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Special Feature

Modification of immune responses to exercise by carbohydrate, glutamine and anti-oxidant supplements

MICHAEL GLEESON and NICOLETTE C BISHOP

School of Sport and Exercise Sciences, University of Birmingham, Edgbaston, Birmingham, England

Summary Immunosuppression in athletes involved in heavy training is undoubtedly multifactorial in origin. Training and competitive surroundings may increase the athlete's exposure to pathogens and provide optimal conditions for pathogen transmission. Heavy prolonged exertion is associated with numerous hormonal and biochemical changes, many of which potentially have detrimental effects on immune function. Furthermore, improper nutrition can compound the negative influence of heavy exertion on immunocompetence. An athlete exercising in a carbohydrate-depleted state experiences larger increases in circulating stress hormones and a greater perturbation of several immune function indices. The poor nutritional status of some athletes may predispose them to immunosuppression. For example, dietary deficiencies of protein and specific micronutrients have long been associated with immune dysfunction. Although it is impossible to counter the effects of all of the factors that contribute to exercise-induced immunosuppression, it has been shown to be possible to minimize the effects of many factors. Athletes can help themselves by eating a well-balanced diet that includes adequate protein and carbohydrate, sufficient to meet their energy requirements. This will ensure a more than adequate intake of trace elements without the need for special supplements. Consuming carbohydrate (but not glutamine or other amino acids) during exercise attenuates rises in stress hormones, such as cortisol, and appears to limit the degree of exercise-induced immunosuppression, at least for non-fatiguing bouts of exercise. Evidence that high doses of anti-oxidant vitamins can prevent exercise-induced immunosuppression is also lacking.

Key words: amino acids, carbohydrate, exercise, immune, leucocytes, nutrition, protein.

Introduction

A heavy schedule of training and competition can lead to chronic immunosuppression in athletes, placing them at a greater risk from opportunistic infections, particularly infections affecting the upper respiratory tract.[1,2] Furthermore, following each bout of prolonged strenuous exercise there follows a period of temporary immunosuppression, which has been termed an 'open window' opportunity for infection.[3] There is an abundance of epidemiological evidence and clinical data to suggest that nutritional deficiencies alter immunocompetence and increase the risk of infection and even medically harmless infections may significantly affect athletic performance. There are many factors that influence exercise-induced immunosuppression (e.g. physical, environmental and psychological stress) and nutrition is also a factor that undoubtedly plays a critical role. This brief review examines the role of nutrition in exercise-induced immunosuppression and in particular the recent research that has examined the effects of carbohydrate and amino acid supplements on immune responses to exercise. Some important questions addressed in the present review include: (i) Are the reported dietary practices of athletes optimal for immune function? (ii) Are there any specific nutritional practices that might impair immune function or exacerbate the temporary immunosuppression that follows an acute bout of prolonged strenuous exercise? (iii) Does feeding of carbohydrate (CHO) or amino acid supplements during and after prolonged exercise lessen the stress on the immune system? (iv) Can nutrient supplements reduce the risk of infection following heavy exertion?

Correspondence: Professor Michael Gleeson, School of Sport and Exercise Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, England. Email: m.gleeson@bham.ac.uk

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Immune function and the nutrition of elite athletes

Nutrient availability has the potential to affect almost all aspects of the immune system,[4–6] because many nutrients are involved in energy metabolism and protein synthesis. Most immune responses involve cell replication and the production of proteins with specific functions (e.g. cytokines, antibodies, acute phase proteins). Potential mechanisms of action include interference with the production of humoral and secretory antibodies, altered cell-mediated immunity, bactericidal capacity of phagocytes, complement formation and the T lymphocyte proliferative response to mitogens.[7] The mechanisms by which nutrient deficiencies affect the immune system have been classified as either 'direct' or 'indirect'. A nutritional deficiency is said to have a direct effect when the nutritional factor being considered has primary activity within the lymphoid system, and an indirect effect when the primary activity affects all cellular material or another organ system that acts as an immune regulator.[8] For example, CHO availability directly affects a number of leucocyte functions, but also exerts an indirect effect on the lymphoid system

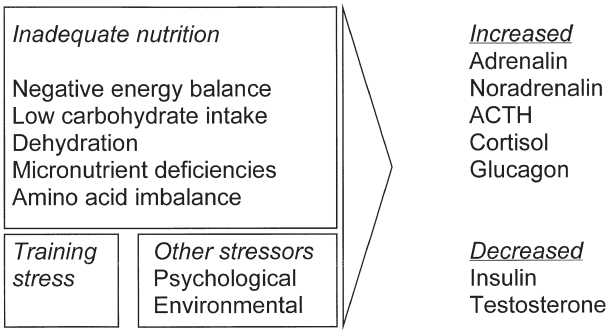

Figure 1 Nutrition and the stress hormone response. Various stressors, including inadequate nutrition, prior exercise with inadequate recovery, psychological anxiety and environmental extremes (e.g. heat, altitude), modify the hormonal response to exercise. Changes in plasma levels of stress hormones, including catecholamines, adrenocorticotrophic hormone (ACTH) and cortisol, are probably mostly responsible for the observed changes in immune function following an acute bout of exercise.

through its influence on circulating levels of the catecholamines, adrenocorticotrophic hormone and cortisol (Fig. 1).

The extent of the impact of a certain nutrient deficiency on the functioning of the immune system depends on the duration of the deficiency, as well as the athlete's nutritional status as a whole. The severity of the deficiency is a further influencing factor, although even a mild deficiency of a single nutrient can result in an altered immune response.[4] Excessive amounts of specific nutrients (e.g. omega-3 polyunsaturated fatty acids, iron and zinc) also have the potential to cause detrimental effects to immune function.[9,10] The 1991 Consensus Conference on Foods, Nutrition and Sports Performance recommended the following dietary advice: 'In the optimum diet for most sports, CHO is likely to contribute about 60–70% of the total energy intake and protein about 12%, with the remainder coming from fat'.[11] Athletes are generally advised to eat a well-balanced diet made up of a variety of foods in sufficient quantity to cover their energy expenditures. However, diets high in protein or CHO or fat, very low energy diets, fasting or megadoses of vitamins and minerals are just some of the many dietary alterations commonly used by athletes. Such dietary extremes may in fact compromise immune function. For example, diets that are excessively high in CHO, favoured by many athletes in order to keep glycogen stores high, are generally low in meat products and thus consequently in protein (which is an important nutrient for immune function) and vitamin B12 (essential for DNA and RNA synthesis). Energy-restricted diets are not uncommon in sports where leanness or low body mass is thought to confer a performance advantage (e.g. gymnastics, figure skating, endurance running) or is required to meet certain bodyweight 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.[12] Even short-term dieting can influence immune function in athletes. For example, it has been shown that a loss of 2 kg body mass over a 2 week period adversely affects macrophage phagocytic function.[13]

Carbohydrate

There is little reason for doubting the importance of adequate CHO availability for maintenance of heavy training schedules and successful athletic performance.[14] It is currently recommended that during periods of heavy training athletes should consume sufficient CHO to cover about 60% of their energy costs.[15] The recommended daily intake is 8–10 g CHO/kg body mass for athletes who train for more than 2 h/day. These recommendations are principally aimed at restoring muscle and liver glycogen stores to ensure sufficient CHO availability for skeletal muscle contraction for training on successive days. However, glucose is also an important fuel for cells of the immune system, including lymphocytes, neutrophils and macrophages. Phagocytes utilize glucose at a rate 10-fold greater than they utilize glutamine when these substrates are both present in the culture medium at normal physiological concentrations.[16] The importance of glucose for the proper functioning of lymphocytes and macrophages is further emphasized in a study that has found that concanavalin-A-stimulated proliferation of these cells *in vitro* is dependent on glucose concentration over the physiological range.[17] Cells of the immune system have very high metabolic rates[18] and this consequently highlights the importance for adequate nutrition for the provision of fuels to maintain immunocompetence.

Some recent studies have examined the impact of dietary CHO on immune responses to exercise. One such study from our lab has investigated the effect of a high (76%) or low (6%) CHO diet on changes in circulating numbers of leucocyte subsets and plasma cortisol and glutamine levels in response to 1 h of cycle ergometer exercise at 70% maximal oxygen uptake (VO_2max).[19] We have found that the low-CHO diet significantly increases the magnitude of the leucocytosis and the rise in the neutrophil:lymphocyte ratio (an accepted indicator of exercise stress) observed during the first 4 h of recovery compared with values obtained with subjects on their normal diets. Furthermore, plasma cortisol correlates negatively with the percentage CHO in the diet and strongly correlates with the post-exercise leucocytosis. On the low-CHO diet, the post-exercise fall in the plasma glutamine concentration is greater than on the normal or high-CHO diet. It was concluded in this paper that the metabolic stress imposed by the low-CHO availability results in increased release of cortisol, which in turn induces the greater neutrophilia. It was further speculated that athletes deficient in CHO are placing themselves at high risk from the other known immunosuppressive effects of cortisol[20] and falls in glutamine availability, including the suppression of antibody production, lymphocyte proliferation and natural killer cell cytotoxic activity (NKCA). In a similar study, it has been observed that exercising (1 h at 75% VO_2max) in a glycogen-depleted state (induced by prior exercise and 2 days on a low-CHO diet) results in a greater fall in circulating lymphocyte numbers at 2 h post-exercise compared with the same exercise performed after 2 days on a high-CHO diet.[21] However, the manipulation of CHO status does not affect the decrease in phytohaemoagglutinin (PHA)-stimulated lymphocyte proliferation that

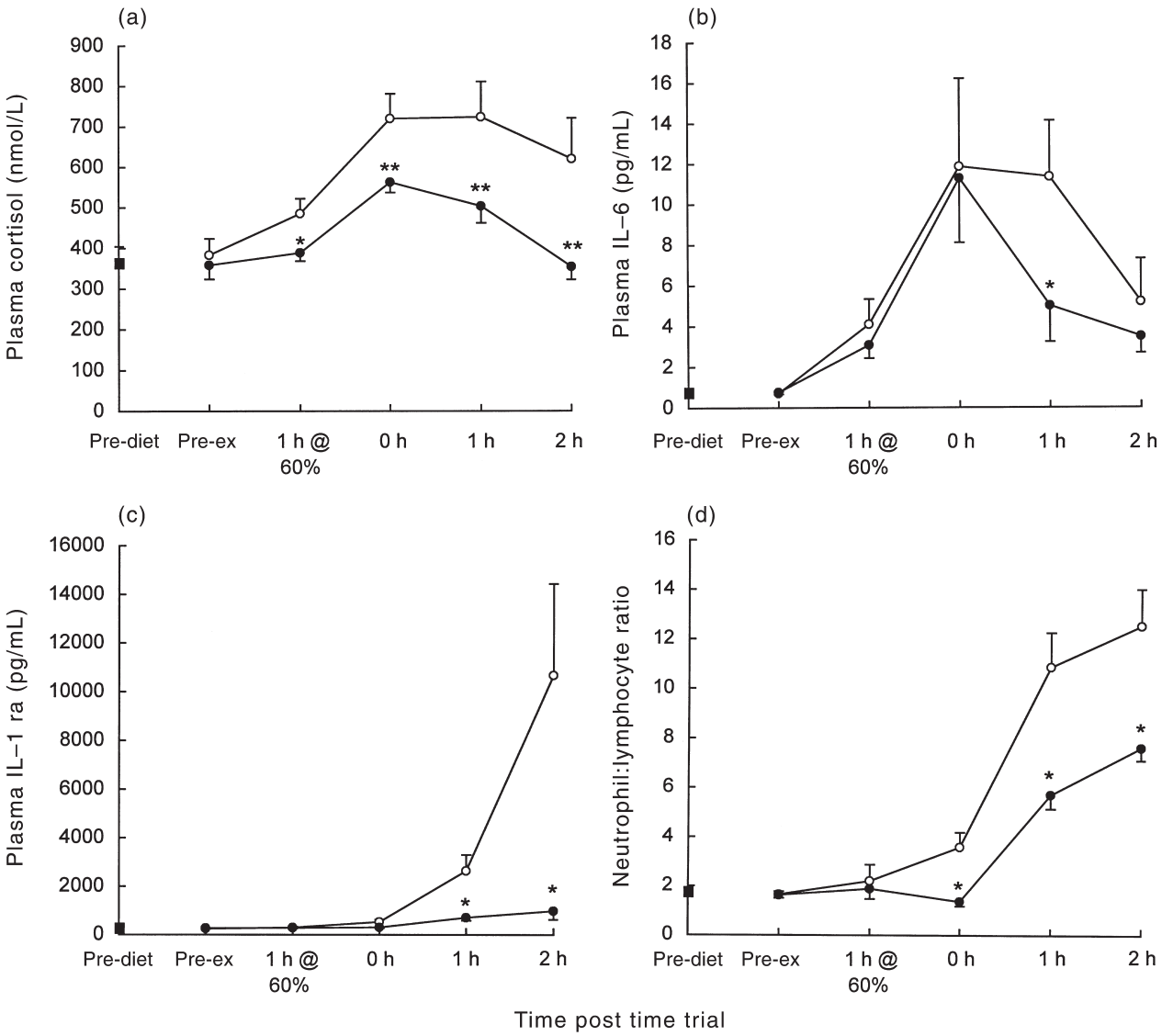

Figure 2 Changes in the concentrations of (a) plasma cortisol, (b) plasma IL-6, (c) plasma interleukin-1 receptor antagonist (IL-1 ra) and (d) the blood neutrophil:lymphocyte ratio after 1 h of cycling at 60% maximal oxygen uptake (VO_2max) immediately followed by a 30 min time trial (work rate around 80% VO_2max). For the 3 days prior to the exercise trial, subjects (n = 12) consumed either a high carbohydrate (CHO) diet (more than 70% of total dietary energy from CHO) or a low CHO diet (less than 10% of total dietary energy from CHO). Data are presented as the mean and SEM. (○), Low CHO diet; (●), high CHO diet; (■), pre-diet. *P < 0.05, **P < 0.01 compared with the low CHO diet.

occurs after exercise. Dietary CHO also modifies the cytokine response to exercise: cyclists who perform 60 min of exercise at 75% VO_2max followed by a time trial (equivalent to 30 min work at 80% VO_2max) exhibit markedly higher plasma cortisol, IL-1 receptor antagonist and IL-6 responses to the exercise when given a low-CHO diet (< 1 g CHO/kg per day) compared with a high-CHO diet (approximately 8 g CHO/kg per day) for 3 days prior to the exercise test (Fig. 2; M Gleeson and NC Bishop, unpubl. data, 2000). There is less information available on the effects of dietary carbohydrate content on immune adaptations associated with exercise training, although one recent study has investigated the effects of endurance training for 7 weeks on CHO-rich (65% of dietary energy from CHO) or fat-rich (62% of dietary energy from fat) diets on natural immunity.[22] The results indicate that diet may influence immune status during periods of exercise training, because NKCA increases on the high-CHO diet compared with the high-fat diet in response to training. The authors concluded that diets rich in fat are detrimental to immune function, but the study could not clarify whether this effect was due to a lack of dietary CHO or an excess of a specific dietary fat component.[22] Some polyunsaturated fatty acids can exert either direct effects (by altering cell membrane fluidity) or indirect effects (as eicosanoid precursors) on immune function, including reduced IL-2 production and suppressed mitogen-induced lymphocyte proliferation.[23,24] Thus, these fatty acids may exert an undesirable influence on immune function during and after exercise.[24]

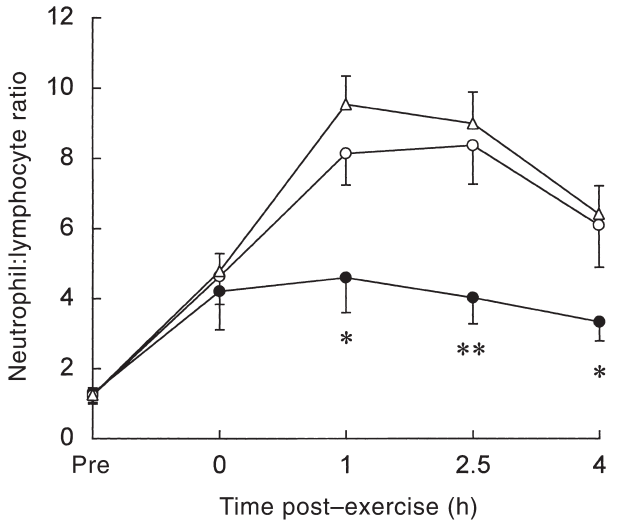

Figure 3 Changes in the neutrophil:lymphocyte ratio following 2 h of cycling at 60% maximal oxygen uptake (VO_2max) when fed a 6% w/v carbohydrate solution (●), the same volume of an artificially sweetened placebo solution (○) or a restricted fluid intake (△). $*P < 0.05$, $**P < 0.01$ compared with the placebo trial. Data are from Bishop *et al.*[26]

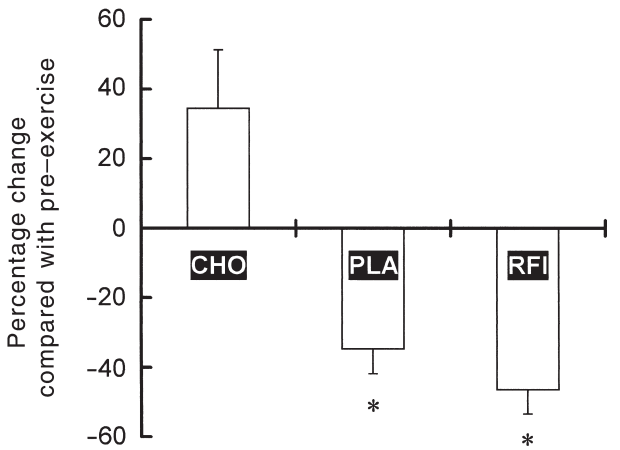

Figure 4 Percentage change (compared with pre-exercise) in the lipopolysaccharide-stimulated neutrophil degranulation response immediately following 2 h of cycling at 60%VO_2max when fed a 6% w/v carbohydrate solution (CHO), the same volume of an artificially sweetened placebo solution (PLA) or a restricted fluid intake (RFI). $*P < 0.05$ compared with pre-exercise. Data are from Bishop *et al.*[26]

Consumption of CHO during exercise also attenuates rises in plasma catecholamines and cortisol and reduces the degree of exercise-induced immunosuppression. For example, in a recent randomized, double-blind, placebo-controlled study, 30 marathon runners ingested 750 mL of a 6% (w/v) CHO or placebo drink immediately prior to a 2.5 h treadmill run at 75–80% VO_2max with a further 250 mL of CHO or placebo drink ingested every 15 min throughout the exercise.[25] Carbohydrate ingestion lowered the plasma cortisol, IL-6 and IL-1 receptor antagonist responses to exercise

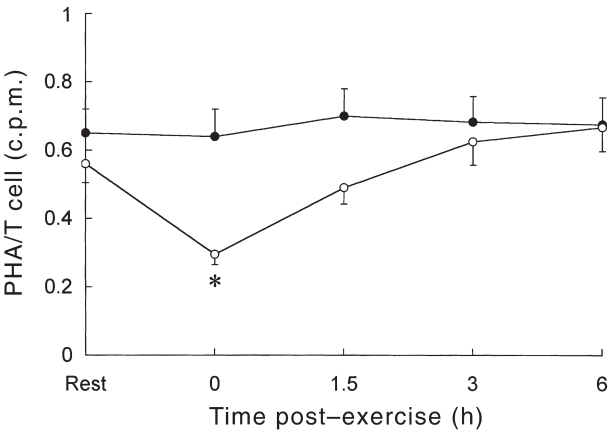

Figure 5 Change in PHA-stimulated lymphocyte proliferation following 2.5 h running when fed a 6% w/v carbohydrate solution (CHO, ●) or the same volume of an artificially sweetened placebo solution (PLA, ○). $*P < 0.05$ compared with CHO. Data are from Henson *et al.*[32]

compared with the placebo treatment. The plasma cortisol concentrations correlated negatively to plasma glucose levels immediately post-exercise. Carbohydrate intake during exercise also attenuates the trafficking of most leucocyte and lymphocyte subsets,[26–29] including the rise in the neutrophil:lymphocyte ratio[26] (Fig. 3), prevents the exercise-induced fall in neutrophil function[30,31] (Fig. 4) and reduces the extent of the diminution of PHA-stimulated T-lymphocyte proliferation (on a per cell basis) following prolonged exercise[32] (Fig. 5).

The consumption of CHO in beverages during exercise may also have an additional benefit in helping to maintain saliva flow rate during exercise. Saliva contains several proteins with antimicrobial properties, including IgA, lysozyme and α-amylase. During periods of heavy training, athletes have been found to have lower concentrations of salivary IgA (s-IgA) and it has been suggested that this may contribute to their increased incidence of upper respiratory tract infection (URTI).[33] Saliva secretion is under neural control. The sympathetic nervous system stimulation that occurs during exercise causes vasoconstriction of the blood vessels to the salivary glands and results in a reduction in saliva secretion. Regular fluid intake during exercise is reported to prevent this effect[34] and a recent study from our lab has confirmed that regular consumption of CHO-containing drinks helps to maintain saliva flow rate and hence s-IgA secretion rate during prolonged exercise compared with a restricted fluid intake regimen.[35]

While CHO feeding during exercise appears to be effective in minimizing some of the immune perturbations associated with prolonged continuous strenuous exercise, it does not prevent the fall in the plasma glutamine concentration and seems less effective for less demanding exercise of an intermittent nature, for example football[27] or rowing[36] training. It is also apparent that CHO feeding is not effective in reducing immune cell trafficking and functional depression when exercise is performed to the point of fatigue.[37]

Protein and amino acids

The daily protein requirement is approximately doubled in athletes compared with the sedentary population. An intake of less than 1.6 g protein/kg body mass per day is likely to be associated with a negative nitrogen balance in athletes who are training hard, particularly endurance athletes.[38] Provided that athletes consume a well-balanced diet that meets their requirement for energy, the increased requirement for protein will be met.[11] In view of this, those individuals at most risk from protein deficiency would be athletes undertaking a programme of food restriction in order to lose weight, vegetarians, and athletes consuming unbalanced diets (e.g. with an excessive amount of CHO at the expense of protein).

It is well accepted that an inadequate intake of protein impairs host immunity with particularly detrimental effects on the T cell system, resulting in increased incidence of opportunistic infections.[4–6,8] One of the most dramatic manifestations of this is widespread atrophy of lymphoid tissue. In humans, protein-energy malnutrition (PEM) has been found to depress the number of mature, fully differentiated T lymphocytes and the *in vitro* response to T-lymphocyte mitogens, although the latter is reversible with nutritional repletion.[39] Additionally, the T-lymphocyte $CD4^+/CD8^+$ ratio is markedly decreased in PEM.[4,39] Essentially all forms of immunity have been shown to be affected by PEM in humans, depending on the severity of the protein deficiency relative to energy intake. These effects include impaired phagocytic cell function, decreased cytokine production and reduced complement formation.[4,39] While it is unlikely that athletes would ever reach a state of such extreme malnutrition unless dieting very severely, the impairment of host defence mechanisms is observed even in moderate protein deficiency.[39]

Too much dietary protein, at the expense of CHO, could also be potentially harmful to immune function. It has been demonstrated that a diet rich in protein (24% protein, 72% fat, 3% CHO) consumed for 4 days results in a 25% lowering of muscle and plasma glutamine levels.[40] Furthermore, consuming a high-protein, low-CHO diet for several days prior to exercise results in an even lower plasma glutamine concentration after exercise.[19] 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.[41] Indeed, glutamine is considered important, if not essential, to lymphocytes and other rapidly dividing cells, including the gut mucosa and bone marrow stem cells. It is also required for optimal macrophage phagocytic activity.[41] Prolonged exercise is associated with a fall in the plasma concentration of glutamine[42–44] and it has been hypothesized that such a decrease could impair immune function. The overtraining syndrome is associated with a chronic reduction in plasma glutamine levels[42] and it has been suggested that this may be partly responsible for the immunosuppression apparent in this condition. Interestingly, there is some evidence that an additional intake of 20–30 g protein/day can restore depressed plasma glutamine levels in overtrained athletes.[45]

Several scientists have suggested that exogenous provision of glutamine supplements may be beneficial by preventing the impairment of immune function following prolonged exercise. Castell *et al.* 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 following the race.[46] However, several recent studies that have investigated the effect of glutamine supplementation during exercise on various indices of immune function have failed to find any beneficial effect.[47–49] A glutamine solution (0.1 g/kg body mass) given at 0, 30, 60 and 90 min following a marathon race prevents the fall in the plasma glutamine concentration, but does not prevent the fall in mitogen-induced lymphocyte proliferation and lymphocyte-activated killer cell activity.[47] Similarly, maintaining the plasma glutamine concentration by consuming glutamine in drinks taken both during and after 2 h of cycling at 60% VO_2max does not affect leucocyte subset trafficking or prevent the exercise-induced fall in neutrophil function.[48] Furthermore, neither glutamine or milk protein (casein) supplements taken during and after exercise in amounts sufficient to prevent the post-exercise fall in the plasma glutamine concentration can prevent the exercise-induced decrease in lymphocyte proliferative response, NKCA and s-IgA secretion rate.[49] Unlike the feeding of CHO during exercise, it seems that glutamine supplements do not affect the immune function perturbations that have been examined to date.

Anti-oxidant vitamins and minerals

Vitamins are essential organic molecules that cannot be synthesized in the body and therefore must be obtained from food. Many vitamins are the precursors of coenzymes involved in energy metabolism and protein or nucleic acid synthesis. Thirteen compounds are now classed as vitamins and fall into two categories: fat-soluble and water-soluble compounds. Several vitamins are essential for normal immune function: fat-soluble vitamins A and E and water-soluble vitamins B_{12} and C. Other vitamins (e.g. B_6 and folic acid) also play important roles in immune function, but are not discussed here because dietary deficiencies in humans are extremely rare.

There are no indications in the literature to suggest that vitamin intake among athletes in general is insufficient.[50,51] Athletes tend to ingest above average quantities of these micronutrients and it may be that, as with dietary protein requirements, any increase in need is countered by increased dietary intake. However, it could be that the requirement for most vitamins is simply not increased in athletes. For example, vitamin loss via sweat during exercise is negligible. However, vitamins with anti-oxidant properties (including vitamins C, E and β-carotene (provitamin A)) may be required in increased quantities in athletes in order to inactivate the products of exercise-induced increased free radical formation and lipid peroxidation.[52,53] Oxygen free radical formation that accompanies the dramatic increase in oxidative metabolism during exercise may inhibit locomotory and bactericidal activity of neutrophils, reduce the proliferation of T and B lymphocytes and inhibit NKCA.[54] Sustained endurance training appears to be associated with an adaptive upregulation of the anti-oxidant defence system.[55] However, such adaptations may be insufficient to protect athletes who train extensively and these individuals should consider increasing their intakes of nutritional anti-oxidants, such as

vitamins C, E and β-carotene, in order to reduce free radical damage.[50,53]

In a study by Peters *et al.* using a double blind placebo research design, it has been determined that a daily supplementation of 600 mg of vitamin C (10-fold of the recommended daily dietary allowance (RDA)) reduces the incidence of symptoms of URTI (68% compared with 33% in age- and sex-matched control runners) after participation in a 90 km ultramarathon.[56] However, the method used to report URTI symptoms resulted in an unrealistically high incidence of URTI in the placebo group. Furthermore, a double-blind, placebo-controlled study has found no effect of vitamin C supplementation (1000 mg/day for 8 days) on the immune response to 2.5 h of running.[57]

Animal studies have shown an increased oxidation of vitamin E during exercise that could result in reduced anti-oxidant protection.[58] Dietary vitamin E stimulates mononuclear cell production of IL-Iβ via its influence on the arachidonic acid metabolic pathways[59] and cytokine production is further facilitated by a vitamin E-influenced inhibition of prostaglandin-E_2 production.[60] Severe vitamin E deficiency results in impaired cell-mediated immunity and decreased antibody synthesis.[60,61]

Vitamin A is also essential for immunocompetence.[61] Vitamin A deficiency in animals and humans results in atrophy of the thymus, decreased lymphocyte proliferation in response to mitogens and increased bacterial binding to respiratory tract epithelial cells and impaired secretory IgA production.[61,62] Consequently, vitamin A-deficient humans have a higher incidence of spontaneous infection.[62] Vitamin A-deficient experimental animals also demonstrate reduced NKCA, lower production of interferon and antibodies, impaired delayed cutaneous hypersensitivity and less effective macrophage activity.[62] However, supplementing ultramarathon runners with vitamin A was found to have an insignificant effect on the incidence of URTI following the 1991 Comrades Marathon.[63]

The trace element selenium is also a component of anti-oxidant defence, because it is a cofactor of glutathione peroxidase/reductase and thus influences the quenching of reactive oxygen species[6] and selenium deficiency can affect all components of the immune system. As such, it is possible that the requirement of selenium is increased in those individuals involved in heavy exercise training. However, any selenium supplement should be taken with caution: supplements of amounts up to the RDA appear non-toxic, yet the safety of larger doses has not been confirmed and intakes of 25 mg (approximately 40 × RDA) have been associated with vomiting, abdominal pain, hair loss and fatigue.[64]

In general, supplementation of individual micronutrients or consumption of large doses of simple anti-oxidant mixtures is not recommended. Athletes should obtain complex mixtures of anti-oxidant compounds from increased consumption of fruits and vegetables. Consuming megadoses of individual vitamins (not uncommon in athletes) is likely to do more harm than good. Because most vitamins function mainly as coenzymes in the body, once these enzyme systems are saturated, the vitamin in free form can have toxic effects. For example 300 mg of vitamin E (as α-tocopherol acetate), given daily to 18 men for a period of 3 weeks, produces a significant depression in the bactericidal activity of peripheral blood leucocytes and mitogen-induced lymphocyte proliferation.[60] Megadoses of vitamin A may impair the inflammatory response and complement formation as well as having other pathological and teratogenic effects.[64]

Conclusions

Both heavy exercise and nutrition exert separate influences on immune function; these influences appear to be greater when exercise stress and poor nutrition act synergistically. Exercise training increases the body's requirement for most nutrients and, in many cases, these increased needs are countered by increased food consumption. However, some athletes adopt an unbalanced dietary regimen and many surveys show that few athletes follow the best dietary pattern for optimal sport nutrition. It seems very likely that the poor nutritional status of some athletes may predispose them to immunosuppression. Although it is impossible to counter the effects of all of the causes that contribute to exercise-induced immunosuppression, it has been shown to be possible to minimize the effects of many factors. Athletes can help themselves by eating a well-balanced diet that includes adequate CHO, protein and micronutrients. Consumption of CHO drinks during training is recommended, because this practice appears to attenuate some of the immunosuppressive effects of prolonged exercise, provided that exercise is not continued to the point of fatigue. Athletes may benefit from an increased intake of anti-oxidants, but the dangers of excessive oversupplementation of micronutrients should be highlighted; many micronutrients given in quantities beyond a certain threshold will in fact reduce immune responses.

References

1 Nieman DC, Johansen LM, Lee JW, Arabatzis K. Infectious episodes in runners before and after the Los Angeles Marathon. *J. Sports Med. Phys. Fitness* 1990; **30**: 316–28.

2 Peters EM, Bateman ED. Ultramarathon running and URTI: An epidemiological survey. *S. Afr. Med. J.* 1983; **64**: 582–4.

3 Pedersen BK, Bruunsgaard H. How physical exercise influences the establishment of infections. *Sports Med.* 1995; **19**: 393–400.

4 Chandra RK. Nutrition and the immune system: An introduction. *Am. J. Clin. Nutr.* 1997; **66**: S460–3.

5 Kubena KS, McMurray DN. Nutrition and the immune system: A review of nutrient–nutrient interactions. *J. Am. Diet Assoc.* 1996; **96**: 1156–64.

6 Scrimshaw NS, SanGiovanni JP. Synergism of nutrition, infection and immunity: An overview. *Am. J. Clin. Nutr.* 1997; **66**: S464–77.

7 Gleeson M, Bishop NC. Immunology. In: Maughan RJ (ed.). *Basic and Applied Sciences for Sports Medicine*. Oxford: Butterworth Heinemann, 1999; 199–236.

8 Cunningham-Rundles S. Effects of nutritional status on immunological function. *Am. J. Clin. Nutr.* 1982; **35**: 1202–10.

9 Bishop NC, Blannin AK, Walsh NP, Robson PJ, Gleeson M. Nutritional aspects of immunosuppression in athletes. *Sports Med.* 1999; **28**: 151–76.

10 Shephard RJ, Shek PN. Heavy exercise, nutrition and immune function: Is there a connection? *Int. J. Sports Med.* 1995; **16**: 491–7.

11 Devlin JT, Williams C. Foods, nutrition and sports performance: A final consensus statement. *J. Sports Sci.* 1991; **9** (Suppl.): iii.

12 Beals KA, Manore MM. The prevalence and consequence of eating disorders in female athletes. *Int. J. Sport Nutr.* 1994; **4**: 175–95.
13 Kono I, Kitao H, Matsuda M, Haga S, Fukushima H, Kashigawa H. Weight reduction in athletes may adversely affect the phagocytic function of monocytes. *Phys. Sportsmed.* 1988; **16**: 56–65.
14 Coyle EF. Timing and method of increased carbohydrate intake to cope with heavy training, competition and recovery. In: Williams C, Devlin J (eds). *Foods, Nutrition and Sports Performance*. London: E & FN Spon, 1992; 35–63.
15 Hawley JA, Dennis SC, Lindsey EH, Noakes TD. Nutritional practices of athletes: Are they sub-optimal? *J. Sports Sci.* 1995; **13**: S75–81.
16 Blannin AK, Walsh NP, Clark AM, Gleeson M. Rates of glutamine and glucose utilisation by quiescent and stimulated human neutrophils in vitro. *J. Physiol.* 1998; **506**: P121.
17 Hume DA, Weidemann MJ. Role and regulation of glucose in proliferating cells. *J. Natl Cancer Inst.* 1979; **62**: 3–8.
18 Calder PC. Fuel utilisation by cells of the immune system. *Proc. Nutr. Soc.* 1995; **54**: 65–82.
19 Gleeson M, Blannin AK, Walsh NP, Bishop NC, Clark AM. Effect of low and high carbohydrate diets on the plasma glutamine and circulating leukocyte responses to exercise. *Int. J. Sport Nutr.* 1998; **8**: 49–59.
20 Cupps TR, Fauci AS. Corticosteroid-mediated immunoregulation in man. *Immunol. Rev.* 1982; **65**: 133–55.
21 Mitchell JB, Pizza FX, Paquet A, Davis JB, Forrest MB, Braun WA. Influence of carbohydrate status on immune responses before and after endurance exercise. *J. Appl. Physiol.* 1998; **84**: 1917–25.
22 Pedersen BK, Helge J, Richter E, RhodeT, Ostrowski K, Kiens B. Training and natural immunity: Effects of diets rich in fat or carbohydrate. *Eur. J. Appl. Physiol.* 2000; **82**: 98–102.
23 Endres S, Meydani SN, Ghorbani R, Schindler R, Dinarello CA. Dietary supplementation with n-3 fatty acids suppresses interleukin-2 production and mononuclear cell proliferation. *J. Leukoc. Biol.* 1993; **54**: 599–603.
24 Konig D, Berg A, Weinstock C, Keul J, Northoff H. Essential fatty acids, immune function and exercise. *Exerc. Immunol. Rev.* 1997; **3**: 1–31.
25 Nehlsen-Cannarella SL, Fagoaga OR, Nieman DC *et al.* Carbohydrate and the cytokine response to 2.5 h of running. *J. Appl. Physiol.* 1997; **82**: 1662–7.
26 Bishop NC, Blannin AK, Rand L, Johnson R, Gleeson M. Effects of carbohydrate and fluid intake on the blood leucocyte responses to prolonged cycling. *J. Sports Sci.* 1999; **17**: 26–7.
27 Bishop NC, Blannin AK, Robson PJ, Walsh NP, Gleeson M. The effects of carbohydrate supplementation on immune responses to a soccer-specific exercise protocol. *J. Sports Sci.* 1999; **17**: 787–96.
28 Nieman DC, Fagoaga OR, Butterworth DE *et al.* Carbohydrate supplementation affects blood granulocyte and monocyte trafficking but not function after 2.5 h running. *Am. J. Clin. Nutr.* 1997; **66**: 153–9.
29 Nieman DC, Henson DA, Garner EB *et al.* Carbohydrate affects natural killer cell redistribution but not function after running. *Med. Sci. Sports Exerc.* 1997; **20**: 1318–24.
30 Bishop NC, Blannin AK, Rand L, Johnson R, Gleeson M. The effects of carbohydrate supplementation on neutrophil degranulation responses to prolonged cycling. *Int. J. Sports Med.* 2000; **21** (Suppl. 1): S78.
31 Nagatomi R, Tamagawa A, Yoshida S, Omori H, Yamauchi Y. Glucose intake prevents the increase in the number and the functional failure of circulating granulocytes that develops during prolonged exercise. Proceedings of Biochemistry of Exercise, Aberdeen. *Clin. Sci.* 1994; **87**: 89.
32 Henson DA, Nieman DC, Parker JCD *et al.* Carbohydrate supplementation and the lymphocyte proliferative response to long endurance running. *Int. J. Sports Med.* 1998; **19**: 574–80.
33 Mackinnon LM. *Advances in Exercise Immunology.* Champaign (IL): Human Kinetics, 1999.
34 Ford J, Trevatt N, Dix CA, Fallowfield JL. The effect of fluid replacement and heat on salivary flow rate and optical density at 280 nm in response to exercise. *J. Sports Sci.* 1997; **15**: 44–5.
35 Bishop NC, Blannin AK, Gleeson M. Effect of carbohydrate and fluid intake during prolonged exercise on saliva flow and IgA secretion. *Med. Sci. Sports Exerc.* 2000; (in press).
36 Nieman DC, Nehlsen-Cannarella SL, Fagoaga OR *et al.* Immune response to two hours of rowing in elite female rowers. *Int. J. Sports Med.* 1999; **20**: 476–81.
37 Bishop NC, Blannin AK, Walsh NP, Gleeson M. Effect of carbohydrate supplementation on human neutrophil degranulation responses following cycling to fatigue. *J. Physiol.* 2000; **523P**: 225P.
38 Lemon PWR. Effect of exercise on protein requirements. In: Williams C, Devlin J (eds). *Foods, Nutrition and Sports Performance*. London: E & FN Spon, 1992; 65–86.
39 Daly JM, Reynolds J, Sigal RK, Shou J, Liberman MD. Effect of dietary protein and amino acids on immune function. *Crit. Care Med.* 1990; **18** (2 Suppl.): S86–93.
40 Greenhaff PL, Gleeson M, Maughan RJ. The influence of an alteration in diet composition on plasma and muscle glutamine levels in man. *Clin. Sci.* 1988; **74**: P20.
41 Ardawi MSM, Newsholme EA. Glutamine metabolism in lymphoid tissues. In: Haussinger D, Sies H (eds). *Glutamine Metabolism in Mammalian Tissues*. Berlin: Springer-Verlag, 1994; 235–46.
42 Parry-Billings M, Budgett R, Koutedakis Y *et al.* Plasma amino acid concentrations in the overtraining syndrome: Possible effects on the immune system. *Med. Sci. Sports Exerc.* 1992; **24**: 1353–8.
43 Rennie MJ, Edwards RHT, Krywawych S, Davies CTM, Halliday D. Effect of exercise on protein turnover in man. *Clin. Sci.* 1981; **61**: 627–39.
44 Walsh NP, Blannin AK, Robson PJ, Gleeson M. Glutamine, exercise and immune function: Links and possible mechanisms. *Sports Med.* 1998; **26**: 177–91.
45 Kingsbury KJ, Kay L, Hjelm M. Contrasting amino acid patterns in elite athletes: Association with fatigue and infection. *Br. J. Sports Med.* 1998; **32**: 25–33.
46 Castell LM, Poortmans JR, Newsholme EA. Does glutamine have a role in reducing infections in athletes? *Eur. J. Appl. Physiol.* 1996; **73**: 488–90.
47 Rohde T, Asp S, Maclean D, Pedersen BK. Competitive sustained exercise in humans, and lymphokine activated killer cell activity – An intervention study. *Eur. J. Appl. Physiol*. 1998; **78**: 448–53.
48 Walsh NP, Blannin AK, Bishop NC, Robson PJ, Gleeson M. Effect of oral glutamine supplementation on human neutrophil lipopolysaccharide-stimulated degranulation following prolonged exercise. *Int. J. Sport Nutr.* 2000; **10**: 39–50.
49 Krzykowski K, Wolsk Petersen W, Ostrowski K *et al.* Effects of glutamine and protein supplementation on exercise-induced decrease in lymphocyte function and salivary IgA. *Int. J. Sports Med.* 2000; (in press).
50 Clarkson PM. Minerals: Exercise performance and supplementation in athletes. In: Williams C, Devlin J (eds). *Foods,*

Nutrition and Sports Performance. London: E & FN Spon, 1992; 113–46.

51 Erp-Baart AMJ, Saris WHM, Binkhorst RA, Elvers JWH. Nation-wide survey on nutritional habits in elite athletes, part II. Mineral and vitamin intake. *Int. J. Sports Med.* 1989; **10**: S11–16.

52 Goldfarb AH. Antioxidants: Role of supplementation to prevent exercise-induced oxidative stress. *Med. Sci. Sports Exerc.* 1993; **25**: 232–6.

53 Packer L. Oxidants, antioxidant nutrients and the athlete. *J. Sports Sci.* 1997; **15**: 353–63.

54 Peters EM. Exercise, immunology and upper respiratory tract infections. *Int. J. Sports Med.* 1997; **18** (1 Suppl.): S69–77.

55 Duthie GG, Jenkinson AM, Morrice PC, Arthur JR. Antioxidant adaptations to exercise. In: Maughan RJ, Shirreffs SM (eds). *Biochemistry of Exercise IX.* Champaign: Human Kinetics, 1996; 465–70.

56 Peters EM, Goetzsche JM, Grobbelaar B, Noakes TD. Vitamin C supplementation reduces the incidence of post-race symptoms of upper respiratory tract infection in ultramarathon runners. *Am. J. Clin. Nutr.* 1993; **57**: 170–4.

57 Nieman DC, Henson DA, Butterworth DE *et al.* Vitamin C supplementation does not alter the immune response to 2.5 hours of running. *Int. J. Sport Nutr.* 1997; **7**: 173–84.

58 Quintanuha AT, Packer L. Vitamin E, physical exercise and tissue oxidative damage. In: Porter R, Whelan J (eds). *Biology of Vitamin E*. London: Pitman, 1983; 56–69.

59 Cannon JG, Meydani SN, Fielding RA *et al.* Acute phase response in exercise II. Association between vitamin E, cytokines, and muscle proteolysis. *Am. J. Physiol.* 1991; **260**: R1235–40.

60 Prasad JS. Effect of vitamin E supplementation on leukocyte function. *Am. J. Clin. Nutr.* 1980; **33**: 606–8.

61 Chandra RK. Nutrition and immunity: Lessons from the past and new insights into the future. *Am. J. Clin. Nutr.* 1991; **53**: 1087–101.

62 Dreisen S. Nutrition and the immune response – A review. *Int. J. Vitamin Nutr. Res.* 1978; **49**: 220–8.

63 Peters EM, Campbell A, Pawley L. Vitamin A fails to increase resistance to upper respiratory infection in distance runners. *S. Afr. J. Sports Med.* 1992; **7**: 3–7.

64 Williams MH. *Nutrition for Fitness and Sport*, 4th edn. Dubuque: Brown and Benchmark, 1995.