Consensus Statement
Immunonutrition and Exercise

Stéphane Bermon[1], Lindy M Castell[2], Philip C Calder[3], Nicolette C Bishop[4], Eva Blomstrand[5], Frank C Mooren[6], Karsten Krüger[6], Andreas N Kavazis[7], John C Quindry[8], David S Senchina[9], David C Nieman[10], Michael Gleeson[11], David B Pyne[12], Cecilia M Kitic[13], Graeme L Close[14], D Enette Larson-Meyer[15], Ascension Marcos[16], Simin N Meydani[17], Dayong Wu[17], Neil P Walsh[18], Ryochi Nagatomi[19].

1 Monaco Institute of Sports Medicine and Surgery, Monaco, and University Côte d'Azur, LAMHESS, Nice, France
2 Green Templeton College, University of Oxford, Oxford, UK.
3 Human Development & Health Academic Unit, Faculty of Medicine, University of Southampton, Southampton, UK.
4 School of Sport, Exercise and Health Sciences, Loughborough University, Leics, UK.
5 Swedish School of Sport and Health Sciences, Stockholm, Sweden
6 Department of Sports Medicine, Justus-Liebig University, Giessen, Germany
7 School of Kinesiology, Auburn University, Auburn, AL, USA;
8 Health and Human Performance, University of Montana, Missoula, MT, USA
9 Kinesiology Program, Biology Department, Drake University, Des Moines, IA, USA
10 Appalachian State University, Human Performance Laboratory, North Carolina Research Campus, Kannapolis, North Carolina, USA
11 School of Sport, Exercise and Health Sciences, Loughborough University, Leics, UK.
12 Australian Institute of Sport, Australia
13 Sport Performance Optimisation Research Team, School of Health Sciences, University of Tasmania, Launceston, Tasmania, Australia
14 Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, UK
15 Nutrition & Exercise Laboratory, Department of Family and Consumer Sciences, University of Wyoming, Laramie, WY, USA
16 Institute of Food Science, Technology and Nutrition (ICTAN), Spanish National Research Council (CSIC), Madrid, Spain
17 Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA, USA
18 School of Sport, Health and Exercise Sciences, Bangor University, Bangor, United Kingdom
19 Laboratory of Health & Sports Science, Tohoku University Graduate School of Biomedical Engineering, Sendai, Japan

Key words: Aging; Biomarkers; Exercise; Immune system; Inflammation; Nutrition; Obesity

Acknowledgements

The co-editors (SB and LC) are very grateful to the authors for the time they have spent not only on providing appropriate articles for the series but also (together with the EIR Board) offering helpful critiques of the full document. The International Association of Athletics Federations (IAAF) is gratefully acknowledged for supporting the editing costs. The help of Anna Carlqvist in dealing with the references is warmly appreciated.

Address for Correspondence:
Stéphane Bermon, Monaco Institute for Sports Medicine and Surgery (IM2S), 11 avenue d'Ostende, 98000, Monaco
Phone: +377 99991000, Email: bermon@unice.fr

CONSENSUS STATEMENT

The first indexed scientific publication about immunonutrition is almost 70 years old (103). Since 1947, more than 10,000 scientific articles have been published in that field representing a consistent body of knowledge. Within this field, exercise (acute or chronic) modalities are of recent interest, and only approximately 400 publications exist so far. One third of them has been published during the last three years showing that immunonutrition and exercise is a fast developing area of research. This can be explained on the one hand by the considerable development of the global sports nutrition market. On the other hand, it is also due to high levels of expectation from both elite athletes and those who are keen on the concept of "Exercise is Medicine". High level athletes are very frequently exposed to high intensity or exhausting training programmes, travel, sleep disturbances, psycho-social and environmental stressors. All these factors are potential immune disruptors sometimes leading to immunodepression and increased likelihood of illness.

In order to minimise these phenomena and to optimise recovery, nutritional interventions are often considered by athletes and their entourages as possible countermeasures to the training-related immunodepression. However, among the numer-

ous nutrients available, only a few of them have so far shown any positive effects in maintaining athlete immune health. Moreover, elderly and overweight/obese individuals who demonstrate increased inflammatory status and immune dysfunction are often prescribed physical training programmes as a countermeasure. In such circumstances, nutrition appears as a possible valuable additional support to these populations. As significant biotechnological progress has been achieved during the last fifteen years, it is of critical importance, when designing an experiment in the field of immunonutrition and exercise, to select adequate biomarkers which fit best to the research aim and the experimental design.

In this consensus statement on immunonutrition and exercise, a panel of knowledgeable contributors from across the globe provides a consensus of updated science, including the background, the aspects for which a consensus actually exists, the controversies and, when possible, suggested directions for future research.

This consensus statement series includes an introduction section (Stephane Bermon and Philip Calder) followed by sections on: carbohydrates (Nicolette Bishop); fatty acids (Philip Calder); branched chain amino acids (Eva Blomstrand); glutamine (Lindy Castell); polyphenols (David Nieman) and herbal supplements (David Senchina); antioxidants (Andreas Kavazis and John Quindry); minerals (Frank Mooren and Karsten Krüger); probiotics and prebiotics (Michael Gleeson and David Pyne); vitamin D (Graeme Close and Enette Larson-Meyer) and bovine colostrum (Cecilia Kitic). It also contains some specific sections on: immunonutrition in competitive athletes and military personnel (Neil Walsh), exercising obese and overweight (Ascension Marcos, and elderly (Simin Meydani and Dayong Wu) individuals; biomarkers used in immunonutrition, and exercise science (Neil Walsh, Simin Meydani, Dayong Wu, and Ryochi Nagatomi).

Carbohydrates are fuel for the immune cells. As far as immune functions are concerned, carbohydrates appear to be more effective when ingested during exercise rather than increasing their relative content in the daily diet. Carbohydrates have been shown to minimise some of the immune perturbations that are associated with strenuous or lasting physical exercise and can be considered as a partial countermeasure for exercise-induced immunodepression. However, carbohydrates have failed so far to demonstrate any reduction in the incidence of upper respiratory tract illness (URTI) after prolonged exercise.

There is evidence from *in vitro*, animal and epidemiological studies that several saturated fatty acids promote inflammatory processes through the omega-6 (n-6) polyunsaturated fatty acids (PUFA) and arachidonic acid pathway. n-6 PUFAs have also shown some immunodepressive effects. These phenomena occur whatever the origin of arachidonic acid: meat, eggs or plants. In untrained individuals, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) appear to decrease the exercise-induced inflammation and muscle soreness. However, most of the studies on fatty acids and immune functions and inflammation in the context of exercise have provided data which is difficult to interpret.

Similar to recommendations in sports nutrition, there is no scientific ground for an athlete to consume an excessive amount of proteins (more than 2 g/kg body weight per day) in order to boost his or her immune system or limit an exercise-induced inflammation. Despite a good rationale for glutamine (Gln) supplementation based on sound biochemical investigation, laboratory-based exercise studies have proved mainly negative in terms of providing any direct enhancement of immune function due to Gln feeding. More studies need to investigate the apparent link between glutamine and the decreased incidence of self-reported URTI. During prolonged exercise branched chain amino acids (BCAA; leucine, isoleucine, valine) are oxidized as substrates and their plasma concentration decreases. An increase in BCAA plasma concentration can help to prevent a decrease in the plasma Gln concentration, and might therefore have the capacity to influence the immune response indirectly. However, the evidence for such an effect is weak.

Zinc (Zn), magnesium (Mg) and iron (Fe) are important minerals for immune function. As these minerals often show reduced concentration or availability during exercise or training, it is important to check that the athlete's diet contains sufficient quantity of these elements. However, there is no evidence showing that supplementing non-deficient athletes might boost the immune system or prevent exercise-induced immunodepression. Selenium (Se) and manganese (Mn) cannot be classified as immunonutrients for exercise.

Prolonged, exhaustive exercise and immune system activation are associated with an increased production of reactive oxygen species (ROS), leading to a potential increase in oxidative stress. However, there is no data to support links between exercise-induced oxidative stress and immune dysfunction or the postulated benefits of dietary antioxidant supplementation in preventing immune dysfunction during exercise, or in reducing the risk of respiratory illness in athletes. Emerging evidence indicating that antioxidant supplementation mitigates important exercise-induced adaptations (including the immune system) contributes to the debate for and against antioxidant supplementation in athletes.

Herbal supplements are widely used by athletes either to improve their performance or to boost their immune system. However, few human *in vivo* studies focusing on specific immune parameters are available and most of the available studies use *ex vivo* or *in vitro* conditions. Results from these studies are conflicting and are often not in full agreement with the purported immunomodulatory claims from the food supplement industry. For example, as therapeutic immunomodulators for athletes, there is some evidence that echinacea may be efficacious whereas the evidence for ginseng is poor. Polyphenols, including flavonoids are mostly found in tea, coffee, fruits and wine. They exhibit strong anti-inflammatory, antioxidant, anti-pathogenic, and immuno-regulatory properties *in vitro.* Epidemiological data in general support that polyphenol-rich plant extracts and unique polyphenol-nutrient mixtures have small but significant effects in increasing antioxidant capacity, with inconsistent, short-term effects on mitigating exercise-induced oxidative stress, inflammation, and immune dysfunction. Quercetin consumed at high doses (500

to 1,000 mg/day) has been linked to reduced incidence of self-reported URTI in athletes.

Probiotics are interesting immunonutrients since they demonstrate immunomodulation properties on both local and systemic (some aspects of both innate and acquired immune responses) immunity. In non-athletic populations, a recent systematic review concluded that probiotic use resulted in a lower incidence of URTI, reduced numbers of illness days, and fewer days of absence from day care/school/work. Despite a lower number of studies, the same benefits seem to exist in athletic populations. Although a daily dose of ~10^{10} live bacteria is widely promoted, there is still some debate about the optimal duration of supplementation and the potential benefits of selecting and mixing specific bacterial strains with or without prebiotics.

Bovine colostrum exhibits antibacterial, anti-inflammatory and anti-viral properties. Several investigations have reported a reduction (not always statistically significant) in self-reported URTI incidence in athletes following a period greater than four weeks of bovine colostrum supplementation. However, the effect of bovine colostrum on illness duration is less conclusive.

A large number of different immune cells and functions are influenced by vitamin D. These effects are mainly mediated through modulation of the expression of several genes. Optimal circulating 25-hydroxy vitamin D concentration is possibly beyond 75 nmol/l as individuals with such a vitamin D concentration demonstrated a lower incidence of URTI than those with an actually recommended vitamin D concentration (of around 50 nmol/l). Optimal vitamin D concentrations for immune cells require further study before they can be recommended to athletes who would like to maintain their immune function at the highest level without compromising their health.

As long as the diet meets the energy demands and provides sufficient macro- and micro- nutrients to support the immune system, there is probably no need for consumption of "immune boosting" supplements. However, there are specific scenarios when elite athletes or military personnel might benefit from nutritional supplements to bolster immunity. More randomized controlled trials in these individuals with sufficient participant numbers and rigorous designs are required to investigate whether the nutritional practices adopted by elite athletes impair immunity and increase infection; and, whether purported "immune boosting" supplements benefit immune health without blunting the desired training adaptations.

Obesity is related to immune dysfunction and chronic low grade inflammation. There is a consistent body of biological evidence attesting to the anti-inflammatory effects of regular physical training in obese or overweight individuals. Indeed, regular physical activity decreases toll-like receptor (TLR)-4 expression and induces shift from M1-type macrophages to M-2 type macrophages; both of these phenomenon promoting anti-inflammatory patterns. The anti-inflammatory effects of regular exercise are also partly mediated through interleukin (IL)-6 production at the muscle level. IL-6 triggers an anti-inflammatory cascade via the induction of the anti-inflammatory cytokines interleukin-1 receptor antagonist (IL-1ra) and IL-10, and also inhibits tumour necrosis factor (TNF)-α and its associated insulin resistance pattern. IL-6 also promotes fat oxidation which is beneficial to obese individuals. These immunological changes associated with training have been proven to be clinically relevant in many studies including obese adults and adolescents.

A decrease in cell-mediated immune function in the elderly (immunosenescence) contributes to higher morbidity and mortality. Nevertheless, aging appears to be linked with an increased inflammatory response. Given the focus on exercise-induced immunodepression in this series and the journal, it seems likely that extreme exercise will exacerbate immune system impairment in aging. Moderate regular exercise, however, may even enhance immune function in the elderly. For example, calisthenic exercise increases the function of natural killer (NK) cells and T-cells in older women. It is not known whether the exercising elderly have specific nutritional needs, although many appear deficient in micronutrients essential for immune function. In addition, increased inflammation, oxidative stress and muscle damage suggest that exercising older adults might require nutrients with immune enhancing and/or anti-inflammatory properties. In terms of energy provision, total calorie intake should be adjusted to avoid conversion of excess caloric intake to body fat. Moreover, glucose tolerance and insulin sensitivity decrease with aging.

Currently, a single marker able to predict the effect of a dietary and/or exercise intervention on different aspects of immune function does not exist. The range of available biomarkers is quite wide from *in vitro* tests to clinical symptoms. However, each biomarker should be carefully chosen according to its intrinsic characteristics (links with causal pathway and clinical endpoint, biological sensitivity and specificity, feasibility, practicality, and cost) as well as the designed study's aim and primary outcome. Mechanistic studies or studies aiming at testing hypotheses at molecular, cellular or immune function levels should rather consider *in vitro* or *ex vivo* biomarkers. Whereas *in vivo* biomarkers or biomarkers relying on patient symptoms should be preferred in experiments describing integrated response or clinical studies.

INTRODUCTION: IMMUNONUTRITION, INFLAMMATION AND EXERCISE

Physical exercise (chronic or acute) influences the immune system and its functions. All immune components or functions, systemic, local or mucosal, innate or adaptive, cellular or cytokine-related are positively or negatively linked with exercise regimens (406). This body of knowledge represents the interdisciplinary field of Exercise Immunology.

Similarly, the diet (macro and micronutrients, as well as non-nutritive components) is known to influence the immune system and its functions. Quantitative aspects (from protein-energy malnutrition to unbalanced Western diets) as well as qualitative aspects (oligo elements, vitamins, mineral, anti-oxi-

dants, plant-derived immunomodulators, probiotics, amino acids, and fatty acids) can either stimulate or inhibit selective immune functions or inflammation. For instance, consumption of dietary fibres reduces chronic inflammation by decreasing lipid oxidation (124). Fibres also interact with the gut microbiota via short-chain fatty acids produced during colonic fermentation (251). Fibres from oats or barley smooth the rate of appearance of glucose in the blood, reducing the glycaemic index and glycaemic load, and as a consequence production of nitric oxide, superoxide and peroxynitrite which are powerful pro-oxidant and pro-inflammatory molecules (80). Whole-grain foods also exert anti-inflammatory properties, such as free radical scavenging, antioxidant enzyme activation, or modification of the redox status of tissues and cells (124). These close interactions between diet and the immune system are the genesis of the term "Immunonutrition" which represents another new interdisciplinary field of basic and applied research.

As the immune system and inflammation, one of its major effectors, are regulated by both exercise and nutrition, it is of particular interest to address how nutrients can affect immunity in an exercise perspective. However, when nutrition is concerned, it appears that the commitment and the goals to achieve are very different when comparing a sedentary overweight individual to a high-level athlete. Indeed, as inappropriate exercise regimens or training programmes may alter some immune functions and promote illnesses (306), elite athletes are always considering diet and nutrition plans as possible countermeasures to the so-called exercise-induced immunodepression. This latter term is more appropriate than the traditionally used term "immunosuppression" which means specific manipulation of the immune system, e.g. via cyclosporin.

However, among the numerous nutrients and foods promoted for their purported immuno-modulating effects, only a very limited number has proved to be effective in maintaining or restoring some immune functions or preventing illnesses. This consensus statement series addresses the issue of macronutrients, probiotics, vitamin D, antioxidants and plant-derived immunomodulators, minerals and some promising dietary compounds as immune support for exercising humans. It also deals with immunonutrition in exercising overweight or elderly individuals and explores the relevance of selected immune/inflammation markers commonly used when designing a nutrition study in exercise immunology.

When the diet is inappropriate (more likely excessive high glycaemic index foods and/or caloric intakes), a part of the innate immune system is overreacting to the excessive amount of visceral fat leading to a chronic, low grade inflammation and potential subsequent inflammation-related diseases. Here, regular exercise is considered as a potential countermeasure to the inflammatory-driven morbidities such as cardiovascular diseases, chronic obstructive pulmonary diseases, colon and breast cancers, insulin resistance, type II diabetes, and some neurodegenerative diseases (307,326).

The anti-inflammatory effects of exercise are achieved through several possible pathways (143). The reduction in visceral fat mass associated with a secondary reduced release of adipokines is one of the main mechanisms. Moreover, following each bout of exercise, the release of high amounts of cortisol and adrenaline associated with an increased production and release of IL 6 and other mediators now often referred to as "myokines" from working skeletal muscles contribute to the generation of an anti-inflammatory "milieu". IL-6 is pleiotropic: it may have different actions in different contexts, and thus may not always act in a manner that could be described as pro-inflammatory.

At the cellular level, a reduced expression of TLR on monocytes and macrophages and a subsequent inhibition of downstream pro-inflammatory cytokines production are observed. Within the adipose tissue quantitative and qualitative changes in monocytes-macrophages are noted. The number of M1-type macrophages is decreased as well as their associated pro-inflammatory cytokines (IL-6 and TNF-α), whereas M2-type macrophage numbers and their anti-inflammatory cytokines (IL-10 and adiponectin) are increased (205). Franceschi and colleagues (133) introduced the concept of 'inflammaging' as part of the spectrum of immunosenescence. Inflammaging is the chronic low-grade inflammatory state present in aging individuals and is believed to be a consequence of a remodelling of the innate and acquired immune system. It is characterized by increased systemic concentrations of pro-inflammatory cytokines such as IL-1, IL-6, and TNF-α (398) as well as increased C-reactive protein (CRP) concentration which are used as clinical markers. Inflammaging increases the risk of morbidities and age-related diseases, and is also associated with increased skeletal muscle wasting, strength loss, and functional impairments. In this particular context, both nutrition (115) and exercise (219) interventions are proposed for elderly or frail individuals as a countermeasure of the aging process and its associated inflammation-related diseases. This provides the rationale for producing the present consensus statements.

Most athletes, whether recreational or elite, and in all parts of the world, use sports foods and supplements. The popularity of certain types of dietary supplements demonstrates that athletes may often be more motivated by an interest in health benefits rather than, for example, direct ergogenic effects. In this series of consensus statements on immunonutrition, athletes are therefore strongly advised not only to seek the advice of a properly qualified nutritionist before embarking on supplementation but also to pay careful attention to the importance of the recommended daily allowance (RDA). It is a common misconception among athletes that, if x g of a product works, then taking double that amount will be even better! In fact, this approach is very likely to lead to health problems rather than to solutions. Readers can find more detailed discussion on this topic in Castell et al. (78).

CARBOHYDRATES

Background

The role of carbohydrate as an ergogenic aid for performance has long been recognised. The recommended daily carbohydrate intake for athletes who train for one to three hours each

day is 6–10 g/kg body mass increasing to 8–12 g/kg body mass for athletes training more than four hours each day, with additional intake of 30-60 g/h during exercise lasting for 1 hour or more (384). This guidance is principally aimed at restoring muscle and liver glycogen stores before exercise and maintaining blood glucose levels during exercise to ensure sufficient glucose availability for skeletal muscle contraction. However, carbohydrate availability also has the potential to limit the degree of exercise-induced immune dysfunction through direct or indirect actions. Directly, glucose acts as a fuel substrate for immune cells (12) therefore it could be argued that post-exercise hypoglycaemia could endanger immune cell function. However, the significance of this alone is questionable given that immune cells do not rely solely on glucose for energy. Conversely, since both catecholamines (adrenaline, noradrenaline) and cortisol are known to have potent modulatory effects on immune function (208) increasing carbohydrate availability may more likely act indirectly by reducing the stress hormone response to the exercise, thereby limiting exercise-induced immune impairments.

Consensus: carbohydrates, exercise and immune function

Dietary carbohydrates

Performing exercise at around 70% VO_2max for at least 1 hour following several days on very low carbohydrate diets (typically less than 10% of dietary energy intake from carbohydrate) is associated with a greater adrenaline and cortisol response, higher circulating neutrophil counts and modest depressions in circulating lymphocyte counts. These effects are diminished when exercising following a high (typically more than 70% of energy intake) carbohydrate diet (36,259). High carbohydrate diets prior to exercise are also associated with a blunted cytokine response (e.g. IL-6, IL-10 and IL-1ra) (37), thought to be related to a reduced need for IL-6 to exert its glucoregulatory actions (309).

In contrast, increasing dietary carbohydrate does not appear to exert any beneficial effects on either resting or post-exercise immune cell functions. A high or a low carbohydrate diet for several days was associated with similar levels of bacterially-stimulated neutrophil degranulation and mitogen-stimulated lymphocyte proliferation before exercise, and resulted in a similar magnitude of impairment post-exercise (36,259).

Carbohydrate supplementation during exercise

Given the established association between cortisol and immune cell function, nutritional measures that attenuate exercise-induced elevations in plasma cortisol have been hypothesized to be effective in minimizing post-exercise immune impairments. Specifically, carbohydrate (compared with placebo) ingestion during exercise is suggested to limit exercise-induced falls in immune function by maintaining plasma glucose levels, thereby blunting the plasma cortisol response. While evidence from the literature largely supports this, there is evidence to suggest that the beneficial effects of consuming carbohydrate during exercise can also occur in the absence of any effect on plasma cortisol levels (35,153). Consuming around 60 g/h of carbohydrate during prolonged exercise attenuates the rise in plasma cytokines (270), attenuates the trafficking of most leucocyte subsets, apart from NK cells (174,285,289), prevents the exercise-induced fall in bacterially-stimulated neutrophil degranulation (38) and increases neutrophil respiratory burst activity (351). In addition, consuming carbohydrate (compared with placebo) during prolonged exercise prevents the decrease in both number and percentage of anti-viral Type 1 helper T cells and the suppression of interferon gamma (IFN-γ) production from these cells (224). Consuming carbohydrate during exercise also diminishes typical post-exercise decreases in T lymphocyte proliferation following mitogen or antigen (influenza) stimulation (34,174), an effect that was still evident 24 hours later (34). This may be partially related to lower T cell apoptosis within stimulated cell cultures when carbohydrate is consumed during exercise (153). Migration of immune cells to infected tissue is crucial to host defence, and carbohydrate ingestion (60 g/h) during prolonged exercise has been shown to attenuate post-exercise falls in T-lymphocyte migration into human rhinovirus-infected airway epithelial tissue (35).

Although carbohydrate feeding during exercise appears to be effective in minimizing some of the immune perturbations associated with prolonged strenuous exercise, it has minimal effect on salivary secretory immunoglobulin A (SIgA) secretion (32,284) or NK cell cytotoxic activity (285) but may increase NK cell responsiveness to IL-2 (254). Consuming carbohydrate seems less effective at minimizing more modest alterations in immune function during intermittent exercise with regular rest intervals (288), resistance exercise (279) and exercise to fatigue (33). Furthermore, consuming more than 60 g carbohydrate per hour has negligible additional benefit (224,350,351) most likely because the maximum rate of exogenous carbohydrate oxidation is around 1 g/min (i.e. 60 g/h; (400)). Finally, carbohydrate supplementation does not influence the decrease in *in vivo* immunity to a novel antigen seen after 2 hours of moderate intensity exercise in non-fasted runners (102).

There is insufficient evidence to date to support any beneficial effect of carbohydrate ingestion on symptoms of upper respiratory illness; one study of 93 runners who consumed placebo or carbohydrate during a marathon reported that, of the sixteen runners who reported illness in the fifteen days after the race, ten had consumed placebo and six had consumed carbohydrate (284).

Conclusion

Carbohydrate ingestion is a partial countermeasure against exercise-induced immune impairment and is more effective when consumed as a supplement during exercise than by increasing dietary content of carbohydrate on a routine basis. However, evidence that carbohydrate ingestion reduces the incidence of URTI after prolonged exercise is currently lacking.

FATTY ACIDS AS IMMUNOMODULATORS

Background

Fatty acids are a major component of most human diets and most fatty acids can be synthesised endogenously in the human body (54). Individual fatty acids are distinguished by

the length of their hydrocarbon chain, and by the absence, presence, number and configuration (*cis* or *trans*) of double bonds within that chain. Saturated and monounsaturated fatty acids can be synthesised *de novo* from precursors such as glucose. The simplest polyunsaturated fatty acids (PUFAs), linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3), cannot be synthesised in humans, but are synthesised in plants. Humans can metabolise these two essential fatty acids further, inserting additional double bonds (desaturation) and extending the hydrocarbon chain (elongation). Through these processes, linoleic acid can be converted to arachidonic acid (20:4n-6) and alpha-linolenic acid to eicosapentaenoic acid (EPA; 20:5n-3). Further metabolism to longer chain, more unsaturated derivatives is possible (e.g., of EPA to docosahexaenoic acid (DHA; 22:6n-3)).

The principal roles of fatty acids are as energy sources and membrane constituents (54). Certain fatty acids have additional, specific roles, such as serving as precursors for the synthesis of bioactive lipid mediators (e.g. prostaglandins), and influencing membrane and intracellular signalling processes, the activation of transcription factors and gene expression (62). Through these different actions, fatty acids are able to influence cellular functions and thus physiological responses, including immune and inflammatory responses (59).

Consensus

There is evidence from *in vitro*, animal and epidemiological studies that several saturated fatty acids promote inflammatory processes (334). Lipid mediators, including prostaglandins and leukotrienes, produced from the omega-6 (n-6) PUFA arachidonic acid are intimately involved in inflammation and many widely used anti-inflammatory drugs target arachidonic acid metabolism (106). Several of the mediators produced from arachidonic acid also suppress cell-mediated immune responses by targeting antigen-presenting cell and helper T-cell activities, acting in part via regulatory T-cells (106). Arachidonic acid is consumed in the diet from meat, eggs and organs such as liver, or it can be synthesised from the plant essential fatty acid linoleic acid. Thus, there is a widely held view that common n-6 PUFAs of both animal and plant origin are pro-inflammatory and immunodepressive. However, strong evidence that variations in dietary intake of linoleic acid do affect inflammation is lacking (197). Older research showed that γ-linolenic acid (18:3n-6) and its derivative dihomo-γ-linolenic acid (20:3n-6), which are both metabolic intermediates between linoleic and arachidonic acids, exert anti-inflammatory effects (360). There is limited exploration of the influence of saturated or n-6 PUFAs on immune function or inflammation in the context of exercise.

Oily fish and fish oil supplements contain the long-chain omega-3 (n-3) PUFAs EPA and DHA (60). EPA and DHA are also found in some algal oils and in krill oil. In each of these sources both the absolute amounts of EPA and DHA and their ratio can vary widely. There is substantial evidence from *in vitro*, animal, epidemiological and human intervention studies that the combination of EPA and DHA exerts anti-inflammatory actions (58,61) and may enhance cell-mediated immune function (59). The effects observed are dose-dependent and may require an intake of >2 g per day the combination of EPA and DHA (58,59,61). Both EPA and DHA can independently exert anti-inflammatory effects (5), and they have been shown to counter the effects of classic inflammatory stimuli like endotoxin as well as saturated fatty acids and n-6 PUFAs (61). EPA and DHA are readily incorporated into cell membranes, partly replacing arachidonic acid. Thus, they result in decreased production of pro-inflammatory and immunosuppressive omega-6-derived lipid mediators (61). In contrast, the analogous mediators produced from EPA are often only weakly bioactive (61). Importantly, both EPA and DHA are substrates for the biosynthesis of potent mediators which resolve inflammation and enhance immune function: these are termed resolvins, protectins and maresins (19). EPA and DHA act through several other mechanisms to decrease inflammatory responses of neutrophils, macrophages and endothelial cells. These mechanisms include reducing activation of the pro-inflammatory transcription factor NFκB and activation of peroxisome proliferator activated receptor γ (61). Within antigen-presenting cells, T-cells and B-cells EPA and DHA act by regulating key signalling events within the cell membrane (184).

Effects of the combination of EPA and DHA, usually as fish oil, or of DHA alone, on inflammation and immune function have been explored in a number of studies involving exercise protocols in both athletes and non-athletes. Several studies report that supplementation with EPA and DHA decreases the degree of muscle soreness induced by a bout of exercise in untrained individuals (201,382,383), although not all studies saw this (151). This effect also occurred with DHA alone at a supplemental intake of 3 g/day (91). Furthermore, in untrained individuals, EPA and DHA have been reported to diminish the exercise-induced elevation in pro-inflammatory cytokines including TNF-α and IL-6 (383). Once again this effect was also seen with DHA alone (108). Thus, the majority of studies suggest that omega-3 PUFAs decrease the inflammatory response induced by exercise in untrained individuals and that this translates to less muscle damage and soreness. In contrast, Gray et al. (2012; (152)) and Da Boit et al. (2015; (99)) both observed no effect of EPA and DHA on the plasma IL-6 response to an exercise bout, although the production of IL-2 by mitogen-stimulated blood mononuclear cells and natural killer cell activity were both enhanced post-exercise with prior omega-3 PUFA treatment in both of these studies. These observations suggest that omega-3 PUFAs might enhance aspects of cell-mediated and innate immunity post-exercise.

Andrade et al. (2007; (10)) reported that elite swimmers who took EPA and DHA for 45 days showed increased production of IL-2 and increased T-cell proliferation when peripheral blood mononuclear cells were stimulated with the mitogen phytohaemagglutinin, although IFN-γ production was decreased, making the findings difficult to interpret. There was no effect of EPA and DHA over 6 weeks on salivary IgA concentration prior to or after an exercise bout in trained cyclists (287). There was also no effect on pre-or post-exercise blood leucocyte numbers or the concentrations of C-reactive protein, IL-1β, IL-6 or IL-8 (287). A study of high dose n-3 PUFAs (3 g/day EPA plus 1.8 g/day DHA) given for 6 weeks to endurance trained males reported no effects on marathon-induced changes in blood leucocyte numbers or blood concentrations of TNF-α, IL-1ra, IL-6 or transforming

growth factor beta (388). Likewise, high dose EPA and DHA (2.2 g/day of each) for 6 weeks had no effect on treadmill exercise induced changes in several blood inflammatory markers in trained males (43). A small study in wheelchair basketball athletes found that EPA and DHA prevented the elevation in plasma IL-6, but not other pro-inflammatory cytokines, induced by an exercise bout, and prevented damage to neutrophils (244). Capo et al. (66) reported few effects of EPA and DHA on several plasma cytokines in elite soccer players undergoing an exercise bout, although there was a weaker TNF-α and IL-6 response of peripheral blood mononuclear cells to endotoxin in the n-3 group PUFA after exercise. This was linked to a lower exercise-induced upregulation of toll-like receptor 4 in the n-3 group (66). Other studies present results from studies of n-3 PUFAs on immune outcomes in athletes that are difficult to interpret (105,346).

Controversies and Future Directions

There is limited exploration of the influence of saturated or n-6 PUFAs on immune function or inflammation in the context of exercise. There are a number of studies of the long chain n-3 PUFAs EPA and DHA, usually in combination, on immune function or inflammation in the context of exercise in both untrained and trained individuals. These studies have used moderate (<1.0 g/day) to high (4 g/day) doses of EPA plus DHA for a period of one week to several months; some publications are not sufficiently clear about the n-3 dose used and many studies have involved a small number of subjects. Few studies have used the same design and the immune and inflammatory outcomes reported are highly variable between studies, although several report measurement of the same common plasma inflammatory cytokines. This makes it difficult to draw firm conclusions. However, EPA and DHA appear to decrease exercise-induced inflammation and muscle damage and soreness in untrained individuals. It is not yet clear whether EPA and DHA affect inflammation or immune function in trained individuals, although some studies suggest they might. Larger studies of duration of several weeks to months are recommended to explore better the effects of omega-3 PUFAs, and other fatty acids, on inflammation and immune function in trained individuals. Immune and inflammatory outcomes to be measured should be more carefully considered (2,3).

AMINO ACIDS

Amino acids are the building blocks for protein. Twenty amino acids build up the body proteins, nine of these are considered essential, that is, they have to be supplied through the diet; the remaining, non-essential, amino acids can be synthesized by the body. Exercise increases the oxidation of amino acids, and both synthesis and degradation of muscle protein are increased after exercise (312). The rate of synthesis can be elevated for up to 72 hours after exercise (258). Recent consensus indicates that amino acid requirements are increased with regular exercise training, suggesting that protein intakes as high as ~150-200% of current recommendations might be necessary for these individuals (313).

Several amino acids also have other important roles, for example serving as substrates in the synthesis of neurotransmitters, stimulating protein synthesis or improving immune function. A list of amino acids involved in immunology and their roles can be found in a comprehensive review by Li et al. (2007; (230)). The most studied amino acids in exercise immunology are Gln, BCAA, alanine and arginine. Gln and BCAA are the most studied in terms of supplementation before and after exercise.

Branched chain amino acids

During prolonged, fatiguing exercise the branched chain amino acids (BCAA) (leucine, isoleucine and valine) are taken up by the working muscle and their plasma concentration decreases (41,397). The BCAA are oxidized to provide energy but, more importantly, the metabolism of BCAA produces nitrogen for Gln synthesis.

Intake of BCAA rapidly increases their plasma and muscle concentration (42,154,260), and it is suggested that this will increase the production of Gln. A fall in the plasma concentration of Gln (p[Gln]) has been observed during/after sustained exercise (104,332), and has been proposed to be linked with exercise-induced immunodepression ((77,336); see Glutamine chapter). BCAA intake could therefore indirectly influence the immune response. However, despite the elevated levels of BCAA in plasma and muscle after intake of these amino acids, the release of Gln from the exercising muscle remains unchanged unless large amounts of BCAA are ingested (42,236,237). In contrast to these findings, chronic supplementation with BCAA to athletes was able to prevent the decrease in p[Gln] and immunodepression following a triathlon or a 30 km race (21). Furthermore, ten weeks of BCAA supplementation to trained cyclists prevented the increase in neutrophil number in trained cyclists which was observed without supplementation (207).

A direct effect of BCAA on cells of the immune system may also be conceivable since these amino acids, in particularly leucine, may stimulate protein synthesis and activate cytokine and antibody production through a direct effect on mTOR signalling (see (230)). However, there is currently no evidence for such an effect, and available data indicate that BCAA are required to maintain the high rate of protein synthesis in these cells rather than to stimulate the immune function (see (57,97)).

Consensus

There are some indications that BCAA intake can reduce exercise-induced immunodepression. However, there is currently not enough data from controlled studies to recommend BCAA ingestion in combination with exercise to enhance immune function.

Glutamine

Background

Glutamine is the most abundant amino acid in the body and was originally classified as a non-essential amino acid (340). However, since the 1990s there has been increasing evidence that Gln becomes “conditionally essential” in specific conditions of stress (73,220).

Gln is synthesized, stored and released predominantly by skeletal muscle and, to a lesser extent, by adipocytes, liver and lung. It is taken up by intestinal cells, such as enterocytes and colonocytes, by the kidney, liver and immune cells such as lymphocytes, macrophages and neutrophils. It has been suggested that Gln supplementation might improve the digestive and defence mechanisms of the intestine (100,410).

Gln is required by rapidly-dividing cells (213), providing nitrogen for purine and pyrimidine nucleotide synthesis, enabling synthesis of new DNA and RNA, for mRNA synthesis and DNA repair. Ardawi and Newsholme (1985; (12,13)) observed a high Gln utilisation by human lymphocytes at rest. Subsequent *in vitro* work (303) showed that when Gln was reduced in culture medium a decrease occurred in the proliferative ability of human lymphocytes. Gln and BCAA (see Amino acids section) are the most studied amino acids in terms of supplementation before and after exercise.

The p[Gln] is increased in athletes after short-term exercise (316). However, after prolonged, exhaustive exercise such as a marathon, the p[Gln] can be decreased by 20-25% (77,104). Similar decreases have been observed after repeated bouts of prolonged exercise (336). In two studies at moderate altitude (athletes, in summer) and high altitude (military personnel, in winter) a significant decrease in p[Gln] occurred after intensive training and coincided with a high incidence of URTI (16,79).

The post-exercise decrease in p[Gln] is often concomitant with a decrease in circulating lymphocyte numbers which transiently increase initially as part of the well-known leucocytosis observed after exhaustive exercise. Immune cell function is also decreased at this stage, for example, in both lymphocytes and NK cells. Rohde et al. (1996; (336)) observed a marked decrease in p[Gln] in triathletes at 2 hours after prolonged exercise, paralleled by changes in lymphokine activated killer (LAK) cell activities. A decrease in p[Gln] in marathon runners coincided with increases in acute phase markers such as the cytokine IL-6 and complement C5a, as well as an increased incidence of self-reported URTI (76,77).

There is some evidence that Gln, or a Gln precursor (BCAA), can lessen the incidence of exercise-induced URTI after marathon running (21,77). However, several studies, mostly laboratory-based, have shown no effect of maintaining a normal or high p[Gln] on various aspects of immune function. These included: LAK cell activity, lymphocyte numbers, some leucocyte subsets, salivary IgA, CD3 T-cell receptors, NK cells, leucocytosis, plasma elastase release from lipopolysaccharide (LPS)-stimulated neutrophils (216,335,337,403); CD8, CD4 with/without CD28 & 9 surface receptors (215), although the latter study also showed less neutrocytosis in the Gln group than the placebo group.

In a recent study Caris et al. (2014; (67)) observed a positive effect of both Gln and carbohydrate in modulating the Th1/Th2 (helper cells) balance after exercise. The post-exercise ratio of $CD4^+$ helper/ $CD8^+$ cytotoxic/suppressor cells has been seen to be higher in athletes provided with Gln rather than placebo after both marathon running (75) and heavy load training (373).

It has been suggested that muscle Gln is not markedly decreased as a result of exercise, although Rennie et al. (1981; (332)) did see a decrease in muscle Gln in their study, which also produced a biphasic response of p[Gln] to 3.75 hours of exercise. The decrease is markedly less than the pathologically low Gln concentrations observed in the muscle of critically ill patients (341). Current opinion considers that, in general, the body has sufficient stores of Gln to replenish post-exercise plasma reductions readily (see (139)). The time frame for this is not known. Interestingly, Hiscock et al. (2002; (180)) measured the intracellular content of Gln in peripheral blood mononucleocytes (PBMC), and found good availability of Gln for the cells after exercise.

The presence of glutaminase, the major degradation enzyme of Gln, was established in human neutrophils by Castell et al. (2004; (74)). There appears to be a link between production of the major neutrophil chemoattractant, IL-8, and Gln. In i*n vitro* studies the provision of Gln results in a decrease in IL-8 production in athletes (73), and in clinical studies in patients with acute pancreatitis (23). Provision of exogenous Gln might therefore lead to a decrease in the requirement for IL-8 secretion to attract more neutrophils to the site of tissue damage, though this is speculative.

IL-6 is probably the most studied cytokine (myokine) in exercise immunology. The plasma concentration of IL-6 increases markedly after strenuous and prolonged exercise and this increase was further enhanced after Gln supplementation (181). This might prove beneficial if, as has been suggested, IL-6 acts as an anti-inflammatory cytokine in exercise (311).

In regard to leucocytosis after endurance exercise, Fehrenbach et al. (1999; (126)) described a possible protective effect of heat-shock protein (HSP) in athletes. There is substantial evidence that Gln is important for HSP generation in both *in vitro* and *in vivo* studies (200,423,426). Zuhl et al., 2014 (433) observed anti-inflammatory effects of Gln via HSP70 on intestinal permeability and peripheral blood mononuclear cells. Raizel et al. (2016; (327)) recently showed that treating rats with oral free L-Gln (with L-alanine or as a dipeptide) induced cytoprotective effects via HSP70 after resistance exercise. HSP facilitates neutrophil activity (179,296): given the presence of glutaminase on the secretory granules of human neutrophils (74), the effect of Gln on the heat shock response might induce changes in neutrophil function.

Consensus

Despite a good rationale for Gln supplementation based on sound biochemical investigation, laboratory-based exercise studies have proved disappointing in terms of providing any direct enhancement of immune function due to Gln feeding (see Controversies). Recently, it has been suggested that there is sufficient Gln availability in body stores to combat post-exercise decreases in immune function after endurance events. Nevertheless, a decrease in p[Gln] may act as a marker for immunodepression and increased incidence of minor illnesses. Thus, a marked decrease in p[Gln] may indicate

decreased immunocompetence, in particular in the individual who is vulnerable to opportunistic infections. There are some indications that provision of Gln or a Gln precursor can lessen the incidence of exercise-induced URTI.

Controversies

Since p[Gln] decreases by approximately 20-25% after prolonged, exhaustive exercise, given its role in some key immune cells, this might be expected to have ramifications for immune function in athletes. There was a sound biochemical and clinical rationale for thinking that Gln provision might be a simple panacea for minor illnesses and for exercise-induced immunodepression. Despite the evidence that Gln or a Gln precursor can lessen the incidence of exercise-induced URTI, several laboratory-based studies have shown no effect of maintaining a normal or high p[Gln] on some specific aspects of immune function.

Future Directions

Data on the effects of supplementation with Gln or Gln precursors on neutrophil function in exercise have become increasingly interesting, and further investigation in humans should prove to be useful. Gln has a role in generating heat shock protein: this might have a protective effect on immunodepression in exercise, and more studies are required. There may also be other aspects of immune function as yet unstudied, which might respond more effectively to the provision of Gln before or after prolonged, exhaustive exercise.

MINERALS

Background

Several minerals are known to exert modulatory effects on immune function, including Zn, Mg, Fe, Se, and Mn. With the exception of Zn and Fe, isolated deficiencies are rare. Regarding exercise, requirements for some of these minerals are certainly higher in athletes compared with sedentary people. On the one hand, exercise has a pronounced effect on mineral metabolism; on the other hand, exercise increases losses in sweat and urine. However, excess intakes of some minerals are known to impair immune function. Earlier reviews have discussed mineral supplementation comprehensively (63,138). The present consensus statement considers supplementation of five specific minerals (Zn, Mg, Fe, Se and Mn) in relation to exercise.

Consensus

Zinc

The essential trace element Zn is an important co-factor of several enzymes and transcription factors and thereby involved in various physiological processes during growth, metabolism, and development. Studies with hereditary diseases of Zn deficiencies such as acrodermatitis enteropathica have demonstrated the importance of proper Zn levels for immune function of both adaptive and non-adaptive systems (187). Severe Zn deficiency in these patients is accompanied by several symptoms including enhanced susceptibility to infections. But even mild Zn deficiency occurring in populations at risk such as elderly people or vegetarians may result in impairment of NK cell lytic activity and T cell mediated functions (319). Intracellular Zn levels in T cells seem to be highly regulated and involved during T cell activation. In macrophages Zn seems to play a part in important anti-inflammatory roles by inhibiting NF-κB signalling. Beside its action on immune cells Zn seems to have direct anti-viral properties via Intercellular Adhesion Molecule (ICAM)-1 receptors on respiratory epithelial cells of the nasal epithelium (186).

The RDA of Zn for men and women in the US is 11 and 8 mg, respectively; in the EU a gender independent value of 10 mg is given. Zn can be found in a wide variety of foods like certain types of sea food such as oysters, crabs and lobsters, red meat, poultry, beans, nuts and whole grains. In contrast, bioavailabilty of Zn is impaired by phytates which are present in whole-grain breads, cereals, and legumes, and by Fe supplementation.

There is considerable mobilization of Zn during exercise into the blood, which is re-distributed soon after termination of exercise. Nevertheless losses of Zn via sweat and urine, in addition to reduced dietary intake, have been identified as major risk factors for Zn deficiency in athletes. Therefore a number of studies reported Zn deficiency (serum levels < 70 µg/dl) in elite athletes, especially in endurance athletes (257). However, the impact of these alterations on athletes' immune system/function remains to be shown. Therefore regular supplementation of Zn cannot be recommended. Nevertheless, there is some evidence from general population studies that Zn supplementation might be effective in the prevention and therapy of the common cold, which represents the major disease form of athletes during transient immunodepression in the early post-exercise period. A recent study presented weak evidence for Zn in the prevention of the common cold in children (6). In addition, recent meta-analysis including 17 trials and a total of 2121 participants presented moderate evidence that oral Zn formulations may shorten the duration of symptoms of the common cold (354). It has been suggested that supplementation should start within 24 hours of the onset of symptoms (367). Based on these studies, a transient supplementation during periods of intensive exercise bouts together with psychological stress such as during competition might be beneficial, especially if a history of recurrent infections exists. Side effects of Zn supplementation include bad taste and nausea.

Magnesium

Mg is an essential biological element which is predominantly located in bones (approx. 52%), in muscle cells (28%), and soft tissue (19%). Serum and red blood cells contain only 0.3% and 0.5%, respectively. In general, Mg is involved as an important regulator in three main physiological processes; 1) enzyme activation, e.g. during energy metabolism, 2) stabilizing membrane function and integrity, 3) cell signalling, e.g. as a natural antagonist of intracellular calcium signals (263). With respect to the function of the immune system Mg seems to be involved in the following steps: cofactor for immunoglobulin synthesis, immune cell adherence, antibody-dependent cytolysis, activation of macrophages. Moreover, Mg deficiency is associated with clinical signs of inflammation, such as immune cell activation and enhanced levels of circulating inflammatory mediators (222).

The concentration of total serum Mg is approximately 0.75-1.1 mmol/l, which is, however, a rather poor indicator of the body's Mg status. Serum acts as a transit pathway between electrolyte uptake and excretion, bone stores and actively metabolising tissues. These processes are affected by a number of hormones such as parathyroid hormone, calcitonin, vitamin D, insulin, glucagon, antidiuretic hormone, aldosterone and sex steroids.

Exercise-induced alterations of serum Mg seem to depend on exercise intensity and duration. After short-term, high-intensity exercise the majority of studies indicated an increase of extracellular Mg; however, after prolonged submaximal exercise most studies reported a hypomagnesaemia (56). It seems unlikely that sweat Mg losses and/or enhanced renal Mg excretion alone account for this decrease in serum. Some authors suggested therefore that, during prolonged exercise, a shift of Mg into the cellular compartment occurs. Longitudinal and cross-sectional studies demonstrated that intensive training periods may be followed by Mg depletion and that athletes are prone to Mg deficiency (348).

Therefore it can be speculated that the exercise-associated changes in immune function especially in the early post-exercise period might be aggravated in Mg-deficient athletes (222). In contrast, it has been demonstrated that Mg supplementation did not prevent exercise-induced alterations of immune parameters in athletes with balanced Mg status (262). Therefore, Mg supplementation can be recommended only after diagnosis of Mg deficiency which relies on both clinical symptoms and laboratory diagnosis (serum Mg < 0.75 mmol/l is considered to be a useful measurement for severe deficiency). Important food sources of Mg are vegetables, fish, nuts, and whole grains. Mg formulations include both inorganic and organic compounds of which the latter seemed to have a better bioavailability.

Iron

Fe is an essential nutrient which is primarily used as a cofactor for enzymes in the mitochondrial respiratory chain, in the citric acid cycle and during DNA synthesis, as well as being the central molecule for binding and transport of oxygen by haemoglobin and myoglobin (414).

For immunity, Fe is important for lymphocyte proliferation and differentiation while it interferes with cell mediated immune effector pathways and cytokine activities (356,414). Furthermore, Fe exerts multiple effects on macrophage polarization and functionality (269).

Changes in Fe status can thus affect the immune response in multiple ways, particularly in the context of infection (82). The RDA is 18 and 8 mg for women and men respectively. Sources of Fe are flesh foods, vegetables and grains. The haem Fe, found in meat products, is best absorbed. In general, male athletes tend to consume at least the RDA for Fe, but female athletes tend to consume somewhat less (166). If this under-supply is combined with heavy Fe loss by menstruation, haemolysis, gastrointestinal bleeding, inflammatory status by heavy physical activity or loss by sweat, Fe balance may be compromised (235,253). Accordingly, Fe deficiencies have been reported mainly in women competing in running, field hockey, cross country skiing, basketball and others (253). In this case, the use of Fe fortified foods and Fe supplements may be considered (51). Therefore, Fe supplementation in combination with vitamin C should be recommended for athletes with Fe deficiency anaemia and monitored carefully for prophylaxis. During infection the supplementation of some minerals like Fe is not recommended because it is suggested that pathogenic microorganisms might benefit (107). However, an immunological effect of Fe supplementation in the context of exercise has not been shown so far.

Selenium and Manganese

Se status may affect the function of cells of both adaptive and innate immunity. Currently, the recommended amounts for adequate Se intake of adults range between 25 and 100 µg/day, with an average of 60 µg/day for men and 53 µg/day for women (328,376). Neither Se deficiencies nor immunological effects of supplementation in athletes have been described yet. For Mn, daily intake through dietary sources provides the necessary amount required for several key physiological processes, including antioxidant defence, energy metabolism, immune function and others. During exercise, Mn might play a role as an antioxidant since a superoxide dismutase in the mitochondrial matrix functions with Mn. There is no evidence for neither deficiency nor supplementation for Se nor Mn in athletes, thus both minerals cannot be classified as immunonutrition during exercise (81). An overview about these minerals, their immune related functions, symptoms of deficiency, deficiencies in sports and recommendations for supplementation is given in Table 1.

Future directions

While there is evidence that regular exercise training of high volume and intensity may be accompanied by deficiencies of certain minerals such as Zn, Mg and Fe, the impact of these alterations on the athlete's immune function needs to be demonstrated. *In vitro* experiments could demonstrate the involvement of these ions in certain immune processes. But it remains to be shown whether these deficiencies are able to aggravate exercise-induced immune responses.

ANTIOXIDANTS

Background

Free radicals are reactive molecules with unpaired electron(s) (158). High levels of free radicals damage cellular components. Antioxidants are chemical compounds and enzymes that exist as a natural means of quenching free radical overproduction. However, moderate levels of radicals and other oxidants are central to the control of gene expression, cell signalling pathway regulation, and physiological modulation of skeletal muscle force production (318). In the context of inflammation in health and disease, genomic, cellular, and physiological outcomes are regulated by fluctuations between free radical species and their antioxidant counterparts. In this section, we outline oxidants (mainly ROS and reactive nitrogen species (RNS)) and antioxidants (with a focus on dietary sources of antioxidants), their effects on exercise, and the interface with the immune system (329).

Table 1: Overview about specific minerals, their immune related functions, symptoms of deficiency, deficiencies in sports and recommendations for supplementation.

	Immune related functions	Deficiency signs or symptoms (general)	Deficiencies in sports	Supplementation
Zinc	Adaptive and non-adaptive immune responses	Enhanced susceptibility to infections	Zn deficiency has been described in elite athletes, especially in endurance athletes	Regular and continuous supplementation cannot be recommended; short-term Zn supplementation might be effective in common cold therapy; transient supplementation during intensive physical/ psychological stress can be considered especially if there is a history of recurrent infections
Magnesium	Cofactor for immunoglobulin synthesis, immune cell adherence, antibody-dependent cytolysis, activation of macrophages	Enhanced neuromuscular excitability, muscle cramps, anxiety, and clinical signs of inflammation, such as immune cell activity, increased circulating inflammatory mediators	Endurance athletes; sports using protective clothing, which increases Mg losses via sweat	Mg supplementation in athletes with balanced Mg status cannot be recommended. Supplementation can be recommended only after diagnosis of Mg deficiency.
Iron	Haemoglobin/ myoglobin synthesis, energy metabolism Lymphocyte proliferation and differentiation macrophage polarization and functionality	Anaemia, cognitive impairment, weakness, immune abnormalities	Mainly women competing in running, field hockey, cross country skiing, basketball	Recommended for athletes with iron deficiency anaemia and monitored carefully for prophylaxis, no data about immunological effects
Selenium	Se acts mainly through selenoproteins, e.g. antioxidant selenoenzymes such as glutathione peroxidases (GPxs) and thioredoxin reductases (TrxRs)	Increased risk of Keshan disease and Kashin-Beck disease (both often occur in conjunction with iodine deficiency or environmental toxins)	Not described	Not described
Manganese	Manganese superoxide dismutase might play a role as an antioxidant	Not described	Not described	Not described

Relevant to immune system interactions, superoxide is one of the strongest cellular oxidants, but it is quickly dismutated to hydrogen peroxide by the enzyme superoxide dismutase. Hydrogen peroxide is a more stable, non-free radical ROS that is permeable to cellular membranes. Despite being a weak oxidizing agent, high local concentrations of hydrogen peroxide are cytotoxic. Toxicity is typically associated with oxidizing chain reactions promoted by Fe (Fenton reaction) centered molecules which produce hydroxyl radicals. Hydroxyl radicals are strong oxidants, highly reactive and, when concentrated, can be the most damaging ROS in biological systems. Central to inflammation, hypochlorite is another well-known ROS product (329). Hypochlorite is formed by the action of the oxidative enzyme myeloperoxidase utilizing hydrogen peroxide. Hypochlorite is produced by neutrophils and macrophages (233,432) and, independent of pathogen defence, can oxidize circulating cholesterol and other humoral factors with deleterious consequences. Moreover, this oxidant readily forms hypochlorous acid, subsequently crossing cell membranes and damaging essentially all cellular constituents with negative effects (71).

Nitric oxide is held to be the main RNS in inflammatory processes and is synthesized enzymatically (nitrogen oxide synthase isoforms) from the amino acid L-arginine. Nitric oxide is a weak reducing agent, but can react with superoxide to produce peroxynitrite. As with ROS, RNS promote health or disease within the context of a dose, duration and the local biochemical environment. In certain scenarios peroxynitrite is a strong oxidizing agent that depletes thiol groups, and damages DNA and proteins (234).

Consensus

Undeniably, participation in acute exercise is associated with a transient production in the ROS/RNS (317). Moreover, while the source of ROS/RNS production is largely thought to be generated within the contracting skeletal muscle (331) there is ample evidence that the immune system is also responsible for exercise-generated radical species (325).

The involvement of the immune system in ROS and RNS production

The rate of oxygen consumption by phagocytes (e.g., neutrophils, eosinophils and mononuclear phagocytes) increases when exposed to certain stimuli (e.g., pathogens, pollutants, etc.). When this occurs, the phagocytes produce high levels of superoxide and collectively these events are known as the "respiratory burst" (14). The purpose of this phenomenon is to

generate powerful microbiocidal agents by the internal defence arm of the immune system. Specifically, macrophages produce NO which plays a critical role in redox-related functions of the immune response (422).

Interaction of exercise and antioxidant content (potential) of the immune system

It is well established that prolonged, high intensity training and competition can result in acute immune impairment in athletes and usually manifests as an increased susceptibility to minor illnesses, particularly URTI (see Section 12). Essential to this understanding is the fact that acute high intensity exercise is associated with upregulation of endogenous antioxidant enzyme transcripts (129). This finding is important and suggestive of the fact that, as with muscle level adaptations, the immune system is resilient and ultimately adapts beneficially to exercise.

Controversies

Advocates of antioxidant supplementation argue that exercise-induced oxidant production cannot be adequately quenched without dietary intervention. While this logic may seem reasonable at first, several arguments contradict the notion that athletes and recreational exercisers require dietary antioxidant supplementation. For example, dietary antioxidant consumption has been proposed to reduce the risk of respiratory illness, but conclusive data to support or oppose this statement are not available (121). Furthermore, links between exercise-induced oxidative stress and immune dysfunction (275), and the postulated benefits of dietary antioxidant supplementation in preventing immune dysfunction during exercise, are not substantiated empirically (276).

In addition, to counteract the proposed need for supplemental antioxidants, there is no conclusive evidence that exercise-induced ROS production is detrimental to human health. By contrast, the fact that exercise elicits both oxidative stress and numerous adaptive health and athletic performance benefits is paradoxical to the idea that supplemental antioxidants are needed. Moreover, regular exercise training promotes fortification of endogenous enzymatic and non-enzymatic antioxidants, a fact that extends to circulating immune cells from exercised individuals (129). The adaptive increase in endogenous antioxidants does not fully quench the ROS/RNS generated during the exercise, but is clearly sufficient to protect against deleterious outcomes due to exercise-induced oxidative stress (194).

According to scientific consensus, therefore, athletes who consume an appropriate energy intake from nutrient-dense foods do not need antioxidant supplementation. Moreover, there is no evidence that exercise in extreme environments necessitates antioxidant supplementation (324). In contrast, one feasible circumstance in which supplemental dietary antioxidants may be warranted is in individual cases of nutrient deficiencies (e.g., antioxidant status below the normal range for good health). This latter instance is a rare exception to our broader understanding of exercise and oxidative stress: nevertheless it has been questioned scientifically by investigating the dietary practices of athletes (396). Importantly, emerging evidence indicates that antioxidant supplementation mitigates important exercise-induced adaptations which now appear to extend to the immune system.

Future directions

The debate for and against antioxidant supplementation in athletes and regular exercisers appears likely to continue despite comprehensive understanding of immunonutrition and exercise-induced oxidative stress. Antioxidant supplementation practices are often driven by business models and consumer biases that seek a convenient means to improve athletic performance, health, and longevity. Accordingly, there is a pressing need for additional research to demonstrate when, and in what context, antioxidant supplementation may be efficacious. Moreover, future work should be mindful of corporate biases and include points for consumer advocacy whenever possible. It is proposed that exercise and nutritional scientists should join with practitioners to educate athletes about the current scientific understanding regarding antioxidant supplements and exercise-induced oxidative stress and inflammation. Education efforts should be strategic and ever mindful of consumer demand for pill-based solutions to complex problems like performance enhancement, a point that is of particular importance to exercise and immunonutrition.

PLANT-DERIVED IMMUNOMODULATORS: HERBAL SUPPLEMENTS

In this article "botanical supplements" ("herbal supplements") refers to single- or multi-organ plant extracts in tablet or liquid form containing a diverse array of phytochemicals, in contrast to "botanicals" ("herbals") which refers to isolated plant compounds or compound groups.

Background

Several plants are used by athletes as dietary/nutritional supplements (Table 2). Quantifying global rates of use is logistically problematical because athletes and researchers differ in their definition of "herbal supplement", and multicomponent preparations or foodstuffs may contain herbs unbeknownst to athletes. Usage surveys sometimes neglect herbal supplements or lump different supplements together as one group (123,211,359). Thus, use is likely to be underestimated.

Athletes consume herbal supplements for both health and performance reasons, and a given supplement often has more than one presumed use. Many herbal supplements consumed by athletes have purported immunomodulatory capacities (Table 2). Presumed immunomodulatory herbal supplements are diverse in terms of taxonomy, plant organs used, and bioactive compounds.

Consensus

Empirical evidence for the immunomodulatory capacities of herbal supplements is often incomplete, equivocal, and/or weak, whether the studies used athletes/non-athletes or exercise/non-exercise models. Ginseng and echinacea possibly serve as the best models for examining immunomodulatory herbal supplements in athletic contexts, because they have been more robustly researched, and bioavailability studies suggest these supplements' bioactive molecules can pass

Table 2: Herbal supplement use by 8424 athletes based on 27 published surveys. Data used for this table were gleaned from a subset of 27 athlete surveys identified by Knapik et al. (211) as containing references to specific herbal supplements (references 9-12, 15, 19, 23, 35, 49, 58, 67, 68, 80, 101, 121, 123, 128, 131, 132, 141, 144, 149, 151-153, 159, and 198 in (211)), but were analyzed differently here. * = Many herbal supplements were quantified only once and were not tabulated: alfalfa, chamomile, ciwujia, evening primrose, goldenseal, green tea, guarana, kava kava, kola nut, peppermint, tea tree oil, and yohimbe. Some surveys also noted "herbal supplements" (4 surveys; 0.9 ± 2.9%) or mixed herbal preparations (4 surveys; 3.5 ± 10.5%). † = "Yes" indicates at least some use among athletes as an immunomodulator, and "no" indicates the supplement is not consumed as an immunomodulator; designations do not connote whether the plant is primarily taken as an immunomodulator (e.g., echinacea) or only secondarily (e.g., ginseng), or efficacy. ‡ Two additional studies did not provide usage statistics. § One additional study did not provide usage statistics.

Supplement*	# surveys reporting use	Average % (± s.d.) of respondents using supplement (across all 27 surveys)	Purported immune-modulator?†
Ginseng	20‡	10.6 ± 17.6%	Yes
Echinacea	14	9.5 ± 18.0%	Yes
Garlic (+/- horseradish)	4§	3.1 ± 11.8%	Yes
Ginkgo	4§	0.9 ± 2.6%	Yes
Spirulina (blue-green algae)	4§	0.4 ± 1.4%	Yes
Ephedra	3	1.7 ± 5.8%	No
St. John's Wort	3	0.3 ± 1.1%	Yes
Flax, Flaxseed	2	0.2 ± 1.0%	No
Tribulus	2	0.1 ± 0%	No

through the gut into the bloodstream in physiologically relevant quantities.

Echinacea is primarily taken by athletes for prevention or treatment of upper respiratory tract infections such as colds or influenza. Recent reviews and meta-analyses from clinical trials with the general population concur that echinacea supplementation may lessen symptom severity or duration, but are equivocal in their assessment of its prevention capabilities (204,349). Results from athlete/exercise studies on echinacea are similar (Table 3) (27,155,353). Though all three studies used *E. purpurea*-based preparations, Table 3 epitomizes the problems in forming conclusions about echinacea supplements (or most herbal supplements for that matter; e.g., few studies, different populations, measurements, exercise interventions, and treatment interventions). One recent review that examined clinical studies, *ex vivo* studies (where blood samples were drawn pre- and post-exercise, but lymphocytes were stimulated *in vitro*), and *in vitro* studies concluded that echinacea supplements may stimulate both innate and adaptive immunity (the former more so) and that alk(yl)amides and caffeic acid derivatives are the likely bioactive molecules (358). In terms of ergogenic potential, echinacea supplementation did not improve endurance capacity or VO_2max in three studies (22,25,375) but did in one (417).

Ginseng is primarily taken by athletes as an ergogenic or adaptogenic aid, either as a standalone supplement or in multicomponent "energy drinks." Recent reviews have discounted its utility as an ergogenic aid (15) and further suggest that any benefits seen in energy drinks are likely attributable to caffeine or sugars and not ginseng (18). Ginsenosides are the presumed immunomodulatory constituents. A review of the immunomodulatory effects of ginseng supplements in athletes has been provided elsewhere (Table 2 in (358)). Though there are more *in vivo* studies on ginseng and its potential immunomodulatory effects than echinacea, outcomes were worryingly inconsistent across studies and often weak, likely owing to the diverse species, extract types, and dosing used. The two studies investigating IL-6 are a good example of this predicament. In one study (202), trained males consumed heat-treated P. *ginseng* supplements for seven days before two 45-minute treadmill runs and demonstrated reduced IL-6 compared to controls a couple hours post-exercise but not a day later. In a separate study (225), untrained male subjects consumed *P. pseudoginseng* for three days before 30 minutes of treadmill running at 60% VO_2max and demonstrated no difference in IL-6 levels post-exercise compared to controls.

Table 3. Representative studies concerning potential immunomodulatory effects of Echinacea supplementation in athletes. Abbreviations: RA = recreationally-active, TR = trained.

	Berg et al. 1998 (27)	Hall et al. 2007 (155)	Schoop et al. 2006 (353)
Population	42 TR ♂	32 RA ♀ + ♂	80 RA ♀ + ♂
Treatment	8 mL/d for 28 d Echinacin (*E. purpurea* juice)	28 d *E. purpurea*	8 wk Echinaforce (*E. purpurea* tablet)
Study Design	Separate treatment and placebo groups	Pre-to-post comparison	Treatment tolerability study
Exercise	Competitive sprint triathlon at Day 28	3, 30s serial Wingate tests at Days 0 and 28	Subjects' own regular training regimens
Immune System-Associated Outcomes	*Vs. control*: no respiratory infections (placebos had some); ↓ serum and urine IL-2R; ↑ urine IL-6; slight changes in NK cells and T-cells	*Vs. pre-treatment:* reduced symptom severity (but not incidence); reduced post-exercise declines in salivary IgA (SIgA)	*Vs. a reference general population*: fewer upper respiratory infections

Owing to the sparse literature available, many athletics-associated claims about herbal supplements have yet to be scientifically addressed and recommendations need be cautious. As therapeutic

immunomodulators for athletes, there is some evidence that echinacea may be efficacious whereas the evidence for ginseng is murky. Scant literature exists on the potential immunomodulatory effects of the other herbal supplements in Table 2 among athletes or even in the general population, and reviews may be found elsewhere (78).

Controversies and Future Directions

For some herbal supplements, data are only available from animal models or *in vitro* work with human or animal cells. While valuable, such data may not directly translate to human clinical outcomes because of bioavailability/pharmacokinetic reasons or species differences. Potential immunomodulatory effects of bystander molecules (such as endotoxin [LPS] from bacteria growing on plant material or incorporated during the extraction process) are concerns for *in vitro* studies and may explain contradictory activities of plant extracts such as dual cytokine-enhancing and -suppressing properties from a single extract (387); some experiments proactively addressed such concerns whereas others did not. Unaccounted "pre-clinical factors" (especially those during plant growth, harvest, and processing) and differences in experimental methods confound cross-study comparisons (357).

Muddying the waters are issues related to the products themselves: herbal supplement labels may not accurately represent actual supplement contents; there can be lot-to-lot variation from a single manufacturer; and stark differences can exist across manufacturers for a single supplement. Supplements may also inadvertently (or, some allege, covertly) contain substances considered banned/"doping agents" (92,135,214,297). Many herbal supplements are not regulated by government agencies.

Thus, one should be cautious in concluding that any given supplement is consistently efficacious, or that there is a signal failure due to a lack of consiliency in the published research. Rather, lack of consiliency represents the Byzantine nature of herbal supplements in "real world" contexts due to the factors just described. It also limits the guidance professionals can provide to athletes concerning herbal supplement efficacy or safety.

Aforementioned pitfalls can guide future work, which will need to be transdisciplinary to account for all pre-clinical and clinical factors that may influence immune outcomes. Few human *in vivo* studies have focused on specific immune parameters such as cell subpopulations or antibody or cytokine profiles. Such work would help illumine mechanisms and provide the additional benefit of linking clinical outcomes with findings from *ex vivo* or *in vitro* studies.

POLYPHENOLS

Introduction to Polyphenols

The plant kingdom uses nearly 50,000 secondary metabolites for defence, attraction, and protection (163). These plant metabolites include approximately 29,000 terpenes, 12,000 alkaloids, and 8,000 phenolics. The 8,000 phenolic compounds or polyphenols are divided into four main classes: flavonoids (~50% of all polyphenols), phenolic acids, lignans, and stilbenes. Flavonoids are further classified into six simple (flavan-3-ols, flavanones, flavones, isoflavones, flavonols, anthocyanins) and two complex subgroups (condensed tannins or proanthocyanidins, derived tannins) (17) (Table 4). In foods, flavonoids, lignans, and stilbenes are usually found as glycosides, and phenolic acids as esters with various polyols, and structural variations influence absorption and bioavailability (429).

Nutritional assessment of dietary polyphenol and flavonoid intake has improved with the development of databases from Phenol Explorer (www. http://phenol-explorer.eu/) and the U.S. Department of Agriculture (http://www.ars.usda.gov/services/docs.htm?docid=24953). Recommendations for dietary polyphenol and flavonoid intake have not yet been established but should be forthcoming as improvements in assessment methods continue. In Europe, the average dietary polyphenol intake has been estimated at 1,187 mg/day (ranging from about 1,700 mg/day in Denmark to 660 mg/day in Greece), with coffee, tea, fruits, and wine as the principal sources (429). In Europe, only ~100 polyphenols are consumed at levels exceeding 1 mg/day, and flavonoids represent 40% of the total polyphenols ingested (429). Dietary flavonoid intake from all foods and beverages among US adults is 251 mg/day, with tea as the primary source (80% of total flavonoid intake) (355). Only 29% consume tea on a given day, and when tea is removed from the analysis, total flavonoid intake falls to about 50 mg/day, reflecting the low intake of fruits and vegetables by US adults (~2 servings/day) (185,355).

Many flavonoids exhibit strong anti-inflammatory, antioxidant, anti-pathogenic, and immuno-regulatory properties when studied using *in vitro* procedures (1,128,241,413). Most flavonoids are poorly absorbed in the human small intestine and undergo extensive biotransformation after ingestion. Thus, *in vitro* data using the original food-based flavonoid molecule has questionable relevance when evaluating bioactive effects following ingestion. A large proportion of ingested plant polyphenols reaches the colon, and microbial degradation transforms the extremely diverse population of dietary polyphenols into a smaller number of metabolites, including simple phenols and derivatives of benzoic acid, phenylacetic acid, mandelic acid, phenylpropionic acid, and cinnamic acid (116,119,120,345). The bacterial transformation of food polyphenols in the colon varies widely depending on the unique gut microbiota composition of the individual as influenced by genotype, diet, lifestyle, and other factors (116). The metabolites created from bacterial degradation can exert local health benefits to colon endothelial cells, modulate the composition of the microbiota, and hence indirectly influence their own metabolism and bioavailability (116). The gut-derived phenolics can be reabsorbed into the portal vein, undergo phase II biotransformation in the liver, enter the systemic circulation and become a part of the so-called "food metabolome", exert a variety of bioactive effects, and then finally be excreted in the urine (119,120,345) (see Figure 1). Recent *in vitro* studies using biotransformed phenolics at physiologically relevant concentrations indicate that degradation of flavonoids to simpler phenolics actually increases their overall anti-inflammatory bioactivity (241,413).

Epidemiological studies support a strong linkage between high versus low dietary polyphenol intake and reduced risk for overall mortality (189) and a wide spectrum of health conditions including neurodegenerative diseases (371), body weight gain (29), systemic inflammation and oxidative stress (11,72), diabetes (391), cardiovascular disease (409), and hypertension (223). A higher intake of flavonoids predicts increased odds of healthy aging (344). A systematic review and meta-analysis showed that flavonoid supplementation (range of 0.2 to 1.2 g/day in 14 selected studies) decreased URTI incidence by 33% compared with control (372). Many flavonoids exert anti-viral effects, modulate NK cell activities and regulatory T (Treg) cell properties, and influence macrophage inflammatory responses (209). High dietary intake of flavonoids has been linked in the Framingham Heart Study Offspring Cohort with decreased systemic inflammation using a cluster of biomarkers (72).

Countermeasure Effects of Polyphenols to Exercise-Induced Physiological Stress

Taken together, cell culture and epidemiological data support the recent focus of investigators on the use of polyphenols as potential countermeasures to exercise-induced inflammation, oxidative stress, immune changes, illness, and delayed onset of muscle soreness (DOMS) (for reviews, see (268,277,278,379)). Multiple dosing strategies have been employed including single and combined purified polyphenols (e.g., quercetin, resveratrol), plant extracts (e.g., green tea, black currant, pomegranates), and increased fruit and vegetable food or juice intake (e.g., blueberries, bananas, tart cherry juice). Most studies incorporate a one to three week polyphenol loading period prior to an exercise stress intervention. Few papers are available for any particular polyphenol or plant extract, and research designs vary in regards to the supplementation regimen, form of exercise stress, and outcome measures (268,277,278,281,282,379). The data in general support that polyphenol-rich plant extracts and unique polyphenol-nutrient mixtures (e.g., quercetin with green tea extract, vitamin C, and fish oil, or freeze-dried blueberry powder with green tea extract) have small but significant effects in increasing anti-oxidant capacity, with inconsistent, short-term effects on mitigating exercise-induced oxidative stress, inflammation, and immune dysfunction. High blueberry and green tea flavonoid versus placebo intake for 17 days was linked to reduced *ex vivo* viral replication in blood samples collected from athletes after a 3-day overreaching, running protocol (1). Large-dose intake of single flavonoids (e.g., 500 to 1,000 mg quercetin) has been linked to reduced URTI in athletes, but has not proved to be a useful alternative to ibuprofen in regards to countering post-exercise pain, inflammation, and soreness for the athlete (277).

Table 4. Polyphenol classes and subclasses, and food sources.

FLAVONOIDS	**Sample Polyphenols**	**Food Sources**
Simple Flavonoids		
Flavan-3-ols	(+)-catechins, (-)-epicatechin (-)-epigallocatechin-3-gallate	Tea, chocolate, tree fruits, grapes
Flavanones	Hesperetin, Naringenin, Eriodictyol	Citrus fruits and juices
Flavones	Luteolin, Apigenin	Parsley, celery seed, oregano
Isoflavones	Daidzein, Genistein, Glycitein	Soybeans, soy-based foods, legumes
Flavonols	Quercetin, Kaempferol, Myricetin, Isorhamnetin	Onions, apples, tea, berries
Anthocyanidins	Cyanidin, Delphinidin, Malvidin, Pelargonidin, Peonidin, Petunidin	Most berries, stone fruits
Complex Flavonoids		
Condensed Tannins (Proanthocyanidins)	Procyanidins Prodelphinidins Propelargonidin	Chocolate, stone fruit (apples, pears), grapes, strawberries, cranberries nut skins, cinnamon, beer, wine barley, legumes
Derived Tannins	Theaflavins Theabrownins Theaflavins	Fermented teas (black, oolong)
PHENOLIC ACIDS		
Hydroxycinnamic acids	5-Caffeoylquinic acid 4-Caffeoylquinic acid 3-Caffeoylquinic acid Ferulic acid 5-Feruloylquinic acid	Coffee, grain products
Hydroxybenzoic acids	5-O-Galloylquinic acid Gallic acid	Tea, wine, mixed fruits
LIGNANS	Secoisolariciresinol Matairesinol Pinoresinol Lariciresinol Sesamolin	Grain products, nuts, dried fruits, carrots, dried basil, berries, seeds, citrus fruits, stone fruits, peppers, zucchini
STILBENES	Resveratrol	Wine, berries, grapes

Source: Adapted from (17): Balentine DA, Dwyer JT, Erdman JW Jr, Ferruzzi MG, Gaine PC, Harnly JM, Kwik-Uribe CL. Recommendations on reporting requirements for flavonoids in research. Am J Clin Nutr 101:1113-1125, 2015; Phenol Explorer (http://phenol-explorer.eu/compounds).

Future Directions

Future studies should focus on the long-term relationship between increased intake of polyphenols, gut-derived phenolics, and systemic and post-exercise inflammation, oxidative stress, anti-viral defence, and immune function in athletes using global and targeted metabolomics (281,282). Intense and prolonged exertion has been related to an enhanced translocation of gut-derived phenolics into the circulation during a 17-day period of polyphenol supplementation (282) (Figure 1). Elevated blood and tissue gut-derived phenolics from chronic, high polyphenol intake over several months may result in subtle but important bioactive effects that translate to improved recovery and ability to train intensively, with reduced rates of illness (1,241,281,282). This is a complex relationship that demands a multi-omics, long-term approach. Research is needed to define optimal dosing regimens and whether

increased intake of foods high in polyphenols such as berries, tea, and coffee results in meaningful bioactive effects without the need for high doses of unique flavonoid mixtures. In the very least, long-term, high polyphenol intake from plants food sources is important for the health of all humans, including and more importantly, in relation to the present series, for athletes.

PROBIOTICS – PREBIOTICS

Background

Probiotic-rich foods and supplements contain non-pathogenic bacteria that colonise the gut purportedly yielding a variety of health benefits that include reduced incidence of respiratory and gastrointestinal illness. There are several possible ways in which probiotics may reduce the risk of respiratory and gastrointestinal illness symptoms. By their growth and metabolism, probiotics help inhibit the growth of other bacteria, antigens, toxins and carcinogens in the gut, and reduce potentially harmful effects. Probiotics can also influence immune function via interaction with immune cells associated with the gut. Prebiotics are non-digestible food ingredients that promote the growth of beneficial microorganisms in the intestines. Probiotics are found in several foods, particularly dairy products such as milk, yoghurt and cheese (134), although concentrations are relatively low. Consequently, there is widespread interest in use of dietary supplements containing probiotics in both the general and sporting communities.

Figure 1

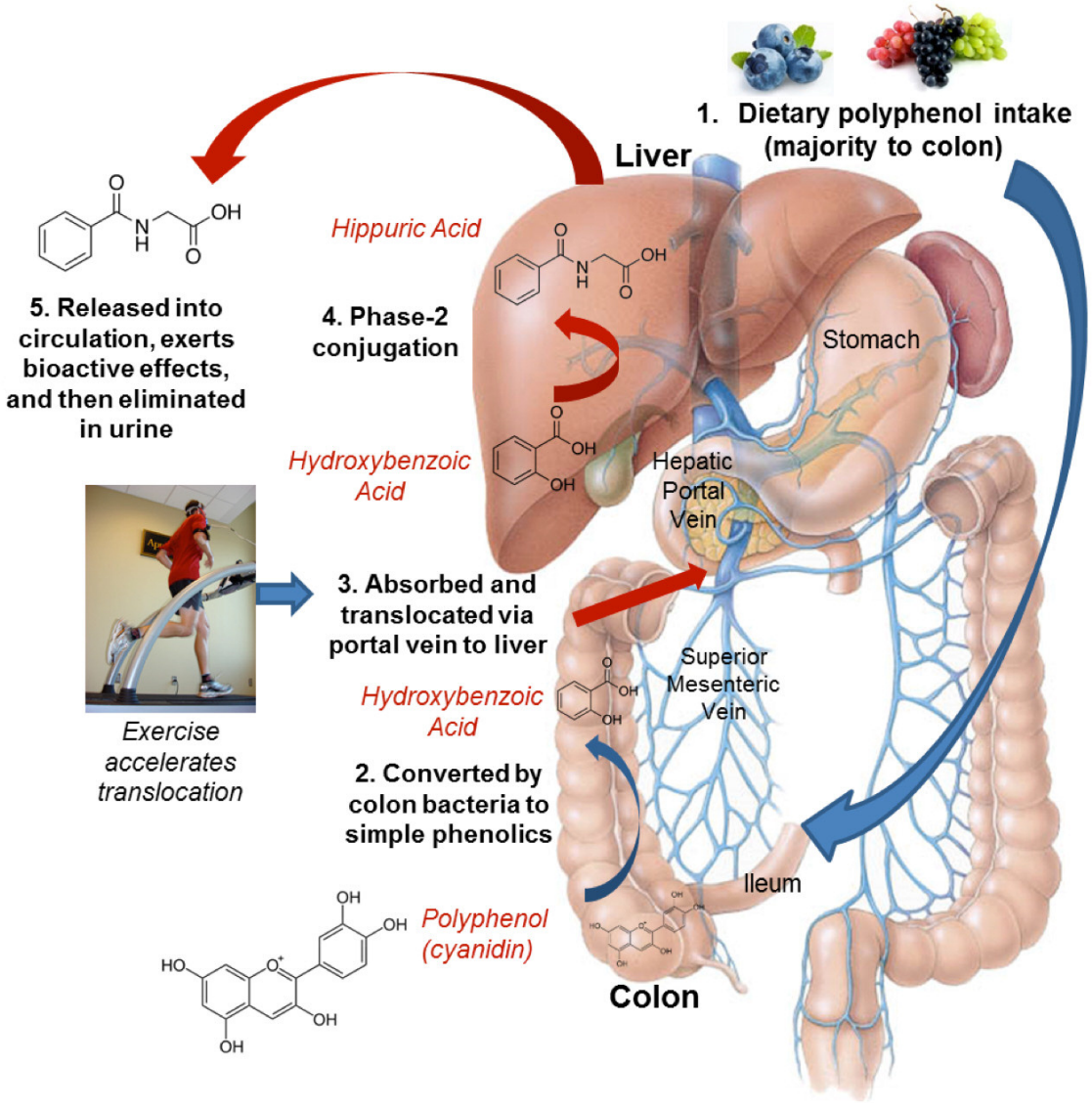

Figure 1 - Cyanidin-3-glucoside (C3G) is a widely consumed dietary anthocyanin, and can be used as a sample polyphenol to show how the body metabolizes flavonoids. After ingestion, the majority of C3G goes to the colon where bacteria degrade it to multiple, simple phenolics including hydroxybenzoic acid. Next, hydroxybenzoic acid and other phenolics are absorbed through the colon to the liver via the superior mesenteric and hepatic portal veins. Prolonged and intensive exercise accelerates the translocation of simple phenolics from the colon to the liver. In the liver, phase II metabolism adds glycine to convert hydroxybenzoic acid to hippuric acid that is released into the circulation. Hippuric acid has been linked to multiple bioactive effects, and is ultimately eliminated from the body through the urine. Many other polyphenols go through a similar biotransformation pathway.
SOURCES: Based on data from references (128) and (281). The contribution of Xiaowei Chen in designing this figure is acknowledged.

Consensus

In clinical practice, probiotics have been used since the early 1900s to manage common gastrointestinal conditions including stomach cramps, irregular bowel movements, excessive flatulence, diarrhoea, and irritable bowel syndrome. In research settings, the focus has been on verifying the clinical benefits of probiotic ingestion and supplementation, and underlying mechanisms of action. Many studies have been conducted on the effects of probiotic use on gastrointestinal problems and URTI in the general population. A recent systematic review (210) of twenty placebo-controlled trials concluded that probiotic use resulted in lower numbers of illness days, shorter illness episodes and fewer days absence from day care/school/work. The most recent Cochrane systematic review of probiotic benefits for URTI using data from randomised controlled trials involving 3,720 non-athletes from 12 studies concluded that probiotics were better than placebo in reducing URTI incidence by ~47%, and the average duration of an acute URTI episode by ~2 days (162).

The most important mechanisms of probiotic action are thought to be via immunomodulation of local immunity (by interaction with gut-associated lymphoid tissue and maintenance of gut barrier function) and systemic immunity (by enhancing some aspects of both innate and acquired immune responses) (31,227). Probiotic intake can increase NK cell cytolytic activity (330), enhance phagocytic activity and microbicidal capacity of granulocytes and monocytes, modify the production of cytokines and elevate levels of specific IgG, IgA and IgM (162), with effects that can extend beyond the gut to distal mucosal sites. Animal studies indicate that regular probiotic ingestion can influence responses in the respiratory tract and improve protection against bacterial and viral pathogens via modulation of lung macrophage and T-cell numbers and functions (131,227,245).

Controversies

To date there are few published studies of the effectiveness of probiotic use in athletes and team sports; a recent comprehensive review (323) identified 15 relevant experimental studies that investigated immunomodulatory and/or clinical outcomes. Of the eight studies that recorded self-reported URTI incidence, five found reduced URTI frequency or fewer days of illness (94,142,167,415,416), and three reported trivial or no effects (141,206,386). A randomised, placebo-controlled trial involving physically active individuals (415) reported that fewer URTI episodes (relative risk ratio 0.73) were experienced in those who ingested daily a *Bifidobacterium* probiotic compared with placebo over a 150-day intervention period. However, a large study of 983 Finnish military recruits failed to show significant clinical benefits for supplementation with a combination of *Lactobacillus rhamnosus* GG (LGG) and *Bifidobacterium animalis ssp. lactis* BB12 for 150 days (203). Studies that examined immunomodulatory effects of probiotics in athletes have reported increased interferon-γ production in whole blood culture (83) and T-cells (94) and better maintenance of secretory IgA during intensive training (142,242). A recent study reported that URTI incidence was unchanged despite reductions in cytomegalovirus (CMV) and Epstein-Barr Virus (EBV) antibody titres after 20 weeks of supplementation with *Lactobacillus casei* (144).

Most studies have examined probiotic effects in small numbers (<50) of recreationally active individuals over periods lasting <6 months. URTI has typically been established by self-report questionnaires and not all studies have used a randomised, placebo controlled design. However, there is now sufficient understanding of the mechanism of action of certain probiotic strains, and enough evidence from trials with athletes and highly physically active people (in addition to 12 studies cited in The Cochrane review (162) on children and adults) to signify that there are mostly positive effects. Similar to other supplements for which health claims are made there is concern of bias in the literature, with a stronger likelihood of publication of studies with positive, as opposed to trivial, equivocal or negative outcomes.

Other potential benefits of probiotics could be reduced risk of gastrointestinal discomfort symptoms and diarrhoea (e.g. so-called runner's trots) during prolonged exercise, reduced endotoxaemia during exercise in the heat, and reduced incidence of gastrointestinal infections – a particular concern when travelling abroad. Further large-scale studies are needed to determine if these potential benefits are real, and to confirm that taking probiotics can reduce the number of training days lost to infection and which strains of probiotics are most effective for athletes. The studies that have shown reduced URTI incidence in athletes have been mostly limited to *Lactobacillus* and *Bifidobacterium* species and used daily doses of ~10^{10} live bacteria. These doses (~10^{10} live bacteria) showing efficacy with athletes are comparable to those used in non-athlete studies (range ~10^{8}-10^{10}) although recommended dosages can be strain-specific. Although probiotic supplements contain similar bacterial species to dairy foods, there is little consensus on the relative effectiveness of commonly used species. As for prebiotics, or combinations of prebiotics and probiotics, there are currently no published studies on their efficacy in athletes for reducing respiratory or gastrointestinal illness symptoms.

Future Directions

Long-term tolerance of probiotic supplementation in highly-trained athletes over several months to years or the benefits, if any, of cycling on and off probiotics, are important questions warranting investigation. The laboratory-based efficacy and field effectiveness of multi-component formulations combining several different probiotics species, or probotics and prebiotics, need evidence-based studies. Pharmaceutical companies are already making a wide range of multi-component formulations. No studies have systematically investigated how the dosage regimens of probiotic supplementation might vary as a function of sex, age, medical history, dietary practices, fitness level and/or training background. Health care practitioners are seeking this information to assist them in prescribing individualised probiotic/prebiotic supplementation programmes.

BOVINE COLOSTRUM

Background

Bovine colostrum is the fluid produced by the mammary glands for 24-72 hours following calving. While antibody

transfer in a human takes place predominantly via the placenta, calves rely on colostrum for the passive transfer of immunoglobulins (Ig). As such, the concentration of Ig and other immune factors, in combination with growth factors and nutrients, is much greater in bovine than human colostrum. For the calf, bovine colostrum is essential for immune system establishment and gastrointestinal growth and differentiation. This has led researchers to explore the potential of bovine colostrum to modulate immune function in humans, particularly in exercise where immune perturbations are common.

Bovine colostrum is an incredibly complex fluid and understanding of its potential molecular function is improving with advances in technology, such as proteomic analysis (8). Bioactive components of bovine colostrum include IgG, lactoferrin, lactoperoxidase, defensins, trypsin inhibitor, micro RNAs, insulin-like growth factor-1 (IGF-1) and transforming growth factor-β (190,343). Following ingestion, many of these components have been shown to survive digestion and exert effects at the level of the gut, or systemically. In cell culture and animal models (porcine, rat and mice) bovine colostrum exhibits anti-bacterial, anti-inflammatory and antiviral properties (425,427). However, the effectiveness of stable, standardised preparations of non-hyperimmunised bovine colostrum to modulate the immune system in healthy, exercising humans is less clear.

Consensus

Exercise is associated with immune perturbations and upper respiratory symptoms (URS) are commonly reported in elite athletes (122). Several investigations have reported URS incidence following a period of bovine colostrum supplementation, with all of them suggesting that bovine colostrum supplementation is associated with a reduction (not always statistically significant) in URS incidence in athletes. A retrospective analysis of training diaries from investigations with healthy, active male participants (n=174) reported a significantly lower incidence of URS with eight weeks of bovine colostrum supplementation of 60 g/day (32%) compared to a whey placebo (48%) [relative risk (RR) of 0.6](49). Similarly, when compared to a whey or skim milk powder placebo, others have reported a trend for a reduction in URS incidence over 8-12 weeks of lower dose colostrum supplementation (10 to 25 g) in trained cyclists [RR 0.4 (362) and 0.3 (363)], elite swimmers (weeks 5-10: RR 0.4) (95), active males (RR 0.6) (198) and marathon runners (mean URS incidence of 0.8 in colostrum group compared to 1.1 in placebo) (96). The ability of bovine colostrum to shorten URS duration is less clear, with some investigations reporting no change (49,198,362) and some a reduction (95,96,363). While the ability of bovine colostrum to shorten symptom duration is unclear, bovine colostrum supplementation (10-60 g) for greater than four weeks appears to reduce self-reported URS incidence by 30 to 60%.

While changes in salivary SIgA have been related to URS (137) the mechanism for a reduction in URS following a period of bovine colostrum supplementation does not appear to relate specifically to increases in SIgA concentration (101,199,363). One investigation reported a significant 79% increase in SIgA concentration after 12 weeks of bovine colostrum supplementation (26 g/day) (d=4.8) (96), although the placebo group also reported a large increase over this timeframe (d=3.0). While some of the increase in SIgA was attributed to competing in a marathon, colostrum accounted for 29% of the variation in SIgA (96). In contrast, studies of similar duration and/or those providing higher doses of bovine colostrum (~60 g/day) have not reported changes in SIgA (198,256,362,363). Other proposed mechanisms for the reduction in URS incidence may be related to minimising the increase in winter salivary bacterial load (198) or a reduction in the suppression of receptor-mediated stimulation of neutrophil oxidative burst (199). There is also evidence that bovine colostrum may reduce the post-exercise decrease in neutrophil function and salivary lysozyme (101), and reduce immunodepression during a period of intensified training (362) although these mechanisms are yet to be confirmed. It is unlikely that bovine colostrum supplementation alters circulating cytokine concentrations following short-term intense exercise (68,362) in athletes who have not undertaken a period of intensified training overload.

Controversies

The gastrointestinal tract is the largest immune organ in the human body and in combination with its intestinal microbiota it not only provides a physical barrier against commensal and pathogenic bacteria, but plays a central role in innate and adaptive immunity (364). In the calf, bovine colostrum is essential for intestinal development, providing a rationale to investigate the potential of bovine colostrum to influence gut health in humans.

Early work in healthy adults demonstrated that bovine colostrum reduced gastrointestinal permeability when co-ingested with non-steroidal anti-inflammatory drugs (314) and reduced systemic endotoxin concentrations in abdominal surgery patients (44), suggestive of maintenance of the intestinal barrier preventing endotoxin translocation across the gut. Only two human studies have investigated if bovine colostrum reduces the increase in intestinal permeability associated with exercise (240,264) and their findings are conflicting, possibly attributable to study differences in markers of intestinal permeability, exercise protocols, colostrum dose and period of supplementation. While animal studies suggest a beneficial effect (321), more controlled human studies are required to determine the impact of bovine colostrum on exercise associated gut permeability and endotoxin translocation.

In combination with influencing gastrointestinal growth, IGF-1 plays a role in immune and neuroendocrine regulation. While only one study has reported autonomic alterations following a period of colostrum supplementation (363), controversy exists as to the possibility of absorption of IGF-1 from bovine colostrum. Only one laboratory has reported increases in IGF-1 following bovine colostrum supplementation periods of eight days and of two weeks (255,256) with others reporting no change (53,90,228), and athletes ingesting 60 g/day not returning any positive doping test (for substances banned in 2002) (217). Mero determined that orally administered IGF-1 alone did not appear in the circulation and concluded that IGF-1 is not absorbed from bovine colostrum (255) although this is not necessarily correct as bovine colostrum contains

numerous components that would keep IGF-1 intact during gastrointestinal transit (315). The IGF-1 content in a 20 g dose of bovine colostrum (343) is approximately equivalent to that contained in three glasses of milk. Although not on the World Anti-Doping Agency's list of banned substances, this governing body does not recommend the ingestion of colostrum.

Future Directions

While bovine colostrum appears to reduce the incidence of URS, the specific mechanism/s for this require further investigation and should include measures of salivary antimicrobial proteins, in addition to SIgA. Well-designed investigations are also necessary to elucidate the potential of bovine colostrum to modulate intestinal permeability and inflammation in exercising humans.

As more is discovered about the minor constituents of bovine colostrum (8), there is the potential to discover novel bioactive proteins, and enhance understanding of the efficacy of already known components. Lactoferrin, isolated from bovine colsotrum, shows particular promise as an immune modulating glycoprotein. The majority of orally administered bovine lactoferrin survives gastric transit (392), exerting its effects through the interaction with gut enterocytes and resident immune cells. In neonatal animal models lactoferrin stimulates crypt cell proliferation (333), increases serum IgG and modulates cytokine secretion of stimulated mesenteric lymph nodes and spleen immune cells (89). Animal and cell culture models support the anti-viral, anti-bacterial immune modulating properties of lactoferrin (399) so it is suprising that there is limited literature investigating the effects of bovine lactoferrin supplementation in healthy humans (266), and no studies investigating potential effects on exercise-induced immune modulation. Benefits of lactoferrin for Fe deficiency anaemia associated with pregnancy (301), and a reported increase in T cell activation in healthy males (unblinded study) (266) support the exploration of bovine lactoferrin supplementation to modulate exercise-induced immune perturbations and/or enhance immune surveillance.

VITAMIN D

Introduction

From a sport and exercise science perspective, interest and research into vitamin D over the last decade has witnessed a remarkable resurgence (420). The reason for this is partly attributable to the re-emergence of the entirely preventable bone disorder rickets (298) but perhaps mainly due to the emerging evidence to suggest a fundamental role of vitamin D in many areas pertinent to the athlete. These include: skeletal muscle function (84), body composition (172), inflammation (421), muscle regeneration (299), and cardiac structure as well as aspects of innate and acquired immunity (168).

Vitamin D is unique in that, unlike other vitamins, it is not primarily obtained from dietary sources; rather it is synthesized via UV irradiation of the skin's dermis. Once in the circulation, either from diet, supplements or UV irradiation, vitamin D is transported to the liver bound to vitamin D binding protein where it is hydroxylated eventually leading to its activation. The hydroxylated compound 25-(OH) D is the major circulating vitamin D metabolite and is therefore the assay of choice when it comes to detecting vitamin D deficiencies. A further hydroxylation step in the kidney (or some tissues directly) is required to produce the active metabolite 1,25-$(OH)_2$ D and it is this active metabolite that is responsible for the multiple biological effects via both genomic and non-genomic mechanisms.

Defining terminology and why deficiencies occur

Although the multiplicity of fundamental biological roles of vitamin D is now appreciated, it is well documented that many individuals, including elite athletes, exhibit vitamin D deficiencies (Figure 2). These deficiencies are clear in athletes who live in temperate (84) as well as sunny climates and train predominantly in both indoor (424) and outdoor environments (84). This worldwide phenomenon of vitamin D deficiencies is partially attributable to poor dietary intakes, although the major reason is more likely a direct consequence of modern sun-shy lifestyles, including the use of appropriately applied high-factor sunscreen creams (125), which significantly restrict vitamin D synthesis.

A major area of confusion in the vitamin D literature arises from a lack of consensus as to what constitutes a genuine vitamin D deficiency and, more recently, the concept of a potentially "optimal" vitamin D concentration for health and athletic performance. It is beyond the scope of this article to explore this debate and therefore an "adequate" concentration will be classed here as >50 nmol/l as defined by the US Institute of Medicine. Moreover, it has also been suggested that what may be optimal for one tissue, such as bone or skeletal muscle, may not be optimal for another, such as immune function. In fact, from an exercise immunology perspective recent research is beginning to indicate that aspects of the immune system may require higher concentrations of vitamin D than has previously been defined as "adequate" for bone health (168).

Vitamin D and the immune system

Emerging research suggests that vitamin D plays a key role in both innate and acquired immunity, most likely exerting its function through gene expression modulation (168,183). In this role 1,25-$(OH)_2$ D functions as part of a heterodimer with its vitamin D receptor (VDR) and the retinoid X receptor modulating the expression of genes with specific vitamin D response elements located in the regulatory region (168). In fact it is estimated that close to 5% of the human genome is modulated by vitamin D (430), which is required in sufficient quantities to work effectively in gene expression modulation.

Many cells of the immune system including monocytes, macrophage, neutrophils and T and B lymphocytes contain the VDR and also express the enzyme, 1-α hydroxylase, which is responsible for hydroxylation of 25-(OH) D to its active 1,25-$(OH)_2$ D form. Activation in immune cells appears to be regulated by circulating concentrations of 25-(OH) D and induced by activation of the toll-like receptor cascade in the presence of pathogenic microbiota (30). In the immune system specifically, vitamin D up-regulates gene expression of broad-spectrum anti-microbial peptides (AMP), important regulators in innate immunity (231,408), and exerts an

Figure 2

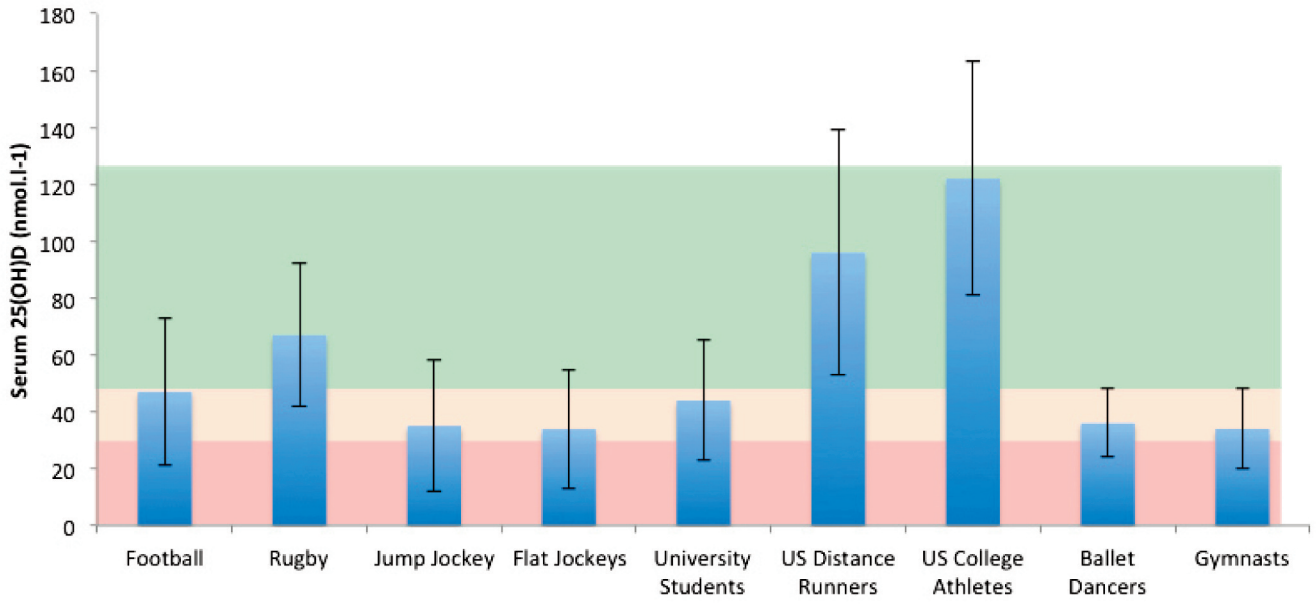

Figure 2 – Vitamin D concentrations of a variety of athletes tested (at rest) internationally. Pink area represents deficiency (<30 nmol/l), yellow area insufficiency (<50 nmol/l) and green area adequate (>50 nmol/l) as defined by the US National Institutes of Health Office of Dietary Supplements. (Data redrawn from (84,157,226,421,424)).

immunomodulatory effect on T and B lymphocytes in acquired immunity (168,431). AMP, including cathelicidin, are important proteins in the innate immune system (148) and help defend against acute illness including tuberculosis, influenza and the common cold (64,65,395). It is further suggested that vitamin D maintains a balance between the inflammatory Th1/Th17 cells and the immunosuppressive Th2/Treg cells to dampen inflammation and tissue damage (178) and to modulate the acquired immune response. Additional studies suggest that vitamin D enhances natural killer cell cytolytic activity (4), and acts to trigger the oxidative burst in activated macrophages (368). A single dose of vitamin D3 (100,000 IU) has been shown to enhance the innate immune response and restrict growth of mycobacteria *in vitro* (248).

Variations in vitamin D concentrations have the potential to influence immune response. A handful of studies in athletes (93,168), military personnel (218) and the general population (28,136,342) have reported negative associations between vitamin D concentration and incidences of URTI. In one study in college athletes, vitamin D concentrations over the winter and spring were negatively associated with documented frequency of acute URTI (157). The breakpoint for contracting single illness appeared to occur at ~95 nmol/l such that all athletes with circulating concentrations lower than this breakpoint had one or more episodes of illness whereas those with higher concentrations had one or fewer episodes. As has been shown in Figure 2, many athletes present with vitamin D concentrations significantly below this proposed breakpoint. A similar study in endurance athletes reported that a greater proportion of athletes maintaining circulating 25-(OH) D concentration <30 nmol/l presented with URS with the fewest symptoms reported in those with 25-(OH) D concentrations >120 nmol/l (171). Athletes with low vitamin D concentrations also had more URS days and higher symptom-severity scores. Randomly-assigned, placebo-controlled studies are needed in athletic populations to confirm the effectiveness of correcting low vitamin D concentrations on aspects of immune health and the prevention of URTI. A recent study in university athletes found evidence that 14-week supplementation with 5000 IU per day of vitamin D_3 during winter training significance increased salivary secretion rates of cathelicidin and SIgA (171).

Consensus

It is now established that many athletes present with deficient D concentrations, especially during the winter months, which result in numerous deleterious consequences. The concept of an "optimal" vitamin D concentration, however, is proving difficult to establish with emerging evidence suggesting that "optimal" vitamin D concentrations may in fact be tissue specific. Recent evidence is suggesting that athletes presenting with "sufficient" vitamin D concentrations (>50 nmol/l) may still be at an increased risk of contracting a URTI compared with athletes presenting with >75 nmol/l (171).

It must be stressed that there is a growing trend for mega dose supplementation in athletes. This is of concern due to some evidence which suggests an increase in all-cause mortality in individuals with high (>140 nmol/l) vitamin D concentrations (118), although cause and effect data to prove this are still lacking.

At present, perhaps the best advice for athletes is to monitor their vitamin D concentration and, in terms of immune health, aim for a target concentration >75 nmol/l. During the summer months athletes should aim to obtain sensible sun exposure, whilst ensuring that they do not suffer from sunburn, and consider a supplement of up to 4000 IU vitamin D_3 per day during the winter months if concentrations drop below 75 nmol/l.

Future directions

Future studies now need to establish if there is in fact an optimum concentration for an athlete's immune health utilizing a research design that will allow cause and effect to be established rather than reliance on correlative data.

IMMUNONUTRITION AND EXERCISE IN SPECIFIC POPULATIONS

Competitive athletes and military personnel

Background

A commonly asked question is, 'do athletes and military personnel have special nutritional requirements to maintain immunity; for example, do they need 'immune-boosting' supplements?' A somewhat simplistic answer is 'no'; so long as the diet meets the energy demands and provides sufficient macro- and micro- nutrients to support the immune system (140,405). The reasoning here is mostly based on the empirical evidence along with some sound logic: support for 'immune-boosting' nutritional supplements largely comes from studies in those with compromised immunity, such as the elderly and clinical patients; not, from young, otherwise healthy, individuals whom, as logic would dictate, might have little to gain from such supplements (229). To accept this viewpoint ignores the proviso about matching energy intake to expenditure and providing sufficient macro- and micronutrients to maintain immunity. Indeed, there are various athlete and military scenarios where energy, macro- and micronutrient intake may be insufficient.

Consensus

In the real world, athletes and warfighters either intentionally or non-intentionally experience deficits in energy intake (e.g. weight-loss diets, restricted rations) and macronutrient intake (e.g. restricted carbohydrate). There is substantial evidence showing that only a few days on restricted energy intake compromises immunity (221,295,401). There may be other times when athletes or warfighters experience both a down-turn in host defence and increased exposure to pathogens, e.g. foreign travel for training camps, competitions and military operations. As such, there are specific scenarios when these individuals might benefit from nutritional supplements to bolster immunity (Table 5). Thus the current, more reasoned, answer to the question, 'do athletes and military personnel have special nutritional requirements to maintain immunity?' is 'yes, sometimes.'

Table 5. Effects of nutritional supplements on the common cold and immunity in athlete and military scenarios

Scenario	Immune health and performance	Supplement	Supporting evidence and knowledge gaps	Refs.
Winter season	Common cold and Influenza season; URS decrease training and performance; low UVB skin exposure decreases vitamin D	Vitamin D_3	Moderate support for vitamin D in athletes/military; recommend 1,000 IU/day D_3 from autumn to spring to maintain sufficiency	168
		Vitamin C	Moderate support in athletes/military; Cochrane review of 5 studies in heavy exercisers (n=598) shows ~50% decrease in URS taking vitamin C (0.25–1.0 g/day); unclear if antioxidants blunt adaptation in well-trained; further support required	173
		Probiotics	Moderate support in athletes with daily dose of ~10^{10} live bacteria; Cochrane review of 12 studies (n=3720) shows ~50% decrease in URS incidence and ~2 d shortening of URS; minor side effects	142, 162
		Glutamine	Limited support; Gln (2 x 5 g), or a Gln precursor, decreased self-reported URTI after endurance events; this dose increases but does not maintain blood Gln nor alter many aspects of immunity; mechanism for therapeutic effect unclear; further studies required	77, 139
Suffering URTI	URS decrease training and performance; particularly in illness prone	Zinc lozenges	Moderate support; Cochrane review shows benefit of zinc acetate lozenges (75 mg) to decrease duration of URS; must be taken < 24 h after onset of URS; side effects include bad taste and nausea	367
		Vitamin C	No support; Cochrane reviews show no benefit of 'initiating' vitamin C supplementation (> 200 mg/day) after onset of URS	114, 173
Foreign travel	Increased URS risk; stress prior to travel may decrease immunity; increased exposure to pathogens; travellers' diarrhoea and risk of dehydration	Probiotics	Moderate support; probiotics can reduce risk of travellers' diarrhoea; probiotics do not decrease episode duration; minor side effects; further studies required	232, 380
Energy deficit	Training with energy deficit decreases performance and immunity	Multi-vitamin/ mineral; probiotics; bovine colostrum etc.	Limited support for supplements to reduce URS and bolster immunity in these scenarios; multivitamin/mineral supplement may provide insurance; unclear if antioxidants blunt adaptation in well-trained; impact of train low CHO on immune health remains unclear; further studies required	45, 110, 132
Train low CHO	CHO restriction/periodization may increase adaptation and performance but decrease immunity			86, 165
Training camp/military operation	Threats to immunity include: increase in physical exertion; other stressors e.g. psychological, altered sleep, heat and/or altitude; limited food choices; energy deficit			140, 406, 407

URS = upper respiratory symptoms; URTI = upper respiratory tract illness; CHO = carbohydrate.

Controversies and future directions

Paradoxically, nutritional strategies currently adopted by endurance athletes, including training with low carbohydrate (Table 5), may benefit training adaptations and performance at the expense of immunity; for example, carbohydrate restriction may increase the immunosuppressive stress hormone response to exercise (86,140,165). Consequently, the rather modest benefits studies show in terms of training adaptations

and performance might, in the long term, be lost if the athlete gets sick more often; *viz*. 'the less sick the more the athlete trains and the better they perform' (246,380,407). Studies are required to investigate whether the nutritional practices adopted by elite athletes impair immunity and increase infection; and, whether purported 'immune-boosting' supplements benefit immune health without blunting the desired training adaptations. Recent Cochrane reviews have noted the low quality of many studies on nutritional supplements to support immune health; specifically, small samples, poor controls and unclear procedures for randomisation and blinding were commonplace (162). Clearly, there is a pressing need for randomized controlled trials in elite athletes and military personnel with sufficient participant numbers; rigorous controls and procedures; appropriate supplementation regimens; and, clinically meaningful *in vivo* measures of immunity (see section on biomarkers)

Overweight and obese exercising humans

Obesity, a state of malnutrition related to excessive intake of energy has been related to immune dysfunction (175). High body fat levels are accompanied by changes in white blood cells, especially an increase in total leucocyte number, with altered differential counts in neutrophils, monocytes and lymphocytes, finding increased values of lymphocytes and neutrophils in boys and girls, respectively. However, low T- and B-cell mitogen-induced proliferation is shown in obesity (247). Both cell-mediated and humoral immunity are affected by obesity, with low antibody production after vaccination (247,361). Moreover, obesity has been characterized as a state of chronic low-grade inflammation, with an excessive amount of adipose tissue as the main determinant of this process (411).

Physical inactivity seems to be a prominent and modifiable risk factor to develop excess weight and obesity. According to epidemiological studies and clinical trials there is evidence addressing the influence of physical activity and fitness on low-grade inflammation in adulthood (160,302), athletes (292,390) and, to a lesser extent, in children and adolescents (412). Regular exercise seems to have proven anti-inflammatory effects in normal subjects (412) but also in overweight and obese individuals (175,411).

Regular training leads to a reduced TLR4 expression baseline, accompanied by a lower percentage of circulating CD14+CD16+ monocytes, which could result in an anti-inflammatory effect (243,385). In the case of obese subjects, macrophages are a potential source of inflammatory processes where the microbiota is also involved leading to lower insulin sensitivity in several tissues (liver, adipose tissue, hypothalamus, muscle) and a state of chronic low-grade inflammation (393). Studies performed in mice fed a high fat diet showed that exercise training reduces visceral adipose tissue followed by a change of M1 macrophage phenotype to M2 macrophages (205).

Cytokines have been shown to play a particular role in the regulation of the metabolism due to exercise, leading to immunomodulation within the adaptation mechanisms involved (272). However, it is important to highlight that the cytokine response depends on the acute or chronic exercise as well as its intensity, duration, the mass of muscle recruited, endurance capacity and idiosyncracy of the person practising exercise (411). The contracting skeletal muscle is a major source of circulating IL-6 in response to acute exercise. During heavy exercise, such as a marathon, there can be up to a 120-fold increase in the IL-6 plasma levels with the duration of the event explaining more than 50% of the variation. The aforementioned plasma IL-6 increase supports the hypothesis that post-exercise cytokine production is related to skeletal muscle and duration of exercise. Nevertheless, IL-6 shows a markedly lower response to acute exercise in trained subjects. The health benefits of long-term regular exercise are ascribed to the anti-inflammatory response elicited by an acute bout of exercise, which is partly mediated by muscle-derived IL-6. This IL-6 increase seems to induce an anti-inflammatory cytokine cascade (IL-1ra and IL-10), and to inhibit the production of pro-inflammatory cytokines, such as TNF-α (305,308). Therefore, the anti-inflammatory effects of exercise may offer protection against TNF-induced insulin resistance.

IL-6 stimulates lipolysis as well as fat oxidation. The increase of IL-6 after acute exercise is linked to increased CRP levels (290). In response to regular physical activity, basal as well as post-exercise plasma levels of IL-6 decrease by mechanisms that might include increased glycogen content, improved antioxidative capacity and improved insulin sensitivity. The lower levels of IL-6 in circulation will subsequently result in lower CRP levels. In untrained subjects, basal plasma IL-6 and CRP levels are elevated via mechanisms that may involve impaired insulin sensitivity and/or increased oxidative stress (305,308). The status of glycogen stores is also an important contributor to IL-6 production with exercise: the lower the glycogen the higher the IL-6 production. It is of particular importance in overweight, obese and diabetic patients especially for those with particular diets.

Adipose tissue is regarded as an active endocrine organ that releases a large number of bioactive mediators (pro-inflammatory cytokines, leptin, adiponectin, peptide YY, among others) modulating not only appetite and metabolism, but also the immune system involving inflammatory processes (339).

The EVASYON study aimed to develop a comprehensive intervention including diet and physical activity and to evaluate its efficacy in adolescents with excess weight and obesity. Some beneficial changes were achieved due to an early reduction of immunological and metabolic markers including leptin, IL-8 and TNF-α, delivered by adipose tissue and whose high levels are considered to be linked to an inflammatory state (339).

In the AFINOS study performed in Spanish adolescents, cardiorespiratory fitness and muscular fitness were shown to be inversely associated with adiponectin and leptin levels. Vigorous physical activity levels have also been inversely associated with leptin (249).

Preliminary evidence from the AFINOS study seemed to indicate that achievement of a healthy weight in this population

group might be the most effective strategy to prevent chronic low-grade inflammation and future cardiovascular and metabolic diseases. Indeed, an active lifestyle and a desirable cardiorespiratory fitness may attenuate these problems.

Therefore, physical exercise has been shown to increase weight management efficacy, being a potential therapeutic approach to modulate low-grade inflammation. Particularly, the encouragement of doing physical activity during adolescence could have important implications for public health, as a specific strategy to avoid high levels of the well-known sedentary habits during this crucial life period. Likewise, other types of physical activity related to muscular fitness (that is, resistance training) might be taken into consideration during adolescence because high levels of muscular fitness have shown negative associations with inflammatory proteins. Therefore, understanding the interrelationships between physical activity, fitness and fatness may be the main way to prevent low-grade inflammation, particularly at these ages (250).

The exercising elderly

It is well documented that aging is associated with a decline in cell-mediated immune function, a phenomenon often called immunosenescence, which contributes to the higher morbidity and mortality from infectious diseases in older population. On the other hand, mounting evidence suggests that aging is associated with an increased inflammatory response (212,347,398). Chronic, low-grade inflammation has been implicated in the pathogenesis of many common degenerative and metabolic diseases associated with aging. Several studies have shown that acute and prolonged or vigorous bouts of exercise cause immunodepression as well as the related increase in incidence of URTI symptoms, and may also induce increased inflammation and oxidative stress (273,365,406). Therefore, extreme exercise may exacerbate the age-associated dysregulation of the aging immune system (286,366,394). However, regular moderate exercise in general causes no such adverse effect, and might even enhance the immune function (274), particularly in older individuals (365,406). Studies showed that calisthenic exercise increased NK activity and T cell function in elderly women (286); primary antibody and delayed-type hypersensitivity (DTH) responses to the novel antigen keyhole limpet haemocyanin (KLH) were lower in older than in young subjects, but these *in vivo* measures of the immune function were improved by exercise in older but not young subjects (150,370). A possible reason behind this observation is that, relative to their young counterparts, the older individuals have a less optimal immune response which is restored by moderate exercise (406).

Proper nutrition, *i.e.*, adequate and well balanced intake of nutrients, is important for normal function of the immune system. Currently no information is available as to whether exercising older persons have unique nutritional needs compared to their young adult counterparts. However, a significant percentage of older adults have low consumption of several micronutrients including the B vitamins, vitamin E and Zn, all of which are needed for the normal function of the immune system (239,300). At the same time, both inflammation and oxidative stress increases with aging suggesting that the older exercising adults might require higher level of nutrients and foods with antioxidant and anti-inflammatory properties. In addition, when conducting the same type of exercise, the older persons are known to more easily suffer from muscle damage and require a longer period to recover from it (127). The exercise-induced muscle damage can initiate an inflammatory response, which could further exacerbate the chronic low-grade inflammation observed in older adults, further suggesting that exercising older adults might require higher level of nutrients and other dietary components with immune enhancing and/or anti-inflammatory properties than non-exercising older adults or their young counterparts. However, previous studies which mainly involve younger adults have indicated that consuming antioxidant supplements, with the possible exception of quercetin, does not help in terms of improving exercise-induced immunodepression, inflammatory response, and URTI (see the antioxidants and polyphenols sections – both in this series). In support of this, studies thus far suggest that antioxidant micronutrient supplementation may not afford protection against muscle damage but, rather, it may interfere with cellular signalling functions of ROS and interrupt training-induced adaptations (50,192). Further studies are needed to determine whether exercising older adults would respond in a different manner from young adults, given the observation that older adults may have higher requirements for nutrients and food components that possess antioxidant and anti-inflammatory properties.

Another nutritional consideration for older adults is the amount of total energy. Since the intensity and duration of exercise are usually less in older persons compared to young adults, the total calorie intake should be adjusted accordingly to avoid conversion of excess calories to body fat. Additionally, the general recommendation to increase calorie intake from carbohydrates should be exercised with caution for older persons, due to the fact that glucose tolerance and insulin sensitivity are decreased with aging.

In summary, information on nutritional needs of exercising older adults whether micro or macro-nutrients is scarce. The age-associated dysregulation of the immune response (suppression of cell-mediated immunity and increased inflammation), together with other age associated changes, and low consumption of nutrient rich foods strongly supports the necessity of further research in this area so that specific recommendations can be made.

BIOMARKERS IN IMMUNONUTRITION

Introduction

In this section the strengths and weaknesses of various biomarkers used in studies by nutritional immunologists are evaluated (Table 6). An important consideration is that exercise immunologists often perform investigative work in the field, away from the rigorously controlled laboratory environment. Consequently, the studies are often limited by a lack of experimental control and the choice of measurement tool(s) is often dictated by convenience, practicality and cost. With this in mind, areas of uncertainty, gaps in knowledge and opportunities for continued research development on immune biomark-

ers are highlighted, particularly research targeted towards the development of technologies applicable in the field. These opportunities include rapid, non-invasive measurements of immunity by portable devices at single time points and even continuous monitoring by wearable technology (e.g. smart contact lenses) may be possible in the not too distant future.

Classification of upper respiratory tract illness

Arguably the most illuminating studies on factors influencing common cold incidence (e.g. psychological stress, sleep) have quarantined individuals before and for up to 7 days after intranasal inoculation with live common cold viruses (rhinovirus, respiratory syncytial virus or coronavirus) and assessed the development of clinical colds (Table 6) (87,88). Although this represents a strong experimental model to identify the effects of nutritional interventions on common cold incidence, there are obvious limitations that have prohibited its adoption by exercise immunologists. These include ethical considerations, as well as cost, requirement for medical facilities and support, together with the fact that athletes are unlikely to participate in a study where ~40% of individuals develop a common cold (191). Another limitation is that studies using the common cold challenge model have not identified whether the increased development of common cold in those under psychological stress (88) or sleep stress (87) is due to a systemic immunodepression or local effects at the nasal mucosa. For these reasons exercise immunologists have relied heavily on subjective self-report of common cold symptoms using either unstandardised health logs, standardised symptom questionnaires (e.g. Jackson) or physician assessment of common cold symptoms (Table 6). In 1958 Jackson et al. reported clinical features of the common cold after infecting >1,000 individuals by nasal instillation of nasal common cold secretions collected from donors (191). In the ~40% of individuals who developed symptoms of a common cold in the 6-day monitoring period, 8 clinical symptoms were incorporated into the questionnaire. Symptoms included headache, sneezing, chilliness and sore throat that appeared in the first 48 h and nasal discharge, nasal obstruction, cough and malaise that appeared later. The 8 clinical symptoms were scored on a 4-point scale from 0 (no symptom) to 3 (severe symptom): Jackson's criteria for a common cold included a total symptom score of ≥14 and a "yes" answer to the dichotomous question, 'do you think that you are suffering from a common cold?' during the 6-day monitoring period (191).

The Wisconsin upper respiratory symptom survey (WURSS; (20)) has also been used widely by exercise immunologists (149,283,374), including nutritional intervention studies (149), as it considers the impact of common cold symptoms on quality of life measures (Table 6). Studies have raised questions about the validity of the physician-verified common cold, highlighting that neither self-reported nor physician-verified common colds should ubiquitously be referred to as infectious (93,374). Notwithstanding these limitations, studies highlight the negative impact of self-reported common cold symptoms on training volume (246) and medal-winning prospects in elite athletes (322,380).

Three key recommendations include: 1) standardising the recording of common cold symptoms in athletes (e.g. incorporating the Jackson common cold scale or the WURSS into a training log); 2) recording the impact of common cold symptoms on training and performance (e.g. discontinued or reduced training); and 3) where possible, incorporating identification of infections from pathological analysis of swabs (Table 6 see next page). Common cold challenge studies show that only ~40% of those inoculated develop symptoms associated with the common cold yet >80% are typically infected (positive virology/specific antibody response) (87,88,191). Thus, an important research question for exercise immunologists is, 'why do less than half of those infected develop symptoms of the common cold? Adopting these recommendations will allow the exercise immunologist to understand more fully the influence of exercise training and nutritional interventions on the development of common cold symptoms and their impact on training and performance in those with and without confirmed infectious aetiology.

***In vivo* immunity**

Where feasible, exercise immunologists are encouraged to use *in vivo* methods for assessing immune responses (3,405,406). By initiating an integrated and highly coordinated immune response in the normal tissue environment, *in vivo* immune methods provide more clinically relevant information that extends beyond *in vitro* assays (Table 6) (87). A weakness of many *in vitro* assays is the requirement to separate immune cells from their normal environment and incubate in artificial culture. Examples of *in vivo* immune methods include assessing: the circulating antibody response to influenza vaccination and hepatitis B vaccination (55); the local skin response to intradermal antigens using delayed type hypersensitivity (DTH) (52) and to topically applied antigens using contact hypersensitivity (CHS) (164). Studies demonstrate that both acute and chronic exercise can increase influenza vaccination success (circulating antibody titre) in those with sub-optimal immunity (e.g. elderly) or where antigen immunogenicity is low; but little is known about the influence of high-level training and nutritional interventions on the success of influenza vaccination in young, healthy athletes. A distinct advantage of the vaccine model (e.g. influenza) is that athletes may be keen to participate in a study where the clinical protection afforded by the vaccine is directly beneficial to them (147,406). Recognised limitations with the vaccine model include that the *ex vivo* T cell response to influenza vaccination has been more strongly related to vaccine protection than the circulating antibody titre that is typically measured (304). Also, the incorporation of repeat antigens in the influenza vaccine elicits a mixture of primary and secondary antibody responses; thus providing limited mechanistic insight (55,406). Using a novel antigen in the DTH method (e.g. keyhole limpet haemocyanin) (369); or CHS method (e.g. diphenylcyclopropenone (DPCP))(109,164) presents the opportunity to assess the influence of exercise as a stressor, and nutritional interventions on both the primary and secondary immune response. The DTH and CHS methods (Table 6) also overcome some other limitations with the vaccine method including: variable immunogenicity (e.g. hepatitis B (177)); annual changes in vaccine composition (e.g. influenza (55)); and, difficulty when comparing the circulating antibody results from different studies using in-house enzyme-linked immunosorbent assays (ELISA) or other technologies (55). Nevertheless,

Table 6. Classification and ratings of biomarkers used in immunonutrition and exercise experiments

Ratings on a continuum where: OOOOO lowest to ●●●●● highest utility. [1]Clinical relevance = considers relation to clinically meaningful outcome; [2]Scientific rating = considers validity (link to immunological mechanism(s)), reliability and diagnostic accuracy (i.e. sensitivity and specificity); [3]Practical status = considers convenience, speed, invasiveness and cost. WURSS = Wisconsin upper respiratory symptom survey; URTI = upper respiratory tract illness; Ig = immunoglobulin; DTH = delayed type hypersensitivity; KLH = keyhole limpet haemocyanin; CHS = contact hypersensitivity; DPCP = Diphenylcyclopropenone; SIgA = secretory IgA; NK = Natural Killer; CTL = Cytotoxic T Lymphocyte; CMV = Cytomegalovirus; HV = Herpes Virus; CVD = Cardiovascular Diseases; CRP = C-Reactive Protein.

Method	Clinical Relevance[1]	Scientific Rating[2]	Practical Status[3]	Overall Rating	Comments	Example Ref.
Upper Respiratory Tract Illness						
Jackson common cold questionnaire – 8 items	●●●●○	●●●●○	●●●●●	●●●●○	Symptoms derived from >1,000 individuals after nasal instillation with common cold	191
WURSS – 21 or 44 items	●●●○○	●●○○○	●●●●○	●●●○○	Considers URTI symptom impact on quality of life. Weaknesses: lengthy to complete, external validity	20
Live common cold challenge (experimental infection)	●●●●●	●●●●○	○○○○○	●●●○○	Strong clinical and scientific utility. Weaknesses: ethics, cost, medical support and quarantine	87, 88
Pathological determination of URTI	●●●●○	●●●●○	●○○○○	●●●○○	Useful partnered with symptomatology to identify infectious *vs*. non-infectious aetiology	93, 374
Physician identified URTI	●●●○○	●●○○○	●●○○○	●●○○○	Weaknesses: unstandardized; studies have questioned utility	93, 374
URTI symptom log	●●○○○	●○○○○	●●●●○	●●○○○	Weaknesses: unstandardized; preference is to use an externally valid questionnaire e.g. Jackson	20, 191
In vivo Immunity						
Ig response to vaccination (influenza, hepatitis B, pneumococcal)	●●●●○	●●●●○	●●●○○	●●●●○	Clinically relevant, beneficial to participant; hepatitis B vaccination allows study of primary and secondary Ig response; weaknesses: influenza vaccination elicits a mixture of primary and secondary Ig responses; variability in vaccine immunogenicity and Ig assays	55 147, 304, 406
DTH skin tests (e.g. KLH, multitest)	●●●●○	●●●●○	●●●○○	●●●●○	Clinically relevant; novel antigens (e.g. KLH) enable investigation of primary and secondary response. Weaknesses: relation to URTI unclear; Merieux multitest no longer available; intradermal injection and skin swellings may be uncomfortable	52, 369
CHS skin tests (e.g. DPCP)	●●●○○	●●●●○	●●●●○	●●●●○	Clinically relevant; novel antigens (e.g. DPCP) enable investigation of primary and secondary response; patches applied to skin (not intradermal); simple measurement (e.g. skin fold). Weaknesses: relation to URTI needs investigating; skin reactions may be uncomfortable	109, 164
Mucosal Immunity						
Saliva SIgA	●●●○○	●●●○○	●●●○○	●●●○○	Clinically relevant; convenient collection. Weaknesses: lack of standardisation; assays are time consuming and costly; relation to URTI in athletes is not definitive; requires individual baseline	145, 271
Tear fluid SIgA	●●●○○	●●●○○	●●●○○	●●●○○	Clinically relevant; shows promise. Weakness: only one study to date	161
Ex vivo/In vitro Immunity						
Phagocytosis and oxidative burst assays	●●○○○	●●●○○	●●●○○	●●●○○	Moderate clinical relevance; scientifically justified useful indicators for body's overall innate immune defence. Phagocytosis and oxidative burst can be measured at the same time using flow cytometric method. Weaknesses: not pathogen-specific; requires analysis of fresh samples in the same day; lack of standardisation in analysis procedure and reference values; limited to within-study comparison	9, 130

Method	Clinical Relevance[1]	Scientific Rating[2]	Practical Status[3]	Overall Rating	Comments	Example Ref.
Cytotoxicity assays (CTL, NK)	●●○○○	●●●○○	●○○○○	●●○○○	Moderate clinical relevance; scientifically justified useful indicators for assessing body's defence against viral and other intracellular infections. Weaknesses: time consuming for target cell labelling; not pathogen-specific for NK; could be pathogen-specific (CTL) but requiring use of *ex vivo* priming and re-challenge; lack of standardisation in analysis procedure and reference values; requires same-day analysis; time consuming and costly; requiring sterile culture condition; conventional methods involving use of radioisotope labelling; limited to within-study comparison	7, 267, 418
Lymphocyte proliferation	●●○○○	●●●○○	●●○○○	●●○○○	Moderate clinical relevance; scientifically justified useful indicators for cell-mediated immune response. Weaknesses: polyclonal reaction but not antigen/pathogen-specific, or could be pathogen-specific but requiring experimental infection or vaccination and *ex vivo* re-challenge; lack of standardisation in analysis procedure and reference values; requires blood samples for relatively rapid processing; requiring sterile culture condition; conventional methods involving use of radioisotope and special equipment for sample harvesting and counting; the alternative fluorescence dye tracking method requires flow cytometer and is more time-consuming; limited to within-study comparison	159
Immune Cell Trafficking and Other Markers						
White blood cell count	●●●●○	●●●○○	●●●●○	●●●○○	High clinical relevance at the presence of symptoms such as fever, localized pain, or cough & sputum representing bacterial infection, scientifically justified as mentioned in the text, sensitivity is high for bacterial infection but low for viral infection, false positive results when asymptomatic and may represent sympathetic activation, classical marker for bacterial infection. Weakness: requires blood samples for relatively rapid processing	24, 261
Left shift (band/segmented neutrophil)	●●●●○	●●●○○	●●●●○	●●●○○	High clinical relevance at the presence of symptoms such as fever, localized pain, or cough & sputum representing bacterial infection, scientifically justified as mentioned in the text, sensitivity is high for bacterial infection but low for viral infection, classical marker for bacterial infection. Weakness: requires blood samples for relatively rapid processing	182
NK cell count	●○○○○	●○○○○	●○○○○	●○○○○	Low clinical relevance, may represent sympathetic activation, limited target cells (CMV, HV infected cells and some type of cancers). Weaknesses: assays are time consuming and costly (flow cytometric analysis) requiring blood samples for relatively rapid processing	238, 252
Lymphocyte count	●●●○○	●●●○○	●●●○○	●●○○○	Moderate clinical relevance, decreased in systemic viral infection or autoimmune diseases due to chemokine mediated recruitment. Weakness: requires blood samples for relatively rapid processing	238
CD4/CD8	●●○○○	●○○○○	●○○○○	●○○○○	Low clinical relevance except for elderly individuals. CD8 previously considered to represent immune suppression but are now considered as a subpopulation of T cells with cytotoxic property. Weaknesses: assays are time consuming and costly (flow cytometric analysis) requiring blood samples for relatively rapid processing	381, 419
N/L Ratio (Neutrophil/Lymphocyte)	●●○○○	●●●○○	●●●○○	●●○○○	Moderate significance for asymptomatic healthy person as a marker of sympathetic nervous system activation, no association with immune responses but relevant to athletic condition, a potential risk factor for hypertension and type-2 diabetes. Weakness: requires blood samples for relatively rapid processing	47, 428
Cytokines production and soluble cytokine receptors	●●●○○	●●●●○	●●●○○	●●●○○	Clinically relevant specific. Weaknesses: blood draw and long processing, costly	117, 338
CRP	●●●○○	●●○○○	●●●●○	●●●○○	Clinically relevant. Established risk factor for CVD	290

determining the clinical significance of DTH and CHS responses, with specific regard to infection, is an important avenue for future research. It is possible that the strength of the cutaneous recall response e.g. to DPCP (109) could be generalised beyond skin immunity to indicate the immune system's general ability to respond to an infectious challenge. Available evidence from clinical studies supports this notion as cutaneous immune measures are impaired in individuals with acute infectious illness (26); they also track immune status and predict mortality in critically ill human immunodeficiency virus (HIV)-infected patients (113).

Mucosal immunity

Assessing immune markers in saliva and tears is of interest to exercise immunologists, particularly those working in the field environment. This is because collection of these fluids is non-invasive, convenient, practical and low cost (Table 6) but also because as many as 95% of all infections are thought to be initiated at the mucosal surfaces (46). This highlights the important role mucosal immunity plays in defence against opportunistic infections such as the common cold (46). SIgA production is the major effector function of the mucosal immune system providing a 'first line of defence'. SIgA is known to exhibit broad-spectrum antimicrobial activity against a range of viral and bacterial pathogens, through inhibition of pathogen adherence and penetration of the mucosal epithelia and by neutralising viruses within the epithelial cells during transcytosis (48).

Exercise and nutritional interventions might conceivably alter mucosal immunity at the level of local B cell antibody synthesis (e.g. via altered autonomic nervous system (ANS) activity or altered nutrient availability) (291) and/or by altering the transport of IgA (e.g. via altered ANS activity or availability of polymeric Ig receptor (69,320)). On this premise, there has been widespread attention and optimism that salivary SIgA may serve as a non-invasive biomarker of mucosal immunity and common cold risk. Research demonstrates that salivary SIgA has some utility for a monitoring application, whereby an individual's normal, healthy reference range is determined under standardised conditions and atypically low values can indicate an increased likelihood of common cold (145,271). The availability of a point-of-care tool for measuring salivary SIgA has made its monitoring in athletes possible (85), highlighting an avenue for continued research efforts. Perhaps not surprisingly, given the large individual variability, research has not convincingly demonstrated the utility of salivary SIgA as a predictive biomarker for the common cold by identifying atypically low values for an on-the-spot application (against the population reference range). Studies have shown a decrease in salivary SIgA lasting for an hour or more after prolonged exercise (146); this is in line with the 'open window hypothesis' (389,402). However, studies have also shown no change (32) or even an increase in salivary SIgA after prolonged exercise (40). Low levels of saliva SIgA during fluid and energy deficits (295) and in those deficient for vitamin D (171) were countered by additional energy intake in one study in military personnel (110) and by vitamin D supplementation in another study in athletes (170). Nevertheless, there are examples of conflicting evidence for mucosal immune responses to nutritional supplementation. Twelve weeks of bovine colostrum supplementation increased salivary SIgA ~80% in athletes in one study (96) but had no effect in another (198). The levels of salivary SIgA and other saliva antimicrobial proteins (AMP) (e.g. lysozyme) reported in the literature are highly variable: key considerations include that multiple glands contribute to saliva composition, and likely to its variable composition. There are also various potential confounders (e.g. diurnal variation (111); psychological stress (193); nutritional status (295) and gender (169)). Those wishing to measure salivary SIgA (and other AMP) in their studies should: standardise the saliva collection method (e.g. 5 min passive drool); standardise the reporting of salivary SIgA (report both SIgA concentration and secretion rate) (40,404); and control potential confounders. With the search for viable alternatives in mind, a recent study demonstrated that tear SIgA (tear is secreted only by the lacrimal gland) has potential as a non-invasive biomarker of mucosal immunity and common cold risk (161). Decreased levels of tear sIgA but no change in salivary SIgA were observed during pathology-confirmed common cold and the week before individuals reported common cold symptoms. The risk of common cold symptoms the following week increased nine-fold in those with low tear SIgA. Further advances in nanotechnology and microfluidics might, in the near future, afford the possibility for on-the-spot tear fluid measurement devices and even continuous bio-monitoring by contact lenses.

***Ex vivo/in vitro* immunity**

To assess if a nutritional intervention is effective in modulating immune response, it is necessary to use appropriate markers as measurable outcomes. A variety of markers has been identified as suitable for assessing different aspects of immune system status and functionality, and different techniques are developed to measure these markers with varied sensitivity, accuracy, feasibility, and relevance for the intended purpose. Investigators can select appropriate markers based on the specific objectives of their studies. These markers, along with the studies for which they are adopted, are usually classified as *in vivo*, *ex vivo*, and *in vitro* markers/studies. In the *in vivo* setting, both nutritional intervention and the assessment of the immune response are conducted using a living subject (human or animal). This approach best reflects the physiological response to the intervention. In the *ex vivo* setting, the intervention is administered to a living subject but the immune function analysis is conducted outside of the body using accessible biological materials. For example, assessing the ability of the immune cells isolated from the blood of study participants to respond to a stimulus which mimics pathogenic or non-pathogenic agents to which the immune cells might be exposed. The incubation is performed in the presence of a synthetic media enriched in serum that can be from an animal, typically foetal calf serum, or from a human subject. This approach, particularly when performed in the presence of a subject's serum, can provide very useful information about how the immune cells might respond when challenged *in vivo*. The *in vitro* setting for both the intervention and assessment are conducted outside of the body using either cells from the participant or cell lines. Thus the key difference between the *ex vivo* and *in vitro* studies is whether the intervention is administered to humans/animals or added to the cell culture. *In vitro* experiments alone have limited value, but

they are usually used as a screening tool to search for candidates of interest for animal or human studies, or for further investigation of the cellular and molecular mechanisms of an intervention that has shown functional efficacy *in vivo*. A brief description is provided below to introduce the most commonly used *ex vivo* and *in vitro* indices for assessing immune function in exercise and nutrition research (Table 6).

In these assays, immune cells are separated from the body (or from stock as cell lines) and maintained in culture medium. Various stimuli, e.g. mitogens, are used to activate cells to make them proliferate, synthesize and release soluble factors, directly attack target cells, or engulf the added foreign particles (microorganisms or inert substances).

Phagocytosis

Phagocytosis is a process by which specialized phagocytic cells (neutrophils and monocytes/macrophages) engulf and internalize solid matter. Phagocytosis is a key mechanism of the innate immune system to contain and kill invading pathogens, or to process them for antigen presentation to T cells, and activating the adaptive immunity. The substrates used for phagocytosis assay include bacteria, yeast, red blood cells, and inert particles. One popular method is to incubate one of these substrates labelled with fluorescence with phagocytic cells and then determine the cellular uptake of these substrates using a flow cytometer. This assay can be coupled to evaluation of oxidative burst and bacterial killing, as described below.

Oxidative (respiratory) burst

This reaction is triggered by phagocytosis or exposure to certain inflammatory mediators causing a dramatic increase in oxidative metabolism which will result in the rapid generation and release of reactive oxygen species by neutrophils and monocytes. These released toxic compounds are used to kill bacteria after phagocytosis. Oxidative burst is commonly measured either using cytochrome C reduction with a photometry method, the chemiluminescence conversion method is used by most people now, or based on changes in fluorescence properties of appropriate substrate compounds, which can be assessed by flow cytometry. The bactericidal assay adds live bacteria to a culture of phagocytes, subsequently measuring phagocytosis and the destruction of pathogens.

Cytotoxicity assay

Activity of both cytotoxic T lymphocytes (CTL) and NK cells can be assessed by a cytotoxicity assay. CTL recognizes cells on the basis of their cell-specific surface antigens. NK cells, a special type of lymphocyte, directly lyse tumour cells or virally infected cells. In the CTL activity assay, target cells can be lymphoblasts, cultured tissue cells, or tumour cells. In NK activity assay, tumour cells serve as target cells. In both assays, target cells are labelled with ^{51}Cr and then incubated with effector cells (e.g. human peripheral blood mononucleocytes (PBMC), or animal spleen cells as a source of NK cells) at different ratios. The percentage of ^{51}Cr released is calculated to represent lysis of target cells which reflects the cytotoxicity of effector cells. Flow cytometry methods are now used as a favourable non-isotope alternative to the classical ^{51}Cr method.

Lymphocyte proliferation

The proliferative response of lymphocytes is frequently used to assess cell-mediated immune response. Cell proliferation can be quantified by measuring incorporation of [^{3}H]-thymidine into DNA, or non-radioactive methods such as bromodeoxyuridine incorporation, and fluorescence dye dilution assays. Common agents used to simulate lymphocyte proliferation are the T-cell mitogens concanavalin A (Con A), phytohaemagglutinin (PHA), and anti-CD3 Ab, all of which stimulate T-cell proliferation. LPS stimulates B-cell proliferation, and pokeweed mitogen (PWM) stimulates proliferation of both T- and B-cells.

Cytokine production

Immune cells produce an array of protein mediators called cytokines, which serve as messengers to regulate immune cell activities or directly exert effector function. Cytokine quantification is helpful for assessing both general state and particular functions of immune system. Agents used to stimulate lymphocyte proliferation as mentioned above as well as some cytokines and growth factors can induce high levels of cytokine production in culture medium which can be conveniently quantified by ELISA. In addition, intracellular cytokine levels in specific cells can be assessed in a mixed cell population (without the need for purification specific cell types) using flow cytometer and a combination of stains for cell surface markers and antibodies (Ab) against the cytokine of interest.

One difficulty in interpreting data obtained from cytokine analysis is that many of them are, at times, redundant and at other times oppose function. This makes it difficult to gain insight into the "real change" in function of the immune system based on the impact of an intervention on relatively few cytokines measured. Assay costs can constrain the numbers of samples measured. Fortunately, in recent years, the emerging "omics" technologies (genomics, proteomics, and metabolomics) have provided the opportunity to obtain a more holistic and integrated view of the basal and stimulated potential for cytokine production using small aliquot of sample and more sophisticated data analysis approaches. Combined with the conventional assays, these newer approaches will provide a more comprehensive and insightful understanding of whether and how nutritional intervention impacts cytokine production and the function of the immune system in general. Furthermore, these approaches will help address and reduce the well-known intra-individual variation observed in response to exercise and nutritional interventions. The "omics" information comes from genomic and epigenetic regulations (alleles, single nucleotide polymorphism, methylation), post-transcriptional regulation (microRNA), and proteomics screening for isomeric forms of protein and post-translational modification. These can be used as tools to identify the intrinsic inter-personal differences and to design personalized interventions to achieve optimal results.

Leucocyte trafficking

Leucocytes or white blood cells, including granulocytes (neutrophils, eosinophils, and basophils), monocytes, and lymphocytes (T-cells, B-cells, NK-cells), have essential roles in host defence. Leucocyte counts have long been used as a simple

clinical marker of inflammation and/or immune function but, because there is a variety of mechanisms which also influence each other, careful consideration is necessary to understand the implication of the observed changes in the leucocyte counts in association with exercise and training.

Acute alteration of leucocyte counts by a single bout of exercise

A single bout of exercise results in changes in the circulating numbers of leucocytes. The direction of change depends on the duration and intensity of the exercise. Short-term exhaustive exercise of less than one hour induces leucocytosis mainly due to increases in the circulating numbers of neutrophils and lymphocytes including NK cells, whereas prolonged exercise beyond one hour induces a marked neutrophilia. After strenuous or prolonged exercise, circulating T-cell and NK cell counts usually go down transiently below baseline within about 30 minutes after exercise (39,310). All these changes may be attenuated by carbohydrate ingestion. Leucocytosis after high intensity running in marathon runners or long distance running (18-20 km) was attenuated by carbohydrate supplementation (188,280).

Leucocyte count in persons with different level of aerobic fitness or with obesity

As a long term effect of exercise training, recent studies demonstrated the association between fitness measurements and leucocyte counts. Maximal metabolic equivalents (METs) achieved during a treadmill exercise test were inversely associated with total leucocytes, neutrophil and basophil counts after adjustment for age, whereas body mass index (BMI) was positively associated with total leucocyte, neutrophil, lymphocyte, monocyte, and basophil counts in a cross sectional survey of non-smoking healthy men (195). The Dose-Response to Exercise in Women Aged 45–75 yr (DREW) study demonstrated an exercise-volume dependent decrease in total leucocyte and neutrophil counts after 6-month moderate intensity exercise intervention for 390 sedentary obese postmenopausal women randomly assigned to different doses of exercise (196). The decrease in the waist circumference, but not the decrease in BMI, correlated with the reduction of leucocyte and neutrophil counts in this study. During a study with a mean follow-up period of 45.6 months, obesity was associated with leucocytosis with low grade elevation of serum acute phase CRP without any recognized cause of leucocytosis other than being obese (176).

Mechanism of alterations in leucocyte trafficking

Haematopoiesis, retention, release and clearance of leucocytes need to be considered to understand leucocyte trafficking. They are known to be differentially regulated across different subsets of leucocytes. Myeloid cells are constantly produced in the bone marrow under the regulation of the local microenvironment, in which stromal cells play the central role. Upon conventional inflammatory response initiated by bacterial infection, monocyte or dendritic cell derived granulocyte macrophage colony-stimulating factor and granulocyte colony-stimulating factor accelerate proliferation of myeloid progenitors as well as their maturation. CXC chemokines, such as IL-8, produced at the site of inflammation or infection, recruit neutrophils from the bone marrow (377). Malnutrition has long been recognized as a factor that suppresses haematopoiesis leading to anaemia and pan-leucopaenia. Recently, protein malnutrition was found to promote adipogenic differentiation of bone marrow stromal cells, which affects haematopoiesis (98).

Chemokine receptor CXCR4 and the corresponding ligand CXCL12 are essential for retention and homing as well as for the clearance of leucocytes. CXCL12 is abundantly expressed in the stromal cells of bone marrow as well as in the endovasculature in various organs such as spleen, liver and lung. CXCL12 expression on bone marrow stromal cells was recently shown to be decreased by noradrenaline released from the sympathetic terminal in the bone marrow via the β adrenergic receptor. Circadian fluctuation of noradrenaline release in the bone marrow is suggested to be the major cause of circadian fluctuation of leucocyte counts, especially neutrophils (352). On the other hand, the corresponding receptor CXCR4 on lymphocyte is known to be under the control of glucocorticoid. Strenuous or exhausting exercise that induces hypothalamic pituitary adrenal axis activation is considered as the major cause of post exercise lymphopaenia (294). Interestingly, senescent neutrophils expressing CXCR4 are redeployed into the bone marrow, where they are subjected to phagocytic elimination (378). Stressful exercise, such as prolonged, exhaustive or high intensity strenuous exercise, may therefore lead to post exercise lymphopaenia.

NK cells (CD56 dim cytotoxic NK subset) and CD8 positive T cells, γ-δ T cells are major cytotoxic leucocytes in the circulation. These cytotoxic effector leucocyte subsets comprise the marginal pool by an adhesion molecule CD11a/ fractalkine CX3CR1-mediated attachment to the endothelium. Adrenaline rapidly attenuates this attachment without affecting the expression of the adhesion molecule to allow demargination and release of these leucocytes into circulation to induce adrenergic leucocytosis through β2 adrenergic stimuli (112). Adrenergic stimulus is not only delivered through bone marrow sympathetic nerve terminal, but also through catecholamines released from either sympathetic nerve terminals in various organs and adrenal medulla.

In summary, during a single bout of exercise with either peripheral sympathetic nerve activation or systemic adrenaline response during prolonged exercise when extensive lipolysis is required, neutrophils and monocytes of myeloid origin will be released from bone marrow by CXCL12 downregulation. Catecholamines would also attenuate CD11a/CXCR1 mediated attachment of marginated cytotoxic leucocytes including NK cells resulting in marked leucocytosis. Neutrophils are known to have a blood half-life of 6.5 hours, and the majority of aged neutrophils would express CXCR4 several hours after the release from bone marrow and will return to bone marrow for clearance by phagocytes. In conditions of exercise with a glucocorticoid response, lymphocytes will be redeployed to secondary lymphoid tissues by enhanced CXCR4 expression and may lead to transient lymphopaenia.
Enhanced sympathetic activity in obese individuals seems to play a central role in the observed leukocytosis similar to the observed elevated blood pressure. The obesity-associated chronic inflammation, through increased leptin production,

enhances sympathetic activity and therefore leukocytosis (156). Therefore, attenuation of obesity-related leucocytosis after sessions of exercise training may either be the result of decreased sympathetic activity through weight reduction or because of anti-inflammatory effect of exercise leading to reduced leptin release or both.

Clinical implication and limitations

Impaired adhesion or excess retention of leucocytes both compromise self-defence as demonstrated in primary immuno-deficiency patients with leucocyte adhesion-deficiency (LAD) syndrome (293). LAD is caused by the mutations of the gene encoding for the β-2 integrin CD18, which is required for firm adhesion of leucocytes to endothelium before transmigration. LAD patients demonstrate marked leucocytosis with high incidence of gingivitis, periodontitis, cutaneous infections without pus formation, life-threatening bacterial infections and delayed wound healing.

The clinical implication of altered leucocyte trafficking by exercise is that, during sympathetic activation, even though the circulating leucocyte number is high, it is possible that immune or inflammatory reaction through leucocytes may be blunted because of attenuation of adhesion through β adrenergic stimuli. This may avoid both systemic and local immune responses and inflammation that affects "fight or flight" actions. However, a similar situation may happen when metabolic demand is high and after prolonged exercise or in obesity with sympathetic activation. Therefore, leucocytosis in such situations may be considered as a new "open window". Previously the term "open window" referred to the occurrence of lymphopaenia or decreased NK cells after strenuous exercise due to retention in secondary lymphoid organs and marginal pool. However, a mild and transient retention to their site of action may not confer impaired immunity. This might be explained by the fact that NK cells have a limited spectrum of cytotoxicity, which is strictly determined by the expression of variant MHC-I (major histocompatibility antigen 1) or loss of MHC-1 due to either viral infection such as CMV or herpes simplex virus or in some tumour cells (70).

Carbohydrate ingestion known to attenuate leucocytosis is likely to be mediated by attenuation of sympathetic drive by reducing the energy demand, which may be an effective way to close the open window, as previously recognized. On the other hand, reduced energy intake as often observed in female athletes' triad or relative energy deficiency in sports, may affect haematopoiesis in starvation or malnutrition (265). Although there is insufficient clinical information related to leucocyte trafficking, it is likely that serious cases may present leucopaenia as well as anaemia, leading to impaired immunity when haematopoiesis is limited. At the earlier phase of reduced energy intake, sympathetic driven lipolysis may attenuate leucocyte adhesion, and this may compromise neutrophil defence as mentioned above.

Conflict of Interest

F. Mooren has received grants from Verla Pharm and Hermes Arzneimittel, Germany.
M. Gleeson has received grants for research projects from GlaxoSmithKline, Nestle, Pepsico and Yakult Honsha.
The other authors have declared no conflict of interests in regard to this article.

REFERENCES

1. Ahmed M, Henson DA, Sanderson MC, Nieman DC, Gillitt ND and Lila MA. The protective effects of a polyphenol-enriched protein powder on exercise-induced susceptibility to virus infection. Phytother Res PTR 28: 1829–1836, 2014.
2. Albers R, Antoine J-M, Bourdet-Sicard R, Calder PC, Gleeson M, Lesourd B, Samartín S, Sanderson IR, Van Loo J, Vas Dias FW and Watzl B. Markers to measure immunomodulation in human nutrition intervention studies. Br J Nutr 94: 452–481, 2005.
3. Albers R, Bourdet-Sicard R, Braun D, Calder PC, Herz U, Lambert C, Lenoir-Wijnkoop I, Méheust A, Ouwehand A, Phothirath P, Sako T, Salminen S, Siemensma A, van Loveren H and Sack U. Monitoring immune modulation by nutrition in the general population: identifying and substantiating effects on human health. Br J Nutr 110 Suppl 2: S1-30, 2013.
4. Al-Jaderi Z and Maghazachi AA. Effects of vitamin D3, calcipotriol and FTY720 on the expression of surface molecules and cytolytic activities of human natural killer cells and dendritic cells. Toxins 5: 1932–1947, 2013.
5. Allaire J, Couture P, Leclerc M, Charest A, Marin J, Lépine M-C, Talbot D, Tchernof A and Lamarche B. A randomized, crossover, head-to-head comparison of eicosapentaenoic acid and docosahexaenoic acid supplementation to reduce inflammation markers in men and women: the Comparing EPA to DHA (ComparED) Study. Am J Clin Nutr 104: 280–287, 2016.
6. Allan GM and Arroll B. Prevention and treatment of the common cold: making sense of the evidence. Can Med Assoc J 186: 190–199, 2014.
7. Allegra S, Deleine C, Michael-Jubely R, Gryson C, Boirie Y, Kantakamalakul W and Vasson M-P. Implementation of the EGFP-K562 flow cytometric NK test: determination of NK cytotoxic activity in healthy elderly volunteers before and after feeding. Cytometry A 69: 992–998, 2006.
8. Altomare A, Fasoli E, Colzani M, Parra XMP, Ferrari M, Cilurzo F, Rumio C, Cannizzaro L, Carini M, Righetti PG and Aldini G. An in depth proteomic analysis based on ProteoMiner, affinity chromatography and nano-HPLC-MS/MS to explain the potential health benefits of bovine colostrum. J Pharm Biomed Anal 121: 297–306, 2016.
9. Amulic B, Cazalet C, Hayes GL, Metzler KD and Zychlinsky A. Neutrophil function: from mechanisms to disease. Annu Rev Immunol 30: 459–489, 2012.
10. Andrade PMM, Ribeiro BG, Bozza MT, Costa Rosa LFB and Tavares do Carmo MG. Effects of the fish-oil supplementation on the immune and inflammatory responses in elite swimmers. Prostaglandins Leukot Essent Fatty Acids 77: 139–145, 2007.
11. Annuzzi G, Bozzetto L, Costabile G, Giacco R, Mangione A, Anniballi G, Vitale M, Vetrani C, Cipriano P, Della Corte G, Pasanisi F, Riccardi G and Rivellese AA. Diets naturally rich in polyphenols improve fasting and postprandial dyslipidemia and reduce oxidative stress: a randomized controlled trial. Am J Clin Nutr 99: 463–471, 2014.
12. Ardawi MS. Glutamine and glucose metabolism in human peripheral lymphocytes. Metabolism 37: 99–103, 1988.

13. Ardawi MS and Newsholme EA. Metabolism in lymphocytes and its importance in the immune response. Essays Biochem 21: 1–44, 1985.
14. Babior BM. The respiratory burst of phagocytes. J Clin Invest 73: 599–601, 1984.
15. Bahrke MS, Morgan WP and Stegner A. Is ginseng an ergogenic aid? Int J Sport Nutr Exerc Metab 19: 298–322, 2009.
16. Bailey DM, Castell LM, Newsholme EA and Davies B. Continuous and intermittent exposure to the hypoxia of altitude: implications for glutamine metabolism and exercise performance. Br J Sports Med 34: 210–212, 2000.
17. Balentine DA, Dwyer JT, Erdman JW, Ferruzzi MG, Gaine PC, Harnly JM and Kwik-Uribe CL. Recommendations on reporting requirements for flavonoids in research. Am J Clin Nutr 101: 1113–1125, 2015.
18. Ballard SL, Wellborn-Kim JJ and Clauson KA. Effects of commercial energy drink consumption on athletic performance and body composition. Phys Sportsmed 38: 107–117, 2010.
19. Bannenberg G and Serhan CN. Specialized pro-resolving lipid mediators in the inflammatory response: An update. Biochim Biophys Acta 1801: 1260–1273, 2010.
20. Barrett B, Locken K, Maberry R, Schwamman J, Brown R, Bobula J and Stauffacher EA. The Wisconsin Upper Respiratory Symptom Survey (WURSS): a new research instrument for assessing the common cold. J Fam Pract 51: 265, 2002.
21. Bassit RA, Sawada LA, Bacurau RF, Navarro F and Costa Rosa LF. The effect of BCAA supplementation upon the immune response of triathletes. Med Sci Sports Exerc 32: 1214–1219, 2000.
22. Baumann CW, Bond KL, Rupp JC, Ingalls CP and Doyle JA. Echinacea purpurea supplementation does not enhance VO2max in distance runners. J Strength Cond Res Natl Strength Cond Assoc 28: 1367–1372, 2014.
23. de Beaux FRCS AC, O'Riordain MD MG, Ross PhD JA, Jodozi PhD L, Carter MD DC and Fearon MD KCH. Glutamine-supplemented total parenteral nutrition reduces blood mononuclear cell interleukin-8 release in severe acute pancreatitis. Nutrition 14: 261–265, 1998.
24. Beier-Holgersen R and Brandstrup B. Influence of postoperative enteral nutrition on cellular immunity. A random double-blinded placebo controlled clinical trial. Int J Colorectal Dis 27: 513–520, 2012.
25. Bellar D, Moody KM, Richard NS and Judge LW. Efficacy of a botanical supplement with concentrated Echinacea purpurea for increasing aerobic capacity. ISRN Nutr 2014: 149549, 2014.
26. Bennett BK, Hickie IB, Vollmer-Conna US, Quigley B, Brennan CM, Wakefield D, Douglas MP, Hansen GR, Tahmindjis AJ and Lloyd AR. The relationship between fatigue, psychological and immunological variables in acute infectious illness. Aust N Z J Psychiatry 32: 180–186, 1998.
27. Berg A, Northoff H, Konig D, Weinstock C, Grathwohl D, Parnham M, Stuhlfauth I and Keul J. Influence of Echinacin (EC31) treatment on the exercise-induced immune response in athletes. J Clin Res 1: 367–380, 1998.
28. Berry DJ, Hesketh K, Power C and Hyppönen E. Vitamin D status has a linear association with seasonal infections and lung function in British adults. Br J Nutr 106: 1433–1440, 2011.
29. Bertoia ML, Rimm EB, Mukamal KJ, Hu FB, Willett WC and Cassidy A. Dietary flavonoid intake and weight maintenance: three prospective cohorts of 124,086 US men and women followed for up to 24 years. Brit Med J 352: i17, 2016.
30. Bikle DD. Vitamin D and immune function: understanding common pathways. Curr Osteoporos Rep 7: 58–63, 2009.
31. Binnendijk KH and Rijkers GT. What is a health benefit? An evaluation of EFSA opinions on health benefits with reference to probiotics. Benef Microbes 4: 223–230, 2013.
32. Bishop NC, Blannin AK, Armstrong E, Rickman M and Gleeson M. Carbohydrate and fluid intake affect the saliva flow rate and IgA response to cycling. Med Sci Sports Exerc 32: 2046–2051, 2000.
33. Bishop NC, Blannin AK, Walsh NP and Gleeson M. Carbohydrate beverage ingestion and neutrophil degranulation responses following cycling to fatigue at 75% VO2 max. Int J Sports Med 22: 226–231, 2001.
34. Bishop NC, Walker GJ, Bowley LA, Evans KF, Molyneux K, Wallace FA and Smith AC. Lymphocyte responses to influenza and tetanus toxoid in vitro following intensive exercise and carbohydrate ingestion on consecutive days. J Appl Physiol 99: 1327–1335, 2005.
35. Bishop NC, Walker GJ, Gleeson M, Wallace FA and Hewitt CRA. Human T lymphocyte migration towards the supernatants of human rhinovirus infected airway epithelial cells: influence of exercise and carbohydrate intake. Exerc Immunol Rev 15: 127–144, 2009.
36. Bishop NC, Walsh NP, Haines DL, Richards EE and Gleeson M. Pre-exercise carbohydrate status and immune responses to prolonged cycling: I. Effect on neutrophil degranulation. Int J Sport Nutr Exerc Metab 11: 490–502, 2001.
37. Bishop NC, Walsh NP, Haines DL, Richards EE and Gleeson M. Pre-exercise carbohydrate status and immune responses to prolonged cycling: II. Effect on plasma cytokine concentration. Int J Sport Nutr Exerc Metab 11: 503–512, 2001.
38. Bishop NC, Walsh NP and Scanlon GA. Effect of prolonged exercise and carbohydrate on total neutrophil elastase content. Med Sci Sports Exerc 35: 1326–1332, 2003.
39. Blannin A. Acute exercise and innate immune function. In: Immune function in sport and exercise. Gleeson M, ed. Churchill Livingstone, 2006.
40. Blannin AK, Robson PJ, Walsh NP, Clark AM, Glennon L and Gleeson M. The effect of exercising to exhaustion at different intensities on saliva immunoglobulin A, protein and electrolyte secretion. Int J Sports Med 19: 547–552, 1998.
41. Blomstrand E, Celsing F and Newsholme EA. Changes in plasma concentrations of aromatic and branched-chain amino acids during sustained exercise in man and their possible role in fatigue. Acta Physiol Scand 133: 115–121, 1988.
42. Blomstrand E and Saltin B. BCAA intake affects protein metabolism in muscle after but not during exercise in humans. Am J Physiol Endocrinol Metab 281: E365-374, 2001.
43. Bloomer RJ, Larson DE, Fisher-Wellman KH, Galpin AJ and Schilling BK. Effect of eicosapentaenoic and docosahexaenoic acid on resting and exercise-induced inflammatory and oxidative stress biomarkers: a randomized, placebo controlled, cross-over study. Lipids Health Dis 8: 36, 2009.
44. Bölke E, Jehle PM, Hausmann F, Däubler A, Wiedeck H, Steinbach G, Storck M and Orth K. Preoperative oral application of immunoglobulin-enriched colostrum milk and mediator response during abdominal surgery. Shock 17: 9–12, 2002.

45. Booth CK, Coad RA, Forbes-Ewan CH, Thomson GF and Niro PJ. The physiological and psychological effects of combat ration feeding during a 12-day training exercise in the tropics. Mil Med 168: 63–70, 2003.
46. Bosch JA, Ring C, de Geus EJC, Veerman ECI and Amerongen AVN. Stress and secretory immunity. Int Rev Neurobiol 52: 213–253, 2002.
47. Bozbay M and Uyarel H. Neutrophil-to-lymphocyte ratio: A novel and simple prognostic marker for infective endocarditis. J Crit Care 30: 822, 2015.
48. Brandtzaeg P. Secretory immunity with special reference to the oral cavity. J Oral Microbiol 5, 2013.
49. Brinkworth GD and Buckley JD. Concentrated bovine colostrum protein supplementation reduces the incidence of self-reported symptoms of upper respiratory tract infection in adult males. Eur J Nutr 42: 228–232, 2003.
50. Brisswalter J and Louis J. Vitamin supplementation benefits in master athletes. Sports Med 44: 311–318, 2014.
51. Brutsaert TD, Hernandez-Cordero S, Rivera J, Viola T, Hughes G and Haas JD. Iron supplementation improves progressive fatigue resistance during dynamic knee extensor exercise in iron-depleted, nonanemic women. Am J Clin Nutr 77: 441–448, 2003.
52. Bruunsgaard H, Hartkopp A, Mohr T, Konradsen H, Heron I, Mordhorst CH and Pedersen BK. In vivo cell-mediated immunity and vaccination response following prolonged, intense exercise. Med Sci Sports Exerc 29: 1176–1181, 1997.
53. Buckley JD, Brinkworth GD and Abbott MJ. Effect of bovine colostrum on anaerobic exercise performance and plasma insulin-like growth factor I. J Sports Sci 21: 577–588, 2003.
54. Burdge GC and Calder PC. Introduction to fatty acids and lipids. World Rev Nutr Diet 112: 1–16, 2015.
55. Burns VE. Using vaccinations to assess in vivo immune function in psychoneuroimmunology. Methods Mol Biol 934: 371–381, 2012.
56. Calbet JA, Mooren FC, Burke LM, Stear SJ and Castell LM. A-Z of nutritional supplements: dietary supplements, sports nutrition foods and ergogenic aids for health and performance: part 24. Br J Sports Med 45: 1005–1007, 2011.
57. Calder PC. Branched-chain amino acids and immunity. J Nutr 136: 288S–293S, 2006.
58. Calder PC. n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. Am J Clin Nutr 83: 1505S–1519S, 2006.
59. Calder PC. The relationship between the fatty acid composition of immune cells and their function. Prostaglandins Leukot Essent Fatty Acids 79: 101–108, 2008.
60. Calder PC. Very long chain omega-3 (n-3) fatty acids and human health. Eur J Lipid Sci Technol 116: 1280–1300, 2014.
61. Calder PC. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. Biochim Biophys Acta 1851: 469–484, 2015.
62. Calder PC. Functional roles of fatty acids and their effects on human health. JPEN J Parenter Enteral Nutr 39: 18S–32S, 2015.
63. Calder PC and Jackson AA. Undernutrition, infection and immune function. Nutr Res Rev 13: 3–29, 2000.
64. Cannell JJ and Hollis BW. Use of vitamin D in clinical practice. Altern Med Rev J Clin Ther 13: 6–20, 2008.
65. Cannell JJ, Vieth R, Umhau JC, Holick MF, Grant WB, Madronich S, Garland CF and Giovannucci E. Epidemic influenza and vitamin D. Epidemiol Infect 134: 1129–1140, 2006.
66. Capó X, Martorell M, Llompart I, Sureda A, Tur JA and Pons A. Docosahexanoic acid diet supplementation attenuates the peripheral mononuclear cell inflammatory response to exercise following LPS activation. Cytokine 69: 155–164, 2014.
67. Caris AV, Lira FS, de Mello MT, Oyama LM and dos Santos RVT. Carbohydrate and glutamine supplementation modulates the Th1/Th2 balance after exercise performed at a simulated altitude of 4500 m. Nutrition 30: 1331–1336, 2014.
68. Carol A, Witkamp RF, Wichers HJ and Mensink M. Bovine colostrum supplementation's lack of effect on immune variables during short-term intense exercise in well-trained athletes. Int J Sport Nutr Exerc Metab 21: 135–145, 2011.
69. Carpenter GH, Proctor GB, Ebersole LE and Garrett JR. Secretion of IgA by rat parotid and submandibular cells in response to autonomimetic stimulation in vitro. Int Immunopharmacol 4: 1005–1014, 2004.
70. Carrillo-Bustamante P, Kesmir C and de Boer RJ. Can selective MHC downregulation explain the specificity and genetic diversity of NK cell receptors? Front Immunol 6: 311, 2015.
71. Carroll L, Davies MJ and Pattison DI. Reaction of low-molecular-mass organoselenium compounds (and their sulphur analogues) with inflammation-associated oxidants. Free Radic Res 49: 750–767, 2015.
72. Cassidy A, Rogers G, Peterson JJ, Dwyer JT, Lin H and Jacques PF. Higher dietary anthocyanin and flavonol intakes are associated with anti-inflammatory effects in a population of US adults. Am J Clin Nutr 102: 172–181, 2015.
73. Castell L. Glutamine supplementation in vitro and in vivo, in exercise and in immunodepression. Sports Med 33: 323–345, 2003.
74. Castell L, Vance C, Abbott R, Marquez J and Eggleton P. Granule localization of glutaminase in human neutrophils and the consequence of glutamine utilization for neutrophil activity. J Biol Chem 279: 13305–13310, 2004.
75. Castell LM and Newsholme EA. The effects of oral glutamine supplementation on athletes after prolonged, exhaustive exercise. Nutrition 13: 738–742, 1997.
76. Castell LM, Poortmans JR, Leclercq R, Brasseur M, Duchateau J and Newsholme EA. Some aspects of the acute phase response after a marathon race, and the effects of glutamine supplementation. Eur J Appl Physiol 75: 47–53, 1997.
77. Castell LM, Poortmans JR and Newsholme EA. Does glutamine have a role in reducing infections in athletes? Eur J Appl Physiol 73: 488–490, 1996.
78. Castell LM, Stear SJ and Burke LM. Nutritional supplements in sport, exercise, and health: An A-Z guide. Abingdon, Oxon, UK: Routledge, 2015.
79. Castell LM, Thake CD and Ensign W. Biochemical markers of possible immunodepression in military training in harsh environments. Mil Med 175: 158–165, 2010.
80. Ceriello A. Postprandial hyperglycemia and diabetes complications: is it time to treat? Diabetes 54: 1–7, 2005.
81. Chen P, Chakraborty S, Mukhopadhyay S, Lee E, Paoliello MMB, Bowman AB and Aschner M. Manganese homeostasis in the nervous system. J Neurochem 134: 601–610, 2015.

82. Cherayil BJ. Iron and immunity: immunological consequences of iron deficiency and overload. Arch Immunol Ther Exp (Warsz) 58: 407–415, 2010.
83. Clancy RL, Gleeson M, Cox A, Callister R, Dorrington M, D'Este C, Pang G, Pyne D, Fricker P and Henriksson A. Reversal in fatigued athletes of a defect in interferon gamma secretion after administration of Lactobacillus acidophilus. Br J Sports Med 40: 351–354, 2006.
84. Close GL, Russell J, Cobley JN, Owens DJ, Wilson G, Gregson W, Fraser WD and Morton JP. Assessment of vitamin D concentration in non-supplemented professional athletes and healthy adults during the winter months in the UK: implications for skeletal muscle function. J Sports Sci 31: 344–353, 2013.
85. Coad S, Gray B, Wehbe G and McLellan C. Physical demands and salivary immunoglobulin A responses of elite Australian rules football athletes to match play. Int J Sports Physiol Perform 10: 613–617, 2015.
86. Cochran AJ, Myslik F, MacInnis MJ, Percival ME, Bishop D, Tarnopolsky MA and Gibala MJ. Manipulating carbohydrate availability between twice-daily sessions of high-intensity interval training over 2 weeks improves time-trial performance. Int J Sport Nutr Exerc Metab 25: 463–470, 2015.
87. Cohen S, Doyle WJ, Alper CM, Janicki-Deverts D and Turner RB. Sleep habits and susceptibility to the common cold. Arch Intern Med 169: 62–67, 2009.
88. Cohen S, Tyrrell DA and Smith AP. Psychological stress and susceptibility to the common cold. N Engl J Med 325: 606–612, 1991.
89. Comstock SS, Reznikov EA, Contractor N and Donovan SM. Dietary bovine lactoferrin alters mucosal and systemic immune cell responses in neonatal piglets. J Nutr 144: 525–532, 2014.
90. Coombes JS, Conacher M, Austen SK and Marshall PA. Dose effects of oral bovine colostrum on physical work capacity in cyclists. Med Sci Sports Exerc 34: 1184–1188, 2002.
91. Corder KE, Newsham KR, McDaniel JL, Ezekiel UR and Weiss EP. Effects of short-term docosahexaenoic acid supplementation on markers of inflammation after eccentric strength exercise in women. J Sports Sci Med 15: 176–183, 2016.
92. Coutinho Moraes DF, Still DW, Lum MR and Hirsch AM. DNA-Based authentication of botanicals and plant-derived dietary supplements: where have we been and where are we going? Planta Med 81: 687–695, 2015.
93. Cox AJ, Gleeson M, Pyne DB, Callister R, Hopkins WG and Fricker PA. Clinical and laboratory evaluation of upper respiratory symptoms in elite athletes. Clin J Sport Med 18: 438–445, 2008.
94. Cox AJ, Pyne DB, Saunders PU and Fricker PA. Oral administration of the probiotic Lactobacillus fermentum VRI-003 and mucosal immunity in endurance athletes. Br J Sports Med 44: 222–226, 2010.
95. Crooks C, Cross ML, Wall C and Ali A. Effect of bovine colostrum supplementation on respiratory tract mucosal defenses in swimmers. Int J Sport Nutr Exerc Metab 20: 224–235, 2010.
96. Crooks CV, Wall CR, Cross ML and Rutherfurd-Markwick KJ. The effect of bovine colostrum supplementation on salivary IgA in distance runners. Int J Sport Nutr Exerc Metab 16: 47–64, 2006.
97. Cruzat VF, Krause M and Newsholme P. Amino acid supplementation and impact on immune function in the context of exercise. J Int Soc Sports Nutr 11: 61, 2014.
98. Cunha MCR, Lima F da S, Vinolo MAR, Hastreiter A, Curi R, Borelli P and Fock RA. Protein malnutrition induces bone marrow mesenchymal stem cells commitment to adipogenic differentiation leading to hematopoietic failure. PloS One 8: e58872, 2013.
99. Da Boit M, Mastalurova I, Brazaite G, McGovern N, Thompson K and Gray SR. The effect of krill oil supplementation on exercise performance and markers of immune function. PloS One 10: e0139174, 2015.
100. Dai Z-L, Li X-L, Xi P-B, Zhang J, Wu G and Zhu W-Y. L-Glutamine regulates amino acid utilization by intestinal bacteria. Amino Acids 45: 501–512, 2013.
101. Davison G and Diment BC. Bovine colostrum supplementation attenuates the decrease of salivary lysozyme and enhances the recovery of neutrophil function after prolonged exercise. Br J Nutr 103: 1425–1432, 2010.
102. Davison G, Kehaya C, Diment BC and Walsh NP. Carbohydrate supplementation does not blunt the prolonged exercise-induced reduction of in vivo immunity. Eur J Nutr 55: 1583–1593, 2016.
103. Davison HM. Nutrition and allergy. J Am Acad Appl Nutr 1: 75, 1947.
104. Décombaz J, Reinhardt P, Anantharaman K, von Glutz G and Poortmans JR. Biochemical changes in a 100 km run: free amino acids, urea, and creatinine. Eur J Appl Physiol 41: 61–72, 1979.
105. Delfan M, Ebrahim K, Baesi F, Mirakhori Z, Ghalamfarsa G, Bakhshaei P, Saboor-Yaraghi AA, Razavi A, Setayesh M, Yousefi M and Jadidi-Niaragh F. The immunomodulatory effects of fish-oil supplementation in elite paddlers: A pilot randomized double blind placebo-controlled trial. Prostaglandins Leukot Essent Fatty Acids 99: 35–40, 2015.
106. Dennis EA and Norris PC. Eicosanoid storm in infection and inflammation. Nat Rev Immunol 15: 511–523, 2015.
107. Diaz-Ochoa VE, Jellbauer S, Klaus S and Raffatellu M. Transition metal ions at the crossroads of mucosal immunity and microbial pathogenesis. Front Cell Infect Microbiol 4: 2, 2014.
108. DiLorenzo FM, Drager CJ and Rankin JW. Docosahexaenoic acid affects markers of inflammation and muscle damage after eccentric exercise. J Strength Cond Res 28: 2768–2774, 2014.
109. Diment BC, Fortes MB, Edwards JP, Hanstock HG, Ward MD, Dunstall HM, Friedmann PS and Walsh NP. Exercise intensity and duration effects on in vivo immunity. Med Sci Sports Exerc 47: 1390–1398, 2015.
110. Diment BC, Fortes MB, Greeves JP, Casey A, Costa RJS, Walters R and Walsh NP. Effect of daily mixed nutritional supplementation on immune indices in soldiers undertaking an 8-week arduous training programme. Eur J Appl Physiol 112: 1411–1418, 2012.
111. Dimitriou L, Sharp NCC and Doherty M. Circadian effects on the acute responses of salivary cortisol and IgA in well trained swimmers. Br J Sports Med 36: 260–264, 2002.
112. Dimitrov S, Lange T and Born J. Selective mobilization of cytotoxic leukocytes by epinephrine. J Immunol 184: 503–511, 2010.
113. Dolan MJ, Clerici M, Blatt SP, Hendrix CW, Melcher GP, Boswell RN, Freeman TM, Ward W, Hensley R and Shearer GM. In vitro T cell function, delayed-type hypersensitivity skin testing, and CD4+ T cell subset phenotyping independently predict survival time in patients infected with human immunodeficiency virus. J Infect Dis 172: 79–87, 1995.

114. Douglas RM, Hemilä H, Chalker E and Treacy B. Vitamin C for preventing and treating the common cold. Cochrane Database Syst Rev CD000980, 2007.
115. Draganidis D, Karagounis LG, Athanailidis I, Chatzinikolaou A, Jamurtas AZ and Fatouros IG. Inflammaging and skeletal muscle: can protein intake make a difference? J Nutr 146: 1940–1952, 2016.
116. Duda-Chodak A, Tarko T, Satora P and Sroka P. Interaction of dietary compounds, especially polyphenols, with the intestinal microbiota: a review. Eur J Nutr 54: 325–341, 2015.
117. Duramad P, McMahon CW, Hubbard A, Eskenazi B and Holland NT. Flow cytometric detection of intracellular TH1/TH2 cytokines using whole blood: validation of immunologic biomarker for use in epidemiologic studies. Cancer Epidemiol Biomarkers Prev 13: 1452–1458, 2004.
118. Durup D, Jørgensen HL, Christensen J, Schwarz P, Heegaard AM and Lind B. A reverse J-shaped association of all-cause mortality with serum 25-hydroxyvitamin D in general practice: the CopD study. J Clin Endocrinol Metab 97: 2644–2652, 2012.
119. van Duynhoven J, Vaughan EE, Jacobs DM, Kemperman RA, van Velzen EJJ, Gross G, Roger LC, Possemiers S, Smilde AK, Doré J, Westerhuis JA and Van de Wiele T. Metabolic fate of polyphenols in the human superorganism. Proc Natl Acad Sci USA 108 Suppl 1: 4531–4538, 2011.
120. Edmands WM, Ferrari P, Rothwell JA, Rinaldi S, Slimani N, Barupal DK, Biessy C, Jenab M, Clavel-Chapelon F, Fagherazzi G, Boutron-Ruault M-C, Katzke VA, Kühn T, Boeing H, Trichopoulou A, Lagiou P, Trichopoulos D, Palli D, Grioni S et al. Polyphenol metabolome in human urine and its association with intake of polyphenol-rich foods across European countries. Am J Clin Nutr 102: 905–913, 2015.
121. Elkington LJ, Gleeson M, Pyne DB, Callister R and Wood LG. Inflammation and immune function: can antioxidants help the endurance athlete? In: Antioxidants in Sport Nutrition. Lamprecht M, ed. . Boca Raton (FL): CRC Press/Taylor & Francis, 2015.
122. Engebretsen L, Steffen K, Alonso JM, Aubry M, Dvorak J, Junge A, Meeuwisse W, Mountjoy M, Renström P and Wilkinson M. Sports injuries and illnesses during the Winter Olympic Games 2010. Br J Sports Med 44: 772–780, 2010.
123. Erdman KA, Fung TS and Reimer RA. Influence of performance level on dietary supplementation in elite Canadian athletes. Med Sci Sports Exerc 38: 349–356, 2006.
124. Fardet A. New hypotheses for the health-protective mechanisms of whole-grain cereals: what is beyond fibre? Nutr Res Rev 23: 65–134, 2010.
125. Faurschou A, Beyer DM, Schmedes A, Bogh MK, Philipsen PA and Wulf HC. The relation between sunscreen layer thickness and vitamin D production after ultraviolet B exposure: a randomized clinical trial. Br J Dermatol 167: 391–395, 2012.
126. Fehrenbach E and Niess AM. Role of heat shock proteins in the exercise response. Exerc Immunol Rev 5: 57–77, 1999.
127. Fell J and Williams D. The effect of aging on skeletal-muscle recovery from exercise: possible implications for aging athletes. J Aging Phys Act 16: 97–115, 2008.
128. de Ferrars RM, Czank C, Zhang Q, Botting NP, Kroon PA, Cassidy A and Kay CD. The pharmacokinetics of anthocyanins and their metabolites in humans. Br J Pharmacol 171: 3268–3282, 2014.
129. Fisher G, Schwartz DD, Quindry J, Barberio MD, Foster EB, Jones KW and Pascoe DD. Lymphocyte enzymatic antioxidant responses to oxidative stress following high-intensity interval exercise. J Appl Physiol 110: 730–77, 2011.
130. Flannagan RS, Jaumouillé V and Grinstein S. The cell biology of phagocytosis. Annu Rev Pathol 7: 61–98, 2012.
131. Forsythe P. Probiotics and lung immune responses. Ann Am Thorac Soc 11 Suppl 1: S33-37, 2014.
132. Fortes MB, Diment BC, Greeves JP, Casey A, Izard R and Walsh NP. Effects of a daily mixed nutritional supplement on physical performance, body composition, and circulating anabolic hormones during 8 weeks of arduous military training. Appl Physiol Nutr Metab 36: 967–975, 2011.
133. Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, Ottaviani E and De Benedictis G. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci 908: 244–254, 2000.
134. Getz L. A healthful dose of bacteria — yogurt is the best probiotic source, but clients do have other options. Today's Dietitian 13: 46, 2011.
135. Gilroy CM, Steiner JF, Byers T, Shapiro H and Georgian W. Echinacea and truth in labeling. Arch Intern Med 163: 699–704, 2003.
136. Ginde AA, Mansbach JM and Camargo CA. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infection in the Third National Health and Nutrition Examination Survey. Arch Intern Med 169: 384–390, 2009.
137. Gleeson M. Mucosal immune responses and risk of respiratory illness in elite athletes. Exerc Immunol Rev 6: 5–42, 2000.
138. Gleeson M. Can nutrition limit exercise-induced immunodepression? Nutr Rev 64: 119–131, 2006.
139. Gleeson M. Dosing and efficacy of glutamine supplementation in human exercise and sport training. J Nutr 138: 2045S–2049S, 2008.
140. Gleeson M. Immunological aspects of sport nutrition. Immunol Cell Biol 94: 117–123, 2016.
141. Gleeson M, Bishop NC, Oliveira M, McCauley T, Tauler P and Lawrence C. Effects of a Lactobacillus salivarius probiotic intervention on infection, cold symptom duration and severity, and mucosal immunity in endurance athletes. Int J Sport Nutr Exerc Metab 22: 235–242, 2012.
142. Gleeson M, Bishop NC, Oliveira M and Tauler P. Daily probiotic's (Lactobacillus casei Shirota) reduction of infection incidence in athletes. Int J Sport Nutr Exerc Metab 21: 55–64, 2011.
143. Gleeson M, Bishop NC, Stensel DJ, Lindley MR, Mastana SS and Nimmo MA. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. Nat Rev Immunol 11: 607–615, 2011.
144. Gleeson M, Bishop NC and Struszczak L. Effects of Lactobacillus casei Shirota ingestion on common cold infection and herpes virus antibodies in endurance athletes: a placebo-controlled, randomized trial. Eur J Appl Physiol 116: 1555–1563, 2016.
145. Gleeson M, McDonald WA, Pyne DB, Cripps AW, Francis JL, Fricker PA and Clancy RL. Salivary IgA levels and infection risk in elite swimmers. Med Sci Sports Exerc 31: 67–73, 1999.
146. Gleeson M and Pyne DB. Respiratory inflammation and infections in high-performance athletes. Immunol Cell Biol 94: 124–131, 2016.

147. Gleeson M, Pyne DB, McDonald WA, Clancy RL, Cripps AW, Horn PL and Fricker PA. Pneumococcal antibody responses in elite swimmers. Clin Exp Immunol 105: 238–244, 1996.
148. Gombart AF, Borregaard N and Koeffler HP. Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. FASEB J 19: 1067–1077, 2005.
149. Grabs V, Nieman DC, Haller B, Halle M and Scherr J. The effects of oral hydrolytic enzymes and flavonoids on inflammatory markers and coagulation after marathon running: study protocol for a randomized, double-blind, placebo-controlled trial. BMC Sports Sci Med Rehabil 6: 8, 2014.
150. Grant RW, Mariani RA, Vieira VJ, Fleshner M, Smith TP, Keylock KT, Lowder TW, McAuley E, Hu L, Chapman-Novakofski K and Woods JA. Cardiovascular exercise intervention improves the primary antibody response to keyhole limpet hemocyanin (KLH) in previously sedentary older adults. Brain Behav Immun 22: 923–932, 2008.
151. Gray P, Chappell A, Jenkinson AM, Thies F and Gray SR. Fish oil supplementation reduces markers of oxidative stress but not muscle soreness after eccentric exercise. Int J Sport Nutr Exerc Metab 24: 206–214, 2014.
152. Gray P, Gabriel B, Thies F and Gray SR. Fish oil supplementation augments post-exercise immune function in young males. Brain Behav Immun 26: 1265–1272, 2012.
153. Green KJ, Croaker SJ and Rowbottom DG. Carbohydrate supplementation and exercise-induced changes in T-lymphocyte function. J Appl Physiol 95: 1216–1223, 2003.
154. van Hall G, MacLean DA, Saltin B and Wagenmakers AJ. Mechanisms of activation of muscle branched-chain alpha-keto acid dehydrogenase during exercise in man. J Physiol 494: 899–905, 1996.
155. Hall H, Fahlman MM and Engels HJ. Echinacea purpurea and mucosal immunity. Int J Sports Med 28: 792–797, 2007.
156. Hall JE, da Silva AA, do Carmo JM, Dubinion J, Hamza S, Munusamy S, Smith G and Stec DE. Obesity-induced hypertension: role of sympathetic nervous system, leptin, and melanocortins. J Biol Chem 285: 17271–17276, 2010.
157. Halliday TM, Peterson NJ, Thomas JJ, Kleppinger K, Hollis BW and Larson-Meyer DE. Vitamin D status relative to diet, lifestyle, injury, and illness in college athletes. Med Sci Sports Exerc 43: 335–343, 2011.
158. Halliwell B and Gutteridge J. Free radicals in biology and medicine. Oxford, UK: Oxford University Press, 2007.
159. Hamer DH, Sempértegui F, Estrella B, Tucker KL, Rodríguez A, Egas J, Dallal GE, Selhub J, Griffiths JK and Meydani SN. Micronutrient deficiencies are associated with impaired immune response and higher burden of respiratory infections in elderly Ecuadorians. J Nutr 139: 113–119, 2009.
160. Hamer M. The relative influences of fitness and fatness on inflammatory factors. Prev Med 44: 3–11, 2007.
161. Hanstock HG, Walsh NP, Edwards JP, Fortes MB, Cosby SL, Nugent A, Curran T, Coyle PV, Ward MD and Yong XHA. Tear fluid SIgA as a noninvasive biomarker of mucosal immunity and common cold risk. Med Sci Sports Exerc 48: 569–577, 2016.
162. Hao Q, Dong BR and Wu T. Probiotics for preventing acute upper respiratory tract infections. Cochrane Database Syst Rev CD006895, 2015.
163. Harnly J. Importance of accurate measurements in nutrition research: dietary flavonoids as a case study. Adv Nutr 7: 375–382, 2016.
164. Harper Smith AD, Coakley SL, Ward MD, Macfarlane AW, Friedmann PS and Walsh NP. Exercise-induced stress inhibits both the induction and elicitation phases of in vivo T-cell-mediated immune responses in humans. Brain Behav Immun 25: 1136–1142, 2011.
165. Hawley JA and Morton JP. Ramping up the signal: promoting endurance training adaptation in skeletal muscle by nutritional manipulation. Clin Exp Pharmacol Physiol 41: 608–613, 2014.
166. Haymes E. Trace minerals and exercise. In: Nutrition in Exercise and Sport.CRC Press, 1998.
167. Haywood BA, Black KE, Baker D, McGarvey J, Healey P and Brown RC. Probiotic supplementation reduces the duration and incidence of infections but not severity in elite rugby union players. J Sci Med Sport 17: 356–360, 2014.
168. He C-S, Aw Yong XH, Walsh NP and Gleeson M. Is there an optimal vitamin D status for immunity in athletes and military personnel? Exerc Immunol Rev 22: 42–64, 2016.
169. He C-S, Bishop NC, Handzlik MK, Muhamad AS and Gleeson M. Sex differences in upper respiratory symptoms prevalence and oral-respiratory mucosal immunity in endurance athletes. Exerc Immunol Rev 20: 8–22, 2014.
170. He C-S, Fraser WD, Tang J, Brown K, Renwick S, Rudland-Thomas J, Teah J, Tanqueray E and Gleeson M. The effect of 14 weeks of vitamin D3 supplementation on antimicrobial peptides and proteins in athletes. J Sports Sci 34: 67–74, 2016.
171. He C-S, Handzlik M, Fraser WD, Muhamad A, Preston H, Richardson A and Gleeson M. Influence of vitamin D status on respiratory infection incidence and immune function during 4 months of winter training in endurance sport athletes. Exerc Immunol Rev 19: 86–101, 2013.
172. Heller JE, Thomas JJ, Hollis BW and Larson-Meyer DE. Relation between vitamin D status and body composition in collegiate athletes. Int J Sport Nutr Exerc Metab 25: 128–135, 2015.
173. Hemilä H and Chalker E. Vitamin C for preventing and treating the common cold. Cochrane Database Syst Rev CD000980, 2013.
174. Henson DA, Nieman DC, Parker JC, Rainwater MK, Butterworth DE, Warren BJ, Utter A, Davis JM, Fagoaga OR and Nehlsen-Cannarella SL. Carbohydrate supplementation and the lymphocyte proliferative response to long endurance running. Int J Sports Med 19: 574–580, 1998.
175. de Heredia FP, Gómez-Martínez S and Marcos A. Obesity, inflammation and the immune system. Proc Nutr Soc 71: 332–8, 2012.
176. Herishanu Y, Rogowski O, Polliack A and Marilus R. Leukocytosis in obese individuals: possible link in patients with unexplained persistent neutrophilia. Eur J Haematol 76: 516–520, 2006.
177. Hernández-Bernal F, Aguilar-Betancourt A, Aljovin V, Arias G, Valenzuela C, de Alejo KP, Hernández K, Oquendo O, Figueredo N, Figueroa N, Musacchio A, Véliz G, García E, Mollineda AD, Juvier AI, Trujillo J, Delahanty A, Ortega D, Cinza Z et al. Comparison of four recombinant hepatitis B vaccines applied on an accelerated schedule in healthy adults. Hum Vaccin 7: 1026–1036, 2011.
178. Hewison M. An update on vitamin D and human immunity. Clin Endocrinol 76: 315–325, 2012.

179. Hinchado MD, Giraldo E and Ortega E. Adrenoreceptors are involved in the stimulation of neutrophils by exercise-induced circulating concentrations of Hsp72: cAMP as a potential "intracellular danger signal." J Cell Physiol 227: 604–608, 2012.
180. Hiscock N, Morgan R, Davidson G, Garcia J, Grace F, Boisseau N, Castell LM, Mackinnon LT, Davies B and Bailey DM. Peripheral blood mononuclear cell glutamine concentration and in vitro proliferation in response to an acute, exercise-induced decrease in plasma glutamine concentration in man. In: Proc Phys Soc 539P, 54P, 2002.
181. Hiscock N, Petersen EW, Krzywkowski K, Boza J, Halkjaer-Kristensen J and Pedersen BK. Glutamine supplementation further enhances exercise-induced plasma IL-6. J Appl Physiol 95: 145–148, 2003.
182. Honda T, Uehara T, Matsumoto G, Arai S and Sugano M. Neutrophil left shift and white blood cell count as markers of bacterial infection. Clin Chim Acta 457: 46–53, 2016.
183. Hossein-nezhad A and Holick MF. Vitamin D for health: a global perspective. Mayo Clin Proc 88: 720–755, 2013.
184. Hou TY, McMurray DN and Chapkin RS. Omega-3 fatty acids, lipid rafts, and T cell signaling. Eur J Pharmacol 785: 2–9, 2016.
185. Hoy MK, Goldman JD and Sebastian RS. Fruit and vegetable intake of US adults estimated by two methods: What we eat in America, National Health and Nutrition Examination Survey 2009-2012. Public Health Nutr 19: 2508–2512, 2016.
186. Hulisz D. Efficacy of zinc against common cold viruses: an overview. J Am Pharm Assoc JAPhA 44: 594–603, 2004.
187. Ibs K-H and Rink L. Zinc-altered immune function. J Nutr 133: 1452S–1456S, 2003.
188. Ihalainen JK, Vuorimaa T, Puurtinen R, Hämäläinen I and Mero AA. Effects of carbohydrate ingestion on acute leukocyte, cortisol, and interleukin-6 response in high-intensity long-distance running. J Strength Cond Res 28: 2786–2792, 2014.
189. Ivey KL, Hodgson JM, Croft KD, Lewis JR and Prince RL. Flavonoid intake and all-cause mortality. Am J Clin Nutr 101: 1012–1020, 2015.
190. Izumi H, Kosaka N, Shimizu T, Sekine K, Ochiya T and Takase M. Bovine milk contains microRNA and messenger RNA that are stable under degradative conditions. J Dairy Sci 95: 4831–4841, 2012.
191. Jackson GG, Dowling HF, Spiesman IG and Boand AV. Transmission of the common cold to volunteers under controlled conditions. I. The common cold as a clinical entity. AMA Arch Intern Med 101: 267–278, 1958.
192. Jackson MJ. Free radicals generated by contracting muscle: by-products of metabolism or key regulators of muscle function? Free Radic Biol Med 44: 132–141, 2008.
193. Jemmott JB, Borysenko JZ, Borysenko M, McClelland DC, Chapman R, Meyer D and Benson H. Academic stress, power motivation, and decrease in secretion rate of salivary secretory immunoglobulin A. Lancet 1: 1400–1402, 1983.
194. Ji LL, Kang C and Zhang Y. Exercise-induced hormesis and skeletal muscle health. Free Radic Biol Med 98: 113–122, 2016.
195. Johannsen NM, Priest EL, Dixit VD, Earnest CP, Blair SN and Church TS. Association of white blood cell subfraction concentration with fitness and fatness. Br J Sports Med 44: 588–93, 2010.
196. Johannsen NM, Swift DL, Johnson WD, Dixit VD, Earnest CP, Blair SN and Church TS. Effect of different doses of aerobic exercise on total white blood cell (WBC) and WBC subfraction number in postmenopausal women: results from DREW. PloS One 7: e31319, 2012.
197. Johnson GH and Fritsche K. Effect of dietary linoleic acid on markers of inflammation in healthy persons: a systematic review of randomized controlled trials. J Acad Nutr Diet 112: 1029–41, 2012.
198. Jones AW, Cameron SJS, Thatcher R, Beecroft MS, Mur LAJ and Davison G. Effects of bovine colostrum supplementation on upper respiratory illness in active males. Brain Behav Immun 39: 194–203, 2014.
199. Jones AW, Thatcher R, March DS and Davison G. Influence of 4 weeks of bovine colostrum supplementation on neutrophil and mucosal immune responses to prolonged cycling. Scand J Med Sci Sports 25: 788–796, 2015.
200. Jordan I, Balaguer M, Esteban ME, Cambra FJ, Felipe A, Hernández L, Alsina L, Molero M, Villaronga M and Esteban E. Glutamine effects on heat shock protein 70 and interleukines 6 and 10: Randomized trial of glutamine supplementation versus standard parenteral nutrition in critically ill children. Clin Nutr 35: 34–40, 2016.
201. Jouris KB, McDaniel JL and Weiss EP. The Effect of Omega-3 Fatty Acid Supplementation on the Inflammatory Response to eccentric strength exercise. J Sports Sci Med 10: 432–438, 2011.
202. Jung HL, Kwak HE, Kim SS, Kim YC, Lee CD, Byurn HK and Kang HY. Effects of Panax ginseng supplementation on muscle damage and inflammation after uphill treadmill running in humans. Am J Chin Med 39: 441–450, 2011.
203. Kalima K, Lehtoranta L, He L, Pitkäniemi J, Lundell R, Julkunen I, Roivainen M, Närkiö M, Mäkelä MJ, Siitonen S, Korpela R and Pitkäranta A. Probiotics and respiratory and gastrointestinal tract infections in Finnish military conscripts - a randomised placebo-controlled double-blinded study. Benef Microbes 7: 463–471, 2016.
204. Karsch-Völk M, Barrett B and Linde K. Echinacea for preventing and treating the common cold. JAMA 313: 618–619, 2015.
205. Kawanishi N, Yano H, Yokogawa Y and Suzuki K. Exercise training inhibits inflammation in adipose tissue via both suppression of macrophage infiltration and acceleration of phenotypic switching from M1 to M2 macrophages in high-fat-diet-induced obese mice. Exerc Immunol Rev 16: 105–118, 2010.
206. Kekkonen RA, Vasankari TJ, Vuorimaa T, Haahtela T, Julkunen I and Korpela R. The effect of probiotics on respiratory infections and gastrointestinal symptoms during training in marathon runners. Int J Sport Nutr Exerc Metab 17: 352–363, 2007.
207. Kephart WC, Wachs TD, Mac Thompson R, Brooks Mobley C, Fox CD, McDonald JR, Ferguson BS, Young KC, Nie B, Martin JS, Company JM, Pascoe DD, Arnold RD, Moon JR and Roberts MD. Ten weeks of branched-chain amino acid supplementation improves select performance and immunological variables in trained cyclists. Amino Acids 48: 779–789, 2016.
208. Khansari DN, Murgo AJ and Faith RE. Effects of stress on the immune system. Immunol Today 11: 170–175, 1990.
209. Kim YS, Sayers TJ, Colburn NH, Milner JA and Young HA. Impact of dietary components on NK and Treg cell function for cancer prevention. Mol Carcinog 54: 669–678, 2015.

210. King S, Glanville J, Sanders ME, Fitzgerald A and Varley D. Effectiveness of probiotics on the duration of illness in healthy children and adults who develop common acute respiratory infectious conditions: a systematic review and meta-analysis. Br J Nutr 112: 41–54, 2014.
211. Knapik JJ, Steelman RA, Hoedebecke SS, Austin KG, Farina EK and Lieberman HR. Prevalence of Dietary Supplement Use by Athletes: Systematic Review and Meta-Analysis. Sports Med 46: 103–123, 2016.
212. Krabbe KS, Pedersen M and Bruunsgaard H. Inflammatory mediators in the elderly. Exp Gerontol 39: 687–699, 2004.
213. Krebs H. Glutamine metabolism in the animal body. In: Glutamine: Metabolism, Enzymology, and Regulation. Mora J, Palacios R, eds. . New York: Academic Press, 1980.
214. Krochmal R, Hardy M, Bowerman S, Lu Q-Y, Wang H-J, Elashoff R and Heber D. Phytochemical assays of commercial botanical dietary supplements. Evid Based Complement Alternat Med 1: 305–313, 2004.
215. Krzywkowski K, Petersen EW, Ostrowski K, Kristensen JH, Boza J and Pedersen BK. Effect of glutamine supplementation on exercise-induced changes in lymphocyte function. Am J Physiol Cell Physiol 281: C1259-1265, 2001.
216. Krzywkowski K, Petersen EW, Ostrowski K, Link-Amster H, Boza J, Halkjaer-Kristensen J and Pedersen BK. Effect of glutamine and protein supplementation on exercise-induced decreases in salivary IgA. J Appl Physiol 91: 832–838, 2001.
217. Kuipers H, van Breda E, Verlaan G and Smeets R. Effects of oral bovine colostrum supplementation on serum insulin-like growth factor-I levels. Nutrition 18: 566–567, 2002.
218. Laaksi I, Ruohola J-P, Tuohimaa P, Auvinen A, Haataja R, Pihlajamäki H and Ylikomi T. An association of serum vitamin D concentrations < 40 nmol/L with acute respiratory tract infection in young Finnish men. Am J Clin Nutr 86: 714–717, 2007.
219. de Labra C, Guimaraes-Pinheiro C, Maseda A, Lorenzo T and Millán-Calenti JC. Effects of physical exercise interventions in frail older adults: a systematic review of randomized controlled trials. BMC Geriatr 15: 154, 2015.
220. Lacey JM and Wilmore DW. Is glutamine a conditionally essential amino acid? Nutr Rev 48: 297–309, 1990.
221. Laing SJ, Oliver SJ, Wilson S, Walters R, Bilzon JLJ and Walsh NP. Neutrophil-degranulation and lymphocyte-subset response after 48 hr of fluid and/or energy restriction. Int J Sport Nutr Exerc Metab 18: 443–456, 2008.
222. Laires MJ and Monteiro C. Exercise, magnesium and immune function. Magnes Res 21: 92–96, 2008.
223. Lajous M, Rossignol E, Fagherazzi G, Perquier F, Scalbert A, Clavel-Chapelon F and Boutron-Ruault M-C. Flavonoid intake and incident hypertension in women. Am J Clin Nutr 103: 1091–1098, 2016.
224. Lancaster GI, Khan Q, Drysdale PT, Wallace F, Jeukendrup AE, Drayson MT and Gleeson M. Effect of prolonged exercise and carbohydrate ingestion on type 1 and type 2 T lymphocyte distribution and intracellular cytokine production in humans. J Appl Physiol 98: 565–571, 2005.
225. Lau W, Liang M, Sokmen B, Spalding T, Quezada L and Chuang W. Effect of Panax notoginseng (Chinese ginseng) and cycling exercise on IL-6 and cortisol in untrained non-diabetic men. Med Sci Sports Exerc 43: S432, 2011.
226. Lehtonen-Veromaa MKM, Möttönen TT, Nuotio IO, Irjala KMA, Leino AE and Viikari JSA. Vitamin D and attainment of peak bone mass among peripubertal Finnish girls: a 3-y prospective study. Am J Clin Nutr 76: 1446–14453, 2002.
227. Lehtoranta L, Pitkäranta A and Korpela R. Probiotics in respiratory virus infections. Eur J Clin Microbiol Infect Dis 33: 1289–1302, 2014.
228. Leppäluoto J, Rasi S, Martikkala V and Puukka M. Bovine colostrum supplementation enhances physical performance on maximal exercise tests. In: International Congress on Sport Science 2000.
229. Lesourd BM. Nutrition and immunity in the elderly: modification of immune responses with nutritional treatments. Am J Clin Nutr 66: 478S–484S, 1997.
230. Li P, Yin Y-L, Li D, Kim SW and Wu G. Amino acids and immune function. Br J Nutr 98: 237–252, 2007.
231. Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schauber J, Wu K, Meinken C, Kamen DL, Wagner M, Bals R, Steinmeyer A, Zügel U, Gallo RL, Eisenberg D, Hewison M, Hollis BW et al. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 311: 1770–1773, 2006.
232. Lomax AR and Calder PC. Probiotics, immune function, infection and inflammation: a review of the evidence from studies conducted in humans. Curr Pharm Des 15: 1428–518, 2009.
233. Love DT, Barrett TJ, White MY, Cordwell SJ, Davies MJ and Hawkins CL. Cellular targets of the myeloperoxidase-derived oxidant hypothiocyanous acid (HOSCN) and its role in the inhibition of glycolysis in macrophages. Free Radic Biol Med 94: 88–98, 2016.
234. Luiking YC, Ten Have GAM, Wolfe RR and Deutz NEP. Arginine de novo and nitric oxide production in disease states. Am J Physiol Endocrinol Metab 303: E1177-1189, 2012.
235. Lukaski HC. Vitamin and mineral status: effects on physical performance. Nutrition 20: 632–644, 2004.
236. MacLean DA, Graham TE and Saltin B. Branched-chain amino acids augment ammonia metabolism while attenuating protein breakdown during exercise. Am J Physiol 267: E1010-1022, 1994.
237. MacLean DA, Graham TE and Saltin B. Stimulation of muscle ammonia production during exercise following branched-chain amino acid supplementation in humans. J Physiol 493: 909–922, 1996.
238. Maecker HT, McCoy JP and Nussenblatt R. Standardizing immunophenotyping for the Human Immunology Project. Nat Rev Immunol 12: 191–200, 2012.
239. Maijó M, Clements SJ, Ivory K, Nicoletti C and Carding SR. Nutrition, diet and immunosenescence. Mech Ageing Dev 136–137: 116–128, 2014.
240. Marchbank T, Davison G, Oakes JR, Ghatei MA, Patterson M, Moyer MP and Playford RJ. The nutriceutical bovine colostrum truncates the increase in gut permeability caused by heavy exercise in athletes. Am J Physiol Gastrointest Liver Physiol 300: G477-484, 2011.
241. Marín L, Miguélez EM, Villar CJ and Lombó F. Bioavailability of dietary polyphenols and gut microbiota metabolism: antimicrobial properties. BioMed Res Int 2015: 905215, 2015.
242. Marinkovic DM, Kostic-Vucicevic MM, Vukasinovic-Vesic MD, Stojmenovic TB, Dikic NV, Andjelkovic MS, Djordjevic BI, Tanaskovic BP and Minic RD. L. helveticus Lafti® L10 supplementation modulates mucosal and humoral immunity in elite athletes: a randomized double-blinded placebo-controlled trial. J Strength Cond Res , 2016. Epub

243. Markofski MM, Flynn MG, Carrillo AE, Armstrong CLH, Campbell WW and Sedlock DA. Resistance exercise training-induced decrease in circulating inflammatory CD14+CD16+ monocyte percentage without weight loss in older adults. Eur J Appl Physiol 114: 1737–1748, 2014.
244. Marques CG, Santos VC, Levada-Pires AC, Jacintho TM, Gorjão R, Pithon-Curi TC and Cury-Boaventura MF. Effects of DHA-rich fish oil supplementation on the lipid profile, markers of muscle damage, and neutrophil function in wheelchair basketball athletes before and after acute exercise. Appl Physiol Nutr Metab 40: 596–604, 2015.
245. Marranzino G, Villena J, Salva S and Alvarez S. Stimulation of macrophages by immunobiotic Lactobacillus strains: influence beyond the intestinal tract. Microbiol Immunol 56: 771–781, 2012.
246. Mårtensson S, Nordebo K and Malm C. High training volumes are associated with a low number of self-reported sick days in elite endurance athletes. J Sports Sci Med 13: 929–933, 2014.
247. Martí A, Marcos A and Martínez JA. Obesity and immune function relationships. Obes Rev 2: 131–140, 2001.
248. Martineau AR, Wilkinson RJ, Wilkinson KA, Newton SM, Kampmann B, Hall BM, Packe GE, Davidson RN, Eldridge SM, Maunsell ZJ, Rainbow SJ, Berry JL and Griffiths CJ. A single dose of vitamin D enhances immunity to mycobacteria. Am J Respir Crit Care Med 176: 208–213, 2007.
249. Martinez-Gomez D, Eisenmann JC, Gomez-Martinez S, Veses A, Romeo J, Veiga OL, Marcos A and AFINOS Study Group. Associations of physical activity and fitness with adipocytokines in adolescents: the AFINOS Study. Nutr Metab Cardiovasc Dis 22: 252–259, 2012.
250. Martinez-Gomez D, Eisenmann JC, Wärnberg J, Gomez-Martinez S, Veses A, Veiga OL, Marcos A and AFINOS Study Group. Associations of physical activity, cardiorespiratory fitness and fatness with low-grade inflammation in adolescents: the AFINOS Study. Int J Obes 34: 1501–1507, 2010.
251. Maslowski KM, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, Schilter HC, Rolph MS, Mackay F, Artis D, Xavier RJ, Teixeira MM and Mackay CR. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature 461: 1282–1286, 2009.
252. McAnulty LS, Nieman DC, Dumke CL, Shooter LA, Henson DA, Utter AC, Milne G and McAnulty SR. Effect of blueberry ingestion on natural killer cell counts, oxidative stress, and inflammation prior to and after 2.5 h of running. Appl Physiol Nutr Metab 36: 976–984, 2011.
253. McClung JP, Gaffney-Stomberg E and Lee JJ. Female athletes: a population at risk of vitamin and mineral deficiencies affecting health and performance. J Trace Elem Med Biol 28: 388–392, 2014.
254. McFarlin BK, Flynn MG, Stewart LK and Timmerman KL. Carbohydrate intake during endurance exercise increases natural killer cell responsiveness to IL-2. J Appl Physiol 96: 271–275, 2004.
255. Mero A, Kähkönen J, Nykänen T, Parviainen T, Jokinen I, Takala T, Nikula T, Rasi S and Leppäluoto J. IGF-I, IgA, and IgG responses to bovine colostrum supplementation during training. J Appl Physiol 93: 732–739, 2002.
256. Mero A, Miikkulainen H, Riski J, Pakkanen R, Aalto J and Takala T. Effects of bovine colostrum supplementation on serum IGF-I, IgG, hormone, and saliva IgA during training. J Appl Physiol 83: 1144–1151, 1997.
257. Micheletti A, Rossi R and Rufini S. Zinc status in athletes: relation to diet and exercise. Sports Med 31: 577–582, 2001.
258. Miller BF, Olesen JL, Hansen M, Døssing S, Crameri RM, Welling RJ, Langberg H, Flyvbjerg A, Kjaer M, Babraj JA, Smith K and Rennie MJ. Coordinated collagen and muscle protein synthesis in human patella tendon and quadriceps muscle after exercise. J Physiol 567: 1021–1033, 2005.
259. Mitchell JB, Pizza FX, Paquet A, Davis BJ, Forrest MB and Braun WA. Influence of carbohydrate status on immune responses before and after endurance exercise. J Appl Physiol 84: 1917–1925, 1998.
260. Moberg M, Apró W, Ekblom B, van Hall G, Holmberg H-C and Blomstrand E. Activation of mTORC1 by leucine is potentiated by branched-chain amino acids and even more so by essential amino acids following resistance exercise. Am J Physiol Cell Physiol 310: C874-884, 2016.
261. Mondello S, Italiano D, Giacobbe MS, Mondello P, Trimarchi G, Aloisi C, Bramanti P and Spina E. Glutamine-supplemented total parenteral nutrition improves immunological status in anorectic patients. Nutrition 26: 677–681, 2010.
262. Mooren FC, Golf SW and Völker K. Effect of magnesium on granulocyte function and on the exercise induced inflammatory response. Magnes Res 16: 49–58, 2003.
263. Mooren FC, Krüger K, Völker K, Golf SW, Wadepuhl M and Kraus A. Oral magnesium supplementation reduces insulin resistance in non-diabetic subjects - a double-blind, placebo-controlled, randomized trial. Diabetes Obes Metab 13: 281–284, 2011.
264. Morrison SA, Cheung SS and Cotter JD. Bovine colostrum, training status, and gastrointestinal permeability during exercise in the heat: a placebo-controlled double-blind study. Appl Physiol Nutr Metab 39: 1070–1082, 2014.
265. Mountjoy M, Sundgot-Borgen J, Burke L, Carter S, Constantini N, Lebrun C, Meyer N, Sherman R, Steffen K, Budgett R and Ljungqvist A. The IOC consensus statement: beyond the female athlete triad--Relative Energy Deficiency in Sport (RED-S). Br J Sports Med 48: 491–497, 2014.
266. Mulder AM, Connellan PA, Oliver CJ, Morris CA and Stevenson LM. Bovine lactoferrin supplementation supports immune and antioxidant status in healthy human males. Nutr Res 28: 583–589, 2008.
267. Murphy WJ, Parham P and Miller JS. NK cells--from bench to clinic. Biol Blood Marrow Transplant 18: S2-7, 2012.
268. Myburgh KH. Polyphenol supplementation: benefits for exercise performance or oxidative stress? Sports Med 44 Suppl 1: S57-70, 2014.
269. Nairz M, Schroll A, Demetz E, Tancevski I, Theurl I and Weiss G. "Ride on the ferrous wheel"--the cycle of iron in macrophages in health and disease. Immunobiology 220: 280–294, 2015.
270. Nehlsen-Cannarella SL, Fagoaga OR, Nieman DC, Henson DA, Butterworth DE, Schmitt RL, Bailey EM, Warren BJ, Utter A and Davis JM. Carbohydrate and the cytokine response to 2.5 h of running. J Appl Physiol 82: 1662–1667, 1997.
271. Neville V, Gleeson M and Folland JP. Salivary IgA as a risk factor for upper respiratory infections in elite professional athletes. Med Sci Sports Exerc 40: 1228–1236, 2008.
272. Nielsen AR and Pedersen BK. The biological roles of exercise-induced cytokines: IL-6, IL-8, and IL-15. Appl Physiol Nutr Metab 32: 833–839, 2007.

273. Nieman DC. Exercise, upper respiratory tract infection, and the immune system. Med Sci Sports Exerc 26: 128–139, 1994.
274. Nieman DC. Exercise, infection, and immunity. Int J Sports Med 15 Suppl 3: S131-141, 1994.
275. Nieman DC. Exercise immunology: nutritional countermeasures. Can J Appl Physiol 26: S45-55, 2001.
276. Nieman DC. Immunonutrition support for athletes. Nutr Rev 66: 310–320, 2008.
277. Nieman DC. Quercetin's bioactive effects in human athletes. Curr Top Nutraceut Res 33–44, 2010.
278. Nieman DC. Flavonoids. In: Nutritional Supplements in Sport, Exercise, and Health, An A-Z Guide. Castell LM, Stear SJ, Burke LM, eds. . Oxford, UK: Routledge, 2015.
279. Nieman DC, Davis JM, Brown VA, Henson DA, Dumke CL, Utter AC, Vinci DM, Downs MF, Smith JC, Carson J, Brown A, McAnulty SR and McAnulty LS. Influence of carbohydrate ingestion on immune changes after 2 h of intensive resistance training. J Appl Physiol 96: 1292–8, 2004.
280. Nieman DC, Fagoaga OR, Butterworth DE, Warren BJ, Utter A, Davis JM, Henson DA and Nehlsen-Cannarella SL. Carbohydrate supplementation affects blood granulocyte and monocyte trafficking but not function after 2.5 h or running. Am J Clin Nutr 66: 153–159, 1997.
281. Nieman DC, Gillitt ND, Knab AM, Shanely RA, Pappan KL, Jin F and Lila MA. Influence of a polyphenol-enriched protein powder on exercise-induced inflammation and oxidative stress in athletes: a randomized trial using a metabolomics approach. PloS One 8: e72215, 2013.
282. Nieman DC, Gillitt ND, Sha W, Meaney MP, John C, Pappan KL and Kinchen JM. Metabolomics-based analysis of banana and pear ingestion on exercise performance and recovery. J Proteome Res 14: 5367–5377, 2015.
283. Nieman DC, Henson DA, Austin MD and Sha W. Upper respiratory tract infection is reduced in physically fit and active adults. Br J Sports Med 45: 987–992, 2011.
284. Nieman DC, Henson DA, Fagoaga OR, Utter AC, Vinci DM, Davis JM and Nehlsen-Cannarella SL. Change in salivary IgA following a competitive marathon race. Int J Sports Med 23: 69–75, 2002.
285. Nieman DC, Henson DA, Garner EB, Butterworth DE, Warren BJ, Utter A, Davis JM, Fagoaga OR and Nehlsen-Cannarella SL. Carbohydrate affects natural killer cell redistribution but not activity after running. Med Sci Sports Exerc 29: 1318–1324, 1997.
286. Nieman DC, Henson DA, Gusewitch G, Warren BJ, Dotson RC, Butterworth DE and Nehlsen-Cannarella SL. Physical activity and immune function in elderly women. Med Sci Sports Exerc 25: 823–831, 1993.
287. Nieman DC, Henson DA, McAnulty SR, Jin F and Maxwell KR. n-3 polyunsaturated fatty acids do not alter immune and inflammation measures in endurance athletes. Int J Sport Nutr Exerc Metab 19: 536–546, 2009.
288. Nieman DC, Nehlsen-Cannarella SL, Fagoaga OR, Henson DA, Shannon M, Davis JM, Austin MD, Hisey CL, Holbeck JC, Hjertman JM, Bolton MR and Schilling BK. Immune response to two hours of rowing in elite female rowers. Int J Sports Med 20: 476–481, 1999.
289. Nieman DC, Nehlsen-Cannarella SL, Fagoaga OR, Henson DA, Utter A, Davis JM, Williams F and Butterworth DE. Effects of mode and carbohydrate on the granulocyte and monocyte response to intensive, prolonged exercise. J Appl Physiol 84: 1252–1259, 1998.
290. Niemelä M, Kangastupa P, Niemelä O, Bloigu R and Juvonen T. Acute changes in inflammatory biomarker levels in recreational runners participating in a marathon or half-marathon. Sports Med - Open 2: 21, 2016.
291. Norderhaug IN, Johansen FE, Schjerven H and Brandtzaeg P. Regulation of the formation and external transport of secretory immunoglobulins. Crit Rev Immunol 19: 481–508, 1999.
292. Northoff H, Weinstock C and Berg A. The cytokine response to strenuous exercise. Int J Sports Med 15 Suppl 3: S167-171, 1994.
293. Notarangelo LD and Badolato R. Leukocyte trafficking in primary immunodeficiencies. J Leukoc Biol 85: 335–343, 2009.
294. Okutsu M, Ishii K, Niu KJ and Nagatomi R. Cortisol-induced CXCR4 augmentation mobilizes T lymphocytes after acute physical stress. Am J Physiol Regul Integr Comp Physiol 288: R591-599, 2005.
295. Oliver SJ, Laing SJ, Wilson S, Bilzon JLJ, Walters R and Walsh NP. Salivary immunoglobulin A response at rest and after exercise following a 48 h period of fluid and/or energy restriction. Br J Nutr 97: 1109–16, 2007.
296. Ortega E, Giraldo E, Hinchado MD, Martínez M, Ibáñez S, Cidoncha A, Collazos ME and García JJ. Role of Hsp72 and norepinephrine in the moderate exercise-induced stimulation of neutrophils' microbicide capacity. Eur J Appl Physiol 98: 250–5, 2006.
297. Outram S and Stewart B. Doping through supplement use: a review of the available empirical data. Int J Sport Nutr Exerc Metab 25: 54–9, 2015.
298. Owens DJ, Fraser WD and Close GL. Vitamin D and the athlete: emerging insights. Eur J Sport Sci 15: 73–84, 2015.
299. Owens DJ, Sharples AP, Polydorou I, Alwan N, Donovan T, Tang J, Fraser WD, Cooper RG, Morton JP, Stewart C and Close GL. A systems-based investigation into vitamin D and skeletal muscle repair, regeneration, and hypertrophy. Am J Physiol Endocrinol Metab 309: E1019-1031, 2015.
300. Pae M, Meydani SN and Wu D. The role of nutrition in enhancing immunity in aging. Aging Dis 3: 91–129, 2012.
301. Paesano R, Pacifici E, Benedetti S, Berlutti F, Frioni A, Polimeni A and Valenti P. Safety and efficacy of lactoferrin versus ferrous sulphate in curing iron deficiency and iron deficiency anaemia in hereditary thrombophilia pregnant women: an interventional study. Biometals 27: 999–1006, 2014.
302. Panagiotakos DB, Kokkinos P, Manios Y and Pitsavos C. Physical activity and markers of inflammation and thrombosis related to coronary heart disease. Prev Cardiol 7: 190–194, 2004.
303. Parry-Billings M, Evans J, Calder PC and Newsholme EA. Does glutamine contribute to immunosuppression after major burns? Lancet 336: 523–525, 1990.
304. Pascoe AR, Fiatarone Singh MA and Edwards KM. The effects of exercise on vaccination responses: a review of chronic and acute exercise interventions in humans. Brain Behav Immun 39: 33–41, 2014.
305. Pedersen BK. The anti-inflammatory effect of exercise: its role in diabetes and cardiovascular disease control. Essays Biochem 42: 105–117, 2006.
306. Pedersen BK and Bruunsgaard H. How physical exercise influences the establishment of infections. Sports Med 19: 393–400, 1995.
307. Pedersen BK and Saltin B. Evidence for prescribing exercise as therapy in chronic disease. Scand J Med Sci Sports 16 Suppl 1: 3–63, 2006.

308. Pedersen BK, Steensberg A, Fischer C, Keller C, Keller P, Plomgaard P, Febbraio M and Saltin B. Searching for the exercise factor: is IL-6 a candidate? J Muscle Res Cell Motil 24: 113–119, 2003.
309. Pedersen BK, Steensberg A and Schjerling P. Muscle-derived interleukin-6: possible biological effects. J Physiol 536: 329–337, 2001.
310. Pedersen BK and Toft AD. Effects of exercise on lymphocytes and cytokines. Br J Sports Med 34: 246–251, 2000.
311. Petersen AMW and Pedersen BK. The role of IL-6 in mediating the anti-inflammatory effects of exercise. J Physiol Pharmacol 57 Suppl 10: 43–51, 2006.
312. Phillips SM, Tipton KD, Aarsland A, Wolf SE and Wolfe RR. Mixed muscle protein synthesis and breakdown after resistance exercise in humans. Am J Physiol 273: E99-107, 1997.
313. Phillips SM and Van Loon LJC. Dietary protein for athletes: from requirements to optimum adaptation. J Sports Sci 29 Suppl 1: S29-38, 2011.
314. Playford RJ, MacDonald CE, Calnan DP, Floyd DN, Podas T, Johnson W, Wicks AC, Bashir O and Marchbank T. Co-administration of the health food supplement, bovine colostrum, reduces the acute non-steroidal anti-inflammatory drug-induced increase in intestinal permeability. Clin Sci 100: 627–633, 2001.
315. Playford RJ, Woodman AC, Clark P, Watanapa P, Vesey D, Deprez PH, Williamson RC and Calam J. Effect of luminal growth factor preservation on intestinal growth. Lancet 341: 843–848, 1993.
316. Poortmans JR, Siest G, Galteau MM and Houot O. Distribution of plasma amino acids in humans during submaximal prolonged exercise. Eur J Appl Physiol 32: 143–147, 1974.
317. Powers SK, Duarte J, Kavazis AN and Talbert EE. Reactive oxygen species are signalling molecules for skeletal muscle adaptation. Exp Physiol 95: 1–9, 2010.
318. Powers SK, Ji LL, Kavazis AN and Jackson MJ. Reactive oxygen species: impact on skeletal muscle. Compr Physiol 1: 941–969, 2011.
319. Prasad AS. Discovery of human zinc deficiency: its impact on human health and disease. Adv Nutr 4: 176–90, 2013.
320. Proctor GB, Garrett JR, Carpenter GH and Ebersole LE. Salivary secretion of immunoglobulin A by submandibular glands in response to autonomimetic infusions in anaesthetised rats. J Neuroimmunol 136: 17–24, 2003.
321. Prosser C, Stelwagen K, Cummins R, Guerin P, Gill N and Milne C. Reduction in heat-induced gastrointestinal hyperpermeability in rats by bovine colostrum and goat milk powders. J Appl Physiol 96: 650–654, 2004.
322. Pyne DB, Hopkins WG, Batterham AM, Gleeson M and Fricker PA. Characterising the individual performance responses to mild illness in international swimmers. Br J Sports Med 39: 752–756, 2005.
323. Pyne DB, West NP, Cox AJ and Cripps AW. Probiotics supplementation for athletes - clinical and physiological effects. Eur J Sport Sci 15: 63–72, 2015.
324. Quindry J, Dumke C, Slivka D and Ruby B. Impact of extreme exercise at high altitude on oxidative stress in humans. J Physiol 594: 5093–5104, 2016.
325. Quindry JC, Stone WL, King J and Broeder CE. The effects of acute exercise on neutrophils and plasma oxidative stress. Med Sci Sports Exerc 35: 1139–1145, 2003.
326. Radak Z, Suzuki K, Higuchi M, Balogh L, Boldogh I and Koltai E. Physical exercise, reactive oxygen species and neuroprotection. Free Radic Biol Med 98: 187–96, 2016.
327. Raizel R, Leite JSM, Hypólito TM, Coqueiro AY, Newsholme P, Cruzat VF and Tirapegui J. Determination of the anti-inflammatory and cytoprotective effects of l-glutamine and l-alanine, or dipeptide, supplementation in rats submitted to resistance exercise. Br J Nutr 116: 470–9, 2016.
328. Rayman MP. Selenium and human health. Lancet 379: 1256–1268, 2012.
329. Rayner BS, Love DT and Hawkins CL. Comparative reactivity of myeloperoxidase-derived oxidants with mammalian cells. Free Radic Biol Med 71: 240–255, 2014.
330. Reale M, Boscolo P, Bellante V, Tarantelli C, Di Nicola M, Forcella L, Li Q, Morimoto K and Muraro R. Daily intake of Lactobacillus casei Shirota increases natural killer cell activity in smokers. Br J Nutr 108: 308–314, 2012.
331. Reid MB and Durham WJ. Generation of reactive oxygen and nitrogen species in contracting skeletal muscle: potential impact on aging. Ann N Y Acad Sci 959: 108–116, 2002.
332. Rennie MJ, Edwards RH, Krywawych S, Davies CT, Halliday D, Waterlow JC and Millward DJ. Effect of exercise on protein turnover in man. Clin Sci 61: 627–639, 1981.
333. Reznikov EA, Comstock SS, Yi C, Contractor N and Donovan SM. Dietary bovine lactoferrin increases intestinal cell proliferation in neonatal piglets. J Nutr 144: 1401–1408, 2014.
334. Rocha DM, Caldas AP, Oliveira LL, Bressan J and Hermsdorff HH. Saturated fatty acids trigger TLR4-mediated inflammatory response. Atherosclerosis 244: 211–215, 2016.
335. Rohde T, Asp S, MacLean DA and Pedersen BK. Competitive sustained exercise in humans, lymphokine activated killer cell activity, and glutamine--an intervention study. Eur J Appl Physiol 78: 448–453, 1998.
336. Rohde T, MacLean DA, Hartkopp A and Pedersen BK. The immune system and serum glutamine during a triathlon. Eur J Appl Physiol 74: 428–434, 1996.
337. Rohde T, MacLean DA and Pedersen BK. Effect of glutamine supplementation on changes in the immune system induced by repeated exercise. Med Sci Sports Exerc 30: 856–862, 1998.
338. Romagnani S. Lymphokine production by human T cells in disease states. Annu Rev Immunol 12: 227–257, 1994.
339. Romeo J, Martinez-Gomez D, Diaz LE, Gómez-Martinez S, Marti A, Martin-Matillas M, Puertollano MA, Veiga OL, Martinez JA, Wärnberg J, Zapatera B, Garagorri JM, Morandé G, Campoy C, Moreno LA, Marcos A and EVASYON Study Group. Changes in cardiometabolic risk factors, appetite-controlling hormones and cytokines after a treatment program in overweight adolescents: preliminary findings from the EVASYON study. Pediatr Diabetes 12: 372–380, 2011.
340. Rose W. The nutritive significance of the amino acids. Physiol Rev 109–136, 1938.
341. Roth E, Funovics J, Mühlbacher F, Schemper M, Mauritz W, Sporn P and Fritsch A. Metabolic disorders in severe abdominal sepsis: glutamine deficiency in skeletal muscle. Clin Nutr 1: 25–41, 1982.
342. Sabetta JR, DePetrillo P, Cipriani RJ, Smardin J, Burns LA and Landry ML. Serum 25-hydroxyvitamin d and the incidence of acute viral respiratory tract infections in healthy adults. PloS One 5: e11088, 2010.

343. Sacerdote P, Mussano F, Franchi S, Panerai AE, Bussolati G, Carossa S, Bartorelli A and Bussolati B. Biological components in a standardized derivative of bovine colostrum. J Dairy Sci 96: 1745–1754, 2013.
344. Samieri C, Sun Q, Townsend MK, Rimm EB and Grodstein F. Dietary flavonoid intake at midlife and healthy aging in women. Am J Clin Nutr 100: 1489–1497, 2014.
345. Sánchez-Patán F, Monagas M, Moreno-Arribas MV and Bartolomé B. Determination of microbial phenolic acids in human faeces by UPLC-ESI-TQ MS. J Agric Food Chem 59: 2241–2247, 2011.
346. Santos VC, Levada-Pires AC, Alves SR, Pithon-Curi TC, Curi R and Cury-Boaventura MF. Effects of DHA-rich fish oil supplementation on lymphocyte function before and after a marathon race. Int J Sport Nutr Exerc Metab 23: 161–169, 2013.
347. Sarkar D and Fisher PB. Molecular mechanisms of aging-associated inflammation. Cancer Lett 236: 13–23, 2006.
348. Saur P, Joneleit M, Tölke H, Pudel V, Niedmann P and Kettler. Evaluation of the magnesium status in athletes. 53:72-78, 2002. German J Sports Med 53: 72–78, 2002.
349. Schapowal A, Klein P and Johnston SL. Echinacea reduces the risk of recurrent respiratory tract infections and complications: a meta-analysis of randomized controlled trials. Adv Ther 32: 187–200, 2015.
350. Scharhag J, Meyer T, Auracher M, Gabriel HH and Kindermann W. Effects of graded carbohydrate supplementation on the immune response in cycling. Med Sci Sports Exerc 38: 286–292, 2006.
351. Scharhag J, Meyer T, Gabriel HHW, Auracher M and Kindermann W. Mobilization and oxidative burst of neutrophils are influenced by carbohydrate supplementation during prolonged cycling in humans. Eur J Appl Physiol 87: 584–587, 2002.
352. Scheiermann C, Kunisaki Y, Lucas D, Chow A, Jang J-E, Zhang D, Hashimoto D, Merad M and Frenette PS. Adrenergic nerves govern circadian leukocyte recruitment to tissues. Immunity 37: 290–301, 2012.
353. Schoop R, Büechi S and Suter A. Open, multicenter study to evaluate the tolerability and efficacy of Echinaforce Forte tablets in athletes. Adv Ther 23: 823–833, 2006.
354. Science M, Johnstone J, Roth DE, Guyatt G and Loeb M. Zinc for the treatment of the common cold: a systematic review and meta-analysis of randomized controlled trials. Can Med Assoc J 184: E551-561, 2012.
355. Sebastian RS, Wilkinson Enns C, Goldman JD, Martin CL, Steinfeldt LC, Murayi T and Moshfegh AJ. A new database facilitates characterization of flavonoid intake, sources, and positive associations with diet quality among US adults. J Nutr 145: 1239–1248, 2015.
356. Seligman PA, Kovar J and Gelfand EW. Lymphocyte proliferation is controlled by both iron availability and regulation of iron uptake pathways. Pathobiology 60: 19–26, 1992.
357. Senchina DS. Athletics and herbal supplements. Am Sci 101: 134–141, 2013.
358. Senchina DS, Hallam JE and Cheney DJ. Multidisciplinary perspectives on mechanisms of activity of popular immune-enhancing herbal supplements used by athletes. Front Biol 8: 78–100, 2013.
359. Senchina DS, Shah NB, Doty DM, Sanderson CR and Hallam JE. Herbal supplements and athlete immune function--what's proven, disproven, and unproven? Exerc Immunol Rev 15: 66–106, 2009.
360. Sergeant S, Rahbar E and Chilton FH. Gamma-linolenic acid, dihommo-gamma linolenic, eicosanoids and inflammatory processes. Eur J Pharmacol 785: 77–86, 2016.
361. Sheridan PA, Paich HA, Handy J, Karlsson EA, Hudgens MG, Sammon AB, Holland LA, Weir S, Noah TL and Beck MA. Obesity is associated with impaired immune response to influenza vaccination in humans. Int J Obes 2005 36: 1072–1077, 2012.
362. Shing CM, Peake J, Suzuki K, Okutsu M, Pereira R, Stevenson L, Jenkins DG and Coombes JS. Effects of bovine colostrum supplementation on immune variables in highly trained cyclists. J Appl Physiol 102: 1113–1122, 2007.
363. Shing CM, Peake JM, Suzuki K, Jenkins DG and Coombes JS. A pilot study: bovine colostrum supplementation and hormonal and autonomic responses to competitive cycling. J Sports Med Phys Fitness 53: 490–501, 2013.
364. Shulzhenko N, Morgun A, Hsiao W, Battle M, Yao M, Gavrilova O, Orandle M, Mayer L, Macpherson AJ, McCoy KD, Fraser-Liggett C and Matzinger P. Crosstalk between B lymphocytes, microbiota and the intestinal epithelium governs immunity versus metabolism in the gut. Nat Med 17: 1585–1593, 2011.
365. Simpson RJ, Kunz H, Agha N and Graff R. Exercise and the regulation of immune functions. Prog Mol Biol Transl Sci 135: 355–380, 2015.
366. Simpson RJ, Lowder TW, Spielmann G, Bigley AB, LaVoy EC and Kunz H. Exercise and the aging immune system. Ageing Res Rev 11: 404–420, 2012.
367. Singh M and Das RR. Zinc for the common cold. Cochrane Database Syst Rev CD001364, 2013.
368. Sly LM, Lopez M, Nauseef WM and Reiner NE. 1alpha,25-Dihydroxyvitamin D3-induced monocyte antimycobacterial activity is regulated by phosphatidylinositol 3-kinase and mediated by the NADPH-dependent phagocyte oxidase. J Biol Chem 276: 35482–93, 2001.
369. Smith AJ, Vollmer-Conna U, Bennett B, Hickie IB and Lloyd AR. Influences of distress and alcohol consumption on the development of a delayed-type hypersensitivity skin test response. Psychosom Med 66: 614–619, 2004.
370. Smith TP, Kennedy SL and Fleshner M. Influence of age and physical activity on the primary in vivo antibody and T cell-mediated responses in men. J Appl Physiol 97: 491–498, 2004.
371. Solanki I, Parihar P, Mansuri ML and Parihar MS. Flavonoid-based therapies in the early management of neurodegenerative diseases. Adv Nutr 6: 64–72, 2015.
372. Somerville VS, Braakhuis AJ and Hopkins WG. Effect of flavonoids on upper respiratory tract infections and immune function: A systematic review and meta-analysis. Adv Nutr 7: 488–497, 2016.
373. Song Q-H, Xu R-M, Zhang Q-H, Shen G-Q, Ma M, Zhao X-P, Guo Y-H and Wang Y. Glutamine supplementation and immune function during heavy load training. Int J Clin Pharmacol Ther 53: 372–376, 2015.
374. Spence L, Brown WJ, Pyne DB, Nissen MD, Sloots TP, McCormack JG, Locke AS and Fricker PA. Incidence, etiology, and symptomatology of upper respiratory illness in elite athletes. Med Sci Sports Exerc 39: 577–586, 2007.
375. Stevenson JL, Krishnan S, Inigo MM, Stamatikos AD, Gonzales JU and Cooper JA. Echinacea-based dietary supplement does not increase maximal aerobic capacity in endurance-trained men and women. J Diet Suppl 13: 324–338, 2016.

376. Stoffaneller R and Morse NL. A review of dietary selenium intake and selenium status in Europe and the Middle East. Nutrients 7: 1494–1537, 2015.
377. Strydom N and Rankin SM. Regulation of circulating neutrophil numbers under homeostasis and in disease. J Innate Immun 5: 304–314, 2013.
378. Suratt BT, Petty JM, Young SK, Malcolm KC, Lieber JG, Nick JA, Gonzalo J-A, Henson PM and Worthen GS. Role of the CXCR4/SDF-1 chemokine axis in circulating neutrophil homeostasis. Blood 104: 565–571, 2004.
379. Sureda A, Tejada S, Bibiloni M del M, Tur JA and Pons A. Polyphenols: well beyond the antioxidant capacity: polyphenol supplementation and exercise-induced oxidative stress and inflammation. Curr Pharm Biotechnol 15: 373–379, 2014.
380. Svendsen IS, Taylor IM, Tønnessen E, Bahr R and Gleeson M. Training-related and competition-related risk factors for respiratory tract and gastrointestinal infections in elite cross-country skiers. Br J Sports Med 50: 809–815, 2016.
381. Takele Y, Adem E, Getahun M, Tajebe F, Kiflie A, Hailu A, Raynes J, Mengesha B, Ayele TA, Shkedy Z, Lemma M, Diro E, Toulza F, Modolell M, Munder M, Müller I and Kropf P. Malnutrition in healthy individuals results in increased mixed cytokine profiles, altered neutrophil subsets and function. PloS One 11: e0157919, 2016.
382. Tartibian B, Maleki BH and Abbasi A. The effects of ingestion of omega-3 fatty acids on perceived pain and external symptoms of delayed onset muscle soreness in untrained men. Clin J Sport Med 19: 115–119, 2009.
383. Tartibian B, Maleki BH and Abbasi A. Omega-3 fatty acids supplementation attenuates inflammatory markers after eccentric exercise in untrained men. Clin J Sport Med 21: 131–137, 2011.
384. Thomas DT, Erdman KA and Burke LM. Position of the Academy of nutrition and dietetics, dietitians of Canada, and the American college of sports medicine: nutrition and athletic performance. J Acad Nutr Diet 116: 501–528, 2016.
385. Timmerman KL, Flynn MG, Coen PM, Markofski MM and Pence BD. Exercise training-induced lowering of inflammatory (CD14+CD16+) monocytes: a role in the anti-inflammatory influence of exercise? J Leukoc Biol 84: 1271–1278, 2008.
386. Tiollier E, Chennaoui M, Gomez-Merino D, Drogou C, Filaire E and Guezennec CY. Effect of a probiotics supplementation on respiratory infections and immune and hormonal parameters during intense military training. Mil Med 172: 1006–1011, 2007.
387. Todd DA, Gulledge TV, Britton ER, Oberhofer M, Leyte-Lugo M, Moody AN, Shymanovich T, Grubbs LF, Juzumaite M, Graf TN, Oberlies NH, Faeth SH, Laster SM and Cech NB. Ethanolic echinacea purpurea extracts contain a mixture of cytokine-suppressive and cytokine-inducing compounds, including some that originate from endophytic bacteria. PloS One 10: e0124276, 2015.
388. Toft AD, Thorn M, Ostrowski K, Asp S, Moller K, Iversen S, Hermann C, Sondergaard SR and Pedersen BK. N-3 polyunsaturated fatty acids do not affect cytokine response to strenuous exercise. J Appl Physiol 89: 2401–2406, 2000.
389. Tomasi TB, Trudeau FB, Czerwinski D and Erredge S. Immune parameters in athletes before and after strenuous exercise. J Clin Immunol 2: 173–178, 1982.
390. Tomaszewski M, Charchar FJ, Przybycin M, Crawford L, Wallace AM, Gosek K, Lowe GD, Zukowska-Szczechowska E, Grzeszczak W, Sattar N and Dominiczak AF. Strikingly low circulating CRP concentrations in ultramarathon runners independent of markers of adiposity: how low can you go? Arterioscler Thromb Vasc Biol 23: 1640–1644, 2003.
391. Tresserra-Rimbau A, Guasch-Ferré M, Salas-Salvadó J, Toledo E, Corella D, Castañer O, Guo X, Gómez-Gracia E, Lapetra J, Arós F, Fiol M, Ros E, Serra-Majem L, Pintó X, Fitó M, Babio N, Martínez-González MA, Sorli JV, López-Sabater MC et al. Intake of total polyphenols and some classes of polyphenols is inversely associated with diabetes in elderly people at high cardiovascular disease risk. J Nutr 146: 767–777, 2016.
392. Troost FJ, Steijns J, Saris WH and Brummer RJ. Gastric digestion of bovine lactoferrin in vivo in adults. J Nutr 131: 2101–2104, 2001.
393. Tsukumo DM, Carvalho BM, Carvalho Filho MA and Saad MJA. Translational research into gut microbiota: new horizons on obesity treatment: updated 2014. Arch Endocrinol Metab 59: 154–160, 2015.
394. Turner JE. Is immunosenescence influenced by our lifetime "dose" of exercise? Biogerontology 17: 581–602, 2016.
395. Urashima M, Segawa T, Okazaki M, Kurihara M, Wada Y and Ida H. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. Am J Clin Nutr 91: 1255–1260, 2010.
396. Urso ML and Clarkson PM. Oxidative stress, exercise, and antioxidant supplementation. Toxicology 189: 41–54, 2003.
397. Van Hall G, MacLean DA, Saltin B and Wagenmakers AJ. Muscle protein degradation and amino acid metabolism during prolonged knee-extensor exercise in humans. Clin Sci 97: 557–567, 1999.
398. Vasto S, Candore G, Balistreri CR, Caruso M, Colonna-Romano G, Grimaldi MP, Listi F, Nuzzo D, Lio D and Caruso C. Inflammatory networks in ageing, age-related diseases and longevity. Mech Ageing Dev 128: 83–91, 2007.
399. Vogel HJ. Lactoferrin, a bird's eye view. Biochem Cell Biol 90: 233–244, 2012.
400. Wagenmakers AJ, Brouns F, Saris WH and Halliday D. Oxidation rates of orally ingested carbohydrates during prolonged exercise in men. J Appl Physiol 75: 2774–2780, 1993.
401. Walrand S, Moreau K, Caldefie F, Tridon A, Chassagne J, Portefaix G, Cynober L, Beaufrère B, Vasson MP and Boirie Y. Specific and nonspecific immune responses to fasting and refeeding differ in healthy young adult and elderly persons. Am J Clin Nutr 74: 670–678, 2001.
402. Walsh NP, Bishop NC, Blackwell J, Wierzbicki SG and Montague JC. Salivary IgA response to prolonged exercise in a cold environment in trained cyclists. Med Sci Sports Exerc 34: 1632–1637, 2002.
403. Walsh NP, Blannin AK, Bishop NC, Robson PJ and Gleeson M. Effect of oral glutamine supplementation on human neutrophil lipopolysaccharide-stimulated degranulation following prolonged exercise. Int J Sport Nutr Exerc Metab 10: 39–50, 2000.
404. Walsh NP, Blannin AK, Clark AM, Cook L, Robson PJ and Gleeson M. The effects of high-intensity intermittent exercise on saliva IgA, total protein and alpha-amylase. J Sports Sci 17: 129–134, 1999.

405. Walsh NP, Gleeson M, Pyne DB, Nieman DC, Dhabhar FS, Shephard RJ, Oliver SJ, Bermon S and Kajeniene A. Position statement. Part two: Maintaining immune health. Exerc Immunol Rev 17: 64–103, 2011.
406. Walsh NP, Gleeson M, Shephard RJ, Gleeson M, Woods JA, Bishop NC, Fleshner M, Green C, Pedersen BK, Hoffman-Goetz L, Rogers CJ, Northoff H, Abbasi A and Simon P. Position statement. Part one: Immune function and exercise. Exerc Immunol Rev 17: 6–63, 2011.
407. Walsh NP and Oliver SJ. Exercise, immune function and respiratory infection: An update on the influence of training and environmental stress. Immunol Cell Biol 94: 132–139, 2016.
408. Wang T-T, Nestel FP, Bourdeau V, Nagai Y, Wang Q, Liao J, Tavera-Mendoza L, Lin R, Hanrahan JW, Mader S, White JH and Hanrahan JH. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J Immunol 173: 2909–2912, 2004.
409. Wang X, Ouyang YY, Liu J and Zhao G. Flavonoid intake and risk of CVD: a systematic review and meta-analysis of prospective cohort studies. Br J Nutr 111: 1–11, 2014.
410. Wang X, Pierre JF, Heneghan AF, Busch RA and Kudsk KA. Glutamine improves innate immunity and prevents bacterial enteroinvasion during parenteral nutrition. J Parenter Enter Nutr 39: 688–697, 2015.
411. Wärnberg J, Cunningham K, Romeo J and Marcos A. Physical activity, exercise and low-grade systemic inflammation. Proc Nutr Soc 69: 400–406, 2010.
412. Wärnberg J, Nova E, Romeo J, Moreno LA, Sjöström M and Marcos A. Lifestyle-related determinants of inflammation in adolescence. Br J Nutr 98 Suppl 1: S116-120, 2007.
413. Warner EF, Zhang Q, Raheem KS, O'Hagan D, O'Connell MA and Kay CD. Common phenolic metabolites of flavonoids, but not their unmetabolized precursors, reduce the secretion of vascular cellular adhesion molecules by human endothelial cells. J Nutr 146: 465–473, 2016.
414. Weiss G. Iron and immunity: a double-edged sword. Eur J Clin Invest 32 Suppl 1: 70–78, 2002.
415. West NP, Horn PL, Pyne DB, Gebski VJ, Lahtinen SJ, Fricker PA and Cripps AW. Probiotic supplementation for respiratory and gastrointestinal illness symptoms in healthy physically active individuals. Clin Nutr 33: 581–587, 2014.
416. West NP, Pyne DB, Cripps AW, Hopkins WG, Eskesen DC, Jairath A, Christophersen CT, Conlon MA and Fricker PA. Lactobacillus fermentum (PCC®) supplementation and gastrointestinal and respiratory-tract illness symptoms: a randomised control trial in athletes. Nutr J 10: 30, 2011.
417. Whitehead MT, Martin TD, Scheett TP and Webster MJ. Running economy and maximal oxygen consumption after 4 weeks of oral Echinacea supplementation. J Strength Cond Res 26: 1928–1933, 2012.
418. Whiteside TL. Measurement of cytotoxic activity of NK/LAK cells. Curr Protoc Immunol Ed John E Coligan Al Chapter 7: Unit 7.18, 2001.
419. Wikby A, Maxson P, Olsson J, Johansson B and Ferguson FG. Changes in CD8 and CD4 lymphocyte subsets, T cell proliferation responses and non-survival in the very old: the Swedish longitudinal OCTO-immune study. Mech Ageing Dev 102: 187–198, 1998.
420. Willis KS, Peterson NJ and Larson-Meyer DE. Should we be concerned about the vitamin D status of athletes? Int J Sport Nutr Exerc Metab 18: 204–224, 2008.
421. Willis KS, Smith DT, Broughton KS and Larson-Meyer DE. Vitamin D status and biomarkers of inflammation in runners. Open Access J Sports Med 3: 35–42, 2012.
422. Wink DA, Hines HB, Cheng RYS, Switzer CH, Flores-Santana W, Vitek MP, Ridnour LA and Colton CA. Nitric oxide and redox mechanisms in the immune response. J Leukoc Biol 89: 873–891, 2011.
423. Wischmeyer PE, Musch MW, Madonna MB, Thisted R and Chang EB. Glutamine protects intestinal epithelial cells: role of inducible HSP70. Am J Physiol 272: G879-884, 1997.
424. Wolman R, Wyon MA, Koutedakis Y, Nevill AM, Eastell R and Allen N. Vitamin D status in professional ballet dancers: winter vs. summer. J Sci Med Sport 16: 388–391, 2013.
425. Xu ML, Kim HJ, Wi GR and Kim H-J. The effect of dietary bovine colostrum on respiratory syncytial virus infection and immune responses following the infection in the mouse. J Microbiol Seoul Korea 53: 661–666, 2015.
426. Xue H, Sufit AJD and Wischmeyer PE. Glutamine therapy improves outcome of in vitro and in vivo experimental colitis models. J Parenter Enter Nutr 35: 188–197, 2011.
427. Yadav R, Angolkar T, Kaur G and Buttar HS. Antibacterial and antiinflammatory properties of bovine colostrum. Recent Pat Inflamm Allergy Drug Discov 10: 49–53, 2016.
428. Yombi JC, Schwab PE and Thienpont E. Neutrophil-to-lymphocyte ratio (NLR) distribution shows a better kinetic pattern than C-reactive protein distribution for the follow-up of early inflammation after total knee arthroplasty. Knee Surg Sports Traumatol Arthrosc 24: 3287–3292, 2016.
429. Zamora-Ros R, Knaze V, Rothwell JA, Hémon B, Moskal A, Overvad K, Tjønneland A, Kyrø C, Fagherazzi G, Boutron-Ruault M-C, Touillaud M, Katzke V, Kühn T, Boeing H, Förster J, Trichopoulou A, Valanou E, Peppa E, Palli D et al. Dietary polyphenol intake in Europe: the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Eur J Nutr 55: 1359–1375, 2016.
430. Zella LA, Shevde NK, Hollis BW, Cooke NE and Pike JW. Vitamin D-binding protein influences total circulating levels of 1,25-dihydroxyvitamin D3 but does not directly modulate the bioactive levels of the hormone in vivo. Endocrinology 149: 3656–3667, 2008.
431. Zittermann A. Vitamin D in preventive medicine: are we ignoring the evidence? Br J Nutr 89: 552–572, 2003.
432. Zschaler J and Arnhold J. Impact of simultaneous stimulation of 5-lipoxygenase and myeloperoxidase in human neutrophils. Prostaglandins Leukot Essent Fatty Acids 107: 12–21, 2016.
433. Zuhl MN, Lanphere KR, Kravitz L, Mermier CM, Schneider S, Dokladny K and Moseley PL. Effects of oral glutamine supplementation on exercise-induced gastrointestinal permeability and tight junction protein expression. J Appl Physiol 116: 183–191, 2014.