responses to exercise. PhD thesis, Loughborough University, 2007.
5. Allgrove JE, Geneen L, Latif S and Gleeson M. Influence of a fed or fasted state on the s-IgA response to prolonged cycling in active men and women. Int J Sport Nutr Exerc Metab 19: 209-221, 2009.
6. Allgrove JE, Gomes E, Hough J and Gleeson M. Effects of exercise intensity on salivary antimicrobial proteins and markers of stress in active men. J Sports Sci 26: 653-661, 2008.

7. Baj Z, Kantorski J, Majewska E, Zeman K, Pokoca L, Fornalczyk E, Tchorzewski H, Sulowska Z and Lewicki R. Immunological status of competitive cyclists before and after the training season. Int J Sports Med 15: 319-324, 1994.
8. Baltgalvis KA, Berger FG, Pena MM, Davis JM and Carson JA. Effect of exercise on biological pathways in ApcMin/+ mouse intestinal polyps. J Appl Physiol 104: 1137-1143, 2008.
9. Baslund B, Lyngberg K, Andersen V, Halkjaer KJ, Hansen M, Klokker M and Pedersen BK. Effect of 8 wk of bicycle training on the immune system of patients with rheumatoid arthritis. J Appl Physiol 75: 1691-1695, 1993.
10. Basterfield L, Reul JM and Mathers JC. Impact of physical activity on intestinal cancer development in mice. J Nutr 135: 3002S-3008S, 2005.
11. Beiter T, Zimmermann M, Fragasso A, Armeanu S, Lauer UM, Bitzer M, Su H, Young WL, Niess AM and Simon P. Establishing a novel single-copy primer-internal intron-spanning PCR (spiPCR) procedure for the direct detection of gene doping. Exerc Immunol Rev 14: 73-85, 2008.
12. Ben Eliyahu S, Page GG and Schleifer SJ. Stress, NK cells, and cancer: Still a promissory note. Brain Behav Immun 21: 881-887, 2007.
13. Ben Eliyahu S, Page GG, Yirmiya R and Shakhar G. Evidence that stress and surgical interventions promote tumor development by suppressing natural killer cell activity. Int J Cancer 80: 880-888, 1999.
14. Ben Eliyahu S, Yirmiya R, Liebeskind JC, Taylor AN and Gale RP. Stress increases metastatic spread of a mammary tumor in rats: evidence for mediation by the immune system. Brain Behav Immun 5: 193-205, 1991.
15. Benschop RJ, Oostveen FG, Heijnen CJ and Ballieux RE. Beta 2-adrenergic stimulation causes detachment of natural killer cells from cultured endothelium. Eur J Immunol 23: 3242-3247, 1993.
16. Bermon S. Airway inflammation and upper respiratory tract infection in athletes: is there a link? Exerc Immunol Rev 13: 6-14, 2007.
17. Bianchini F, Kaaks R and Vainio H. Weight control and physical activity in cancer prevention. Obes Rev 3: 5-8, 2002.
18. Bishop NC, Blannin AK, Armstrong E, Rickman M and Gleeson M. Carbohydrate and fluid intake affect the saliva flow rate and IgA response to cycling. Med Sci Sports Exerc 32: 2046-2051, 2000.
19. Bishop NC and Gleeson M. Acute and chronic effects of exercise on markers of mucosal immunity. Front Biosci 14: 4444-4456, 2009.
20. Bishop NC, Walker GJ, Bowley LA, Evans KF, Molyneux K, Wallace FA and Smith AC. Lymphocyte responses to influenza and tetanus toxoid in vitro following intensive exercise and carbohydrate ingestion on consecutive days. J Appl Physiol 99: 1327-1335, 2005.
21. Bishop NC, Walker GJ, Scanlon GA, Richards S and Rogers E. Salivary IgA responses to prolonged intensive exercise following caffeine ingestion. Med Sci Sports Exerc 38: 513-519, 2006.
22. Bjermer L and Anderson SD. Bronchial hyperresponsiveness in athletes: mechanisms for development. Eur Respir Mon 33: 19-34, 2005.
23. Blannin AK, Robson PJ, Walsh NP, Clark AM, Glennon L and Gleeson M. The effect of exercising to exhaustion at different intensities on saliva immunoglobulin A, protein and electrolyte secretion. Int J Sports Med 19: 547-552, 1998.

24. Bosch JA, Ring C, de Geus EJ, Veerman EC and Amerongen AV. Stress and secretory immunity. Int Rev Neurobiol 52: 213-253, 2002.
25. Brenner IK, Shek PN and Shephard RJ. Infection in athletes. Sports Med 17: 86-107, 1994.
26. Brown JD and Siegel JM. Exercise as a buffer of life stress: a prospective study of adolescent health. Health Psychol 7: 341-353, 1988.
27. Bruunsgaard H, Galbo H, Halkjaer-Kristensen J, Johansen TL, MacLean DA and Pedersen BK. Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. J Physiol 499 (Pt 3): 833-841, 1997.
28. Burniston JG. Adaptation of the rat cardiac proteome in response to intensity-controlled endurance exercise. Proteomics 9: 106-115, 2009.
29. Buettner P, Mosig S, Lechtermann A, Funke H and Mooren FC. Exercise affects the gene expression profiles of human white blood cells. J Appl Physiol 102: 26-36, 2007.
30. Campbell JP, Edwards KM, Ring C, Drayson MT, Bosch JA, Inskip A, Long JE, Pulsford D and Burns VE. The effects of vaccine timing on the efficacy of an acute eccentric exercise intervention on the immune response to an influenza vaccine in young adults. Brain Behav Immun 24: 236-242, 2010.
31. Campbell PT, Wener MH, Sorensen B, Wood B, Chen-Levy Z, Potter JD, McTiernan A and Ulrich CM. Effect of exercise on in vitro immune function: a 12-month randomized, controlled trial among postmenopausal women. J Appl Physiol 104: 1648-1655, 2008.
32. Carins J and Booth C. Salivary immunoglobulin-A as a marker of stress during strenuous physical training. Aviat Space Environ Med 73: 1203-1207, 2002.
33. Carpenter GH, Proctor GB, Anderson LC, Zhang XS and Garrett JR. Immunoglobulin A secretion into saliva during dual sympathetic and parasympathetic nerve stimulation of rat submandibular glands. Exp Physiol 85: 281-286, 2000.
34. Carpenter GH, Proctor GB, Ebersole LE and Garrett JR. Secretion of IgA by rat parotid and submandibular cells in response to autonomimetic stimulation in vitro. Int Immunopharmacol 4: 1005-1014, 2004.
35. Ceddia MA, Voss EW, Jr. and Woods JA. Intracellular mechanisms responsible for exercise-induced suppression of macrophage antigen presentation. J Appl Physiol 88: 804-810, 2000.
36. Ceddia MA and Woods JA. Exercise suppresses macrophage antigen presentation. J Appl Physiol 87: 2253-2258, 1999.
37. Chatterton RT, Jr., Vogelsong KM, Lu YC, Ellman AB and Hudgens GA. Salivary alpha-amylase as a measure of endogenous adrenergic activity. Clin Physiol 16: 433-448, 1996.
38. Chen J, Zhao X, Fritsche J, Yin P, Schmitt-Kopplin P, Wang W, Lu X, Haring HU, Schleicher ED, Lehmann R and Xu G. Practical approach for the identification and isomer elucidation of biomarkers detected in a metabonomic study for the discovery of individuals at risk for diabetes by integrating the chromatographic and mass spectrometric information. Anal Chem 80: 1280-1289, 2008.
39. Chen YW, Nader GA, Baar KR, Fedele MJ, Hoffman EP and Esser KA. Response of rat muscle to acute resistance exercise defined by transcriptional and translational profiling. J Physiol 545: 27-41, 2002.

40. Chiang LM, Chen YJ, Chiang J, Lai LY, Chen YY and Liao HF. Modulation of dendritic cells by endurance training. Int J Sports Med 28: 798-803, 2007.
41. Chorell E, Moritz T, Branth S, Antti H and Svensson MB. Predictive metabolomics evaluation of nutrition-modulated metabolic stress responses in human blood serum during the early recovery phase of strenuous physical exercise. J Proteome Res 8: 2966-2977, 2009.
42. Chubak J, McTiernan A, Sorensen B, Wener MH, Yasui Y, Velasquez M, Wood B, Rajan KB, Wetmore CM, Potter JD and Ulrich CM. Moderate-intensity exercise reduces the incidence of colds among postmenopausal women. Am J Med 119: 937-942, 2006.
43. Coffman RL, Chatelain R, Leal LM and Varkila K. Leishmania major infection in mice: a model system for the study of CD4+ T-cell subset differentiation. Res Immunol 142: 36-40, 1991.
44. Connolly PH, Caiozzo VJ, Zaldivar F, Nemet D, Larson J, Hung SP, Heck JD, Hatfield GW and Cooper DM. Effects of exercise on gene expression in human peripheral blood mononuclear cells. J Appl Physiol 97: 1461-1469, 2004.
45. Corthay A. A three-cell model for activation of naive T helper cells. Scand J Immunol 64: 93-96, 2006.
46. Coussens LM and Werb Z. Inflammation and cancer. Nature 420: 860-867, 2002.
47. Cox AJ, Gleeson M, Pyne DB, Callister R, Fricker PA and Scott RJ. Cytokine gene polymorphisms and risk for upper respiratory symptoms in highly-trained athletes. Exerc Immunol Rev 16: 8-21, 2010.
48. Cox AJ, Gleeson M, Pyne DB, Callister R, Hopkins WG and Fricker PA. Clinical and laboratory evaluation of upper respiratory symptoms in elite athletes. Clin J Sport Med 18: 438-445, 2008.
49. Cox AJ, Gleeson M, Pyne DB, Saunders PU, Callister R and Fricker PA. Respiratory symptoms and inflammatory responses to Difflam throat-spray intervention in half-marathon runners: a randomised controlled trial. Br J Sports Med 44: 127-133, 2010.
50. Cox AJ, Gleeson M, Pyne DB, Saunders PU, Clancy RL and Fricker PA. Valtrex therapy for Epstein-Barr virus reactivation and upper respiratory symptoms in elite runners. Med Sci Sports Exerc 36: 1104-1110, 2004.
51. Cox AJ, Pyne DB, Saunders PU, Callister R and Gleeson M. Cytokine responses to treadmill running in healthy and illness-prone athletes. Med Sci Sports Exerc 39: 1918-1926, 2007.
52. Davis JM, Kohut ML, Jackson DA, Colbert LH, Mayer EP and Ghaffar A. Exercise effects on lung tumor metastases and in vitro alveolar macrophage antitumor cytotoxicity. Am J Physiol 274: R1454-R1459, 1998.
53. Davison G, Allgrove J and Gleeson M. Salivary antimicrobial peptides (LL-37 and alpha-defensins HNP1-3), antimicrobial and IgA responses to prolonged exercise. Eur J Appl Physiol 106: 277-284, 2009.
54. Demarzo MM, Martins LV, Fernandes CR, Herrero FA, Perez SE, Turatti A and Garcia SB. Exercise reduces inflammation and cell proliferation in rat colon carcinogenesis. Med Sci Sports Exerc 40: 618-621, 2008.
55. Dhabhar FS and Viswanathan K. Short-term stress experienced at time of immunization induces a long-lasting increase in immunologic memory. Am J Physiol Regul Integr Comp Physiol 289: R738-R744, 2005.
56. Diffee GM, Seversen EA, Stein TD and Johnson JA. Microarray expression analysis of effects of exercise training: increase in atrial MLC-1 in rat ventricles. Am J Physiol Heart Circ Physiol 284: H830-H837, 2003.

57. Dimitriou L, Sharp NC and Doherty M. Circadian effects on the acute responses of salivary cortisol and IgA in well trained swimmers. Br J Sports Med 36: 260-264, 2002.
58. Drummond MJ, McCarthy JJ, Fry CS, Esser KA and Rasmussen BB. Aging differentially affects human skeletal muscle microRNA expression at rest and after an anabolic stimulus of resistance exercise and essential amino acids. Am J Physiol Endocrinol Metab 295: E1333-E1340, 2008.
59. Edwards AJ, Bacon TH, Elms CA, Verardi R, Felder M and Knight SC. Changes in the populations of lymphoid cells in human peripheral blood following physical exercise. Clin Exp Immunol 58: 420-427, 1984.
60. Edwards KM, Burns VE, Allen LM, McPhee JS, Bosch JA, Carroll D, Drayson M and Ring C. Eccentric exercise as an adjuvant to influenza vaccination in humans. Brain Behav Immun 21: 209-217, 2007.
61. Edwards KM, Campbell JP, Ring C, Drayson MT, Bosch JA, Downes C, Long JE, Lumb JA, Merry A, Paine NJ and Burns VE. Exercise intensity does not influence the efficacy of eccentric exercise as a behavioural adjuvant to vaccination. Brain Behav Immun 24: 623-630, 2010.
62. Engebretsen L, Steffen K, Alonso JM, Aubry M, Dvorak J, Junge A, Meeuwisse W, Mountjoy M, Renstrom P and Wilkinson M. Sports injuries and illnesses during the Winter Olympic Games 2010. Br J Sports Med 44: 772-780, 2010.
63. Fabbri M, Smart C and Pardi R. T lymphocytes. Int J Biochem Cell Biol 35: 1004-1008, 2003.
64. Fahlman MM and Engels HJ. Mucosal IgA and URTI in American college football players: a year longitudinal study. Med Sci Sports Exerc 37: 374-380, 2005.
65. Fairey AS, Courneya KS, Field CJ, Bell GJ, Jones LW and Mackey JR. Randomized controlled trial of exercise and blood immune function in postmenopausal breast cancer survivors. J Appl Physiol 98: 1534-1540, 2005.
66. Fehrenbach E, Zieker D, Niess AM, Moeller E, Russwurm S and Northoff H. Microarray technology--the future analyses tool in exercise physiology? Exerc Immunol Rev 9: 58-69, 2003.
67. Ferguson LR. Dissecting the nutrigenomics, diabetes, and gastrointestinal disease interface: from risk assessment to health intervention. OMICS 12: 237-244, 2008.
68. Fiatarone MA, Morley JE, Bloom ET, Benton D, Solomon GF and Makinodan T. The effect of exercise on natural killer cell activity in young and old subjects. J Gerontol 44: M37-M45, 1989.
69. Fleshner M. Exercise and neuroendocrine regulation of antibody production: protective effect of physical activity on stress-induced suppression of the specific antibody response. Int J Sports Med 21 Suppl 1: S14-S19, 2000.
70. Fleshner M. Translational research using in vivo measures of primary antibody responses. Brain Behav Immun 19: 309-310, 2005.
71. Fleshner M, Deak T, Nguyen KT, Watkins LR and Maier SF. Endogenous glucocorticoids play a positive regulatory role in the anti-keyhole limpet hemocyanin in vivo antibody response. J Immunol 166: 3813-3819, 2001.
72. Fleshner M, Nguyen KT, Mazzeo RS and Roth DA. Voluntary exercise potentiates, whereas forced exercise suppresses anti-KLH responses. Soc Neurosci Abstr 23: 1997.
73. Flynn MG, McFarlin BK, Phillips MD, Stewart LK and Timmerman KL. Toll-like receptor 4 and CD14 mRNA expression are lower in resistive exercise-trained elderly women. J Appl Physiol 95: 1833-1842, 2003.

74. Fondell E, Lagerros YT, Sundberg CJ, Lekander M, Balter O, Rothman KJ and Balter K. Physical activity, Stress, and Self-Reported Upper Respiratory Tract Infection. Med Sci Sports Exerc 2010. Doi: 10.1249/MSS.0b013e3181edf108.
75. Fox PC, van der Ven PF, Sonies BC, Weiffenbach JM and Baum BJ. Xerostomia: evaluation of a symptom with increasing significance. J Am Dent Assoc 110: 519-525, 1985.
76. Francis JL, Gleeson M, Pyne DB, Callister R and Clancy RL. Variation of salivary immunoglobulins in exercising and sedentary populations. Med Sci Sports Exerc 37: 571-578, 2005.
77. Fricker PA. Infectious problems in athletes: an overview. In: Medical Problems in Athletes, edited by Fields KB and Fricker PA. Malden MA: Blackwell Sciences, 1997, p. 3-5.
78. Fricker PA, Gleeson M, Flanagan A, Pyne DB, McDonald WA and Clancy RL. A clinical snapshot: Do elite swimmers experience more upper respiratory illness than nonathletes? Clin Exerc Physiol 2: 155-158, 2000.
79. Fricker PA, McDonald WA, Gleeson M and Clancy RL. Exercise-associated hypogammaglobulinemia. Clin J Sport Med 9: 46-48, 1999.
80. Fricker PA and Pyne DB. Why do athletes seem prone to infection? Med Today 6: 66, 2005.
81. Friedenreich CM, Gregory J, Kopciuk KA, Mackey JR and Courneya KS. Prospective cohort study of lifetime physical activity and breast cancer survival. Int J Cancer 124: 1954-1962, 2009.
82. Friedman EM, Becker KA, Overstreet DH and Lawrence DA. Reduced primary antibody responses in a genetic animal model of depression. Psychosom Med 64: 267-273, 2002.
83. Friedmann T, Rabin O and Frankel MS. Ethics. Gene doping and sport. Science 327: 647-648, 2010.
84. Fry RW, Morton AR, Crawford GP and Keast D. Cell numbers and in vitro responses of leucocytes and lymphocyte subpopulations following maximal exercise and interval training sessions of different intensities. Eur J Appl Physiol Occup Physiol 64: 218-227, 1992.
85. Gabriel H and Kindermann W. Flow cytometry. Principles and applications in exercise immunology. Sports Med 20: 302-320, 1995.
86. Gabriel H, Schmitt B, Urhausen A and Kindermann W. Increased CD45RA+CD45RO+ cells indicate activated T cells after endurance exercise. Med Sci Sports Exerc 25: 1352-1357, 1993.
87. Gannon G, Shek PN and Shephard RJ. Natural killer cells: modulation by intensity and duration of exercise. Exerc Immunol Rev 1: 26-48, 1995.
88. Gazda LS, Smith T, Watkins LR, Maier SF and Fleshner M. Stressor exposure produces long-term reductions in antigen-specific T and B cell responses. Stress 6: 259-267, 2003.
89. Gleeson M. Mucosal immune responses and risk of respiratory illness in elite athletes. Exerc Immunol Rev 6: 5-42, 2000.
90. Gleeson M and Bishop NC. The T cell and NK cell immune response to exercise. Ann Transplant 10: 43-48, 2005.
91. Gleeson M, Ginn E and Francis JL. Salivary immunoglobulin monitoring in an elite kayaker. Clin J Sport Med 10: 206-208, 2000.

92. Gleeson M, Hall ST, McDonald WA, Flanagan AJ and Clancy RL. Salivary IgA subclasses and infection risk in elite swimmers. Immunol Cell Biol 77: 351-355, 1999.
93. Gleeson M, McDonald WA, Cripps AW, Pyne DB, Clancy RL and Fricker PA. The effect on immunity of long-term intensive training in elite swimmers. Clin Exp Immunol 102: 210-216, 1995.
94. Gleeson M, McDonald WA, Cripps AW, Pyne DB, Clancy RL, Fricker PA and Wlodarczyk JH. Exercise, stress and mucosal immunity in elite swimmers. In: Advances in Mucosal Immunity, edited by Mestecky J. New York: Plenum Press, 1995, p. 571-574.
95. Gleeson M, McDonald WA, Pyne DB, Cripps AW, Francis JL, Fricker PA and Clancy RL. Salivary IgA levels and infection risk in elite swimmers. Med Sci Sports Exerc 31: 67-73, 1999.
96. Gleeson M and Pyne DB. Special feature for the Olympics: effects of exercise on the immune system: exercise effects on mucosal immunity. Immunol Cell Biol 78: 536-544, 2000.
97. Gleeson M, Pyne DB, Austin JP, Lynn FJ, Clancy RL, McDonald WA and Fricker PA. Epstein-Barr virus reactivation and upper-respiratory illness in elite swimmers. Med Sci Sports Exerc 34: 411-417, 2002.
98. Gleeson M, Pyne DB and Callister R. The missing links in exercise effects on mucosal immunity. Exerc Immunol Rev 10: 107-128, 2004.
99. Grant RW, Mariani RA, Vieira VJ, Fleshner M, Smith TP, Keylock KT, Lowder TW, McAuley E, Hu L, Chapman-Novakofski K and Woods JA. Cardiovascular exercise intervention improves the primary antibody response to keyhole limpet hemocyanin (KLH) in previously sedentary older adults. Brain Behav Immun 22: 923-932, 2008.
100. Gray AB, Telford RD, Collins M and Weidemann MJ. The response of leukocyte subsets and plasma hormones to interval exercise. Med Sci Sports Exerc 25: 1252-1258, 1993.
101. Green KJ and Rowbottom DG. Exercise-induced changes to in vitro T-lymphocyte mitogen responses using CFSE. J Appl Physiol 95: 57-63, 2003.
102. Green KJ, Rowbottom DG and Mackinnon LT. Exercise and T-lymphocyte function: a comparison of proliferation in PBMC and NK cell-depleted PBMC culture. J Appl Physiol 92: 2390-2395, 2002.
103. Hack V, Strobel G, Weiss M and Weicker H. PMN cell counts and phagocytic activity of highly trained athletes depend on training period. J Appl Physiol 77: 1731-1735, 1994.
104. Handschin C and Spiegelman BM. The role of exercise and PGC1alpha in inflammation and chronic disease. Nature 454: 463-469, 2008.
105. Hanson LA, Bjorkander J and Oxelius VA. Selective IgA Deficiency. In: Primary and secondary immunodeficiency disorders, edited by Chandra RK. Edinburgh: Churchill Livingstone, 1983, p. 62-64.
106. Helenius I, Lumme A and Haahtela T. Asthma, airway inflammation and treatment in elite athletes. Sports Med 35: 565-574, 2005.
107. Hilberg T, Deigner HP, Moeller E, Claus RA, Ruryk A, Glaser D, Landre J, Brunkhorst FM, Reinhart K, Gabriel HH and Russwurm S. Transcription in response to physical stress--clues to the molecular mechanisms of exercise-induced asthma. FASEB J 19: 1492-1494, 2005.
108. Hittel DS, Hathout Y and Hoffman EP. Proteomics and systems biology in exercise and sport sciences research. Exerc Sport Sci Rev 35: 5-11, 2007.

109. Ho CS, Lopez JA, Vuckovic S, Pyke CM, Hockey RL and Hart DN. Surgical and physical stress increases circulating blood dendritic cell counts independently of monocyte counts. Blood 98: 140-145, 2001.
110. Hoene M and Weigert C. The stress response of the liver to physical exercise. Exerc Immunol Rev 16: 163-183, 2010.
111. Hoffman-Goetz L. Physical activity and cancer prevention: animal-tumor models. Med Sci Sports Exerc 35: 1828-1833, 2003.
112. Hoffman-Goetz L, Pervaiz N and Guan J. Voluntary exercise training in mice increases the expression of antioxidant enzymes and decreases the expression of TNF-alpha in intestinal lymphocytes. Brain Behav Immun 23: 498-506, 2009.
113. Hoffman-Goetz L, Pervaiz N, Packer N and Guan J. Freewheel training decreases pro- and increases anti-inflammatory cytokine expression in mouse intestinal lymphocytes. Brain Behav Immun 24: 1105-1115, 2010.
114. Holloway KV, O'Gorman M, Woods P, Morton JP, Evans L, Cable NT, Goldspink DF and Burniston JG. Proteomic investigation of changes in human vastus lateralis muscle in response to interval-exercise training. Proteomics 9: 5155-5174, 2009.
115. Hong S and Mills PJ. Effects of an exercise challenge on mobilization and surface marker expression of monocyte subsets in individuals with normal vs. elevated blood pressure. Brain Behav Immun 22: 590-599, 2008.
116. Housh TJ, Johnson GO, Housh DJ, Evans SL and Tharp GD. The effect of exercise at various temperatures on salivary levels of immunoglobulin A. Int J Sports Med 12: 498-500, 1991.
117. Huberman E and Sachs L. Mutability of different genetic loci in mammalian cells by metabolically activated carcinogenic polycyclic hydrocarbons. Proc Natl Acad Sci U S A 73: 188-192, 1976.
118. Ibfelt T, Petersen EW, Bruunsgaard H, Sandmand M and Pedersen BK. Exercise-induced change in type 1 cytokine-producing CD8+ T cells is related to a decrease in memory T cells. J Appl Physiol 93: 645-648, 2002.
119. Jadeski L and Hoffman-Goetz L. Exercise and in vivo natural cytotoxicity against tumour cells of varying metastatic capacity. Clin Exp Metastasis 14: 138-144, 1996.
120. Jonsdottir IH, Hellstrand K, Thoren P and Hoffmann P. Enhancement of natural immunity seen after voluntary exercise in rats. Role of central opioid receptors. Life Sci 66: 1231-1239, 2000.
121. Ju J, Nolan B, Cheh M, Bose M, Lin Y, Wagner GC and Yang CS. Voluntary exercise inhibits intestinal tumorigenesis in Apc(Min/+) mice and azoxymethane/dextran sulfate sodium-treated mice. BMC Cancer 8: 316, 2008.
122. Kappel M, Tvede N, Galbo H, Haahr PM, Kjaer M, Linstow M, Klarlund K and Pedersen BK. Evidence that the effect of physical exercise on NK cell activity is mediated by epinephrine. J Appl Physiol 70: 2530-2534, 1991.
123. Kasapis C and Thompson PD. The effects of physical activity on serum C-reactive protein and inflammatory markers: a systematic review. J Am Coll Cardiol 45: 1563-1569, 2005.
124. Kawai T, Morita K, Masuda K, Nishida K, Sekiyama A, Teshima-Kondo S, Nakaya Y, Ohta M, Saito T and Rokutan K. Physical exercise-associated gene expression signatures in peripheral blood. Clin J Sport Med 17: 375-383, 2007.
125. Keegan TH, Glaser SL, Clarke CA, Dorfman RF, Mann RB, DiGiuseppe JA, Chang ET and Ambinder RF. Body size, physical activity, and risk of Hodgkin's lymphoma in women. Cancer Epidemiol Biomarkers Prev 15: 1095-1101, 2006.

126. Keller C, Keller P, Giralt M, Hidalgo J and Pedersen BK. Exercise normalises overexpression of TNF-alpha in knockout mice. Biochem Biophys Res Commun 321: 179-182, 2004.
127. Kimura F, Aizawa K, Tanabe K, Shimizu K, Kon M, Lee H, Akimoto T, Akama T and Kono I. A rat model of saliva secretory immunoglobulin: a suppression caused by intense exercise. Scand J Med Sci Sports 18: 367-372, 2008.
128. Kizaki T, Takemasa T, Sakurai T, Izawa T, Hanawa T, Kamiya S, Haga S, Imaizumi K and Ohno H. Adaptation of macrophages to exercise training improves innate immunity. Biochem Biophys Res Commun 372: 152-156, 2008.
129. Klentrou P, Cieslak T, MacNeil M, Vintinner A and Plyley M. Effect of moderate exercise on salivary immunoglobulin A and infection risk in humans. Eur J Appl Physiol 87: 153-158, 2002.
130. Koch A. Immune response to resistance exercise. Am J Lifestyle Med 4: 244-252, 2010.
131. Kohut ML, Boehm GW and Moynihan JA. Moderate exercise is associated with enhanced antigen-specific cytokine, but not IgM antibody production in aged mice. Mech Ageing Dev 122: 1135-1150, 2001.
132. Kohut ML, Cooper MM, Nickolaus MS, Russell DR and Cunnick JE. Exercise and psychosocial factors modulate immunity to influenza vaccine in elderly individuals. J Gerontol A Biol Sci Med Sci 57: M557-M562, 2002.
133. Kohut ML, Davis JM, Jackson DA, Colbert LH, Strasner A, Essig DA, Pate RR, Ghaffar A and Mayer EP. The role of stress hormones in exercise-induced suppression of alveolar macrophage antiviral function. J Neuroimmunol 81: 193-200, 1998.
134. Kohut ML, Davis JM, Jackson DA, Jani P, Ghaffar A, Mayer EP and Essig DA. Exercise effects on IFN-beta expression and viral replication in lung macrophages after HSV-1 infection. Am J Physiol 275: L1089-L1094, 1998.
135. Koenigsrainer I, Zieker D, Loeffler M, Buehler S, Walter M, Beckert S, Glatzle J, Northoff H, Nadalin S and Koenigsrainer A. Influence of exhaustive exercise on the immune system in solid organ transplant recipients. Exerc Immunol Rev 16: 184-193, 2010.
136. Krueger K and Mooren FC. T cell homing and exercise. Exerc Immunol Rev 13: 37-54, 2007.
137. Laing SJ, Gwynne D, Blackwell J, Williams M, Walters R and Walsh NP. Salivary IgA response to prolonged exercise in a hot environment in trained cyclists. Eur J Appl Physiol 93: 665-671, 2005.
138. Lamm ME. Current concepts in mucosal immunity. IV. How epithelial transport of IgA antibodies relates to host defense. Am J Physiol 274: G614-G617, 1998.
139. Lancaster GI, Halson SL, Khan Q, Drysdale P, Wallace F, Jeukendrup AE, Drayson MT and Gleeson M. Effects of acute exhaustive exercise and chronic exercise training on type 1 and type 2 T lymphocytes. Exerc Immunol Rev 10: 91-106, 2004.
140. Lancaster GI, Khan Q, Drysdale P, Wallace F, Jeukendrup AE, Drayson MT and Gleeson M. The physiological regulation of toll-like receptor expression and function in humans. J Physiol 563: 945-955, 2005.
141. LaPerriere A, Ironson G, Antoni MH, Schneiderman N, Klimas N and Fletcher MA. Exercise and psychoneuroimmunology. Med Sci Sports Exerc 26: 182-190, 1994.
142. Lee R, West D, Phillips SM and Britz-McKibbin P. Differential metabolomics for quantitative assessment of oxidative stress with strenuous exercise and nutritional intervention: thiol-specific regulation of cellular metabolism with N-acetyl-L-cysteine pretreatment. Anal Chem 82: 2959-2968, 2010.

143. Lehmann R, Zhao X, Weigert C, Simon P, Fehrenbach E, Fritsche J, Machann J, Schick F, Wang J, Hoene M, Schleicher ED, Haring HU, Xu G and Niess AM. Medium chain acylcarnitines dominate the metabolite pattern in humans under moderate intensity exercise and support lipid oxidation. PLoS One 5: e11519, 2010.
144. Li TL and Gleeson M. The effect of collection methods on unstimulated salivary immunoglobulin A, total protein, amylase and cortisol. Bull Phys Ed 36: 17-30, 2004.
145. Li TL and Gleeson M. The effect of single and repeated bouts of prolonged cycling and circadian variation on saliva flow rate, immunoglobulin A and alpha-amylase responses. J Sports Sci 22: 1015-1024, 2004.
146. Li TL and Gleeson M. The effects of carbohydrate supplementation during repeated bouts of prolonged exercise on saliva flow rate and immunoglobulin A. J Sports Sci 23: 713-722, 2005.
147. Liao HF, Chiang LM, Yen CC, Chen YY, Zhuang RR, Lai LY, Chiang J and Chen YJ. Effect of a periodized exercise training and active recovery program on antitumor activity and development of dendritic cells. J Sports Med Phys Fitness 46: 307-314, 2006.
148. Libicz S, Mercier B, Bigou N, Le Gallais D and Castex F. Salivary IgA response of triathletes participating in the French Iron Tour. Int J Sports Med 27: 389-394, 2006.
149. Lowder T, Padgett DA and Woods JA. Moderate exercise protects mice from death due to influenza virus. Brain Behav Immun 19: 377-380, 2005.
150. Lowder T, Padgett DA and Woods JA. Moderate exercise early after influenza virus infection reduces the Th1 inflammatory response in lungs of mice. Exerc Immunol Rev 12: 97-111, 2006.
151. Lu Q, Ceddia MA, Price EA, Ye SM and Woods JA. Chronic exercise increases macrophage-mediated tumor cytolysis in young and old mice. Am J Physiol 276: R482-R489, 1999.
152. Mackinnon LT. Immunoglobulin, antibody and exercise. Exerc Immunol Rev 2: 1-34, 1996.
153. Mackinnon LT, Chick TW, Van As A and Tomasi TB. Decreased secretory immunoglobulins following intense endurance exercise. Sports Training Med Rehabil 1: 209-218, 1989.
154. Mackinnon LT and Jenkins DG. Decreased salivary immunoglobulins after intense interval exercise before and after training. Med Sci Sports Exerc 25: 678-683, 1993.
155. MacNeil B and Hoffman-Goetz L. Chronic exercise enhances in vivo and in vitro cytotoxic mechanisms of natural immunity in mice. J Appl Physiol 74: 388-395, 1993.
156. MacNeil B and Hoffman-Goetz L. Effect of exercise on natural cytotoxicity and pulmonary tumor metastases in mice. Med Sci Sports Exerc 25: 922-928, 1993.
157. Madden KS and Felten DL. Experimental basis for neural-immune interactions. Physiol Rev 75: 77-106, 1995.
158. Mahoney DJ, Safdar A, Parise G, Melov S, Fu M, MacNeil L, Kaczor J, Payne ET and Tarnopolsky MA. Gene expression profiling in human skeletal muscle during recovery from eccentric exercise. Am J Physiol Regul Integr Comp Physiol 294: R1901-R1910, 2008.
159. Maier SF, Nguyen KT, Deak T, Watkins LR and Fleshner M. Acute stress suppresses KLH-specific but not mitogenic (ConA) proliferative response: evidence for reduced T cell expansion. Soc Neurosci Abstr 23: 1997.

160. Matthews CE, Ockene IS, Freedson PS, Rosal MC, Merriam PA and Hebert JR. Moderate to vigorous physical activity and risk of upper-respiratory tract infection. Med Sci Sports Exerc 34: 1242-1248, 2002.
161. McCarthy DA and Dale MM. The leucocytosis of exercise. A review and model. Sports Med 6: 333-363, 1988.
162. McCarthy DA, Macdonald I, Grant M, Marbut M, Watling M, Nicholson S, Deeks JJ, Wade AJ and Perry JD. Studies on the immediate and delayed leucocytosis elicited by brief (30-min) strenuous exercise. Eur J Appl Physiol Occup Physiol 64: 513-517, 1992.
163. McDowell SL, Chaloa K, Housh TJ, Tharp GD and Johnson GO. The effect of exercise intensity and duration on salivary immunoglobulin A. Eur J Appl Physiol Occup Physiol 63: 108-111, 1991.
164. McDowell SL, Hughes RA, Hughes RJ, Housh TJ and Johnson GO. The effect of exercise training on salivary immunoglobulin A and cortisol responses to maximal exercise. Int J Sports Med 13: 577-580, 1992.
165. McFarlin BK, Flynn MG, Campbell WW, Craig BA, Robinson JP, Stewart LK, Timmerman KL and Coen PM. Physical activity status, but not age, influences inflammatory biomarkers and toll-like receptor 4. J Gerontol A Biol Sci Med Sci 61: 388-393, 2006.
166. McFarlin BK, Flynn MG, Campbell WW, Stewart LK and Timmerman KL. TLR4 is lower in resistance-trained older women and related to inflammatory cytokines. Med Sci Sports Exerc 36: 1876-1883, 2004.
167. McFarlin BK, Flynn MG, Phillips MD, Stewart LK and Timmerman KL. Chronic resistance exercise training improves natural killer cell activity in older women. J Gerontol A Biol Sci Med Sci 60: 1315-1318, 2005.
168. McTiernan A. Mechanisms linking physical activity with cancer. Nat Rev Cancer 8: 205-211, 2008.
169. Meyerhardt JA, Heseltine D, Niedzwiecki D, Hollis D, Saltz LB, Mayer RJ, Thomas J, Nelson H, Whittom R, Hantel A, Schilsky RL and Fuchs CS. Impact of physical activity on cancer recurrence and survival in patients with stage III colon cancer: findings from CALGB 89803. J Clin Oncol 24: 3535-3541, 2006.
170. Miccheli A, Marini F, Capuani G, Miccheli AT, Delfini M, Di Cocco ME, Puccetti C, Paci M, Rizzo M and Spataro A. The influence of a sports drink on the postexercise metabolism of elite athletes as investigated by NMR-based metabolomics. J Am Coll Nutr 28: 553-564, 2009.
171. Michishita R, Shono N, Inoue T, Tsuruta T and Node K. Effect of exercise therapy on monocyte and neutrophil counts in overweight women. Am J Med Sci 339: 152-156, 2010.
172. Mizutani K, Sonoda S, Hayashi N, Takasaki A, Beppu H, Saitoh E and Shimpo K. Analysis of protein expression profile in the cerebellum of cerebral infarction rats after treadmill training. Am J Phys Med Rehabil 89: 107-114, 2010.
173. Moldoveanu AI, Shephard RJ and Shek PN. Exercise elevates plasma levels but not gene expression of IL-1beta, IL-6, and TNF-alpha in blood mononuclear cells. J Appl Physiol 89: 1499-1504, 2000.
174. Molteni R, Ying Z and Gomez-Pinilla F. Differential effects of acute and chronic exercise on plasticity-related genes in the rat hippocampus revealed by microarray. Eur J Neurosci 16: 1107-1116, 2002.

175. Monninkhof EM, Elias SG, Vlems FA, van dT, I, Schuit AJ, Voskuil DW and van Leeuwen FE. Physical activity and breast cancer: a systematic review. Epidemiology 18: 137-157, 2007.
176. Mooren FC, Lechtermann A and Volker K. Exercise-induced apoptosis of lymphocytes depends on training status. Med Sci Sports Exerc 36: 1476-1483, 2004.
177. Moraska A, Campisi J, Nguyen KT, Maier SF, Watkins LR and Fleshner M. Elevated IL-1beta contributes to antibody suppression produced by stress. J Appl Physiol 93: 207-215, 2002.
178. Moraska A and Fleshner M. Voluntary physical activity prevents stress-induced behavioral depression and anti-KLH antibody suppression. Am J Physiol Regul Integr Comp Physiol 281: R484-R489, 2001.
179. Moynihan JA, Ader R, Grota LJ, Schachtman TR and Cohen N. The effects of stress on the development of immunological memory following low-dose antigen priming in mice. Brain Behav Immun 4: 1-12, 1990.
180. Muller W. Dissecting the cytokine network. Cell Immunol 244: 162-164, 2006.
181. Murphy EA, Davis JM, Brown AS, Carmichael MD, Van Rooijen N, Ghaffar A and Mayer EP. Role of lung macrophages on susceptibility to respiratory infection following short-term moderate exercise training. Am J Physiol Regul Integr Comp Physiol 287: R1354-R1358, 2004.
182. Murphy K, Travers P and Walport M. Janeway's Immunobiology. NY: Garland Science Publishing, 2008.
183. Nakamura S, Kobayashi M, Sugino T, Kajimoto O, Matoba R and Matsubara K. Effect of exercise on gene expression profile in unfractionated peripheral blood leukocytes. Biochem Biophys Res Commun 391: 846-851, 2010.
184. Nehlsen-Cannarella SL, Nieman DC, Jessen J, Chang L, Gusewitch G, Blix GG and Ashley E. The effects of acute moderate exercise on lymphocyte function and serum immunoglobulin levels. Int J Sports Med 12: 391-398, 1991.
185. Nelson M, Popp H, Sharpe K and Ashenden M. Proof of homologous blood transfusion through quantification of blood group antigens. Haematologica 88: 1284-1295, 2003.
186. Neubauer O, Reichhold S, Nersesyan A, Koenig D and Wagner KH. Exercise-induced DNA damage: is there a relationship with inflammatory responses? Exerc Immunol Rev 14: 51-72, 2008.
187. Neville V, Gleeson M and Folland JP. Salivary IgA as a risk factor for upper respiratory infections in elite professional athletes. Med Sci Sports Exerc 40: 1228-1236, 2008.
188. Nichol KE, Poon WW, Parachikova AI, Cribbs DH, Glabe CG and Cotman CW. Exercise alters the immune profile in Tg2576 Alzheimer mice toward a response coincident with improved cognitive performance and decreased amyloid. J Neuroinflammation 5: 13, 2008.
189. Nielsen AR, Hojman P, Erikstrup C, Fischer CP, Plomgaard P, Mounier R, Mortensen OH, Broholm C, Taudorf S, Krogh-Madsen R, Lindegaard B, Petersen AM, Gehl J and Pedersen BK. Association between interleukin-15 and obesity: interleukin-15 as a potential regulator of fat mass. J Clin Endocrinol Metab 93: 4486-4493, 2008.
190. Nielsen AR, Mounier R, Plomgaard P, Mortensen OH, Penkowa M, Speerschneider T, Pilegaard H and Pedersen BK. Expression of interleukin-15 in human skeletal muscle effect of exercise and muscle fibre type composition. J Physiol 584: 305-312, 2007.

191. Nielsen AR and Pedersen BK. The biological roles of exercise-induced cytokines: IL-6, IL-8, and IL-15. Appl Physiol Nutr Metab 32: 833-839, 2007.
192. Nieman DC. Is infection risk linked to exercise workload? Med Sci Sports Exerc 32: S406-S411, 2000.
193. Nieman DC. Current perspective on exercise immunology. Curr Sports Med Rep 2: 239-242, 2003.
194. Nieman DC, Buckley KS, Henson DA, Warren BJ, Suttles J, Ahle JC, Simandle S, Fagoaga OR and Nehlsen-Cannarella SL. Immune function in marathon runners versus sedentary controls. Med Sci Sports Exerc 27: 986-992, 1995.
195. Nieman DC, Dumke CI, Henson DA, McAnulty SR, McAnulty LS, Lind RH and Morrow JD. Immune and oxidative changes during and following the Western States Endurance Run. Int J Sports Med 24: 541-547, 2003.
196. Nieman DC, Dumke CL, Henson DA, McAnulty SR, Gross SJ and Lind RH. Muscle damage is linked to cytokine changes following a 160-km race. Brain Behav Immun 19: 398-403, 2005.
197. Nieman DC, Henson DA, Austin MD and Brown VA. Immune response to a 30-minute walk. Med Sci Sports Exerc 37: 57-62, 2005.
198. Nieman DC, Henson DA, Dumke CL, Lind RH, Shooter LR and Gross SJ. Relationship between salivary IgA secretion and upper respiratory tract infection following a 160-km race. J Sports Med Phys Fitness 46: 158-162, 2006.
199. Nieman DC, Henson DA, Fagoaga OR, Utter AC, Vinci DM, Davis JM and Nehlsen-Cannarella SL. Change in salivary IgA following a competitive marathon race. Int J Sports Med 23: 69-75, 2002.
200. Nieman DC, Johanssen LM, Lee JW and Arabatzis K. Infectious episodes in runners before and after the Los Angeles Marathon. J Sports Med Phys Fitness 30: 316-328, 1990.
201. Nieman DC and Nehlsen-Cannarella SL. Exercise and Infection. In: Exercise and Disease, edited by Watson RR and Eisinger M. Boca Raton, LA: CRC Publishers, 1992, p. 121-148.
202. Nieman DC, Nehlsen-Cannarella SL, Markoff PA, Balk-Lamberton AJ, Yang H, Chritton DB, Lee JW and Arabatzis K. The effects of moderate exercise training on natural killer cells and acute upper respiratory tract infections. Int J Sports Med 11: 467-473, 1990.
203. Nieman DC, Tan SA, Lee JW and Berk LS. Complement and immunoglobulin levels in athletes and sedentary controls. Int J Sports Med 10: 124-128, 1989.
204. Nocon M, Hiemann T, Muller-Riemenschneider F, Thalau F, Roll S and Willich SN. Association of physical activity with all-cause and cardiovascular mortality: a systematic review and meta-analysis. Eur J Cardiovasc Prev Rehabil 15: 239-246, 2008.
205. Northoff H, Symons S, Zieker D, Schaible EV, Schaefer K, Thoma S, Loeffler M, Abbasi A, Simon P, Niess AM and Fehrenbach E. Gender- and menstrual phase dependent regulation of inflammatory gene expression in response to aerobic exercise. Exerc Immunol Rev 14: 86-103, 2008.
206. Okutsu M, Suzuki K, Ishijima T, Peake J and Higuchi M. The effects of acute exercise-induced cortisol on CCR2 expression on human monocytes. Brain Behav Immun 22: 1066-1071, 2008.
207. Oliver SJ, Laing SJ, Wilson S, Bilzon JL, Walters R and Walsh NP. Salivary immunoglobulin A response at rest and after exercise following a 48 h period of fluid and/or energy restriction. Br J Nutr 97: 1109-1116, 2007.

208. Ornish D, Magbanua MJ, Weidner G, Weinberg V, Kemp C, Green C, Mattie MD, Marlin R, Simko J, Shinohara K, Haqq CM and Carroll PR. Changes in prostate gene expression in men undergoing an intensive nutrition and lifestyle intervention. Proc Natl Acad Sci U S A 105: 8369-8374, 2008.
209. Ortega E, Forner MA and Barriga C. Exercise-induced stimulation of murine macrophage chemotaxis: role of corticosterone and prolactin as mediators. J Physiol 498 (Pt 3): 729-734, 1997.
210. Ortega E, Rodriguez MJ, Barriga C and Forner MA. Corticosterone, prolactin and thyroid hormones as hormonal mediators of the stimulated phagocytic capacity of peritoneal macrophages after high-intensity exercise. Int J Sports Med 17: 149-155, 1996.
211. Paffenbarger RS, Jr., Lee IM and Leung R. Physical activity and personal characteristics associated with depression and suicide in American college men. Acta Psychiatr Scand Suppl 377: 16-22, 1994.
212. Paffenbarger RS, Jr., Lee IM and Wing AL. The influence of physical activity on the incidence of site-specific cancers in college alumni. Adv Exp Med Biol 322: 7-15, 1992.
213. Palmer FM, Nieman DC, Henson DA, McAnulty SR, McAnulty L, Swick NS, Utter AC, Vinci DM and Morrow JD. Influence of vitamin C supplementation on oxidative and salivary IgA changes following an ultramarathon. Eur J Appl Physiol 89: 100-107, 2003.
214. Peake J, Nosaka K and Suzuki K. Characterization of inflammatory responses to eccentric exercise in humans. Exerc Immunol Rev 11: 64-85, 2005.
215. Peake J and Suzuki K. Neutrophil activation, antioxidant supplements and exercise-induced oxidative stress. Exerc Immunol Rev 10: 129-141, 2004.
216. Peake JM. Exercise-induced alterations in neutrophil degranulation and respiratory burst activity: possible mechanisms of action. Exerc Immunol Rev 8: 49-100, 2002.
217. Pedersen BK. Edward F. Adolph distinguished lecture: muscle as an endocrine organ: IL-6 and other myokines. J Appl Physiol 107: 1006-1014, 2009.
218. Pedersen BK. The diseasome of physical inactivity--and the role of myokines in muscle--fat cross talk. J Physiol 587: 5559-5568, 2009.
219. Pedersen BK and Febbraio MA. Muscle as an endocrine organ: focus on muscle-derived interleukin-6. Physiol Rev 88: 1379-1406, 2008.
220. Pedersen BK and Fischer CP. Beneficial health effects of exercise--the role of IL-6 as a myokine. Trends Pharmacol Sci 28: 152-156, 2007.
221. Pedersen BK and Hoffman-Goetz L. Exercise and the immune system: regulation, integration, and adaptation. Physiol Rev 80: 1055-1081, 2000.
222. Pedersen BK, Steensberg A, Fischer C, Keller C, Ostrowski K and Schjerling P. Exercise and cytokines with particular focus on muscle-derived IL-6. Exerc Immunol Rev 7: 18-31, 2001.
223. Peters C, Loetzerich H, Niemeier B, Schule K and Uhlenbruck G. Influence of a moderate exercise training on natural killer cytotoxicity and personality traits in cancer patients. Anticancer Res 14: 1033-1036, 1994.
224. Peters EM and Bateman ED. Ultramarathon running and upper respiratory tract infections. An epidemiological survey. S Afr Med J 64: 582-584, 1983.
225. Petersen AM and Pedersen BK. The anti-inflammatory effect of exercise. J Appl Physiol 98: 1154-1162, 2005.

226. Phillips AC, Carroll D, Evans P, Bosch JA, Clow A, Hucklebridge F and Der G. Stressful life events are associated with low secretion rates of immunoglobulin A in saliva in the middle aged and elderly. Brain Behav Immun 20: 191-197, 2006.
227. Ploeger HE, Takken T, de Greef MH and Timmons BW. The effects of acute and chronic exercise on inflammatory markers in children and adults with a chronic inflammatory disease: a systematic review. Exerc Immunol Rev 15: 6-41, 2009.
228. Pohjanen E, Thysell E, Jonsson P, Eklund C, Silfver A, Carlsson IB, Lundgren K, Moritz T, Svensson MB and Antti H. A multivariate screening strategy for investigating metabolic effects of strenuous physical exercise in human serum. J Proteome Res 6: 2113-2120, 2007.
229. Potteiger JA, Chan MA, Haff GG, Mathew S, Schroeder CA, Haub MD, Chirathaworn C, Tibbetts SA, Mcdonald J, Omoike O and Benedict SH. Training status influences T-cell responses in women following acute resistance exercise. J Strength Cond Res 15: 185-191, 2001.
230. Proctor GB, Garrett JR, Carpenter GH and Ebersole LE. Salivary secretion of immunoglobulin A by submandibular glands in response to autonomimetic infusions in anaesthetised rats. J Neuroimmunol 136: 17-24, 2003.
231. Puglisi MJ and Fernandez ML. Modulation of C-reactive protein, tumor necrosis factor-alpha, and adiponectin by diet, exercise, and weight loss. J Nutr 138: 2293-2296, 2008.
232. Putlur P, Foster C, Miskowski JA, Kane MK, Burton SE, Scheett TP and McGuigan MR. Alteration of immune function in women collegiate soccer players and college students. J Sports Sci Med 3: 234-243, 2004.
233. Pyne DB, Baker MS, Fricker PA, McDonald WA, Telford RD and Weidemann MJ. Effects of an intensive 12-wk training program by elite swimmers on neutrophil oxidative activity. Med Sci Sports Exerc 27: 536-542, 1995.
234. Pyne DB and Gleeson M. Effects of intensive exercise training on immunity in athletes. Int J Sports Med 19 Suppl 3: S183-S191, 1998.
235. Pyne DB, McDonald WA, Gleeson M, Flanagan A, Clancy RL and Fricker PA. Mucosal immunity, respiratory illness, and competitive performance in elite swimmers. Med Sci Sports Exerc 33: 348-353, 2001.
236. Rabinovich RA, Figueras M, Ardite E, Carbo N, Troosters T, Filella X, Barbera JA, Fernandez-Checa JC, Argiles JM and Roca J. Increased tumour necrosis factor-alpha plasma levels during moderate-intensity exercise in COPD patients. Eur Respir J 21: 789-794, 2003.
237. Radom-Aizik S, Zaldivar F, Jr., Leu SY and Cooper DM. A brief bout of exercise alters gene expression and distinct gene pathways in peripheral blood mononuclear cells of early- and late-pubertal females. J Appl Physiol 107: 168-175, 2009.
238. Radom-Aizik S, Zaldivar F, Jr., Leu SY and Cooper DM. Brief bout of exercise alters gene expression in peripheral blood mononuclear cells of early- and late-pubertal males. Pediatr Res 65: 447-452, 2009.
239. Radom-Aizik S, Zaldivar F, Jr., Leu SY, Galassetti P and Cooper DM. Effects of 30 min of aerobic exercise on gene expression in human neutrophils. J Appl Physiol 104: 236-243, 2008.
240. Radom-Aizik S, Zaldivar F, Jr., Oliver S, Galassetti P and Cooper DM. Evidence for microRNA involvement in exercise-associated neutrophil gene expression changes. J Appl Physiol 109: 252-261, 2010.

241. Rankinen T, Roth SM, Bray MS, Loos R, Perusse L, Wolfarth B, Hagberg JM and Bouchard C. Advances in exercise, fitness, and performance genomics. Med Sci Sports Exerc 42: 835-846, 2010.
242. Reid VL, Gleeson M, Williams N and Clancy RL. Clinical investigation of athletes with persistent fatigue and/or recurrent infections. Br J Sports Med 38: 42-45, 2004.
243. Ricardo JS, Cartner L, Oliver SJ, Laing SJ, Walters R, Bilzon JL and Walsh NP. No effect of a 30-h period of sleep deprivation on leukocyte trafficking, neutrophil degranulation and saliva IgA responses to exercise. Eur J Appl Physiol 105: 499-504, 2009.
244. Richardson MR, Lai X, Dixon JL, Sturek M and Witzmann FA. Diabetic dyslipidemia and exercise alter the plasma low-density lipoproteome in Yucatan pigs. Proteomics 9: 2468-2483, 2009.
245. Rivier A, Pene J, Chanez P, Anselme F, Caillaud C, Prefaut C, Godard P and Bousquet J. Release of cytokines by blood monocytes during strenuous exercise. Int J Sports Med 15: 192-198, 1994.
246. Roberts JA. Viral illnesses and sports performance. Sports Med 3: 298-303, 1986.
247. Robson PJ, Blannin AK, Walsh NP, Castell LM and Gleeson M. Effects of exercise intensity, duration and recovery on in vitro neutrophil function in male athletes. Int J Sports Med 20: 128-135, 1999.
248. Rogers CJ, Berrigan D, Zaharoff DA, Hance KW, Patel AC, Perkins SN, Schlom J, Greiner JW and Hursting SD. Energy restriction and exercise differentially enhance components of systemic and mucosal immunity in mice. J Nutr 138: 115-122, 2008.
249. Rogers CJ, Colbert LH, Greiner JW, Perkins SN and Hursting SD. Physical activity and cancer prevention: pathways and targets for intervention. Sports Med 38: 271-296, 2008.
250. Rogers CJ, Zaharoff DA, Hance KW, Perkins SN, Hursting SD, Schlom J and Greiner JW. Exercise enhances vaccine-induced antigen-specific T cell responses. Vaccine 26: 5407-5415, 2008.
251. Ronsen O, Pedersen BK, Oritsland TR, Bahr R and Kjeldsen-Kragh J. Leukocyte counts and lymphocyte responsiveness associated with repeated bouts of strenuous endurance exercise. J Appl Physiol 91: 425-434, 2001.
252. Rossen RD, Butler WT, Waldman RH, Alford RH, Hornick RB, Togo Y and Kasel JA. The proteins in nasal secretion. II. A longitudinal study of IgA and neutralizing antibody levels in nasal washings from men infected with influenza virus. JAMA 211: 1157-1161, 1970.
253. Rovio S, Kareholt I, Helkala EL, Viitanen M, Winblad B, Tuomilehto J, Soininen H, Nissinen A and Kivipelto M. Leisure-time physical activity at midlife and the risk of dementia and Alzheimer's disease. Lancet Neurol 4: 705-711, 2005.
254. Safdar A, Abadi A, Akhtar M, Hettinga BP and Tarnopolsky MA. miRNA in the regulation of skeletal muscle adaptation to acute endurance exercise in C57Bl/6J male mice. PLoS One 4: e5610, 2009.
255. Sari-Sarraf V, Reilly T and Doran DA. Salivary IgA response to intermittent and continuous exercise. Int J Sports Med 27: 849-855, 2006.
256. Sari-Sarraf V, Reilly T, Doran DA and Atkinson G. The effects of single and repeated bouts of soccer-specific exercise on salivary IgA. Arch Oral Biol 52: 526-532, 2007.
257. Schwellnus MP, Kiessig M, Derman W and Noakes T. Fusafungine reduces symptoms of upper respiratory tract infections (URTI) in runners after a 56km race. Med Sci Sports Exerc S396: 1997.

258. Shek PN, Sabiston BH, Buguet A and Radomski MW. Strenuous exercise and immunological changes: a multiple-time-point analysis of leukocyte subsets, CD4/CD8 ratio, immunoglobulin production and NK cell response. Int J Sports Med 16: 466-474, 1995.
259. Shek PN, Sabiston BH, Paucod JC and Vidal D. Strenuous exercise and immune changes. In: Accord Franco-Canadien, Vol. 3. Physical exercise, hyperthermia, immune system and recovery sleep in man, edited by Buguet A and Radomski MW. La Tronche, France: Centre de recherches du Service de Sante des Armees, 1994, p. 121-137.
260. Shek PN and Shephard RJ. Physical exercise as a human model of limited inflammatory response. Can J Physiol Pharmacol 76: 589-597, 1998.
261. Shephard RJ. Physical activity, training and the immune response. Carmel, IN: Cooper Publishing Group, 1997.
262. Shephard RJ. Adhesion molecules, catecholamines and leucocyte redistribution during and following exercise. Sports Med 33: 261-284, 2003.
263. Shephard RJ. Development of the discipline of exercise immunology. Exerc Immunol Rev 16: 194-222, 2010.
264. Shephard RJ. The history of exercise immunology. In: The history of exercise physiology, edited by Tipton C. Champaign, IL: Human Kinetics, 2010.
265. Shephard RJ and Futcher R. Physical activity and cancer: how may protection be maximized? Crit Rev Oncog 8: 219-272, 1997.
266. Shephard RJ, Gannon G, Hay JB and Shek PN. Adhesion molecule expression in acute and chronic exercise. Crit Rev Immunol 20: 245-266, 2000.
267. Shephard RJ, Kavanagh T, Mertens DJ, Qureshi S and Clark M. Personal health benefits of Masters athletics competition. Br J Sports Med 29: 35-40, 1995.
268. Shephard RJ and Shek PN. Associations between physical activity and susceptibility to cancer: possible mechanisms. Sports Med 26: 293-315, 1998.
269. Shephard RJ and Shek PN. Effects of exercise and training on natural killer cell counts and cytolytic activity: a meta-analysis. Sports Med 28: 177-195, 1999.
270. Shinkai S, Shore S, Shek PN and Shephard RJ. Acute exercise and immune function. Relationship between lymphocyte activity and changes in subset counts. Int J Sports Med 13: 452-461, 1992.
271. Sim YJ, Yu S, Yoon KJ, Loiacono CM and Kohut ML. Chronic exercise reduces illness severity, decreases viral load, and results in greater anti-inflammatory effects than acute exercise during influenza infection. J Infect Dis 200: 1434-1442, 2009.
272. Simpson RJ, McFarlin BK, McSporran C, Spielmann G, Hartaigh B and Guy K. Toll-like receptor expression on classic and pro-inflammatory blood monocytes after acute exercise in humans. Brain Behav Immun 23: 232-239, 2009.
273. Sloan RP, Shapiro PA, Demeersman RE, McKinley PS, Tracey KJ, Slavov I, Fang Y and Flood PD. Aerobic exercise attenuates inducible TNF production in humans. J Appl Physiol 103: 1007-1011, 2007.
274. Smith A, Vollmer-Conna U, Bennett B, Wakefield D, Hickie I and Lloyd A. The relationship between distress and the development of a primary immune response to a novel antigen. Brain Behav Immun 18: 65-75, 2004.
275. Smith AJ, Vollmer-Conna U, Bennett B, Hickie IB and Lloyd AR. Influences of distress and alcohol consumption on the development of a delayed-type hypersensitivity skin test response. Psychosom Med 66: 614-619, 2004.

276. Smith JA, Gray AB, Pyne DB, Baker MS, Telford RD and Weidemann MJ. Moderate exercise triggers both priming and activation of neutrophil subpopulations. Am J Physiol 270: R838-R845, 1996.
277. Smith TP, Kennedy SL and Fleshner M. Influence of age and physical activity on the primary in vivo antibody and T cell-mediated responses in men. J Appl Physiol 97: 491-498, 2004.
278. Snyder BK, Roghmann KJ and Sigal LH. Effect of stress and other biopsychosocial factors on primary antibody response. J Adolesc Health Care 11: 472-479, 1990.
279. Sonna LA, Wenger CB, Flinn S, Sheldon HK, Sawka MN and Lilly CM. Exertional heat injury and gene expression changes: a DNA microarray analysis study. J Appl Physiol 96: 1943-1953, 2004.
280. Speck RM, Courneya KS, Masse LC, Duval S and Schmitz KH. An update of controlled physical activity trials in cancer survivors: a systematic review and meta-analysis. J Cancer Surviv 4: 87-100, 2010.
281. Speirs RL, Herring J, Cooper WD, Hardy CC and Hind CR. The influence of sympathetic activity and isoprenaline on the secretion of amylase from the human parotid gland. Arch Oral Biol 19: 747-752, 1974.
282. Spence L, Brown WJ, Pyne DB, Nissen MD, Sloots TP, McCormack JG, Locke AS and Fricker PA. Incidence, etiology, and symptomatology of upper respiratory illness in elite athletes. Med Sci Sports Exerc 39: 577-586, 2007.
283. Spence RR, Heesch KC and Brown WJ. Exercise and cancer rehabilitation: a systematic review. Cancer Treat Rev 36: 185-194, 2010.
284. Starkie R, Ostrowski SR, Jauffred S, Febbraio M and Pedersen BK. Exercise and IL-6 infusion inhibit endotoxin-induced TNF-alpha production in humans. FASEB J 17: 884-886, 2003.
285. Starkie RL, Angus DJ, Rolland J, Hargreaves M and Febbraio MA. Effect of prolonged, submaximal exercise and carbohydrate ingestion on monocyte intracellular cytokine production in humans. J Physiol 528: 647-655, 2000.
286. Starkie RL, Rolland J, Angus DJ, Anderson MJ and Febbraio MA. Circulating monocytes are not the source of elevations in plasma IL-6 and TNF-alpha levels after prolonged running. Am J Physiol Cell Physiol 280: C769-C774, 2001.
287. Steensberg A, Toft AD, Bruunsgaard H, Sandmand M, Halkjaer-Kristensen J and Pedersen BK. Strenuous exercise decreases the percentage of type 1 T cells in the circulation. J Appl Physiol 91: 1708-1712, 2001.
288. Steerenberg PA, van Asperen IA, van Nieuw AA, Biewenga A, Mol D and Medema GJ. Salivary levels of immunoglobulin A in triathletes. Eur J Oral Sci 105: 305-309, 1997.
289. Steppich B, Dayyani F, Gruber R, Lorenz R, Mack M and Ziegler-Heitbrock HW. Selective mobilization of CD14(+)CD16(+) monocytes by exercise. Am J Physiol Cell Physiol 279: C578-C586, 2000.
290. Stewart LK, Flynn MG, Campbell WW, Craig BA, Robinson JP, McFarlin BK, Timmerman KL, Coen PM, Felker J and Talbert E. Influence of exercise training and age on CD14+ cell-surface expression of toll-like receptor 2 and 4. Brain Behav Immun 19: 389-397, 2005.
291. Strazdins L, Meyerkort S, Brent V, D'Souza RM, Broom DH and Kyd JM. Impact of saliva collection methods on sIgA and cortisol assays and acceptability to participants. J Immunol Methods 307: 167-171, 2005.

292. Sugiura H, Nishida H, Sugiura H and Mirbod SM. Immunomodulatory action of chronic exercise on macrophage and lymphocyte cytokine production in mice. Acta Physiol Scand 174: 247-256, 2002.
293. Suzui M, Kawai T, Kimura H, Takeda K, Yagita H, Okumura K, Shek PN and Shephard RJ. Natural killer cell lytic activity and CD56(dim) and CD56(bright) cell distributions during and after intensive training. J Appl Physiol 96: 2167-2173, 2004.
294. Suzuki K, Nakaji S, Yamada M, Totsuka M, Sato K and Sugawara K. Systemic inflammatory response to exhaustive exercise. Cytokine kinetics. Exerc Immunol Rev 8: 6-48, 2002.
295. Suzuki K, Totsuka M, Nakaji S, Yamada M, Kudoh S, Liu Q, Sugawara K, Yamaya K and Sato K. Endurance exercise causes interaction among stress hormones, cytokines, neutrophil dynamics, and muscle damage. J Appl Physiol 87: 1360-1367, 1999.
296. Swann JB, Vesely MD, Silva A, Sharkey J, Akira S, Schreiber RD and Smyth MJ. Demonstration of inflammation-induced cancer and cancer immunoediting during primary tumorigenesis. Proc Natl Acad Sci U S A 105: 652-656, 2008.
297. Thalacker-Mercer AE, Dell'Italia LJ, Cui X, Cross JM and Bamman MM. Differential genomic responses in old vs. young humans despite similar levels of modest muscle damage after resistance loading. Physiol Genomics 40: 141-149, 2010.
298. Tharp GD and Barnes MW. Reduction of saliva immunoglobulin levels by swim training. Eur J Appl Physiol Occup Physiol 60: 61-64, 1990.
299. Thomas NE, Leyshon A, Hughes MG, Davies B, Graham M and Baker JS. The effect of anaerobic exercise on salivary cortisol, testosterone and immunoglobulin (A) in boys aged 15-16 years. Eur J Appl Physiol 107: 455-461, 2009.
300. Timmerman KL, Flynn MG, Coen PM, Markofski MM and Pence BD. Exercise training-induced lowering of inflammatory (CD14+CD16+) monocytes: a role in the anti-inflammatory influence of exercise? J Leukoc Biol 84: 1271-1278, 2008.
301. Timmons BW and Cieslak T. Human natural killer cell subsets and acute exercise: a brief review. Exerc Immunol Rev 14: 8-23, 2008.
302. Timmons BW, Tarnopolsky MA and Bar-Or O. Sex-based effects on the distribution of NK cell subsets in response to exercise and carbohydrate intake in adolescents. J Appl Physiol 100: 1513-1519, 2006.
303. Tiollier E, Gomez-Merino D, Burnat P, Jouanin JC, Bourrilhon C, Filaire E, Guezennec CY and Chennaoui M. Intense training: mucosal immunity and incidence of respiratory infections. Eur J Appl Physiol 93: 421-428, 2005.
304. Tomasi TB, Trudeau FB, Czerwinski D and Erredge S. Immune parameters in athletes before and after strenuous exercise. J Clin Immunol 2: 173-178, 1982.
305. Tuomilehto J, Lindstrom J, Eriksson JG, Valle TT, Hamalainen H, Ilanne-Parikka P, Keinanen-Kiukaanniemi S, Laakso M, Louheranta A, Rastas M, Salminen V and Uusitupa M. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 344: 1343-1350, 2001.
306. Tvede N, Heilmann C, Halkjaer-Kristensen J and Pedersen BK. Mechanisms of B-lymphocyte suppression induced by acute physical exercise. J Clin Lab Immunol 30: 169-173, 1989.
307. Tvede N, Kappel M, Klarlund K, Duhn S, Halkjaer-Kristensen J, Kjaer M, Galbo H and Pedersen BK. Evidence that the effect of bicycle exercise on blood mononuclear cell proliferative responses and subsets is mediated by epinephrine. Int J Sports Med 15: 100-104, 1994.

308. Verde T, Thomas S and Shephard RJ. Potential markers of heavy training in highly trained distance runners. Br J Sports Med 26: 167-175, 1992.
309. Vieira VJ, Valentine RJ, Wilund KR, Antao N, Baynard T and Woods JA. Effects of exercise and low-fat diet on adipose tissue inflammation and metabolic complications in obese mice. Am J Physiol Endocrinol Metab 296: E1164-E1171, 2009.
310. Vieira VJ, Valentine RJ, Wilund KR and Woods JA. Effects of diet and exercise on metabolic disturbances in high-fat diet-fed mice. Cytokine 46: 339-345, 2009.
311. Walker FR, Hodyl NA and Hodgson DM. Neonatal bacterial endotoxin challenge interacts with stress in the adult male rat to modify KLH specific antibody production but not KLH stimulated ex vivo cytokine release. J Neuroimmunol 207: 57-65, 2009.
312. Walsh NP, Bishop NC, Blackwell J, Wierzbicki SG and Montague JC. Salivary IgA response to prolonged exercise in a cold environment in trained cyclists. Med Sci Sports Exerc 34: 1632-1637, 2002.
313. Walsh NP, Blannin AK, Clark AM, Cook L, Robson PJ and Gleeson M. The effects of high-intensity intermittent exercise on saliva IgA, total protein and alpha-amylase. J Sports Sci 17: 129-134, 1999.
314. Wang C, Miller SM, Egleston BL, Hay JL and Weinberg DS. Beliefs about the causes of breast and colorectal cancer among women in the general population. Cancer Causes Control 21: 99-107, 2010.
315. Wessner B, Gryadunov-Masutti L, Tschan H, Bachl N and Roth E. Is there a role for microRNAs in exercise immunology? A synopsis of current literature and future developments. Exerc Immunol Rev 16: 22-39, 2010.
316. West NP, Pyne DB, Kyd JM, Renshaw GM, Fricker PA and Cripps AW. The effect of exercise on innate mucosal immunity. Br J Sports Med 44: 227-231, 2010.
317. West NP, Pyne DB, Renshaw G and Cripps AW. Antimicrobial peptides and proteins, exercise and innate mucosal immunity. FEMS Immunol Med Microbiol 48: 293-304, 2006.
318. West-Wright CN, Henderson KD, Sullivan-Halley J, Ursin G, Deapen D, Neuhausen S, Reynolds P, Chang E, Ma H and Bernstein L. Long-term and recent recreational physical activity and survival after breast cancer: the California Teachers Study. Cancer Epidemiol Biomarkers Prev 18: 2851-2859, 2009.
319. Wetmore CM and Ulrich CM. Mechanisms associating physical activity with cancer incidence: exercise and immune function in: Cancer Prevention and Management through Exercise and Weight Control, edited by McTiernan. Boca Raton: CRC Taylor and Francis, 2005, p. 157-176.
320. Whitham M, Laing SJ, Dorrington M, Walters R, Dunklin S, Bland D, Bilzon JL and Walsh NP. The influence of an arduous military training program on immune function and upper respiratory tract infection incidence. Mil Med 171: 703-709, 2006.
321. Williams AG and Folland JP. Similarity of polygenic profiles limits the potential for elite human physical performance. J Physiol 586: 113-121, 2008.
322. Wolin KY, Yan Y, Colditz GA and Lee IM. Physical activity and colon cancer prevention: a meta-analysis. Br J Cancer 100: 611-616, 2009.
323. Woodland DL, Hogan RJ and Zhong W. Cellular immunity and memory to respiratory virus infections. Immunol Res 24: 53-67, 2001.
324. Woods JA. Exercise and resistance to neoplasia. Can J Physiol Pharmacol 76: 581-588, 1998.

325. Woods JA, Ceddia MA, Kozak C and Wolters BW. Effects of exercise on the macrophage MHC II response to inflammation. Int J Sports Med 18: 483-488, 1997.
326. Woods JA, Ceddia MA, Wolters BW, Evans JK, Lu Q and McAuley E. Effects of 6 months of moderate aerobic exercise training on immune function in the elderly. Mech Ageing Dev 109: 1-19, 1999.
327. Woods JA, Davis JM, Mayer EP, Ghaffar A and Pate RR. Exercise increases inflammatory macrophage antitumor cytotoxicity. J Appl Physiol 75: 879-886, 1993.
328. Woods JA, Davis JM, Mayer EP, Ghaffar A and Pate RR. Effects of exercise on macrophage activation for antitumor cytotoxicity. J Appl Physiol 76: 2177-2185, 1994.
329. Woods JA, Evans JK, Wolters BW, Ceddia MA and McAuley E. Effects of maximal exercise on natural killer (NK) cell cytotoxicity and responsiveness to interferon-alpha in the young and old. J Gerontol A Biol Sci Med Sci 53: B430-B437, 1998.
330. World Cancer Research Fund. Food, Nutrition, Physical Activity and the Prevention of Cancer: a Global Perspective. Washington DC: AICR 2007.
331. Yan B, A J, Wang G, Lu H, Huang X, Liu Y, Zha W, Hao H, Zhang Y, Liu L, Gu S, Huang Q, Zheng Y and Sun J. Metabolomic investigation into variation of endogenous metabolites in professional athletes subject to strength-endurance training. J Appl Physiol 106: 531-538, 2009.
332. Yang KD, Chang WC, Chuang H, Wang PW, Liu RT and Yeh SH. Increased complement factor H with decreased factor B determined by proteomic differential displays as a biomarker of tai chi chuan exercise. Clin Chem 56: 127-131, 2010.
333. Zahm SH, Hoffman-Goetz L, Dosemeci M, Cantor KP and Blair A. Occupational physical activity and non-Hodgkin's lymphoma. Med Sci Sports Exerc 31: 566-571, 1999.
334. Zieker D, Fehrenbach E, Dietzsch J, Fliegner J, Waidmann M, Nieselt K, Gebicke-Haerter P, Spanagel R, Simon P, Niess AM and Northoff H. cDNA microarray analysis reveals novel candidate genes expressed in human peripheral blood following exhaustive exercise. Physiol Genomics 23: 287-294, 2005.
335. Zieker D, Zieker J, Dietzsch J, Burnet M, Northoff H and Fehrenbach E. CDNA-microarray analysis as a research tool for expression profiling in human peripheral blood following exercise. Exerc Immunol Rev 11: 86-96, 2005.
336. Zielinski MR, Muenchow M, Wallig MA, Horn PL and Woods JA. Exercise delays allogeneic tumor growth and reduces intratumoral inflammation and vascularization. J Appl Physiol 96: 2249-2256, 2004.
337. Zolla L. Proteomics studies reveal important information on small molecule therapeutics: a case study on plasma proteins. Drug Discov Today 13: 1042-1051, 2008.

Position Statement Part two: Maintaining immune health

Neil P. Walsh[1], Michael Gleeson[2], David B. Pyne[3], David C. Nieman[4], Firdaus S. Dhabhar[5], Roy J. Shephard[6], Samuel J. Oliver[1], Stéphane Bermon[7], Alma Kajeniene[8]

[1] School of Sport, Health and Exercise Sciences, Bangor University, UK.
[2] School of Sport, Exercise and Health Sciences, Loughborough University, UK.
[3] Department of Physiology, Australian Institute of Sport, Australia.
[4] Human Performance Labs, North Carolina Research Campus and Appalachian State University, USA.
[5] Department of Psychiatry and Behavioural Sciences and Stanford Institute for Immunity, Transplantation, and Infection, Stanford University, USA.
[6] Faculty of Physical Education and Health, University of Toronto, Canada.
[7] Monaco Institute of Sports Medicine and Surgery (IM_2S), Monaco.
[8] Kaunas Sports Medicine Center and Kaunas University of Medicine, Lithuania.

CONSENSUS STATEMENT

The physical training undertaken by athletes is one of a set of lifestyle or behavioural factors that can influence immune function, health and ultimately exercise performance. Others factors including potential exposure to pathogens, health status, lifestyle behaviours, sleep and recovery, nutrition and psychosocial issues, need to be considered alongside the physical demands of an athlete's training programme.

The general consensus on managing training to maintain immune health is to start with a programme of low to moderate volume and intensity; employ a gradual and periodised increase in training volumes and loads; add variety to limit training monotony and stress; avoid excessively heavy training loads that could lead to exhaustion, illness or injury; include non-specific cross-training to offset staleness; ensure sufficient rest and recovery; and instigate a testing programme for identifying signs of performance deterioration and manifestations of physical stress. Inter-individual variability in immunocompetence, recovery, exercise capacity, non-training stress factors, and stress tolerance likely explains the different vulnerability of athletes to illness. Most athletes should be able to train with high loads provided their programme includes strategies devised to control the overall strain and stress. Athletes, coaches and medical personnel should be alert to periods of increased risk of illness (e.g. intensive training weeks, the taper period prior to competition, and during competition) and pay particular attention to recovery and nutritional strategies.

Correspondence:
Neil Walsh; email: n.walsh@bangor.ac.uk; telephone: +44 1248 383480

Although exercising in environmental extremes (heat, cold, altitude) may increase the stress response to acute exercise and elevate the extent of leukocyte trafficking it does not appear to have marked effects on immune function other than a depression of cell-mediated immunity when training at altitude. The available evidence does not support the contention that athletes training and competing in cold (or hot) conditions experience a greater reduction in immune function compared with thermoneutral conditions. Nevertheless, it remains unknown if athletes who regularly train and compete in cold conditions report more frequent, severe or longer-lasting infections. Research should identify whether the airway inflammation associated with breathing large volumes of cold dry air or polluted air impairs airway defences and whether athletes (and their physicians) wrongly interpret the sore throat symptoms that accompany exercising in cold or polluted air as an infection.

Elite athletes can benefit from immunonutritional support to bolster immunity during periods of physiological stress. Ensuring adequate energy, carbohydrate and protein intake and avoiding deficiencies of micronutrients are key to maintaining immune health. Evidence is accumulating that some nutritional supplements including flavonoids such as quercetin and *Lactobacillus* probiotics can augment some aspects of immune function and reduce illness rates in exercise-stressed athletes. Limited data are non-supportive or mixed for use of N-3 polyunsaturated fatty acids, β-glucans, bovine colostrums, ginseng, echinacea or megadoses of vitamin C by athletes.

Relatively short periods of total sleep deprivation in humans (up to 3 consecutive nights without sleep) do not influence the risk of infection, and the reported increase in natural killer cell activity with this duration of total sleep deprivation would seem to rule out the possibility of an "open-window" for respiratory infections. Very little is known about the effects of more prolonged sleep disruption and repeated sleep disturbances on immune function and infection incidence, although recent studies have highlighted the importance of sleep quantity (total duration of sleep per night) and quality (number of awakenings per night) to protect against the common cold in healthy adults.

Short- or long-term exercise can activate different components of a physiological stress response. Prolonged intense exercise may induce negative health consequences, many of which may be mediated by physiological pathways activated by chronic stress. Psychological stress is likely additive to the effects of physical stress and whereas short exposures to both physical or psychological stress can have a beneficial effect on immune function, chronic exposure to stress exerts detrimental effects on immune function and health. However, regular moderate exercise could be an important factor in ameliorating the negative health effects of chronic stress via the optimization and maintenance of the survival-promoting physiological changes induced by the short-term or acute stress response. Further research on mechanisms mediating the salubrious effects of exercise, and on the relationship between exercise and the psychosocial stress-status of an individual, is likely to be helpful for more fully and widely harnessing the health benefits of exercise.

It is agreed by everyone that prevention of infection is always superior to treatment and this is particularly true in athletes residing in countries with limited medical facilities. Although there is no single method that completely eliminates the risk of contracting an infection, there are several effective ways of reducing the number of infectious episodes incurred over a given period. These means of reducing infection risk include appropriate management of training loads, use of appropriate recovery strategies, good personal hygiene, avoiding contact with large crowds, young children and sick people, good nutrition, getting adequate good quality sleep and limiting other life stresses to a minimum. Part two of the position statement includes sections on: training considerations (David Pyne); nutritional countermeasures to exercise-induced immune perturbations (David Nieman); effects of stress on immune function (Firdaus Dhabhar); sleep disruption and immune function (Roy Shephard); environmental extremes and the immune response to exercise (Neil Walsh and Samuel Oliver) and finally, prevention and treatment of common infections (Stéphane Bermon and Alma Kajeniene).

Key Words: exercise; sport; immune; leukocyte; pathogen; infection; training; overtraining; overreaching; adaptation; diet; supplement; stress; in vivo; sleep; environment; treatment; prevention

TRAINING CONSIDERATIONS

Background

There is considerable incentive for athletes, coaches, and teams to implement practical strategies that limit the risk of training-related perturbations in immune function. The physical training undertaken by athletes is one of a set of lifestyle or behavioural factors that can influence immune function, health and ultimately exercise performance. Other factors including health status, lifestyle behaviours, pathogen transmission, nutrition and psychosocial issues, need to be considered alongside the physical demands of an athlete's training programme. Guidelines on prescribing training to keep athletes healthy are sought-after in the sporting community.

The challenge of preparing guidelines for prescribing training in the absence of specific experimental studies has been acknowledged (8, 134). There are only a few training studies that have directly examined the relationship between training loads and patterns of illness in highly trained athletes, and the effectiveness of various training and lifestyle interventions – see reviews (85, 171) and the respiratory infections and exercise section in part one of this position statement. It is difficult to study elite athletes in their regular training environment especially during preparations for major competition. Experimental control of training, lifestyle and dietary practices, and other confounders such as time missed with injury can be problematic. Investigators have generally used moderately active individuals, often volunteers in graduate research programmes, as participants in exercise immunology studies. The predominance of short cross-sectional studies of the acute effects of exercise rather than long-term prospective studies of athletes in training over weeks, months or years is another issue (85). The limited number of experimental studies makes it difficult to develop definitive practical guidelines for athletes, coaches, clinicians and team officials.

To overcome the shortage of studies, clinicians and scientists working with athletes need to translate and apply selected findings of studies in related fields. Research areas including clinical immunology, nutritional immunology, sports medicine, exercise physiology, psychoneuroimmunology and sports psychology should yield useful insights. Moderate physical activity may enhance immune function and reduce infection incidence mainly in less fit subjects, and pre-event fitness status can also influence the risk of illness (185). However, results from studies involving sedentary or only moderately active individuals may not easily translate to highly trained athletes. Guidelines for maintaining good health (as discussed later in this part of the position statement) and training will also depend on the experience, skills and knowledge of coaches, athletes, clinicians and scientists.

In most sports it is accepted that there exists a dose-response relationship between training and performance (7). Athletes in endurance sports generally require high training volumes to develop the background necessary for success in high-level competition. Sudden increases in either training volume or intensity, or in combination, may place additional pressure on immune function. Post-exercise immune function dysfunction is most pronounced when the exercise is continuous, prolonged (>1.5 h), of moderate to high intensity (55–75% maximal O_2 uptake), and performed with minimal nutritional support (85) (as discussed in the following section). The risk of developing symptoms of non-functional overreaching (short-term decrements in performance capacity where the athlete is unable to recover fully after sufficient rest) or overtraining (long-term decrements that may take several weeks or months to resolve) (131) can be increased by monotonous training without alternating hard and easy training days, a lack of a complete rest day once per week, increasing loads when the total load is already high, and too many competitions (171). In terms of planning and monitoring, integrated indices of training loads in a multivariate model are likely to be more highly correlated with illness than individual factors such as training load, volume or intensity (72). An imbalance between training loads and recovery is also a major contributor to the onset of fatigue, overtraining and illness (141). A well planned recovery programme is essential if athletes are to stay healthy and be ready to perform at their best.

Consensus

The general consensus on managing training to maintain immune health is to start with a programme of low to moderate volume and intensity; employ a gradual and periodised increase in training volumes and loads; add variety to limit training monotony and stress; avoid excessive training distances that could lead to exhaustion, illness or injury (75); include non-specific cross training to offset staleness; ensure sufficient rest and recovery; and instigate a testing programme for identifying signs of performance deterioration and manifestations of physical stress (85, 171). Inter-individual variability in recovery, exercise capacity, non-training stress factors, and stress tolerance likely explains the differential vulnerability of athletes to illness (172). Most athletes should be able to train with high loads provided their programme includes strategies devised to control the overall strain and stress (Table 1). Athletes should be encouraged to undertake intensive training in

the knowledge that variations in performance and fatigue are symptoms to be expected and respected, and not necessarily problems to overcome (206). Athletes, coaches and medical personnel should be alert to these periods of increased risk of illness (e.g. intensive training weeks, the taper period prior to competition, and during competition) and pay particular attention to recovery and nutritional strategies (151).

Table 1. Suggested strategies for modifying training and recovery activities to limit the risk of training-induced impairments in immune health.

Training Descriptor	**Comment**
Frequency	Increase the frequency of shorter training sessions rather than enduring fewer but longer sessions.
Volume	Reduce the overall weekly training volume and/or volume of individual training sessions.
Intensity	Avoid prolonged intensive training sessions or activities. Employ shorter sharper (spike) sessions mixed with lower-intensity work.
Load (volume x intensity)	Systematically manipulate the training volume and/or intensity to manage the degree of training load.
Load increments	Reduce the size of increments in frequency, volume, intensity and load of training e.g. increases of 5-10% per week rather than 15-30%.
Load sequencing – weekly microcycle	Undertake two or three easy-moderate training sessions after each high intensity session rather than the traditional pattern of simply alternating hard – easy sessions.
Load sequencing – multi-week macrocyle	Plan an easier recovery or adaptation week every 2nd or 3rd week rather than using longer 3 – 6 week cycles with increasing loads.
Recovery – session/week	Implement recovery activities immediately after the most intensive or exhaustive training sessions.
Recovery - season	Permit athletes at heightened risk of illness a longer period of passive and active recovery (several weeks) after completion of a season or major competition.

Controversies

Studies are often limited by: using participants with moderate fitness rather than highly trained athletes; poor description or omission of training details; absence of a suitable control group; and, a modest sample size that reduces statistical power. Changes in immune function after exercise are often transient and small in magnitude (106). Although a substantial amount of research has been conduct-

ed, several important questions remain unanswered. Are different guidelines needed for (previously) sedentary individuals, moderately active and highly-trained athletes? How much exercise or training is too much? Should guidelines be general or sports-specific? Which are the best clinical signs and symptoms of overtraining or impending illness (37)? Which diagnostic tests are useful in monitoring immune status (3)? A section in part one of this position statement highlights the strengths and weaknesses of various methods used to assess immune status and the challenges associated with interpreting the clinical significance of results from these tests. What is the relative effectiveness of other tactics such as nutritional countermeasures (see section that follows), sleep (see sleep disruption section in this part of the position statement) and recovery interventions (111, 181)? Given limitations in time, money and resources, coaches are often unable to implement every strategy and a process of prioritising training, recovery and behavioural interventions is necessary.

Future directions

A systematic programme of clinical and experimentally controlled research is needed to formulate evidence-based training guidelines or recommendations to maintain immune health in athletes. Studies are needed with both recreational and elite athletes. Modelling studies of responses to physical training (16) should shed light on the relative influence of training volume, intensity and loads on the immune system. Molecular biology is already yielding some insights for identifying athletes more at risk of illness (36) and should further our understanding of how the immune system responds to various types of training. For a more detailed account of a role for "omics" in exercise immunology, readers are directed to the "omics" section in part one of this position statement. Studies should also address how individual variations in the risk of illness relate to training (172). A combination of field-based diagnostic technology, experimental research, insightful analytical approaches (99), and the clinical/practical experience of physicians and athletes/coaches is likely to be the most effective approach for managing the training and immunity of athletes.

NUTRITIONAL COUNTERMEASURES TO EXERCISE-INDUCED IMMUNE PERTURBATIONS

Background

Nutrition, exercise, mental stress, and other lifestyle factors influence immune function and the risk of certain types of infection such as upper respiratory tract infections (URTI). In contrast to moderate physical activity, prolonged and intensive exertion by athletes causes numerous changes in immunity in multiple body compartments and an increased risk of URTI (150). Elite athletes must train intensively to compete at the highest levels and they can benefit from immunonutritional support to bolster immunity during periods of physiological stress (151). Non-athletes engaging in moderate physical activity programmes do not require nutritional supplements, and can obtain all needed nutrients from a healthy and balanced diet.

Each acute bout of heavy exertion leads to physiological stress and transient but clinically significant changes in immunity and host pathogen defence, with elevations in stress hormones, pro- and anti-inflammatory cytokines, and reactive oxygen species (85, 148). Natural killer cell activity, various measures of T and B cell function, upper airway neutrophil function, salivary IgA concentration, granulocyte oxidative burst activity, skin delayed-type hypersensitivity response, and major histocompatibility complex (MHC) II expression in macrophages are suppressed for at least several hours during recovery from prolonged, intense endurance exercise (as discussed in detail in part one of this position statement). These immune changes occur in several compartments of the immune system and body (e.g., the skin, upper respiratory tract mucosal tissue, lung, blood, muscle, and peritoneal cavity).

During the "open window" of impaired immunity (which may last between three and 72 hours, depending on the immune measure), pathogen resistance is lowered, increasing the risk of subclinical and clinical infection (150). Epidemiological studies indicate that athletes engaging in marathon and ultramarathon race events and/or very heavy training are at increased risk of URTI (150) (as described in the section on respiratory infections and exercise in part one of this position statement). Together, these epidemiological and exercise immunology studies support the viewpoint that heavy exercise workloads increase URTI risk through altered immune function.

Consensus

Various nutritional agents have been tested for their capacity to attenuate immune changes and inflammation following intensive exercise, thus lowering the magnitude of physiologic stress and URTI risk. This strategy is similar to the immunonutritional support provided to patients recovering from trauma and surgery, and to the frail elderly (151). Some question the value of using immunonutritional support for athletes because blocking the transient immune changes, oxidative stress, and inflammation following heavy exertion interferes with important signaling mechanisms for training adaptations (88, 182). Another viewpoint is that efficacious nutritional supplements only partially block exercise-induced immune dysfunction, inflammation, and oxidative stress, analogous to the beneficial use of ice packs to reduce swelling following mild injuries (209, 225). This debate will hopefully spur additional research on the overall value of immunonutritional support for athletes.

Table 2 summarizes published findings on a variety of supplements, with a focus on those investigated by several different research groups on human athletes. Results for most nutritional supplements tested as countermeasures to exercise-induced inflammation, oxidative stress, and immune dysfunction following heavy exertion have been disappointing. Early studies focused on large dose vitamin and/or mineral supplements, and no consistent countermeasure benefit has been observed (41, 42, 87, 157, 158). A series of studies dating back to the mid-1990s have shown that carbohydrate supplement ingestion before and/or during prolonged exercise attenuates increases in blood neutrophil and monocyte counts, stress hormones, and anti-inflammatory cytokines such as interleukin (IL)-6, IL-10, and IL-1ra, but has little effect on decrements in salivary IgA output and T cell

and natural killer cell function (26, 41, 85, 149, 153). Thus, carbohydrate ingestion during heavy exercise has emerged as an effective but partial countermeasure to immune dysfunction, with favourable effects on measures related to stress hormones and inflammation, but with limited effects on markers of innate or adaptive immunity. Glutamine and amino acid supplements are not recommended because the best studies show no benefits when compared to placebo, perhaps due to abundant storage pools within the body that cannot be sufficiently depleted by exercise (85, 86, 113).

Controversies and future directions

The growing realization that extra vitamins, minerals, and amino acids do not provide countermeasure benefits for healthy and well-fed athletes during heavy train-

Table 2. Summary of rationale and findings for selected immunonutritional supplements.

Immunonutritional Supplement	Proposed Rationale	Recommendation Based On Current Evidence
Vitamin E	Quenches exercise-induced reactive oxygen species (ROS) and augments immunity	Not recommended; may be pro-oxidative with heavy exertion
Vitamin C	Quenches ROS and augments immunity	Not recommended; not consistently different from placebo
Multiple vitamins and minerals	Work together to quench ROS and reduce inflammation	Not recommended; not different from placebo; balanced diet is sufficient
Glutamine	Important immune cell energy substrate that is lowered with prolonged exercise	Not recommended; body stores exceed exercise-lowering effects
Branched chain amino acids (BCAAs)	BCAAs (valine, isoleucine, and leucine) are the major nitrogen source for glutamine synthesis in muscle	Not recommended; data inconclusive, and rationale based on glutamine is faulty
Carbohydrate	Maintains blood glucose during exercise, lowers stress hormones, and thus counters immune dysfunction	Recommended; up to 60 g per hour of heavy exertion helps dampen immune inflammatory responses, but not immune dysfunction
Bovine colostrums	Mix of immune, growth, and hormonal factors improve immune function and the neuroendocrine system, and lower illness risk	Jury still out, with mixed results
Probiotics	Improve intestinal microbial flora, and thereby enhance gut and systemic immune function	Jury still out, with mixed results
N-3 PUFAs (fish oil)	Exerts anti-inflammatory effects post-exercise	Not recommended; no different from placebo
β-glucan	Receptors found on immune cells, and animal data show supplementation improves innate immunity and reduces infection rates	Not recommended; human study with athletes showed no benefits
Herbal supplements (e.g., Ginseng, Echinacea)	Contain bioactive molecules that augment immunity and counter infection	Not recommended; humans studies do not show consistent support within an athletic context
Quercetin	*In vitro* studies show strong anti-inflammatory, anti-oxidative, and anti-pathogenic effects. Animal data indicate increase in mitochondrial biogenesis and endurance performance, reduction in illness	Recommended, especially when mixed with other flavonoids and nutrients; human studies show strong reduction in illness rates during heavy training and mild stimulation of mitochondrial biogenesis and endurance performance in untrained subjects; anti-inflammatory and anti-oxidative effects when mixed with green tea extract and fish oil

ing has shifted the focus to other types of nutritional components. *In vitro*/cell culture, animal, and epidemiological research indicate that advanced supplements such as probiotics, bovine colostrum, β-glucan, flavonoids and polyphenols such as quercetin, resveratrol, curcumin, and epigallicatechin-3-gallate (EGCG), N-3 polyunsaturated fatty acids (N-3 PUFAs or fish oil), herbal supplements, and unique plant extracts (e.g., green tea extract, blackcurrant extract, tomato extract with lycopene, anthocyanin-rich extract from bilberry, polyphenol-rich pomegranate fruit extract), warrant well-conducted studies with athletes to determine if they are effective countermeasures to exercise-induced immune dysfunction and risk of URTI (6, 124, 144, 152, 155). Limited data are non-supportive or mixed for use of N-3 PUFAs (156), probiotics (221), bovine colostrums (202), ginseng (196), or Echinacea (196) by athletes.

An evolving hypothesis is that the immune system is so diverse that a mixture of these advanced supplements, perhaps within a carbohydrate beverage, will probably perform better than one supplement by itself (6, 156). The "pharma" approach of using large doses of a single molecule is not as effective as a "cocktail" strategy for nutritional supplements.

A secondary hypothesis is that the primary immune target of nutrient supplements should be the nonspecific, innate arm of the immune system to enhance immunosurveillance against a wide variety of pathogens in athletes. If the nutritional supplement improves natural killer cell, macrophage, and granulocyte function before and/or after heavy exertion, then risk of infection is more effectively countered than when the target is the slower moving adaptive immune components (154, 155, 159).

Some nutritional supplements exert impressive effects *in vitro* and in animal-based models, but then fail when studied under double-blinded, placebo-controlled conditions in human athletes. A prime example is β-glucan, a polysaccharide found in the bran of oat and barley cereal grains, the cell wall of baker's yeast, certain types of fungi, and many kinds of mushrooms. Rodent studies indicate that oat β-glucan supplements offset the increased risk of infection associated with exercise stress through augmentation of macrophage and neutrophil function, but these results were not upheld in a study of human cyclists (144, 159).

The physiologic effects of dietary polyphenols such as quercetin, EGCG, curcumin, lycopene, resveratrol, luteolin, and tiliroside are of great current interest to exercise immunologists due to their antioxidative, anti-inflammatory, anti-pathogenic, cardioprotective, anticarcinogenic, and mitochondrial stimulatory activities (151, 152). Several recent quercetin supplementation studies in human athletes have focused on potential influences as a countermeasure to post-exercise inflammation, oxidative stress, and immune dysfunction, in improving endurance performance, and in reducing illness rates following periods of physiologic stress (162). When quercetin supplementation is combined with other polyphenols and food components such as green tea extract, isoquercetin, and fish oil, a substantial reduction in exercise-induced inflammation and oxidative stress occurs in athletes, with chronic augmentation of innate immune function (155). Quercetin

supplementation (1,000 mg/day for two to three weeks) also reduces illness rates in exercise-stressed athletes (154). Animal studies support a role for quercetin as an exercise mimetic for mitochondrial biogenesis, and recent data in untrained human subjects indicate modest enhancement in skeletal muscle mitochondrial density and endurance performance (162). Quercetin has multiple bioactive effects that support athletic endeavour, and research continues to define optimal dosing regimens and adjuvants that amplify these influences (152, 162).

Summary remarks

Endurance athletes must train hard for competition and are interested in strategies to keep their immune systems robust and to avoid illness despite the physiologic stress they experience. The ultimate goal is to provide athletes with a sports drink or supplement bar containing carbohydrate and a cocktail of advanced supplements that will lower infection risk, exert significant and measurable influences on their innate immune systems, and attenuate exercise-induced oxidative stress and inflammation. The athlete can combine this strategy with other approaches that help maintain immunity and health.

EFFECTS OF STRESS ON IMMUNE FUNCTION – IMPLICATIONS FOR THE EFFECTS OF EXERCISE ON HEALTH

Understanding the psychological, biological, and health effects of exercise in the context of stress and stress physiology is important for several reasons: First, the process of exercising induces a physiological stress response and increases circulating concentrations of noradrenaline (norepinephrine), adrenaline (epinephrine), cortisol, and other stress-related factors including cytokines (93, 166). An acute or short-term stress response can have beneficial effects. However, intense prolonged exercise may induce negative health consequences, many of which may be mediated by physiological pathways activated by chronic stress (85). Secondly, exercise, when performed under the appropriate conditions, could be a factor in ameliorating the deleterious health effects of chronic stress and increased allostatic load (viz. the physiological cost that results from ongoing adaptive efforts to maintain homeostasis in response to stressors) (128, 223). A novel and important mechanism mediating the salubrious effects of exercise could be through its optimization of the beneficial, survival-promoting effects of the short-term or acute stress response (44). Thirdly, the psychosocial stress status of an individual may be important for determining whether a given exercise regimen is salubrious or harmful.

Although the word "stress" generally has negative connotations, stress is a familiar and ubiquitous aspect of life, being a stimulant for some, and a burden for many. Numerous definitions have been proposed for stress, each focusing on aspects of an internal or external challenge/stimulus, on stimulus perception, or on a physiological response to the stimulus (190). An integrated definition proposes that *stress is a constellation of events, consisting of a stimulus (stressor), that precipitates a reaction in the brain (stress perception), that activates physio-*

logical fight or flight systems in the body (stress response) (46). The stress response induces the release of the principal stress hormones (noradrenaline, adrenaline, and cortisol/corticosterone) as well as a myriad of neurotransmitters, hormones, peptides, cytokines and other factors. Since virtually every cell in the body expresses receptors for one or more of these factors, all cells and tissues can receive biological signals that alert them regarding the presence of a stressor. The only way that a stressor can affect brain, body, and health is by inducing biological changes through a physiological stress response.

Although stress can be harmful when it is chronic or long lasting (43, 82, 128), a short-term fight-or-flight stress response has salubrious adaptive effects (44, 45, 50). Therefore, the duration of stress is an important factor in determining its effects on immune function and health. *Acute stress* has been defined as stress that lasts for a period of minutes to hours, and *chronic stress* as stress that persists for several hours per day for weeks or months (46). Dysregulation of the circadian cortisol rhythm is one marker that is related to the deleterious effects of chronic stress (46, 192). It is important to note that there are significant individual differences in stress perception, processing, and coping that mediate differences in the intensity and duration of a physiological response to a given stressor (32, 49, 50, 92). It is known that chronic or long-term stressors can have adverse effects on health, many of which may be mediated through immune mechanisms. However, it is important to recognize that a psycho-physiological stress response is one of nature's fundamental survival mechanisms (44). Without a fight-or-flight stress response, a lion has no chance of catching a gazelle, just as the gazelle has no chance of escape. During such short-term stress responses observed in nature, physiological systems act in synchrony to enable survival. Therefore, it was hypothesized that just as the stress response prepares the cardiovascular, musculoskeletal and neuroendocrine systems for fight or flight, under certain conditions, stress may also prepare the immune system for challenges (e.g. wounding or infection) that may be imposed by a stressor (e.g. predator or surgical procedure) (48, 50). Short duration stressors induce a redistribution of immune cells within the body and immune function is significantly enhanced in organs like the skin to which leukocytes traffic during acute stress. Studies have also identified mechanisms involving dendritic cell, neutrophil, macrophage, and lymphocyte trafficking, maturation, and function through which acute stressors may enhance innate as well as adaptive immunity.

Effects of acute versus chronic stress on immune cell distribution

Effective immunoprotection requires rapid redistribution and recruitment of leukocytes into sites of surgery, wounding, infection, or vaccination. Numerous studies have shown that stress and stress hormones induce significant changes in absolute numbers and relative proportions of leukocytes in the blood (9, 48, 52, 69, 194). An acute stress-induced redistribution of leukocytes within different body compartments is perhaps one of the most under-appreciated effects of stress (51). Acute stress induces an initial increase followed by a decrease in blood mononuclear leukocyte numbers (48, 187). Stress conditions that result in activation of the sympathetic nervous system induce an increase in circulating leukocyte numbers (both mononuclear and polymorphonuclear cells). These conditions

may occur during the beginning of a stress response, very short duration stress (order of minutes), mild psychological stress, or during exercise. In contrast, stress conditions that result in the activation of the hypothalamic-pituitary-adrenal axis induce a decrease in circulating mononuclear cell (viz. lymphocyte and monocyte) numbers. These conditions often occur during the later stages of a stress response, exposure to long duration acute stressors (order of hours), or during severe stress or prolonged and/or intense exercise. An elegant example comes from Schedlowski et al. who measured changes in blood T cell and natural killer (NK) cell numbers as well as plasma catecholamine and cortisol levels in parachutists 2 hours before, immediately after, and 1 hour after a jump (193). Results showed a significant increase in T cell and NK cell numbers immediately (minutes) after the jump that was followed by a significant decrease an hour later. An early increase in plasma catecholamines preceded early increases in lymphocyte numbers, whereas the more delayed rise in plasma cortisol preceded the later decrease in lymphocyte numbers (193). Importantly, changes in NK cell activity and antibody-dependent cell-mediated cytotoxicity closely paralleled changes in blood NK cell numbers, thus suggesting that changes in leukocyte numbers may be an important mediator of apparent changes in leukocyte "functional activity." A similar profile of changes in lymphocyte and monocyte numbers has been characterized in patients experiencing surgery stress and has been related to successful postsurgical recovery (187).

Thus, an acute stress response induces biphasic changes in blood leukocyte numbers. Soon after the beginning of stress (order of minutes) or during mild acute stress, or exercise, the body's "soldiers" (leukocytes), exit their "barracks" (spleen, lung, marginated pool and other organs) and enter the "boulevards" (blood vessels and lymphatics). This results in an increase in blood leukocyte numbers, the effect being most prominent for NK cells and polymorphonuclear granulocytes. As the stress response continues, leukocytes exit the blood and take position at potential "battle stations" (such as the skin, lung, gastro-intestinal and urinary-genital tracts, mucosal surfaces, and lymph nodes) in preparation for immune challenges which may be imposed by the actions of the stressor (45, 48, 50). Such a redistribution of leukocytes results in a decrease in blood mononuclear leukocyte numbers. Thus, acute stress induces a redistribution of several leukocyte subsets from the barracks, through the boulevards, and to potential battle stations within the body. It is important to note that in addition to leukocyte redistribution, acute stressors also enhance immune function through additional mechanisms involving dendritic cell, neutrophil, macrophage, and lymphocyte trafficking, maturation, and function (215).

In contrast to acute stress, chronic stress induces deleterious changes in leukocyte numbers. First, exposure to chronic stress results in lower resting-state immune cell numbers that would imply a diminished capacity to mount immune responses (46). Secondly, exposure to chronic stress decreases the magnitude of acute stress-induced immune cell redistribution (46). In effect, chronic stress reduces the number of "soldiers" in the body's army, and reduces the capacity of the remaining leukocytes to mobilize from "boulevards to battle stations" during a fight-or-flight response.

Acute stress psychophysiology as an endogenous adjuvant

It has been proposed that a psycho-physiological stress response is nature's fundamental survival mechanism that could be harnessed therapeutically to augment immune function during vaccination, wound healing or infection (54). These adjuvant-like immuno-enhancing effects of acute stress may have evolved because many stressful situations (aggression, accident) result in immune activation (wounding, infection) and vice versa. Interestingly, in modern times, many medical procedures involving immune activation (vaccination, surgery) also induce a stress response. In keeping with the above hypothesis, studies have shown that patients undergoing knee surgery, who show a robust and adaptive immune cell redistribution profile during the acute stress of surgery, also show significantly enhanced recovery (187). Similarly, an elegant series of adjuvant effects of acute mental stress or exercise can enhance vaccine-induced humoral and cell-mediated immunity in human subjects (60, 62) (for review see: (61)). Although acute stress- (or exercise-) induced immunoenhancement may serve to increase immunoprotection during vaccination, infection, or wounding, it may also exacerbate immunopathology if the enhanced immune response is directed against innocuous or self-antigens, or becomes dysregulated following prolonged activation as seen during chronic stress.

Numerous studies have been conducted to elucidate mechanisms mediating acute stress-induced enhancement of immune function. Viswanathan and Dhabhar (216) used a subcutaneously implanted surgical sponge model to elucidate the effects of stress on the kinetics, magnitude, subpopulation, and chemoattractant specificity of leukocyte trafficking to a site of immune activation or surgery. Results showed that an acute stress-induced increase in leukocyte trafficking coupled with specific chemokines and cytokines released during the initiation cascades of inflammation can alter the course of different (innate versus adaptive, early versus late, acute versus chronic) protective or pathological immune responses (216). Since the skin is one target organ to which leukocytes traffic during stress, studies were conducted to examine whether skin immunity is enhanced when immune activation/antigen exposure occurs following a stressful experience. Studies showed that acute stress experienced at the time of novel or primary antigen exposure results in a significant enhancement of the ensuing skin immune response (54). Compared to controls, mice restrained for 2.5 hours before primary immunization with keyhole limpet haemocyanin (KLH) showed a significantly enhanced immune response when re-exposed to KLH nine months later. This immunoenhancement was mediated by an increase in numbers of memory and effector helper T cells in sentinel lymph nodes at the time of primary immunization. Further analyses showed that the early stress-induced increase in T cell memory was followed by a robust increase in infiltrating lymphocyte and macrophage numbers months later at a novel site of antigen re-exposure. Enhanced leukocyte infiltration was driven by increased levels of the Type-1 cytokines, interleukin (IL)-2, interferon-γ (IFN-γ) and tumour necrosis factor-α observed at the site of antigen re-exposure. Other investigators have similarly reported stress-induced enhancement of Type-1 cytokine driven cell-mediated immunity (13, 189, 222) and Type-2 cytokine driven humoral immunity (Type-2 cytokines include for example IL-4 and IL-10) (30, 222). Viswanathan et al. (215)

further showed that important interactive components of innate (dendritic cells and macrophages) and adaptive (surveillance T cells) immunity are mediators of the stress-induced enhancement of a primary immune response. Although much work remains to be done, to identify further molecular, cellular, and physiological mechanisms, studies have also identified endocrine and immune mediators of these effects showing that corticosterone and adrenaline are important systemic mediators and IFN-γ is an important local mediator of immunoenhancement induced by acute stress (47, 53).

Effects of chronic stress on immune function

The immuno-suppressive and dysregulatory effects of chronic stress have been reviewed extensively (2, 33, 64, 82, 101). Chronic stress is known to dysregulate immune responses (82) by altering the cytokine balance from Type-1 to Type-2 cytokine-driven responses (83) and accelerating immunosenescence (65), and to suppress immunity by decreasing numbers (46), trafficking (46), and function of protective immune cells while increasing regulatory/suppressor T cells (192). Through these effects, chronic stressors are thought to exacerbate pro-inflammatory diseases and increase susceptibility to infections and cancer (44). Exercise and cancer is discussed in detail in part one of the position statement.

Importance of relationship between stress and exercise

Understanding the psychological, physiological, and health effects of exercise in the context of stress and stress physiology is critical for several important reasons: First, the process of exercising invariably induces a physiological stress response and results in higher circulating concentrations of noradrenaline, adrenaline, cortisol, other stress-related factors, and even cytokines (93, 166). Exercise-induced pain, exhaustion, or injury could also induce psychological stress. Moreover, intense prolonged exercise (85) or exercising under extreme environmental conditions (218), may lead to chronic exposure to stress hormones which may make the individual susceptible to the deleterious health effects of chronic stress. Thus, short- or long-term exercise can activate different components of a physiological stress response. The relative concentrations of exercise-induced stress-related hormones, cytokines and other factors induced in the body are likely to depend on a host of factors including the genetic makeup, psycho-physiological health, and fitness of the individual, as well as the type, intensity, duration, and chronicity of exercise. Since immune cells and organs have receptors for, and respond to, the myriad of stress-related physiological factors that are released during exercise, many effects of exercise on the immune system are likely to be mediated by these factors. Secondly, when performed under appropriate conditions, exercise could be a significant factor in ameliorating the deleterious health effects of chronic stress (169, 223). The type, intensity, duration and frequency of exercise and the conditions under which it should be performed in order to effectively reduce the stress burden of different individuals need to be understood and defined clearly. It is likely that one would need different strokes for different folks, i.e., running could serve as a "de-stressor" for some while others would benefit from aerobics, swimming, dancing or yoga. The most desirable results are likely to arise when the physical as well as psychosocial aspects of the exercise are matched with factors such as the fitness, capability, temperament, personality, etc., of the exercis-

ing individual. Thirdly, the psychosocial stress status of an individual may affect the relationship between exercise and health positively or negatively. For example, a chronically stressed individual may react differently to the effects of exercise, and may have lower thresholds for exercise-induced wear and tear compared to someone who is not chronically stressed. This is an area of research that is ripe for investigation and is relevant for the well-being of recreational and elite athletes, as well as armed forces and other professions for whom exercise is a critical aspect of training and job-performance.

Conclusion

Exercise and stress are intricately linked. Exercise induces a physiological stress response. Intense and/or prolonged exercise may induce negative health consequences, many of which may be mediated by physiological pathways activated by chronic stress. However, moderate exercise could be an important factor in ameliorating the negative health effects of chronic stress. Moreover, the stress status of an individual could in turn affect the degree and extent of the salubrious effects of exercise. One important mechanism mediating the salubrious effects of exercise could be the optimization and maintenance of the survival-promoting physiological changes induced by the short-term or acute stress response. Further research into the effects of exercise and stress on immune function and health, on mechanisms mediating the salubrious effects of exercise, and on the relationship between exercise and the psychosocial stress-status of an individual, is likely to be helpful for harnessing the health benefits of exercise more fully and widely.

SLEEP DISRUPTION AND IMMUNE FUNCTION

Background

There seems quite a close interaction between immune function and sleep. In laboratory animals the intracerebral infusion of interleukin (IL)-1, interferon-γ (IFN-γ) or tumour necrosis factor-α (TNF-α) tends to induce sleep (112, 164), and studies of circulating cytokine levels in patients with excessive daytime sleepiness suggest that these same factors influence human sleep patterns (91, 214). Associations have also been observed between abnormalities of immune function and various forms of sleep disruption of interest to the exercise scientist. Issues include sleep deprivation, shift work, and disturbances of the circadian rhythm associated with global travel. However, it has been difficult to determine whether the observed changes in immune responses reflect a disturbance of sleep per se, disturbances of the circadian periodicity of hormone secretions (114, 145, 213), a general stress response, or a cognitive reaction to loss of sleep.

The following is a brief review of the impact of various types of sleep disturbance upon immune responses, noting the practical significance for the physically active individual.

Sleep deprivation

Sleep deprivation may be acute (for example, because of the anxiety associated with international competition, or the demands of extended military combat (20)), or chronic (due to pain, or the obstructed breathing associated with severe obesity or airway congestion due to respiratory infection). Although abnormalities of immune function have been described in these various situations, they reflect in part such factors as overall anxiety, very prolonged exercise, and a shortage or excess of food rather than a direct influence of sleep deprivation upon the immune system.

Animal studies have failed to demonstrate consistent immunological responses, perhaps because of problems in enforcing wakefulness in rats and mice. In laboratory studies of humans, some authors have noted alterations of immune function after 4-5 hours of sleep disturbance, but others have not seen changes unless participants remained awake for several days. One study found that keeping healthy volunteers awake between 22:00 and 03:00 led to decreases in both total natural killer (NK) cell activity and activity per NK cell, total lymphokine activated killer cell activity and activity per precursor cell (CD16+56+ cells and CD25+ cells), together with a decrease in concanavalin-stimulated IL-2 production (100). After a night of recovery sleep, NK cell activity was restored, but IL-2 levels remained depressed. By using actigraphy to monitor sleep, a recent study showed decreased NK cell mobilization in response to a cognitive stress test in healthy women who had experienced disrupted sleep (224). Indeed, wrist-mounted actigraph movement monitors may present a simple and inexpensive method to monitor sleep quantity and quality in athletes and soldiers. Sleep deprivation from 23:00 to 03:00 has also been shown to induce markers of inflammation, particularly in women; this is thought to be secondary to an activation of nuclear factor-kappa B, and an up-regulation of pro-inflammatory genes (103). In consequence, increases in lipopolysaccharide-stimulated production of IL-6 and TNF-α have been observed (102), together with increased levels of C-reactive protein (132). CD4+, CD16+, CD56+ and CD57+ lymphocyte counts were decreased after one night without sleep (57), in a manner reminiscent of exposure to other forms of stress (166). More prolonged sleep deprivation leads to increases in leukocyte, granulocyte and monocyte counts and the proportion of lymphocytes in the S phase of the cell cycle (57), with enhanced NK cell activity, interferon production and IL-1 and IL-2 like activity, and increased levels of C-reactive protein (57, 132, 165). However, some authors have found that the increase of NK cell activity is a relatively late phenomenon, seen after 64 h (57) but not 40 h of sleep deprivation (138). Recovery of the various immune parameters follows a similar pattern to the restoration of neuro-behavioural function, suggesting a relationship between immunological change and biological pressures to sleep.

Laboratory studies have also shown small decrements in parameters such as maximal oxygen intake (39) and endurance exercise performance (163) following one or more nights without sleep. One practical consequence is that an individual who attempts to maintain a given submaximal exercise intensity must use a larger fraction of maximal aerobic power, thereby potentially exaggerating normal immune responses to vigorous exercise.

Shift work

Shift work is of two main types- an 8-h rotating shift (which requires repeated displacements of the individual's circadian rhythm), and prolonged periods of night work (which increase a person's total exposure to light, often with disruptions of normal social life). Adverse effects seem linked mainly to prolonged periods of night work (40). Such employment is associated with an increased risk of breast, prostate and colon cancers (34, 40). Plainly, the socio-economic, demographic, dietary and lifestyle characteristics of shift workers could contribute to this risk. Exposure to light during the night hours decreases body concentrations of melatonin, thus stimulating the hypothalamic-pituitary-gonadal axis, and causing an increased production of testosterone and/or oestrogen (95, 207). Other investigators have postulated that prolonged night work alters the balance of cytokines that regulate tumour growth. In their view, a chronic decrease in NK cells and cytotoxic, tumour-infiltrating lymphocytes leads to a decreased production of tumour inhibiting cytokines (IL-1, IL-2, IFN-γ and TNF-α) and an increased production of tumour stimulating cytokines such as IL-10 (12, 24, 56, 123).

Disturbances of circadian rhythm

Athletes need to adjust their circadian rhythms as a consequence of latitudinal travel. The normal, free-running cycle has a length of 25-27 h. Disturbances are thus greater for an eastward displacement of 6 h (where the circadian clock must be adjusted by moving 18 h forward) than for a corresponding westward journey (where the circadian balance is restored by a 6-h shift). Various determinants of physical performance show a circadian fluctuation (198), and such characteristics may be less than optimal during the daytime until adjustment is complete. However, for many athletes the temporary disturbance of cognitive function is more important than any deterioration of physical performance. Current attempts to speed circadian adjustments are based on pre-travel exposure to bright light at the new hour of waking, immediate adoption of the new schedule of meals and exercise on arrival, and (for some physicians) the ingestion of melatonin (73). Given the known interactions between cytokines and sleepiness, there seems scope for future studies that attempt to speed circadian adaptations by manipulating cytokine levels.

The normal circadian variation of immune responses reflects parallel changes in hormone secretion (213). Total circulating lymphocytes present essentially a mirror image of plasma cortisol concentrations, peaking around 20:00-21:00 when cortisol is at its nadir. Most authors also agree that circulating counts for individual leukocyte subsets are highest during sleep, although the timing of peak concentrations is disputed. Haus et al. (96) and Ritchie et al. (183) reported increased eosinophil, monocyte, lymhocyte, T and B cell counts between 24:00 and 02:00. Others also found the largest numbers of B and NK cells in the early morning (70, 80). On the other hand, Abo et al. (1) and Bertouch et al. (10) found the acrophase for B cells in the evening, with the T cell and the CD4+/CD8+ ratios conforming to a similar pattern (70, 71, 109). Plasma IL-6 concentrations rise with the onset of sleep (176). Plasma IL-1 concentrations peak around midnight, followed by a peaking of IL-2 and a decline of NK cell activity, these various changes apparent-

ly being linked to the onset of slow-wave sleep. Responsiveness to pokeweed mitogen but not phytohaemagglutinin is increased during the sleeping hours (136, 137, 139). The maximum stimulation of cytolytic activity by IFN-γ is seen in the early morning, but the inhibitory effect of cortisol peaks at night; moreover, oral melatonin given around 18:00 augments the response to IFN-γ (79). There are also circadian variations in serum immunoglobulin concentrations (178) and the *in vitro* production of cytokines in whole blood (98, 168).

Clinical significance and future directions

Stimulation of inflammatory processes in those experiencing chronic sleep disruption may increase the risk of chronic disorders such as atherosclerosis, diabetes mellitus, Crohn's disease, and rheumatoid arthritis (208). Suggestions that immune disturbances increase the risk of cancer in shift workers also merit further exploration.

Sleep deprivation appears to reduce the antibody response to viruses in experimental animals and very prolonged periods of total sleep deprivation (typically about 20 consecutive days without sleep) result in lethal bloodstream infection and mortality in animals (21, 67, 211). However, much shorter periods of total sleep deprivation in humans (e.g. 3 consecutive nights without sleep) do not seem to influence the risk of infection, and the reported increase in NK cell activity with this duration of total sleep deprivation (57) would seem to rule out the possibility of an "open-window" for respiratory infections (147).

There is a pressing need to study whether disturbances to sleep quantity (total duration of sleep per night) or quality (number of awakenings per night) may have an adverse effect on immune health of the athlete or soldier. One recent study showed little effect of one night of total sleep deprivation on selected immune indices at rest and after exercise (181). However, very little is known about the effects of more prolonged sleep disruption or repeated sleep disturbances on immune function and infection incidence. One recent landmark study, albeit in healthy adults, showed that those who self-reported poor sleep quantity and/or quality exhibited increased symptoms of the common cold after intra-nasal inoculation with rhinovirus (31). Adults who slept for less than 7 h per night were almost 3-times more likely to develop symptoms of the common cold than those who slept more than 8 h per night. These findings highlight the importance of sleep quantity and quality in protecting humans against upper respiratory tract infections. Athlete and military support staff should consider monitoring sleep quantity and quality using a small, inexpensive and non-invasive movement sensor such as an actigraph. The utility of pharmacological and non-pharmacological interventions to improve sleep quantity and/or quality in those who frequently experience sleep disruption should be investigated alongside objective measures of immune status and infection incidence.

ENVIRONMENTAL EXTREMES AND THE IMMUNE RESPONSE TO EXERCISE

Background

Athletes, military personnel, mountaineers and those in physically demanding occupations are often required to reside in, or to perform vigorous physical activity in, adverse environmental conditions. Potential adverse conditions include extremes of heat and humidity, cold, high altitude and air pollutants. Lay people commonly believe that a hot bath or sauna can have therapeutic effects for all manner of ailments and that getting cold and wet increases the incidence of the common cold. Leading exercise immunologists have suggested that physical activity performed in stressful environments poses a greater than normal threat to immune function (199, 201), but this remains controversial (218).

This section summarises what we do and do not know about the immune response to exercise in environmental extremes, outlining some controversies and directions for future research. For a comprehensive review, readers are directed elsewhere (218).

Heat stress and immune function

Consensus

Exercising in hot conditions in which core temperature rises by ≥1°C compared with thermoneutral conditions (where core temperature rise is <1°C) augments anticipated increases in circulating stress hormones including catecholamines and cytokines, with associated elevations in circulating leukocyte counts (38, 180). Controlled studies that have clamped the rise in core temperature by undertaking moderate intensity endurance exercise in cool water demonstrate a significant contribution of the rise in core temperature to the development of the leukocytosis and cytokinaemia of exercise (38, 180). However, with the exception of a reduction in stimulated lymphocyte responses after exercise in the heat (197), and in exertional heat illness (EHI) patients (core temperature >40°C) (59), laboratory studies show a limited effect of exercise in the heat on: neutrophil function, monocyte function, natural killer cell activity (NKCA) and mucosal immunity (116-118, 129, 135, 205). Therefore, most of the available evidence does not support the contention that exercising in the heat poses a greater threat to immune function compared with thermoneutral conditions. It is also worth mentioning that individuals exercising in the heat tend to fatigue sooner (compared with performing the same exercise in thermoneutral conditions), so that their exposure to exercise stress in the heat tends to be self-limiting (89).

Controversies and future directions

The findings from tightly restricted laboratory studies that have evoked only modest increases in core temperature (peak <39°C) become somewhat redundant when one considers that core temperature often exceeds 40°C in athletes and soldiers whilst exercising in the heat (59, 184). Although field studies provide the opportunity to investigate the effects of severe heat stress on immune function, these studies often lack adequate experimental control. Somewhat surprisingly, clinically significant outcomes such as *in vivo* immune responses and infection

incidence have not been compared between athletes and soldiers training in hot and humid conditions and those training in thermoneutral conditions. In this regard, the next best evidence we have comes from studies showing that whole-body heating with saunas reduces upper respiratory tract infection (URTI) incidence (66) and hot water immersion improves clinical outcomes for cancer patients (105).

Without doubt the most exciting ongoing controversy in this sub-discipline of exercise-immunology centres on whether the immune system is involved in the aetiology of exertional heat stroke (EHS). Unlike the more mild EHI, EHS is a life threatening acute heat illness characterised by hyperthermia (core temperature >40°C) and neurological abnormalities that can develop after exposure to high ambient temperature and humidity (142). The putative involvement of immune dysregulation in the aetiology of EHS was first described in the exercise immunology literature by Shephard and Shek (201) and more recently refined by Lim and Mackinnon (120). During exercise-heat stress, gastrointestinal ischaemia can result in damage to the intestinal mucosa and leakage of lipopolysaccharide (LPS) into the portal circulation. The LPS is typically neutralized firstly by the liver and secondly by monocytes and macrophages. However, these defences may become overwhelmed, resulting in increased LPS in the peripheral circulation; the increase in circulating LPS may be exacerbated if immune function is impaired during heavy training (e.g. via decreased anti-LPS antibodies) (15). In turn, a sequence of events ensues involving LPS binding to its binding protein, the transfer of LPS to its receptor complex, toll-like receptor-4, with subsequent nuclear factor-kappa B activation and translation and production of inflammatory mediators including interleukin (IL)-1β, tumour necrosis factor alpha (TNF-α), IL-6 and inducible nitric oxide synthase (195). These events can lead to the systemic inflammatory response syndrome (SIRS), intravascular coagulation and eventually to multi-organ failure. This is an attractive model, particularly for cases of EHS that are otherwise difficult to explain, because the pyrogenic cytokines (e.g. IL-1β, and TNF-α) can alter thermoregulation (IL-1 induces fever) and cause cardiovascular instability resulting in collapse of the athlete or soldier (Figure 1).

Authors often cite support for an involvement of immune dysregulation in the aetiology of EHS from studies showing the following: circulating LPS levels in ultramarathon runners similar to florid sepsis (15); improved heat tolerance in heat-stressed animals treated with corticosteroids and antibiotics to prevent increases in circulating LPS (77, 78); cytokinaemia in EHS patients (17); symptoms of heat stroke in animals receiving IL-1 or TNF-α (122); enhanced survival in heat-stressed animals receiving IL-1 receptor antagonist (27) and important roles for heat shock proteins (e.g. HSP72) in cellular acquired thermal tolerance (126). In addition, recent work in rats shows that experimentally induced inflammation (via intramuscular injection of turpentine) compromises heat tolerance, further supporting a role for immune dysregulation in heat stroke (121).

However attractive an immune model of heat illness appears, there are many inconsistencies and gaps in knowledge that require elucidation. For example,

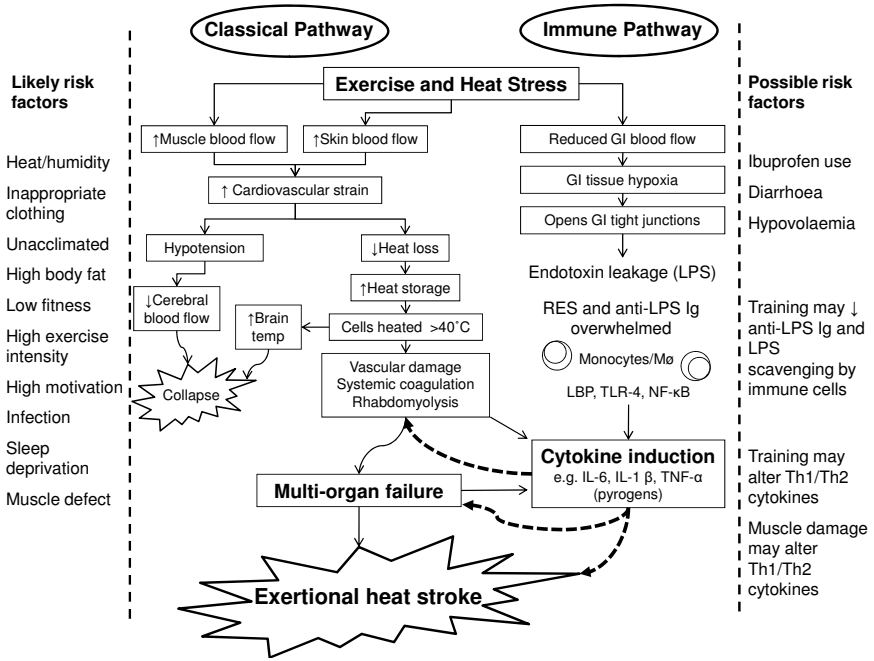

Figure 1. Classical and immune pathways of exertional heat stroke (EHS). GI = gastrointestinal; LPS = lipopolysaccharide; RES = reticuloendothelial system; Ig = immunoglobulin; Mø = macrophage; LBP = lipopolysaccharide binding protein; TLR-4 = toll-like receptor-4; NF-κB = nuclear factor-kappa B. Solid arrows indicate likely links in pathway; broken arrows indicate unsubstantiated in EHS aetiology.

there exists great variability in circulating LPS and cytokine levels in heat stroke and EHS casualties (15, 17, 23, 218). There is no consensus about the level of circulating LPS associated with clinical manifestations of EHS, although Moore et al. (140) have suggested a threshold of 60 $pg.ml^{-1}$. In light of this, it seems unreasonable that one widely cited paper presents pre-exercise circulating LPS in ultra-distance triathletes of 81 $pg.ml^{-1}$; it would be reasonable to assume that triathletes attend a race without initial clinical manifestations of heat illness (15). Similarly, studies reporting cytokinaemia in heat stroke and EHS patients show large variability in responses between patients and levels that are more often than not below the magnitude seen during SIRS and sepsis (17). Lack of experimental control in field studies and delay in admitting patients to hospital for blood collection add to the confusing picture regarding cytokines and heat stroke pathology. It is quite conceivable that the cytokinaemia of EHS is instrumental in the recovery from EHS, but this idea needs substantiating (119). On a more critical note, studies reporting raised circulating LPS and cytokines in end-stage heat stroke tell us very little about a putative involvement of the immune system in the aetiology of heat stroke. Prospective studies in humans are required to examine the extent of any immune dysregulation prior to collapse (218). An important yet unanswered question is whether the time course of LPS leakage from the gut, the resulting

cytokinaemia, altered thermoregulation and cardiovascular instability during exercise-heat stress coincide with the development of EHS. Human studies have shed some light on this, albeit using an experimental model of endotoxaemia that did not involve exercise-heat stress (133, 212). Infusing 2 $ng.kg^{-1}$ *Escherichia coli* endotoxin evoked maximal circulating TNF concentration 60-90 min after infusion and maximal body temperature 180 min after infusion (212). A decrease in blood pressure, which would be expected to contribute to the collapse in an EHS casualty, was not observed until 120 min after endotoxin infusion. Given the time course of these responses, an involvement of immune dysregulation in EHS during relatively short duration exercise (e.g. <60 min) appears less likely. A significant proportion of EHI cases, particularly in military personnel, occur in exercise bouts lasting <60 min (59, 175). The more traditional predisposing factors for EHS (Figure 1) such as high heat load, effort unmatched to fitness and underlying illness (175) alongside a recently proposed muscle defect causing excessive endogenous heat production likely play a prominent role in EHS aetiology (174).

Cold stress and immune function

Consensus

The term 'colds' may come from the popular belief that cold exposure causes URTI (25, 200). To date, there is no conclusive evidence to support a direct effect of prolonged cold exposure on URTI incidence. Reports from a number of Antarctic studies have shown little evidence of URTI among personnel except immediately after the visit of supply ships, when new strains of virus are imported into the community (76, 200), although the extent of cold exposure among study participants may have been relatively small.

Current consensus is that a continuum exists for the effects of passive body cooling on immune function. Very mild decreases in core temperature (~0.5°C) have little or even stimulatory effects on immune function (19, 115) but modest (~1°C) (35) and severe (~4°C) (220) decreases in core temperature have depressive effects on immune function. Compared with exercise in thermoneutral conditions, exercise in cold air conditions is associated with similar, or slightly lower, core temperature and neuro-endocrine activation (217) and similar immune modulation (179, 217, 218).

Controversies and future directions

Although lay people believe that getting cold and wet causes the common cold, this remains controversial because evidence from studies where participants were inoculated intra-nasally with cold viruses after cold exposure does not support such a belief (58). Nevertheless, more recent, novel work indicates that cooling body parts such as the feet increases self-reporting of cold symptoms (104). The authors claim this is due to reflex vasoconstriction in the upper airways and an associated reduction in respiratory defence. To settle this controversy, more experimental work is required that overcomes the limitations of existing studies. For example, published investigations have not mimicked the typical exposure to the common cold (58), have been limited by a small number of participants (58) or did not involve appropriate virology to quantify common cold incidence objectively after cold exposure (104).

To summarise, the limited evidence does not support the contention that athletes training and competing in cold conditions experience a greater reduction in immune function vs. those exercising under thermoneutral conditions. Nevertheless, it remains unknown if athletes who regularly train and compete in cold conditions report more frequent, severe or longer-lasting infections. Research should identify whether the airway inflammation associated with breathing large volumes of cold dry air (81) or polluted air (55) impairs airway defences (both ciliary function and immune responses) and whether athletes wrongly interpret as an URTI the symptoms of sore throat or exercise-induced bronchospasm that accompany exercising in cold or polluted air. As soldiers are often required to spend prolonged periods of activity interspersed with inactivity in cold and wet conditions they are particularly susceptible to hypothermia (core temperature ≤35°C) and associated reductions in immune function. The influence of hypothermia on *in vivo* immune function, wound healing and infection risk warrants further enquiry.

Altitude stress and immune function

Consensus

Athletes often train, and sometimes compete, at modest altitude (up to 2500 m) whereas mountaineers and occupational groups (e.g. high altitude miners and soldiers) often perform at high altitude (4000 m or higher). Upper respiratory and gastrointestinal tract symptoms are common in lowlanders who travel to high altitude (108, 143, 191, 203) and there are some reports that elite athletes experience increased URTI symptoms during and immediately after training camps at modest altitude (5, 90). Anecdotal reports of impaired wound healing in mountaineers at high altitude (170) are supported by laboratory studies in animals showing that breathing hypoxic air (12% $O_2 \approx 4000$ m) impairs wound healing after intradermal injection with *Escherichia coli* (110). The small number of investigations that have examined immune function in humans working and training at altitude (Table 3) indicate that NKCA and humoral immunity are either unaffected or enhanced (11, 28, 29, 68, 108, 130, 173). In contrast, cell mediated immunity is consistently reported to be impaired at altitude, with studies indicating decreases in CD4+: CD8+ T-lymphocyte ratio (68, 226) and T-lymphocyte proliferation (68, 173). Increased sympathetic nervous activity and hypothalamic–pituitary–adrenal axis activity are thought to play a prominent role in immune modulation at altitude (188).

Controversies and future directions

Although a small body of evidence supports the commonly held belief that high altitude exposure increases URTI (191, 203) this remains controversial because there exists some overlap in the symptoms of acute mountain sickness and URTI. Given the acknowledged immune alterations with exercise performed at sea level (85) and the additional stress responses to exercise with increasing altitude (127) an appealing hypothesis is that a continuum of responses exists whereby exercise with increasing altitude is associated with a greater degree of immune depression (127, 218). Unfortunately, only limited information from well controlled laboratory and field studies is available in this regard. Relatively little is known about the influence of altitude on innate immune function (Table 3) and the studies to date typically have not employed adequate experimental control (97). It is quite conceivable that other stressors experienced by athletes and mountaineers at alti-

Table 3. Immune function and infection symptoms during sojourns and athletic training in hypoxia. M = male; F = female; SL = sea level; TR = temporary altitude resident; Ig = immunoglobulin; NKCA = natural killer cell activity; URT = upper respiratory tract; LHTL = live high train low; IHT = intermittent hypoxia training, CRP = C-reactive protein. ND = no difference.

Reference	Participants	Hypoxic exposure and activity/training	Immune function and infection symptoms
Chohan et al.(29)	10 altitude natives (M), 8 TR (M) and 31 SL (M).	Natives and TR at 3692m. TR resided at 3692m for 2 years. Activity unknown.	↑ Serum Ig response to inoculation with T-cell dependent vaccine in natives and TR *vs*. SL.
Chohan and Singh (28)	24 altitude natives (M), 45 TR (M) and 66 SL (M).	Natives and TR at 3692m. TR resided at 3692m for 2 years. Activity unknown.	↑ T-lymphocyte function in natives and TR *vs*. SL residents.
Meehan et al.(130)	7 (M). No controls.	28 days of progressive decompression to 7620m in a chamber. Minimal activity.	ND in nasal IgA: protein, nasal lysosome: protein, CD4+:CD8+ ratio, lymphocyte function or NKCA.
Biselli et al.(11)	18 TR (M) and 18 SL controls (M).	20 days at 4930m. Activity level unknown.	ND serum Ig [G, A, M] and B-cell response to vaccine (T-cell independent) *vs*. control.
Bailey et al.(5)	10 elite runners TR and 19 SL controls (12M: 7F).	28 days at 1640m. Training at same relative exercise intensity in both groups.	↑ URT and gastrointestinal symptoms in runners at altitude *vs*. SL controls.
Pyne et al.(173)	10 elite swimmers TR (5M: 5F) and 8 staff controls (M).	21 days at 2102m. 3 sessions per day for swimmers (~5.5 h/day). Staff <4 h/week.	ND in infections or lymphocyte proliferation between groups. ↓ T-lymphocyte proliferation and ↑ B-cell proliferation *vs*. pre in both groups.
Hitomi et al.(97)	7 M. No controls.	7 days. IHT 2 h/day at 4500m. Activity unknown.	↑ neutrophil function *vs*. pre following IHT.
Tiollier et al.(210)	6 LHTL elite cross country (3M: 3F) and 5 elite controls (2M: 3F).	18 days. LHTL 11 h/day for 6 days each at 2500, 3000 and 3500m. Both groups trained at 1200m with matched load (~3h/day). 5 control athletes at 1200m.	ND in saliva [IgA] between groups. ↓saliva [IgA] in LHTL group at 2500 and 3500m *vs*. pre.
Facco et al.(68)	13 F. No controls.	21 days at 5050m. 1.5 h exercise 3-5 days/week.	ND in NKCA, ↓CD4+:CD8+ ratio and lymphocyte proliferation *vs*. pre.
Kleessen et al.(108)	7 Mountaineers (5M: 2F). No controls.	47 day altitude expedition where 29 days >5000m.	ND in serum Ig [G, A, M] and total faecal bacteria. ↑ CRP and gram-negative faecal bacteria *vs*. pre. ↓ Bifidobacteria (anti-microbial capacity) *vs*. pre.
Zhang et al.(226)	8 LHTL university soccer players (M) and 8 SL controls	28 days. LHTL 10h/day at equivalent of 3000m. Both groups trained at SL.	↑ URT symptoms in LHTL *vs*. control (2 LHTL with symptoms *vs*. 0 in control). ↓CD4+:CD8+

tude contribute to the observed alterations in infection incidence and immune function (e.g. raised physical and psychological stress, cold exposure and nutritional restriction).

In summary, although high altitude exposure has limited effects on humoral immunity, a number of studies have shown decreased cell-mediated immunity at high altitude. There is a need for tightly controlled laboratory and field studies employing exercising normoxia controls, resting hypoxia controls and clinically relevant *in vivo* immune methods to elucidate further the effects of altitude on immune health.

PREVENTION AND TREATMENT OF COMMON INFECTIONS

Background

Several studies (84, 160, 161, 167) have suggested that athletes are at increased risk of respiratory tract infections (URTI). For a more detailed account, readers are directed to the section on respiratory infections and exercise in part one of this

position statement. Exercise-induced suppression of some immune functions after intense and/or prolonged exercise and during strenuous training periods may explain the so-called "open window theory" and J-shaped curve paradigm, respectively. Regular sharing of the same training and living facilities within a team may also contribute to this increased frequency or duration of URTI (84). Moreover, the increased exposure to foreign (or new) pathogens while travelling put the athlete at a higher risk of gastrointestinal infections (GI) (14). Thus, acute URTI is the most common reason for presenting to a sports medicine clinic (74, 146), and it is the most common medical condition affecting athletes at both the summer and winter Olympic Games (94, 177).

Consensus

It is agreed by everyone that prevention is always superior to treatment and this is particularly true in athletes residing in countries with limited medical facilities. However, there is no single intervention that completely eliminates the risk of contracting an infection, but there are several effective ways of reducing the number, duration and severity of infectious episodes incurred over a period. Most of the following practical guidelines, driven by common sense, can be understood by everyone who keeps in mind the contagious nature of viruses, bacteria and fungi.

Practical guidelines for prevention of infections among athletes

- Check that your athletes are updated on all vaccines needed at home and for foreign countries should they travel abroad for training and competition.
- Minimize contacts with infected/sick people, young children, animals and potentially contaminated objects.
- Keep at distance from people who are coughing, sneezing or have a "runny nose", and when appropriate wear or ask them to wear a disposable mask.
- Wash hands regularly, before meals, and after direct contact with potentially contagious people, animals, blood, secretions, public places and bathrooms. Carry alcohol-based gel with you where lavatories are not available or not clean enough.
- Use disposable paper towels and limit hand to mouth/nose contact when suffering from URTI or GI symptoms.
- Do not share drinking bottles, cups, towels, etc.
- While competing or training abroad, prefer cold beverage from sealed bottles, avoid crude vegetables, and meat. Wash and peel fruits before eating.
- Quickly isolate a team member with infection symptoms and move out his/her roommate.
- Protect airways from being directly exposed to very cold and dry air during strenuous exercise, by using a face mask.
- Ensure adequate level of carbohydrate intake before and during strenuous or prolonged exercise in order to limit the extent and severity of the exercise-induced immunodepression phase (see nutritional countermeasures section in this part of the position statement).
- Wear proper out-door clothing and avoid getting cold and wet after exercise.
- Get at least 7 hours sleep per night (31) (see sleep disruption section in this part of the position statement).
- Avoid crash dieting and rapid weight loss.

- Wear flip-flop or thongs when going to the showers, swimming pool and locker rooms in order to avoid dermatological diseases.
- Keep other life stresses to a minimum.

Should infection occur, the athlete and his or her entourage must use some basic guidelines for exercise during infectious episodes (186) before being referred to a physician.

Guidelines for exercise during episodes of URTI or GI in athletes

- *First day of illness:*

No strenuous exercise or competitions when experiencing URTI symptoms like sore throat, coughing, runny or congested nose. No exercise when experiencing symptoms like muscle/joint pain and headache, fever and generalized feeling of malaise, diarrhoea or vomiting. Drink plenty of fluids, keep from getting wet and cold, and minimize life-stress.
Consider use of topical therapy with nasal drainage, decongestants and analgesics if feverish. Report illness to a team physician or health care personnel and keep away from other athletes if you are part of a team training or travelling together.

- *Second day:*

If body temperature >37.5-38 °C, or increased coughing, diarrhoea or vomiting: no training. If no fever or malaise and no worsening of "above the neck" symptoms: light exercise (pulse <120 bpm) for 30-45 min, indoors during winter and by yourself.

- *Third day:*

If fever and URTI or GI symptoms are still present: consult your physician. In GI cases, antibiotics should be taken if unformed stools occur more than four times a day or for fever, blood, pus, or mucus in stools. Quinolones should be avoided whenever possible because of an increased risk of tendinopathy. If no fever or malaise and no worsening of initial symptoms: moderate exercise (pulse <150 bpm) for 45-60 min, preferably indoors and by yourself.

- *Fourth day:*

If no symptom relief: do not try to exercise but make an office visit to your doctor. Stool cultures or examination for ova and parasites should generally be reserved for cases that last beyond 10 to 14 days. If first day of improved condition, follow the guidelines below (186):

Guidelines for return to exercise after infections

- Wait one day without fever and with improvement of URTI or GI symptoms before returning to exercise.
- Stop physical exercise and consult your physician if a new episode with fever or worsening of initial symptoms or persistent coughing and exercise-induced breathing problems occur.
- Use the same number of days to step up to normal training as spent off regular training because of illness.
- Observe closely your tolerance to increased exercise intensity and take an extra day off if recovery is incomplete.

- Use proper outdoor clothing and specific cold air protection for airways when exercising in temperatures below –10°C the first week after URTI.

Controversies

The first one is infectious mononucleosis (IM). Indeed, strenuous physical training performed during the initial or convalescence phase of Epstein Barr virus infection can be associated with increased morbidity, relapse, delayed recovery, and splenic rupture. This last occurrence is rare (0.1% of IM) on the athletic field and rarely fatal now (22). Most splenic ruptures occur between 4 days and 4 weeks after onset and very few occur beyond week 5 (63). Four recent reviews (4, 107, 125, 219) suggested that all spleens that rupture are enlarged, but it is important to note that splenomegaly is found in 50% of IM and that physical examination is quite insensitive to detect an enlarged at-risk spleen reliably. Although return to sport after IM is still a topic of debate, we recommend First, a week without febrile episodes or systemic symptoms and a substantial decrease in serum viral antibody titres and liver enzymes before starting light exercise; Secondly, exclude the possibility of hepatosplenomegaly in an athlete returning to contact sports, by performing abdominal ultrasound or CT scan; Thirdly, observe the tolerance of each training session and its recovery and discontinue the exercise if relapse or worsening while waiting for a consultation with the physician.

The second is about the diagnosis of viral myocarditis, which is the reason for sudden cardiac death in 5-22% of athletes under 35 years of age (see review (18)). For the purpose of prevention it is thus recommended to stop elite sport for 4 weeks after an unspecific infection. As some athletes experience up to six colds or viral (and probably unspecific) infections per year, one can understand why this recommendation is rarely implemented. Thus, it is important to take subtle discomforts seriously and initiate further evaluation when viral infection is strongly suspected particularly in spring and summer (Parvovirus B19, Herpes virus 6, Echovirus, Coxsackie, Poliovirus). Electrocardiogram, laboratory parameters, serologic markers, and echocardiography are helpful in diagnosis of myocarditis, but are not specific. Magnetic resonance imaging of the heart has become an important tool, but is not affordable by all. The cost-benefit ratio of myocarditis diagnosis in athletes remains a matter of controversy.

Future directions

As a high proportion of episodes of respiratory symptoms in athletes have not been associated with identification of a respiratory pathogen (37, 204), other potentially treatable causes of upper respiratory symptoms should be considered, particularly in athletes with recurrent symptoms. A better understanding of this phenomenon could lead to significant changes in the prevention and management of common infections in athletes.

REFERENCES

1. Abo T, Kawate T, Itoh K and Kumagai K. Studies on the bioperiodicity of the immune response. I. Circadian rhythms of human T, B, and K cell traffic in the peripheral blood. J Immunol 126: 1360-1363, 1981.
2. Ader R. Psychoneuroimmunology IV. San Diego: Academic Press, 2006.
3. Albers R, Antoine JM, Bourdet-Sicard R, Calder PC, Gleeson M, Lesourd B, Samartin S, Sanderson IR, Van Loo J, Vas Dias FW and Watzl B. Markers to measure immunomodulation in human nutrition intervention studies. Br J Nutr 94: 452-481, 2005.
4. Auwaerter PG. Infectious mononucleosis: return to play. Clin Sports Med 23: 485-97, xi, 2004.
5. Bailey DM, Davies B, Romer L, Castell L, Newsholme E and Gandy G. Implications of moderate altitude training for sea-level endurance in elite distance runners. Eur J Appl Physiol Occup Physiol 78: 360-368, 1998.
6. Bakker GC, van Erk MJ, Pellis L, Wopereis S, Rubingh CM, Cnubben NH, Kooistra T, van Ommen B and Hendriks HF. An antiinflammatory dietary mix modulates inflammation and oxidative and metabolic stress in overweight men: a nutrigenomics approach. Am J Clin Nutr 91: 1044-1059, 2010.
7. Banister EW, Morton RH and Clarke JR. Clinical dose-response effects of exercise. In: The physiology and pathophysiology of exercise tolerance, edited by Steinacker JM and Wand SA. New York: Plenum, 1997, p. 297-290.
8. Barnett A, Cerin E, Reaburn P and Hooper S. The effects of training on performance and performance-related states in individual elite athletes: a dynamic approach. J Sports Sci 28: 1117-1126, 2010.
9. Benschop RJ, Rodriguez-Feuerhahn M and Schedlowski M. Catecholamine-induced leukocytosis: early observations, current research, and future directions. Brain Behav Immun 10: 77-91, 1996.
10. Bertouch JV, Roberts-Thomson PJ and Bradley J. Diurnal variation of lymphocyte subsets identified by monoclonal antibodies. Br Med J (Clin Res Ed) 286: 1171-1172, 1983.
11. Biselli R, Le Moli S, Matricardi PM, Farrace S, Fattorossi A, Nisini R and D'Amelio R. The effects of hypobaric hypoxia on specific B cell responses following immunization in mice and humans. Aviat Space Environ Med 62: 870-874, 1991.
12. Blask DE. Melatonin, sleep disturbance and cancer risk. Sleep Med Rev 13: 257-264, 2009.
13. Blecha F, Barry RA and Kelley KW. Stress-induced alterations in delayed-type hypersensitivity to SRBC and contact sensitivity to DNFB in mice. Proc Soc Exp Biol Med 169: 239-246, 1982.
14. Boggess BR. Gastrointestinal infections in the traveling athlete. Curr Sports Med Rep 6: 125-129, 2007.
15. Bosenberg AT, Brock-Utne JG, Gaffin SL, Wells MT and Blake GT. Strenuous exercise causes systemic endotoxemia. J Appl Physiol 65: 106-108, 1988.
16. Bosquet L, Montpetit J, Arvisais D and Mujika I. Effects of tapering on performance: a meta-analysis. Med Sci Sports Exerc 39: 1358-1365, 2007.
17. Bouchama A, Parhar RS, el Yazigi A, Sheth K and al Sedairy S. Endotoxemia and release of tumor necrosis factor and interleukin 1 alpha in acute heatstroke. J Appl Physiol 70: 2640-2644, 1991.

18. Brennan FH, Stenzler B and Oriscello R. Diagnosis and management of myocarditis in athletes. Curr Sports Med Rep 2: 65-71, 2003.
19. Brenner IK, Castellani JW, Gabaree C, Young AJ, Zamecnik J, Shephard RJ and Shek PN. Immune changes in humans during cold exposure: effects of prior heating and exercise. J Appl Physiol 87: 699-710, 1999.
20. Brenner IK, Severs YD, Rhind SG, Shephard RJ and Shek PN. Immune function and incidence of infection during basic infantry training. Mil Med 165: 878-883, 2000.
21. Brown H and Dougherty T. The diurnal variation of blood leucocytes in normal and adrenalectomized mice. Endocrinology 58: 365-375, 1956.
22. Burroughs KE. Athletes resuming activity after infectious mononucleosis. Arch Fam Med 9: 1122-1123, 2000.
23. Camus G, Poortmans J, Nys M, Deby-Dupont G, Duchateau J, Deby C and Lamy M. Mild endotoxaemia and the inflammatory response induced by a marathon race. Clin Sci (Lond) 92: 415-422, 1997.
24. Carrillo-Vico A, Reiter RJ, Lardone PJ, Herrera JL, Fernandez-Montesinos R, Guerrero JM and Pozo D. The modulatory role of melatonin on immune responsiveness. Curr Opin Investig Drugs 7: 423-431, 2006.
25. Castellani JW, IK MB and Rhind SG. Cold exposure: human immune responses and intracellular cytokine expression. Med Sci Sports Exerc 34: 2013-2020, 2002.
26. Chen YJ, Wong SH, Wong CK, Lam CW, Huang YJ and Siu PM. The effect of a pre-exercise carbohydrate meal on immune responses to an endurance performance run. Br J Nutr 100: 1260-1268, 2008.
27. Chiu WT, Kao TY and Lin MT. Increased survival in experimental rat heatstroke by continuous perfusion of interleukin-1 receptor antagonist. Neurosci Res 24: 159-163, 1996.
28. Chohan IS and Singh I. Cell mediated immunity at high altitude. Int J Biometeorol 23: 21-30, 1979.
29. Chohan IS, Singh I, Balakrishnan K and Talwar GP. Immune response in human subjects at high altitude. Int J Biometeorol 19: 137-143, 1975.
30. Cocke R, Moynihan JA, Cohen N, Grota LJ and Ader R. Exposure to conspecific alarm chemosignals alters immune responses in BALB/c mice. Brain Behav Immun 7: 36-46, 1993.
31. Cohen S, Doyle WJ, Alper CM, Janicki-Deverts D and Turner RB. Sleep habits and susceptibility to the common cold. Arch Intern Med 169: 62-67, 2009.
32. Cohen S and Hamrick N. Stable individual differences in physiological response to stressors: implications for stress-elicited changes in immune related health. Brain Behav Immun 17: 407-414, 2003.
33. Cohen S, Miller GE and Rabin BS. Psychological stress and antibody response to immunization: a critical review of the human literature. Psychosom Med 63: 7-18, 2001.
34. Costa G, Haus E and Stevens R. Shift work and cancer - considerations on rationale, mechanisms, and epidemiology. Scand J Work Environ Health 36: 163-179, 2010.
35. Costa RJ, Smith AH, Oliver SJ, Walters R, Maassen N, Bilzon JL and Walsh NP. The effects of two nights of sleep deprivation with or without energy restriction on immune indices at rest and in response to cold exposure. Eur J Appl Physiol 109: 417-428, 2010.
36. Cox AJ, Gleeson M, Pyne DB, Callister R, Fricker PA and Scott RJ. Cytokine gene polymorphisms and risk for upper respiratory symptoms in highly-trained athletes. Exerc Immunol Rev 16: 8-21, 2010.

37. Cox AJ, Gleeson M, Pyne DB, Callister R, Hopkins WG and Fricker PA. Clinical and laboratory evaluation of upper respiratory symptoms in elite athletes. Clin J Sport Med 18: 438-445, 2008.
38. Cross MC, Radomski MW, VanHelder WP, Rhind SG and Shephard RJ. Endurance exercise with and without a thermal clamp: effects on leukocytes and leukocyte subsets. J Appl Physiol 81: 822-829, 1996.
39. Davis GM, Plyley MJ, Gottesman ST, Shephard RJ and Goode RC. Variations in cardiorespiratory and strength parameters during moderate exercise and sleep deprivation. Can J Appl Spts Sci 7: 236-237, 1982.
40. Davis S and Mirick DK. Circadian disruption, shift work and the risk of cancer: a summary of the evidence and studies in Seattle. Cancer Causes Control 17: 539-545, 2006.
41. Davison G and Gleeson M. Influence of acute vitamin C and/or carbohydrate ingestion on hormonal, cytokine, and immune responses to prolonged exercise. Int J Sport Nutr Exerc Metab 15: 465-479, 2005.
42. Davison G and Gleeson M. The effect of 2 weeks vitamin C supplementation on immunoendocrine responses to 2.5 h cycling exercise in man. Eur J Appl Physiol 97: 454-461, 2006.
43. Dhabhar FS. Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection versus immunopathology. Allergy Asthma Clin Immunol 4: 2-11, 2008.
44. Dhabhar FS. Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection and immunopathology. Neuroimmunomodulation 16: 300-317, 2009.
45. Dhabhar FS and McEwen BS. Stress-induced enhancement of antigen-specific cell-mediated immunity. J Immunol 156: 2608-2615, 1996.
46. Dhabhar FS and McEwen BS. Acute stress enhances while chronic stress suppresses cell-mediated immunity in vivo: a potential role for leukocyte trafficking. Brain Behav Immun 11: 286-306, 1997.
47. Dhabhar FS and McEwen BS. Enhancing versus suppressive effects of stress hormones on skin immune function. Proc Natl Acad Sci U S A 96: 1059-1064, 1999.
48. Dhabhar FS and McEwen BS. Bidirectional effects of stress & glucocorticoid hormones on immune function: Possible explanations for paradoxical observations. In: Psychoneuroimmunology, edited by Ader R, Felten DL and Cohen N. San Diego: Academic Press, 2001, p. 301-338.
49. Dhabhar FS, McEwen BS and Spencer RL. Adaptation to prolonged or repeated stress--comparison between rat strains showing intrinsic differences in reactivity to acute stress. Neuroendocrinology 65: 360-368, 1997.
50. Dhabhar FS, Miller AH, McEwen BS and Spencer RL. Differential activation of adrenal steroid receptors in neural and immune tissues of Sprague Dawley, Fischer 344, and Lewis rats. J Neuroimmunol 56: 77-90, 1995.
51. Dhabhar FS, Miller AH, McEwen BS and Spencer RL. Effects of stress on immune cell distribution. Dynamics and hormonal mechanisms. J Immunol 154: 5511-5527, 1995.
52. Dhabhar FS, Miller AH, McEwen BS and Spencer RL. Stress-induced changes in blood leukocyte distribution. Role of adrenal steroid hormones. J Immunol 157: 1638-1644, 1996.

53. Dhabhar FS, Satoskar AR, Bluethmann H, David JR and McEwen BS. Stress-induced enhancement of skin immune function: A role for gamma interferon. Proc Natl Acad Sci U S A 97: 2846-2851, 2000.
54. Dhabhar FS and Viswanathan K. Short-term stress experienced at time of immunization induces a long-lasting increase in immunologic memory. Am J Physiol Regul Integr Comp Physiol 289: R738-R744, 2005.
55. Diamond G, Legarda D and Ryan LK. The innate immune response of the respiratory epithelium. Immunol Rev 173: 27-38, 2000.
56. Dimitrov S, Lange T, Tieken S, Fehm HL and Born J. Sleep associated regulation of T helper 1/T helper 2 cytokine balance in humans. Brain Behav Immun 18: 341-348, 2004.
57. Dinges DF, Douglas SD, Zaugg L, Campbell DE, McMann JM, Whitehouse WG, Orne EC, Kapoor SC, Icaza E and Orne MT. Leukocytosis and natural killer cell function parallel neurobehavioral fatigue induced by 64 hours of sleep deprivation. J Clin Invest 93: 1930-1939, 1994.
58. Douglas RC, Jr., Couch RB and Lindgren KM. Cold doesn't affect the "common cold" in study of rhinovirus infections. JAMA 199: 29-30, 1967.
59. DuBose DA, Wenger CB, Flinn SD, Judy TA, Dubovtsev AI and Morehouse DH. Distribution and mitogen response of peripheral blood lymphocytes after exertional heat injury. J Appl Physiol 95: 2381-2389, 2003.
60. Edwards KM, Burns VE, Adkins AE, Carroll D, Drayson M and Ring C. Meningococcal A vaccination response is enhanced by acute stress in men. Psychosom Med 70: 147-151, 2008.
61. Edwards KM, Burns VE, Carroll D, Drayson M and Ring C. The acute stress-induced immunoenhancement hypothesis. Exerc Sport Sci Rev 35: 150-155, 2007.
62. Edwards KM, Burns VE, Reynolds T, Carroll D, Drayson M and Ring C. Acute stress exposure prior to influenza vaccination enhances antibody response in women. Brain Behav Immun 20: 159-168, 2006.
63. Eichner ER. Sports medicine pearls and pitfalls--defending the spleen: return to play after infectious mononucleosis. Curr Sports Med Rep 6: 68-69, 2007.
64. Epel ES. Psychological and metabolic stress: a recipe for accelerated cellular aging? Hormones (Athens) 8: 7-22, 2009.
65. Epel ES, Blackburn EH, Lin J, Dhabhar FS, Adler NE, Morrow JD and Cawthon RM. Accelerated telomere shortening in response to life stress. Proc Natl Acad Sci U S A 101: 17312-17315, 2004.
66. Ernst E, Pecho E, Wirz P and Saradeth T. Regular sauna bathing and the incidence of common colds. Ann Med 22: 225-227, 1990.
67. Everson CA. Sustained sleep deprivation impairs host defense. Am J Physiol 265: R1148-R1154, 1993.
68. Facco M, Zilli C, Siviero M, Ermolao A, Travain G, Baesso I, Bonamico S, Cabrelle A, Zaccaria M and Agostini C. Modulation of immune response by the acute and chronic exposure to high altitude. Med Sci Sports Exerc 37: 768-774, 2005.
69. Fauci AS and Dale DC. The effect of in vivo hydrocortisone on subpopulations of human lymphocytes. J Clin Invest 53: 240-246, 1974.
70. Fernandes G. Chronobiology of immune functions: cellular and humoral aspects. In: Biological rhythms in clinical and laboratory medicine, edited by Touitou Y and Haus E. Heidelberg: Springer Verlag, 1994, p. 493-503.

71. Fernandes G, Carandente F, Halberg E, Halberg F and Good RA. Circadian rhythm in activity of lympholytic natural killer cells from spleens of Fischer rats. J Immunol 123: 622-625, 1979.
72. Foster C. Monitoring training in athletes with reference to overtraining syndrome. Med Sci Sports Exerc 30: 1164-1168, 1998.
73. French J. Circadian rhythms, jet lag and the athlete. In: Current Therapy in Sports Medicine, edited by Torg J and Shephard RJ. St. Louis: Mosby, 1995, p. 596-600.
74. Fricker PA. Infectious problems in athletes: an overview. In: Medical Problems in Athletes, edited by Fields KB and Fricker PA. Malden: Blackwell Science, 1997, p. 3-5.
75. Fry RW, Morton AR and Keast D. Periodisation of training stress--a review. Can J Sport Sci 17: 234-240, 1992.
76. Gard S. Respiratory virus infections other than influenza. Arch Environ Health 17: 543-546, 1968.
77. Gathiram P, Wells MT, Brock-Utne JG and Gaffin SL. Prophylactic corticosteroid increases survival in experimental heat stroke in primates. Aviat Space Environ Med 59: 352-355, 1988.
78. Gathiram P, Wells MT, Brock-Utne JG, Wessels BC and Gaffin SL. Prevention of endotoxaemia by non-absorbable antibiotics in heat stress. J Clin Pathol 40: 1364-1368, 1987.
79. Gatti G, Carignola R, Masera R, Satori ML, Salvadori A, Magro E and Angeli A. Circadian-stage specified effects of melatonin on human natural killer (NK) cell activity: in vivo and in vitro studies. Ann Rev Chronopharmacol 5: 25-28, 1989.
80. Gatti G, Carvalho R, Delponte D, Masera R, Carignola R, Caradente F and Angeli A. Circadian changes of human natural killer cells and their in vitro susceptibility to cortisol inhibition. Ann Rev Chronopharmacol 3: 75-78, 1987.
81. Giesbrecht GG. The respiratory system in a cold environment. Aviat Space Environ Med 66: 890-902, 1995.
82. Glaser R and Kiecolt-Glaser JK. Stress-induced immune dysfunction: implications for health. Nat Rev Immunol 5: 243-251, 2005.
83. Glaser R, MacCallum RC, Laskowski BF, Malarkey WB, Sheridan JF and Kiecolt-Glaser JK. Evidence for a shift in the Th-1 to Th-2 cytokine response associated with chronic stress and aging. J Gerontol A Biol Sci Med Sci 56: M477-M482, 2001.
84. Gleeson M. The scientific basis of practical strategies to maintain immunocompetence in elite athletes. Exerc Immunol Rev 6: 75-101, 2000.
85. Gleeson M. Immune function in sport and exercise. J Appl Physiol 103: 693-699, 2007.
86. Gleeson M. Dosing and efficacy of glutamine supplementation in human exercise and sport training. J Nutr 138: 2045S-2049S, 2008.
87. Gleeson M, Nieman DC and Pedersen BK. Exercise, nutrition and immune function. J Sports Sci 22: 115-125, 2004.
88. Gomez-Cabrera MC, Domenech E, Romagnoli M, Arduini A, Borras C, Pallardo FV, Sastre J and Vina J. Oral administration of vitamin C decreases muscle mitochondrial biogenesis and hampers training-induced adaptations in endurance performance. Am J Clin Nutr 87: 142-149, 2008.
89. Gonzalez-Alonso J, Teller C, Andersen SL, Jensen FB, Hyldig T and Nielsen B. Influence of body temperature on the development of fatigue during prolonged exercise in the heat. J Appl Physiol 86: 1032-1039, 1999.

90. Gore CJ, Hahn A, Rice A, Bourdon P, Lawrence S, Walsh C, Stanef T, Barnes P, Parisotto R, Martin D and Pyne D. Altitude training at 2690m does not increase total haemoglobin mass or sea level VO2max in world champion track cyclists. J Sci Med Sport 1: 156-170, 1998.
91. Gozal D, Serpero LD, Kheirandish-Gozal L, Capdevila OS, Khalyfa A and Tauman R. Sleep measures and morning plasma TNF-alpha levels in children with sleep-disordered breathing. Sleep 33: 319-325, 2010.
92. Gunnar M and Quevedo K. The neurobiology of stress and development. Annu Rev Psychol 58: 145-173, 2007.
93. Hackney AC. Exercise as a stressor to the human neuroendocrine system. Medicina (Kaunas) 42: 788-797, 2006.
94. Hanley DF. Medical care of the US Olympic Team. JAMA 236: 147-148, 1976.
95. Hansen J. Risk of breast cancer after night- and shift work: current evidence and ongoing studies in Denmark. Cancer Causes Control 17: 531-537, 2006.
96. Haus E, Lakatua DJ, Swoyer J and Sackett-Lundeen L. Chronobiology in hematology and immunology. Am J Anat 168: 467-517, 1983.
97. Hitomi Y, Miyamura M, Mori S, Suzuki K, Kizaki T, Itoh C, Murakami K, Haga S and Ohno H. Intermittent hypobaric hypoxia increases the ability of neutrophils to generate superoxide anion in humans. Clin Exp Pharmacol Physiol 30: 659-664, 2003.
98. Hohagen F, Timmer J, Weyerbrock A, Fritsch-Montero R, Ganter U, Krieger S, Berger M and Bauer J. Cytokine production during sleep and wakefulness and its relationship to cortisol in healthy humans. Neuropsychobiology 28: 9-16, 1993.
99. Hopkins WG, Marshall SW, Batterham AM and Hanin J. Progressive statistics for studies in sports medicine and exercise science. Med Sci Sports Exerc 41: 3-13, 2009.
100. Irwin M, McClintick J, Costlow C, Fortner M, White J and Gillin JC. Partial night sleep deprivation reduces natural killer and cellular immune responses in humans. FASEB J 10: 643-653, 1996.
101. Irwin MR. Human psychoneuroimmunology: 20 years of discovery. Brain Behav Immun 22: 129-139, 2008.
102. Irwin MR, Carrillo C and Olmstead R. Sleep loss activates cellular markers of inflammation: sex differences. Brain Behav Immun 24: 54-57, 2010.
103. Irwin MR, Wang M, Ribeiro D, Cho HJ, Olmstead R, Breen EC, Martinez-Maza O and Cole S. Sleep loss activates cellular inflammatory signaling. Biol Psychiatry 64: 538-540, 2008.
104. Johnson C and Eccles R. Acute cooling of the feet and the onset of common cold symptoms. Fam Pract 22: 608-613, 2005.
105. Katschinski DM, Wiedemann GJ, Longo W, d'Oleire FR, Spriggs D and Robins HI. Whole body hyperthermia cytokine induction: a review, and unifying hypothesis for myeloprotection in the setting of cytotoxic therapy. Cytokine Growth Factor Rev 10: 93-97, 1999.
106. Kendall A, Hoffman-Goetz L, Houston M, MacNeil B and Arumugam Y. Exercise and blood lymphocyte subset responses: intensity, duration, and subject fitness effects. J Appl Physiol 69: 251-260, 1990.
107. Kinderknecht JJ. Infectious mononucleosis and the spleen. Curr Sports Med Rep 1: 116-120, 2002.

108. Kleessen B, Schroedl W, Stueck M, Richter A, Rieck O and Krueger M. Microbial and immunological responses relative to high-altitude exposure in mountaineers. Med Sci Sports Exerc 37: 1313-1318, 2005.
109. Knapp MS and Pownall R. Lymphocytes are rhythmic: is this important? Br Med J (Clin Res Ed) 289: 1328-1330, 1984.
110. Knighton DR, Halliday B and Hunt TK. Oxygen as an antibiotic. The effect of inspired oxygen on infection. Arch Surg 119: 199-204, 1984.
111. Koenig D, Grathwohl D, Weinstock C, Northoff H and Berg A. Upper respiratory tract infection in athletes: influence of lifestyle, type of sport, training effort, and immunostimulant intake. Exerc Immunol Rev 6: 102-120, 2000.
112. Krueger JM and Majde JA. Sleep as a host defense: its regulation by microbial products and cytokines. Clin Immunol Immunopathol 57: 188-199, 1990.
113. Krzywkowski K, Petersen EW, Ostrowski K, Kristensen JH, Boza J and Pedersen BK. Effect of glutamine supplementation on exercise-induced changes in lymphocyte function. Am J Physiol Cell Physiol 281: C1259-C1265, 2001.
114. Kusaka Y, Kondou H and Morimoto K. Healthy lifestyles are associated with higher natural killer cell activity. Prev Med 21: 602-615, 1992.
115. Lackovic V, Borecky L, Vigas M and Rovensky J. Activation of NK cells in subjects exposed to mild hyper- or hypothermic load. J Interferon Res 8: 393-402, 1988.
116. Laing SJ, Blackwell J, Gwynne D, Walters R and Walsh NP. Neutrophil degranulation response to 2 hours of exercise in a 30 degrees C environment. Aviat Space Environ Med 76: 1068-1073, 2005.
117. Laing SJ, Gwynne D, Blackwell J, Williams M, Walters R and Walsh NP. Salivary IgA response to prolonged exercise in a hot environment in trained cyclists. Eur J Appl Physiol 93: 665-671, 2005.
118. Laing SJ, Jackson AR, Walters R, Lloyd-Jones E, Whitham M, Maassen N and Walsh NP. Human blood neutrophil responses to prolonged exercise with and without a thermal clamp. J Appl Physiol 104: 20-26, 2008.
119. Leon LR, Blaha MD and DuBose DA. Time course of cytokine, corticosterone, and tissue injury responses in mice during heat strain recovery. J Appl Physiol 100: 1400-1409, 2006.
120. Lim CL and Mackinnon LT. The roles of exercise-induced immune system disturbances in the pathology of heat stroke: the dual pathway model of heat stroke. Sports Med 36: 39-64, 2006.
121. Lim CL, Wilson G, Brown L, Coombes JS and Mackinnon LT. Pre-existing inflammatory state compromises heat tolerance in rats exposed to heat stress. Am J Physiol Regul Integr Comp Physiol 292: R186-R194, 2007.
122. Lin MT, Liu HH and Yang YL. Involvement of interleukin-1 receptor mechanisms in development of arterial hypotension in rat heatstroke. Am J Physiol 273: H2072-H2077, 1997.
123. Lucey DR, Clerici M and Shearer GM. Type 1 and type 2 cytokine dysregulation in human infectious, neoplastic, and inflammatory diseases. Clin Microbiol Rev 9: 532-562, 1996.
124. Lyall KA, Hurst SM, Cooney J, Jensen D, Lo K, Hurst RD and Stevenson LM. Short-term blackcurrant extract consumption modulates exercise-induced oxidative stress and lipopolysaccharide-stimulated inflammatory responses. Am J Physiol Regul Integr Comp Physiol 297: R70-R81, 2009.

125. Macknight JM. Infectious mononucleosis: ensuring a safe return to sport. Phys Sportsmed 30: 27-41, 2002.
126. Maloyan A, Palmon A and Horowitz M. Heat acclimation increases the basal HSP72 level and alters its production dynamics during heat stress. Am J Physiol 276: R1506-R1515, 1999.
127. Mazzeo RS. Altitude, exercise and immune function. Exerc Immunol Rev 11: 6-16, 2005.
128. McEwen BS. Protective and damaging effects of stress mediators. N Engl J Med 338: 171-179, 1998.
129. McFarlin BK and Mitchell JB. Exercise in hot and cold environments: differential effects on leukocyte number and NK cell activity. Aviat Space Environ Med 74: 1231-1236, 2003.
130. Meehan R, Duncan U, Neale L, Taylor G, Muchmore H, Scott N, Ramsey K, Smith E, Rock P and Goldblum R. Operation Everest II: alterations in the immune system at high altitudes. J Clin Immunol 8: 397-406, 1988.
131. Meeusen R, Duclos M, Gleeson M, Rietjems J, Steinacker JM and Urhausen A. Prevention, diagnosis and treatment of the Overtraining Syndrome. Eur J Sport Sci 6: 1-14, 2006.
132. Meier-Ewert HK, Ridker PM, Rifai N, Regan MM, Price NJ, Dinges DF and Mullington JM. Effect of sleep loss on C-reactive protein, an inflammatory marker of cardiovascular risk. J Am Coll Cardiol 43: 678-683, 2004.
133. Michie HR, Manogue KR, Spriggs DR, Revhaug A, O'Dwyer S, Dinarello CA, Cerami A, Wolff SM and Wilmore DW. Detection of circulating tumor necrosis factor after endotoxin administration. N Engl J Med 318: 1481-1486, 1988.
134. Midgley AW, McNaughton LR and Jones AM. Training to enhance the physiological determinants of long-distance running performance: can valid recommendations be given to runners and coaches based on current scientific knowledge? Sports Med 37: 857-880, 2007.
135. Mitchell JB, Dugas JP, McFarlin BK and Nelson MJ. Effect of exercise, heat stress, and hydration on immune cell number and function. Med Sci Sports Exerc 34: 1941-1950, 2002.
136. Moldofsky H. Central nervous system and peripheral immune functions and the sleep-wake system. J Psychiatry Neurosci 19: 368-374, 1994.
137. Moldofsky H. Sleep and the immune system. Int J Immunopharmacol 17: 649-654, 1995.
138. Moldofsky H, Lue FA, Davidson JR and Gorczynski R. Effects of sleep deprivation on human immune functions. FASEB J 3: 1972-1977, 1989.
139. Moldofsky H, Lue FA, Eisen J, Keystone E and Gorczynski RM. The relationship of interleukin-1 and immune functions to sleep in humans. Psychosom Med 48: 309-318, 1986.
140. Moore GE, Holbein ME and Knochel JP. Exercise-associated collapse in cyclists is unrelated to endotoxemia. Med Sci Sports Exerc 27: 1238-1242, 1995.
141. Mujika I. The influence of training characteristics and tapering on the adaptation in highly trained individuals: a review. Int J Sports Med 19: 439-446, 1998.
142. Muldoon S, Deuster P, Brandom B and Bunger R. Is there a link between malignant hyperthermia and exertional heat illness? Exerc Sport Sci Rev 32: 174-179, 2004.
143. Murdoch DR. Symptoms of infection and altitude illness among hikers in the Mount Everest region of Nepal. Aviat Space Environ Med 66: 148-151, 1995.

144. Murphy EA, Davis JM, Carmichael MD, Mayer EP and Ghaffar A. Benefits of oat beta-glucan and sucrose feedings on infection and macrophage antiviral resistance following exercise stress. Am J Physiol Regul Integr Comp Physiol 297: R1188-R1194, 2009.
145. Nakachi K and Imai K. Environmental and physiological influences on human natural killer cell activity in relation to good health practices. Jpn J Cancer Res 83: 798-805, 1992.
146. Nieman DC. Exercise, upper respiratory tract infection, and the immune system. Med Sci Sports Exerc 26: 128-139, 1994.
147. Nieman DC. Upper respiratory tract infections and exercise. Thorax 50: 1229-1231, 1995.
148. Nieman DC. Immune response to heavy exertion. J Appl Physiol 82: 1385-1394, 1997.
149. Nieman DC. Influence of carbohydrate on the immune response to intensive, prolonged exercise. Exerc Immunol Rev 4: 64-76, 1998.
150. Nieman DC. Is infection risk linked to exercise workload? Med Sci Sports Exerc 32: S406-S411, 2000.
151. Nieman DC. Immunonutrition support for athletes. Nutr Rev 66: 310-320, 2008.
152. Nieman DC. Quercetin's bioactive effects in human athletes. Curr Topic Nutraceut Res 8: 33-44, 2010.
153. Nieman DC, Henson DA, Gojanovich G, Davis JM, Murphy EA, Mayer EP, Pearce S, Dumke CL, Utter AC, McAnulty SR and McAnulty LS. Influence of carbohydrate on immune function following 2 h cycling. Res Sports Med 14: 225-237, 2006.
154. Nieman DC, Henson DA, Gross SJ, Jenkins DP, Davis JM, Murphy EA, Carmichael MD, Dumke CL, Utter AC, McAnulty SR, McAnulty LS and Mayer EP. Quercetin reduces illness but not immune perturbations after intensive exercise. Med Sci Sports Exerc 39: 1561-1569, 2007.
155. Nieman DC, Henson DA, Maxwell KR, Williams AS, McAnulty SR, Jin F, Shanely RA and Lines TC. Effects of quercetin and EGCG on mitochondrial biogenesis and immunity. Med Sci Sports Exerc 41: 1467-1475, 2009.
156. Nieman DC, Henson DA, McAnulty SR, Jin F and Maxwell KR. n-3 polyunsaturated fatty acids do not alter immune and inflammation measures in endurance athletes. Int J Sport Nutr Exerc Metab 19: 536-546, 2009.
157. Nieman DC, Henson DA, McAnulty SR, McAnulty L, Swick NS, Utter AC, Vinci DM, Opiela SJ and Morrow JD. Influence of vitamin C supplementation on oxidative and immune changes after an ultramarathon. J Appl Physiol 92: 1970-1977, 2002.
158. Nieman DC, Henson DA, McAnulty SR, McAnulty LS, Morrow JD, Ahmed A and Heward CB. Vitamin E and immunity after the Kona Triathlon World Championship. Med Sci Sports Exerc 36: 1328-1335, 2004.
159. Nieman DC, Henson DA, McMahon M, Wrieden JL, Davis JM, Murphy EA, Gross SJ, McAnulty LS and Dumke CL. Beta-glucan, immune function, and upper respiratory tract infections in athletes. Med Sci Sports Exerc 40: 1463-1471, 2008.
160. Nieman DC, Johanssen LM and Lee JW. Infectious episodes in runners before and after a roadrace. J Sports Med Phys Fitness 29: 289-296, 1989.
161. Nieman DC, Johanssen LM, Lee JW and Arabatzis K. Infectious episodes in runners before and after the Los Angeles Marathon. J Sports Med Phys Fitness 30: 316-328, 1990.

162. Nieman DC, Williams AS, Shanely RA, Jin F, McAnulty SR, Triplett NT, Austin MD and Henson DA. Quercetin's influence on exercise performance and muscle mitochondrial biogenesis. Med Sci Sports Exerc 42: 338-345, 2010.
163. Oliver SJ, Costa RJ, Laing SJ, Bilzon JL and Walsh NP. One night of sleep deprivation decreases treadmill endurance performance. Eur J Appl Physiol 107: 155-161, 2009.
164. Opp MR, Kapas L and Toth LA. Cytokine involvement in the regulation of sleep. Proc Soc Exp Biol Med 201: 16-27, 1992.
165. Palmblad J, Cantell K, Strander H, Froberg J, Karlsson CG, Levi L, Granstrom M and Unger P. Stressor exposure and immunological response in man: interferon-producing capacity and phagocytosis. J Psychosom Res 20: 193-199, 1976.
166. Pedersen BK and Hoffman-Goetz L. Exercise and the immune system: regulation, integration, and adaptation. Physiol Rev 80: 1055-1081, 2000.
167. Peters EM. Exercise, immunology and upper respiratory tract infections. Int J Sports Med 18 Suppl 1: S69-S77, 1997.
168. Petrovsky N, McNair P and Harrison LC. Circadian rhythmicity of interferon-gamma production in antigen-stimulated whole blood. Chronobiologia 21: 293-300, 1994.
169. Phillips AC, Burns VE and Lord JM. Stress and exercise: Getting the balance right for aging immunity. Exerc Sport Sci Rev 35: 35-39, 2007.
170. Pugh L and Ward M. Some effects of high altitude on man. Lancet 271: 1115-1121, 1956.
171. Pyne DB, Gleeson M, McDonald WA, Clancy RL, Perry C, Jr. and Fricker PA. Training strategies to maintain immunocompetence in athletes. Int J Sports Med 21 Suppl 1: S51-S60, 2000.
172. Pyne DB, Hopkins WG, Batterham AM, Gleeson M and Fricker PA. Characterising the individual performance responses to mild illness in international swimmers. Br J Sports Med 39: 752-756, 2005.
173. Pyne DB, McDonald WA, Morton DS, Swigget JP, Foster M, Sonnenfeld G and Smith JA. Inhibition of interferon, cytokine, and lymphocyte proliferative responses in elite swimmers with altitude exposure. J Interferon Cytokine Res 20: 411-418, 2000.
174. Rae DE, Knobel GJ, Mann T, Swart J, Tucker R and Noakes TD. Heatstroke during endurance exercise: is there evidence for excessive endothermy? Med Sci Sports Exerc 40: 1193-1204, 2008.
175. Rav-Acha M, Hadad E, Epstein Y, Heled Y and Moran DS. Fatal exertional heat stroke: a case series. Am J Med Sci 328: 84-87, 2004.
176. Redwine L, Hauger RL, Gillin JC and Irwin M. Effects of sleep and sleep deprivation on interleukin-6, growth hormone, cortisol, and melatonin levels in humans. J Clin Endocrinol Metab 85: 3597-3603, 2000.
177. Reeser JC, Willick S and Elstad M. Medical services provided at the Olympic Village polyclinic during the 2002 Salt Lake City Winter Games. Wisc Med J 102: 20-25, 2003.
178. Reinberg A, Schuller E, Clench J and Smolensky MH. Circadian and circannual rhythms of leukocytes, proteins and immunoglobulins. In: Recent advances in the chronobiology of allergy and immunology, edited by Smolensky MH, Reinberg A and McGovern JP. Oxford: Pergamon Press, 1980, p. 251-259.

179. Rhind SG, Gannon GA, Shek PN, Brenner IK, Severs Y, Zamecnik J, Buguet A, Natale VM, Shephard RJ and Radomski MW. Contribution of exertional hyperthermia to sympathoadrenal-mediated lymphocyte subset redistribution. J Appl Physiol 87: 1178-1185, 1999.
180. Rhind SG, Gannon GA, Shephard RJ, Buguet A, Shek PN and Radomski MW. Cytokine induction during exertional hyperthermia is abolished by core temperature clamping: neuroendocrine regulatory mechanisms. Int J Hyperthermia 20: 503-516, 2004.
181. Ricardo JS, Cartner L, Oliver SJ, Laing SJ, Walters R, Bilzon JL and Walsh NP. No effect of a 30-h period of sleep deprivation on leukocyte trafficking, neutrophil degranulation and saliva IgA responses to exercise. Eur J Appl Physiol 105: 499-504, 2009.
182. Ristow M, Zarse K, Oberbach A, Kloting N, Birringer M, Kiehntopf M, Stumvoll M, Kahn CR and Bluher M. Antioxidants prevent health-promoting effects of physical exercise in humans. Proc Natl Acad Sci U S A 106: 8665-8670, 2009.
183. Ritchie AW, Oswald I, Micklem HS, Boyd JE, Elton RA, Jazwinska E and James K. Circadian variation of lymphocyte subpopulations: a study with monoclonal antibodies. Br Med J (Clin Res Ed) 286: 1773-1775, 1983.
184. Roberts WO. Exercise-associated collapse in endurance events: A classification system. Phys Sportsmed 17: 49-55, 1989.
185. Romeo J, Warnberg J, Pozo T and Marcos A. Physical activity, immunity and infection. Proc Nutr Soc 69: 390-399, 2010.
186. Ronsen O. Prevention and management of respiratory tract infections in athletes. New Stud Athlet 20: 49-56, 2005.
187. Rosenberger PH, Ickovics JR, Epel E, Nadler E, Jokl P, Fulkerson JP, Tillie JM and Dhabhar FS. Surgical stress-induced immune cell redistribution profiles predict short-term and long-term postsurgical recovery. A prospective study. J Bone Joint Surg Am 91: 2783-2794, 2009.
188. Rostrup M. Catecholamines, hypoxia and high altitude. Acta Physiol Scand 162: 389-399, 1998.
189. Saint-Mezard P, Chavagnac C, Bosset S, Ionescu M, Peyron E, Kaiserlian D, Nicolas JF and Berard F. Psychological stress exerts an adjuvant effect on skin dendritic cell functions in vivo. J Immunol 171: 4073-4080, 2003.
190. Sapolsky RM. The influence of social hierarchy on primate health. Science 308: 648-652, 2005.
191. Sarnquist FH. Physicians on Mount Everest. A clinical account of the 1981 American Medical Research Expedition to Everest. West J Med 139: 480-485, 1983.
192. Saul AN, Oberyszyn TM, Daugherty C, Kusewitt D, Jones S, Jewell S, Malarkey WB, Lehman A, Lemeshow S and Dhabhar FS. Chronic stress and susceptibility to skin cancer. J Natl Cancer Inst 97: 1760-1767, 2005.
193. Schedlowski M, Jacobs R, Stratmann G, Richter S, Hadicke A, Tewes U, Wagner TO and Schmidt RE. Changes of natural killer cells during acute psychological stress. J Clin Immunol 13: 119-126, 1993.
194. Schwab CL, Fan R, Zheng Q, Myers LP, Hebert P and Pruett SB. Modeling and predicting stress-induced immunosuppression in mice using blood parameters. Toxicol Sci 83: 101-113, 2005.
195. Selkirk GA, McLellan TM, Wright HE and Rhind SG. Mild endotoxemia, NF-kappaB translocation, and cytokine increase during exertional heat stress in trained and untrained individuals. Am J Physiol Regul Integr Comp Physiol 295: R611-R623, 2008.

196. Senchina DS, Shah NB, Doty DM, Sanderson CR and Hallam JE. Herbal supplements and athlete immune function--what's proven, disproven, and unproven? Exerc Immunol Rev 15: 66-106, 2009.
197. Severs Y, Brenner I, Shek PN and Shephard RJ. Effects of heat and intermittent exercise on leukocyte and sub-population cell counts. Eur J Appl Physiol Occup Physiol 74: 234-245, 1996.
198. Shephard RJ. Sleep, biorhythms and human performance. Sports Med 1: 11-17, 1984.
199. Shephard RJ. Immune changes induced by exercise in an adverse environment. Can J Physiol Pharmacol 76: 539-546, 1998.
200. Shephard RJ and Shek PN. Cold exposure and immune function. Can J Physiol Pharmacol 76: 828-836, 1998.
201. Shephard RJ and Shek PN. Immune dysfunction as a factor in heat illness. Crit Rev Immunol 19: 285-302, 1999.
202. Shing CM, Hunter DC and Stevenson LM. Bovine colostrum supplementation and exercise performance: potential mechanisms. Sports Med 39: 1033-1054, 2009.
203. Singh I, Chohan IS, Lal M, Khanna PK, Srivastava MC, Nanda RB, Lamba JS and Malhotra MS. Effects of high altitude stay on the incidence of common diseases in man. Int J Biometeorol 21: 93-122, 1977.
204. Spence L, Brown WJ, Pyne DB, Nissen MD, Sloots TP, McCormack JG, Locke AS and Fricker PA. Incidence, etiology, and symptomatology of upper respiratory illness in elite athletes. Med Sci Sports Exerc 39: 577-586, 2007.
205. Starkie RL, Hargreaves M, Rolland J and Febbraio MA. Heat stress, cytokines, and the immune response to exercise. Brain Behav Immun 19: 404-412, 2005.
206. Steinacker JM, Lormes W, Lehmann M and Altenburg D. Training of rowers before world championships. Med Sci Sports Exerc 30: 1158-1163, 1998.
207. Stevens RG. Light-at-night, circadian disruption and breast cancer: assessment of existing evidence. Int J Epidemiol 38: 963-970, 2009.
208. Suarez EC. Self-reported symptoms of sleep disturbance and inflammation, coagulation, insulin resistance and psychosocial distress: evidence for gender disparity. Brain Behav Immun 22: 960-968, 2008.
209. Sureda A, Tauler P, Aguilo A, Cases N, Llompart I, Tur JA and Pons A. Influence of an antioxidant vitamin-enriched drink on pre- and post-exercise lymphocyte antioxidant system. Ann Nutr Metab 52: 233-240, 2008.
210. Tiollier E, Schmitt L, Burnat P, Fouillot JP, Robach P, Filaire E, Guezennec C and Richalet JP. Living high-training low altitude training: effects on mucosal immunity. Eur J Appl Physiol 94: 298-304, 2005.
211. Toth LA, Tolley EA and Krueger JM. Sleep as a prognostic indicator during infectious disease in rabbits. Proc Soc Exp Biol Med 203: 179-192, 1993.
212. van Deventer SJ, Buller HR, ten Cate JW, Aarden LA, Hack CE and Sturk A. Experimental endotoxemia in humans: analysis of cytokine release and coagulation, fibrinolytic, and complement pathways. Blood 76: 2520-2526, 1990.
213. Veldhuis JD, Johnson ML, Iranmanesh A and Lizarralde G. Rhythmic and nonrhythmic modes of anterior pituitary hormone release in man. In: Biological rhythms in clinical and laboratory medicine, edited by Touitou Y and Haus E. Heidelberg: Springer Verlag, 1994, p. 277-291.

214. Vgontzas AN, Papanicolaou DA, Bixler EO, Kales A, Tyson K and Chrousos GP. Elevation of plasma cytokines in disorders of excessive daytime sleepiness: role of sleep disturbance and obesity. J Clin Endocrinol Metab 82: 1313-1316, 1997.
215. Viswanathan K, Daugherty C and Dhabhar FS. Stress as an endogenous adjuvant: augmentation of the immunization phase of cell-mediated immunity. Int Immunol 17: 1059-1069, 2005.
216. Viswanathan K and Dhabhar FS. Stress-induced enhancement of leukocyte trafficking into sites of surgery or immune activation. Proc Natl Acad Sci U S A 102: 5808-5813, 2005.
217. Walsh NP, Bishop NC, Blackwell J, Wierzbicki SG and Montague JC. Salivary IgA response to prolonged exercise in a cold environment in trained cyclists. Med Sci Sports Exerc 34: 1632-1637, 2002.
218. Walsh NP and Whitham M. Exercising in environmental extremes: a greater threat to immune function? Sports Med 36: 941-976, 2006.
219. Waninger KN and Harcke HT. Determination of safe return to play for athletes recovering from infectious mononucleosis: a review of the literature. Clin J Sport Med 15: 410-416, 2005.
220. Wenisch C, Narzt E, Sessler DI, Parschalk B, Lenhardt R, Kurz A and Graninger W. Mild intraoperative hypothermia reduces production of reactive oxygen intermediates by polymorphonuclear leukocytes. Anesth Analg 82: 810-816, 1996.
221. West NP, Pyne DB, Peake JM and Cripps AW. Probiotics, immunity and exercise: a review. Exerc Immunol Rev 15: 107-126, 2009.
222. Wood PG, Karol MH, Kusnecov AW and Rabin BS. Enhancement of antigen-specific humoral and cell-mediated immunity by electric footshock stress in rats. Brain Behav Immun 7: 121-134, 1993.
223. Woods JA, Vieira VJ and Keylock KT. Exercise, inflammation, and innate immunity. Immunol Allergy Clin North Am 29: 381-393, 2009.
224. Wright CE, Erblich J, Valdimarsdottir HB and Bovbjerg DH. Poor sleep the night before an experimental stressor predicts reduced NK cell mobilization and slowed recovery in healthy women. Brain Behav Immun 21: 358-363, 2007.
225. Yfanti C, Akerstrom T, Nielsen S, Nielsen AR, Mounier R, Mortensen OH, Lykkesfeldt J, Rose AJ, Fischer CP and Pedersen BK. Antioxidant supplementation does not alter endurance training adaptation. Med Sci Sports Exerc 42: 1388-1395, 2010.
226. Zhang Y, Hu Y and Wang F. Effects of a 28-day "living high--training low" on T-lymphocyte subsets in soccer players. Int J Sports Med 28: 354-358, 2007.

A Review of Sex Differences in Immune Function after Aerobic Exercise

Trevor L. Gillum[1], Matthew R. Kuennen[3], Suzanne Schneider[2], and Pope Moseley[4]

[1] California Baptist University, Riverside, CA 92504,
[2] University of New Mexico, Albuquerque, NM 87122,
[3] West Texas A&M University, Canyon, Texas 79016,
[4] Department of Internal Medicine, University of New Mexico, Albuquerque, NM 87122

ABSTRACT

When menstrual phase and oral contraceptives are controlled for, males and females display marked differences in immune response to an exercise stress. In highly controlled research studies, sex differences in immune cell changes, cytokine alterations, along with morbidity and mortality after inoculation are apparent. Exercise has been hypothesized to serve as a model of various clinical stresses by inducing similar hormonal and immunological alterations. Thus, a greater understanding of sex differences in post exercise non-specific immune function may provide insight into more effective clinical approaches and treatments. This paper reviews the recent evidence supporting sex differences in post exercise immune response and highlights the need for greater control when comparing the post exercise immune response between sexes.

Key Words: Immune Function, Sex, Cytokines, Aerobic Exercise.

INTRODUCTION

Exercise as a model to assess immune function

Exercise modulates the non-specific (innate) (52) and specific (acquired or adaptive) (12) arms of the immune system with an intensity dependent response. Moderate bouts of exercise have been shown to enhance immunity (51). However, intense exercise depresses the immune system (8, 52). More specifically, during

Address for Correspondence:
Trevor Gillum, Department of Kinesiology, California Baptist University
8432 Magnolia Avenue, Riverside, CA 92504
Phone: (951) 343-4950, Email: tgillum@calbaptist.edu

moderate and intense bouts of exercise there are transient increases in circulating pro- and anti- inflammatory cytokine levels (55), concentration of lymphocytes and lymphocyte sub-sets (46), and macrophage activity (22). Recently, researchers (9, 53, 75, 76,) have suggested there are sex differences in the immune response to moderate and intense exercise.

Exercise has been hypothesized to serve as a model for certain clinical stresses. In a review article, Dr. BK Pederson wrote:

> "Physical exercise can be regarded as a prototype of physical stress. Many clinical physical stressors (e.g. surgery, trauma, burn, sepsis) induce a pattern of hormonal and immunological responses that have similarities to that of exercise (60)."

Clinical physical injury, similar to exercise injury, displays marked sex differences (4). For example, females have higher levels of mortality than males in response to burns of similar size (31). Females have a lower incidence of multiple organ dysfunction syndrome (MODS) and sepsis in response to shock compared to males (17). It is thought that the disparity in sex outcomes results from interactions of sex hormones with various aspects of the immune system. Since exercise induces similar immune responses, it may provide a useful model to study sex differences in immune response to clinical stressors. However, to understand this relationship, studies that control for menstrual phase, oral contraceptive (OC) use, and fitness levels between men and women are needed. The focus of this narrative review will be to discuss what is currently known about sex differences in non-specific immune responses to aerobic exercise. This review will discuss both animal and human studies that have examined the post exercise immune response.

Sex Difference in Immune Function in Non-Exercising Conditions

Several aspects of immunity have marked sex differences in non-exercising conditions. T cells, macrophages, and monocytes possess estrogen receptors (4) with two different subtypes, ERα and ERβ (61). ERα is mainly found in the uterus and mammary glands, while ERβ prevails in the central nervous, cardiovascular, and immune systems (32). Through these receptors, estrogen led to greater survival against herpes simplex virus 1 (HSV-1) in inoculated rats (9). In addition, in vitro stimulation of lymphocytes with phytohemagglutinin, a toxin used to elicit cytokine production from immune competent cells, found that females produce more Th2 (IL-4, IL-10) cytokines than males (29). Th2 cytokines are responsible for secretion of antibodies and this may play a role in the higher incidence of autoimmune diseases in women (85). Furthermore, females have a higher percentage of T lymphocytes within the total lymphocyte pool (5), and have more active circulating polymorphonuclear leukocytes (neutrophils) and macrophages (64, 65). Overall, physiologic levels of estrogen stimulate humoral and cell-mediated immune responses, but large increases in estrogen (either from pregnancy or supraphysiologic doses) can suppress cell-mediated immunity (54). Taken together, results imply that females of reproductive age have a more active immune system than age matched males. This could account for females having a lower incidence of, and mortality rates from, certain types of infection (bacteria septlcemai, pneumonia/influenza, bacterial meningitis) (28) and lower rates of atherosclerosis (79). Similarly, this could also explain the increased incidence of autoimmune diseases.

Sex Difference in Immune Response to Exercise: Inoculation Studies

Inoculating animals with viruses has previously been used as a model to study upper respiratory infections in animals by inducing illness (33). Inoculation purposefully infects the animal by transferring the causative agent into the animal. In this manner, whole body responses can be measured after inducing a specific illness. With this methodology, female mice experienced lower mortality after intranasal inoculation with herpes simplex virus 1 (HSV-1) at rest and after exercise than males. HSV-1 was delivered after the third bout of running to exhaustion or after 3 non-exercising control sessions. Though exercise resulted in greater morbidity (illness symptoms) than control, both sexes experienced the same degree of morbidity. Despite males and females having a similar rate of infection by HSV-1 after inoculation, fewer females died (9). Similarly, female mice that exercised at a moderate intensity had a greater macrophage resistance to HSV-1 than their male counterparts (8). However, both males and females experienced suppressed macrophage function after exhaustive exercise, and experienced this suppression to a similar degree. Thus, it is plausible that the decreased mortality after HSV-1 inoculation seen in female mice may be due to increased macrophage function. Since more females survived HSV-1 inoculation than males, the presence of estrogen could be an important determinant of this response. However, ovariectomized mice supplemented with estrogen experienced higher mortality than intact female mice after HSV-1 inoculation (7). Despite the better protection of intact mice, there was only a trend ($p=0.1$) toward intact females having greater macrophage resistance than the estrogen treated ovariectomized group. Therefore, the authors suggested that antiviral macrophage resistance is not responsible for the lower mortality (7). Since estrogen supplementation did not restore the protective effects of intact mice, other female hormones could be responsible for this added fortification of female mice. Taken together, animal research with HSV-1 inoculation demonstrates that male and female mice are equally susceptible to an infection at rest or after exhaustive exercise. However, more females survived. The greater macrophage activity may be responsible for this effect, but future studies should incorporate other immune parameters. The mechanism behind greater female survival with HSV-1 may be related to other ovarian hormones besides estrogen. It should be noted that the results from the experiments above were performed by a single research group and have yet to be replicated by others.

Sex Difference in the Cytokine Response to Exercise

The local response to a tissue injury involves the release of cytokines. Cytokines are released from the site of inflammation. The local response of cytokine release is supplemented by the release of cytokines from the liver, termed the acute phase response. The acute phase cytokines are TNF-α, IL-1β, and IL-6. These pro-inflammatory cytokines cause the movement of lymphocytes, neutrophils, and monocytes to the injured site. These leukocytes ultimately infiltrate the damaged muscle and serve to repair the tissue (2). Initially, exercise leads to increased release of pro-inflammatory cytokines (TNF-α, IL-1β,) and this is counteracted quickly by the release of cytokine inhibitors (IL-1ra, TNF receptors) and anti-inflammatory cytokines (IL-10), which limit the inflammatory response of exercise (60).

With chronic exercise and training, there is a decrease in cytokine production during an acute bout of exercise (69). Decreased cytokine release may contribute to immunosuppression and lead to a greater risk of bacteria and infection that is often evident in endurance-trained athletes (51). However, this decrease in inflammation could be a key link between exercise and health through a possible reduction in the risk of chronic disease.

Generally, cytokines are released after prolonged exercise or exercise that causes muscle damage (10, 60). The intensity and duration of exercise, along with fitness level, determines the cytokine profile (30). Interestingly, exercise does not cause an alteration in pro-inflammatory gene expression in peripheral blood mononucleated cells (PBMC) (81), suggesting that this is not a primary site for cytokine release. Recently, researchers demonstrated IL-6 is released from the exercising muscle (38, 67). IL-6 can increase 100 fold after exercise making it the most responsive cytokine to exercise and perhaps underscoring its biological significance. IL-6 has been shown to regulate metabolic factors such as glucose uptake and fatty acid oxidation (59). Recently, IL-6 released from the exercising muscle has been shown to have anti-inflammatory properties through its up-regulation of anti-inflammatory cytokines IL-1ra (56) and IL-10 (55), in addition to inhibiting TNF-α release (66). For a detailed review of IL-6 and exercise, see *Febbraio, 2005* (21).

Sex differences in the regulation of cytokines have been previously demonstrated in non-exercising conditions. After lymphocytes were stimulated with phytohemaglutinin, a toxin used to elicit cytokine production from immune competent cells, a greater Th1 profile, characterized by increased release of IFN-γ and IL-2, was shown in lymphocytes drawn from men compared to women. Women possessed a greater Th2 cytokine release (IL-4, IL-10) than men, but there were no differences across the menstrual cycle (29). Th2 cytokines are responsible for humoral mediated immunity and lead to increased secretion of antibodies. Similarly, IL-1 release from mononucleated cells is lower in males and is menstrual phase dependent in females (44). More specifically, the balance of the IL-1 family (IL-1-α, IL-β - agonist, IL-1ra - antagonist) is menstrual phase dependent. The ratio of agonist (IL-1-α, IL-β) to antagonist (IL-1ra) was equal during the follicular stage, but the agonist was ~45% higher in the luteal phase. Thus, the activity of IL-1α/β was greater in the luteal phase. IL-1β may influence reproductive functions like endometrial development and preparing the birth canal for parturition. IL-1β has also been shown to block luteinizing hormone and ovulation in rats (28). After trauma-hemorrhage injury, ovariectomized mice had decreased cytokine expression (IL-2, IL-3, and IFN-γ) from macrophages compared to ovariectomized mice treated with 17-β estradiol. The estradiol treated group maintained cytokine release after injury and this suggests that estrogen is capable of preventing immunosuppression that had been previously demonstrated with male mice and enhancing survival (41).

Currently, there are a handful of studies that have compared the cytokine response to exercise between sexes. There was no difference reported in serum IL-10, IL-1ra, IL-6, and IL-8 between men and women immediately and 1.5 hours after completing a marathon (50). The in-vitro production of IL-1, IFN-γ, and IL-4

from cultured whole blood showed no differences between sexes in response to continuous incremental cycling at 55%, 70%, and 85% VO_{2peak} (49). Similarly, 90 minutes of cycling at 65% VO_{2max} resulted in no difference in serum IL-6 levels between men and women (75). There was however, a trend ($p=0.06$) of increased IL-6 in women who took OC and those who were not taking OC and exercising in the follicular phase (75). The change in IL-6 values could be due to altered carbohydrate (CHO) oxidation rates. It was shown that whole body CHO oxidation during 50 min of cycling at 70-90% of lactate threshold is higher in the follicular phase (89). This higher rate of CHO oxidation could have lead to a greater depletion of CHO. In response to low CHO availability, IL-6 production will increase (38). In contrast, Edwards found that 60 minutes after a maximal cycling test, female IL-6 values were greater than men (18), although there were no differences between sexes at baseline, immediately, or 30 minutes post exercise. At 60 minutes post exercise, the male IL-6 values decreased towards baseline while the female values continued to rise. The exercise-induced IL-6 response is directly linked to the duration and intensity of exercise, along with the number of muscle fibers recruited (increased release) and the fitness level of subjects (decreased response) (57). Thus, methodological differences could account for the current disparity in the literature regarding IL-6.

At the transcriptional level, Northoff et al found a sex and menstrual phase difference in mRNA inflammatory gene expression in response to a 60 min run at 93% of the individual's anaerobic threshold (53). Women in the luteal phase demonstrated a greater condition of pro-inflammation than women in the follicular phase or men immediately after exercise. This pro-inflammatory state was characterized by an increase in inflammatory genes (interferon-γ, IL-12 receptor β1, and prostaglandin D2 receptor) and a decrease in anti-inflammatory genes (IL-6, IL1R2, IL1-ra) in PBMC. The authors state that the increase pro-inflammatory condition in the luteal phase could be a "mechanism designed to end a very early pregnancy in case of major external stress input. After all, human females get a new chance to conceive in the next month and nature may prefer to destabilize a pregnancy under influence of stress rather than carry it on under high risk." Furthermore, women in the luteal phase regulated over 200 genes (129 genes up-regulated, 143 genes down-regulated), while women in the follicular phase regulated 80 genes (48/32) and men regulated only 63 genes (34/29). Interestingly, post exercise IL-6 mRNA was down- regulated in the luteal phase, while up-regulated in the follicular phase after exercise. Future studies that control for menstrual cycle are needed to assess the expression of the specific proteins before any conclusions can be drawn.

Thus, in limited research on aerobic exercise, it appears the overall cytokine response to exercise is not markedly different between sexes. However, few studies controlled for either menstrual phase or oral contraception. Some work has demonstrated a greater up-regulation of inflammation (129 genes up-regulated, 143 genes down-regulated) in the luteal phase at the transcriptional level after exercise (53). Potential sex differences in IL-6 may exist after maximal exercise (18) and further research is needed to confirm the IL-6 response at longer time points after exercise while controlling for menstrual phase and oral contraceptive use.

Sex Differences in Leukocyte Response to Exercise

Moderate aerobic exercise results in a transient increase in both innate (monocytes, macrophages, neutrophils, NK cells) and specific (B and T lymphocytes) cells of the immune system. The effector cells of the innate immune system are monocytes, macrophages, neutrophils, and a subset of lymphocytes called natural killer (NK) cells. These cells represent the first line of defense against infections by neutralizing microbes or pathogens through phagocytosis (monocytes, macrophages, neutrophils) or by directly lysing the pathogen (NK cells). T cells recognize specific antigens presented to them to create memory cells, and B cells secrete antibodies to kill extracelluar pathogens. B cells are fundamental for eradicating bacterial infections. The number of total leukocytes, lymphocytes, granulocytes (neutrophils), and monocytes increase in a biphasic response (46). The immediate increase of leukocytes is characterized by increases in lymphocytes, monocytes, macrophages, and neutrophils, and is then followed by a delayed response of additional neutrophils 2 hours post exercise (46, 87).

Both the duration and intensity of exercise combine to determine the specific increase in leukocytes with exercise. Exercising for up to 30 minutes leads to increased lymphocytes (CD4+T cells, CD8+T cells, CD19+ B cells, CD16+ NK cells, CD56+ NK cells), which return to baseline values within 10-30 minutes after cessation of exercise (46). Longer duration exercise requires longer time periods for leukocytes to return to baseline. Specifically, CD8+ lymphocytes increase more with exercise than CD4+ cells (60). CD8+ lymphocytes can directly kill foreign or infected cells, whereas CD4+ are helper cells that mainly produce cytokines to magnify the immune response. Also, memory lymphocytes are recruited into the circulation more so than naïve lymphocytes (27). Memory cells are more likely than naïve cells to relocate to non-lymphoid tissues or possible locations of infection, like the vasculature of the skin, lung, liver, and gut.

The increases in epinephrine release and cardiac output associated with exercise are thought to contribute to the exercise-induced leukocytosis through de-margination from vascular pools and immune organs (24, 26, 80). The delayed increase in neutrophils may be mediated by an increase in Granulocyte colony-stimulating factor (G-CSF) more so than epinephrine or cardiac output (87). Epinephrine release in response to submaximal exercise has been shown to be sex dependent, with males demonstrating a greater release compared to mid-follicular females (11, 15, 34). However, an overall greater expression of β_2-adrenergic receptors on lymphocyte has been found in women compared to men (43, 84). The majority of previous research suggests there are no post exercise sex differences in leukocytes (1, 49), lymphocytes (1, 49), natural killer cells (6, 48) monocytes (1) or neutrophils (1). However, the above studies did not control for menstrual cycle phase, oral contraceptives, or matching male and female subjects for activity or fitness level.

In one of the few studies to examine immune cell changes that controlled for menstrual phase, oral contraception, and fitness, Timmons *et al* showed that women taking OC had a greater post exercise increase in lymphocytes and neutrophils compared to men and non-OC users after 90 min of cycling at 65% of VO_{2max}

(75). Women taking OC experienced cycle specific (follicular and luteal phases that corresponded to triphasic OC) exercise induced changes in total leukocytes, neutrophils, monocytes, and lymphocytes, whereas non OC users had no fluctuations across the menstrual cycle. The increase in immune cells after exercise were greater in OC users on days taking the pill, and these increases were always greater than the post-exercise changes seen in men. There were no differences in total leukocytes, neutrophils, and monocytes between men and regularly menstruating women not taking OC. However, non-OC users had a greater post exercise increase in lymphocytes than men. Taken together, this study demonstrated immune cell changes between men and women that are specific to OC use. There was a greater increase in immune cells after exercise in the high progesterone phase of women taking OC than men and non OC using women. Also, non OC using women had more lymphocytes circulating post exercise than men.

Since there were no changes in lymphocyte number across the menstrual cycle in non-OC users, sex hormones probably do not account for sex differences. While the authors corrected for exercise-induced changes in plasma volume, there was no mention of correcting for contraceptive induced changes in plasma volume. Previous research has found an increase in plasma volume in women taking OC (83). A difference in plasma volume between woman taking OC and those who did not could influence the results not only of the previous study, but also much of the preceding literature.

Thus, with moderate to intense aerobic exercise, the circulating leukocyte populations change dramatically. However, the majority of research suggests that there is no difference between sexes in the leukocyte response to aerobic exercise. Currently, Timmons *et al* is the only study to control for OC use, and the only study to show a difference between men, OC, and non OC users. Future research is warranted.

Sex Differences in Natural Killer Cell Response to Exercise

Natural Killer (NK) cells are a subset of lymphocytes produced in the bone marrow and are part of the innate immune system. NK cells kill virally infected cells or tumor cells through direct cytolytic mechanisms, without activation. NK cells account for 10-15% of circulating blood mononuclear cells. During exercise, NK cells are transiently increased by 186- 344% of initial resting value, following both maximal and sub-maximal bouts (63). NK cells are the most responsive leukocyte to exercise due to their catecholamine sensitivity (25). The magnitude of increase in NK cells is more responsive to the intensity than duration of exercise. Generally, NK cell number and activity will decline only in intense exercise lasting at least 1 hour (58). At rest, men have a higher NK cell activity despite no difference in NK cell numbers than regularly menstruating women or women using OC. Women using OC had the lowest NK cell activity (88). Furthermore, IL-1 release from monocytes, an activator of NK cell activity, has been shown to be both sex and menstrual phase dependent (44).

Previous research supports the notion that there are no sex differences in NK cell number or activity in response to incremental or continuous exercise (6, 48).

However, neither study controlled for menstrual phase or OC use. In contrast, adolescent girls not taking OC and tested in the mid-follicular phase had a greater increase in NK cell count than adolescent boys during (77) and after (78) cycling exercise for 60 min at 70% VO_2**.** Also, NK cell subset expression was significantly different between sexes (77). NK cells can be divided into 2 unique groups: $CD56^{dim}$, representing 90% of the circulating NK cells, and $CD56^{bright}$ cells that are more responsible for inflammation (13). The ratio of $CD56^{dim}$: $CD56^{bright}$ have been shown to play a role in reproduction as the concentration of NK cells in the uterine mucosa changes across the menstrual cycle and with pregnancy (40). For an in depth review of NK cell subset changes with exercise see *Timmons, 2008* (74). NK cell activity was not assessed in either study. Since results from Yovel 2001 (88) suggest there is both a sex and OC effect on NK cell activity at rest, future controlled studies are needed to quantify NK cell activity during and after exercise in an adult population.

Sex Differences in Neutrophil Response to Exercise

Neutrophils are a large subset of granulocytes, comprising ~90% of all granulocytes. Granulocytes are characterized by the granules in their cytoplasm and consist also of basophils and eosinophils. Neutrophils are members of the innate immune system. They are part of the acute inflammatory response and are the first cells recruited from the blood to the site of injury or infection (5). Neutrophils attack microbes that have entered the circulation by phagocytosing the microbe or releasing oxidative bursts to destroy the pathogen. Neutrophils also produce cytokines to recruit more neutrophils and other immune cells to the site of injury and enhance both specific and innate immunity. Granulocytes are higher in the luteal phase compared to the follicular phase (19) and have been shown to increase during pregnancy (82). There is evidence that with pregnancy there is a decrease in cell-mediated immunity (36). As a compensation mechanism, the pregnant women increase activity of the innate system, most notably granulocytes.

Acute exercise causes a mild inflammatory response to repair damaged tissue, which is characterized first by neutrophil infiltration, followed by macrophage infiltration several hours later (23). While the current data on sex differences in neutrophil infiltration after exercise are equivocal (45, 70, 71), generally females rats have a blunted post exercise inflammatory response that leads to less neutrophils infiltrating skeletal muscles and less muscle soreness (70, 72). From animal studies, it seems that estrogen is limiting neutrophil infiltration by acting as a cell membrane stabilizer and antioxidant. However, data from human studies are less compelling. For a review of sex differences in neutrophil infiltration *see Point – Counterpoint, Tiidus & Hubal 2009 (35, 73).*

Higher numbers of circulating neutrophils were observed both at rest and after 90 min of cycle ergometry in women taking OC compared to men and non-users. Furthermore, the greatest increase in neutrophils after exercise in OC users was seen in the luteal phase when estradiol levels were lowest (75). Since estrogen has been shown to inhibit the inflammatory response to exercise (70, 72), it makes sense that neutrophils would be highest when estrogen was lowest. Previously,

data from males suggested that increased IL-6 levels during exercise lead to increases in cortisol, which ultimately are responsible for exercise neutrophilia (68). However, data from sex comparison studies suggest there is no correlation between IL-6 levels and cortisol during exercise (18, 75). OC users had higher cortisol and neutrophil levels compared to men and non-users, but equivalent resting and post exercise levels of IL-6 (75). This could potentially highlight differences in regulation of anti-inflammatory mediators between men and women and future research should be conducted to understand this response.

Potential Mechanisms of Action for Sex Differences in Immune Response to Exercise Given the post-exercise sex differences in immune function, estrogen may be responsible for this disparity. However, results from a few well-controlled studies suggest other physiologic variables account for the sex discrepancies. The sex differences in IL-6 during maximal exercise could potentially be mediated by a difference in the amount of adipose tissue (42). Mohamed-Ali showed that adipose tissue released IL-6 (47). Furthermore, increases in catecholamines during exercise are related to IL-6 release from adipose tissue (39). Thus, the greater IL-6 response in women could be due to their greater fat content (18).

The disparity in post-exercise leukocyte and neutrophil responses between women who took OC, non-OC users, and men could be related to differences in growth hormone and cortisol levels. Both growth hormone (3) and cortisol levels (75) are higher in women taking OC. Furthermore, both growth hormone (37) and cortisol (14) have been shown to increase circulating neutrophil levels. However, in Timmons *et al* (75), cortisol levels did not differ between menstrual cycle phases, only between groups. Thus, cortisol alone could not be responsible for the increased post exercise immune cell response of the OC users. Exercise induced leukocytosis seen in both men and women appear to be associated with the increased circulating catecholamines (60). Thus, as noted by Timmons *et al*, the greater increase in lymphocytes in women during exercise may be due to their greater density of lymphocyte β_2-adrenergic receptors (84, 43). Furthermore, the number of β_2-adrenergic receptors on lymphocytes decreases over 10 wks of aerobic training (62). Thus differences in training also may be responsible for some of the sex differences reported in studies that did not control for fitness.

Intact female mice had lower mortality rates to post-exercise HSV-1 inoculation compared to males or ovariectomized females (7, 8). Yet, when estrogen was replaced after ovariectomy, ovariectomized females were still more susceptible than the intact group. Therefore, the authors concluded that physiologic doses of estrogen (1μg/day) are not responsible for the enhanced immunity seen in intact female animals. Further research is warranted to confirm this finding and to identify the cause for the greater immune response of the female animals. Similarly, 8 days of supplementing men with estradiol had no effect on resting or post exercise cortisol, IL-6, or neutrophil counts after 90 min of cycle ergometry at 60% of aerobic capacity (76). This study reinforces the suggestion that estrogen alone is not responsible for immune sex differences, and could potentially point to a difference in the expression of estrogen receptors (ER) on cells throughout the body. Both males and females have ERα and ERβ in skeletal muscle, with ERα mRNA

Table 1. Sex differences in immune function in studies that controlled for menstrual phase and OC use.

Author	N size	Exercise	Immune changes
Timmons, 2005	12 women (6 OC users, 6 NOC users), 12 men	90 min cycling, 65 % VO_{2max}	38% > lymphocyte increase post exercise in NOC women compared to men.
Northoff, 2008	9 women, 12 men	60 min treadmill run, 93% AT	>Pro-inflammatory gene expression in LP compared to men or FP.
Brown, 2004	89 female mice, 86 male mice.	3 consecutive days of treadmill running after HSV-1 inoculation until volitional fatigue.	>morbidity for males (28%) compared to females (16%).
Brown, 2006	36 female mice, 36 male mice	3 days of moderate (90 min) or exhaustive (volitional fatigue) treadmill running after HSV-1 inoculation.	>macrophage antiviral resistance in moderately exercised females compared to males.
Gonzalez, 1998	9 women	80 min walking, 32% VO_{2max} in cold (-5°C) environment.	41% decrease in IL1-β after exercise in LP compared to FP. No change in IL-6 or TNFα.
Timmons, 2006a	25 girls, 33 boys	60 min cycling, 70% VO_{2max}.	>Leukocyte count at 30 & 60 min post exercise in T5 boys compared to T4/5 girls. >NK cell response immediately post exercise in T4/5 girls compared to T3/4 boys.
Timmons, 2006b	11 girls, 11 boys.	60 min cycling, 70% VO_{2max}.	> Lymphocyte count in girls at 30 min (29%) and 60 min (23%) of exercise. $CD56^{dim}$ cells (105%) and $CD56^{dim}$ expressed as proportions (67%) greater in girls. $CD56^{bright}$ cell counts 82% greater in girls but not $CD56^{bright}$ proportions.
Ferrandez, 1999	60 female, 60 male mice	Swimming until exhaustion	>chemotaxis index in females compared to age matched male mice

OC – oral contraceptive user. NOC – non oral contraceptive user. LP – Luteal Phase. FP-Follicular Phase. T5 – Tanner stage 5. T4/5 – Tanner stage 4 and 5.

180 fold greater than ERβ (86). Females exhibit greater ERβ expression in the lungs than men (20), while ERβ mRNA is higher on adipocytes in women (16). Taken together, these data suggests a sex difference not only in ER quantities, but also a site-specific preferential expression of ER isotypes.

Future Research Considerations and Conclusions

When menstrual phase and oral contraceptives are controlled, males and females display marked differences in immune response to exercise (Table 1). Sex differences in immune cell changes, cytokine alterations, along with morbidity and mortality are apparent after submaximal and maximal aerobic exercise stressors. The primary mechanism for many of the sex differences does not appear to involve the presence of estrogen. Thus, future research should clarify which specific ovarian-related changes are responsible for these immune response differences and their specific actions. Future work should address the impact of site-specific ER isotypes on the post exercise immune response, as this may mediate sex differences. Also, while transcriptional evidence suggests a menstrual and sex-dependent effect on the cytokine response to running (53), there have been no studies that have examined serum cytokine responses in a similar, well controlled manner. Studies examining cytokines should carefully control intensity with regards to metabolic thresholds, as the exercising muscle may be a main source of serum cytokines. By using exercise to model the stress responses to certain clinical traumas, this avenue of research may provide valuable insight into new approaches and sex-specific treatments.

REFERENCES

1. Barriga C, Pedrera MI, Maynar M, Maynar J, Ortega E. Efffect of submaximal physical exercise performed by sedentary men and women on some parameters of the immune system. Rev Esp Fisiol. 49(2): 79-85. 1993.

2. Belcastro AN, Arthurn GD, Albisser TA, Raj DA. Heart, liver, and skeletal muscle myeloperoxidase activity during exercise. J App Physiol.. 80: 1331-35. 1996.

3. Bemben DA, Boileau RA, Bahr JM, Neson RA, Misner JE. Effects of oral contraceptives on hormonal and metabolic responses during exercise. Med Sci Sports Exerc. 24: 434-41. 1992.

4. Bird MD, Karavitis J, Kovacs EJ. Sex differences and estrogen modulation of the cellular immune response after injury. Cell Immunol. 252: 57-67. 2008.

5. Bouman A, Schipper M, Heineman M, Faas MM. Gender difference in the non-specific and specific immune response in humans. Am J Reprod Immunol. 52: 19-26. 2004.

6. Brahmi Z, Thomas JE, Park M, Doddeswell IR. The effects of acute exercise on natural killer-cell activity of trained and sedentary human subjects. J Clin Immunol. 5: 321-28. 1985.

7. Brown AS, Davis MJ, Murphy AE, Carmichael DM, Carson AJ, Ghaffar A, Mayer PE. Susceptibility to HSV-1 infection and exercise stress in female mice: role of estrogen. J App Physiol. 103: 1592-97. 2007.

8. Brown AS, Davis MJ, Murphy EA, Carmichael DM, Carson JA, Ghaffar A, Mayer PE. Gender differences in macrophage antiviral function following exercise stress. Med Sci Sports Exerc. 38(5): 859-86. 2006.

9. Brown AS, Davis MJ, Murphy EA, Carmichael DM, Ghaffar A, Mayer PE. Gender differences in viral infection after repeated exercise stress. Med Sci Sports Exerc. 36(8): 1290-1295. 2004.

10. Bruunsgaard H, Glabo H, Halkjaer-Kristensen J, Johansen T, Maclean D, Pederson B. Exercise induced increase in interleukin-6 is related to muscle damage. J Physiol (Lond). 499: 833-41. 1997.

11. Carter SL, Rennie C, and Tarnopolsky MA. Substrate utilization during endurance exercise in men and women after endurance training. Am J Physiol Endocrinol Metab. 280: E898–E907. 2001.

12. Ceddia MA, Woods JA. Exercise suppresses macrophage antigen presentation, J Appl Physiol 87: 2253–2258. 1999.

13. Cooper MA, Fehniger TA, and Caligiuri MA. The biology of human natural killer-cell subsets. Trends Immunol. 22: 633–640. 2001.

14. Cronstein BN, Kimmel SC, Levin RI, Martiniuk R, Weissmann G. A mechanism for the anti-inflammatory effects of corticosteroids: the glucocorticoid receptor regulates leukocyte adhesion to endothelial cells and expression of endothelial-leukocyte adhesion molecule-1 and intercellular adhesion 1. Proc Natl Acad Sci USA. 89: 9991-5. 1992.

15. Davis SN, Galassetti P, Wasserman DH, and Tate D. Effects of gender on neuroendocrine and metabolic counterregulatory responses to exercise in normal man. J Clin Endocrinol Metab. 85: 224–230. 2000.

16. Dieudonne MN, Leneveu MC, Giudicelli Y, and Pecquery R. Evidence for functional estrogen receptors alpha and beta in human adipose cells: regional specificities and regulation by estrogens. Am J Physiol Cell Physiol. 286: C655–C661. 2004.

17. Dolecek R. Endocrine changes after burn trauma--a review. Keio J Med. 38:262–276. 1989.

18. Edwards KM, Burns VE, Ring C, Carroll D. Individual differences in the interleukin-6 response to maximal and submaximal exercise tasks. J Sports Sci. 24(8): 855-8. 2006.

19. Faas M, Bouman A, Moes J, Heineman MJ, de Leij L, Schuiling G. The immune response during the luteal phase of the ovarian cycley: a TH2 type response? Fertil Steril. 74: 1008-13. 2000.

20. Fasco MJ, Hurteau GJ, and Spivack SD. Gender-dependent expression of alpha and beta estrogen receptors in human nontumor and tumor lung tissue. Mol Cell Endocrinol 188: 125–140. 2002.

21. Febbraio MA, Pederson BK. Contraction induced myokine production and release: is skeletal muscle an endocrine organ? Exerc Sport Sci Rev. 33(3): 114-9. 2005.

22. Ferrandez MD, De La Fuente M. Effects of age, sex, and physical exercise on the phagocytotic process of murine peritoneal macrophages. Acta Physiol Scand. 166: 47-53. 1999.

23. Fielding, RA, Manfredi TJ, Ding W, Fiatarone MA, Evans WJ, and Cannon JG. Neutrophil and IL-beta accumulation in skeletal muscle. Am J Physiol Regulatory Integrative Comp Physiol. 265: R166-R172. 1993.

24. Foster NK, Maryn JB, Rangno Re, Hogg JC, Pardy Rl. Leukocytosis of exercise: role of cardiac output and catecholamines. J App Physiol. 61: 2218-23. 1986.

25. Fry RW, Morton AR, Crawford GP, Keast D. Cell numbers and in vitro responses of leukocyte and lymphocyte subpopulations following maximal exercise and interval training session of different intensities. Eur J App Physiol. 64: 218-227. 1992.

26. Gader AM, Cash JD. The effect of adrenaline, noradrenalin, isoprenaline and salbutamol on the resting levels of white blood cells in man. Scand J Haematol. 14: 5-10. 1975.

27. Gannon GA, Rhind S, Shek PN, Shephard RJ. Naïve and memory T cell subsets and differentially mobilized during physical stress. Int J Sports Med. 23: 223-29. 2002.

28. Gannon JG, St. Pierre B. Gender differences in host defense mechanisms. J Psychiatr Res. 31(1): 99-113. 1997.

29. Giron - Gonzalea JA, Moral FJ, Elvira J, Garcia-Gil D, Guerrero F, Gavilan I, Escobar L. Consistent production of a higher TH1:TH2 cytokine ratio by stimulated T cells in men compared to women. Eur J Endocrinol. 143(1): 31-36. 2000.

30. Gokhale R, Chandrashekara S, Vasanthakumar K. Cytokine response to strenuous exercise in athletes and non-athletes—an adaptive response. Cytokine. 40(2): 123-7. 2007.

31. Gregory MS, Faunce DE, Duffner LA, Kovacs EJ. Gender difference in cell-mediated immunity after thermal injury is mediated, in part, by elevated levels of interleukin-6. J Leukoc Biol. 67:319-326. 2000.

32. Gustafsson JA. Novel aspects of estrogen actions. J Soc Gynecol Investig. Jan-Feb; 7(1 Suppl): S8-9. 2000.

33. Hardy RD, Jafri HS, Olsen K, Hatfield J, et al. Mycoplasma pneumoniae induces respiratory infection, airway hyperreactivity, and pulmonary inflammation: a murine model of infection-associated chronic reactive airway disease. Infect Immun. 70(2): 649-54. 2002.

34. Horton TJ, Pagliassotti MJ, Hobbs K, and Hill JO. Fuel metabolism in men and women during and after long-duration exercise. J App Physiol.. 85: 1823–1832. 1998.

35. Hubal MJ, Clarkson P. Counterpoint: Estrogen and Sex do not Significantly Influence Post-Exercise Indexes of Muscle Damage, Inflammation, and Repair. J Appl Physiol. 106: 1012-1014. 2009.

36. Jamieson DJ, Theiler RN, Rasmussen SA. Emerging infections and pregnancy. Emerg Infect Dis. 12(11): 1638-43. 2006.

37. Kappel M, Hansen MB, Diamant M, Jorgensen JO, Gyhrs A, Pederson BK. Effects of an acute bolus growth hormone infusion on the immune system. Horm Metab Res. 25: 579-85. 1993.

38. Keller C, Steensberg A, Pilegaard H, Osada T, Saltin B, Pedersen BK, and Neufer PD. Transcriptional activation of the IL-6 gene in human contracting skeletal muscle: influence of muscle glycogen content. FASEB J. 15: 2748–2750. 2001.

39. Keller P, Keller C, Robinson LE, Perderson BK. Epinephrine infusion increases adipose interleukin-6 gene expression and systemic levels in humans. J App Physiol. 97: 1309- 12. 2004.

40. King A, Burrows T, Verma S, Hiby S, and Loke YW. Human uterine lymphocytes. Hum Reprod Update. 4: 480–485, 1998.

41. Knoferl MW, Jarrar D, Angele MK, Ayala A, et al. 17 beta-Estradiol normalizes immune responses in ovariectomized females after trauma-hemorrhage. Am J Physiol Lung Cell Mol Physiol. 281: C1131-C1138. 2001.

42. Kulwara M, Venkatraman J, Awad A, Pendergast D. Effect of dietary fat intake and exercise on inflammatory mediators of the immune system in sedentary men and women. J Am Coll Nutr. 23(4): 331-40. 2004.

43. Landmann R. Beta-adrenergicreceptors in human leukocyte subpopulations. Eur J Am Coll Nutr. 1:30 -36. 1992.

44. Lynch EA, Dinarello CA, Gannon JG. Gender differences in IL-1 alpha, IL-1 Beta, and IL-1 receptor agonist from mononuclear cells and urinary excretion. J Immunol. 53(1): 300-06. 1994.

45. MacIntyre DL, Reid WD, Lyster DM, McKenzie DC. Different effects of strenuous eccentric exercise on the accumulation of neutrophils in muscle in women and men. Eur J Appl Physiol. 81: 47–53, 2000.

46. McCarthy DA, Dale MM. The leukocytosis of exercise. A review and model. Sports Med. 6: 333-363. 1988.

47. Mohamed-Ali V, Goodrick S, Rawesh A, et al. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor alpha, in vivo. J Clin Endocrinol Metab. 82: 4196-4200. 1997.

48. Moyna MN, Acker GR, Weber KM, Fulton JR, Robettson RJ, Goss FL, Rabin BS. Exercise-induced alterations in natural killer cell number and function. Eur J of App Physiol. 74: 227-33. 1996.

49. Moyna NM, Acker GR, Fulton JR, Weber K, Goss FL, Robertson RJ, Tollerud DJ, Rabin BS. Lymphocyte function and cytokine production during incremental exercise in active and sedentary males and females. Int J Sports Med. 17(8): 585-91. 1996a.

50. Nieman DC, Henson DA, Smith LL, Utter AC, Vinci DM, Davis MJ, Kaminsky DE, Shute M. Cytokine changes after a marathon race. J App Physiol. 91: 109-14. 2001.

51. Nieman DC. Exercise, upper respiratory tract infection, and the immune system. Med Sci Sports Exerc. 26(2): 128-39. 1994.

52. Nieman, DC. Immune response to heavy exertion. J App Physiol. 82: 1385-94. 1997.

53. Northoff H, Symon S, Zieker D, Schaible E, Schafer K, et al. Gender – and – menstrual phase dependent regulation of inflammatory gene expression in response to aerobic exercise. Exerc Immunol Rev. 14:86-103. 2008.

54. Olsen NJ, Kovacs MA. Gonadal steroids and immunity. Endocr Rev. 17: 369-84. 1996.

55. Ostrowski K, Rohde T, Asp S, Schjerling P, Pederson BK. Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. J Physiol. 515: 287-91. 1999.

56. Ostrowski K, Schjerling P, Pedersen BK. Physical activity and plasma interleukin-6 in humans—effect of intensity of exercise. Eur J Appl Physiol 83: 512–515, 2000.

57. Pedersen BK, Febbraio M. Muscle derived interleukin 6 – a possible link between skeletal muscle, adipose tissue, liver, and brain. Brain Behav Immun. 19(5): 371-6. 2005.

58. Pedersen, BK. Exercise and cytokines. Immunol Cell Biol. 78: 532-35. 2000.

59. Pedersen BK, Akerstrom CA, Nielson AR, Fischer C. Role of myokines in exercise and metabolism. J App Physiol. 103: 1093-98. 2007.

60. Pedersen BK, Hoffman-Goetz L. Exercise and the immune system: Regulation, integration, and adaptation. Annu Rev Physiol. 80: 1055-81. 2000.

61. Samy TS, Schwacha MG, Cioffi WG, Bland IH, et al. Androgen and estrogen receptors in splenic T lymphocytes: effect of flutamide and trauma-hemorrhage. Shock. 14: 465-70. 2000.

62. Schaller K, Mechau D, Brosse-Scharmann H, Weiss M, Liesen H. Increased training load and the -adrenergic receptor system on human lymphocytes. J App Physiol. 87(1): 317-24. 1999.

63. Shephard RJ, Shek PN. Effects of exercise and training on natural killer cell counds and cytolytic activity. Sports Med. 28(3): 177-95. 1999.

64. Spitzer JA, Zhang P. Gender differences in neutrophil function and cytokine-induced neutrophil chemoattractant generation in endotoxic rats. Inflammation. 20: 485-498. 1996a.

65. Spitzer JA, Zhang P. Protein tyrosine kinase activity and the influence of gender in phagocytosis and tumor necrosis factor secretion in alveolar macrophages and lung-recruited neutrophils. Shock. 6: 426-33. 1996b.

66. Starkie R, Ostrowski SR, Jauffred S, Febbraio M, Pedersen BK. Exercise and IL-6 infusion inhibit endotoxin-induced TNF-alpha production in humans. FASEB J 17: 884–886. 2003.

67. Steensberg A, Febbraio MA, Osada T, Schjerling P, van Hall G, Saltin B, and Pedersen BK. Interleukin-6 production in contracting human skeletal muscle is influenced by pre-exercise muscle glycogen content. J Physiol. 537: 633–639. 2001.

68. Steensberg A, Fischer CP, Keller C, et al. IL-6 enhances plasma IL-1ra, IL-10, and cortisol in humans. Am J Physiol Cell Physiol. 280: C769-74. 2003.

69. Stewart LK, Flynn MG, Campbell WW, Craig BA, Robinson JP, McFarlin BK, Timmerman KL, Coen PM, Felker J, Talbert E. Influence of exercise training and age on CD14+ cell-surface expression of toll-like receptor 2 and 4. Brain Behav Immun. 19: 389–397, 2005.

70. Stupka N, Lowther S, Chorneyko K, Bourgeois JM, Hogben C, and Tarnopolsky MA. Gender differences in muscle inflammation after eccentric exercise. J Appl Physiol. 89: 2325–2332. 2000.

71. Stupka N, Tarnopolsky MA, Yardley NJ, Phillips SM. Cellular adaptation to repeated eccentric exercise-induced muscle damage. J Appl Physiol. 91: 1669–1678. 2001.

72. Tiidus PM and Bombardier E. Oestrogen attenuates post-exercise myeloperoxidase activity in skeletal muscle of male rats. Acta Physiol Scand, 166: 85–90. 1999.

73. Tiidus PM, Enns DL. Point:Counterpoint: Estrogen and sex do/do not influence post-exercise indexes of muscle damage, inflammation, and repair. J Appl Physiol. 106: 1010-1012, 2009.

74. Timmons BW, Cieslak T. Human natural killer cell subsets and acute exercise: a brief review. Exerc Immunol Rev. 14: 8-23. 2008.

75. Timmons BW, Hamadeh MJ, Devries MC, Tarnopolsky MA. Influence of gender, menstrual phase, and oral contraceptive use on immunological changes in response to prolonged cycling. J App Physiol. 99: 979-85. 2005.

76. Timmons BW, Hamadeh MJ, Tarnopolsky MA. No effect of short-term 17-β estradiol supplementation in healthy men on systemic inflammatory responses to exercise. Am J Physiol Regul Integr Comp Physiol. 291: R285-R290. 2006.

77. Timmons BW, Tarnopolsky MA, Bar-Or B. Sex based effects on the distribution of NK cell subsets in response to exercise and carbohydrate intake in adolescents. J App Physiol. 100: 1513-19. 2006b.

78. Timmons BW, Tarnopolsky MA. Snider DP, Bar-Or O. Immunologcal changes in response to exercise: Influence of age, puberty, and gender. Med Sci Sports Exerc. 38(2): 293-304. 2006a.

79. Tinahones FJ, J.M. Gomez-Zumaquero, L. Garrido-Sanchez, E. Garcia-Fuentes, G. Rojo-Martinez and I. Esteva et al., Influence of age and sex on levels of anti-oxidized LDL antibodies and anti-LDL immune complexes in the general population, J Lipid Res. 46: 452–457. 2005.

80. Tvede N, Kappel M, Klarlund K, Duhn S, Halkjaer-Kristensen J, et al. Evidence that the effect of bicycle exerciseon blood mononuclear cell proliferative responses and subsets is mediated by epinephrine. Int J Sports Med. 15: 100-04. 1994.

81. Ullum H, Martin P, Diamant M, Palmo J, Halkjaer-kristensen J, Pederson B. Bicycle exercise enhances plasma IL-6 but does not change IL-1α, IL-1β, or TNF-α pre-mRNA in BMNC. J App Physiol. 77: 93-97. 1994.

82. Veenstra van Nieuwenhoven Al, Heineman MJ, Faas MM. The immunology of successful pregnancy. Hum Reprod Update. 9: 347-57. 2003.

83. Walters WA, Lim YL. Haemodynamic changes in women taking oral contraceptives. Br J Obstet Gynaecol. 77(11): 1007-12. 2005.

84. Wheeldon NM, Newnham DM, Coutie WJ, Peters JA, McDevitt DG, Lipworth BJ. Influence of sex-steroid hormones on the regulation of lymphocyte beta 2-adrenoreceptors during the menstrual cycle. Br J Clin Pharmacol. 37: 583-88. 1994.

85. Whitacre CC, Reingold SC, O'Looney PA. A gender gap in autoimmunity. Science. 283(5406): 1277-8. 1999.

86. Wiik A, Glenmark B, Ekman M, Esbjornsson-Liljedahl M, Johansson O, Bodin K, Enmark E, and Jansson E. Oestrogen receptor beta is expressed in adult human skeletal muscle both at the mRNA and protein level. Acta Physiol Scand. 179: 381–387. 2003.

87. Yamada M, Katsuhiko S, Kudo S, Totsuka M, Nakaji S, Sugawara K. Raised plasma G-CSF and IL-6 after exercise may play a role in neutrophil mobilization into the circulation. J App Physiol. 92: 1789-94. 2002.

88. Yovel G, Shakhar K, Ben-Eilyahu S. The effect of sex, menstrual cycle, and oral contraceptives on the number and activity of natural killer cells. Gynecol Oncol. 81(2): 254-62. 2001.

89. Zderic TW, Coggan AR, Ruby, BC. Glucose kinetics and substrate oxidation during exercise in the follicular and luteal phases. J App Physiol. 90: 447-53. 2001.

Sex differences in immune variables and respiratory infection incidence in an athletic population

Michael Gleeson, Nicolette Bishop, Marta Oliveira, Tracey McCauley and Pedro Tauler

Gleeson, Bishop, McCauley and Oliveira are with the School of Sport, Exercise and Health Sciences, Loughborough University, United Kingdom.
Tauler is with the Department of Fundamental Biology and Health Sciences, University of the Balearic Islands, Palma de Mallorca, Spain

ABSTRACT

The purpose of this study was to examine sex differences in immune variables and upper respiratory tract infection (URTI) incidence in 18-35 year-old athletes engaged in endurance-based physical activity during the winter months. Eighty physically active individuals (46 males, 34 females) provided resting venous blood samples for determination of differential leukocyte counts, lymphocyte subsets and whole blood culture multi-antigen stimulated cytokine production. Timed collections of unstimulated saliva were also made for determination of saliva flow rate, immunoglobulin A (IgA) concentration and IgA secretion rate. Weekly training and illness logs were kept for the following 4 months. Training loads averaged 10 h/week of moderate-vigorous physical activity and were not different for males and females. Saliva flow rates, IgA concentration and IgA secretion rates were significantly higher in males than females (all $P < 0.01$). Plasma IgA, IgG and IgM concentrations and total blood leukocyte, neutrophil, monocyte and lymphocyte counts were not different between the sexes but males had higher numbers of B cells ($P < 0.05$) and NK cells ($P < 0.001$). The production of interleukins 1β, 2, 4, 6, 8 and 10, interferon-γ and tumour necrosis factor-α in response to multi-antigen challenge were not significantly different in males and females (all $P > 0.05$). The average number of weeks with URTI symptoms was 1.7 ± 2.1 (mean ± SD) in males and 2.3 ± 2.5 in females ($P = 0.311$). It is concluded that most aspects of immunity are similar in men and women in an athletic population and that the observed differences in a few immune variables are not sufficient to substantially affect URTI incidence. Sex differences in immune function among athletes probably do not need to be considered in future mixed gender studies on exercise, infection and immune function unless the focus is on mucosal immunity or NK cells.

Keywords: exercise training, leukocytes, immunoglobulins, cytokines, illness

Corresponding author:
Prof Michael Gleeson, School of Sport, Exercise and Health Sciences, Loughborough University, Loughborough, Leicestershire LE11 3TU, United Kingdom
Tel. 00 44(0)1509226345, Fax. 00 44(0)1509226301, E-mail: M.Gleeson@lboro.ac.uk

INTRODUCTION

Resistance to infection is strongly influenced by the effectiveness of the immune system in protecting the host against pathogenic micro-organisms. Within the general healthy human population there is a range of immuno-competency due to genetic differences, age and lifestyle habits. The sex of the individual also affects immune function. In females, oestrogens and progesterone modulate immune function (32) and thus immunity is influenced by the menstrual cycle and pregnancy (21). Consequently, sex-based differences in responses to infection, trauma and sepsis are evident (4). Women are generally more resistant to viral infections and tend to have more autoimmune diseases than men (4). Oestrogens are generally immune enhancing, whereas androgens, including testosterone, exert suppressive effects on both humoral and cellular immune responses. Females have higher levels of plasma immunoglobulin (Ig) than men and exhibit more vigorous responses to exogenous antigens, indicating a higher level of humoral immunity in females than in males (6). In females, there is increased expression of some cytokines in peripheral blood and vaginal fluids during the follicular phase of the menstrual cycle and with use of hormonal contraceptives (7). In the luteal phase of the menstrual cycle, blood leukocyte counts are higher than in the follicular phase (12), mononuclear cell expression of the heterodimeric transcription factor 1 (a key regulator of the innate immune response) is lower (35), and the immune response is shifted towards a T helper (Th) 2-type response (12). The expression of pro-inflammatory and anti-inflammatory genes in response to exercise is also influenced by the menstrual cycle and there are distinct differences in gene expression between women in the luteal phase and men (29). Thus, in the general population, there are differences in some aspects of immune function between men and women that appear to result in women getting fewer viral infections

Prolonged strenuous exercise has been associated with a transient depression of immune function (16, 17) and a heavy schedule of training and competition can lead to immune impairment in athletes. This is associated with an increased susceptibility to infections, particularly upper respiratory tract infections (URTI) (5, 13, 18, 28, 34). However, it is not clear whether any substantial sex differences exist in any aspect of immune function in an athletic population or whether any such differences affect URTI risk.

The aims of the present study were to determine if sex differences exist in resting immune variables including saliva immunoglobulin A (secretory IgA (SIgA)) secretion, plasma immunoglobulin concentrations, numbers of circulating leukocyte and lymphocyte subsets and cytokine production by antigen-stimulated whole blood culture in an athletic population. We also wished to determine if the incidence of URTI was different in male and female athletes during a period of winter training and competition. Our study population was a group of university athletes on a single campus site so that environment and pathogen exposure were likely to be similar for all subjects.

METHODS

Subjects

One hundred and eight healthy university students who were engaged in regular sports training (predominantly endurance-based activities such as running, cycling, swimming, triathlon, team games and racquet sports) volunteered to participate in the study. Subjects ranged from recreationally active to Olympic triathletes and their average self-reported training loads ranged averaged 9 h/week. Subjects were required to complete a comprehensive health-screening questionnaire prior to starting the study and had not taken any medication in the 4 weeks prior to the study. All subjects were fully informed about the rationale for the study and of all experimental procedures to be undertaken. Subjects provided written consent to participate in the study, which had earlier received the approval of Loughborough University ethical advisory committee. Subjects were enrolled after having fulfilled all inclusion criteria, and presenting none of the exclusion criteria (determined by both questionnaire and interview).

Subjects could be included if they were currently healthy, had been involved in endurance training for at least 2 years, engaged in at least 3 sessions and at least 3 h of total moderate/high intensity training time per week and were between 18-35 years of age. Subjects representing one or more of the following criteria were excluded from participation: Smoking or use of any medication, abnormal haematology (e.g. erythrocyte or leukocyte counts outside the normal range), suffered from or had a history of cardiac, hepatic, renal, pulmonary, neurological, gastrointestinal, haematological or psychiatric illness.

Sample size estimation (14) of 41 subjects per gender group was based on an expected rate of 2.0 ± 1.0 URTI episodes (mean ± SD) during the winter months (27), a target difference of 30% in number of episodes (effect size 0.6), statistical power of 80% and a type I error of 5%. We initially recruited 108 volunteers to account for an estimated 25% drop-out rate over the study period. Of these 108 subjects, 50 were female and 58 were male and 80 subjects (34 females, 46 males) completed the study. Their baseline characteristics as shown in Table 1. Self-reported weekly training loads (mean ± SD) were similar in males and females (9.7 ± 4.7 and 8.7 ± 3.8 h/week, respectively, $P = 0.339$). Reasons for dropout were given as foreign travel, injury or persistent illness (preventing subjects from performing training) or due to undisclosed reasons.

Laboratory visit

The study began in November 2008. Subjects arrived at the laboratory in the morning at 08.30-10:30 following an overnight fast of approximately 12 h. Each subject was asked to empty their bladder before body mass and height were recorded. Information about the study was given to them and they then signed an informed consent form. Subjects then sat quietly for 10 min and completed a health screen questionnaire, training habits questionnaire and inclusion/exclusion criteria questionnaire before providing a saliva sample. With an initial swallow to empty the mouth, unstimulated whole saliva was collected by expectoration into a pre-weighed vial (7 ml-capacity plastic Bijou tubes with screw top) for 2 min

with eyes open, head tilted slightly forward and making minimal orofacial movement. Saliva flow rate (ml/min) was determined by weighing with saliva density assumed to be 1.0 g/ml (9). All saliva samples were stored at –20°C until analysis. Subsequently, a venous blood sample (11 ml) was obtained by venepuncture from an antecubital vein and blood was collected into two Vacutainer tubes (Becton Dickinson, Oxford, UK) containing either K_3EDTA or heparin. Haematological analysis was immediately carried out on the EDTA sample as detailed below.

Questionnaires

During the 4-month subsequent study period subjects were requested to continue with their normal training programs and they completed a health (URTI symptoms) questionnaire on a weekly basis. Supplements (vitamins and minerals, etc.) were not permitted during this period. Subjects were not required to abstain from medication when they were suffering from illness symptoms but they were required, on a weekly basis, to report any unprescribed medications taken, visits to the doctor and any prescribed medications.

The illness symptoms listed on the questionnaire were: sore throat, catarrh in the throat, runny nose, cough, repetitive sneezing, fever, persistent muscle soreness, joint aches and pains, weakness, headache and loss of sleep. The non-numerical ratings of light, moderate or severe (L, M or S, respectively) of severity of symptoms were scored as 1, 2 or 3, respectively to provide a quantitative means of data analysis (15) and the total symptom score for every subject each week was calculated by multiplying the total number of days each symptom was experienced by the numerical ratings of L, M or S symptoms of 1, 2 or 3, respectively. In any given week a total symptom score ≥12 was taken to indicate that a URTI was present. This score was chosen as to achieve it a subject would have to record at least 3 moderate symptoms lasting for 2 days or 2 moderate symptoms lasting for at least 3 days in a given week. A single URTI episode was defined as a period during which the weekly total symptom score was ≥12 and separated by at least one week from another week with a total symptom score ≥12. Subjects were also asked to rate the impact of illness symptoms on their ability to train (normal training maintained, training reduced or training discontinued; L, M or S, respectively). Subjects were also asked to fill in a standard short form International Physical Activity Questionnaire (IPAQ; http://www.ipaq.ki.se/downloads.htm) at weekly intervals, thus providing quantitative information on training loads in metabolic equivalent (MET)-h/week (11).

Blood cell counts

Blood samples in the K_3EDTA vacutainer (4 ml) were used for haematological analysis (including haemoglobin, haematocrit and total and differential leukocyte counts) using an automated cell-counter ($A^c.T^{TM}$5diff haematology analyser, Beckman Coulter, High Wycombe, UK). The intra-assay coefficient of variation for all measured variables was less than 3.0%.

Lymphocyte subsets

Lymphocyte subsets (CD3, CD4, CD8, CD19, CD56) to enumerate total T cells, T-helper cells, T-cytotoxic cells, B cells and NK cells, respectively were deter-

mined in whole blood samples by three-colour flow cytometry (Becton Dickinson FACS-Calibur) with CellQuest analysis software (Becton Dickinson Biosciences, Oxford, UK) as described previously (25). Forward scatter versus side scatter plots were used to gate on the lymphocyte population by morphology and 10,000 lymphocyte events were acquired per analysis. Estimations of the absolute $CD3^+$, CD3+CD4+, CD3+CD8+, NK cell (CD3-CD56+) and B cell (CD3-CD19+) numbers were derived from the total lymphocyte count.

Monocyte TLR4 expression

The cell surface expression of toll-like receptor 4 (TLR4) in heparinised whole blood was quantified (geometric mean fluorescence intensity) by flow cytometry as described by Oliveira and Gleeson (31).

Antigen-stimulated cytokine production

Stimulated whole blood culture production of cytokines (IFN-γ, tumour necrosis factor (TNF)-α, interleukin (IL)-1β, IL-2, IL-4, IL-6, IL-8 and IL-10) was determined as follows: 2 ml of heparinised whole blood was added to 2 ml of RPMI medium (Sigma Chemicals, Poole, UK) with added stimulant at a dilution of 1:4000. The stimulant was a commercially available multi-antigen vaccine (Pediacel Vaccine, Sanofi Pasteur, UK) containing diphtheria, tetanus, acellular pertussis, poliomyelitis and haemophilus influenzae type b antigens. Whole blood was cultured at 37°C and 5% CO_2 for 24 h. After centrifugation at 1500 g for 10 min at 4ºC, supernatants were collected and stored frozen at -80°C prior to analysis of cytokine concentrations using an Evidence Investigator System using the cytokine biochip array EV3513 (Randox, County Antrim, UK). The stimulant dilution of 1:4000 used in this study was based on a previous pilot experiment which established the dose response curve for the measured cytokines over the dilution range of 1:200 – 1:20000. The 1:4000 dilution increased production of all cytokines by at least 4-fold above that of unstimulated whole blood culture, but induced less than 50% of the cytokine production elicited by the highest dose.

Plasma immunoglobulins

The remaining blood in the K_3EDTA tube was centrifuged at 1500 *g* for 10 min at 4 ºC within 10 min of sampling. The plasma obtained was immediately stored at –80 ºC prior to analysis of immunoglobulins A, G and M (immunoturbidometric assay on Pentra 400 autoanalyser, Horiba, France using the manufacturer's calibrators and controls). The intra-assay coefficient of variation for immunoglobulins A, G and M was 3.2%, 1.9% and 2.3%, respectively.

Saliva IgA

Duplicate saliva samples were analysed for SIgA using an ELISA kit (Salimetrics, Philadelphia, USA). The intra-assay coefficient of variation for SIgA was 3.6%. The SIgA secretion rate was calculated by multiplying the SIgA concentration by the saliva flow rate.

Statistical Analysis

Self-reported training load (h/week), average IPAQ scores (MET-h/week), anthropometric and haematological variables were compared between males and

females using unpaired t tests for normally distributed data. The blood leukocyte, neutrophil, monocyte, eosinphil and lymphocyte counts, lymphocyte subset counts, concentrations of secreted cytokines, sIgA concentrations and secretion rates were compared between males and females using unpaired t tests for normally distributed data or nonparametric Mann-Whitney tests for data that were not normally distributed. Statistical significance was accepted at $P < 0.05$. Data are expressed as mean ± SD.

RESULTS

Anthropometric and haematological variables

There was no significant difference in age between males and females (Table 1) but males were taller, heavier and had higher BMI than females (all $P < 0.01$). Males had higher RBC count, haematocrit and haemoglobin concentration than females (all $P < 0.001$).

Training loads

Analysis of the IPAQ questionnaires indicated that the weekly training loads were relatively stable between and within the gender groups over the 4 months of the study (Figure 1) and were equivalent to an average of about 11 h of moderate-vigorous activity per week. The self-reported training loads at the start of the study

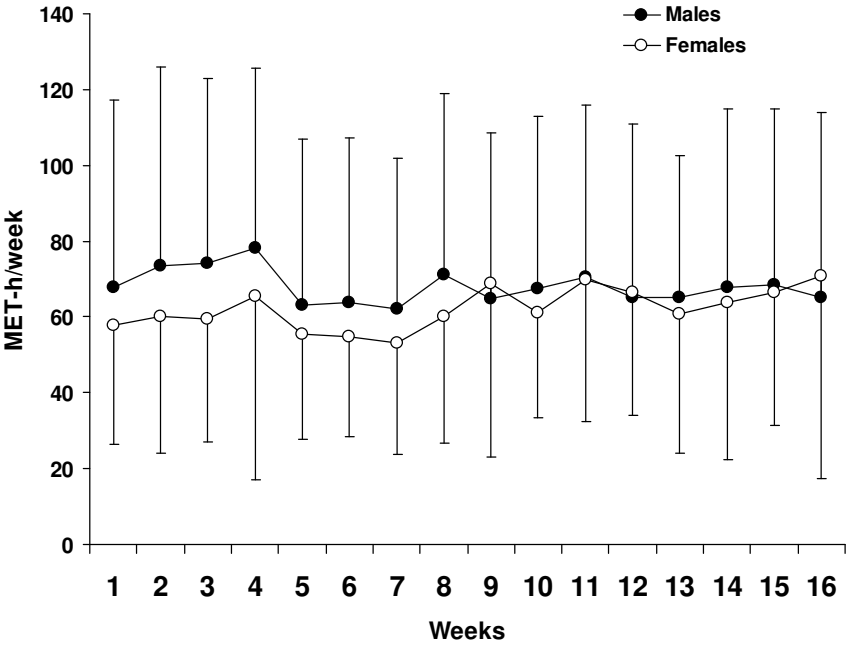

FIGURE 1 – Training loads in MET-h/week over the 4-month study period for men (n=46) and women (n=34) who completed the study. Data are mean ± SD.

and the average IPAQ scores in MET-h/week over the 16 weeks of the study were not significantly different between males and females (Table 1).

Table 1. – Anthropometric, training and haematological variables in male and female athletes

	Males (n=46)	Females (n=34)	P
Age (years)	22.9 ± 4.1	22.1 ± 4.0	0.425
Height (cm)	1.81 ± 0.06	1.68 ± 0.06	<0.001
Body mass (kg)	78.0 ± 10.5	62.6 ± 5.8	<0.001
BMI (kg/m^2)	23.8 ± 2.6	22.3 ± 2.3	0.005
Training load (h/week)	9.7 ± 4.7	8.7 ± 3.8	0.339
IPAQ (MET-h/week)	68.2 ± 39.0	63.4 ± 26.3	0.542
RBC count (x10^{12}/L)	5.01 ± 0.42	4.38 ± 0.38	<0.001
Haematocrit (%)	43.1 ± 2.8	38.7 ± 2.6	<0.001
Haemoglobin (g/L)	146 ± 9	130 ± 10	<0.001

Values are expressed as mean (±SD).

Plasma immunoglobulins and salivary variables

There were no differences between the sexes for plasma concentrations of IgA, IgG and IgM (Table 2). Saliva flow rates, SIgA concentration and SIgA secretion rates (Table 2) were significantly higher in males than females (all $P < 0.01$). For male and female subjects combined, neither the concentration of SIgA nor its secretion rate were related to the plasma IgA concentration ($r = -0.122$ and $r = 0.059$, respectively; both $P > 0.05$).

Table 2. Plasma immunoglobulins and salivary variables in male and female athletes

	Males (n=46)	Females (n=34)	P
Plasma IgA (g/L)	1.52 ± 0.52	1.60 ± 0.50	0.842
Plasma IgG (g/L)	10.16 ± 3.11	10.73 ± 1.71	0.246
Plasma IgM (g/L)	1.40 ± 0.70	1.41 ± 0.70	0.888
Total Ig (g/L)	12.99 ± 3.99	13.74 ± 2.29	0.360
Saliva flow rate (ml/min)	0.50 ± 0.23	0.36 ± 0.20	0.008
SIgA concentration (mg/L)*	180 ± 116	123 ± 53	0.009
SIgA secretion rate (µg/min)*	81.4 ± 55.5	43.8 ± 29.4	<0.001

Values are expressed as mean (±SD). Asterisks indicate data sets that were not normally distributed.

Blood leukocytes, lymphocyte subsets and monocyte TLR4 expression

Total blood leukocyte, neutrophil, monocyte and lymphocyte counts were not significantly different (Table 3) but males had higher numbers of B cells ($P < 0.05$) and NK cells ($P < 0.001$) as illustrated in Table 4. Monocyte TLR4 expression tended to be lower in males (geometric mean fluorescence intensity: 26.1 ± 13.6 in females, 20.5 ± 12.3 in males, $P = 0.062$).

Table 3. Blood leukocyte counts in male and female athletes

	Males (n=46)	Females (n=34)	P
Leukocyte count ($x10^9$/L)	5.66 ± 1.32	5.89 ± 1.60	0.477
Neutrophil count ($x10^9$/L)	2.71 ± 1.05	3.20 ± 1.25	0.062
Monocyte count ($x10^9$/L)	0.51 ± 0.17	0.47 ± 0.14	0.212
Eosinophil count ($x10^9$/L)	0.19 ± 0.12	0.18 ± 0.13	0.753
Lymphocyte count ($x10^9$/L)	2.17 ± 0.53	1.97 ± 0.60	0.127

Values are expressed as mean (±SD).

Table 4. Blood lymphocyte subset counts in male and female athletes

	Males (n=46)	Females (n=34)	P
CD3+ cell count ($x10^9$/L)	1.28 ± 0.45	1.24 ± 0.43	0.718
CD3+CD4+ cell count ($x10^9$/L)	0.68 ± 0.25	0.70 ± 0.23	0.729
CD3+CD8+ cell count ($x10^9$/L)	0.53 ± 0.28	0.48 ± 0.20	0.335
CD3-CD19+ cell count ($x10^9$/L)	0.23 ± 0.13	0.18 ± 0.09	0.048
CD3-CD56+ cell count ($x10^9$/L)	0.30 ± 0.17	0.16 ± 0.07	<0.001

Values are expressed as mean (±SD).

Antigen stimulated cytokine production

The production of interleukins 1β, 2, 4, 6, 8 and 10, IFN-γ and TNF-α by multi-antigen stimulated whole blood culture were not significantly different in males and females (Table 5).

URTI incidence and severity and duration of URTI symptoms

The average number of weeks with URTI symptoms was 1.7 ± 2.1 in males and 2.3 ± 2.5 in females ($P = 0.311$). For weeks when an URTI episode was present (i.e. total symptom severity score of 12 or more), the mean total symptom severity score was 22 ± 7 and 22 ± 11 in males and females, respectively and the mean duration of symptoms was 3.6 ± 1.5 and 3.4 ± 1.5 days in males and females, respectively.

Table 5. Antigen stimulated cytokine production by whole blood culture in male and female athletes.

	Males (n=46)	Females (n=34)	P
IL-1β production (pg/ml)*	9.1 ± 9.9	5.9 ± 4.8	0.114
IL-2 production (pg/ml)*	140 ± 227	118 ± 138	0.996
IL-4 production (pg/ml)*	3.4 ± 4.1	4.6 ± 7.6	0.981
IL-6 production (pg/ml)*	167 ± 133	135 ± 124	0.375
IL-8 production (pg/ml)*	1178 ± 738	897 ± 653	0.133
IL-10 production (pg/ml)*	4.0 ± 5.3	3.8 ± 4.6	0.680
IFN-γ production (pg/ml)*	31 ± 59	26 ± 53	0.431
TNF-α production (pg/ml)*	27 ± 46	17 ± 25	0.166

Values are expressed as mean (±SD). Asterisks indicate data sets that were not normally distributed.

DISCUSSION

The main findings of the present study were that most aspects of immunity are not different between males and female athletes but a few that could potentially influence URTI risk – SIgA concentration and secretion rate, numbers of circulating B cells and NK cells – are lower in women than in men in an athletic population. However, these differences are not sufficient to substantially affect URTI incidence. In contrast monocyte TLR4 expression tended to be higher in females which may compensate for other aspects of their immune function being lower (19). Sex differences in immune function among athletes therefore probably do not need to be considered in future mixed gender studies on exercise, infection and immune function, unless the focus of the study is on mucosal immunity or NK cells.

Low SIgA concentration or secretion rate has been identified as a risk factor for development of URTI in physically active individuals (13, 18, 20, 27). It has been suggested that SIgA levels are a surrogate marker of host protection and the suppression of SIgA after prolonged exercise or heavy training is itself a probable consequence of altered T lymphocyte function (10). Females generally have lower unstimulated saliva flow rates than males (33), whereas SIgA concentration in unstimulated saliva has been reported to be unaffected by sex among relatively large cohorts of healthy young adults (24, 36, 37). A previous small scale study reported lower SIgA concentration and secretion rate in females (n=8) than in males (n=8) among subjects of mixed fitness (3). Two small scale studies on elite swimmers have also reported lower SIgA concentrations in females compared with males (n= 11 females, n = 15 males (18); n = 5 females, n= 7 males (2)); but, to our knowledge, our investigation is the first large scale study to report a sex difference in SIgA secretion in athletes from a range of endurance-based sports. Despite the markedly lower SIgA concentration and secretion rate in females, the

incidence of URTI was not significantly influenced by sex so the clinical significance of the sex difference in SIgA secretion is unclear.

In the general population, women have been reported to have fewer blood monocytes and NK cells, more CD4+ cells and more neutrophils than men (6, 38) and women appear to suffer from fewer viral infections than men (4). Most URTI are of viral origin but in the present study URTI incidence was not significantly different between the sexes. It is possible that the same training load could have a greater depressive effect on humoral immunity (lower SIgA and numbers of circulating B cells) for women than for men (that is not evident in the normal, more sedentary population) but this possibility needs to be resolved by future research. Such an effect may be responsible for the reversal of the usual situation of higher immune function in females into the opposite situation in our athlete cohort. A limitation of the present study is that the phase of the menstrual cycle (when blood and saliva samples were taken) was not determined and we did not establish whether the females were taking oral contraceptives. It is possible that the high training loads of some of the female endurance athletes in our study could have caused them to be amenorrhoic and one would expect that this would make their immune variables more similar to that of men. This aside, menstrual cycle phase was not found to affect resting SIgA responses in endurance trained female athletes (8).

In healthy normal adults, small differences in single selected markers of immune function may not be clinically important. There are two main reasons for this. Firstly, there is a considerable degree of redundancy in the immune system, such that a small change in the functional capacity of one component of immune function may be compensated for by a change in the functional capacity of another. Secondly, there may be a certain amount of excess capacity in some aspects of immune function, particularly for those functions that are assessed using *in vitro* challenges using a high concentration of stimulant (1). Thus, it cannot be stated with any degree of certainty that small differences in one or more aspects of immune function will influence an individual's susceptibility to infection. Indeed for many aspects of immune function (e.g. blood neutrophil count and oxidative burst activity), it is not even known if the normal variation seen in the healthy adult population is a factor that influences the ability to fight infections (23). More substantial differences in one or more aspects of immune function are probably more likely to affect infection risk although this also depends on the degree of exposure to pathogens and the experience of previous exposure. However, for some immune cell functions a sufficiently large variation or change has been related to altered host defence and susceptibility to disease. For example, some studies indicate that susceptibility to infections and cancer is greater in individuals who possess low NK cell activity compared with individuals with moderate to high NK cell activity (22, 26, 30).

Associations between URTI risk and blood immune parameters have not been extensively examined, though an impaired IFN-γ production in unstimulated whole blood culture has been reported in fatigued and illness-prone endurance athletes (10). However, the relevance of this measure of immune function to

infection risk is unclear as cytokine production in the unstimulated state is very low compared with the response to an infectious agent or antigen challenge. Immune functions in females are influenced by endogenous oestrogenic effects (6, 32). In addition, endogenous hormones during the menstrual cycle in female subjects and exogenous hormones in the form of contraceptives or of hormone replacement therapy, affect immune functions such as cytokine production (21), which requires female subjects to be classified as premenopausal (with and without contraceptives) or postmenopausal (with or without hormone replacement therapy). However, Burrows et al. (8) found no differences in SIgA concentration or secretion rate in a group of highly trained female endurance runners over the phases of the menstrual cycle and there was no relationship between SIgA and progesterone concentrations. In the present study the whole blood culture production of measured cytokines in response to multi-antigen challenge was not different in females compared with males. Blood leukocyte, neutrophil, monocyte and lymphocyte counts were also similar in athletic men and women. Circulating numbers of T cells and CD4+ and CD8+ subsets similar as well so it is important to emphasise that most aspects of immunity measured in our study were not different between the sexes. The lower number of circulating B cells and NK cells in females in the present study cannot necessarily be interpreted as meaning lower immune function because it may be that activated cells have moved out of the circulation into the skin, lung, gut, lymph nodes etc. Thus, sex differences in immune function among athletes probably do not need to be considered in future mixed gender studies on exercise, infection and immune function, unless the focus of the study is mucosal immunity or NK cells.

ACKNOWLEDGEMENTS

This study was sponsored by Yakult Honsha Co., Ltd., Japan and GlaxoSmithKline, UK. Pedro Tauler received a “José Castillejo” grant from the Spanish Ministry of Science and Education.

REFERENCES

1. Albers R, Antoine JM, Bourdet-Sicard R, Calder PC, Gleeson M, Lesourd B, Samartin S, Sanderson IR, Van Loo J, Vas Dias FW, and Watzl B. Markers to measure immunomodulation in human nutrition intervention studies. Br J Nutr 94: 452-481, 2005.

2. Allgrove JE. Factors influencing the mucosal immune response to exercise. PhD thesis, Loughborough University 102-121, 2007.

3. Allgrove JE, Geneen L, Latif S, and Gleeson M. Influence of a fed or fasted state on the s-IgA response to prolonged cycling in active men and women. Int J Sport Nutr Exerc Metab 19: 209-221, 2009.

4. Beery TA. Sex differences in infection and sepsis. Crit Care Nurs Clin North Am 15: 55-62, 2003.

5. Bishop NC. Exercise and infection risk. In: Immune function in sport and exercise, edited by Gleeson M. Edinburgh: Elsevier, 2005, p. 1-14.

6. Bouman A, Schipper M, Heineman MJ, and Faas MM. Gender difference in the non-specific and specific immune response in humans. Am J Reprod Immunol 52: 19-26, 2004.

7. Brabin L. Interactions of the female hormone environment, susceptibility to viral infections, and disease progression. AIDS Patient Care STDS 16: 211-221, 2002.

8. Burrows M, Bird SR, and Bishop NC. The menstrual cycle and its effect on the immune status of female endurance runners. J Sports Sci 20: 339-344, 2002.

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

10. Clancy RL, Gleeson M, Cox A, Callister R, Dorrington M, D'Este C, Pang G, Pyne D, Fricker P, and Henriksson A. Reversal in fatigued athletes of a defect in interferon gamma secretion after administration of Lactobacillus acidophilus. Br J Sports Med 40: 351-354, 2006.

11. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF, and Oja P. International physical activity questionnaire: 12-country reliability and validity. Med Sci Sports Exerc 35: 1381-1395, 2003.

12. Faas M, Bouman A, Moesa H, Heineman MJ, de Leij L, and Schuiling G. The immune response during the luteal phase of the ovarian cycle: a TH2-type response. Fertil Steril 74: 1008-1013, 2000.

13. Fahlman MM, and Engels HJ. Mucosal IgA and URTI in American college football players: A year longitudinal study. Med Sci Sports Exerc 37: 374-380, 2005.

14. Faul F, Erdfelder E, Lang A-G, and Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioural and biomedical sciences. Behav Res Methods 39: 175-191, 2007.

15. Fricker PA, Pyne DB, Saunders PU, Cox AJ, Gleeson M, and Telford RD. Influence of training loads on patterns of illness in elite distance runners. Clin J Sport Med 15: 246-252, 2005.

16. Gleeson M. In: Immune function in sport and exercise, edited by Gleeson M. Edinburgh: Elsevier, 2005, p. 67-138.

17. Gleeson M. Exercise and immune function. J Appl Physiol 103: 693-699, 2007.

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

19. Gleeson M, McFarlin B, and Flynn M. Exercise and Toll-like receptors. Exerc Immunol Rev 12: 34-53, 2006.

20. Gleeson M, Pyne DB, Austin JP, Francis JL, Clancy RL, McDonald WA, and Fricker PA. Epstein-Barr virus reactivation and upper respiratory illness in elite swimmers. Med Sci Sports Exerc 34: 411-417, 2002.

21. Haus E, and Smolensky MH. Biologic rhythms in the immune system. Chronobiol Int 16: 581-622, 1999.

22. Imai K, Matsuyama S, Miyake S, Suga K, and Nakachi K. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. Lancet 356: 1795-1799, 2000.

23. Keil D, Luebke RW, and Pruett SB. Quantifying the relationship between multiple immunological parameters and host resistance: probing the limits of reductionism. J Immunol 167: 4543-4552, 2001.

24. Kugler J, Hess M, and Haake D. Secretion of salivary immunoglobulin A in relation to age, saliva flow, mood states, secretion of albumin, cortisol, and catecholamines in saliva. J Clin Immunol 12: 45-49, 1992.

25. Lancaster GI, Halson SL, Khan Q, Drysdale P, Wallace F, Jeukendrup AE, Drayson MT, and Gleeson M. Effects of acute exhaustive exercise and chronic exercise training on type 1 and type 2 lymphocytes. Exerc Immunol Rev 10: 91-106, 2004.

26. Levy SM, Herberman RB, Lee J, Whiteside T, Beadle M, Heiden L, and Simons A. Persistently low natural killer cell activity, age, and environmental stress as predictors of infectious morbidity. Nat Immun Cell Growth Regul 10: 289-307, 1991.

27. Neville V, Gleeson M, and Folland JP. Salivary IgA as a risk factor for upper respiratory infections in elite professional athletes. Med Sci Sports Exerc 40: 1228-1236, 2008.

28. Nieman DC, Johanssen LM, Lee IW, and Arabatzis K. Infectious episodes in runners before and after the Los Angeles Marathon. J Sports Med Phys Fitness 30: 316-328, 1990.

29. Northoff H, Symons S, Zieker D, Schaible EV, Schafer K, Thoma S, Loffler M, Abbasi A, Simon P, Niess AM, and Fehrenbach E. Gender- and menstrual phase dependent regulation of inflammatory gene expression in response to aerobic exercise. Exerc Immunol Rev 14: 86-103, 2008.

30. Ogata K, An E, Shioi Y, Nakamura K, Luo S, Yokose N, Minami S, and Dan K. Association between natural killer cell activity and infection in immunologically normal elderly people. Clin Exp Immunol 124: 392-397, 2001.

31. Oliveira M, and Gleeson M. The influence of prolonged cycling on monocyte Toll-like receptor 2 and 4 expression in healthy men. Eur J Appl Physiol 109: 251-257, 2010.

32. Paavonen T. Hormonal regulation of immune responses. Ann Med 26: 255-258, 1994.

33. Percival RS, Challacombe SJ, and Marsh PD. Flow rates of resting whole and stimulated parotid saliva in relation to age and gender. J Dent Res 73: 1416-1420, 1994.

34. Peters EM, and Bateman ED. Ultramarathon running and URTI: an epidemiological survey. S Afr Med J 64: 582-584, 1983.

35. Schaible E, Boehringer A, Callau D, Niess AM, and Simon P. Exercise and menstrual cycle dependent expression of a truncated alternative splice variant of HIF1 in leukocytes. Exerc Immunol Rev 15: 145-156, 2009.

36. Van Anders SM. Chewing gum has large effects on salivary testosterone, estradiol, ans secretory immunoglobulin A assays in women and men. Psychoneuroendocrinol 35: 305-309, 2010.

37. Van Anders SM. Gonadal steroids and salivary IgA in healthy young women and men. Am J Human Biol 22: 348-352, 2010.

38. Willemsen G, Carroll D, Ring C, and Drayson M. Cellular and mucosal immune rections to mental and cold stress: associations with gender and cardiovascular reactivity. Psychophysiol 39: 222-228, 2002.

Plasma adenosine triphosphate and heat shock protein 72 concentrations after aerobic and eccentric exercise.

Kishiko Ogawa[1], Ryosuke Seta[2], Takahiko Shimizu[3], Shoji Shinkai[1], Stuart K Calderwood[4], Koichi Nakazato[2], Kazue Takahashi[2]

[1] Research Team for Social Participation and Health Promotion, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan
[2] Nippon Sport Science University, Tokyo, Japan
[3] Research Team for Molecular Gerontology, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan
[4] Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, USA

ABSTRACT

The endolysosome pathway has been proposed for secretion of heat shock protein (Hsp)72 with a regulatory role for extracellular adenosine triphosphate (ATP). Here, we tested the hypothesis that extracellular ATP mediates the increase in plasma Hsp72 after exercise. We measured plasma ATP, Hsp72, cathepsin D, norepinephrine, free fatty acid, glucose, and myoglobin in 8 healthy young males (mean±SE: age, 22.3±0.3 years; height, 171.4±0.8 cm; weight, 68.8±3.1 kg; body mass index, 23.5±1.1 kg/cm2; VO^2 max, 44.1±3.8 mL/kg/min) before and at 0, 10, 30, and 60 min after aerobic exercise (cycling) and elbow flexor eccentric exercise. Subjects cycled for 60 min at 70-75% VO_2 max (mean±SE; 157.4±6.9 W). Eccentric strength exercise consisted of flexing the elbow joint to 90° with motion speed set at 30°/sec at extension and 10°/sec at flexion. Subjects performed 7 sets of 10 eccentric actions with a set interval of 60 sec. The motion range of the elbow joint was 90°-180°. Compared with the levels of Hsp72 and ATP in plasma after bicycle exercise, those after eccentric exercise did not change. A significant group × time interaction was not observed for Hsp72 or ATP in plasma. A significant correlation was found between Hsp72 and ATP in plasma (r=0.79, P<0.05), but not between Hsp72 and norepinephrine (r=0.64, P=0.09) after bicycle exercise. A significant correlation between ATP and norepinephrine in plasma was found (r=0.89 P<0.01). We used stepwise multiple-regression analysis to determine independent predictors of exercise-induced elevation of eHsp72. Candidate predictor variables for the stepwise multiple-regression analysis were time (Pre, Post, Post10, Post30, Post60), exercise type (aerobic, eccentric), ATP, cathe-

Corresponding to;
Kishiko Ogawa, Research Team for Social Participation and Health Promotion,
Tokyo Metropolitan Institute of Gerontology, 35-2 Sakaecho, Itabashi,
Tokyo 173-0015, Japan
Tel:+81-3-3964-3241 ext.3129, Fax:+81-3-3579-4776, E-mail: kishiko@tmig.or.jp

psin D, norepinephrine, epinephrine, glucose, and FFA. In the regression model for Hsp72 in plasma, increased ATP and glucose were the strongest predictors of increased Hsp72 (ATP: R^2=0.213, β=0.473, P=0.000; ATP and glucose: R^2=0.263, β=0.534, P=0.000). Collectively, these results imply that ATP in plasma is a trigger of Hsp72 release after exercise.

Key words: Endolysosome, ABC-family transporter, Cathepsin D

INTRODUCTION

Heat shock proteins (Hsp) are highly conserved proteins that are expressed both constitutively and under stressful conditions. In particular, those in the 70-kDa family are released from various cell types, including glia cells (22), human peripheral blood mononuclear cells (14), and cancer cells (33), after *in vitro* challenge with cytokines or heat stress; and also from human brain (29), leukocytes (15), and hepatosplanchnic tissue (13) during and/or after exercise. Walsh et al. first described an exercise-induced increase of extracellular Hsp (eHsp)72 (50). Subsequently, other exercise-related studies have shown that the concentration of Hsp72 in serum or plasma (i.e. eHsp72) is dependent on the duration and intensity of exercise (17), that eHsp72 elevation is accompanied by parallel increases in cytokine levels (51) and in biomarkers for oxidative stress (16), and that a specific vitamin E isoform attenuates the exercise-induced increase of eHsp72 (18, 39). It is clear that extracellular Hsps can play a role as pro-inflammatory immune effectors (10, 36). However, it is unclear whether eHsp72 plays a role as a pro-inflammatory mediator or for chaperoning proteins to prevent aggregation or proteolysis of damaged proteins due to exercise.

The mechanism of excretion out of possible intracellular storage sites is controversial. Recent work from several groups has suggested that Hsps are released by both passive (necrotic) and active mechanisms (3, 43). During exercise, the release of Hsp72 from damaged cells only partially contributes to circulating eHsp72. A comparative study between endurance exercise of different intensities and durations revealed that bouts of running with the highest eHsp72 levels in plasma were associated with the most pronounced creatine kinase concentrations, a prominent marker of tissue damage (24). On the other hand, release from injured tissue can largely be excluded because eHsp72 increases after exercise even in the absence of enhanced plasma creatine kinase levels (30). Moreover, despite missing signs of liver cell damage, hepatosplanchnic release of Hsp72 has been measured after exercise (13). At present, active secretory processes, rather than passive release due to cell damage, are considered to be responsible for Hsp72 release during exercise (30).

The classical pathway can be excluded because Hsp72 lacks a peptide leader sequence that targets the protein for secretion (8). Active secretion via exosomes and lipid rafts may be an alternative secretory mechanism (6, 8). Inhibition of Hsp72 release from peripheral blood mononuclear cells (PBMCs) by monensin (Na^+ ionophore), methyl-β-cyclodextrin (disrupts membrane rafts), or methylamine (inhibits endocytosis) suggests that Hsp72 is transported via the Golgi region into lysosomal lipid rafts prior to exocytosis (6). In the non-classical protein

transport pathway, lipid rafts are specialized membrane microdomains that are formed within the exoplasmic leaflet of the Golgi membrane, and may play a role in Hsp72 exocytosis (6). However, the effect is controversial due to cytotoxicity.

Exosome-mediated Hsp72 secretion is also a potential mechanism in the exercise-induced eHsp72 response. Accumulated intracellular Hsp72 in the leukocytes or other tissues due to exercise may be actively secreted through exosomes into circulation. Hsp72 release from whole blood cells and isolated PBMCs (31) and an increase of exosomal Hsp72 content in PBMCs after experimental heat shock (31) have been found. Exosomes, which are small membrane vesicles secreted by various cell types, including B cells (9), T cells (5), dendritic cells (44), mast cells (46), epithelial cells (49), and PBMCs (31), may provide a secretory pathway allowing cells to actively release specific Hsps. Lancaster *et al.* demonstrated that exosomes gradually increase in both culture medium (RPMI 1640, 0% fetal bovine serum) and PBMC cell cultures under basal incubation (37°C) in a time-dependent manner, and concomitantly the Hsp70 content of exosomes increases, but not significantly (31). Bausero *et al.* suggested that Hsp72 is released within the exosomes via a non-classical protein transport pathway in an intracellular calcium-dependent fashion (4), but not due to extracellular calcium.

Recent studies have implicated the endolysosome pathway for secretion of Hsp72 (35). Hsp72 secretion involves the entry of Hsp72 into endolysosomes through adenosine triphosphate (ATP)-binding cassette (ABC)-family transporters, where they co-localize with intravesicular cathepsin D. These organelles are then transported to the cell surface. Subsequent fusion of Hsp72 containing endolysosomes with the cell surface results in the localization of the lysosomal marker, i.e., lysosomal-associated membrane protein (LAMP) 1 in the plasma membrane and release of Hsp72 along with other protein such as cathepsin D. Although the cell signals involved in triggering stress-induced Hsp72 release through this lysosomal pathway are unknown, recent data suggests a regulatory role for extracellular ATP (34).

The type of exercise strongly influences the increase in Hsp72 in blood. For instance, in aerobic exercises, such as treadmill running, serum Hsp72 increases several fold both during and after the exercise (50). In contrast, eccentric exercises such as elbow flexion, do not induce an increase in eHsp72 (24). However, downhill running has been shown to increase eHsp72 (42). This difference in Hsp72 levels is seen despite both aerobic and eccentric exercises inducing muscle damages. This may be explained by the lysosome mechanism. Extracellular ATP regulates Hsp72 release from ABC-family transporters, and, thus, muscle damage does not contribute to increased eHsp72; instead, eHsp72 increases with extracellular ATP. Therefore, we presently tested the hypothesis that extracellular ATP mediates the increase in plasma Hsp72 after exercise.

METHODS

Subjects

Eight healthy untrained male subjects (mean±SE: age, 22.3±0.3 years; height, 171.4±0.8 cm; weight, 68.8±3.1 kg; body mass index, 23.5±1.1 kg/cm^2; VO_2 max, 44.1±3.8 mL/kg/min) participated in the study. None of the subjects per-

formed strenuous exercise for at least one week before the experiment. All subjects were informed of the purpose and risks of the study before giving written informed consent. This study was conducted in accordance with the Declaration of Helsinki, and its protocol was approved by the Ethics Committee at Tokyo Metropolitan Institute of Gerontology.

Experimental protocol

Preliminary tests

Maximal oxygen uptake (VO_2 max) test was carried out one week before to determine the workload required to elicit 70% VO_2 max. The graded maximal exercise test involved four 5-min bouts of exercise on an electronically braked cycle ergometer (Lode Excalibur, Gronigen, Netherlands). Pedal cadence was maintained at 60 revolutions/min and expired gasses were measured continuously using an automated mass spectrometer for respiratory analysis system (Arco systems, Chiba, Japan). A continuous, incremental cycling test to volitional exhaustion was performed. The initial workload was set at 50 W, with work rate increasing by 50 W every 4 min until 200 W, and by 10 W every 1 min until exhaustion. Expired gases were measured continuously to derive VO_2 max.

Aerobic exercise tests

All subjects cycled for 60 min at 70% VO_2 max (mean±SE: 157.4±6.9 W) in warm conditions (ambient temperature, 24-25°C; relative humidity, 45%). The subjects reported to the laboratory, then they rested in a sitting position for 30 min, and had blood samples taken (Pre). Subjects were then moved to the cycle ergometer and commenced exercise. There was a 3- to 5-min warm-up period of cycling at 30-45% of VO_2 max, immediately followed by 60 min at 70-75% of VO_2 max in warm conditions. The subjects then had a 60-min rest recovery phase in warm conditions after exercise. Blood samples were obtained immediately after the exercise (post) and at 10, 30, and 60 min after exercise. The subjects were permitted to drink a maximum of 400 mL of commercial bottled water during exercise testing.

Eccentric exercise

All subjects participated in a second trial. On arrival at the laboratory, the subjects rested in a sitting position for 30 min, and had blood samples taken (Pre). Subjects were then moved and placed on an isokinetic machine (Biodex Multi-Joint System 3, Biodex Medical Systems; Shirley, NY, USA). The elbow joint angle was flexed to 90° and compulsory eccentric strength was loaded, with motion speed set at 30°/sec at extension and 10°/sec at flexion. Subjects performed 7 sets of 10 eccentric actions with a set interval of 60 sec. The motion range of the elbow joint was 90°-180°. The subjects then had a 60-min recovery phase in warm conditions after exercise. Blood samples were obtained immediately after the exercise (post) and at 10, 30, and 60 min after exercise.

All exercise bouts including preliminary testing were performed between 09:00 and 15:00. The trials were separated by at least 1 week to ensure complete recovery between trials. Except for the last 48 h before each trial, when exercise was regulated by the study protocol, the subjects completed their regular training program and usual daily activities during the study period. During the study peri-

od, the subjects maintained their normal diet, but their food intake was limited for 2 h before exercise testing. All subjects wore similar uniforms during exercise testing.

Blood sampling and analysis

For analysis of eHsp72, cathepsin D, and ATP, whole blood was placed in a tube containing 30 μl of EDTA and spun at 1000 × *g* at 4°C for 10 min, and the supernatant was stored at -80°C until analysis. Enzyme-linked immunosorbent assay (ELISA) kits were used to measure the plasma concentrations of Hsp72 (Stressgen Biotechnologies Co.; Victoria, BC, Canada), cortisol (Immuno-Biological Laboratories Co. Ltd.; Tokyo, Japan), and IL-6 (R&D Systems; Minneapolis, MN, USA). Cathepsin D activity was quantified with a Cathepsin D Assay kit (Fluorimetric) (AnaSpec; San Jose, CA, USA). Briefly, after 5-FAM fluorescence reference standards and samples were simultaneously incubated at 37°C for 10 min, 50 μL of the fluorogenic peptide 5-FAM/QXL^{TM} 520 was added as a substrate. After mixing the reagents completely by shaking the plate gently for 30 sec, measurements of lysis (unquenched MCA peptide) were obtained with a microtiter plate fluorometer (SpectraMax Gemini XS; Molecular Devices, Sunnyvale, CA, USA; excitation: 490 nm; emission: 520 nm). Activity values were expressed in relative fluorescence units. ATP in plasma samples was determined using the luciferin-luciferase technique. Briefly, plasma was diluted 1 part in 100 in sterile, doubly distilled water. Diluted plasma was then assayed immediately using a commercially available firefly luminescent assay kit (BA100, Toyo Bnet, Tokyo, Japan) using an internal standard procedure. All samples were assayed in duplicate. The coefficient of variation of 9 duplicate resting plasma samples was 7%. Norepinephrine was measured using high-performance liquid chromatography. The plasma concentrations of free fatty acid (FFA) and glucose were measured using an immunoenzyme technique and UV hexokinase technique, respectively, and the serum concentration of myoglobin was measured using a radio immunoassay technique (SRL Co.; Tokyo, Japan).

Statistics

A statistics software package was used for all statistical calculations (SPSS ver.17; Tokyo, Japan). We compared the plasma concentrations of eHsp72, ATP, cathepsin D, and norepinephrine between cycling and elbow flexor exercise using a two-way ANOVA (time × groups) with repeated measures. When the analyses indicated a significant difference, Tukey's post-hoc test was used to locate the difference. Pearson correlation analysis was used to identify the association among eHsp72, ATP, cathepsin D, norepinephrine, and myoglobin. We used stepwise multiple-regression analysis to determine independent predictors of exercise-induced elevation of eHsp72. Candidate predictor variables for the stepwise multiple-regression analysis were time (Pre, Post, Post10, Post30, Post60), exercise type (aerobic, eccentric), ATP, cathepsin D, norepinephrine, epinephrine, glucose, and FFA. The level of probability to reject the null hypothesis was set at $P<0.05$ (two-tailed). All comparative data are expressed as means±SE.

RESULTS

Changes in plasma levels of Hsp72, ATP, cathepsin D, and norepinephrine after aerobic and eccentric exercise.

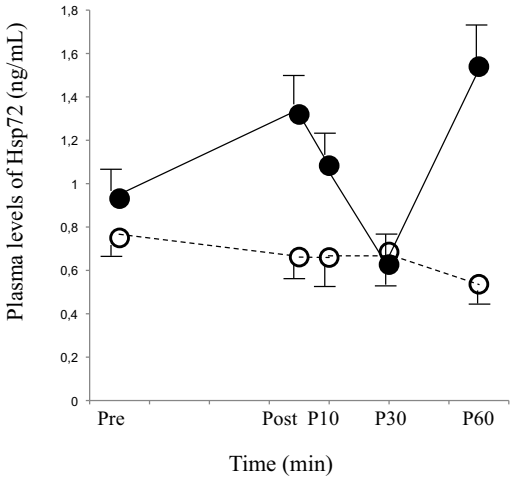

Fig. 1A. Changes in eHsp72 during two types of exercise. •; cycling exercise (aerobic). o; elbow flexor (eccentric). Pre, before exercise; Post, immediately after exercise; P10, 10 min after exercise; P30, 30 min after exercise; P60, 60 min after exercise.

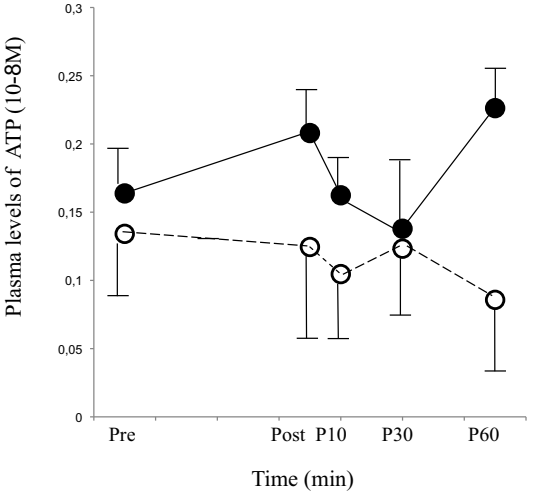

Fig. 1B. Changes in ATP during two types of exercise. •; cycling exercise (aerobic). o; elbow flexor (eccentric). Pre, before exercise; Post, immediately after exercise; P10, 10 min after exercise; P30, 30 min after exercise; P60, 60 min after exercise.

Cycling aerobic exercise, but not eccentric exercise, resulted in an increase of circulating Hsp72 (Fig. 1A). However, a significant group × time interaction was not observed for Hsp72 in plasma.

It has been proposed that extracellular ATP contributes to the induction of eHsp72 during and after stress exposure by an endolysosome mechanism (34). To examine the possible role of extracellular ATP in mediating the elevation of plasma Hsp72 after exercise, subjects underwent both bicycle ergometer exercise and elbow flexor exercise. Compared with the levels of ATP in plasma after bicycle exercise, those after eccentric exercise did not change. A significant group × time interaction was not observed for ATP in plasma (Fig. 1B).

In the lysosomal pathway, if ABC-family transporter co-localization with intravesicular cathepsin D involves the release of Hsp72 into the extracellular space, then cathepsin D may also be released (35). To determine whether cathepsin D mediates the elevation of plasma Hsp72 after exercise, plasma levels of cathepsin D after both aerobic and eccentric exercise were measured. Cathepsin D increased after aerobic exercise, but not after eccentric exercise. A significant group × time interaction

was observed for cathepsin D in plasma after both types of exercise (Fig. 1C; P<0.05).

Norepinephrine has often been demonstrated to induce eHsp72 during stressor exposure; Jonson *et al.* proposed that increases in norepinephrine acting upon α1 adrenergic receptors results in a calcium flux within the cell and a subsequent release of Hsp72 within exosomes (27). To examine the effect of norepinephrine on the exercise-induced increase in eHsp72, the changes in norepinephrine after the two types of exercise were analyzed by 2-way ANOVA with repeated measures. A significant group × time interaction was observed for norepinephrine in plasma after both types of exercise (Fig. 1D; *P*<0.01).

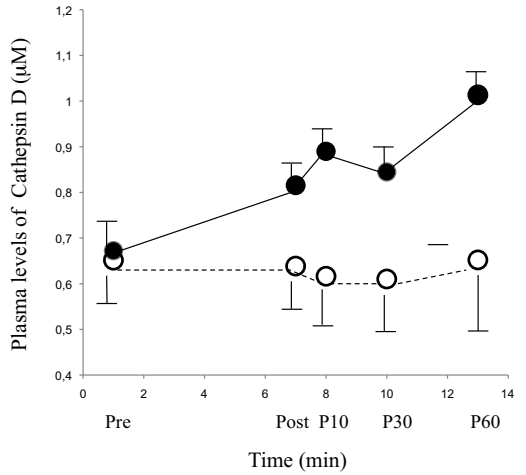

Fig. 1C. Changes in cathepsin D during two types of exercise. •; cycling exercise (aerobic). o; elbow flexor (eccentric). Pre, before exercise; Post, immediately after exercise; P10, 10 min after exercise; P30, 30 min after exercise; P60, 60 min after exercise.

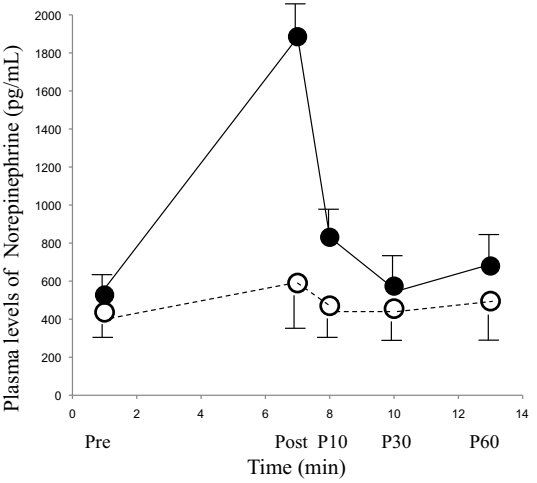

Fig. 1D. Changes in norepinephrine during two types of exercise. •; cycling exercise (aerobic). o; elbow flexor (eccentric). Pre, before exercise; Post, immediately after exercise; P10, 10 min after exercise; P30, 30 min after exercise; P60, 60 min after exercise.

Correlation analyses

The cycling exercise

A significant correlation was found between Hsp72 and ATP in plasma immediately after and 10 min after bicycle exercise (Table 1; r=0.79 and r=0.78 *P*<0.05, respectively), but not between eHsp72 and norepinephrine (Table 1). Significant correlations between ATP and norepinephrine in plasma were found immediately after exercise (r=0.89, *P*<0.01). There were no significant correlations between cathepsin D and other variables after exercises.

The eccentric exercise

After the elbow flexor lengthening contraction, significant negative correlations were found between eHsp72 and cathepsin D immediately after the exercise (r=–0.77, *P*<0.05). Significant correla-

ATP

	Post		Post 10		Post 30		Post 60	
	r	*P*	r	*P*	r	*P*	r	*P*
eHsp72								
Post	0,792	0,019 *						
Post 10	0,776	0,024 *	0,621	0,100				
Post 30	0,367	0,371	0,432	0,285	0,502	0,205		
Post 60	-0,251	0,548	-0,313	0,450	-0,340	0,410	0,434	0,282

Norepinephrine

	Post		Post 10		Post 30		Post 60	
	r	*P*	r	*P*	r	*P*	r	*P*
eHsp72								
Post	0,636	0,090						
Post 10	0,543	0,165	0,238	0,571				
Post 30	0,018	0,967	-0,279	0,503	-0,209	0,620		
Post 60	-0,453	0,259	-0,451	0,262	-0,291	0,485	0,029	0,946

Table 1. Correlation between eHsp72 and ATP or norepinephrine in plasma after aerobic exercise. *; significant differences $P<0.05$ by Pearson correlations. Post, immediately after exercise; P10, 10 min after exercise; P30, 30 min after exercise; P60, 60 min after exercise.

tion between ATP and norepinephrine in plasma was found immediately after the exercise ($r=0.71$, $P<0.05$).

Multiple-regression analysis

To further determine potential associations of the elevation of eHsp72 after exercise, we used a stepwise multiple-regression analysis to determine independent predictors of exercise-induced elevation of eHsp72. Candidate predictor variables for the stepwise multiple-regression analysis were time (Pre, Post, Post10, Post30, Post60), exercise type (aerobic, eccentric), ATP, cathepsin D, norepinephrine, epinephrine, glucose, and FFA. In the regression model for eHsp72 in plasma, increased ATP and glucose were the strongest predictors of increased eHsp72 (ATP: $R^2=0.213$, $\beta=0.473$, $P<0.001$; ATP and Glucose: $R^2=0.263$, $\beta=0.534$, $P<0.001$).

DISCUSSION

The present study demonstrated that circulating levels of ATP are associated with plasma levels of Hsp72. It has been proposed that lysosome exocytosis is a possible mechanism of Hsp release from cells (34); a schematic model involves the activity of ABC-family transmembrane transporters and the participation of purinergic receptors. Extracellular ATP binding causes the opening of purinergic receptor channels, and the entry of Hsp72 into the secretory compartment of lysosomes through ABC-family transporters. The lysosomes are then transported to the cell surface. Subsequent fusion of Hsp72 containing lysosomes with the cell surface results in release of Hsp72 (35). We postulated that circulating levels of ATP stimulated during exercise lead to lysosome exocytosis with the release of Hsp72. In the present study, the plasma levels of ATP were associated with the elevation of eHsp72 after bicycle exercise, which, at least in part, supports our hypothesis on the mechanism of Hsp release—that circulating ATP is a necessary

factor to induce secretion of Hsp72 during aerobic exercise. This may be the reason that both marathon running (15) and downhill running (42) induce increases in eHsp72, whereas eHsp72 does not increase after elbow flexion (24). This shows that exercise-induced elevation of eHsp72 is not caused only by muscle damage, and also raises the possibility that circulating ATP plays a role in the elevation of eHsp72 during exercise.

It is well known that erythrocytes function as O_2 sensors, contributing to the regulation of skeletal muscle blood flow and O_2 delivery. This is caused by the release of ATP during exercise depending on the number of unoccupied O_2 binding sites in the hemoglobin molecule (20). It is also known that muscle contraction-derived ATP can affect adrenergic transmission by acting on purinergic receptors on sympathetic nerve endings, in order that elevated peripheral sympathetic nervous activity and the resultant increased neurovascular levels of norepinephrine evoke vasoconstriction and serve to maintain blood pressure and perfusion to vital organs (32). ATP-sensitive P2X purinoceptors have been shown to enhance norepinephrine exocytosis in cultured cervical ganglion neurons and cardiac synaptosomes (47, 45). Recent evidence suggests that the vasodilatory and sympatholytic functions of intraluminal ATP are mediated via endothelial P2 receptors (38). The source of ATP in plasma remains unclear, but skeletal muscle may release ATP during contractions (19, 38). Endothelial (7) and skeletal muscle cells (23) may release ATP in response to mechanical stress. The present study demonstrated that ATP in plasma was positively and strongly associated with plasma norepinephrine levels after both types of exercise, results that accord well with previous investigations regarding the relationship of ATP and norepinephrine in plasma. However, it has been suggested that human skeletal muscle does not release Hsp72 into the blood during exercise, since the increase in eHsp72 in serum precedes the increase of Hsp72 mRNA and protein in muscle (50), and also because eHsp72 can be found in arterial, but not venous, blood flow in the contracting leg (13).

The P2X receptor is ubiquitously expressed and belongs to a family of ligand-gated channels that are activated by extracellular ATP (11). When activated by ATP, the ionotropic P2X receptors ($P2X_1$-$P2X_7$) form nonselective ion channels permeable to Na^+, K^+ and, primarily, to Ca^{2+} (40). Among the P2X receptors, $P2X_7$ receptors are expressed in humans, including in glia cells (41), macrophages (26), and lymphocytes (21), but not in skeletal muscle (11). The human $P2X_6$ receptor, however, is heavily expressed in skeletal muscle (40). As previous studies have shown, exercise induces increases in the circulating levels of eHsp72 from human hepatosplanchnic tissue (13), from human brain (29), and from leukocytes (25). These results lead us to speculate that $P2X_7$ receptors (and not other P2X receptors) are related to the mechanism of release, and that cells or tissues where the receptors are expressed are the source for Hsp72 release into circulation in response to exercise.

Although secretion mechanisms may vary between cell types, it has been demonstrated that in human LPS-activated monocytes, secretory lysosomes are the site of ATP-induced IL-1β processing; ATP also triggers exocytosis of these organelles with secretion of IL-1β and caspase-1 (2). Calderwood *et al.* suggested that Hsp70 release is a form of leaderless secretion, and its mechanisms of release resemble IL-1β in that they require the activity of ABC-family transmembrane

transporters and the likely participation of $P2X_7$ receptors (8, 34). Regarding IL-1β secretion through lysosome-related vesicles, Andrei *et al.* demonstrated that IL-1β is contained in part within organelles co-fractionating with Rab-7-positive structures and displaying ultrastructural features of late endosomes and dense vesicles; a fraction of IL-1β-containing organelles contains the endolysosomal protein cathepsin D or the lysosomal marker LAMP-1 (1). We were particularly interested in the mechanism of release of eHsp72. We therefore hypothesized that the plasma concentration of cathepsin D should be positively correlated with eHsp72 in plasma if endolysosomes are associated with the mechanism of release of eHsp72 during exercise. However, the present results show that cathepsin D was not associated with eHsp72 in plasma after aerobic exercise, although the concentration of cathepsin D in plasma gradually increased after aerobic exercise but not after eccentric exercise. Thus, we could not confirm that the endolysosome is involved in the mechanism of eHsp72 release. In general, IL-1β does not increase after exercise, whereas eHsp72 increases after exercise. Even though both mechanisms of release are similar, there should be some differences. Mambula *et al.* also observed that IL-1β does not increase in cultured prostate cancer cell (LNCaP) medium after heat shock, whereas eHsp72 in the same medium increases (33).

Cathepsin D takes part in the digestion of exhausted and denatured cellular proteins or proteins showing abnormal structures, and those which enter the cell via endocytosis (37). Dohm *et al.* observed that the proportion of free cathepsin D activity is increased in exercised rats, and suggested that lysosomal enzymes may be involved in increased muscle protein degradation (12). After the eccentric exercise, we did not observe a relationship between cathepsin D and myoglobin. However, both cathepsin D and myoglobin were negatively correlated with eHsp72 respectively after the elbow flexor lengthening contraction. Increased Hsp70 mRNA and Hsp70 expression in human skeletal muscle 2 h after a single bout of treadmill running and 48 h after lengthening resistance exercise have been observed, respectively (48, 50). Previous studies indicate that Hsp72, myoglobin (24, 28), and cathepsin D (37) are independently involved in muscle damage after exercise, but it is not clear what the significance of the relationship among cathepsin D, myoglobin, and Hsp72 is, especially in regards to plasma levels. Further investigations are needed.

In conclusion, we demonstrated that ATP in plasma is associated with eHsp72 in plasma after aerobic exercise, suggesting that extracellular ATP may be a trigger of Hsp72 release. In terms of the endolysosomal mechanism, we measured cathepsin D as a lysosomal enzyme. However, cathepsin D was not associated with eHsp72 in plasma after aerobic exercise, although the concentration of cathepsin D in plasma gradually increased after aerobic exercise but not after eccentric exercise. Exercise thus results in an increase of extracellular ATP, which is a signal for modulating sympathetic nerve activity, and may be a trigger for releasing Hsp72.

ACKNOWLEDGEMENTS

We thank Yumi Shiga, Aya Ikeda, Mihoko Namiki and Taro Fukaya for skilled technical assistance and Haruko Sawada from Tokyo Metropolitan Institute of

Gerontology for her skillful managing supports. This study was supported by a Grant-in-Aid for the Scientist (1403 B: 21300261) of the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES

1. Andrei C, Dazzi C, Lotti L, Torrisi MR, Chimini G, Rubartelli A. 1999. The secretory route of the leaderless protein interleukin 1beta involves exocytosis of endolysosome-related vesicles. Mol Biol Cell. 10(5):1463-75.
2. Andrei C, Margiocco P, Poggi A, Lotti LV, Torrisi MR, Rubartelli A. 2004. Phospholipases C and A2 control lysosome-mediated IL-1 beta secretion:Implications for inflammatory processes. Proc Natl Acad Sci U S A. 101(26):9745-50.
3 Basu S, Binder RJ, Suto R, Anderson KM, Srivastava PK.2000 Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-kappa B pathway. Int Immunol. 12(11):1539-46.
4. Bausero MA, Gastpar R, Multhoff G, Asea A. 2005. Alternative mechanism by which IFN-gamma enhances tumor recognition: active release of heat shock protein 72. J Immunol. 175(5):2900-12.
5. Blanchard N, Lankar D, Faure F, Regnault A, Dumont C, Raposo G, Hivroz C. 2002. TCR activation of human T cells induces the production of exosomes bearing the TCR/CD3/zeta complex. J Immunol. 168(7):3235-41.
6. Broquet AH, Thomas G, Masliah J, Trugnan G, Bachelet M. 2003. Expression of the molecular chaperone Hsp70 in detergent-resistant microdomains correlates with its membrane delivery and release. J Biol Chem. 278(24):21601-6.
7. Burnstock G. 1999. Release of vasoactive substances from endothelial cells by shear stress and purinergic mechanosensory transduction. J Anat. 194 (Pt3):335-42.
8. Calderwood SK, Mambula SS, Gray PJ Jr, Theriault JR. 2007. Extracellular heat shock proteins in cell signaling. FEBS Lett. 581(19):3689-94.
9. Clayton A, Turkes A, Navabi H, Mason MD, Tabi Z. 2005. Induction of heat shock proteins in B-cell exosomes. J Cell Sci. 118(Pt 16):3631-8.
10. Daniels GA, Sanchez-Perez L, Diaz RM, Kottke T, Thompson J, Lai M, Gough M, Karim M, Bushell A, Chong H, Melcher A, Harrington K, Vile RG. 2004. A simple method to cure established tumors by inflammatory killing of normal cells. Nat Biotechnol. 22(9):1125-32.
11. Di Girolamo M, Dani N, Stilla A, Corda D. 2005. Physiological relevance of theendogenous mono(ADP-ribosyl)ation of cellular proteins. FEBS J. 272(18):4565-75.
12. Dohm GL, Kasperek GJ, Tapscott EB, Beecher GR. 1980. Effect of exercise on synthesis and degradation of muscle protein. Biochem J. 188(1):255-62.
13. Febbraio MA, Ott P, Nielsen HB, Steensberg A, Keller C, Krustrup P, Secher NH, Pedersen BK. 2002. Exercise induces hepatosplanchnic release of heat shock protein 72 in humans. J Physiol. 544(Pt 3):957-62.
14. Fehrenbach E, Passek F, Niess AM, Pohla H, Weinstock C, Dickhuth HH, Northoff H., 2000. HSP expression in human leukocytes is modulated by endurance exercise. Med Sci Sports Exerc. 32(3):592-600.

15. Fehrenbach E, Niess AM, Schlotz E, Passek F, Dickhuth HH, Northoff H., 2000. Transcriptional and translational regulation of heat shock proteins in leukocytes of endurance runners. J Appl Physiol. 89(2):704-10.
16. Fehrenbach E, Northoff H. 2001. Free radicals, exercise, apoptosis, and heat shock proteins. Exerc Immunol Rev. 7:66-89.
17. Fehrenbach E, Niess AM, Voelker K, Northoff H, Mooren FC., 2005. Exercise intensity and duration affect blood soluble HSP72. Int J Sports Med. 26(7):552-7.
18. Fischer CP, Hiscock NJ, Basu S, Vessby B, Kallner A, Sjöberg LB, Febbraio MA, Pedersen BK. 2006. Vitamin E isoform-specific inhibition of the exercise-induced heat shock protein 72 expression in humans. J Appl Physiol. 100(5):1679-87.
19. Forrester T. 1972. An estimate of adenosine triphosphate release into the venous effluent from exercising human forearm muscle. J Physiol. 224(3):611-28.
20. González-Alonso J, Olsen DB, Saltin B. 2002. Erythrocyte and the regulation of human skeletal muscle blood flow and oxygen delivery: role of circulating ATP. Circ Res. 91(11):1046-55.
21. Gu BJ, Zhang WY, Bendall LJ, Chessell IP, Buell GN, Wiley JS. 2000. Expression of P2X(7) purinoceptors on human lymphocytes and monocytes: evidence for nonfunctional P2X(7) receptors. Am J Physiol Cell Physiol. 279(4):C1189-97.
22. Guzhova I, Kislyakova K, Moskaliova O, Fridlanskaya I, Tytell M, Cheetham M, Margulis B., 2001. In vitro studies show that Hsp70 can be released by glia and that exogenous Hsp70 can enhance neuronal stress tolerance. Brain Res. 914(1-2):66-73.
23. Hellsten Y, Frandsen U. 1997. Adenosine formation in contracting primary rat skeletal muscle cells and endothelial cells in culture. J Physiol. 504 (Pt 3):695-704.
24. Hirose L, Nosaka K, Newton M, Laveder A, Kano M, Peake J, Suzuki K. 2004. Changes in inflammatory mediators following eccentric exercise of the elbow flexors. Exerc Immunol Rev. 10:75-90.
25. Hunter-Lavin C, Davies EL, Bacelar MM, Marshall MJ, Andrew SM, Williams JH. 2004. Hsp70 release from peripheral blood mononuclear cells. Biochem Biophys Res Commun. 324(2):511-7.
26. Jiang LH, Mackenzie AB, North RA, Surprenant A. 2000. Brilliant blue G selectively blocks ATP-gated rat P2X(7) receptors. Mol Pharmacol. 58(1):82-8.
27. Johnson JD, Fleshner M. 2006. Releasing signals, secretory pathways, and immune function of endogenous extracellular heat shock protein 72. J Leukoc Biol. 79(3):425-34.
28. Kayani AC, Morton JP, McArdle A. 2008. The exercise-induced stress response in skeletal muscle: failure during aging. Appl Physiol Nutr Metab. 33(5):1033-41.
29. Lancaster GI, Møller K, Nielsen B, Secher NH, Febbraio MA, Nybo L. 2004. Exercise induces the release of heat shock protein 72 from the human brain in vivo. Cell Stress Chaperones. 9(3):276-80.
30. Lancaster GI, Febbraio MA. 2005. Mechanisms of stress-induced cellular HSP72 release: implications for exercise-induced increases in extracellular HSP72. Exerc Immunol Rev. 11:46-52.
31. Lancaster GI, Febbraio MA. 2005. Exosome-dependent trafficking of HSP70: a novel secretory pathway for cellular stress proteins. J Biol Chem. 280(24):23349-55.
32. Li J, King NC, Sinoway LI. 2005. Interstitial ATP and norepinephrine concentrations in active muscle. Circulation. 111(21):2748-51.
33. Mambula SS, Calderwood SK. 2006. Heat induced release of Hsp70 from prostate carcinoma cells involves both active secretion and passive release from necrotic cells. Int J Hyperthermia. 22(7):575-85.

34. Mambula SS, Calderwood SK. 2006. Heat shock protein 70 is secreted from tumor cells by a nonclassical pathway involving lysosomal endosomes. J Immunol. 177(11):7849-57.
35. Mambula SS, Stevenson MA, Ogawa K, Calderwood SK. 2007. Mechanisms for Hsp70 secretion: crossing membranes without a leader. Methods. 43(3):168-75.
36. Millar DG, Garza KM, Odermatt B, Elford AR, Ono N, Li Z, Ohashi PS. 2003. Hsp70 promotes antigen-presenting cell function and converts T-cell tolerance to autoimmunity in vivo. Nat Med. 9(12):1469-76.
37. Minarowska A, Gacko M, Karwowska A, Minarowski Ł. 2008. Human cathepsin D. Folia Histochem Cytobiol. 46(1):23-38.
38. Mortensen SP, González-Alonso J, Nielsen JJ, Saltin B, Hellsten Y. 2009. Muscle interstitial ATP and norepinephrine concentrations in the human leg during exercise and ATP infusion. J Appl Physiol. 107(6):1757-62.
39. Niess AM, Fehrenbach E, Schlotz E, Sommer M, Angres C, Tschositsch K, Battenfeld N, Golly IC, Biesalski HK, Northoff H, Dickhuth HH. 2002. Effects of RRR-alpha-tocopherol on leukocyte expression of HSP72 in response to exhaustive treadmill exercise. Int J Sports Med. 23(6):445-52.
40. North RA. 2002. Molecular physiology of P2X receptors. Physiol Rev. 82(4):1013-67.
41. Pannicke T, Fischer W, Biedermann B, Schadlich H, Grosche J, Faude F, Wiedemann P, Allgaier C, Illes P, Burnstock G, and Reichenbach A. 2000. P2x7 receptors in Muller glial cells from the human retina. J Neurosci 20: 5965-5972.
42. Peake J, Nosaka K, Suzuki K. 2005. Characterization of inflammatory responses to eccentric exercise in humans. Exerc Immunol Rev. 11:64-85.
43. Schild H, Rammensee HG. gp96--the immune system's Swiss army knife. 2000. Nat Immunol. 1(2):100-1.
44. Segura E, Amigorena S, Théry C. 2005. Mature dendritic cells secrete exosomes with strong ability to induce antigen-specific effector immune responses. Blood Cells Mol Dis. 35(2):89-93.
45. Sesti C, Broekman MJ, Drosopoulos JH, Islam N, Marcus AJ, Levi R. 2002. EctoNucleotidase in cardiac sympathetic nerve endings modulates ATP-mediated feedback of norepinephrine release. J Pharmacol Exp Ther. 300(2):605-11.
46. Skokos D, Botros HG, Demeure C, Morin J, Peronet R, Birkenmeier G, Boudaly S, Mécheri S. 2003. Mast cell-derived exosomes induce phenotypic and functional maturation of dendritic cells and elicit specific immune responses in vivo. J Immunol. 170(6):3037-45.
47. Sneddon P, Westfall TD, Todorov LD, Mihaylova-Todorova S, Westfall DP, Kennedy C. 1999. Modulation of purinergic neurotransmission. Prog Brain Res. 1999;120:11-20.
48. Thompson HS, Scordilis SP, Clarkson PM, Lohrer WA. 2001. A single bout of eccentric exercise increases HSP27 and HSC/HSP70 in human skeletal muscle. Acta Physiol Scand. 171(2):187-93.
49. van Niel 27. van Niel G, Raposo G, Candalh C, Boussac M, Hershberg R, Cerf-Bensussan N, Heyman M. 2001. Intestinal epithelial cells secrete exosome-like vesicles. Gastroenterology. 121(2):337-49.
50. Walsh RC, Koukoulas I, Garnham A, Moseley PL, Hargreaves M, Febbraio MA. 2001. Exercise increases serum Hsp72 in humans. Cell Stress Chaperones. 6(4):386-93.

51. Yamada PM, Amorim FT, Moseley P, Robergs R, Schneider SM. 2007. Effect of heat acclimation on heat shock protein 72 and interleukin-10 in humans. J Appl Physiol. 103(4):1196-204.

Killer cell immunoglobulin-like receptors and exercise

Diana V. Maltseva[1], Dmitry A. Sakharov[1], Evgeny A. Tonevitsky[1], Hinnak Northoff[2] and Alexander G. Tonevitsky[1]

[1] Department of molecular physiology, Russian Research institute of physical education and sport, Moscow, Russia
[2] Institute of clinical and experimental transfusion medicine (IKET), University of Tübingen, Tübingen, Germany

ABSTRACT

Exercise can alter human health in both beneficial (e. g. reduced risk of infection and of atherosclerosis) and adverse (e. g. anaphylaxis, exercise-induced asthma, and exacerbation of chronic illness) ways. Hitherto, the mechanisms linking exercise and health are not fully understood, but may rest on the capability of exercise to both increase circulating immune cells and modulate their activity. Natural killer (NK) cells, a major component of innate immunity, are one of the most sensitive populations of immune cells to exercise stress. NK cells play an important role in the detection and elimination of tumours and virus-infected cells. To mediate NK cell functions, there is an array of activating and inhibitory receptors with distinct specificities on their surface. Killer-cell immunoglobulin-like receptors (KIRs) which bind to MHC class I are a key example of receptors expressed by NK cells. The combination of MHC class I and KIR variants influences resistance to infections, susceptibility to autoimmune diseases, as well as complications of pregnancy. It is suggested that KIRs may also determine a considerable part of the effects of physical activity on human health. In this review we discuss KIRs in more detail, their role in the onset of human diseases, and the influence of acute exercise on KIR gene expression.

Key words: Killer cell immunoglobulin-like receptors (KIRs), NK cells, exercise, stress response

Address for correspondence:
Dmitry A. Sakharov MSc, PhD, Department of molecular physiology, Russian Research institute of physical education and sport, 105005, Elizavetinsky lane 10, Moscow, Russia
E-mail: dimitri_sakharov@mail.ru, Phone: +7-499-261-4991, Fax: +7-499-261-9404

INTRODUCTION

It is known that exercise as brief in duration as 6 min can mobilize leukocytes (44). Thus, such physical activity-related increase in circulating innate immune cells can happen many times in the daily lives of humans (10). The striking sensitivity of natural killer (NK) cells to exercise stress provides strong support that these cells may be implicated as a potential link between regular physical activity and overall health status (55). NK cells use many types of cell-surface receptors to recognize and to destroy virally-infected or malignantly transformed cells without prior sensitization (9, 37, 58). Inhibitory receptors of NK cells bind major histocompatibility complex (MHC) class I and thus protect healthy, class I-expressing cells from inappropriate NK cell aggression. Activating receptors specifically recognize various molecules that are upregulated on cells stressed by infection or malignant transformation, many of which are MHC class I related. In man, the largest family of receptors for MHC class I ligands expressed by NK cells (and small subsets of T cells) are the killer-cell immunoglobulin-like receptors (KIRs). The KIR family contains multiple inhibitory and activating members (3, 4). The combination of MHC class I and KIR variants influences resistance to viral infections, nonviral pathogens, susceptibility to autoimmune diseases, complications of pregnancy, as well as outcome of haematopoietic stem-cell transplantation (4, 19, 30, 37).

KIRs are categorized on the basis of structural features of the extracellular domain (2D or 3D reflecting the number of immunoglobulin-like domains) and the length of the cytoplasmic tail (L or S for long and short, respectively) (4, 25). KIR function can be predicted from the length of the cytoplasmic domain: long-tailed KIRs are generally inhibitory, whereas all short-tailed KIRs are activating. The only exception to this rule is KIR2DL4, which is a unique activating receptor with a long cytoplasmic domain.

Variability in organization of the *KIR* gene complex

Gene families that encode immunoglobulin-like receptors are located within the leukocyte-receptor complex. The boundaries of the *KIR* locus on chromosome region 19q13.4 are the *KIR3DL3* and *KIR3DL2* genes (17, 61, 67). Between these conserved genes lies a variable set of *KIRs*, commonly containing 7–12 genes. Numerous haplotypes with different content of *KIRs* are present in the human population (62, 67). Haplotypes with identical gene content are further differentiated by polymorphisms of the component genes (37). For some genes over 50 different alleles have been described (56). The consequences of variable gene content and allelic polymorphism are that unrelated individuals rarely have identical *KIR* genotypes and that ethnic populations differ markedly in their distribution of *KIR* genotype frequencies (37, 46).

Despite the extreme variability, some systematic features in the organization of the *KIR* gene complex can be defined. All haplotypes contain at least one *KIR* gene encoding an activating receptor (61). Among the stimulatory *KIR* genes, *KIR2DS4* is much more frequently found in the Caucasian population than any other stimulatory *KIR*. It is suggested that *KIR2DS4* carries out a specific function, which cannot be fully compensated by replacement with one of the other

stimulatory *KIRs*. Four *KIR* genes are held in common by virtually all haplotypes: *KIR3DL3, KIR2DL4, KIR3DL2*, and the pseudogene *KIR3DP1* (17, 61, 67). According to their gene content all haplotypes can be divided in two groups, A and B (Fig. 1) (61, 62). The simpler group A haplotypes have a common organization of seven genes and two pseudogenes but are distinguished by allele combination (62). In contrast to the A haplotypes, the B haplotypes have a more variable gene content. More than 20 different B haplotypes have been described, which in addition to genes that are present in group A haplotypes, include *KIR* genes that are unique to group B haplotypes: *KIR2DL5A (KIR2DL5B), KIR2DS1, KIR2DS2, KIR2DS3* and *KIR2DS5* (4, 61, 62). Genes *KIR2DL2* (an allele of *KIR3DL3*) and KIR3DS1 (an allele of *KIR3DL1*) are also specific to group B haplotypes. Most group-B-specific *KIR* genes encode activating receptors. In general, group B haplotypes contain more genes that encode activating *KIRs* than do group A haplotypes. All human populations have both group A and group B haplotypes, although their frequencies vary (37). In Caucasians group A and group B haplotypes are present at an approximately equal frequency (58% group A haplotypes, and 42% group B haplotypes). It is worth noting that the diversity of group A haplotypes is mainly due to allelic polymorphisms, including copy number variation, whereas group B haplotypes are both polymorphic and polygenic (56, 61). The variety of *KIRs* in copy number variation can lead to changes of transcripts levels through gene dosage (23, 56, 69). Such a high level of diversity probably reflects strong pressure from pathogens on the human NK/T cell immune response (19, 56).

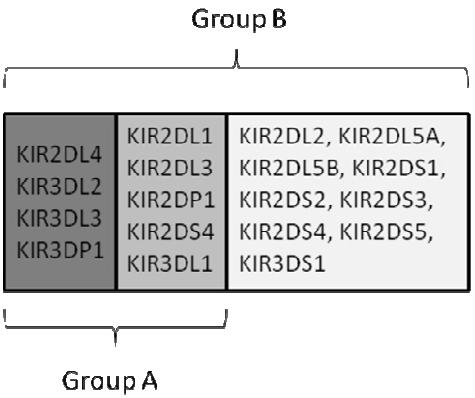

Figure 1. Group organisation of human *KIR* haplotypes. Activating *KIR* genes containing the gene name "S", inhibitory – "L", pseudogenes – "P". *KIR* genes, which are conservative for virtually all haplotypes, are in grey. Genes that can be present in both group A and group B *KIR* haplotypes are in light grey. Genes (and/or alleles) that are specific to group B *KIR* haplotypes are in white.

The *KIR* gene sequences, including intergenic regions, are highly conserved with exception of *KIR2DL4* (67). The high level of homology could facilitate non-reciprocal recombination, an evolutionary mechanism that can delete, duplicate or recombine genes (37). Such mechanisms may be behind a variation in number of immunoglobulin exons in some members of the *KIR* family, a generation of novel hybrid genes, as well as gain and loss of genes (4, 56, 67). Based on the genomic sequences, three hybrid genes exist: *KIR2DL5A/3DP1* (termed *KIR2DL5B*), *KIR2DL1/2DS1,* and *KIR2DL3/2DP1* (26, 56). Recombination processes may be facilitated by repeated elements, which exhibit dense clustering within *KIR* gene introns (56). It was suggested that such plasticity of the *KIR* complex allows a relatively rapid form of natural selection (4).

Regulation of *KIR* gene expression

KIR expression is restricted to NK cells and small subsets of T cells (2, 32, 63). The pattern of *KIR* gene expression is quite complex. The expression of a particular *KIR* (or its alleles) is largely independent of the expression of any other *KIRs* (7, 64). Moreover, each NK cell clone expresses only a portion of the set of KIRs encoded in a given individual's genome. The *KIR* genes are seemingly expressed stochastically, with the exceptions of *KIR2DL4*, which is expressed by all NK cells (37, 64). However, NK cell clones maintain a once established *KIR* expression pattern through multiple cell divisions (64). Thus, every NK cell stably expresses an apparently random combination of the available *KIR* genes. This combinatorial expression of genes is unique in human biology, and is essential to create a diverse and sensitive repertoire of NK cell specificities.

To comprehend how the *KIR* repertoire is generated and maintained, it is crucial to understand the regulation of *KIR* gene expression. Some explorations of *KIR* promoter regions were accomplished (52, 57, 68). Initially, close examination of the *KIR* region showed that the sequences upstream of the transcribed region are highly homologous (>91%), with the exception of the *KIR2DL4* gene, suggesting similar transcription regulation among the genes (59, 67). However, lately, *KIR* promoters were divided into four differently regulated groups, two of which control clonally expressed *KIR* genes, while one is unique for *KIR2DL4* (the only *KIR* gene transcribed in all NK cells), and one for the weakly expressed *KIR3DL3* (5, 52, 57). The differences in these promoters, including variations of transcription-factor binding sites, could explain altered patterns of expression. More recently it was established that *KIR* genes have two promoters: a distal promoter with weaker activity and proximal promoter (13, 23, 53). The latter one is bidirectional which leads to competing forward and reverse promoter activities resulting in a synthesis of sense and antisense transcripts, respectively. The *KIR2DL4* is unique because it is the only *KIR* gene lacking the repeat region and containing an activating element in the first intron (57, 67). It has been established that transcription starts with *KIR2DL4* which opens up the *KIR* locus, ensuring access of the transcription machinery to other *KIR* genes (37, 57). Then, using an unknown mechanism, NK cells express different combinations of *KIR* genes (64). It appears that transcription of *KIRs* occurs in a stochastic manner. However, the subset of *KIR* genes that are expressed by a particular NK cell becomes fixed through methylation in the 5' area of unexpressed *KIR* genes, and the pattern of expression is passed on to daughter cells during cell division (7, 43).

Cytotoxic T cells express *KIRs* in a similar manner to NK cells (49, 63, 65), but the transcriptional control of *KIR* expression differs between NK and T cells (68). This fact emphasizes the biological significance of *KIR* expression in T cells. Although *KIR* expression correlates with T cell differentiation – even naïve T lymphocytes have the transcriptional machinery to support the activation of the minimal *KIR* promoter – it was established that epigenetic mechanisms such as DNA methylation also play an important role in determining *KIR* expression in T cell subsets (24). It is noteworthy that signalling through KIRs expressed by T cells differs from KIR signalling in NK cells (50).

Human diseases and combination of MHC class I and KIR variants

NK cells play a role in the innate immune response that occurs in the early phase of infection. In particular, they are important for helping to clear viral infection (35). They kill infected cells, secrete inflammatory cytokines and interact with dendritic cells to determine a moment when an adaptive immune response should start (31). In functioning as NK cell receptors for MHC class I molecules, KIRs work together with the conserved lectin-like receptors CD94-NKG2A (NK group 2, member A; an inhibitory receptor) and CD94-NKG2C (an activating receptor) (64). All NK cells are non-responsive towards healthy autologous cells, a tolerance that involves the interaction of at least one autologous human leukocyte antigen (HLA) class I isoform with an inhibitory KIR or CD94-NKG2A. It is interesting to note that inhibitory signalling can not only prevent NK cell-mediated cytotoxicity, but also interfere with adhesion of NK cells to target cells (38). The balance of signals from activating and inhibitory receptors can be influenced by changes in surface expression levels of ligands on the target cells, which can alter the overall activation threshold of NK cells. Therefore, despite the supposed stochastic nature of KIR expression and the independent inheritance of *KIR* genes and genes encoding HLA, some regulatory link between the HLA repertoire and KIR expression evidently exists (4, 41, 69).

The lectin-like receptors have a broader view and recognize complexes of HLA-E and peptides cleaved from the leader sequences of HLA-A, HLA-B, HLA-C and HLA-G (30). Receptors of the KIR family are expressed on later stages of NK cell development than CD94-NKG2 (37). In contrast to CD94-NKG2, individual KIRs recognize distinct subsets of the classical human MHC-I molecules (30, 41). Together, the different inhibitory KIRs possess the capability to recognize 100% of the known HLA-C allotypes and subsets of HLA-A and HLA-B allotypes (41). The inhibitory KIR2DL2/2DL3 and the KIR2DL1 molecules are receptors for two mutually exclusive groups of HLA-C allotypes, HLA-C1 and HLA-C2, respectively (4, 32). HLA-C2 with KIR2DL1 is the combination expected to provide the strongest inhibition, and is apparently associated with lung cancer (1, 37). KIR3DL1 binds with HLA-B Bw4 allotypes (5, 34, 41). An increased frequency of KIR3DL1 and its ligand has been observed in kidney cancer patients compared with normal controls (1). Different alleles of *KIR3DL1* vary in terms of cell surface expression and strength of inhibitory signalling (4). KIR2DL4 interacts with HLA-G, which is upregulated in some tumour cells and under conditions of inflammation. KIR3DL2 is only known to recognize HLA-A3 and HLA-A11 allotypes (41). The ligands for KIR2DL5 and KIR3DL3 remain to be determined.

Based upon the high homology between the extracellular domains of activating and inhibitory KIR receptors (~99%), it was reported that activating KIRs recognize the same HLA molecules as their inhibitory counterparts, but with significantly weaker affinities (41, 51). However, the activating KIR-HLA affinities may be enhanced by specific peptides presented on the HLA molecules (41). Such enhancement has been observed for KIR2DS1 under its interaction with Epstein-Barr virus-infected cells, KIR3DL1 binding with HLA-B, and KIR3DL2 recognizing HLA-A3/-A11 (15, 51, 54). Interestingly, an activating signal generated by

a weaker interaction of KIR2DS1 with HLA-C2 can mute a stronger inhibitory signal from KIR2DL1 (37, 41). It appears that this effect may be explained by the same mechanism. Alternatively, activating KIRs may bind entirely distinct ligands and may be involved in the recognition of pathogen structures (41, 61). Thus, KIR2DS4 has been shown to recognize a non-MHC-I polypeptide on the surface of melanoma cells. Along this line, it was suggested that activating KIR receptors are involved in MHC-independent recognition of herpes simplex virus-infected cells (39).

A number of studies have reported associations between distinct *KIR*/*HLA* compound genotypes with susceptibility or resistance to viral infections. It was ascertained that homozygosity for both *KIR2DL3* and group HLA-C1 allotypes, providing lower inhibitory signals, is associated with increased resistance to hepatitis C virus infection (18). A lower frequency of *KIR2DL2* and/or *KIR2DL3* in combination with HLA-C1 ligands was found in patients with chronic hepatitis B compared with healthy controls (12). For infection with HIV, the progress to AIDS is slower in patients who have activating *KIR3DS1* in combination with HLA-B *Bw4-801* and an inhibitory *KIR3DL1*004* allele in combination with HLA-B *Bw4* (27, 29).

While haplotypes containing multiple activating *KIRs* may mediate a protective NK cell response against infectious disease, these same haplotypes may also predispose for autoimmune disease (28, 37). It has been found that activating *KIR2DS1* and/or *KIR2DS2* genes and group B *KIR* haplotypes are present in higher frequency in patients with certain autoimmune diseases than in healthy individuals (37). In the case of psoriatic arthritis, individuals carrying (activating) *KIR2DS1* and/or K*IR2DS2* genes show increased susceptibility to the onset of the disease, but only when one or both ligands of their homologous inhibitory receptors *KIR2DL1* and *KIR2DL2* (or *KIR2DL3*) are missing (28, 34). Absence of ligands for inhibitory KIRs could potentially lower the threshold for NK and/or T cell activation mediated through activating receptors, thereby contributing to pathogenesis. It was inferred that the trend for susceptibility to develop psoriatic arthritis increases when genotypes are ordered by their ability to confer the most inhibition (protection) to the most activation (34). Further, an influence of *KIR/HLA-C* gene combinations on type I diabetes and scleroderma was shown (37). Interestingly, acute coronary syndrome and rheumatoid vasculitis were associated with expression of *KIR2DS2* by clonally expanded populations of $CD4^{+}CD28^{null}$ T cells (70). In these diseases T cells expressing *KIR* genes are directly implicated in the disease mechanism. This fact brings up the question about a role of NK-cell responses for KIR-associated autoimmunity (37). It is noteworthy that an array of studies have described *KIR*/*HLA* compound genotypes that are associated with susceptibility to certain cancers (37, 41).

Since the interactions of KIRs with cognate HLA ligands can dramatically influence overall responsiveness of NK and T cells expressing these receptors, they have the potential to influence both the innate and adaptive immune response. Since we know that exercise has effects on both parts of the immune response, the question arises, what roles KIRs may play in the effects of physical activity on human health.

KIR gene expression and exercise

A rapid increase in circulating numbers of lymphocytes, in particular NK cells, with the onset of exercise is a well-documented phenomenon (10, 21, 44, 50) as is a change in the gene expression profile following exercise (6, 8, 11, 42, 48). However, there are only a handful of studies providing information about the effect of exercise on *KIR* genes.

In a recent study, Radom-Aizik et al. tested the alteration of gene expression in peripheral blood mononuclear cells of early- and late-pubertal girls using Affymetrix GeneChip technology (42). These authors found that four *KIR* genes, encoding three inhibitory receptors, KIR2DL3, KIR3DL1, and KIR3DL2, and one activating receptor KIR2DL4, had higher expression after exercise (2.3-3.0 fold). Blood samples were drawn before and after exercise consisting of ten 2-min bouts of constant-workrate cycle ergometry (the workrate was roughly halfway between the anaerobic threshold and peak oxygen uptake). Only insignificant differences in fold changes of *KIR* gene expression between the two groups of girls was observed. Earlier, Büttner et al. (6) had found that the *KIR2DS4* (activating) gene was upregulated more than 1.3 fold in their microarray analysis of exercise-induced changes of gene expression profiles of blood leukocytes. Only young men participating in leisure time sports were recruited for this study. The participants performed a strenuous treadmill exercise test at ~80% of maximal oxygen uptake (VO_{2max}) until exhaustion. In contrast to this work, Connolly et al. (8) reported down regulation of the *KIR2DS4* gene after 30 min exercise at ~80% of VO_{2max} in blood samples of untrained men. More recently, our laboratory has investigated the impact of high intensity exercise on gene expression by blood leukocytes. We examined the transcription response of male athletes after a ramp type treadmill test with an incremental step protocol, where the workrate is progressively increased until exhaustion, using GeneChip Human Gene 1.0 ST Arrays (unpublished data). In this kind of test, athletes perform at an exercise intensity above the anaerobic threshold for a rather long time (4-6 min). The results of our microarray analysis indicate that some genes of the *KIR* locus are upregulated more than 1.8 fold. Unfortunately, we cannot extract isolated data for individual *KIR* genes from our results due to the fact that *KIR*-specific probes on these arrays are common for several *KIR* genes.

Thus, so far, existing data are quite restricted and not entirely consistent. One can suggest that the dissimilarity of results may be caused by differences in gender and exercise intensity or duration. Thus, it was reported that the expression level of the *KIR3DL3* which is present in all haplotypes, was higher in females than in males (60).Thereto, the *KIR3DL3* transcript was detected in the $CD56^{bright}$ subset of NK cells as opposed to $CD56^{dim}$ NK cells. Consequently, a different mobilization of these two NK cell subsets during exercise (50) might result in various effects on *KIR3DL3* gene expression. Our preliminary exploration allows us to suggest that training levels of participants may also bias changes of *KIR* expression after exercise (47). It is important to note that global changes in NK cell numbers among total lymphocytes after exercise were not taken into account in all above mentioned studies. The number of NK cells may significantly vary in blood samples of different individuals both before and after exercise (14). In addi-

tion, the dissimilarity of results may be related to the high degree of polymorphism in the *KIR* gene family. As mentioned above, allelic variation was observed for most *KIR* genes (62) and some of the promoter polymorphisms lead to loss of transcription factor binding sites and affect the frequency of gene expression (23).

At this point we like to stress that exercise can obviously induce transcription of both, genes encoding activating and genes encoding inhibitory KIRs. Since KIRs are the major set of receptors determining the functional activity of NK cells, it is justifiable to infer that modulation of *KIR* expression by exercise may have the potential to influence the functional state of NK cells in both directions: activation or inhibition. This may seem to be a paradox or it could be arbitrary, merely reflecting the proposed stochastic nature of *KIR* expression. We would, however, argue that it also looks suspiciously like a mirror of the known dichotomous overall effects of exercise on the immune system. These are namely immune enhancement expressed as increased resistance to infection and certain cancers and immunosuppression expressed as increased susceptibility to infection following exhaustive exercise and as reduced chronic low grade inflammation with regular exercise. As we know, the effects of regular moderate exercise are highly beneficial to health, and there is solid evidence to suggest that NK cells may play an important role in this. After all, it is their task to kill virus infected cells and cancer cells, and improvement of NK activity through exercise has been documented *in vitro* (9, 16, 20, 33, 45). NK cells are also important producers of interferon (IFN)-γ, a cytokine which has the potential to amplify inflammatory cytokines (9). Suppression of IFN-γ release through exercise has also been shown (66). In addition, recently, persuasive evidence was provided that the *KIR* genotype predicts the capacity of NK cells to provide IFN-γ in response to various stimuli (19). Thus, in spite of the proposed stochastic nature of *KIR* expression it is tempting to speculate that the proven modulation of the functional state of NK cells through exercise may somehow be related to the observed modulation of *KIR* expression through exercise.

Thus, we like to hypothesize that, in the end, modulation of *KIR* gene expression by exercise may be involved in mediating the beneficial effects of chronic moderate exercise on our health. To test this hypothesis, the possible existence of discriminating regulatory mechanisms for different *KIRs* would be a key point to explore. When looking for possible triggers of *KIR* expression, different candidate molecules may be considered: (i) cytokines, which may be locally effective, although *KIR* gene expression seems to be largely independent of systemic cytokine levels (47); (ii) low molecular weight compounds/metabolites released into plasma upon exercise (22); (iii) proteins released from defective or stressed muscle, or, (iv) transcription factors activated through heat or hypoxia. In context of the latter it is noteworthy that a recognition site for heat shock transcription factor 1, which is involved in induction of heat shock proteins, was found in the *KIR2DL1/S1* promoter (56).

Knowledge of such mechanisms could greatly increase our understanding of the effects of physical exercise on chronic inflammatory diseases and might help to optimize exercise prescriptions to confer health benefits or even open up new opportunities to use exercise as adjunct to therapy in fighting infection, cancer or autoimmune disease.

Based on the structural complexity and high diversity of the *KIR* region, it seems that an individual's *KIR* repertoire may be very relevant for his or her gene expression response to exercise. A recent study revealed an influence of the *KIR* genotype on the ability of NK cells to respond to nonviral infections (19). Other studies emphasize the importance of distinguishing between alleles of *KIRs,* as well as between alleles of genes encoding their specific HLA ligands in disease studies (23, 29, 71). Therefore, the *KIR-HLA* compound genotype deserves consideration in *KIR*-exercise-disease associated research.

CONCLUDING REMARKS

Undoubtedly, much more work is needed to clarify the exact change of *KIR* gene expression patterns in response to physical activity and to determine what kind of conditions can influence this change (e.g. exercise intensity and duration, sex, puberty, training status, ageing). Two of the most intriguing questions also remain open: (i) what is the trigger of *KIR* expression changes in response to exercise and (ii) does the *KIR* genotype exhibit a great influence on the extent of *KIR* gene transcription activation? In the light of huge interest in the clinical role of NK cells and mounting evidence of the broad medical relevance of *KIRs*, to gain an insight into these questions is a fruitful task for the future studies. Changes in *KIR* gene expression caused by exercise may turn out to be a relevant immunotherapeutic marker reflecting peculiarities of the organism, which may be exploited for individual optimization of a programme of regular training or an adjunct exercise therapy.

ACKNOWLEDGEMENT

The authors are supported by the Russian Ministry of Science Grant No. 14.740.11.0117 and 16.740.11.0449.

REFERENCES

1. Al Omar S, Middleton D, Marshall E, Porter D, Xinarianos G, Raji O, Field JK, Christmas SE. Associations between genes for killer immunoglobulin-like receptors and their ligands in patients with solid tumors. Hum Immunol 71: 976–981, 2010.
2. Anfossi N, Pascal V, Vivier E and Ugolini S. Biology of T memory type 1 cells. Immunol Rev 181: 269–278, 2001.
3. Barrow AD, Trowsdale J. The extended human leukocyte receptor complex: diverse ways of modulating immune responses. Immunol Rev 224: 98–123, 2008.
4. Bashirova AA, Martin MP, McVicar DW, Carrington M. The killer immunoglobulin-like receptor gene cluster: tuning the genome for defense. Annu Rev Genomics Hum Genet 7: 277–300, 2006.
5. Bergen J, Stewart CA, van den Elsen PJ, Trowsdale J. Structural and functional differences between the promoters of independently expressed killer cell Ig-like receptors. Eur J Immunol 35: 2191–2199, 2005.

6. Büttner P, Mosig S, Lechtermann A, Funke H, Mooren FC. Exercise affects the gene expression profiles of human white blood cells. J Appl Physiol 102: 26–36, 2007.
7. Chan HW, Kurago ZB, Stewart CA, Wilson MJ, Martin MP, Mace BE, Carrington M, Trowsdale J and Lutz CT. DNA methylation maintains allele-specific KIR gene expression in human natural killer cells. J Exp Med 197: 245–255, 2003.
8. Connolly PH, Caiozzo VJ, Zaldivar F, Nemet D, Larson J, Hung SP, Heck JD, Hatfield GW, Cooper DM. Effects of exercise on gene expression in human peripheral blood mononuclear cells. J Appl Physiol 97: 1461–1469, 2004.
9. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. Trends Immunol 22: 633–640, 2001.
10. Cooper DM, Radom-Aizik S, Schwindt C, Zaldivar F, Jr., Dangerous exercise: lessons learned from dysregulated inflammatory responses to physical activity. J Appl Physiol 103: 700–709, 2007.
11. Fehrenbach E, Zieker D, Niess AM, Moeller E, Russwurm S, Northoff H. Microarray technology — the future analyses tool in exercise physiology? Exerc Immunol Rev 9: 58–69, 2003.
12. Gao X, Jiao Y, Wang L, Liu X, Sun W, Cui B, Chen Z, Zhao Y. Inhibitory KIR and specific HLA-C gene combinations confer susceptibility to or protection against chronic hepatitis B. Clin Immunol 137: 139–146, 2010.
13. Gardiner CM. Killer cell immunoglobulin-like receptors on NK cells: the how, where and why. Int J Immunogenet 35: 1–8, 2008.
14. Goebel MU, Mills PJ. Acute psychological stress and exercise and changes in peripheral leukocyte adhesion molecule expression and density. Psychosom Med 62: 664–670, 2000.
15. Hansasuta P, Dong T, Thananchai H, Weekes M, Willberg C, Aldemir H, Rowland-Jones S, Braud VM. Recognition of HLA-A3 and HLA-A11 by KIR3DL2 is peptide-specific. Eur J Immunol 34: 1673–1679, 2004.
16. Horn P, Kalz A, Lim CL, Pyne D, Saunders P, Mackinnon L, Peake J, Suzuki K. Exercise-recruited NK cells display exercise-associated eHSP-70. Exerc Immunol Rev 13: 100–111, 2007.
17. Hsu KC, Liu XR, Selvakumar A, Mickelson E, O'Reilly RJ, Dupont B. Killer Ig-like receptor haplotype analysis by gene content: evidence for genomic diversity with a minimum of six basic framework haplotypes, each with multiple subsets. J Immunol 169: 5118–5129, 2002.
18. Khakoo SI, Thio CL, Martin MP, Brooks CR, Gao X, Astemborski J, Cheng J, Goedert JJ, Vlahov D, Hilgartner M, Cox S. Little A.M., Alexander G.J., Cramp M.E., O'Brien S.J., Rosenberg W.M., Thomas D.L., Carrington M., HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. Science 305: 872–874, 2004.
19. Korbel DS, Norman PJ, Newman KC, Horowitz A, Gendzekhadze K, Parham P, Riley EM. Killer Ig-like receptor (KIR) genotype predicts the capacity of human KIR-positive CD56^{dim} NK cells to respond to pathogen-associated signals. J Immunol. 182: 6426–6434, 2009.
20. Krause SW, Gastpar R, Andreesen R, Gross C, Ullrich H, Thonigs G, Pfister K, Multhoff G. Treatment of colon and lung cancer patients with ex vivo heat shock protein 70-peptide-activated, autologous natural killer cells: a clinical phase I trial. Clin Cancer Res 10: 3699–3707, 2004.

21. Krüger K, Mooren FC. T cell homing and exercise. Exerc Immunol Rev 13: 37–54, 2007.
22. Lewis GD, Farrell L, Wood MJ, Martinovic M, Arany Z, Rowe GC, Souza A, Cheng S, McCabe EL, Yang E, Shi X, Deo R, Roth FP, Asnani A, Rhee EP, Systrom DM, Semigran MJ, Vasan RS, Carr SA, Wang TJ, Sabatine MS, Clish CB, Gerszten RE. Metabolic signatures of exercise in human plasma. Sci Transl Med 2: 33ra37, 2010.
23. Li H, Pascal V, Martin MP, Carrington M, Anderson SK. Genetic control of variegated KIR gene expression: polymorphisms of the bi-directional KIR3DL1 promoter are associated with distinct frequencies of gene expression. PLoS Genet 4: e1000254, 2008.
24. Li G, Yu M, Weyand CM, and Goronzy JJ. Epigenetic regulation of killer immunoglobulin-like receptor expression in T cells. Blood 114: 3422–3430, 2009.
25. Marsh SG, Parham P, Dupont B, Geraghty DE, Trowsdale J, Middleton D, Vilches C, Carrington M, Witt C, Guethlein LA, Shilling H, Garcia CA, Hsu KC, Wain H. Killer-cell immunoglobulin-like receptor (KIR) nomenclature report, 2002. Immunogenetics 55: 220–226, 2003.
26. Martin MP, Bashirova A, Traherne J, Trowsdale J, Carrington M. Cutting edge: expansion of the KIR locus by unequal crossing over. J Immunol 171: 2192–2195, 2003.
27. Martin MP, Gao X, Lee JH, Nelson GW, Detels R, Goedert JJ, Buchbinder S, Hoots K, Vlahov D, Trowsdale J, Wilson M, O'Brien SJ, Carrington M. Epistatic interaction between KIR3DS1 and HLA-B delays the progression to AIDS. Nat Genet 31: 429–434, 2002.
28. Martin MP, Nelson G, Lee JH, Pellett F, Gao X, Wade J, Wilson MJ, Trowsdale J, Gladman D, Carrington M. Cutting edge: susceptibility to psoriatic arthritis: influence of activating killer Ig-like receptor genes in the absence of specific HLA-C alleles. J Immunol 169: 2818–2822, 2002.
29. Martin MP, Qi Y, Gao X, Yamada E, Martin JN, Pereyra F, Colombo S, Brown EE, Shupert WL, Phair J, Goedert JJ, Buchbinder S, Kirk GD, Telenti A, Connors M, O'Brien SJ, Walker BD, Parham P, Deeks SG, McVicar DW, Carrington M. Innate partnership of HLA-B and KIR3DL1 subtypes against HIV-1. Nat Genet 39: 733–740, 2007.
30. Moffett-King A. Natural killer cells and pregnancy. Nature Rev Immunol 2: 656–663, 2002.
31. Moretta A. Natural killer cells and dendritic cells: rendezvous in abused tissues. Nat Rev Immunol 2: 957–964, 2002.
32. Moretta A, Tambussi G, Bottino C, Tripodi G, Merli A, Ciccone E, Pantaleo G and Moretta L. A novel surface antigen expressed by a subset of human CD3–CD16+ natural killer cells. Role in cell activation and regulation of cytolytic function. J Exp Med 171: 695–714, 1990.
33. Multhoff G. Activation of natural killer cells by heat shock protein 70. Int J Hyperthermia 25: 169–175, 2002.
34. Nelson GW, Martin MP, Gladman D, Wade J, Trowsdale J, Carrington M. Cutting edge: heterozygote advantage in autoimmune disease: hierarchy of protection/susceptibility conferred by HLA and killer Ig-like receptor combinations in psoriatic arthritis. J Immunol 173: 4273–4276, 2004.
35. Orange JS. Human natural killer cell deficiencies and susceptibility to infection. Microbes Infect 4: 1545–1558, 2002.

36. Ostrowski K, Rohde T, Asp S, Schjerling P, Pedersen BK. Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. J Physiol 515(Pt 1): 287–291, 1999.
37. Parham P. MHC class I molecules and KIRs in human history, health and survival. Nat Rev Immunol 5: 201–214, 2005.
38. Pegram HJ, Andrews DM, Smyth MJ, Darcy PK, Kershaw MH. Activating and inhibitory receptors of natural killer cells. Immunol Cell Biol: 1–9, 2010.
39. Pietra G, Semino C, Cagnoni F, Boni L, Cangemi G, Frumento G, Melioli G. Natural killer cells lyse autologous herpes simplex virus infected targets using cytolytic mechanisms distributed clonotypically. J Med Virol 62: 354–363, 2000.
40. Ploeger HE, Takken T, de Greef MH, Timmons BW. The effects of acute and chronic exercise on inflammatory markers in children and adults with a chronic inflammatory disease: a systematic review. Exerc Immunol Rev 15: 6–41, 2009.
41. Purdy AK, Campbell KS. Natural killer cells and cancer: regulation by the killer cell Ig-like receptors (KIR). Cancer Biol Ther. 8: 2211–2220, 2009.
42. Radom-Aizik S, Zaldivar F, Jr., Leu SY and Cooper DM. A brief bout of exercise alters gene expression and distinct gene pathways in peripheral blood mononuclear cells of early- and late-pubertal females. J Appl Physiol 107: 168–175, 2009.
43. Santourlidis S, Trompeter HI,Weinhold S, Eisermann B, Meyer KL, Wernet P and Uhrberg M, Crucial role of DNA methylation in determination of clonally distributed killer cell Ig-like receptor expression patterns in NK cells. J Immunol 169: 4253–4261, 2002.
44. Schwindt CD, Zaldivar F, Wilson L, Leu SY, Wang-Rodriguez J, Mills PJ, Cooper DM. Do circulating leucocytes and lymphocyte subtypes increase in response to brief exercise in children with and without asthma? Br J Sports Med 41: 34–40, 2007.
45. Shephard RJ, Shek PN. Effects of exercise and training on natural killer cell counts and cytolytic activity: a meta-analysis. Sports Med 28: 177–195, 1999.
46. Shilling HG, Guethlein LA, Cheng NW, Gardiner CM, Rodriguez R, Tyan D, Parham P. Allelic polymorphism synergizes with variable gene content to individualize human KIR genotype. J Immunol 168: 2307–2315, 2002.
47. Shleptsova VA, Grebenyuk ES, Khaustova SA, Obraztsova NP, Shkurnikov MU, Sakharov DA, Tonevitsky EG. Expression of genes encoding natural killer cell receptors KIR2DL3 and KIR2DS2 after exercise. Bull Exp Biol Med 149: 755–758, 2010.
48. Simon P, Fehrenbach E, Niess AM. Regulation of immediate early gene expression by exercise: short cuts for the adaptation of immune function. Exerc Immunol Rev 12: 112–131, 2006.
49. Snyder MR, Muegge LO, Offord C, O'Fallon WM, Bajzer Z, Weyand CM, Goronzy JJ. Formation of the killer Ig-like receptor repertoire on $CD4^{+}CD28^{null}$ T cells. J Immunol 168: 3839–3846, 2002.
50. Snyder MR, Nakajima T, Leibson PJ, Weyand CM, Goronzy JJ. Stimulatory killer Ig-like receptors modulate T cell activation through DAP12-dependent and DAP12-independent mechanisms. J Immunol 173: 3725–3731, 2004.
51. Stewart CA, Laugier-Anfossi F, Vély F, Saulquin X, Riedmuller J, Tisserant A, Gauthier L, Romagné F, Ferracci G, Arosa FA, Moretta A, Sun PD, Ugolini S, Vivier E. Recognition of peptide-MHC class I complexes by activating killer immunoglobulin-like receptors. Proc Natl Acad Sci U S A 102: 13224–13229, 2005.

52. Stewart CA, Van Bergen J and Trowsdale J. Different and divergent regulation of the KIR2DL4 and KIR3DL1 promoters. J Immunol 170: 6073–6081, 2003.
53. Stulberg MJ, Wright PW, Dang H, Hanson RJ, Miller JS, Anderson SK. Identification of distal KIR promoters and transcripts. Genes Immun 8: 124–130, 2007.
54. Thananchai H, Gillespie G, Martin MP, Bashirova A, Yawata N, Yawata M, Easterbrook P, McVicar DW, Maenaka K, Parham P, Carrington M, Dong T, Rowland-Jones S. Cutting Edge: Allele-specific and peptide-dependent interactions between KIR3DL1 and HLA-A and HLA-B. J Immunol 178: 33–37, 2007.
55. Timmons BW, Cieslak T. Human natural killer cell subsets and acute exercise: a brief review. Exerc Immunol Rev 14: 8–23, 2008.
56. Traherne JA, Martin M, Ward R, Ohashi M, Pellett F, Gladman D, Middleton D, Carrington M, Trowsdale J. Mechanisms of copy number variation and hybrid gene formation in the KIR immune gene complex. Hum Mol Genet 19: 737–751, 2010.
57. Trompeter H-I, Go´mez-Lozano N, Santourlidis S, Eisermann B, Wernet P, Vilches C and Uhrberg M. Three Structurally and Functionally Divergent Kinds of Promoters Regulate Expression of Clonally Distributed Killer Cell Ig-Like Receptors (KIR), of KIR2DL4, and of KIR3DL3. J Immunol 174: 4135–4143, 2005.
58. Trowsdale J. Genetic and functional relationships between MHC and NK receptor genes. Immunity 15: 363–374, 2001.
59. Trowsdale J, Barten R, Haude A, Stewart CA, Beck S, Wilson MJ. The genomic context of natural killer receptor extended gene families. Immunol Rev 181: 20–38, 2001.
60. Trundley AE, Hiby SE, Chang C, Sharkey AM, Santourlidis S, Uhrberg M, Trowsdale J, Moffett A. Molecular characterization of KIR3DL3. Immunogenetics 57: 904–916, 2006.
61. Uhrberg M, Parham P and Wernet P. Definition of gene content for nine common group B haplotypes of the Caucasoid population: KIR haplotypes contain between seven and eleven genes. Immunogenetics 54: 221–229, 2002.
62. Uhrberg M, Valiante NM, Shum BP, Shilling HG, Lienert-Weidenbach K, Corliss B, Tyan D, Lanier LL, Parham P. Human diversity in killer cell inhibitory receptor genes. Immunity 7: 753–763, 1997.
63. Uhrberg M, Valiante NM, Young NT, Lanier LL, Phillips JH, Parham P. The repertoire of killer cell Ig-like receptor and CD94:NKG2A receptors in T cells: clones sharing identical TCR rearrangement express highly diverse killer cell Ig-like receptor patterns. J Immunol 166: 3923–3932, 2001.
64. Valiante NM, Uhrberg M, Shilling HG, Lienert-Weidenbach K, Arnett KL, D'Andrea A, Phillips JH, Lanier LL and Parham P. Functionally and structurally distinct NK cell receptor repertoires in the peripheral blood of two human donors. Immunity 7: 739–751, 1997.
65. Vely F, Peyrat M, Couedel C, Morcet J, Halary F, Davodeau F, Romagne F, Scotet E, Saulquin X, Houssaint E, Schleinitz N, Moretta A, Vivier E. Bonneville M. Regulation of inhibitory and activating killer-cell Ig-like receptor expression occurs in T cells after termination of TCR rearrangements. J Immunol 166: 2487–2494, 2001.
66. Weinstock C, Koenig D, Harnischmacher R, Keul J, Berg A and Northoff H. Effect of Exhaustive Exercise Stress on the Cytokine response. Med Sci Sports Exerc 29: 345-354, 1997.

67. Wilson MJ, Torkar M, Haude A, Milne S, Jones T, Sheer D, Beck S, Trowsdale J. Plasticity in the organization and sequences of human KIR/ILT gene families. Proc Natl Acad Sci U S A 97: 4778–4783, 2000.
68. Xu J, Vallejo AN, Jiang Y, Cornelia MW, Goronzy JJ. Distinct transcriptional control mechanisms of Killer immunoglobulin-like receptors in Natural Killer (NK) and in T cells. J Biol Chem 280: 24277–24285, 2005.
69. Yawata M, Yawata N, Draghi M, Little AM, Partheniou F, Parham P. Roles for HLA and KIR polymorphisms in natural killer cell repertoire selection and modulation of effector function. J Exp Med 203: 633–645, 2006.
70. Yen JH, Moore BE, Nakajima T, Scholl D, Schaid DJ, Weyand CM, Goronzy JJ. Major histocompatibility complex class I-recognizing receptors are disease risk genes in rheumatoid arthritis. J Exp Med 193: 1159–1167, 2001.
71. Yindom LM, Leligdowicz A, Martin MP, Gao X, Qi Y, Zaman SM, van der Loeff MS, van Tienen C, Jaye A, Aveika A, Worwui A, Diatta M, Vincent T, Whittle HC, Rowland-Jones SL, Walton R, Carrington M. Influence of *HLA* class I and *HLA-KIR* compound genotypes on HIV-2 infection and markers of disease progression in a Manjako community in West Africa. J Virol 84: 8202–8208, 2010.

Instructions for authors of EIR

EIR usually solicits papers from authors with acknowledged expertise in the field to be covered. Unsolicited papers will be considered and can also be accepted. All papers are subject to a peer review process.

Usually the manuscripts will fit into one of two major categories: i. a review which thoroughly covers the area indicated in the heading and includes structuring and critical discussion of existing knowledge and, if possible, the ideas of the authors about potential practical consequences and future developments. Mere mentioning and listing of existing literature is not considered to be a good review. The review can be long, if necessary, or short, if the field covered by the heading is relatively new or very focussed. ii. a paper showing original data accompanied by an extended, review-type discussion.

The general format of the review is somewhat flexible. A review must however have an abstract, an introduction and a conclusion around the main sections. Reviews with three or more sections should list the headings of the sections in form of a bullet point table at the end of the introduction. Longer sections should also give a short interim summary at their end.
If substantial amounts of the authors‘ own new data are to be shown, a section on methods and on results must be included. Data will only be accepted, if methods are stated clearly and appropriate statistical evaluation of results is given.

Other types of papers, eg true meta-analyses of a circumscribed sector of literature or papers focussing on new ideas or hypotheses may also be considered. Interested authors, please contact the editorial board.

For reference style use the one as applied by J. Appl. Physiol., with references listed in alphabetical order. In text use ref. numbers in brackets. When giving more than 1 reference in one bracket, use numerical order.

A short running head should appear after the title, followed by the authors and their respective affiliations. The full address of correspondence should include an e-mail address of the correspondent author. Up to five key words should be added after the abstract.

Send manuscript to Hinnak Northoff, Derek Zieker or one of the other editors. Please use e-mail for all communications including manuscript submission (word or pdf-file) if possible and paste "EIR" in the subject field of your mailing program.

Prof. Dr. Hinnak Northoff
Editor EIR
Institute of clinical and experimental
Transfusion Medicine (IKET)
University of Tübingen
Otfried-Müller-Str. 4/1
D-72076 Tübingen
Tel.: + 49-7071-2981601
Fax: + 49-7071-295240
E-mail: hinnak.northoff@med.uni.tuebingen.de

Dr. Derek Zieker
Managing Editor, EIR
Institute of clinical and experimental
Transfusion Medicine (IKET)
University of Tübingen
Otfried-Müller-Str. 4/1
D-72076 Tübingen
Tel.: + 49-7071-2981657
Fax: + 49-7071-295240
derek.zieker@med-uni-tuebingen.de