

This article was downloaded by: [University of Limerick]

On: 31 January 2009

Access details: Access Details: [subscription number 788797399]

Publisher Routledge

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Sports Sciences

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713721847>

Exercise, nutrition and immune function

Michael Gleeson ^a; David C. Nieman ^b; Bente K. Pedersen ^c

^a School of Sport and Exercise Sciences, Loughborough University, Loughborough, UK ^b Department of Health, Leisure and Exercise Science, Appalachian State University, Boone, NC, USA and ^c Department of Infectious Diseases, University of Copenhagen, Copenhagen Ø, Denmark

Online Publication Date: 01 January 2004

To cite this Article Gleeson, Michael, Nieman, David C. and Pedersen, Bente K.(2004)'Exercise, nutrition and immune function',Journal of Sports Sciences,22:1,115 — 125

To link to this Article: DOI: 10.1080/0264041031000140590

URL: <http://dx.doi.org/10.1080/0264041031000140590>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Exercise, nutrition and immune function

MICHAEL GLEESON,^{1*} DAVID C. NIEMAN² and BENTE K. PEDERSEN³

¹School of Sport and Exercise Sciences, Loughborough University, Loughborough LE11 3TU, UK, ²Department of Health, Leisure and Exercise Science, Appalachian State University, Boone, NC 28608, USA and ³Department of Infectious Diseases, University of Copenhagen, Blegdamsvej 9, DK-2100, Copenhagen Ø, Denmark

Accepted 7 August 2003

Strenuous bouts of prolonged exercise and heavy training are associated with depressed immune cell function. Furthermore, inadequate or inappropriate nutrition can compound the negative influence of heavy exertion on immunocompetence. Dietary deficiencies of protein and specific micronutrients have long been associated with immune dysfunction. An adequate intake of iron, zinc and vitamins A, E, B6 and B12 is particularly important for the maintenance of immune function, but excess intakes of some micronutrients can also impair immune function and have other adverse effects on health. Immune system depression has also been associated with an excess intake of fat. To maintain immune function, athletes should eat a well-balanced diet sufficient to meet their energy requirements. An athlete exercising in a carbohydrate-depleted state experiences larger increases in circulating stress hormones and a greater perturbation of several immune function indices. Conversely, consuming 30–60 g carbohydrate · h⁻¹ during sustained intensive exercise attenuates rises in stress hormones such as cortisol and appears to limit the degree of exercise-induced immune depression. Convincing evidence that so-called ‘immune-boosting’ supplements, including high doses of antioxidant vitamins, glutamine, zinc, probiotics and *Echinacea*, prevent exercise-induced immune impairment is currently lacking.

Keywords: exercise, immunity, leucocytes, macronutrients, micronutrients, training.

Immune function and the nutrition of elite athletes

The immune system protects against, recognizes, attacks and destroys elements that are foreign to the body. The immune system can be divided into two broad functions: innate (natural and non-specific) and acquired (adaptive and specific) immunity, which work together synergistically. The attempt of an infectious agent to enter the body immediately activates the innate system. This so-called ‘first-line of defence’ comprises three general mechanisms with the common goal of restricting the entry of microorganisms into the body: (1) physical/structural barriers (skin, epithelial linings, mucosal secretions); (2) chemical barriers (pH of bodily fluids and soluble factors such as lysozymes and complement proteins); and (3) phagocytic cells (e.g. neutrophils and monocytes/macrophages). Failure of the innate system and the resulting infection activates the acquired system, which aids recovery from infection. Monocytes or macrophages ingest, process and present foreign material (antigens) to lymphocytes. This

is followed by clonal proliferation of T- and B-lymphocytes that possess receptors that recognize the antigen, engendering specificity and ‘memory’ that enable the immune system to mount an augmented cell-mediated and humoral response when the host is reinfected by the same pathogen. Critical to the activation and regulation of immune function is the production of cytokines, including interferons, interleukins and colony-stimulating factors. For further details of the normal immune response, see Gleeson and Bishop (1999). A fundamental characteristic of the immune system is that it involves multiple functionally different cell types, which permits a large variety of defence mechanisms. Assessing immune function status, therefore, requires a thorough methodological approach targeting a large spectrum of immune system parameters. However, currently no instruments are available to predict the cumulative effects of several small changes in immune system parameters on host resistance to infection (Keil *et al.*, 2001).

A heavy schedule of training and competition can lead to immune impairment in athletes, which is associated with an increased susceptibility to infections, particularly upper respiratory tract infections (URTI) (Peters and Bateman, 1983; Nieman *et al.*, 1990). This

* Author to whom all correspondence should be addressed.
e-mail: m.gleeson@lboro.ac.uk

exercise-induced immune dysfunction seems to be mostly due to the immunosuppressive actions of stress hormones such as adrenaline and cortisol. Nutritional deficiencies can also impair immune function and there is a vast body of evidence that many infections are increased in prevalence or severity by specific nutritional deficiencies (Scrimshaw and SanGiovanni, 1997; Calder and Jackson, 2000). However, it is also true that excessive intakes of individual micronutrients (e.g. *n*-3 polyunsaturated fatty acids, iron, zinc, vitamins A and E) can impair immune function and increase the risk of infection (Chandra, 1997). As most athletes will be aware, even medically harmless infections can result in a decrement in athletic performance.

Avoiding nutrient deficiencies

The key to maintaining an effective immune system is to avoid deficiencies of the nutrients that play an essential role in immune cell triggering, interaction, differentiation or functional expression. Malnutrition decreases immune defences against invading pathogens and makes the individual more susceptible to infection (Calder and Jackson, 2000; Calder *et al.*, 2002). Infections with certain pathogens can also affect nutritional status by causing appetite suppression, malabsorption, increased nutrient requirements and increased losses of endogenous nutrients.

Protein and energy

It is well accepted that an inadequate intake of protein impairs host immunity with particularly detrimental effects on the T-cell system, resulting in an increased incidence of opportunistic infections (Chandra, 1997; Scrimshaw and SanGiovanni, 1997; Calder *et al.*, 2002). It is not surprising that protein deficiency impairs immunity because immune defences are dependent on rapid cell replication and the production of proteins with important biological activities, such as immunoglobulins, acute phase proteins and cytokines. In humans, protein-energy malnutrition has been found to depress the number of mature, fully differentiated T-lymphocytes and the *in vitro* proliferative response to mitogens, although the latter is reversible with nutritional repletion (Daly *et al.*, 1990; Reynolds *et al.*, 1990). Additionally, in protein-energy malnutrition the T-lymphocyte CD4+/CD8+ (helper/suppressor cell) ratio is markedly decreased and phagocytic cell function, cytokine production and complement formation are all impaired. Essentially, all forms of immunity have been shown to be affected by protein-energy malnutrition in humans, depending on the severity of the protein deficiency relative to energy intake. Although it is

unlikely that athletes would ever reach a state of such extreme malnutrition unless dieting very severely, some impairment of host defence mechanisms is observed even in moderate protein deficiency (Daly *et al.*, 1990). Among the athletic population, individuals at most risk from protein deficiency are those undertaking a programme of food restriction to lose weight, vegetarians and athletes consuming unbalanced diets (e.g. with an excessive amount of carbohydrate at the expense of protein). Often, deficiencies in protein and energy will be accompanied by deficiencies in micronutrients. Energy-restricted diets are common in sports where leanness or low body mass is thought to confer a performance or aesthetic advantage (e.g. gymnastics, figure skating, endurance running) or is required to meet certain body weight criteria (e.g. boxing, martial arts, weightlifting, rowing). Indeed, this has led to the identification of a new subclinical eating disorder, anorexia athletica, which has been associated with an increased susceptibility to infection (Beals and Manore, 1994). Even short-term dieting can influence immune function in athletes. For example, it has been shown that a loss of 2 kg of body mass over 2 weeks adversely affects macrophage phagocytic function (Kono *et al.*, 1988).

Vitamins and minerals

Several vitamins are essential for normal immune function. Deficiencies of fat-soluble vitamins A and E and water-soluble vitamins folic acid, B6, B12 and C impair immune function and decrease the body's resistance to infection (Scrimshaw and SanGiovanni, 1997; Calder and Jackson, 2000; Calder *et al.*, 2002). Correcting existing deficiencies with specific vitamin supplements can be effective in restoring immune function to normal (Calder and Jackson, 2000).

Several minerals are known to exert modulatory effects on immune function, including zinc, iron, magnesium, manganese, selenium and copper, yet with the exception of zinc and iron, isolated deficiencies are rare. Field studies consistently associate iron deficiency with increased morbidity from infectious disease (Sherman, 1992). Furthermore, exercise has a pronounced effect on both zinc and iron metabolism (Gleeson, 2000). Requirements for these minerals are certainly higher in athletes than sedentary individuals because of increased losses in sweat and urine. However, excesses of some minerals (particularly iron and zinc) can impair immune function and increase susceptibility to infection (Chandra, 1984; Sherman, 1992; Gleeson, 2000). Hence, supplements should be taken only as required and regular monitoring of iron status (serum ferritin and blood haemoglobin) and zinc status (erythrocyte zinc) is probably a good idea. The efficacy of zinc

supplementation as a treatment for the common cold has been investigated in at least 11 studies that have been published since 1984. The findings have been equivocal and recent reviews of this topic have concluded that further research is necessary before the use of zinc supplements to treat the common cold can be recommended (Macknin, 1999; Marshall, 2000). Although there is only limited evidence that taking zinc supplements reduces the incidence of URTI (McElroy and Miller, 2002), in the studies that have reported a beneficial effect of zinc in treating the common cold (i.e. reduction of symptom duration and/or severity) it has been emphasized that zinc must be taken within 24 h of the onset of symptoms to be of any benefit. Potential problems with zinc supplements include nausea, bad taste reactions, lowering of high-density lipoprotein cholesterol, depression of some immune cell functions (e.g. neutrophil oxidative burst) and interference with the absorption of copper (Gleeson, 2000).

Eating the right amount and type of fat

Relatively little is known about the potential contribution of dietary fatty acids to the regulation of exercise-induced modification of immune function. Two groups of polyunsaturated fatty acids (PUFA) are essential to the body: the omega-6 (*n*-6) series, derived from linoleic acid, and the omega-3 (*n*-3) series, derived from linolenic acid. These fatty acids cannot be synthesized in the body and therefore must be derived from the diet. There are reports that diets rich in either of these polyunsaturated fatty acids improve the conditions of patients suffering from diseases characterized by an over-active immune system, such as rheumatoid arthritis; that is, they have anti-inflammatory effects (Calder, 1996; Calder *et al.*, 2002). It has been suggested that high intakes of arachidonic acid relative to intakes of fatty acids of the *n*-3 group may exert an undesirable influence on inflammation and immune function during and after exercise (Konig *et al.*, 1997). However, a recent study showed that *n*-3 PUFA supplementation did not influence the exercise-induced elevation of pro- or anti-inflammatory cytokines (Toft *et al.*, 2000). More research is needed on the effects of altering essential fatty acid intake on immune function after exercise and during periods of heavy training.

A recent study that investigated the effects of endurance training for 7 weeks on a carbohydrate-rich diet (65% of dietary energy from carbohydrate) or a fat-rich diet (62% of dietary energy from fat) concluded that diet composition during training may influence natural immunity since natural killer (NK) cell activity increased on the carbohydrate-rich diet compared with

the fat-rich diet in response to training (Pedersen *et al.*, 2000). The results of this study suggest that a fat-rich diet is detrimental to immune function compared with a carbohydrate-rich diet, but do not clarify whether this effect is due to a lack of dietary carbohydrate or an excess of a specific dietary fat component.

Are megadoses of vitamins needed?

Moderately increasing the intake of some vitamins (notably vitamins A and E) above the amounts normally recommended may enhance immune function in the very young (Coutsoudis *et al.*, 1992) and the elderly (Meydani *et al.*, 1990), but is probably not effective in young adults. Consuming megadoses of individual vitamins, which appears to be a common practice in athletes, can impair immune function and have other toxic effects (Calder *et al.*, 2002; Food Standards Agency, 2003). For example, 300 mg of vitamin E given daily to men (the UK reference nutrient intake for men is 4 mg·day⁻¹; COMA, 1991) for 3 weeks significantly depressed phagocyte function and lymphocyte proliferation (Prasad, 1980). In a recent exercise study, supplementation of athletes with 600 mg·day⁻¹ vitamin E for 2 months before an Ironman triathlon event resulted in elevated oxidative stress and inflammatory cytokine responses during the triathlon compared with placebo (D.C. Nieman *et al.*, unpublished). In elderly people (*n* = 652), a daily 200-mg vitamin E supplement increased the severity of infections, including total illness duration, duration of fever and restriction of physical activity (Graat *et al.*, 2002). Recently, vitamin E supplementation (600 mg·day⁻¹) in patients with ischaemic heart disease has been demonstrated to have either no effect on all-cause mortality (MRC/BHF Heart Protection Study, 2002) or to increase the number of cases who died compared with placebo (Waters *et al.*, 2002). Megadoses of vitamin A may impair the inflammatory response and complement formation as well as having other pathological effects, including causing an increased risk of foetal abnormalities when consumed by pregnant women (Food Standards Agency, 2003).

Vitamins with antioxidant properties including vitamins A, C, E and β -carotene (provitamin A) may be required in increased quantities in athletes to inactivate the products of exercise-induced lipid peroxidation (Packer, 1997). However, there are no convincing data to demonstrate an effect of nutritional antioxidants on muscle damage or delayed-onset muscle soreness. Increased oxygen free-radical formation that accompanies the dramatic rise in oxidative metabolism during exercise could potentially inhibit immune responses (Peters, 1997; Petersen and Pedersen, 2002). Reactive

oxygen species inhibit locomotory and bactericidal activity of neutrophils, reduce the proliferation of T- and B-lymphocytes and inhibit natural killer cell cytotoxic activity. Sustained endurance training appears to be associated with an adaptive up-regulation of the antioxidant defence system (Duthie *et al.*, 1996). However, such adaptations may be insufficient to protect athletes who train extensively (Clarkson, 1992; Packer, 1997).

Vitamin C (ascorbic acid) is found in high concentrations in leucocytes and has been implicated in a variety of anti-infective functions, including promotion of T-lymphocyte proliferation, prevention of corticosteroid-induced suppression of neutrophil activity and inhibition of virus replication (Peters, 2000). It is also a major water-soluble antioxidant that is effective as a scavenger of reactive oxygen species in both intracellular and extracellular fluids. Vitamin C is also required for the regeneration of the reduced form of the lipid-soluble antioxidant, vitamin E. The UK reference nutrient intake (RNI) for vitamin C is $40 \text{ mg} \cdot \text{day}^{-1}$ (COMA, 1991).

In a study by Peters *et al.* (1993), using a double-blind placebo research design, it was determined that daily supplementation of 600 mg (15 times the RNI) of vitamin C for 3 weeks before a 90-km ultramarathon reduced the incidence of symptoms of URTI (68% compared with 33% in age- and sex-matched control runners) in the 2 weeks after the race. In a follow-up study, Peters *et al.* (1996) randomly divided participants in a 90-km ultramarathon ($n = 178$) and their matched controls ($n = 162$) into four treatment groups receiving one of 500 mg vitamin C alone, 500 mg vitamin C plus 400 IU vitamin E (1 IU is equivalent to 0.67 mg), 300 mg vitamin C plus 300 IU vitamin E plus 18 mg β -carotene, or placebo. As runners were requested to continue with their usual habits in terms of dietary intake and the use of nutritional supplements, total vitamin C intake of the four groups was 1004, 893, 665 and 585 $\text{mg} \cdot \text{day}^{-1}$, respectively. The study confirmed previous findings of a lower incidence of symptoms of URTI in those runners with the highest mean daily intake of vitamin C and also indicated that the combination of water-soluble and fat-soluble antioxidants was not more successful in attenuating the post-exercise infection risk than vitamin C alone. This study certainly provides some support for the notion that megadoses of vitamin C reduce URTI risk in endurance athletes. However, some similar studies have not been able to replicate these findings: Himmelstein *et al.* (1998), for example, reported no difference in URTI incidence among 44 marathon runners and 48 sedentary individuals randomly assigned to a 2-month regimen of $1000 \text{ mg} \cdot \text{day}^{-1}$ vitamin C or placebo. Furthermore, a subsequent double-blind, placebo-

controlled study found no effect of vitamin C supplementation ($1000 \text{ mg} \cdot \text{day}^{-1}$ for 8 days) on the immune response to 2.5 h running (Nieman *et al.*, 1997a), although a larger dose of vitamin C supplementation ($1500 \text{ mg} \cdot \text{day}^{-1}$ for 7 days before the race and on race day) did reduce the cortisol and cytokine response to a 90-km ultramarathon race (Nieman *et al.*, 2000). However, in the latter study, no difference in URTI incidence was found between participants on vitamin C and placebo treatments; also, the participants consumed carbohydrate during the race *ad libitum* and this was retrospectively estimated.

In a more recent randomized, double-blind, placebo-controlled study, ingestion of 1500 mg vitamin C $\cdot \text{day}^{-1}$ for 7 days before an ultramarathon race with consumption of vitamin C in a carbohydrate beverage during the race (participants in the placebo group consumed the same carbohydrate beverage without added vitamin C) did not affect oxidative stress, cytokines or immune function during or after the race (Nieman *et al.*, 2002a). In contrast, it has recently been reported that 7 days supplementation with vitamin C ($800 \text{ mg} \cdot \text{day}^{-1}$) before a downhill treadmill run reduced the exercise-induced rise in plasma interleukin (IL)-6, monocyte respiratory burst and natural killer cell numbers compared with a placebo treatment (Hurst *et al.*, 2001). Nieman *et al.* (2002a) summarized the available literature on vitamin C supplementation and immune responses to exercise and concluded that vitamin C supplementation before prolonged intensive exercise 'does not have a consistent effect on blood measures of oxidative stress and muscle damage and that any linkage to immune perturbations remains speculative and more than likely improbable'. It should be noted that consumption of doses in excess of 1000 mg can cause abdominal pain and diarrhoea (Food Standards Agency, 2003), although there are insufficient data on adverse effects to set a safe upper level for vitamin C intake.

Nutritional manipulations to decrease exercise-induced immune impairment in athletes

Since exercise-induced immune function impairment appears mainly to be caused by elevated concentrations of stress hormones, nutritional strategies that effectively reduce the stress hormone response to exercise should limit the degree of exercise-induced immune dysfunction (Nieman and Pedersen, 2000). There is certainly considerable experimental evidence to support this notion, although it is not clear if the magnitude of such effects is sufficient to affect infection risk.

Carbohydrate intake before and during exercise

In recent years, several studies have examined the impact of dietary carbohydrate on hormonal and immune responses to exercise. These studies (Gleeson *et al.*, 1998; Mitchell *et al.*, 1998; Bishop *et al.*, 2001b) have found that when individuals perform prolonged exercise after several days on very low carbohydrate diets (typically <10% of dietary energy intake from carbohydrate), the magnitude of the stress hormone (e.g. adrenaline and cortisol) and cytokine (e.g. IL-6, IL-1ra and IL-10) response is markedly higher than on normal or high carbohydrate diets. It has been speculated that athletes deficient in carbohydrate are placing themselves at risk from the known immunosuppressive effects of cortisol, including the suppression of antibody production, lymphocyte proliferation and natural killer cell cytotoxic activity. Mitchell *et al.* (1998) observed that exercising (1 h at 75% $\dot{V}O_{2max}$) in a glycogen-depleted state (induced by prior exercise and 2 days on a low carbohydrate diet) resulted in a greater fall in circulating lymphocyte numbers 2 h after exercise compared with the same exercise performed after 2 days on a high carbohydrate diet. However, the manipulation of carbohydrate status did not affect the decrease in mitogen-stimulated lymphocyte proliferation that occurred after exercise.

Consumption of carbohydrate during exercise also attenuates rises in plasma catecholamines, adrenocorticotrophic hormone, growth hormone, cortisol and cytokines (Nehlsen-Cannarella *et al.*, 1997; Nieman, 1998). Carbohydrate intake during exercise also attenuates the trafficking of most leucocyte and lymphocyte subsets, including the rise in the neutrophil:lymphocyte ratio (Nieman *et al.*, 1997b; Bishop *et al.*, 1999a), prevents the exercise-induced fall in neutrophil function (Bishop *et al.*, 2000b) and reduces the extent of the diminution of mitogen-stimulated T-lymphocyte proliferation (Henson *et al.*, 1998) following prolonged exercise. Very recently, it was shown that consuming 30–60 g carbohydrate $\cdot h^{-1}$ during 2.5 h of strenuous cycling prevented both the decrease in the number and percentage of interferon (IFN)- γ -positive T-lymphocytes and the suppression of IFN- γ production from stimulated T-lymphocytes observed on the placebo control trial (Lancaster *et al.*, 2003). Interferon- γ production is critical to anti-viral defence and it has been suggested that the suppression of IFN- γ production may be an important mechanism leading to an increased risk of infection after prolonged exercise bouts (Northoff *et al.*, 1998).

Compared with placebo, carbohydrate ingestion during a 3-h treadmill run attenuated plasma concentrations of IL-1ra, IL-6 and IL-10, as well as muscle gene expression for IL-6 and IL-8 (Nieman *et al.*,

2003). The 3-h treadmill run in both the carbohydrate and placebo trials induced gene expression within the muscle for two primary pro-inflammatory cytokines, IL-1 β and TNF- α . Interleukin-6 and IL-8, which are often considered to be components of the secondary inflammatory cascade, were also expressed, but to a lesser extent in the carbohydrate trial. Anti-inflammatory indicators, including plasma IL-1ra, IL-10 and cortisol, were also decreased with carbohydrate feeding. These results suggest that carbohydrate ingestion attenuates the secondary but not the primary pro-inflammatory cascade, decreasing the need for immune responses related to anti-inflammation. However, when carbohydrate is ingested during prolonged exercise, the release of IL-6 from working muscles can be totally inhibited (Febbraio *et al.*, 2003) and the exercise-induced expression of several metabolic genes are blunted compared with exercise in the fasted state (Pilegaard *et al.*, 2002). Infusion of IL-6 in humans stimulates cortisol secretion (with plasma cortisol reaching similar values to those observed during exercise and with a similar time-course) and induces lipolysis as well as eliciting a strong anti-inflammatory response (Pedersen *et al.*, 2003; Starkie *et al.*, 2003). Thus, although carbohydrate ingestion during exercise attenuates the IL-6 response and so reduces the magnitude of the cortisol-induced lymphocytopenia, it will, at the same time, inhibit lipolysis, reduce the anti-inflammatory effects of exercise and attenuate the expression of several metabolic genes in the exercised muscle. In other words, it is possible that carbohydrate ingestion during exercise sessions could limit adaptation to training. However, it can also be argued that carbohydrate intake during training allows the athlete to work harder and for longer and as yet there is no evidence that physiological and performance adaptations are impaired by carbohydrate intake during training sessions. Further research is needed to determine how nutrient intake might affect the transcriptional regulation of metabolic genes in skeletal muscle and what, if any, consequences this has for training adaptation.

While carbohydrate feeding during exercise appears to be effective in minimizing some of the immune perturbations associated with prolonged continuous strenuous exercise, it appears less effective for less demanding exercise of an intermittent nature, for example football (Bishop *et al.*, 1999b) or rowing (Nieman *et al.*, 1999) training. It is also apparent that carbohydrate feeding is not as effective in reducing immune cell trafficking and functional depression when continuous prolonged exercise is performed to the point of fatigue (Bishop *et al.*, 2001a). Pre-exercise feeding of carbohydrate does not seem to be very effective in limiting exercise-induced leucocytosis or depression of

neutrophil function (Lancaster *et al.*, 2001). Also, there is no evidence that the beneficial effect of feeding carbohydrate on immune responses to exercise translates into a reduced incidence of URTI after prolonged exercise such as marathon races. Although a trend for a beneficial effect of carbohydrate ingestion on post-race URTI was reported in a study of 98 marathon runners (Nieman *et al.*, 2002b), this did not achieve statistical significance and larger-scale studies are needed to investigate this possibility.

Fluid intake during exercise

The consumption of beverages during exercise not only helps prevent dehydration (which is associated with an increased stress hormone response) but also helps to maintain saliva flow rate during exercise. Saliva contains several proteins with antimicrobial properties, including immunoglobulin-A (IgA), lysozyme and α -amylase. Saliva secretion usually falls during exercise. Regular fluid intake during exercise is reported to prevent this effect and a recent study (Bishop *et al.*, 2000a) has confirmed that regular consumption of lemon-flavoured carbohydrate-containing drinks helps to maintain saliva flow rate and hence saliva IgA secretion rate during prolonged exercise compared with a restricted fluid intake regimen.

Glutamine supplements

Glutamine is the most abundant free amino acid in human muscle and plasma and is utilized at very high rates by leucocytes to provide energy and optimal conditions for nucleotide biosynthesis (Ardawi and Newsholme, 1983, 1994). Indeed, glutamine is considered important, if not essential, to lymphocytes and other rapidly dividing cells, including the gut mucosa and bone marrow stem cells. Prolonged exercise is associated with a fall in the plasma concentration of glutamine and it has been hypothesized that such a decrease could impair immune function (Parry-Billings *et al.*, 1992; Castell, 2003).

It has been suggested that exogenous provision of glutamine supplements may be beneficial by maintaining the plasma glutamine concentration and hence preventing the impairment of immune function after prolonged exercise. Castell *et al.* (1996) have provided the only prophylactic evidence that an oral glutamine supplement (5 g in 330 ml water) consumed immediately after and 2 h after a marathon reduces the incidence of URTI (in the 7 days after the race). However, it is unlikely that this amount of glutamine supplementation could actually have prevented the post-exercise fall in the plasma glutamine concentra-

tion. Provision of glutamine has been shown to have a beneficial effect on gut function, morbidity and mortality and on some aspects of immune cell function in clinical studies of diseased or traumatized patients. However, several recent studies that have investigated the effect of large amounts of glutamine supplementation during and after exercise on the exercise-induced fall in lymphokine-activated killer cell activity, neutrophil function and mitogen-stimulated lymphocyte proliferation have failed to find any beneficial effect (Rohde *et al.*, 1998; Walsh *et al.*, 2000). Very recently, Bassit *et al.* (2002) reported that supplementation of branched-chain amino acids (BCAA) (6 g \cdot day⁻¹ for 15 days) before a triathlon or 30-km run prevented the approximately 40% decline in mitogen-stimulated lymphocyte proliferation observed in the placebo control group after exercise. Supplementation with BCAA prevented the post-exercise fall in plasma glutamine concentration and was also associated with increased lymphocyte IL-2 and IFN- γ production. More research is needed to resolve these conflicting findings of BCAA and glutamine supplementation on the immune responses to exercise.

Dietary immunostimulants

β -Carotene (pro-vitamin A) acts both as an antioxidant and an immunostimulant, increasing the number of T-helper cells in healthy humans (Alexander *et al.*, 1985) and stimulating natural killer cell activity when added *in vitro* to human lymphatic cultures (Watson *et al.*, 1991). Furthermore, elderly men who had been taking β -carotene supplements (50 mg on alternate days) for 10–12 years were reported to have significantly higher natural killer cell activity than elderly men on placebo (Santos *et al.*, 1996). However, supplementing runners with β -carotene was found to have an insignificant effect on the incidence of URTI after a 90-km ultramarathon (Peters *et al.*, 1992). Furthermore, intakes of supplements in excess of 7 mg \cdot day⁻¹ are not advised because of a possible increased risk of lung cancer in smokers (Food Standards Agency, 2003).

Several herbal preparations are reputed to have immunostimulatory effects and consumption of products containing *Echinacea purpurea* is widespread among athletes. However, few controlled studies have examined the effects of dietary immunostimulants on exercise-induced changes in immune function. In one recent double-blind, placebo-controlled study, the effect of a daily oral pre-treatment for 28 days with pressed juice of *E. purpurea* was investigated in 42 triathletes before and after a sprint triathlon (Berg *et al.*, 1998). A sub-group of athletes was also treated with

magnesium as a reference for supplementation with a micronutrient important for optimal muscular function. The most important finding was that during the 28-day pre-treatment period, none of the athletes in the *Echinacea* group fell ill, compared with three individuals in the magnesium group and four in the placebo group who became ill. Pre-treatment with *Echinacea* appeared to reduce the release of soluble IL-2 receptor before and after the race and increased the exercise-induced rise in IL-6.

Several experiments have demonstrated that *E. purpurea* extracts do indeed demonstrate significant immunomodulatory activities. Among the many pharmacological properties reported, macrophage activation has been demonstrated most convincingly (Stimpel *et al.*, 1984; Steinmuller *et al.*, 1993). Phagocytotic indices and macrophage-derived cytokine concentrations have been shown to be *Echinacea*-responsive in a variety of assays and activation of polymorphonuclear leucocytes and natural killer cells has also been reasonably demonstrated (Barrett, 2003). Changes in the numbers and activities of T- and B-lymphocytes have been reported, but are less certain. Despite this cellular evidence of immunostimulation, the pathways leading to enhanced resistance to infectious disease have not been described adequately. Several dozen human experiments, including a number of blind randomized trials, have reported health benefits. The most robust data come from trials testing *E. purpurea* extracts in the treatment for acute URTI. Although suggestive of modest benefit, these trials are limited both in size and in methodological quality. In a recent randomized, double-blind, placebo-controlled trial, administering unrefined *Echinacea* at the onset of symptoms of URTI in 148 college students did not provide any detectable benefit or harm compared with placebo (Barrett *et al.*, 2002). Hence, while there is a great deal of moderately good-quality scientific data on *Echinacea*, its effectiveness in treating illness or in enhancing human health has not yet been proven beyond a reasonable doubt.

Probiotics are food supplements that contain 'friendly' gut bacteria. There is now a reasonable body of evidence that regular consumption of probiotics can modify the population of the gut microflora and influence immune function (Calder *et al.*, 2002). Some studies have shown that probiotic intake can improve rates of recovery from rotavirus diarrhoea, increase resistance to enteric pathogens and promote anti-tumour activity; there is even some evidence that probiotics may be effective in alleviating some allergic and respiratory disorders in young children (see Kopp-Hoolihan, 2001, for a review). However, to date, there are no published studies of the effectiveness of probiotic use in athletes.

Summary and recommendations

1. Both heavy exercise and nutrition exert separate influences on immune function; these influences appear to be stronger when exercise stress and poor nutrition act synergistically.
2. Dietary deficiencies of energy, protein and specific micronutrients are associated with depressed immune function and increased susceptibility to infection. An adequate intake of iron, zinc and vitamins A, E, B6 and B12 is particularly important for the maintenance of immune function. Athletes need to avoid micronutrient deficiencies.
3. To maintain immune function, athletes should eat a well-balanced diet sufficient to meet their energy requirements. This should ensure an adequate intake of protein and micronutrients.
4. For athletes on energy-restricted diets, vitamin supplements are desirable.
5. An athlete exercising in a carbohydrate-depleted state experiences larger increases in circulating stress hormones and a greater perturbation of several immune function indices.
6. Consumption of carbohydrate ($30\text{--}60\text{ g}\cdot\text{h}^{-1}$) in drinks during prolonged exercise is recommended, as this practice appears to attenuate some of the immunosuppressive effects of prolonged exercise. However, the clinical significance of this has to be determined.
7. Consumption of megadoses of vitamins and minerals is not advised. Excess intakes of some micronutrients (e.g. iron, zinc, vitamin E) can impair immune function.
8. High fat diets suppress some aspects of immune cell function.
9. Convincing evidence that so-called 'immune-boosting' supplements, such as high doses of antioxidant vitamins, glutamine, zinc, probiotics and *Echinacea*, prevent exercise-induced immune impairment is currently lacking. Current evidence regarding the efficacy of *Echinacea* extracts, zinc lozenges and probiotics in preventing or treating common infections is limited and there is insufficient evidence to recommend these supplements at this time.
10. It is still debatable as to whether antioxidant supplements are required or are desirable for athletes. There is conflicting evidence of the effects of high-dose vitamin C in reducing the post-exercise incidence of URTI and this practice has not been shown to prevent exercise-induced immune impairment.
11. Glutamine supplementation is beneficial to immune function in the clinical setting but has not proved effective in abolishing the post-exercise impairment of immune cell function.

References

- Alexander, M., Newmark, H. and Miller, R.G. (1985). Oral beta-carotene can increase the number of CD4+ cells in human blood. *Immunology Letters*, **9**, 221–224.
- Ardawi, M.S.M. and Newsholme, E.A. (1983). Glutamine metabolism in lymphocytes of the rat. *Biochemical Journal*, **212**, 835–842.
- Ardawi, M.S.M. and Newsholme, E.A. (1994). Glutamine metabolism in lymphoid tissues. In *Glutamine Metabolism in Mammalian Tissues* (edited by D. Haussinger and H. Sies), pp. 235–246. Berlin: Springer-Verlag.
- Barrett, B. (2003). Medicinal properties of *Echinacea*: critical review. *Phytomedicine*, **10**, 66–86.
- Barrett, B.P., Brown, R.L., Locken, K., Maberry, R., Bobula, J.A. and D'Alessio, D. (2002). Treatment of the common cold with unrefined *Echinacea*: a randomized, double-blind, placebo-controlled trial. *Annals of Internal Medicine*, **137**, 939–946.
- Bassit, R.A., Sawada, L.A., Bacurau, R.F.P., Navarro, F., Martins, E., Santos, R.V.T., Caperuto, E.C., Rogeri, P. and Costa-Rosa, L.F.B.P. (2002). Branched-chain amino acid supplementation and the immune response of long-distance athletes. *Nutrition*, **18**, 376–379.
- Beals, K.A. and Manore, M.M. (1994). The prevalence and consequence of eating disorders in female athletes. *International Journal of Sport Nutrition*, **4**, 175–195.
- Berg, A., Northoff, H. and Konig, D. (1998). Influence of Echinacin (E31) treatment on the exercise-induced immune response in athletes. *Journal of Clinical Research*, **1**, 367–380.
- Bishop, N.C., Blannin, A.K., Rand, L., Johnson, R. and Gleeson, M. (1999a). Effects of carbohydrate and fluid intake on the blood leucocyte responses to prolonged cycling. *Journal of Sports Sciences*, **17**, 26–27.
- Bishop, N.C., Blannin, A.K., Robson, P.J., Walsh, N.P. and Gleeson, M. (1999b). The effects of carbohydrate supplementation on neutrophil degranulation responses to a soccer-specific exercise protocol. *Journal of Sports Sciences*, **17**, 787–796.
- Bishop, N.C., Blannin, A.K., Armstrong, E., Rickman, M. and Gleeson, M. (2000a). Carbohydrate and fluid intake affect the saliva flow rate and IgA response to cycling. *Medicine and Science in Sports and Exercise*, **32**, 2046–2051.
- Bishop, N.C., Blannin, A.K., Rand, L., Johnson, R. and Gleeson, M. (2000b). The effects of carbohydrate supplementation on neutrophil degranulation responses to prolonged cycling. *International Journal of Sports Medicine*, **21**(suppl. 1), S73.
- Bishop, N.C., Blannin, A.K., Walsh, N.P. and Gleeson, M. (2001a). Carbohydrate beverage ingestion and neutrophil degranulation responses following cycling to fatigue at 75% $\dot{V}O_{2max}$. *International Journal of Sports Medicine*, **22**, 226–231.
- Bishop, N.C., Walsh, N.P., Haines, D.L., Richards, E.E. and Gleeson, M. (2001b). Pre-exercise carbohydrate status and immune responses to prolonged cycling: II. Effect on plasma cytokine concentration. *International Journal of Sport Nutrition and Exercise Metabolism*, **11**, 503–512.
- Calder, P.C. (1996). Fatty acids, dietary lipids and lymphocyte functions. *Biochemical Society Transactions*, **23**, 302–309.
- Calder, P.C. and Jackson, A.A. (2000). Undernutrition, infection and immune function. *Nutrition Research Reviews*, **13**, 3–29.
- Calder, P.C., Field, C.J. and Gill, H.S. (2002). *Nutrition and Immune Function*. Oxford: CABI Publishing.
- Castell, L. (2003). Glutamine supplementation *in vitro* and *in vivo*, in exercise and in immunodepression. *Sports Medicine*, **33**, 323–345.
- Castell, L.M., Poortmans, J.R. and Newsholme, E.A. (1996). Does glutamine have a role in reducing infections in athletes? *European Journal of Applied Physiology*, **73**, 488–490.
- Chandra, R.K. (1984). Excessive intake of zinc impairs immune responses. *Journal of the American Medical Association*, **52**, 1443–1446.
- Chandra, R.K. (1997). Nutrition and the immune system: an introduction. *American Journal of Clinical Nutrition*, **66**, 460S–463S.
- Clarkson, P.M. (1992). Minerals: exercise performance and supplementation in athletes. In *Foods, Nutrition and Sports Performance* (edited by C. Williams and J. Devlin), pp. 113–146. London: E & FN Spon.
- COMA (1991). *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom*. Report of the Panel on Dietary Reference Values, Committee on Medical Aspects of Food and Nutrition Policy. London: HMSO.
- Coutsoudis, A., Kiepiela, P., Coovadia, H.M. and Broughton, M. (1992). Vitamin A supplementation enhances specific IgG antibody levels and total lymphocyte numbers while improving morbidity in measles. *Paediatric Infectious Disease Journal*, **11**, 203–209.
- Daly, J.M., Reynolds, J., Sigal, R.K., Shou, J. and Liberman, M.D. (1990). Effect of dietary protein and amino acids on immune function. *Critical Care Medicine*, **18**(suppl. 2), S86–S93.
- Duthie, G.G., Jenkinson, A.McE., Morrice, P.C. and Arthur, J.R. (1996). Antioxidant adaptations to exercise. In *Biochemistry of Exercise IX* (edited by R.J. Maughan and S.M. Shirreffs), pp. 465–470. Champaign, IL: Human Kinetics.
- Febbraio, M.A., Steensberg, A., Keller, C., Starkie, R.L., Nielsen, H.B., Krstrup, P., Ott, P., Secher, N.H. and Pedersen, B.K. (2003). Glucose ingestion attenuates interleukin-6 release from contracting skeletal muscle in humans. *Journal of Physiology*, **549**, 607–612.

- Food Standards Agency (2003). Safe upper levels for vitamins and minerals. Report of Expert Group on Vitamins and Minerals (<http://www.foodstandards.gov.uk>).
- Gleeson, M. (2000). Minerals and exercise immunology. In *Nutrition and Exercise Immunology* (edited by D.C. Nieman and B.K. Pedersen), pp. 137–154. Boca Raton, FL: CRC Press.
- Gleeson, M. and Bishop, N.C. (1999). Immunology. In *Basic and Applied Sciences for Sports Medicine* (edited by R.J. Maughan), pp. 199–236. Oxford: Butterworth Heinemann.
- Gleeson, M., Blannin, A.K., Walsh, N.P., Bishop, N.C. and Clark, A.M. (1998). Effect of low and high carbohydrate diets on the plasma glutamine and circulating leucocyte responses to exercise. *International Journal of Sport Nutrition*, **8**, 49–59.
- Graat, J.M., Schouten, E.G. and Kok, F.J. (2002). Effect of daily vitamin E and multivitamin–mineral supplementation on acute respiratory tract infections in elderly persons: a randomised control trial. *Journal of the American Medical Association*, **288**, 715–721.
- Henson, D.A., Nieman, D.C., Parker, J.C.D., Rainwater, M.K., Butterworth, D.E., Warren, B.J., Utter, A., Davis, J.M., Fagoaga, O.R. and Nehlsen-Cannarella, S.L. (1998). Carbohydrate supplementation and the lymphocyte proliferative response to long endurance running. *International Journal of Sports Medicine*, **19**, 574–580.
- Himmelstein, S.A., Robergs, R.A., Koehler, K.M., Lewis, S.L. and Qualls, C.R. (1998). Vitamin C supplementation and upper respiratory tract infection in marathon runners. *Journal of Exercise Physiology*, **1**, 1–17.
- Hurst, T.L., Bailey, D.M., Powell, J.R. and Williams, C. (2001). Immune function changes in downhill running subjects following ascorbic acid supplementation. *Medicine and Science in Sports and Exercise*, **33**(suppl. 5), ISEI abstract 2.
- Keil, D., Luebke, R.W. and Pruett, S.B. (2001). Quantifying the relationship between multiple immunological parameters and host resistance: probing the limits of reductionism. *Journal of Immunology*, **167**, 4543–4552.
- Konig, D., Berg, A., Weinstock, C., Keul, J. and Northoff, H. (1997). Essential fatty acids, immune function and exercise. *Exercise Immunology Review*, **3**, 1–31.
- Kono, I., Kitao, H., Matsuda, M., Haga, S., Fukushima, H. and Kashigawa, H. (1988). Weight reduction in athletes may adversely affect the phagocytic function of monocytes. *Physician and Sportsmedicine*, **16**, 56–65.
- Kopp-Hoolihan, L. (2001). Prophylactic and therapeutic uses of probiotics: a review. *Journal of the American Dietetic Association*, **101**, 229–238.
- Lancaster, G.I., Jentjens, R.L.P.G., Moseley, L., Jeukendrup, A.E. and Gleeson, M. (2001). Effect of timing and amount of pre-exercise feeding of carbohydrate on blood neutrophil responses to time trial cycling. *Medicine and Science in Sports and Exercise*, **33**(suppl. 5), ISEI abstract 7.
- Lancaster, G.I., Khan, Q., Drysdale, P.T., Jeukendrup, A.E., Drayson, M.T. and Gleeson, M. (2003). Effect of feeding different amounts of carbohydrate during prolonged exercise on human T-lymphocyte intracellular cytokine production. *Journal of Physiology*, **548P**, 98.
- Macknin, M.L. (1999). Zinc lozenges for the common cold. *Cleveland Clinical Journal of Medicine*, **66**, 27–32.
- Marshall, I. (2000). Zinc for the common cold. *Cochrane Database Systematic Reviews*, 2000(2), CD001364.
- McElroy, B.H. and Miller, S.P. (2002). Effectiveness of zinc gluconate glycine lozenges (Cold-Eeze) against the common cold in school-aged subjects: a retrospective chart review. *American Journal of Therapeutics*, **9**, 472–475.
- Meydani, S.N., Barklund, P.M. and Liu, S. (1990). Vitamin E supplementation enhances cell mediated immunity in elderly subjects. *American Journal of Clinical Nutrition*, **52**, 557–563.
- Mitchell, J.B., Pizza, F.X., Paquet, A., Davis, J.B., Forrest, M.B. and Braun, W.A. (1998). Influence of carbohydrate status on immune responses before and after endurance exercise. *Journal of Applied Physiology*, **84**, 1917–1925.
- MRC/BHF Heart Protection Study (2002). MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20,536 high-risk individuals: a randomised placebo-controlled trial. *Lancet*, **360**, 23–33.
- Nehlsen-Cannarella, S.L., Fagoaga, O.R., Nieman, D.C., Henson, D.A., Butterworth, D.E., Schmitt, R.L., Bailey, E.M., Warren, B.J., Utter, A. and Davis, J.M. (1997). Carbohydrate and the cytokine response to 2.5 h of running. *Journal of Applied Physiology*, **82**, 1662–1667.
- Nieman, D.C. (1998). Influence of carbohydrate on the immune responses to intensive, prolonged exercise. *Exercise Immunology Review*, **4**, 64–76.
- Nieman, D.C. and Pedersen, B.K. (2000). *Nutrition and Exercise Immunology*. Boca Raton, FL: CRC Press.
- Nieman, D.C., Johansen, L.M., Lee, J.W. and Arabatzis, K. (1990). Infectious episodes in runners before and after the Los Angeles Marathon. *Journal of Sports Medicine and Physical Fitness*, **30**, 316–328.
- Nieman, D.C., Henson, D.A., Butterworth, D.E., Warren, B.J., Davis, J.M., Fagoaga, O.R. and Nehlsen-Cannarella, S.L. (1997a). Vitamin C supplementation does not alter the immune response to 2.5 hours of running. *International Journal of Sport Nutrition*, **7**, 173–184.
- Nieman, D.C., Henson, D.A., Garner, E.B., Butterworth, D.E., Warren, B.J., Utter, A., Davis, J.M., Fagoaga, O.R. and Nehlsen-Cannarella, S.L. (1997b). Carbohydrate affects natural killer cell redistribution but not function after running. *Medicine and Science in Sports and Exercise*, **20**, 1318–1324.
- Nieman, D.C., Nehlsen-Cannarella, S.L., Fagoaga, O.R., Henson, D.A., Shannon, M., Davis, J.M., Austin, M.D., Hisey, C.L., Holbeck, J.C., Hjertman, J.M., Bolton, M.R. and Schilling, B.K. (1999). Immune response to two hours of rowing in elite female rowers. *International Journal of Sports Medicine*, **20**, 476–481.
- Nieman, D.C., Peters, E.M., Henson, D.A., Nevines, E.I. and Thompson, M.M. (2000). Influence of vitamin C supplementation on cytokine changes following an ultramarathon. *Journal of Interferon and Cytokine Research*, **20**, 1029–1035.

- Nieman, D.C., Henson, D.A., McAnulty, S.R., McAnulty, L., Swick, N.S., Utter, A.C., Vinci, D.M., Opiela, S.J. and Morrow, J.D. (2002a). Influence of vitamin C supplementation on oxidative and immune changes after an ultramarathon. *Journal of Applied Physiology*, **92**, 1970–1977.
- Nieman, D.C., Henson, D.A., Fagoaga, O.R., Utter, A.C., Vinci, D.M., Davis, J.M. and Nehlsen-Cannarella, S.L. (2002b). Change in salivary IgA following a competitive marathon race. *International Journal of Sports Medicine*, **23**, 69–75.
- Nieman, D.C., Davis, J.M., Henson, D.A., Walberg-Rankin, J., Shute, M., Dumke, C.L., Utter, A.C., Vinci, D.M., Carson, J.A., Brown, A., Lee, W.J., McAnulty, S.R. and McAnulty, L.S. (2003). Carbohydrate ingestion influences skeletal muscle cytokine mRNA and plasma cytokine levels after a 3-h run. *Journal of Applied Physiology*, **94**, 1917–1925.
- Northoff, H., Berg, A. and Weinstock, C. (1998). Similarities and differences of the immune response to exercise and trauma: the IFN-gamma concept. *Canadian Journal of Physiology and Pharmacology*, **76**, 497–504.
- Packer, L. (1997). Oxidants, antioxidant nutrients and the athlete. *Journal of Sports Sciences*, **15**, 353–363.
- Parry-Billings, M., Budgett, R., Koutedakis, Y., Blomstrand, E., Brooks, S., Williams, C., Calder, P.C., Pillings, S., Baigre, R. and Newsholme, E.A. (1992). Plasma amino acid concentrations in the overtraining syndrome: possible effects on the immune system. *Medicine and Science in Sports and Exercise*, **24**, 1353–1358.
- Pedersen, B.K., Helge, J., Richter, E., Rhode, T., Ostrowski, K. and Kiens, B. (2000). Training and natural immunity: effects of diets rich in fat or carbohydrate. *European Journal of Applied Physiology*, **82**, 98–102.
- Pedersen, B.K., Steensberg, A., Keller, P., Keller, C., Fischer, C., Hiscock, N., van Hall, G., Plomgaard, P. and Febbraio, M.A. (2003). Muscle-derived interleukin-6: lipolytic, anti-inflammatory and immune regulatory effects. *Pflügers Archives*, **446**, 9–16.
- Peters, E.M. (1997). Exercise, immunology and upper respiratory tract infections. *International Journal of Sports Medicine*, **18** (suppl. 1), S69–S77.
- Peters, E.M. (2000). Vitamins, immunity and infection risk in athletes. In *Nutrition and Exercise Immunology* (edited by D.C. Nieman and B.K. Pedersen), pp. 109–135. Boca Raton, FL: CRC Press.
- Peters, E.M. and Bateman, E.D. (1983). Ultramarathon running and URTI: an epidemiological survey. *South African Medical Journal*, **64**, 582–584.
- Peters, E.M., Campbell, A. and Pawley, L. (1992). Vitamin A fails to increase resistance to upper respiratory infection in distance runners. *South African Journal of Sports Medicine*, **7**, 3–7.
- Peters, E.M., Goetzsche, J.M., Grobbelaar, B. and Noakes, T.D. (1993). Vitamin C supplementation reduces the incidence of post-race symptoms of upper respiratory tract infection in ultramarathon runners. *American Journal of Clinical Nutrition*, **57**, 170–174.
- Peters, E.M., Goetzsche, J.M., Joseph, L.E. and Noakes, T.D. (1996). Vitamin C is effective as combinations of anti-oxidant nutrients in reducing symptoms of upper respiratory tract infections in ultramarathon runners. *South African Journal of Sports Medicine*, **11**, 23–27.
- Petersen, E.W. and Pedersen, B.K. (2002). Exercise and immune function – effect of nutrition. In *Nutrition and Immune Function* (edited by P.C. Calder, C.J. Field and H.S. Gill), pp. 347–355. Oxford: CABI Publishing.
- Pilegaard, H., Keller, C., Steensberg, A., Helge, J.W., Pedersen, B.K., Saltin, B. and Neuffer, P.D. (2002). Influence of pre-exercise muscle glycogen content on exercise-induced transcriptional regulation of metabolic genes. *Journal of Physiology*, **541**, 261–271.
- Prasad, J.S. (1980). Effect of vitamin E supplementation on leucocyte function. *American Journal of Clinical Nutrition*, **33**, 606–608.
- Reynolds, J., Shou, J., Sigal, R., Ziegler, M. and Daly, J.M. (1990). The influence of protein malnutrition on T cell, natural killer cell, and lymphokine-activated killer cell function, and on biological responsiveness to high-dose interleukin-2. *Cellular Immunology*, **128**, 569.
- Rohde, T., Asp, S., Maclean, D. and Pedersen, B.K. (1998). Competitive sustained exercise in humans, and lymphokine activated killer cell activity – an intervention study. *European Journal of Applied Physiology*, **78**, 448–453.
- Santos, M.S., Meydani, S.N., Leka, L., Wu, D., Fotouhi, N., Meydani, M., Hennekens, C.H. and Gaziano, J.M. (1996). Natural killer cell activity in elderly men is enhanced by beta-carotene supplementation. *American Journal of Clinical Nutrition*, **64**, 772–777.
- Scrimshaw, N.S. and SanGiovanni, J.P. (1997). Synergism of nutrition, infection and immunity: an overview. *American Journal of Clinical Nutrition*, **66**, 464S–477S.
- Sherman, A.R. (1992). Zinc, copper and iron nutriture and immunity. *Journal of Nutrition*, **122**, 604–609.
- Starkie, R., Ostrowski, S.R., Jauffred, S., Febbraio, M. and Pedersen, B.K. (2003). Exercise and IL-6 infusion inhibit endotoxin-induced TNF-alpha production in humans. *FASEB Journal*, **17**, 884–886.
- Steinmuller, C., Roesler, J., Grottrup, E., Franke, G., Wagner, H. and Lohmann-Matthes, M.L. (1993). Polysaccharides isolated from plant cultures of *Echinacea purpurea* enhance the resistance of immunosuppressed mice against systemic infections with *Candida albicans* and *Listeria monocytogenes*. *International Journal of Immunopharmacology*, **15**, 605–614.
- Stimpel, M., Proksch, A., Wagner, H. and Lohmann-Matthes, M.L. (1984). Macrophage activation and induction of macrophage cytotoxicity by purified polysaccharide fractions from the plant *Echinacea purpurea*. *Infection and Immunology*, **46**, 845–849.
- Toft, A.D., Ostrowski, K., Asp, S., Moller, K., Iversen, S., Hermann, C., Sondergaard, S.R. and Pedersen, B.K. (2000). The effects of n-3 PUFA on the cytokine response to strenuous exercise. *Journal of Applied Physiology*, **89**, 2401–2405.

- Walsh, N.P., Blannin, A.K., Bishop, N.C., Robson, P.J. and Gleeson, M. (2000). Oral glutamine supplementation does not attenuate the fall in human neutrophil lipopolysaccharide-stimulated degranulation following prolonged exercise. *International Journal of Sport Nutrition*, **10**, 39–50.
- Waters, D.D., Alderman, E.L., Hsia, J., Howard, B.V., Cobb, F.R., Rogers, W.J., Ouyang, P., Thompson, P., Tardif, J.C., Higginson, L., Bittner, V., Steffes, M., Gordon, D.J., Proschan, M., Younes, N. and Verter, J.I. (2002). Effects of hormone replacement therapy and antioxidant vitamin supplements on coronary atherosclerosis in postmenopausal women: a randomized controlled trial. *Journal of the American Medical Association*, **288**, 2432–2440.
- Watson, R.R., Prabhala, R.H., Plezia, P.M. and Alberts, D.S. (1991). Effect of beta-carotene on lymphocyte subpopulation in elderly humans: evidence for a dose response relationship. *American Journal of Clinical Nutrition*, **53**, 90–94.