Development of the discipline of exercise immunology

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INTRODUCTION

Interest in the influence of exercise upon the human white cell population dates back more than a 100 years. Thus, when introducing the third meeting of the International Society of Exercise Immunology in Brussels, Dr. Bente Klarlund-Pedersen noted that Schulte had already described an exercise-induced leukocytosis as early as 1893. However, for much of the following century interest remained strictly clinical, with physicians assessing the possible changes in vulnerability to bacterial and viral diseases that were induced by various forms of physical activity. In the absence of specific remedies, bed rest was a common medical recommendation for infectious disease, and if the patient recovered from the immediate infection there was often a substantial residual loss of physical condition. Army hospitals in particular were thus anxious to know whether recovery would be compromised if physical activity were to be encouraged during convalescence. Prominent concerns of this era were the influence of exercise upon anterior poliomyelitis and viral hepatitis. The paralysis resulting from the anterior poliomyelitis virus was generally localized to body parts that had been active, and it seemed most likely to develop in those who continued to engage in vigorous exercise in the face of early symptoms (46, 57, 119, 120). Data on viral hepatitis also suggested a need for rest in the acute phase of the disease (1, 65, 115, 128), although most authors concluded that in this condition exercise could be resumed during convalescence, provided that the patient was no longer severely jaundiced (5, 32, 136).

During the 1970s, sports physicians began to enquire whether exercise-induced modifications of immune function were responsible for the high frequency of minor infections seen in competitors around the time of major competitions (1, 65, 115, 128). Concerns were also expressed that such infections might have an adverse impact on athletic performance (23, 38), exacerbate any tendency to exer-

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cise-induced bronchospasm (51), and (if normal activity was maintained) lead to a fatal viral myocarditis (15, 146). In apparent confirmation of these fears, Swedish orienteers had an epidemic of sudden exercise-related deaths between 1979 and 1992, but such incidents stopped quite abruptly in 1992, suggesting that some factor other than myocarditis, possibly blood doping, may have been involved (146, 147).

Dr. Ernst Jokl (photo page 205) was an early proponent of the idea that participation in competitive sport could adversely affect immune function (59). His clinical arguments were vigorously criticized by some exercise scientists such as Dr. Bengt Eriksson of Göteborg and Dr. H.B. Simon (135). However, towards the end of the 1980s, support for Dr. Jokl's views came in the form of controlled studies of long-distance runners conducted by Dr. Edith Peters in South Africa (106) and Dr. David Nieman (photo page 206) and his colleagues at Appalachian State University (95); both noted that race participants showed a substantial increase of respiratory symptoms in the first few weeks after the event. Many authors attempted to link these observations to the changes in cellular and humoral parameters seen during and following various types of physical activity, both acutely, and following several months of training. A detailed account of this phase in the evolution of exercise immunology has been published recently (126).

The modern, experimental phase of exercise immunology began in the 1980s, with involvement of a relatively small group of laboratories. However, over the two following decades there was an exponential expansion of interest and resulting discoveries. The present article examines some of the factors that have encouraged the rapid growth of exercise immunology, marked by an ever-growing output of peer-reviewed papers, the development of an international society specializing in exercise immunology, and the founding of a well-respected journal. Brief comment is made on some of the key personalities involved and a few landmark discoveries are highlighted. This article concludes by noting some future research opportunities and challenges in exercise immunology.

FACTORS UNDERLYING THE RAPID DEVELOPMENT OF EXERCISE IMMUNOLOGY

The Second World War stimulated the development of potent vaccines, antibiotics and chemotherapeutic treatments for many infectious diseases, so that by the second half of the twentieth century interest in the relationships between exercise, training and bacterial and viral diseases was tending to wane. One exception to this generality was the problem of upper respiratory infections. These remained a significant issue for athletes, who feared a deterioration in performance (23, 38, 44) and possible death from viral myocarditis if they decided to compete despite an infection (15, 146, 147). Such considerations were likely important in driving early investigations into various cellular and humoral aspects of exercise immunology. Other influential forces have included major advances in technology, the demands of cold-war military tactics, the potential to use exercise as an experimental model of sepsis, and a growing interest in the areas of psychoneuroimmunology and stress research.

Acute infections and their treatment. Fears that upper respiratory infections might impair competitive performance piqued the interest of several drug companies, who sought to develop protective immunoglobulin preparations (144, 145). Beginning in the 1960s, there was also considerable public health (37, 83, 100) and religious (90) interest in encouraging a healthy, active lifestyle in order to stem the epidemic of coronary heart disease. It was thus important for exercise scientists to understand, and if possible to counter any adverse influence of such activity upon resistance to infections.

Although most governmental initiatives promoted regular, moderate physical activity of the type advocated by the American College of Sports Medicine and the Canadian Society of Exercise Physiology, many lay activists decided that if a little exercise was good, more strenuous exercise was better. The healthy lifestyle movement thus spawned mass participation in marathon and ultra-marathon runs. Some events attracted 40-80,000 participants, many of whom were ill prepared to run over such distances. It was quickly noted that participants apparently had an increased incidence of upper respiratory illness relative to their peers who decided not to enter a given event (95), raising questions about the wisdom of allowing unbridled exercise, and stimulating interest in the possibility of averting an adverse response by the use of dietary supplements and medications.

More recently, questions have arisen about the place of exercise programmes in the fight against the world-wide epidemic of Human Immunodeficiency Virus (HIV) infection. In particular, information was needed as to whether exercise would attenuate or compound the depression of immune function associated with this disease. Exercise seemed a potentially valuable element in therapy, given its ability to stimulate NK cell function, to counter depression (which itself depressed immune function), and to reverse the deterioration of general physique seen in many HIV patients. Dr. Arthur LaPerriere (photo page 207) and his colleagues have played a major role in these enquiries. The response to a moderate training programme has generally proven favourable, with no further depression of T cell numbers; counts of both $CD4^+$ and $CD4^+CD45^+$ cells were increased in exercisers; augmentation of the latter cell sub-set was particularly important, given its potential to activate $CD8^+$ cytotoxic cells. LaPerriere and associates concluded that the response to a moderate exercise programme was at least as good as might be anticipated from contemporary AZT treatment (66). Other observers have claimed enhanced skin responses to the Multitest Mérieux antigen in exercisers (122). However, immune benefits have not always been seen, and much further research is needed (132).

Technological advances. In many areas of biological science, the progress of knowledge has been propelled not by human curiosity or serendipity, but rather by the advent of some new technology or the sudden availability of research funding offered by government or industry hopeful of an early payback of "usable" information. The enormous strides made by cardio-respiratory physiologists during and immediately following World War II provides one obvious example. In the 1940s, high altitude research laboratories replaced the straw levers and smoked drums of the traditional physiology laboratory by recording galvanometers linked to sophisticated electronic transducers of respiratory and intravascular pressures, and continuously recording infra-red, paramagnetic and mass-spectroscopic indicators of gas concentrations were substituted for the laborious chemical analysis of gas samples.

Similar forces have played an important role in shaping the development of exercise immunology. Technical developments have facilitated rapid progress in our understanding of both cellular and the humoral aspects of this sub-discipline. Analysis of leukocytes has been greatly speeded and facilitated with introduction of the Coulter Counter, the FACScan cell sorter, radioactive techniques that measure cell toxicity accurately, and the application of molecular biology techniques to examine cell metabolism. Likewise, in animal laboratories, technological advances in mouse models of both exercise and immune responses, and the ready availability of mouse reagents have stimulated study of the immune system and how it is affected by exercise. Unfortunately, much of the new equipment and related reagents has remained relatively expensive, so that only a limited number of exercise scientists associated with major hospitals, military research laboratories and national sports training facilities have been able to exploit the more expensive components of the new technology.

Automated cell counting. The idea of automated cell counting dates back to 1934 (81). Wallace Coulter developed a device to detect the number and size of particles suspended in a fluid. This was patented in 1953 (22), and became widely available in the late 1960s. Changes in electrical conductance were detected as the cell-containing fluid was drawn through a small aperture. One difficulty was to allow the particles to pass the orifice in a reasonable time; electrophoresis and electrolysis proved unsuccessful, and eventually multiple apertures were used. Counts showed a good correlation with manual enumeration of total leukocytes, lymphocytes and neutrophils (6).

Fluorescence activated cell sorting. Early B cell counts were based on the rosetting technique (12, 49), and unfortunately this did not distinguish clearly between B and NK cells. Development of the fluorescence activated cell sorter and appropriate monoclonal antibodies has allowed much more accurate and automated sorting of cell sub-types; in the case of B cells, exercise-induced increases in count were seen to be much smaller than those that had been estimated by the rosetting technique (28). The basic idea of the automated cell sorter dates back to 1969 (29), and a commercial apparatus became available by the early 1970s (Becton-Dickinson Fluorescence Activated Cell Sorter, FACS ®). Electronic devices sorted cells by volume (39), and absorbing dyes were quickly replaced by fluorescent markers linked to monoclonal antibodies (40, 58, 80). Light from one or more laser beams was directed onto the stream of suspended cells. The forward scatter reflected cell size; lateral scatter reflected the surface form of the cells, membrane folding and granularity, and the intensities of green, orange and red fluorescence emitted by the monoclonal markers. After photomultiplication, data for scatter and fluorescence were gated, plotted, and compared with responses to calibration beads. Development of an ever-growing range of monoclonal antibodies has allowed precise study of surface antigens, and exercise-induced changes in surface characteristics during and following exercise (40, 80, 118). This has allowed assessments of adhesion molecule expression, and determinations of the proportion of mature cells in the circulating blood.

The introduction of tritiated thymidine incorporation methodology has allowed accurate assessment of *in vitro* lymphocyte proliferation rates (79, 134). Beginning with Hedfors and associates (50), exercise immunologists studied the functional activity of B cells in terms of the *in-vitro* pokeweed mitogen-stimulated production of immunoglobulins. Other early studies of cellular activity were based on insulin binding (64), glucose metabolism (11), and the direct observation of phagocytosis in animals (34). One promising development has been to monitor the intracellular generation of superoxide anions using FACscan equipment (110).

Humoral responses. Early investigators studied changes in the production of antigens in response to the injection of foreign erythrocytes (43), neutrophils (67) or various toxoids (30, 33, 69). Progress in the humoral aspects of exercise immunology has been spurred by the development of techniques such as gas-liquid chromatography, enzyme-linked immuno-absorbent assay (ELISA), western blot technologies, and most recently the transcription of cytokine mRNAs, using RNA isolation kits and the molecular biology technique of reverse transcription polymerase chain reaction assay (82, 114).

Cold-war military tactics. Western military commanders became very interested in the possible immuno-suppressant effects of prolonged exercise at the height of the "cold war." The tactical wisdom of that era was that the front-line strength of NATO troops was inadequate to resist a sustained Soviet attack. The game plan of the western generals was thus for their troops to fight a continuous rearguard action for 4-5 days, until they could be reinforced by troops flown from the United States (14). In the event of such extended combat, the defenders would likely be exposed to a combination of immune stressors, including very prolonged exercise, sleep deprivation, possible shortages of food and the anxieties associated with battle.

Exercise as a possible model of sepsis. Another strand of enquiry viewed exercise as a precipitator of the inflammatory response, and this seemed an important topic to investigate because it might throw some light upon the seemingly intractable problem of clinical sepsis (16). Dr. G. Camus and colleagues at the University of Brussels, Dr. Hinnak Northoff (photo page 207) and colleagues at the University of Tübingen, and Dr. Pang Shek at the University of Toronto have all made major contributions to this area of investigation. By 1997, interest in the topic was such that an International Colloquium on *"Immune Responses to Inflammation and Trauma: A Physical Training Model"* was hosted in Toronto; the group photo from this meeting includes many of those most active in exercise immunology in 1997 (photo page 199).

Fourth row (left to right): P. Vasiliou, M. Suzui, D. Smith, A. Carvalho, S. Shinkai, B. Bateman, A. Moldoveanu, R. Moore, D. Koenig, A. Berg, H. Northoff, V. Natale, P. Binder

Third row: N. Klentrou, S. Rhind, J. Landolt, A. Bellerby, S. Woods, I. Brenner, C. Bogard, J. Zamecnik, Z. Suntres, D. Saunders, A. Omri.

Second Row: B.K. Pedersen, J. Marshall, D. Nieman, O. Ronsen, A. LaPerriere, S. Nehlsen-Cannarella, P. Tiidus, M. Miles, E. Simins, J. Dickstein, R. Shippee.

Front row: B. Sabiston, M. Radomski, R. Shephard, P. Shek, A. Buguet, J. Hay

Other areas stimulating research in exercise immunology. Individual investigators have inevitably tended to believe that their particular area of research has made a major contribution to the development of exercise immunology, and there have undoubtedly been many influences other than those already noted. One such candidate is the rapidly growing area of psychoneuroimmunology, the complex two-way interaction between the pysche and immune function that modulates many chronic diseases and conditions (123). Fatiguing exercise, particularly under conditions of international competition or military combat, is undoubtedly very stressful. Indeed, one growing strand of thought urged by Dr. Bente Pedersen of the University of Copenhagen (photo page 206) and Dr. Laurie Hoffman-Goetz of the University of Waterloo, ON (photo page 206), has argued that heavy exercise affects the immune system much as a variety of other stressors, providing a useful general model of the immune response to stress (101). This issue has stimulated particular interest in military laboratories, since soldiers are often expected to undertake prolonged exercise in the face of other stressors such as extremes of heat and cold, hyperbaric environments and sleep deprivation (127).

EMERGENCE OF EXERCISE IMMUNOLOGY AS A SPECIFIC SUB-DISCIPLINE IN THE EXERCISE SCIENCES

Relatively few exercise science laboratories were involved in experimental immunology during the 1980s. Early pioneers included, among others, Dr. Hoffman-Goetz at the University of Waterloo, ON; Dr. Laurel Mackinnon of the University of Queensland, Brisbane; Dr. David Nieman, Dr. Sandra Nehlsen-Cannarella and colleagues, a collaboration between Appalachian State University and Loma Linde University in California; Dr. Bente Pedersen and colleagues at the University of Copenhagen; Drs. Shephard, Shek and Shinkai at the University of Toronto; Dr. Heinz Liesen in Paderborn, Westphalia; Dr. Gerd Uhlenbruck in Cologne; Dr. Hinnak Northoff in Tübingen; and Dr. Holger Gabriel, now at the University of Jena (40, 54, 69, 73, 97, 98, 105, 134). Particularly since the early 1990s, there have been major research contributions from other investigators, including Dr. Mark Davis, of the University of South Carolina, Dr. Jeff Woods, formerly of the University of South Carolina, and now at the University of Illinois (Urbana), Dr. Monika Fleshner, now at the University of Colorado (Boulder) and Marian Kohut, now at Iowa State University (7, 26, 148-150).

The emergence of exercise immunology as a specific sub-discipline of the exercise sciences began in the early 1990s, as FACScan equipment became more widely available, and an ever-growing number of scientific papers addressed various cellular and humoral aspects of exercise immunology. The founding of a professional society, the establishment of a specialist research journal, and the publication of several comprehensive textbooks all helped carry the process forward. Dr. David Nieman has frequently presented slides at professional meetings illustrating the exponential growth of research in exercise immunology that began around 1990 (Figure 1). The course of this trend can also be examined by pairing the terms "immunology" and "exercise or physical activity or fitness" in the HealthStar-Ovid data base. The total number of articles detected by the latter approach is substantially smaller than that seen in Dr. Nieman's graphs, which cover a wide field of interests ranging from the epidemiology of upper respiratory

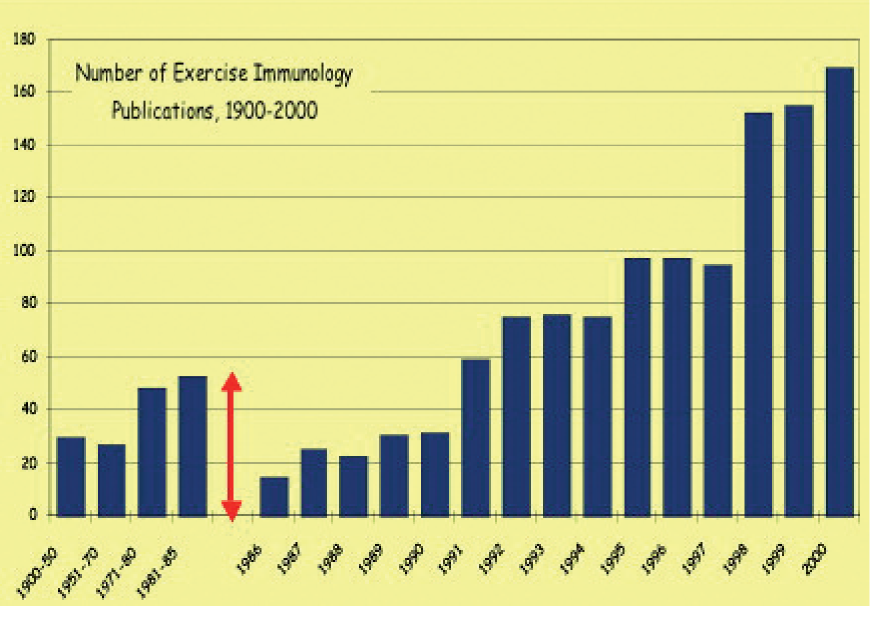


Figure 1. Illustrating the striking growth in the number of peer-reviewed articles in various areas of exercise immunology that began around 1988. (Illustration: Courtesy of Dr. David Nieman

infections to detailed research on cytokines and adhesion molecules. However, both approaches demonstrate the same trend of a rapid increase of interest during the 1990s. Dr. Nieman is no longer updating his graphs, but the alternative method of analysis suggests that for the moment interest may have reached a plateau.

Dr. David Nieman offered a sterling service to those entering the field of exercise immunology by circulating, on a biennial basis and at no charge to recipients, a detailed bibliography that provided extended abstracts and summaries of relevant papers; the first volume, published in 1993, listed and cross-referenced 360 articles from 1902 to 1991. Subsequent biennial updates carried this resource through to 1999, when electronic documentation such as PubMed and HealthStar Ovid made further editions redundant.

Systematic textbooks played an important role in defining and consolidating the field of exercise immunology. The first text written for students of exercise immunology was published by Laurel Mackinnon (74); a much expanded edition of this same book was published seven years later (72). Hoffman-Goetz and MacNeil contributed a chapter examining the possible impact of exercise on immune function in relation to cancer in a more general text on Exercise and Disease (56), and four years later Dr. Hoffman-Goetz edited a multi-authored book devoted entirely to Exercise and Immune Function (53). The following year, I authored a research monograph with an extensive bibliography that synthesized available knowledge to date (132). Finally, a text focusing on nutritional aspects of the exercise-immunology relationship was published by David Nieman and Bengt Pedersen (91).

DEVELOPMENT OF THE INTERNATIONAL SOCIETY OF EXERCISE IMMUNOLOGY

A number of universities in Germany had shown a strong interest in exercise immunology during the late 1980s, and in November 1989 Dr. Heinrich Liesen (photo page 205) of the Institute for Sports Medicine at the University of Paderborn hosted a seminal International Symposium on Sports and the Immune System under the aegis of the German Federation of Sports Medicine. This meeting celebrated the "presentation" of the Institute for Sports Medicine of the University of Paderborn, and also marked the 60th birthday of Professor Gerhard Uhlenbruck of the University of Cologne, a collaborator with Dr. Liesen and an early investigator in exercise immunology (69). Interest in the meeting was strong, and the desire was expressed to repeat such exchanges of the latest information on a regular basis. Senior investigators thus met immediately following the meeting, and formulated plans for the establishment of an inter-disciplinary International Society of Exercise and Immunology.

The Heinz-Nixdorf Foundation, a funding agency associated with a major computer manufacturer in Paderborn had provided generous financial support for the 1989 symposium, and through the good offices of Dr. Liesen this same Foundation was persuaded to provide continuing funding as an International Society was developed. Despite his well-established research credentials and demonstrated administrative ability, Dr. Liesen modestly declined presidency of the new organization, and after some discussion among the *ad hoc* group who were laying the groundwork for the new Society, a consensus developed that Dr. Arthur LaPerriere should serve as the first President. It was decided that the Society would meet biennially, that the first meeting would be in Paderborn, and that Dr. LaPerriere's Presidency would extend from 1993-1995. Subsequent Presidents have included Dr. Bente Klarlund-Petersen (1995-1997, Dr. David Nieman (1997-2001), Dr. Ingabjorg Jonsdottir (2001-2003), Dr. Jeff Woods (2003-2005). Dr. Ryoichi Nagatomi (2005-2007), Dr. Michael Gleeson (2007-2009) and Dr. Maree Gleeson (2009-2011).

Because of the strong initial support from the Heinz Nixdorf Foundation, it was initially resolved that meetings would alternate between Paderborn and various international destinations. However, this plan was later abandoned, in part because those arranging meetings found it more practical to organize meeting rooms near to their home base. The Society thus met in Brussels (1995), returned to Paderborn (1997) and then visited in turn Rome (1999), Baltimore (2001), Copenhagen (2003), Monaco (2005), Sendai, Japan (2007) and Tübingen, Germany (2009). Meetings were generally well attended and well-received, the one contentious gathering being in Brussels (where the meeting was followed by an unfortunate financial dispute between the local organizers and the Society).

Between the biennial meetings, interest was maintained by regional gatherings. In Germany, the principal forum for such interaction was the German Federation of Sports Medicine. In the United States, Dr. David Nieman organized seminars, specific symposia and an "interest group" through the annual meetings of the American College of Sports Medicine. In July of 1997, Canadian investigators from the Faculty of Physical Education & Health of the University of Toronto and what was then the Defence and Civil Institute of Environmental Medicine at Downsview, ON, hosted an International Colloquium on *"Immune Responses to Inflammation and Trauma: A Physical Training Model"*. Papers from this last meeting were published as a special supplement to the Canadian Journal of Physiology and Pharmacology (125). Finally, the PsychoNeuroImmunology Research Society has developed a growing interest in issues of physical activity and exercise, stimulated in part by a special issue of Brain, Behavior & Immunity edited by Dr. Jeff Woods in September of 2005 (151).

DEVELOPMENT OF A SPECIALIST RESEARCH JOURNAL

The International Society of Exercise and Immunology launched a peer-reviewed specialist research journal (the Exercise Immunology Review) in 1995. It began under my editorship, with Dr. Northoff as my associate editor. The Society decided to publish a single volume each year, with an initial focus upon invited in-depth reviews by emerging leaders in the discipline. Because of the specialized nature of the topic, the total circulation was limited (less than 300 copies), but perhaps because of the novelty of the topic, the distinguished nature of the contributors and a concentration upon in-depth reviews, the "Impact Factor" for the journal rapidly topped figures for established sports science journals. In the year 2000, the Impact values for the four top journals in Exercise and Sports Science were the Exercise Immunology Review (2.9), the American Journal of Sports Medicine (2.3), Medicine & Science in Sports & Exercise (2.1) and the Journal of Applied Physiology (2.1). By 2005, the Impact Factor for the Exercise Immunology Review stood at 3.47, and by 2007 it had reached 4.44. The alternative Eigen Factor measure of the journal's influence showed a similar picture of high acceptance (Figure 2).

A commercial publisher (Human Kinetics) produced the first five issues, but sales did not reach their expectations, and Dr. Northoff thus arranged very efficiently for subsequent issues to be published under the aegis of the University of Tübingen and the Exercise Immunology Work Group of the German Society of Sports

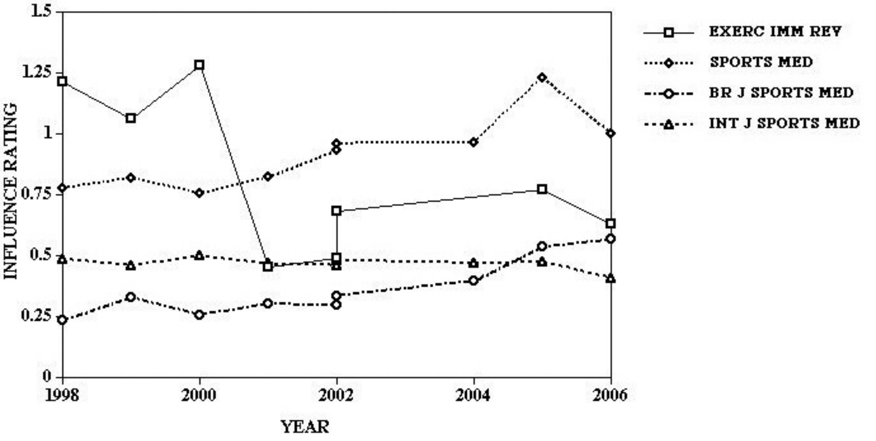


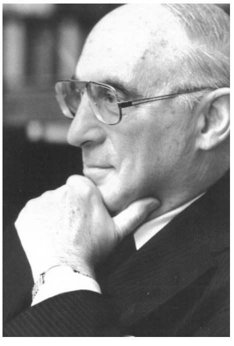
Figure 2. Eigen Factor measures of influence rating for the Exercise Immunology Review compared with three other journals in Sports Medicine and Exercise Science over the period 1998 to 2006.

Medicine and Prevention. In 1998, Dr. Northoff became the lead editor, a task he has now fulfilled very ably for some 12 years. Associate editors became Dr.A. Berg of Freiberg and myself. Dr. Mike Gleeson replaced me as second associate editor, beginning in 2001. The first two issues prepared under Dr. Northoff's guidance focused on a specific theme- nutrients modifying immune responses, and cellular damage, respectively. However, it proved challenging to coordinate the writing of five or more reviews on related topics, and subsequent issues have covered a diverse range of topics. A second innovation proposed by Dr. Northoff was to allow the inclusion, at the discretion of the author and the editor, of recent original research relevant to a given review.

The current mission statement of the Exercise Immunology Review (2008) provides both a good snap-shot of the journal and a concise definition of the field of Exercise Immunology:
"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 modification 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."

KEY PERSONALITIES IN THE EVOLTION OF EXERCISE IMMUNOLOGY

Space will not permit detailed sketches of all the many scientists who have helped to bring exercise immunology to its current stage of development. We here focus on just a few names who were among the pioneers and/or contributed specifically to development of the International Society and its journal. Most of these individuals have had a broad range of career interests, but we focus here on their contributions to exercise immunology.



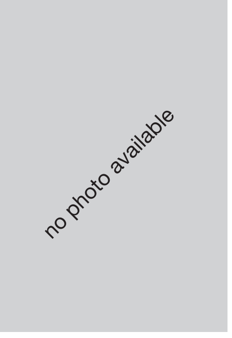
Dr. Ernst Jokl. Dr. Ernst Jokl was born in Germany, and later emigrated to South Africa, before finding his niche as Sports Physician at the University of Kentucky in Lexington, KY. A long interest in sports medicine saw him become one of the seven "founding fathers" of the American College of Sports Medicine. As physician to the University teams, he became acutely aware of the problems caused by upper respiratory infections in high performance sports, and he was an early clinical protagonist of the view that exercise modified immune function (59).

Dr. Heinz Liesen. Dr. Heinz Liesen founded the Institute of Sports Medicine at the University of Paderborn, in Westphalia. He has had an active interest in many aspects of sports performance, particularly soccer and golf. In the context of this article, he organized the first International Symposium on Exercise Immunology (Paderborn, 1989), and he played a vital background role in founding the International Society of Exercise Immunology. His scientific contributions to exercise immunology have received greater recognition in German-speaking countries than in North America. Notably, he and his colleagues have studied interactions between catecholamines and adhesion molecules (121), demonstrating by a study of splenectomized patients that the spleen plays little role in the sequestration of NK cells (8). He wrote an important early review of the area in collaboration with Dr. Gerd Uhlenbruck (69).

Dr. David Nieman. Dr. David Nieman has for many years been Professor of Health, Leisure and Exercise Science at Appalachian State University. He has also been one of the main driving forces behind the growth of interest in Exercise Immunology in North America, usually chairing sessions on this topic at the American College of Sports Medicine. Particular scientific contributions, often undertaken in collaboration with Dr. Sandra Nehlsen-Cannarella, have included the demonstration of a six-fold increase in self-reported upper respiratory infections following participation in a marathon run (95), exploration of the changes in blood leukocyte levels associated with endurance and other forms of exercise (96), and development of the hypothesis of a j-shaped relationship between exercise dose and susceptibility to infection (93).

Dr. Bente Pedersen. Dr. Bente Klarlund Pedersen is Professor in the Department of Infectious Diseases and the Muscle Research Centre at the University of Copenhagen. She served as president of the International Society for Exercise Immunology from 1995-1997, and is one of the leading investigators in exercise immunology in Scandinavia. Particular contributions have included the concept that the immune response to exercise provides a useful general model of immune responses to stress, and her demonstration that muscle production of a cytokine (interleukin-6) plays a major role in the regulation of metabolism during exercise (101).

Dr. Laurie Hoffman-Goetz. Dr. Laurie Hoffman-Goetz is Professor in the Department of Health Studies and Gerontology, a division of the Faculty of Applied Health Sciences at the University of Waterloo, Ontario. One of the early pioneers in exercise immunology. her primary interest has been the interaction between exercise and susceptibility to cancer. In part because the University of Waterloo is at some distance from a major teaching hospital, and in part because she has wished to study the question experimentally rather than epidemiologically, much of her research has concentrated on animal models of carcinogenesis (see below).



Dr. Laurel Mackinnon. Dr. Laurel Mackinnon is Associate Professor in the School of Human Movement Studies at the University of Queensland, Brisbane, NSW. Perhaps her most important contribution to exercise immunology has been in defining the scope of the topic by writing its first systematic textbook (72, 74). Many of her scientific investigations have been completed in association with the Australian National Institute of Sport. She has had opportunity to study the cellular and humoral responses of Australia's top national athletes over months of rigorous training, noting resulting changes in serum immunoglobulins and an increased risk of upper respiratory infections (77).

Dr. Arthur LaPerriere. Dr. Arthur LaPerriere, first President of the International Society of Exercise Immunology, has a strong interest in the field of psychomeuroimmunology. He carried out important early research on responses of the immune system to exercise in patients infected with the Human Immunodeficiency Virus (HIV).

Dr. Hinnak Northoff. Dr. Hinnak Northoff is the Medical Director of the Centre for Clinical Transfusion Medicine GmbH of the German Red Cross, serving that organization well through the crisis associated with the world-wide public perception of "tainted" blood transfusions. In addition to his clinical and administrative responsibilities, he has served the exercise immunology community faithfully through his careful editing of the Exercise Immunology Review (above). He has also found time for some innovative research in exercise immunology, particularly in connection with the concept that exercise can be considered as a model of sepsis (98).

Dr. Roy Shephard. Dr. Roy Shephard was Professor of Applied Physiology at the University of Toronto for 34 years, developing a strong graduate programme in the Exercise Sciences within the School of Physical & Health Education. A substantial number of the post-doctoral fellows and Ph.D. candidates under Dr. Shephard's supervision pursued research in exercise immunology, usually working in collaboration with Dr. Pang Shek and colleagues at the Defence and Civil Institute of Environmental Medicine (Downsview, ON). This laboratory offered facilities for examining many aspects of leukocyte responses to exercise, the expression of adhesion molecules, and molecular aspects of cytokine secretion, together with environmental chambers to explore interactions between exercise and climatic extremes (82, 114, 131, 132, 134). Two of the post-doctoral fellows (Drs. Shinkai and Suzui) have carried their interest in exercise immunology back to Japan. Other contributions have included establishment of the Exercise Immunology Review, organization and editing of the International Colloquium on Exercise as a possible model of sepsis (125), and writing of the first detailed research textbook on Exercise, Immunity and Training (132).

Other early leaders in the International Society of Exercise Immunology. Other early leaders in the Society, not mentioned elsewhere, can be gleaned from the composition of the Editorial Board of the Exercise Immunology Review: members to 2009 have included Drs. V. Brusasco (Genoa), K. de Meirleer (Brussels), M. Fabbrio (Melbourne), L. Fitzgerald (London, England), D. Keast (Perth), L. Jonsdottir (Göteborg), M. Gleeson (Loughborough, UK), R. Nagatomi (Tohoku, Japan), J. Peake (Waseda, Japan), and J. Woods (Urbana/Champaign, IL).

MAJOR DISCOVERIES IN EXERCISE IMMUNOLOGY

Space again limits the number of major discoveries that can be considered. Note is taken of the accurate identification of NK cells, the epidemiology of exercise and upper respiratory infection, the glutamine hypothesis, influence of adhesion molecules as modulators of cell counts, the concept of a cytokine cascade, the role of cytokines in metabolic regulation, and exercise as a model of sepsis and of the stress response.

Accurate identification of NK cell counts and activity. NK cells were originally identified by rosetting, a form of negative selection whereby B cells were removed by adherence to nylon wool, and T cells by panning with anti-CD3 (21). More recently, a form of positive selection became available. The NK cells were coated with biotinylated specific antibody, and passed through a column containing the biotin binding substance avidin linked to polyacrimide beads (10), sometimes using ferric oxide coating and a magnet to complete the separation (113). Cytotoxicity was traditionally assessed by the release of ^{51}Cr from myeloid tumor cells (99, 109), but faster flow cytometric methods are now available (18). These advances have allowed examination of the influence of cell counts, trafficking, prostaglandins (104) and endogenous opioids (35) on NK cell toxicity. In general, NK cell toxicity seems to mirror the corresponding cell counts, increasing during exercise, and remaining depressed for 6-24 hours following activity.

Epidemiology of exercise and upper respiratory infections. The first epidemiological studies in the late 1980s established that the risk of developing respiratory symptoms was increased 2-6 fold by participation in a marathon run (95, 106). Heavy training also increased this risk (48, 71, 96), but adverse effects from a single event were unlikely if the distance covered was less than a marathon (95, 96). Many athletes themselves perceived an optimal level of training, and if they exceeded this level, their risk of infections appeared to increase (132). These observations led David Nieman to formulate the provocative hypothesis of a j-shaped relationship between the amount of exercise performed and the risk of infection (92, 93), although critics were quick to point out the dangers of inferring the shape of a dose-response relationship by pooling studies that differed in amounts and types of exercise, populations and rigour of analysis (129).

Most of the early studies relied on symptom reporting, and it was pointed out that further progress depended on distinguishing infections from inflammatory or allergic responses that also caused respiratory symptoms during exercise (111). Application of modern quality criteria showed that to the year 2000 only 7 of 28 epidemiological studies met current criteria of excellence, and not all of Bradford Hill's markers of a causal association were satisfied (129). Nevertheless, the likelihood of reporting an increased risk of infection following exhausting exercise seemed to be just as great in weak as in well-designed investigations. Further support for an adverse effect of prolonged and vigorous physical activity has come from animal studies showing that stressful exercise can increase susceptibility to both respiratory infections and influenza virus in mice models (25, 61, 84), in part through a stress-hormone modulated reduction in activity of alveolar macrophages (62).

Circulating concentrations of NK cells were early shown to increase during exercise (28), but multiple sampling showed a substantial decrease following vigorous exercise (134). Some investigators argued that this offered an immunological explanation of the susceptibility to upper respiratory infections. In most studies, counts have been depressed for only 6-24 hours, and this seems a rather short period to cause any substantial increase in risk; one study did report a 7 day decrease in NK cell count (124), but an explanation of this aberrant finding is still awaited. An alternative hypothesis is that exhausting exercise depresses immunoglobulin secretion. As early as 1982, low levels of nasal IgA were found in cross country skiers, with a further depression noted during intensive training (140). Similar findings were observed in swimmers (138). Depression of serum immunoglobulins was also demonstrated in Russian athletes as early as 1983 (107), and subsequently confirmed by studies in Germany (138) and at the Australian Institute of Sport (75). Currently, this seems the most convincing explanation of any exercise-induced increase in vulnerability to upper respiratory infections.

The preventive administration of polyvalent immunoglobulins was advocated by Weiss (143, 144), and uncontrolled studies on the German National Boxing team prior to the 1992 Olympic Games claimed benefit from such therapy. Kohut and associates have underlined the role of exercise in enhancing the effectiveness of influenza and other vaccines (60, 63). Other reports have suggested that the oral administration of thymomodulin (42) and the nasal instillation of IgA (52) can protect against upper respiratory infections. However, one Swedish report found that although nasal instillation of IgA increased salivary IgA concentrations, it did not lead to any decrease in respiratory symptoms (70).

The glutamine hypothesis. Newsholme aroused early interest with his hypothesis that the exercise-induced suppression of immune function reflected a decrease in plasma glutamine (88). Glutamine is certainly needed for leukocyte metabolism. Studies from the Oxford laboratory found a 20% fall of plasma glutamine following a marathon run, and a controlled trial showed a reduced risk of immuno-suppression in those who consumed two drinks each containing 5 g of glutamine, (19 vs. 51% reported infections in those given a maltodextrin placebo) (17). A lack of glutamine can undoubtedly impair immune function in malnourished populations, but glutamine lack seems less plausible in athletes who are eating a high protein diet. Subsequent research has generally failed to support either a substantial drop in plasma glutamine levels with prolonged exercise or benefit from glutamine supplements (76, 116),

Adhesion molecules and trafficking as modulators of circulating leukocyte counts. Exercise-induced increases in neutrophil count (2, 27) were noted even before the introduction of automated counters. It was quickly recognized that this reflected a movement of cells from reservoir sites, particularly in the lungs and bone marrow, in response to changes in cortisol and complement concentrations (24, 117). As understanding of the immune system developed, it became apparent that many of the changes in cell count observed during and following exercise were due to a modulation of cell adherence and the demargination of leukocytes (142). Examination of differential responses to exercise began with Rabin and associates (112), spurring an extensive study of the hormonal modulation of adhesion molecules (131). Thus, FACScan studies of surface antigens showed that the well-recognized exercise-induced increases in monocyte counts (2, 3, 27, 31) represented a trafficking of cells from tissue depots, with circulating monocyte numbers subsequently declining as the cells migrated to sites of tissue injury (41). Those with access to sheep models have been able to explore the distribution of lymphocytes between the blood, the thymus, bone marrow and lymph ducts (47). Trafficking is an important area of study, since samples taken from the blood stream represent only a very small (and often a biased) fraction of the total leukicyte population.

The cytokine cascade and metabolic regulation. Cytokines act in minute concentrations, and the cellular response often depends on the presence of other cytokines or cell stimulants. Other complicating factors are a short half-life, strong binding to receptors, a potential for modification of receptor sites (68, 114), and possible neutralization by circulating inhibitors. Thus, detection of exercise-induced changes has been challenging, and the *in vitro* production of cytokines by mitogen or phytohemagglutinin stimulated cells has not always mirrored the changes observed *in vivo*. Dr. Bente Pedersen made the important observation that *in vivo*, cytokines were secreted as a cascade, one cytokine eliciting secretion of the next element in the chain (103). Factors identified as modifying the pattern of cytokine production have included the relative numbers of T_{h1} and T_{h2} cells, the body temperature and any prostaglandins secreted in response to tissue injury.

Dr. Nehlsen-Cannarella and colleagues (85) suggested that one possible mediator of impaired immune function might be a depletion of muscle and liver glycogen reserves during prolonged exercise. Such depletion could conceivably lead to a depletion of plasma glutamine, as originally postulated by Eric Newsholme. The effects of a 6% carbohydrate supplement were thus tested during 2.5 hours of high intensity treadmill running. Immediately and 1.5 hours post-exercise, larger increases of IL-6 and IL-1 receptor antagonist were observed in the placebo controls, suggesting that the carbohydrate attenuated the release of cytokines in the inflammatory cascade. More recently, Bente Pedersen and her associates have confirmed the involvement of certain cytokines in metabolic regulation; during exercise, the IL-6 gene is activated in muscle, and the corresponding cytokine is released into the blood as glycogen stores became depleted. TNF-α also seems to be involved, with a stimulation of lipolysis and cortisol production (102, 108).

Exercise as a model of sepsis. An exercise-related increase of acute phase reactants was an early finding of German investigators (45); this pointed to a temporary enhancement of lytic activity, and spurred interest in the associated production of the cytokine IL-6, with development of what seemed an aseptic inflammatory response (98). Further interest in exercise, particularly eccentric exercise, as a potential model of septic responses to inflammation and trauma was shown in Belgium (Camus and colleagues) (16). It was argued that such a reaction, probably initiated by an increased macrophage production of IL-1, could temporarily depress the immune response to infections. Classical T_{h1} cells have a pro-inflammatory action, but advances in the identification of surface markers revealed a second population (T_{h2}cells) with an anti-inflammatory action; it was thus argued that the relative proportion of the two cell types influenced the likelihood of seeing a septic-type reaction. A parallel to clinical sepsis was seen in the penetration of the gut endothelium by gram-negative micro-organisms during exhausting exercise (13), with a resulting increase in plasma concentrations of lipopolysaccharides. Interest peaked in 1997, with the Toronto colloquium devoted to this theme (55). Although some similarities between strenuous exercise and sepsis were identified, the colloquium also underlined important differences between exercise and clinical sepsis (125).

One problem hampering such research has been that the exercise model cannot ethically be pushed to the point of inducing a septic reaction, at least in humans. Any inflammatory response thus remains sub-clinical and well ordered. Analysis has also proven difficult, many of the key reactants having low concentrations, short half-lives, and neutralizing factors in the circulation. Possibly, molecular biologists may help to resolve these problems by the intracellular assay of cytokine production.

Exercise as a model of stress. There are many points of similarity between strenuous exercise (particularly athletic competition) and responses to other environmental stressors, including modulations of hypothalamic activity and the stress hormones epinephrine and cortisol (19, 101). Exercise has thus been proposed as a model of the general immune response to stress (101). Exercise interacts with any other co-existing stressors, so that an otherwise acceptable intensity of effort becomes excessive if it is undertaken in an adverse environment. Defence laboratories, in particular, have been concerned about interactions between exercise and the immune response at high altitude, during deep diving, and under very hot and very cold conditions (132).

FUTURE CHALLENGES TO EXERCISE IMMUNOLOGY

Future challenges for the exercise immunologist seem likely to include a need to change the transmission of information through scientific meetings and the journal, demands for further study of exercise in relation to aging and cancer, evaluation of the potential to prevent upper respiratory infections by regular moderate exercise, and applications of immune technology in the prevention of athletic doping.

Organizational aspects. The future is likely to present a number of organizational challenges to Exercise Immunology. Rising air fares will probably preclude the attendance of young investigators at future international meetings, and there will thus be a need to develop both national and regional chapters, and other techniques for the on-line discussion of emerging issues. The journal will need to consider producing more than a single issue in any one year, and will also wish to move to instantaneous on-line publication once a paper has received editorial approval. The ever-increasing complexity of investigative techniques may finally call for a certification of both laboratories and investigators qualified to carry out the necessary analyses.

Aging. Current problems of an aging population will undoubtedly encourage a building upon existing knowledge of interactions between aging, exercise and immune function, either in animals or in elderly people (36, 133). Investigators will need to allow for many concomitant factors that can influence immune function in the elderly, including poor nutrition, a build up of chronic infection, a depressed mood state and degenerative changes in various body organs. Given that aging is marked by progressive DNA damage and apoptosis (20), there is likely to be increasing interest in the DNA damage and release of reactive species caused by strenuous exercise (87, 118), and investigators will want to explore the relationship of such responses to the deteriorations of immune function associated with aging and carcinogenesis.

One intriguing recent discovery has been the finding that aging is associated with increased levels of inflammation, and increased concentrations of the pro-inflammatory cytokines IL-1beta and TNF-α; these contribute to hippocampal neurodegeneration in transgenic mice models of Alzheimer's Disease. A promising avenue for future enquiry is thus suggested by the action of regular wheel exercise in reducing the level of these cytokines, and delaying one component of the brain aging process (89).

Cancer. It is now generally accepted that exercise reduces the risk of several types of neoplasm (130). Many possible mechanisms have been discussed, possibly including increased activity of macrophages, NK cells and related cytokines. Certainly, animal experiments suggest that an exercise-induced stimulation of NK cell function decreases the risk of metastasis (78). Given the prevalence of cancer, research into mechanisms underlying the benefits of exercise are likely to continue into the next decade, although if the immune system is involved, it will be necessary to explain why exercise only protects against some types of cancer.

To date, the progress of human studies has been hampered by the slow nature of the carcinogenic process, and the absence of any unifying theory. Research seems likely to continue on small mammals, where tumor cells can be implanted (4) and tumors induced by injection of potent carcinogens (139, 141) or massive numbers of tumor cells (78). However, there will be continuing difficulties in comparing such massive challenges to the gradual process of carcinogenesis seen in most human tumors. It will also be necessary to consider such issues as the stress of enforced exercise, and differences of lifespan and body size between humans and animal models.

Acute upper respiratory infections. There is growing evidence that an appropriately chosen moderate dose of exercise can enhance both cellular and humoral resistance to upper respiratory infections (94, 96, 137). Given the lack of specific remedies for the common cold, it thus appears worthwhile to explore these responses carefully, and to determine how far effects can be maximized in the contexts of public health and prevention of the common cold.

Athletic doping. Finally, exercise immunologists are likely to make an increasing contribution to the unmasking of athletic doping. One troublesome form of cheating has been homologous red blood cell transfusion, and a flow cytometric technique of detecting such transfusions has now been developed, based on the presence of foreign antigens (86). There are also increasing fears that athletes may abuse gene transfer technology to gain competitive advantage. However, the development of a polymerase chain reaction technique now allows the presence of transgenic DNA to be detected in whole blood (9).

REFERENCES

1. Aguirre G, Frontera WR, Colon LR, Amy E, Micheo W, Correa JJ, and Camunas JF. Profile of health services utilization during the XVII Central American and

Caribbean Sport Games: Delegation of Puerto Rico, Ponce 1993. Puerto Rico Health Sci J 13: 273-278, 1994.
2. Ahlborg B and Ahlborg G. Exercise leukocytosis with and withot beta-adrenergic blockade. Acta Med Scand 187: 241-246, 1970.
3. Anderssen KL. Leukocyte response to brief, severe exercise. J Appl Physiol 7: 671-674, 1955.
4. Baracos VE. Exercise inhibits progressive growth of the Morris hepatoma 7777 in male and female rats. Can J Physiol Pharmacol 67: 864-870, 1989.
5. Barker MH, Capps RB, and Allen FW. Acute infectious hepatitis in the Mediterranean Theater. JAMA 128: 997, 1945.
6. Barnard DF, Barnard SA, Carter AB, Machin SJ, Patterson KG, and Yardumian A. Detection of important abnormalities of the differential count using the Coulter STKR blood counter. J Clin Pathol 42: 772-776, 1989.
7. Barnes CA, Forster MJ, Fleshner M, Ahanotu EN, Laudenslager ML, Mazzeo RS, Maier SF, and Lal H. Exercise does not modify spatial memory, brain autoimmunity, or antibody response in aged F-344 rats. Neurobiol Aging 12: 47-53, 1991.
8. Baum M, Geitner T, and Liesen H. The role of the spleen in the leucocytosis of exercise: consequences for physiology and pathophysiology. Int J Sports Med 17: 604-607, 1996.
9. Beiter T, Zimmerman M, Fragasso A, Armeanu S, KLauer 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. Ex Immunol Rev 14: 71-85, 2008.
10. Berenson RJ, Bensinger WI, and Kalamaaz D. Positive selection of viable cell populations using avidin-biotin immunoabsorption. J Immunol Methods 91: 11-19, 1986.
11. Bieger WP, Michel G, Barwich D, Biehl K, and Wirth A. Diminished insulin receptors on monocytes and erythrocytes in hypertriglyceridemia. Metab Clin Exp 33: 982-987, 1984.
12. Bieger WP, Weiss M, Michel G, and Weicker H. Exercise-induced monocytosis and modulation of monocyte function. Int J Sports Med 1: 30-36, 1980.
13. Bosenberg AT, Brock-Utne JG, Gaffin SL, Well MTB, and Blake TW. Strenuous exercise causes systemic endotoxemia. J Appl Physiol 65: 106-108, 1988.
14. 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.
15. Burch GF. Viral diseases of the heart. Acta Cardiol 34: 5-9, 1979.
16. Camus G, Deby-Dupont G, DuChateau J, Deby A, Pincemail J, and Lamy M. Are similar inflammatory factors involved in strenuous exercise and sepsis? Intensive Care Med 24: 602-610, 1994.
17. Castell LM and Newsholme EA. Glutamine and the effects of exhaustive exercise upon the immune response. Can J Physiol Pharm 76: 524-532, 1998.
18. Cavarec L, Quillet-Mary A, Fradelizi D, and Conjeaud H. An improved double fluorescence flow cytometry method for the quantification of killer cell/target cell conjugate formation. J Immunol Meth 130: 251-261, 1990.
19. Clow A and Hucklebridge F. The impact of psychological stress on immune function in the athletic population. Ex Immunol Rev 7: 5-17, 2001.
20. Concordet JP and Ferry A. Physiological programmed cell death in thymocytes is induced by physical stress (exercise). Am J Physiol 265: C626-C629, 1993.

21. Cosentino LM and Cathcart MK. A multi-step isolation scheme for obtaining human natural killer cells. J Immunol Meth 103: 195-204, 1987.
22. Coulter WH. Means for counting particles suspended in a fluid, U.S. Patent Office 1953.
23. Daniels WL, Sharp DS, Wright JE, Vogel JA, Friman G, Beisel WR, and Knapik JJ. Effects of virus infection on physical performance in man. Mil Med 150: 8-14, 1985.
24. Davis JM and Gallin JI. The neutrophil. In: Cellular functions in Immunity and Inflammation, edited by Oppenheim JJ, Rosentreich DL and Potter M. New York, NY.: Elsevier-North Holland, 1981, p. 77.
25. Davis JM, Kohut ML, Colbert LH, Jackson DA, Ghaffar A, and Mayer EP. Exercise, alveolar macrophage function, and susceptibility to respiratory infection. J Appl Physiol 83: 1461-1466, 1997.
26. Davis MA, Kendrick ZV, and Paolone AM. Measurement of antibody response to influenza virus vaccine in aerobically trained mice. Med Sci Sports Exerc 18: S9 (abstract), 1986.
27. De Lanne R, Barnes JR, and Brouha L. Hematological changes during muscular activity and recovery. J Appl Physiol 15: 31-36, 1960.
28. Deuster PA, Curiale AM, Cowan ML, and Finkelman FD. Exercise-induced changes in populations of peripheral blood mononulear cells. Med Sci Sports Exerc: 20: 276-280, 1988.
29. Dittrich W and Gohde W. Impulsfluoremetrie bei Einzelzellen in Suspensionen [Impulse fluorometry of single cells in suspension]. Z Naturforsch - Teil B 24: 360-361, 1969.
30. Douglas JH. The effects of physical training in the immunological response to diphtheria toxid immunization. J Sports Med 14: 48-54, 1974.
31. Dùner H and Pernow B. Histamine and leukocytes in blood during muscular work in man. Scand J Lab Clin Invest 10: 394-396, 1958.
32. Edlund A. The effect of defined physical exercise in the early convalescence of viral hepatitis. Scand J Infect Dis 3: 189-196, 1971.
33. Eskola J, Ruuskanen O, Soppi E, Viljanen MK, Järvinen M, Toivonen H, and Kouvailainen K. Effect of sport stress on lymphocyte transformation and antibody formation. Clin Exp Immunol 32: 339-345, 1978.
34. Fehr HG, Lötzerich H, and Michna H. The influence of physical exercise on peritoneal macrophage functions: Phagocytosis and histochemical studies. Int J Sports Med 9: 77-81, 1988.
35. Fiatarone MA, Morley JE, Bloom ET, Benton D, Makinodan T, and Solomon GF. Endogenous opioids and the exercise-induced augmentation of natural killer cell activity. J Lab Clin Med 112: 544-552, 1988.
36. 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.
37. Fox SM and Haskell WL. Physical activity and the prevention of coronary heart disease. Bull NY Acad Med 44: 950-965, 1968.
38. Friman G, Wright J, Ilbäck N-G, Beisel WR, White JD, Sharp DS, Stephen EL, Daniels WL, and Vogel JA. Does fever or myalgia indicate reduced physical performance capacity in viral infections? Acta Med Scand 217: 353-361, 1985.
39. Fulwyler MJ. Electronic separation of biological cells by volume. Science 150: 910-911, 1965.

40. Gabriel H and Kindermann W. Flow cytometry. Principles and applications in exercise immunology. Sports Med 20: 302-320, 1995.
41. Gabriel H, Urhausen A, Brechtel L, Muller HJ, and Kindermann W. Alterations of regular and mature monocytes are distinct, and dependent on intensity and duration of exercise. Eur J Appl Physiol 69: 179-181, 1994.
42. Garagiola U, Buzzetti M, Cardella E, Confalonieri F, Giani E, Polini V, Ferrante P, Marcuso R, Montanari M, Grossi E, and Pecori A. Immunological patterns during regular intensive training in athletes: Quantification and evaluation of a preventive pharmacological approach. J Int Med Res 23: 85-95, 1995.
43. Good RA and Fernandes G. Enhancement of immunological and resistance to tumor growth in Balb C mice by exercise. Fed Proc 40: 1040, 1981.
44. Grimby G. Exercise in man during pyrogen-induced fever. Scand J Lab Clin Invest 14 (Suppl. 67), 1962.
45. Haralambie G. Serum glycoproteins and physical exercise. Clin Chem Acta 26: 287-291, 1969.
46. Hargreaves ER. Poliomyelitis: Effect of exertion during the paralytic stage. BMJ 2: 1021-1022, 1948.
47. Hay JB and Andrade WH. Lymphocyte recirculation, exercise and immune responses. Can J Physiol Pharm 76: 490-496, 1998.
48. Heath GW, Ford ES, Craven TE, Macera CA, Jackson KL, and Pate RR. Exercise and the incidence of upper respiratory tract infections. Med Sci Sports Exerc 23: 152-157, 1991.
49. Hedfors E, Biberfeld P, and Wahren J. Mobilization to the blood of human non-T and K lymphocytes during physical exercise. J Clin Lab Invest 1: 159-162, 1978.
50. Hedfors E, Holm G, Ivansen M, and Wahren J. Physiological variation of blood lymphocyte reactivity: T-cell subsets, immunoglobulin production, and mixed-lymphocyte reactivity. Clin Immunol Immunopathol 27: 9-14, 1983.
51. Heir T, Aanestad G, Carlsen KH, and Larsen S. Respiratory tract infection and bronchial responsiveness in elite athletes and sedentary control subjects. Scand J Med Sci Sports 5: 94-99, 1995.
52. Hemmingsson P and Hammarstrom L. Nasal administration of immunoglobulin as effective prophylaxis against infections in elite cross-country skiers. Scand J Infect Dis 25: 783-785, 1993.
53. Hoffman-Goetz L. Exercise and Immune Function. Boca Raton FL.: CRC Press, 1996.
54. Hoffman-Goetz L, Keir R, Thorne R, Houston ME, and Young C. Chronic exercise stress in mice depresses splenic T lymphocyte mitogenesis in vitro. Clin Exp Immunol 66: 551-557, 1986.
55. Hoffman-Goetz L, Kubes P, and Shephard RJ. Immune response to inflammation and trauma: A physical training model. Can J Physiol Pharm 76: 469-597, 1998.
56. Hoffman-Goetz L and Macneil B. Exercise, natural immunity, and cancer; causation, correlation or conundrum? In: Exercise and Disease, edited by Watson RR and Eisinger M. Boca Raton, FL.: CRC Press, 1992, p. 37-62.
57. Horstmann DM. Acute poliomyelitis. Relation of physical activity to the time of onset and to the course of the disease. JAMA 142: 236-239, 1950.
58. Hulett HR, Bonner WA, Sweet RG, and Herzenberg LA. Development and application of a rapid cell counter. Clin Chem 19: 813-816, 1973.
59. Jokl E. The immunological status of athletes. J Sports Med Phys Fitness 14: 165-167, 1974.

60. Kohut ML, Arnston BA, Lee W, Rozenboom M, Yoon KJ, Cunnick JE, and McElhaney JE. Moderate exercise improves antibody response to influenza immunization in older adults. Vaccine 22: 2298-2306, 2004.
61. Kohut ML, Boehm GW, and Moynihan JA. Prolonged exercise suppresses antigen-specific cytokine response to upper respiratory infection. J Appl Physiol 90: 678-684, 2001.
62. Kohut ML, Davis JM, Jackson DA, Colbert LH, Strasner A, Essig DA, and Pate RR. The role of stress hormones in exercise-induced suppression of alveolar macrophage antiviral function. J Neuroimmunol 81: 193-200, 1998.
63. Kohut ML, Lee W, Martin A, Arnston BA, Russell DW, Ekkekakis P, Yoon KJ, Bishop A, and Cunnick JE. The exercise-induced enhancement of influenza immunity is mediated in part by improvements in psychosocial factors in older adults. Brain Behav Immun 19: 357-366, 2005.
64. Koivisto VA, Soman VR, and Felig P. Effects of exercise on insulin-binding to monocytes in obesity. Metabolism 29: 168-171, 1980.
65. Kujala UM, Heinonen OJ, Lehto M, Jarvinen M, and Bergfeld JA. Equipment, drugs and problems of the competition and team physician. Sports Med 6: 197-209, 1988.
66. LaPerriere A, Fletcher MA, Antoni MH, Klimas NG, Ironson G, and Schneiderman N. Aerobic exercise training in AIDS risk group. Int. J Sports Med 12: S53-S57, 1991.
67. Lewicki R, Tchorzewski H, Denys A, Kowalska M, and Golinska A. Effect of physical exercise on some parameters of immunity in conditioned sportsmen. Int J Sports Med 8: 309-314, 1987.
68. Lewicki R, Tchorzewski H, Majewska E, Nozak Z, and Baj Z. Effect of maximal physical exercise on T lymphocyte subpopulations and on interleukin 1 (IL-1) and interleukin 2 (IL-2) production in vitro. Int J Sports Med 9: 114-117, 1988.
69. Liesen H and Uhlenbruck G. Sports Immunology. Sport Sci Rev 1 (1): 94-116, 1992.
70. Lindberg K and Berglund B. Effect of treatment with nasal IgA on the incidence of infectious disease in world-class canoeists. Int J Sports Med 17: 235-238, 1996.
71. Linde F. Running and upper respiratory infections. Scand J Sports Sci 9: 21-23, 1987.
72. Mackinnon LT. Advances in Exercise Immunology. Champaign, IL.: Human Kinetics, 1999.
73. Mackinnon LT. Changes in some cellular immune parameters following exercise training. Med Sci Sports Exerc 18: 596-597, 1986.
74. Mackinnon LT. Exercise and Immunology. Champaign, IL.: Human Kinetics, 1992.
75. Mackinnon LT. Immunoglobulin, antibody and exercise. Exerc Immunol Rev 2: 1-34, 1996.
76. Mackinnon LT and Hooper SL. Plasma glutamine and upper respiratory tract infection during intensified training in swimmers. Med Sci Sports Exerc 28: 285-290, 1996.
77. Mackinnon LT, Hooper SL, Jones S, Gordon RD, and Bachman AW. Humoral, immunological and hematological responses to intensified training in swimmers. Med Sci Sports Exerc 29: 1637-1645, 1997.
78. 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.
79. Maurer HR. Potential pitfalls of 3H thymidine techniques to measure cell proliferation. Cell Tiss Kinet 14: 111-120, 1981.

80. Melamed MR, Mullaney PF, and Shapiro HM. An Historical Review of the Development of Flow Cytometers and Sorters. In: Flow cytometry and sorting (2nd ed.), edited by Melamed MR, Lindmo T and Mendelsohn ML. Hoboken, NJ.: Wiley, 1990, p. 1-832.
81. Moldovan A. Photoelectric technique for the counting of microscopical cells. Science 80: 188-189, 1934.
82. Moldoveanu AI, Shephard RJ, and Shek PN. Exercise elevates plasma levels but not gene expression of IL-1b, IL-6, and TNF-α in blood mononuclear cells. J Appl Physiol 89: 1499-1504, 1995.
83. Morris JN, Adams C, Shave SPN, Sirey C, Epstein L, and Sheehan DJ. Vigorous exercise in leisure time and the incidence of coronary heart disease. Lancet 1: 333-339, 1973.
84. Murphy EA, Davis JM, Carmichael MD, Gange JD, and Mayer EP. Exercise stress increases susceptibility to influenza infection. Brain Behav Immun 22: 1152-1155, 2008.
85. Nehlsen-Cannarella SL, Fagoaga OR, Nieman DC, Henson DA, Butterworth DE, Schmitt RL, Bailey EM, Warren BL, and Davis JM. Carbohydrate and the cytokine response to 2.5 hours of running. J Appl Physiol 82: 1662-1667, 1997.
86. Nelson M, Popp H, Sharpe K, and Ashenden M. Proof of homologous blood transfusion through quantification of blood group antigens. Haematol 88: 1284-1295, 2003.
87. Neubauer O, Reichold S, Nerseyan A, König D, and Wagner K-H. Exercise-induced DNA damage: Is there a relationship with inflammatory responses? Exerc Immunol Rev 14: 51-72, 2008.
88. Newsholme EA, Crabtree B, and Ardawi M. Glutamine metabolism in lymphocytes. Its biochemical, physiological and clinical importance. Quart J Exp Physiol 70: 473-489, 1985.
89. Nichol KE, Poon WW, Parachikova AI, Cribbs 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 Neuroinflamm Apr 9; 5: 13, 2008.
90. Nieman D. The Adventist healthstyle. Hagerstown, MD: Review and Herald Publishing Association, 1992.
91. Nieman D and Pedersen BK. Nutrition and Exercise Immunology. Boca Raton, FL.: CRC Press, 2000.
92. Nieman DC. Exercise immunology: Practical applications. Int J Sports Med 18 (Suppl. 1): S91-S100, 1997.
93. Nieman DC. Exercise, infection, and immunity. Int J Sports Med (Suppl. 3): S131-S141, 1994.
94. Nieman DC, Henson DA, Gusewitch G, Warren BJ, Dotson RC, Butterworth DE, and Nehlsen-Cannarella SL. Physical activity and immune function in elderly women. Med Sci Sports Exerc 25: 823-831, 1993.
95. Nieman DC, Johanssen LM, and Lee JW. Infectious episodes in runners before and after a road race. J Sports Med Phys Fitness 29: 289-296, 1989.
96. 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.
97. Nieman DC, Nehlsen-Cannarella SL, Markoff PA, Balk-Lamberton AJ, Yang H, Chritton DBW, 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.

98. Northoff H and Berg A. Immunological mediators as parameters of the reaction to strenuous exercise. Int J Sports Med 12 (Suppl. 1): S9-S15, 1991.
99. Ortaldo JR. Cytotoxicity by natural killer cells: analysis of large granular lymphocytes. Meth Enzymol 132: 445-457, 1986.
100. Paffenbarger RS. Physical activity and fatal heart attack: Protection or selection? In: Exercise in Cardiovascular Health and Disease, edited by Amsterdam EA, Wilmore JH and de Maria AN. New York, NY.: Yorke Books, 1977, p. 35-49.
101. Pedersen BK and Hoffman-Goetz L. Exercise and the Immune System- regulation, integration and adaptation. Physiol Rev 80: 1055-1081, 2000.
102. Pedersen BK, Steensberg A, Fischer C, Keller C, Keller P, Plomgaard P, Wolsk-Petersen E, and Febbraio M. The metabolic role of IL-6 produced during exercise: is IL-6 an exercise factor? Proc Nutr Soc 63: 263-267, 2004.
103. Pedersen BK and Toft AD. Effects of exercise on lymphocytes and cytokines. Br J Sports Med 34: 246-251, 2000.
104. Pedersen BK, Tvede N, K. K, Christensen LDH, F.R., Galbo H, Kharazami A, and Halkjaer-Kristensen J. Indomethacin in vitro and in vivo abolishes post exercise suppression of natural killer cell activity in peripheral blood. Int J Sports Med 11: 127-131, 1990.
105. Pedersen BK, Tvede NR, Hansen FR, Andersen V, Bendix T, Bendtzen K, Galbo H, Haahr PH, Klarlund K, Sylvest J, Thomsen BS, and Halkjaer-Kristensen J. Modulation of natural killer cell activity in peripheral blood by physical exercise. Scand J Immunol 27: 673-678, 1988.
106. Peters EM and Bateman ED. Ultramarathon running and upper respiratory tract infections. An epidemiological survey. S Afr Med J 64: 582-584, 1983.
107. Petrova IV, Kuzmin SN, Kurshakova TS, Suzdalnitsky RS, and Pershin BB. Fagotsitarnaia aktivnost' neitrofilov i gumoral'nye faktory obshchego i mestnogo immuniteta pri intensivnykh fizicheskikh nagruzkakh. [Neutrophil phagocytic acticity and the humoral factors of general and local immunity under intensive physical loading]. Z Mikrobiol, Epidemiol Immunobiol 12: 53-57, 1983.
108. Plomgaard P, Fischer CP, Ibfelt T, Pedersen BK, and van Hall G. Tumor necrosis factor-alpha modulates human in vivo lipolysis. J Clin Endocrinol Metab 93: 543-549, 2008.
109. Pross HF, Baines MG, Rubin P, Shragge P, and Patterson MS. Spontaneous human lymphocyte-mediated cytotoxicity against tumor target cells IX. The quantitation of natural killer cell activity. J Clin Immunol 1: 51-63, 1981.
110. Pyne DB, Baker PA, Frickere WA, McDonald WA, Telford RD, and Weidemann MJ. Effects of an intensive 12-week training program by elite swimmers on neutrophil oxidative activity. Med Sci Sports Exerc 27: 536-542, 1995.
111. Pyne DB and Gleeson M. Effects of intensive exercise training on immunity in athletes. Int J Sports Med 19 (Suppl. 3): S191-S194., 1998.
112. Rabin BS, Moyna NM, Acker GR, and Robertson RJ. Exercised induced alterations of cytokines by Th1 and Th2 lymphocytes. Med Sci Sports Exerc 27: S177, 1995.
113. Rasmussen AM, Smeland EB, Erikstein BK, Caignault L, and Funderud S. A new method for detachment of dynabeads from positively selected B lymphocytes. J Immunol Meth 146: 195-202, 1992.

114. Rhind SG, Shek PN, and Shephard RJ. The impact of exercise on cytokines and receptor expression. Exerc Immunol Rev 1: 97-148, 1995.
115. Roberts JA. Viral illness and sports performance. Sports Med 3: 296-303, 1986.
116. Rohde T, Ullum H, Rasmussen JP, Kristensen JH, Newsholme E, and Pedersen BK. Effects of glutamine on the immune system: influence of muscular exercise and HIV infection. J Appl Physiol 79: 146-150, 1995.
117. Rother K. Leukocyte mobilizing factor: A new biological activity derived from the third component of complement. Eur J Immunol 2: 550-558, 1972.
118. Russack V. Image cytometry: current applications and future trends. Clin Lab Sci 31: 1-34, 1994.
119. Russell RW. Paralytic poliomyelitis. The early symptoms and the effect of physical activity on the course of the disease. BMJ 1: 465-471, 1949.
120. Russell RW. Poliomyelitis. The pre-paralytic stage, and the effects of physical activity on the severity of the paralysis. BMJ 2: 1023-1028, 1947.
121. Schaller K, Mechau D, Scharmann HG, Weiss M, Baum M, and Liesen H. Increased training load and the beta-adrenergic-receptor system on human lymphocytes. J Appl Physiol 87: 317-324, 1999.
122. Schlenzig C, Jäger H, and Rieder H. Einfluss von Sporttherapie auf die zelluläre immunabwehr und die Psyche HIV-infizierter Männer [Influence of sport therapy upon cellular immune function and the pysche of HIV infected men]. Dtsche Z Sportmed 41: 156-160, 1990.
123. Schubert C and Schüssler G. (Psychoneuroimmology: An update). Z, Psychom Med Psychother 55: 1-2, 2009.
124. 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 Santé des Armées, 1994, p. 121-137.
125. Shek PN and Shephard RJ. Physical exercise as a human model of limited inflammatory response. Can J Physiol Pharm 76: 589-597, 1998.
126. Shephard RJ. History of Exercise Immunology. In: History of Exercise Physiology, edited by Tipton CM. Champaign, IL.: Human Kinetics, 2010.
127. Shephard RJ. Immune changes induced by exercise in an adverse environment. Can J Physiol Pharm 76: 539-546, 1998.
128. Shephard RJ. Medical surveillance of endurance sport. In: Endurance in Sport, edited by Shephard RJ and Åstrand P-O. Oxford, U.K.: Blackwell Scientific, 2000, p. 653-666.
129. Shephard RJ. Special feature for the Olympics: effects of exercise on the immune system: overview of the epidemiology of exercise immunology. Immunol Cell Biol 78: 485-495, 2000.
130. Shephard RJ and Futcher R. Physical activity and cancer: how may protection be maximized? Crit Rev Oncogen 8: 219-272, 1997.
131. Shephard RJ, Gannon G, Hay JB, and Shek PN. Adhesion molecule expression in acute and chronic exercise. Crit Rev Immunol 20: 245-266, 2000.
132. Shephard RJ. Physical activity, training and the immune response. Carmel, IN.: Cooper Publishing Group, 1997.
133. Shinkai S, Konishi M, and Shephard RJ. Aging and immune response to exercise. Can J Physiol Pharm 76: 562-572, 1998.

134. 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.
135. Simon HB. The immunology of exercise: a brief review. JAMA 252: 2735-2738, 1984.
136. Swift WE, Gardner HT, Moore D, Streitfeld FH, and Havens WP. Clinical course of viral hepatitis and the effect of exercise during convalescence. Am J Med 8: 614, 1950.
137. Tharp GD and Barnes MW. Basketball exercise and secretory immunoglobulin A. Eur J Appl Physiol 63: 312-314, 1991.
138. Tharp GD and Barnes MW. Reduction of saliva immunoglobulin levels by swim training. Eur J Appl Physiol 60: 61-64, 1990.
139. Thompson HJ, Ronan AM, Ritacco KA, and Tagliaferro AR. Effect of type and amount of dietary fat on the enhancement of mammary tumorigenesis by exercise. Cancer Res 49: 1904-1908, 1989.
140. 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.
141. Uhlenbruck G and Order U. Perspectiven, Probleme und Prioritäten: Sportimmunologie- die nächsten 75 Jahre [Perspectives, problems and priorities: Sports immunology- the next 75 years. Dtsche Z Sportmed 38: 40-47, 1987.
142. Weicker H and Werle E. Interaction between hormones and the immune system. Int J Sports Med 12 (Suppl. 1): S30-S37, 1991.
143. Weiss M. Anamnestische, klinische und laborchemische Daten von 1300 Sporttauglichkeitsuntersuchungen im Hinblick auf Infekte und deren Prophylaxe bei Leistungssportlern (Anamnestic, clinical and lab-chemical data in 1300 studies of sport participants in relation to infections and their prophylaxis in competitive athletes). Dtsche Z Sportmed 33: 399-402, 1993.
144. Weiss M. Infektprophylaxe mit polyvalent Immunoglobulinen: Diskussion anlässlich der Anwendung vor der Olympiade 1992 bei der deutschen Box-Nationalstaffel (Prophylaxis of infections with polyvalent immunoglobulin in top athletes: A discusssion on the occasion of its application in the German boxing team before the Olympic Games of 1992). Dtsche Z Sportmed 33: 466-471, 1993.
145. Weiss M, Fuhrmansky J, Lulay R, and Weicker H. Haufigkeit und Ursache von Immunoglobulin-mangeln bei Sportlern (Frequency and cause of immunoglobulin lack in athletes). Dtsche Z Sportmed 36: 146-153, 1985.
146. Wentworth P, Jentz LA, and Croal AE. Analysis of sudden unexpected death in Southern Ontario with emphasis on myocarditis. Can Med Assoc J 120: 676-680, 1979.
147. Wesslen L, Pahlson C, Lindquist O, Hjelm E, Gnarpe J, Larsson E, Baandrup U, Eriksson L, Fohlman J, Engstrand L, Linglof T, Nystrom-Rosander C, Gnarpe H, Magnius L, Rolf H, C., and Friman G. An increase in sudden unexpected cardiac deaths among young Swedish orienteers during 1979-1992. Eur Heart J 17: 902-910, 1996.
148. Woods JA, Davis JM, Kohut ML, Ghaffar A, Mayer EP, and Pate RR. Effects of exercise on the immune response to cancer. Med Sci Sports Exerc 26: 1109-1115, 1994.
149. Woods JA, Davis JM, Mayer EP, Ghaffar A, and Pate RR. Effects of exercise on macrophage activation for antitumor cytotoxicity. J Appl Physiol: 2177-2185, 1994.

150. Woods JA, Davis JM, Mayer EP, Ghaffar A, and Pate RR. Exercise increases inflammatory macrophage antitumor cytotoxicity. J Appl Physiol 75: 879-886, 1993.
151. Woods JA (ed). Physical activity, behavior, immunity and health. Brain Behav Immun 19 (5): 369-488, 2005.