REVIEW

Nutritional modulation of exercise-induced immunodepression in athletes: a systematic review and meta-analysis

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Background: Heavy exercise induces marked immunodepression that is multifactorial in origin. Nutrition can modulate normal immune function.

Objective: To assess the efficacy of nutritional supplements in exercise-induced immunodepression in athletes.

Design: Systematic review.

Review methods: Randomised and/or controlled trials of athletes undertaking nutritional supplements to minimise the immunodepression after exercise were retrieved. The primary outcome measure was incidence of upper respiratory tract (URT) illness symptoms after exercise, and secondary outcomes included cortisol, cell counts, plasma cytokine concentration, cell proliferative response, oxidative burst, natural killer cell activity and immunoglobulins. When data were available for a pooled estimate of the effect of intervention, meta-analyses were conducted for direct comparisons.

Results: Forty-five studies were included (1603 subjects). The studies were heterogeneous in terms of exercise interventions, selection of athletes, settings and outcomes. The overall methodological quality of most of the trials was poor. Twenty studies addressed carbohydrate supplementation, eight glutamine, 13 vitamin C and four others interventions. Three trials assessed the effect of intervention on prevention of URT infections. The pooled rate ratio for URT infections after vitamin C supplementation against placebo was 0.49 (0.34–0.71). Carbohydrate supplementation attenuated the increase in cortisol and neutrophils after exercise; vitamin C attenuated the decrease in lymphocytes after exercise. No other interventions had significant or consistent effect on any of the studied outcomes.

Conclusions: Although the prevention of URT infections by vitamin C was supported by two trials, further studies are needed. The available evidence failed to support a role for other nutritional supplements in preventing exercise-induced immune suppression. Larger trials with clinically relevant and uniform end points are necessary to clarify the role of these nutritional interventions.

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Background

In contrast to moderate or intermittent physical activity, prolonged and intensive exertion causes numerous changes in immunity that reflect physiological stress and suppression. Athletes undertaking regular strenuous exercise are at increased risk of upper respiratory tract (URT) infection during periods of heavy exercise and for 2 weeks following race events (Peters and Bateman, 1983; Peters, 1990). It has been suggested that the relationship between exercise and

URT infections follows a 'J' curve, with moderate and regular exercise improving the ability to resist infections (Nieman *et al.*, 1993, 1998b, 2004) and heavy acute or chronic exercise decreasing it (Nieman *et al.*, 1990; Nieman, 1994; Castell *et al.*, 1996). The evidence has shown that prolonged periods of intense training may lead to high numbers of neutrophil and low numbers of lymphocytes in blood counts (Nieman *et al.*, 1995, 1998a; Pedersen *et al.*, 1997), impaired phagocytosis (Nieman *et al.*, 1997a) and neutrophilic function (Nieman *et al.*, 2000a), decreased oxidative burst activity (Nieman *et al.*, 1997a), natural killer cell cytolytic activity (NKCA) (Shinkai *et al.*, 1993; Nieman, 1997) and mucosal immunoglobulin levels (Nieman *et al.*, 2002a). Intense training or endurance exercise may also enhance release of pro-inflammatory cytokines such as tumour necrosis factor alfa (TNF-alfa), interleukin-1b (IL-1b) and interleukin-6 (IL-6), followed closely by anti-inflammatory cytokines, such as interleukin-10 (IL-10) and interleukin-1 receptor antagonist (IL-1ra) (Castell *et al.*, 1997; Nieman *et al.*, 2001).

The research on the relationship between nutrition and sport has been focused mainly on the role of dietary nutrients as ergogenic aids. The concept of 'immunonutrition' (Grimble, 2001) and more recently the concept of 'functional foods' (Contor, 2001) refers to the ability of certain nutrients to modulate immune status if they are provided in sufficient amounts, in general far more often in excess of the usual nutritional requirements. Because many nutrients are involved in energy metabolism and protein synthesis, they can modulate immune cell activity and function. Potential mechanisms of action include the ability of the cells to proliferate and differentiate, the production of proteins with specific functions, including chemokines, cytokines or antibodies, phagocytic activity, immune cell mediated immunity and complement formation and activity.

Non-systematic review articles on the efficacy of nutritional interventions in modulating exercise-induced immunodepression recommend that athletes eat a balanced diet that meets their energy requirements and ensures adequate intake of protein and micronutrients (Gleeson *et al.*, 2001, 2004). Besides the consumption of carbohydrates before and during exercise, no clear benefit from any nutritional intervention has appeared in the literature. We are not aware of any systematic reviews relating nutritional interventions in athletes to exercise-induced immune depression.

Objectives

The focus of this review is the effects of nutritional supplements on exercise-induced immunological changes in competitive athletes.

Methods

Randomised controlled trials were identified by searching the following data sources: SPORTDiscus (1975 to May

2005), EBM Reviews – Cochrane Central Register of Controlled Trials 2nd Quarter 2005, EBM Reviews – Cochrane Database of Systematic Reviews 2nd Quarter 2005, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) (1966 to May 2005) and reference lists. The first two levels of the optimal search strategy (Robinson and Dickersin, 2002) were combined with the following subject-specific search terms: 'exercise', 'immune', 'nutrition', 'athletes', 'glutamine', 'carbohydrate', 'vitamin', 'probiotic', 'protein'. Further details of the search strategy are given in Appendix A. The reference lists of all primary studies were reviewed to identify trials not retrieved by the electronic and manual searches. All of the titles and abstracts that were identified by the search and that appeared relevant were selected for a full text review. No restrictions for language were imposed. One hundred and thirty-one publications were identified as potentially relevant. Two investigators (AM, JF) applied the inclusion criteria independently, and where disagreements occurred, they were resolved by consensus. The full text of each paper was obtained. All studies that appeared initially to meet the inclusion criteria, but on closer examination failed to, are detailed in the table of excluded studies (Table 1). From these, 45 were suitable for inclusion (Tables 2–5).

Description of the studies

We included randomised or placebo-controlled trials of athletes undertaking nutritional supplements to minimise immunodepression after exercise.

Criteria for considering studies for the review

Participants

Healthy, competitive athletes or well-trained sportsmen or women, performing acute strenuous exercise. No restrictions for sex or age were made.

Intervention

We considered all studies reporting on efforts to minimize physiological immunodepression after exercise by means of nutritional intervention (e.g., with glutamine, carbohydrates (CHO), vitamins or antioxidants).

Outcome measures

The primary outcome measure was URT infections, while secondary outcomes included immunological host resistance measures such as differential cell counts (lymphocytes/neutrophils); plasma cytokines concentrations; blood mononuclear cells (BMNC)/ lymphocyte proliferative response; oxidative burst/phagocytic activity; NKCA; immunoglobulins.

Table 1 Characteristics of excluded studies

Study reference	Participants and exercise	Supplementation	Outcomes	Results	Reason for exclusion
Gleeson <i>et al.</i> (1998)	12 untrained males; cycling for 60 min at 70% of maximal oxygen uptake on two separate occasions, 3 days apart	High CHO diet ($75 \pm 8\%$ CHO) vs a low CHO diet ($7 \pm 4\%$ CHO)	Cortisol Glutamine Neutrophils	The low-CHO diet was associated with a larger rise in cortisol, greater fall in glutamine, and a larger neutrophilia	Subjects were untrained males
Pedersen <i>et al.</i> (2000)	20 men; endurance training performed 3–4 times a week for 7 weeks	CHO-rich diet vs fat-rich diet	Lymphocyte subsets NK cell activity	No effect on the percentages of CD3+, CD4+, CD8+, CD19+ or CD14+ cells. NK-cell activity increased in the group on the carbohydrate-rich diet and decreased in the group on the fat-rich diet	Subjects were untrained males
Bishop <i>et al.</i> (2001a)	9 males; cycled at 75% VO ₂ max until fatigue	CHO (5% w/v) beverage ingestion before and at 15 min intervals during the exercise	Cortisol Cell counts LPS-stimulated neutrophil degranulation	No effects	Subjects were untrained males

Abbreviations: BCAA-branched chain amino acid; CHO- carbohydrate; ConA: concavalin A; IL: interleukin; INF: interferon; LPS-Lipopolysaccharide; NK-natural killer; PLA-placebo; SG-supplemented group; TNF: tumoral necrosis factor; URT- Upper-respiratory-tract ; PBMC-peripheral blood mononuclear cells.

Studies

Both controlled and randomised clinical trials involving at least one test treatment and one control treatment, concurrent enrolment, follow-up of both the test-treated and the control-treated groups, and in which the intervention was selected by a random process.

Quality assessment of included studies and data extraction

Two reviewers assessed the trials for inclusion by looking at the Methods section of each paper while remaining unaware of the study's results, conclusion and authors. Furthermore, each reviewer independently applied written inclusion criteria to the Methods section of each study to determine the adequacy of subject randomisation, blinding, baseline comparability, outcome measures and the handling of withdrawals/dropouts.

The methodological quality of the included trials was assessed with particular emphasis on allocation concealment and randomisation, which was ranked using the Cochrane Collaboration approach. This led to each trial being attributed a quality score out of a maximum of 17 points (see Appendix B). Trial data were extracted by one reviewer and checked by another. We asked six researchers to provide additional information on trial methodology (method of randomisation) and results (usually requests for data not presented in the original reports).

Quantitative data synthesis

When data were available for a pooled estimate of the impact of intervention, it was intended that meta-analyses would be

conducted for direct comparisons. Effect of interventions on the incidence of URT infections after exercise in athletes was summarised as rate ratios (relative risks) and pooled using the DerSimonian and Laird random-effects model. Effect measures are presented with 95% confidence intervals. We have presented weighted mean differences (WMD) and 95% confidence intervals for continuous outcomes for each randomised controlled trial and analysed them in relevant subgroups according to the specific nutritional intervention used. In order to determine whether combining the results was appropriate, a heterogeneity test (χ^2 statistic) was performed. We used a fixed-effects model to estimate the pooled effect when no evidence of heterogeneity was detected (Whitehead and Whitehead, 1991). However, if evidence of heterogeneity was found to be present, we used a random-effects model (DerSimonian and Laird, 1986). Analysis and forest plots were produced using *RevMan 4.2.5* software (2004). The final manuscript was reviewed by the members of Ga²len involved in the 'Sports, Allergy and Asthma' Work Package.

Results

Selection of trials

We included 45 studies, comprising six distinct intervention arms (namely, with carbohydrates, vitamins or antioxidants, glutamine or branched-chain amino acids, plant sterols, polyunsaturated fatty acids and bovine colostrum) that include a total of 1603 subjects (1049 in intervention groups, 544 in placebo groups plus 10 in a crossover design) from four sports activity categories: swimmers, runners, cyclists and football players. A flow chart of studies assessed and excluded at various stages of the review is provided in

Table 2 Glutamine supplementation studies included in the systematic review

Study ID	Participants and exercise	Supplementation	Outcomes	Results
Castell <i>et al.</i> (1996)	151 marathon and ultra-marathon runners	Glutamine (5 g/L-glutamine in 330 ml water) drink immediately after and 2 h after exercise	URT infections	The percentage of participants free of infections in the intervention group was significantly greater than the placebo group (80.8 ± 4.2 vs 48.8 ± 7.4) No effect
Castell <i>et al.</i> (1997)	18 male runners; Brussels Marathon	330 ml water containing 5 g of glutamine and again 1 h later	Cell counts, IL-1alpha, IL-2, IL-6, plasma glutamine, alanine and BCAA, neopterin	
Rohde <i>et al.</i> (1998)	16 male runners; Copenhagen Marathon	Glutamine (100 mg/kg) in CHO free lemonade administered at 0, 30, 60, and 90-min post-race	Lymphocyte subsets (CD4, CD8, CD16), neutrophils, lymphocyte proliferative response (PHA), LAK cell activity	No effect on LAK cell activity, proliferative responses, exercise-induced changes in concentrations or percentages of any of the lymphocyte subsets
Bassit <i>et al.</i> (2000)	12 elite triathletes: swimming 1.5 km, cycling 40 km and running 10 km	BCAA twice a day (6.0 g: 60%L-leucine, 20%L-valine, 20%L-isoleucine) 30 days before the triathlon and 1 week after	Symptoms of infection Lymphocyte proliferative response (ConA, LPS)	Lower incidence of symptoms of infection Increased proliferative response
Walsh <i>et al.</i> (2000)	7 well-trained males; 2 sessions separated for 1 week of cycle ergometry at 60% of peak O ₂ uptake	250 ml of sugar-free lemon drink consumed at 15-min intervals until 90 min of exercise, then 250 ml lemon flavored glutamine drink 1.2% (w/v) consumed at 15-min intervals for the remaining exercise and 2-h recovery period.	IL-1, IL-2, TNF-alpha, INF-gamma Cortisol, neutrophil elastase release, plasma elastase, oxidative burst	Increased production of IL-1 and 2, TNF-alpha and IFN-gamma No effect
Krzywkowski <i>et al.</i> (2001a)	10 elite athletes; 2 trials separated for 2 weeks of 2 h of cycle ergometry at 75% of peak oxygen uptake	Glutamine (3.5 g in 500 ml water) or placebo (3.5 g maltodextrin in 500 ml water) consumed at 60 min of exercise and then plus four times at 45 min intervals	Cell counts, BMNC proliferative response to PHA, NK cell activity	No effect
Krzywkowski <i>et al.</i> (2001b)	11 athletes; 3 sessions separated for 2 weeks of 2 h of cycle ergometry at 75% of peak oxygen uptake	Glutamine (3.5 g in 500 ml water), glutamine-rich protein (13.7 g protein from sodium caseinate containing 1.23 g glutamine in 375 ml water) or placebo (3.5 g maltodextrin in 500 ml water). Placebo and glutamine beverages supplementation consumed at 60 min of exercise and then at 45 min intervals; the protein beverage was consumed at onset of exercise and then at 1 h intervals	Saliva secretory IgA	No effect
Bassit <i>et al.</i> (2002)	12 elite triathletes: swimming 1.5 km, cycling 40 km, and running 10 km 24 runners ran 30 km in 2 h	BCAA twice a day (6.0 g: 60%L-leucine, 20%L-valine, 20%L-isoleucine) 30 days before the race and 1 week after Runners included were supplemented 15 days before the test	Lymphocyte proliferative response (ConA, LPS) Plasma and <i>in vitro</i> production by PBMC of TNF-alpha, IL-4, INF-gamma, IL-2	More pronounced decrease in the production of IL-4 Decreased lymphocyte proliferative response
Hiscock <i>et al.</i> (2003)	8 highly trained males; 3 session separated for 2 weeks of 2 h of cycle ergometry at 75% of peak O ₂ uptake	Glutamine (3.5 g in 500 ml water), glutamine-rich protein (13.7 g protein from sodium caseinate containing 1.23 g glutamine in 375 ml water), or placebo (3.5 g maltodextrin in 500 ml water). Placebo and glutamine beverage supplementation consumed at 60 minutes of exercise and then at 45 min intervals; the protein beverage was consumed at onset of exercise and then at 1 h intervals	Plasma IL-6, plasma glutamine	Plasma IL-6 increased 11-fold in the placebo group, 14-fold in the glutamine-rich protein and 18-fold in the glutamine group Glutamine and protein supplementation attenuate the glutamine decreased after exercise

Abbreviations: BMNC- blood mononuclear cells; CHO, carbohydrate; ConA: concavalin A; IFN-gamma, interferon gamma; LAK: lymphokine-activated killer; NK-natural killer; PHA – phytohemagglutinin; TNF-alpha, tumor necrosis factor-alpha; URT- Upper-respiratory-tract.

Table 3 Carbohydrate supplementation studies included in the systematic review

Study ID	Participants and exercise	Supplementation	Outcomes	Results
Nieman <i>et al.</i> (1997c)	30 runners; 2.5 h of high-intensity running (approximately 75% VO ₂ max)	0.75 l of 6% CHO before running and then 0.25 l of carbohydrate or placebo fluid every 15 min during exercise	Cell counts, NK activity, cortisol	Carbohydrate attenuated the increase in cortisol and increased glucose concentration and NK cell count.
Nieman <i>et al.</i> (1997a)	30 runners; 2.5 h of high-intensity running (approximately 75% VO ₂ max)	0.75 l of 6% CHO before running and then 0.25 l of carbohydrate every 15 min during exercise	Cortisol, cell counts, phagocytosis/oxidative burst	No effect on NK activity Carbohydrate increased glucose concentrations, attenuated the increase in cortisol, blood neutrophils and monocytes. No effects on blood granulocyte/monocyte phagocytosis or oxidative burst activity
Nehlsen-Cannarella <i>et al.</i> (1997)	30 runners; 2.5 h of high-intensity running (approximately 75% VO ₂ max)	0.75 l of 6% CHO before running and then 0.25 l of carbohydrate every 15 min	Lymphocyte proliferative response (ConA, PHA, PWM), IL-1ra; IL-6, cortisol, NK cells	No effect on Con A-, PHA-, or PWM-induced lymphocyte proliferation Smaller increase of IL-6 and IL-1ra immediately and 1.5 h post-run, respectively. Post-run plasma cortisol and IL-6 levels correlated positively with levels of IL-1ra. Significant effect on blood concentration of NK cells but no effect on NK cell activity
Henson <i>et al.</i> (1998)	30 runners; 2.5 h of high-intensity running (approximately 75% VO ₂ max)	0.75 l of 6% CHO before running and then 0.25 l of carbohydrate every 15 min during exercise	Cortisol, lymphocyte proliferative response (ConA, PHA, PWM)	No effect on blood granulocyte and monocyte phagocytosis or oxidative burst activity Cortisol higher in the placebo group immediately post-run
Henson <i>et al.</i> (1999)	10 triathletes; 2.5 h of running and cycling (approximately 75% VO ₂ max)	6% CHO beverage	Cortisol, lymphocyte proliferative response (ConA, PHA), IL-1beta, NK cell activity	No effect on lymphocyte proliferative response Reduced lymphocytosis Lower cortisol concentrations Reduced lymphocytosis
Bishop <i>et al.</i> (1999)	8 university team football players; 90 min specific exercise bouts (3 days apart)	CHO (400 ml lemon flavoured glucose solution 6%w/v 10 min before and 5 min after each 45 min of exercise)	Cortisol, neutrophils, lymphocytes, LPS elastase release/neutrophyl, salivary IgA	No effects on lymphocyte proliferative response or IL-1beta Reduced NK cell activity No effect: cortisol, lymphocyte counts, salivary IgA and degranulation response of neutrophils to LPS.
Starkie <i>et al.</i> (2000)	6 endurance trained men; Cycling 2 h at 70% of peak O ₂ uptake	Total of 9 ml/kg of an 8% CHO	Monocyte LPS stimulated production of: IL-1alpha-, TNF-alpha- and IL-6	Neutrophil counts 14% higher after the placebo trial than the carbohydrate trial No effect
Nieman <i>et al.</i> (2001)	98 runners; Charlotte Marathon and Grandfather Mountain Marathon	1 l/h of a 6% CHO beverage administered during and for 1.5 h after marathon	Cortisol, plasma IL-10, IL-1ra, IL-6, IL-8	Attenuated the increases in cortisol, IL-10 and IL-1ra
Bishop <i>et al.</i> (2001b)	12 male cyclists; cycling for 1 h at 60% W _{max} immediately followed by a time-trial ensuring an energy expenditure equivalent to cycling for 30 min at 80% W _{max} ; subjects repeated the protocol after 7 days	Diet ensuring either greater than 70% (HIGH) or less than 10% (LOW) of daily energy intake from CHO for the 3 days before exercise	Cortisol/glucose, cell counts, plasma elastase and LPS elastase release/neutrophyl	HIGH compared with the LOW trial was associated with higher plasma glucose concentration, lower plasma cortisol concentration, and lower circulating neutrophil count.
Starkie <i>et al.</i> (2001)	7 trained man; 60 min of exercise at a workload corresponding to each individual's lactate threshold on four randomised occasions (2 cycle ergometer, 2 treadmill)	Total fluid intake of 12 ml (kg/BM) of a 6.4% CHO solution during and after exercise	IL-6 gene expression in muscle biopsies, plasma IL-6	No differences for LPS-stimulated neutrophil degranulation per cell. Smaller increase in IL-6 during both cycling and running exercise

Table 3 Continued

Study ID	Participants and exercise	Supplementation	Outcomes	Results
Koch <i>et al.</i> (2001)	10 males; 2 sessions, 14 days apart, high intensity exercise, short rest interval squat workout	1.0 g/kg body mass CHO 10 min prior to and 10 min following exercise	Cortisol, leucocyte subsets, lymphocyte proliferative response (PHA)	No effect on leukocyte subsets or cortisol Lymphocyte proliferation was less depressed
Bacurau <i>et al.</i> (2002)	12 male athletes cycled for 20 min at a velocity corresponding to 90% of that obtained at the anaerobic threshold and rested for 20 min; repeated six times	CHO (95% glucose polymers and 5% fructose) at 10% (w/v), 1 g/kg/h every 20 min, starting at the 10th minute of the first exercise period	Cortisol, BMNC proliferative response to PHA, cytokines (IL-1, IL-2, TNF-alpha, IFN-gamma)	All of the changes, except IFN gamma production, were prevented by the ingestion of CHO
Bishop <i>et al.</i> (2002)	6 male soccer players; 2 exercise trials, 7 days apart, on each occasion, they completed 6 × 15-min periods of intermittent running	CHO beverages immediately before and at 15-min intervals during the exercise	Cortisol, glucose, plasma IL-6, TNF-alpha, LPS elastase release/neutrophil	Higher glucose, lower cortisol, IL-6, and fewer neutrophils. No effect on TNF-alpha Less decrease in LPS-stimulated elastase release
Scharhag <i>et al.</i> (2002)	14 cyclists; cycling for 4 h in a steady state at 70% of their individual anaerobic threshold	6 and 12% CHO beverages	Neutrophil oxidative burst activity	the decrease of the neutrophil oxidative burst was attenuated
Nieman <i>et al.</i> (2003)	16 marathon runners; two sessions 4–6 weeks apart of 3 h treadmill at 70% maximal O ₂ uptake	12 ml/kg of 6% CHO 15–30 min before running and then over 3 h (4 ml/kg) every 15 min	Cortisol, cell counts, plasma IL-6, IL-10, IL-1ra, IL-8, salivary sIgA, gene expression of 13 cytokines in muscle biopsies: IL-1a, IL-1b, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p35, IL-12p40, IFN-gama, IL-15, TNF-alpha	Plasma IL-6, IL-10, IL-1ra, cortisol, but not IL-8, were significantly lower Attenuated the increase in mRNA for IL-6 and IL-8
McAnulty <i>et al.</i> (2003)	16 marathon runners; two sessions 4–6 weeks apart of 3 h treadmill at 70% maximal O ₂ uptake	12 ml/kg of 6% CHO 15 to 30 min before running and then over 3 h (4 ml/kg) every 15 min	Cortisol, lipid peroxidation (F2-isoprostanes and lipid hydroperoxides, plasma antioxidant potential)	Attenuated the increase in cortisol No effect on antioxidant potential and lipid peroxidation
Lancaster <i>et al.</i> (2003)	Group A: 8 male cyclists; 1-h of high-intensity cycling Group B: 10 cyclists; 1-h of high-intensity cycling	Group A: 75 g CHO as a glucose solution at either 15 or 75 min before exercise Group B: 25 g or 200 g CHO as a glucose solution or a placebo 45 min before exercise	Cortisol, BMNC proliferative response to LPS, neutrophil/lymphocyte ratio, IL-6	No effect
Chan <i>et al.</i> (2003)	9 males; resistance training bout	1 g/kg body mass CHO 10 min before and 10 min after exercise	Cell counts, cortisol, glucose, BMNC PHA stimulated production of IL-2, IL-5	Attenuated the decrease of IL-2 and IL-5 Attenuated the increase in cortisol
Bishop <i>et al.</i> (2003)	9 trained cyclists; cycled for 2 h at 75% VO ₂ max on two occasions	CHO (6.4%) beverage ingestion before (5 ml × kg ⁻¹), during (2 ml × kg ⁻¹), and after (5 ml × kg ⁻¹) the exercise	Cortisol, cell counts, elastase LPS-stimulated release/neutrophil, elastase total neutrophil content	Fewer numbers of circulating neutrophils No effect on total neutrophil elastase content LPS-stimulated neutrophil elastase release fell post exercise by approximately 47% on the PLA trial with no significant change from the CHO trial
Nieman <i>et al.</i> (2004)	30 strength-trained subjects; lifting weights for 2 h (10 exercises, 4 sets each, 10 repetitions)	10 ml × kg(−1) × h(−1) 6% CHO beverage	Cortisol, cell counts, plasma IL-6, IL-10, IL-1ra, IL-8, muscle glycogen content and gene expression of IL-1alpha, IL-1beta, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p35, IL-12p40, IL-15, IFN-gamma, TNF-alpha	No effect

Abbreviations: BM: body mass; BMNC- blood mononuclear cells; CHO, carbohydrate; ConA: concavalin A; LPS-lipopolysaccharide; PBL-peripheral blood lymphocytes; PHA – phytohemagglutinin; ; PUFA: polyunsaturated fatty acids; NK-natural killer; URT- Upper-respiratory-tract.

Table 4 Vitamin supplementation studies included in the systematic review

Study ID	Participants and exercise	Supplementation	Outcomes	Results
Peters <i>et al.</i> (1993)	92 runners; '90 km Comrades Marathon'	600 mg vit. C daily for 21 days before race	URT infections	Significantly more symptoms of URT infection after the race in the placebo group
Peters <i>et al.</i> (1996)	178 runners; '90 km Comrades Marathon'	500 mg vit. C (C, $n=86$), 500 mg vit. C plus 400 UI vit. E (CE, $n=90$), 300 mg vit. C 300 UI vit. E plus 18 mg beta-carotene (CEB, $n=73$) daily for 21 days before race	URT infections	Significantly more symptoms of URT infection after the race in the placebo group vit. C alone as effective as combinations with vit. E or beta-carotene in preventing URT infections symptoms
Nieman <i>et al.</i> (1997b)	30 runners; 2.5 h of high-intensity running (75–80% maximal O ₂ uptake)	Vitamin C (2 × 500 mg tablets daily) for 8 days before the race	Cortisol, cell counts, NK cell activity, lymphocyte proliferative response, IL-6, phagocytosis, oxidative burst	No effect
Himmelstein <i>et al.</i> (1998)	44 marathon runners; 48 sedentary subjects	1000 mg vitamin C daily for 2 months prior and 1 month after marathon	URT infections	No effect
Singh <i>et al.</i> (1999)	10 trained women runners; treadmill run at 65–70% of their VO ₂ max.	Zinc (25 mg) or vitamin E (400 IU) or placebo, twice a day, 3 days before and 1 day after exercise	Cortisol IL-6	No effect
Nieman <i>et al.</i> (2000b)	29; Comrades 90 km marathon	500 mg/day vit C vs 1500 mg/day vit C vs placebo for 7 days before the race, on the day of the race, and for 2 days following completion	Cortisol, adrenaline, IL-10, IL-1ra, TNF-alpha, IL-1beta, IL-6, IL-8	Serum cortisol significantly lower in the vit C1500 group than in combined P and vit C-500 group The increases in plasma IL-6, IL-10, IL-1ra, and IL-8 were attenuated in runners ingesting 1500 mg, but not 500 mg, vitamin C supplements
Petersen <i>et al.</i> (2001)	20 male recreational runners; 5% downhill 90-min treadmill run at 75% VO ₂ max	Antioxidants (500 mg of vitamin C and 400 mg of vitamin E) for 14 days before and 7 days after exercise	Plasma vitamins, creatine kinase, lymphocytes, NK cell, cytokines (IL-1ra, IL-6).	No effect
Peters <i>et al.</i> (2001a)	29; 90 km Comrades Marathon	500 mg/day vit C vs 1500 mg/day vit C vs placebo for 7 days before the race, on the day of the race, and for 2 days following completion	Cortisol, adrenaline, IL-10, IL-1Ra	Serum cortisol significantly lower in the VC-1500 group than in combined P and VC-500 group The increases in plasma IL-10, IL-1ra, and IL-8 were attenuated in runners ingesting 1500 mg, but not 500 mg, vitamin C supplements
Peters <i>et al.</i> (2001b)	16; 90 km Comrades Marathon	Vitamin C (2 × 500 mg tablets daily) for 7 days before the race, on the day of the race, and for 2 days following completion.	Cortisol, cell counts, IL-6, TNF-alpha, C-reactive protein	Significantly lower serum cortisol and C-reactive protein
Krause <i>et al.</i> (2001)	10 athletes; biathlon	2 g vitamin C daily for 1 week prior to biathlon	Neutrophil phagocytosis , neutrophil bactericidal ability	No effect
Tauler <i>et al.</i> (2002)	20 duathlon athletes; runners and cyclists training 14 hours/week	90 days supplementation with antioxidant mixture of vitamin E (500 mg/day) and beta-carotene (30 mg/day) and in the last 15 days also with vitamin C (1 g/day)	Neutrophil glutathione peroxidase, glutathione reductase, superoxidedismutase and catalase activity	Significantly higher glutathione versus glutathione disulfide ratio in neutrophils (about 20%), enhanced antioxidant enzyme activity of superoxide dismutase and catalase in neutrophils

Table 4 Continued

Study ID	Participants and exercise	Supplementation	Outcomes	Results
Nieman <i>et al.</i> (2002b)	28 ultramarathon runners; Umstead 90 km Ultramarathon	Vitamin C (3 × 500 mg tablets daily) for 7 days before the race	Plasma oxidative measures (lipid hydroperoxide and F(2)-isoprostane), cell counts, plasma cytokines (IL-6, IL-10, IL-1Ra, IL-8), lymphocyte proliferative response, cortisol	No effect
Palmer <i>et al.</i> (2003)	28 ultramarathon runners; Umstead 90 km Ultramarathon	Vitamin C (3 × 500 mg tablets daily) for 7 days before the race	Plasma oxidative measures (lipid hydroperoxide and F(2)-isoprostane), salivary IgA	No effect
Robson <i>et al.</i> (2003)	12 trained runners; 2-h treadmill run at 65% VO ₂ max.	3-week period in which subjects ingested baseline multivitamin-mineral complex; 7 days prior to tests ingested daily 18 mg beta-carotene, plus 900 mg vitamin C, plus 90 mg vitamin E	Neutrophil oxidative burst activity	Oxidative burst activity was significantly higher following the 7-day supplementation period

Abbreviations: NK-natural killer; URT- Upper-respiratory-tract.

Figure 1. Excluded and included study details are presented in Table 1 and Tables 2–5, respectively.

Description of included studies

Overall, the quality of the reported trials was unsatisfactory. Using the Cochrane Collaboration approach for allocation concealment (2004), all included trials were allocated a grade D, indicating that this score has not been assigned by the authors. None reported intention-to-treat analyses or randomisation methods. Compliance with the intervention and reasons for failure to comply were seldom described. None of the studies referred to intention-to-report adverse events related to the intervention. The quality rating score gave a median of 7 out of 14 (min = 5, max = 11). In studies where subjects, method description and results were the same and exactly coincident, only one study was considered in the meta-analysis.

Upper respiratory tract infections

Three studies (Peters *et al.*, 1993, 1996; Himmelstein *et al.*, 1998), including 219 marathon runners, addressed the incidence of URT infections. In the study of Himmelstein *et al.* (1998), there were no significant differences in the incidence of URT infections after exercise when the subjects consumed 1000 mg vitamin C daily for 2 months before and 1 month after the marathon. In this study, reasons for withdrawals were described as well as adherence to the protocol. The evaluation of URT infection symptoms was made using a semiquantitative list of respiratory symptoms. Two other studies assessed the impact of vitamin C supplementation, either alone or combined with vitamin E or beta-carotene, on the URT infection symptoms for 14 days after the race (Peters *et al.*, 1993, 1996). In the study of Peters *et al.* (1993), the intervention group consumed 600 mg of vitamin C for 21 days prior to the ultra-marathon, and the URT infections were assessed for 14 days after the race. In this study, the number of runners of the placebo group reporting development of URT infection symptoms after the race was 68%, which was significantly more than that reported by the vitamin C-supplemented group (33%). In the study of Peters *et al.* (1996), athletes were supplemented with vitamin C alone (500 mg/day) or combined with vitamin E (400 UI/day) or beta-carotene (18 mg/day) during the 21 days before the race. Significantly more symptoms of URT infection in the placebo group were observed (40.4%) compared with vitamin C (15.9%) or combinations of vitamins C and E (25.5%) or vitamins C, E and beta-carotene (20.0%). Vitamin C alone was as effective as when combined with vitamin E or beta-carotene in preventing URT infection symptoms. When the data from the studies was pooled, the rate ratio for URT infections after vitamin C supplementation against placebo was 0.49 (0.34–0.71) (Figure 2). In two

Table 5 Colostrum, plant sterols and polyunsaturated fatty acids supplementation studies included in the systematic review

Study ID	Participants and exercise	Supplementation	Outcomes	Results
Mero (1997)	9 male sprinters/jumpers; three strength and speed training period of 8 days separated by 13 days	125 ml/day bovine colostrum supplement containing IGF-I and IgG vs 25-ml/day mixed with placebo vs placebo	Saliva IgA, serum IGF-I, IgG	Increase serum IGF No effects on IgA/IgG
Bouic <i>et al.</i> (1999)	17 marathon runners; ultra-marathon in Cape Town, South Africa	Plant sterols and sterolins (BSS:BSSG mixture)	Cortisol, lymphocytes subsets, neutrophils, IL-6	Decreased IL-6 and cortisol Attenuated neutrophilia, lymphopenia and leukocytosis Increased CD3+ CD4+
Toft <i>et al.</i> (2000)	20 male runners; The Copenhagen Marathon, 1998	3.6 g n-3 PUFA plus 21.6 mg tocopherol for 6 weeks prior to marathon	Plasma TNF-alpha, IL-6, TGF-beta, IL-1ra	No effect

Abbreviations: IGF-I: insulin-like growth factor I; NK-natural killer; PUFA: polyunsaturated fatty acids.

Figure 1 Systematic review flow chart summary.

additional studies, symptoms of infections after glutamine or branched-chain amino acids supplementation were assessed (Castell *et al.*, 1996; Bassit *et al.*, 2002). Questionnaire scores included infection symptoms other than respiratory, such as gastrointestinal complaints. Provision of glutamine or branched-chain amino acids containing drinks decreased the incidence of infection symptoms during the week that

followed different types of exhaustive exercise (Castell *et al.*, 1996; Bassit *et al.*, 2002) compared to placebo.

Cortisol

In 24 of the included trials (Tables 2–5) ($n = 282$), cortisol was one of the outcomes. CHO was the supplemented nutritional intervention in 15 of those trials. Cortisol data were only available in chart form, making data extraction for CHO intervention meta-analysis impossible in eight studies (Nehlsen-Cannarella *et al.*, 1997; Nieman *et al.*, 1997b, 2001, 2003; Bishop *et al.*, 2001b, 2002; Bacurau *et al.*, 2002; Lancaster *et al.*, 2003). In all but one (Lancaster *et al.*, 2003), the CHO intervention had a significant effect, decreasing post-exercise cortisol levels compared to placebo. Four of the eight trials included reported attenuated increase of cortisol after exercise for the treatment group ($n = 96$) compared with controls ($n = 92$) (Figure 3). Subjects included in these trials were runners or cyclists, in contrast to the back squatters or weight lifters (Koch *et al.*, 2001; Chan *et al.*, 2003; Nieman *et al.*, 2004) or football players (Bishop *et al.*, 1999) in the studies where the CHO intervention had no significant effect on cortisol. The study by Lancaster *et al.* (2003), was the only one that examined the effect of CHO ingestion during the pre-exercise period on subsequent hormonal and immune responses to exercise. Statistical heterogeneity was excessive ($I^2 = 92.7\%$), and we were not able to identify a particular trial causing this excess variability. Pooling with such a high degree of heterogeneity is not advisable. However, had pooled data been used, the mean difference in serum cortisol after exercise between placebo and CHO intervention would have shown $-44.90 \text{ ng ml}^{-1}$ (95% CI: -78.12 to $-11.68 \text{ ng ml}^{-1}$) (Figure 3). Four studies (Nieman *et al.*, 2000b, 2002b; Peters *et al.*, 2001a, b), all including ultra-marathon runners, addressed the effect of vitamin C supplementation on cortisol. Two studies (Nieman *et al.*, 2000b; Peters *et al.*, 2001a) referred to the same subjects and

Review: Nutritional countermeasures to the exercise induced immunosuppression
Comparison: 01 URT infections
Outcome: 01 URT infections

Figure 2 Effectiveness of nutritional interventions (vitamin C) compared with placebo on incidence of upper respiratory tract infections after exercise. Data are presented as relative risk for each study (boxes), 95% CIs (horizontal lines) and summary as relative risk with 95% CI (diamonds).

Review: Nutritional countermeasures to the exercise induced immunosuppression
Comparison: 02 Cortisol
Outcome: 01 Cortisol

Figure 3 Effectiveness of nutritional interventions compared with placebo on serum cortisol levels after exercise. Data are presented as weighted mean differences for each study (boxes), 95% CIs (horizontal lines) and summary weighted mean differences with 95% CI (diamonds).

only one was included. In the study of Peters *et al.* (2001a), vitamin C had a significant effect on cortisol in ultramarathon runners when ingested in a dose greater than or equal

to 1000 mg/day for 7 days before race and 2 days after. In the study of Nieman *et al.* (2002b), 1500 mg of vitamin C daily for the 7 days preceding the race had no effect on cortisol.

The WMD on serum cortisol for vitamin C intervention against placebo was $-122.36 \text{ ng ml}^{-1}$ (95% CI -611.11 to $-366.38 \text{ ng ml}^{-1}$) (Figure 3).

In two additional interventions, a mixture of plant sterols and sterolins attenuated the cortisol increase (Bouic *et al.*, 1999) and a 1.2% glutamine drink had no effect compared with placebo (Walsh *et al.*, 2000) in runners. An inhibitory effect of combined vitamin C and vitamin E supplementation (for 4 weeks) on plasma IL-6 and cortisol responses to prolonged exercise has been reported, although in physically active non-athletes (Fischer *et al.*, 2004). Acute supplementation with zinc and vitamin E did not have an effect on the cortisol response to exercise in eumenorrheic runners (Singh *et al.*, 1999).

Cell counts

When cell counts after exercise were available, those most immediately following exercise were chosen. Twenty-six trials were retrieved. Three (Nehlsen-Cannarella *et al.*, 1997; Nieman *et al.*, 1997c; Henson *et al.*, 1998) were not included in the analysis because they referred to the same

subjects as others (Nieman *et al.*, 1997a; Scharhag *et al.*, 2002). In another three studies (Bishop *et al.*, 2002, 2003; Robson *et al.*, 2003) data were only available in chart form, making data extraction impossible. Eighteen trials (Figures 4 and 5) (225 active plus 209 controls) reported counts of lymphocytes or neutrophils in a way that allowed data extraction. Glutamine supplementation had no significant effect on lymphocyte or neutrophil counts, while CHO had a mild effect on attenuation of the increase of neutrophils after exercise (WMD = -1.45 ; 95% CI -2.35 to -0.54) (Figure 4 and 5) but no effect on lymphocytes (WMD = -0.21 ; 95% CI -0.44 to 0.01). Although vitamin C significantly increased lymphocyte counts after exercise (WMD = 0.30 ; 95% CI 0.00 – 0.61), it did not have an effect on neutrophils (Figure 5) (WMD = 0.05 ; 95% CI -1.09 to 1.19) compared to placebo.

Plasma cytokines

In twenty-three of the included studies (Tables 2–5), cytokines or chemokines was one of the outcomes. There was no tendency towards a predominant effect on the part of

Review: Nutritional countermeasures to the exercise induced immunosuppression
Comparison: 03 Cell counts
Outcome: 01 Lymphocytes

Figure 4 Effectiveness of nutritional interventions compared with placebo on lymphocytes counts (10^9 l^{-1}) after exercise. Data are presented as weighted mean differences for each study (boxes), 95% CIs (horizontal lines) and summary weighted mean differences with 95% CI (diamonds).

Review: Nutritional countermeasures to the exercise induced immunosuppression
Comparison: 03 Cell counts
Outcome: 02 Neutrophils

Figure 5 Effectiveness of nutritional interventions compared with placebo on neutrophils counts (10^9 L^{-1}) after exercise. Data are presented as weighted mean differences for each study (boxes), 95% CIs (horizontal lines) and summary weighted mean differences with 95% CI (diamonds).

any nutritional supplement. Carbohydrate intake before or during exercise had a major effect in attenuating increases in IL-6, IL-10, IL-2, IL-8 or IL-1ra in six studies (Nehlsen-Cannarella *et al.*, 1997; Nieman *et al.*, 2001, 2003; Starkie *et al.*, 2001; Bacurau *et al.*, 2002; Bishop *et al.*, 2002) but failed to show any significant effect in nine studies (Henson *et al.*, 1999; Starkie *et al.*, 2000; Petersen *et al.*, 2001; Nieman *et al.*, 2001, 2003, 2004; Bishop *et al.*, 2002; Lancaster *et al.*, 2003; Chan *et al.*, 2003). Vitamin C intake had inconsistent effects on plasma increases of IL-6, IL-10, IL-1ra and IL-8. Doses of 1500 mg of vitamin C daily were reported to attenuate the increase in these cytokines after exercise (Nieman *et al.*, 2000b; Peters *et al.*, 2001a) or have no effect (Nieman *et al.*, 2002b) at lower doses of 1000 or 500 mg daily (Nieman *et al.*, 1997b; Peters *et al.*, 2001b). Of the two studies where glutamine was given as a supplement, one study showed glutamine to augment the increase of plasma IL-6 (Hiscock *et al.*, 2003) after exercise, while the other (Castell *et al.*, 1997) did not. Polyunsaturated fatty acids had no effect on plasma TNF- α , IL-6, TGF- β and IL-1ra (Toft *et al.*, 2000). The plant sterols and sterolins B-sitosterol (BSS)

and B-sitosterol glucoside (BSSG) mixture attenuated the increase of IL-6 after exercise (Bouic *et al.*, 1999).

NK cell cytolytic activity, neutrophil oxidative burst, phagocytosis and salivary immunoglobulin A (IgA)

Five studies (Nieman *et al.*, 1997b,c; Rohde *et al.*, 1998; Henson *et al.*, 1999; Krzywowski *et al.*, 2001a) assessed NKCA, expressing it in lytic units or adjusting for NK cell counts. NKCA was affected by the intervention in one study (Henson *et al.*, 1999), where triathletes consumed a 6% CHO beverage. The interventions in the other four studies had no effect on NKCA. Two trials showed increased neutrophil oxidative burst activity after a 3-week supplementation period with multivitamin-minerals, including beta-carotene and vitamins C and E (Scharhag *et al.*, 2002) and a 12% CHO beverage (Robson *et al.*, 2003), but this was not observed in the other four studies where vitamin C (Nieman *et al.*, 1997b; Krause *et al.*, 2001), glutamine (Walsh *et al.*, 2000) or CHO (Nieman *et al.*, 1997a) were used. Lipopolysaccharide-

stimulated neutrophil elastase release was investigated in five trials (Bishop *et al.*, 1999, 2001b, 2002, 2003; Walsh *et al.*, 2000), but only in one (Bishop *et al.*, 2002), in which CHO was ingested before and during the exercise, was there an attenuation of the decrease in the elastase release per neutrophil. From the seven trials (Nehlsen-Cannarella *et al.*, 1997; Henson *et al.*, 1998, 1999; Rohde *et al.*, 1998; Koch *et al.*, 2001; Krzykowski *et al.*, 2001a) that assessed the lymphocyte proliferative response, only one (Koch *et al.*, 2001) found a less depressed proliferative response when CHO was used. None of the interventions had any effect on salivary IgA in the five trials in which IgA was one of the outcomes (Mero, 1997; Bishop *et al.*, 1999; Krzykowski *et al.*, 2001b; Nieman *et al.*, 2003; Palmer *et al.*, 2003).

Reviewers' conclusions

Limitations of the studies

The methodological quality of most of the included trials was poor. The low median score for the quality rating is not surprising considering that the two major classified items, randomisation and blinding, were not reported. Low-quality trials, particularly those not reporting allocation concealment and those not reporting blinding, are known to favour interventions (Moher *et al.*, 1998; Sterne *et al.*, 2001). The highest scores found were the result of summing individual items. The use of such scores, however, could be arguable (Juni *et al.*, 1999), reflecting the difficulties in determining fair quality rates. Evaluating the suppression of one immunological parameter at a time runs the risk of disregarding changes in host resistance as a whole. This reductionist approach, taken in most of the studies conducted, fails to bring to light the impact of the interventions on overall host resistance. A decreased NKCA function does not necessarily mean an increased susceptibility to infection if, for instance, it is counterbalanced by an increased number of neutrophils (Keil *et al.*, 2001). A validated mathematical model (Keil *et al.*, 1999) should be used to estimate and predict the relationship between multiple immunological parameters and host resistance. In addition, clinical end points, such as incidence of infections, should be used.

Discussion

Glutamine

The plasma concentration of the 'conditionally essential' amino acid glutamine, the most abundant in the human body, is lowered by stress or strenuous physical activity. This is most likely due to increased cellular needs that exceed the supply available from diet or muscle. The plasma glutamine response to exercise is biphasic. It is characterised by an increased concentration during short-term exercise, followed by a decrease after more prolonged exercise (Rennie *et al.*, 1981; Castell and Newsholme, 1998). This and knowledge

about glutamine effects on *in vitro* immunity responses led to the 'glutamine hypothesis' as one explanation of immunodepression after exercise (Newsholme and Parry-Billings, 1990). It has been postulated that restoring glutamine levels after prolonged exercise to physiological levels may help the immune system to resist infections. Although oral provision of glutamine, or branched chain amino acids as a glutamine precursor, abolishes the post-exercise plasma glutamine decrease, it has no consistent effect on lymphocyte or neutrophil counts (Rohde *et al.*, 1998; Walsh *et al.*, 2000; Peters *et al.*, 2001a; Krzykowski *et al.*, 2001a), salivary IgA (Krzykowski *et al.*, 2001b), oxidative burst activity (Walsh *et al.*, 2000), NKCA (Rohde *et al.*, 1998; Walsh *et al.*, 2000) or plasma IL-6 (Castell *et al.*, 1997; Hiscock *et al.*, 2003) after exercise. Although it was suggested that glutamine could enhance Th1-type immune response (Bassit *et al.*, 2002) in swimmers submitted to 4 weeks of intensive training, there was no significant difference in glutamine levels between athletes who developed URT infection and those who did not (Mackinnon and Hooper, 1996). In two studies (Castell *et al.*, 1996; Bassit *et al.*, 2002), infection symptoms after prolonged strenuous exercise in athletes supplemented with glutamine was significantly lower than in the placebo group. The study of Castell *et al.* (1996) included data from eight separate studies. Both studies included symptoms questionnaires assessing not only URT symptoms but also others such as vomiting or diarrhoea. The available data do not provide evidence to support the glutamine hypothesis.

Carbohydrate

It has been speculated that athletes exercising in a low CHO status are placing themselves at an increased risk of exercise-induced immunodepression. Cells of the immune system have high metabolic rates and CHO availability can theoretically influence the immune response to exercise and the ability to maintain immunocompetence. Subjects performing exercise on diets for several days with less than 10% of energy intake from CHO have larger stress hormone (cortisol, adrenaline) and plasma cytokine (IL1ra, IL6, IL10) responses than those on normal or high CHO diets (Mitchell *et al.*, 1998; Bishop *et al.*, 2001c). There are no trials addressing the efficacy of CHO supplementation on preventing URT infections. Attenuation of serum cortisol increases, both in runners and cyclists (Nieman *et al.*, 1997c, 2003, 2004; Bishop *et al.*, 1999, 2003; Koch *et al.*, 2001; Bacurau *et al.*, 2002; McAnulty *et al.*, 2003; Chan *et al.*, 2003) does take place after supplementation (about 11/h of a 6% carbohydrate beverage), but whether this reduces URT infections remains to be proven. There is no evidence supporting effects of CHO supplementation on lymphocyte counts, cytokines or salivary IgA after exercise (Table 3, Figures 4 and 5).

Antioxidants and vitamins

Exercise can produce an imbalance between the free radicals and reactive oxygen species (ROS) produced and the

antioxidant defence system. The antioxidant defence mechanism prevents the generation of ROS or intercepts any that is formed. The dose-response between vitamin intake and immune stimulatory effect is not linear. In fact, megadoses of vitamins may even impair immune function in athletes (Prasad, 1980). Although the cellular antioxidant mechanism adapts efficiently to acute and chronic exercise (Mena *et al.*, 1991), extreme physical exercise causes oxidative damage to athletes, which is reflected by increases in plasma levels of malondialdehyde (Marzatico *et al.*, 1997), urine 8-hydroxideoxyguanosine (Radak *et al.*, 2000) and erythrocyte catalase activity (Tauler *et al.*, 1999), which could relate to exercise-induced immunodepression. Vitamin C supplementation augmented the increase in lymphocyte counts after exercise (Petersen *et al.*, 2001; Peters *et al.*, 2001b), attenuated the serum cortisol increase in ultramarathon runners (Peters *et al.*, 2001a,b; Nieman *et al.*, 2002b) and attenuated the increases in inflammatory cytokines (Nieman *et al.*, 1997b, 2000b; Peters *et al.*, 2001a,b), when ingested in doses greater than or equal to 1000 mg/day, but not on 500 mg/day. However, the risks of vitamin C excessive intake may outweigh any potential benefits, and none of the trials assessed adverse events. High-dose vitamin C intake can cause DNA damage, suggesting a possible link to increased cancer susceptibility (Gerster, 1999). Vitamin C intake in excess of 1000 mg/day has been associated with kidney oxalate stone formation (Baxmann *et al.*, 2003), impaired absorption of copper and excessive absorption of iron (Gerster, 1999). The phagocytic ability of neutrophils is one of the most important host defences against infections. Its antimicrobial mechanisms are activated when neutrophils undergo the oxidative burst and produce more ROS. The natural antioxidant systems that protect neutrophils could be depleted after intensive exercise, and the oxidative burst cannot then be carried out in an effective way. The supplementation with vitamin C or beta-carotene plus vitamin C and E failed, however, to show consistent results in improving oxidative burst activity (Nieman *et al.*, 1997b; Krause *et al.*, 2001; Robson *et al.*, 2003).

Other interventions

Interventions where only one trial was available included n-3 polyunsaturated fatty acids (Toft *et al.*, 2000) (no effects), a plant sterols mixture (Bouic *et al.*, 1999) (which decreased IL-6 and cortisol and attenuated both the increase in neutrophil and the decrease in lymphocyte counts) and bovine colostrum (Mero, 1997) (no effect).

If the excluded papers were considered for the analysis, the reported results would not be different for the CHO interventions.

Limitations and strengths of the review

In common with all meta-analyses, this systematic review may have included studies in which the interventions and

characteristics of the human subjects were too dissimilar for comparison, resulting in questionable conclusions on the supplementation used. The athletes included were from different sports and exercised in different environmental and stress conditions (e.g. during winter or summer); this makes any generalisations open to question. Another problem was the wide range of reported outcome measures. Exercise-induced immunodepression is multifactorial, depending on mechanisms related to neuro-immune-endocrine systems. We tried to compose clinically useful and comparable outcome categories from the trials. Measurements for the number of neutrophils or lymphocytes and NKCA in the blood does not, however, reflect the performance of the immune system as a whole. The immune system has many overlapping components, sometimes with shared redundant functions. Although the change of one or more immune functions may affect the host ability to resist infections, it is not possible to predict the overall effects of small to moderate changes in immune parameters on host resistance. The most useful outcome from a clinical point of view, the incidence of URT infections, was only used in two of the included studies. In these, diagnosis was based on URT symptoms assessed by questionnaire or telephone interview.

The present study has important strengths. Because of a comprehensive search strategy, omission of important trials seems unlikely. To increase reliability we used two independent reviewers for study selection. We provided both a comprehensive summary of controlled trials and a quantitative estimate of the relative importance of specific nutritional countermeasures, such as CHO, glutamine and vitamins, with respect to specific immune parameters.

Implications for practice and future research

Besides improving methodological issues, such as randomisation procedures, allocation concealment, selection of athletes, and reporting of adverse events, future studies should make an effort to consider the immune system as a whole (Gleeson, 2005). Trials should also refer to the exercise training strategy as exercise itself is an important immunomodulator (Espersen *et al.*, 1996). Questions to be addressed include: (i) Are the athletes who show more 'immunodepression' more prone to URT infections during the weeks following exercise? (ii) Which are the relevant outcomes to assess exercise-induced immunodepression? (iii) Is a decrease in nonspecific immunity after intense exercise a normal protective response, with mild immunodepression being an attempt to limit inflammation? (iv) When should this be considered pathological?

Although the prevention of URT infections by vitamin C was supported by two trials, further studies are needed to provide unequivocal proof of effect. Consumption of carbohydrate beverages during exercise appears to attenuate some of the immunodepressive effects of prolonged exercise, but confirmation of any clinical significance in this awaits further

research. There is no evidence to support a role for any other nutritional supplements in preventing exercise-induced immunodepression or in protecting against infections.

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References

- Bacurau RF, Bassit RA, Sawada L, Navarro F, Martins Jr E, Costa Rosa LF (2002). Carbohydrate supplementation during intense exercise and the immune response of cyclists. *Clin Nutr* 21, 423–429.
- Bassit RA, Sawada LA, Bacurau RF, Navarro F, Costa Rosa LF (2000). The effect of BCAA supplementation upon the immune response of triathletes. *Med Sci Sports Exerc* 32, 1214–1219.
- Bassit RA, Sawada LA, Bacurau RF, Navarro F, Martins Jr E, Santos RV *et al.* (2002). Branched-chain amino acid supplementation and the immune response of long-distance athletes. *Nutrition* 18, 376–379.
- Baxmann AC, De OGM, Heilberg IP (2003). Effect of vitamin C supplements on urinary oxalate and pH in calcium stone-forming patients. *Kidney Int* 63, 1066–1071.
- Bishop NC, Blannin AK, Robson PJ, Walsh NP, Gleeson M (1999). The effects of carbohydrate supplementation on immune responses to a soccer-specific exercise protocol. *J Sports Sci* 17, 787–796.
- Bishop NC, Blannin AK, Walsh NP, Gleeson M (2001a). Carbohydrate beverage ingestion and neutrophil degranulation responses following cycling to fatigue at 75% VO₂ max. *Int J Sports Med* 22, 226–231.
- Bishop NC, Gleeson M, Nicholas CW, Ali A (2002). Influence of carbohydrate supplementation on plasma cytokine and neutrophil degranulation responses to high intensity intermittent exercise. *Int J Sport Nutr Exerc Metab* 12, 145–156.
- Bishop NC, Walsh NP, Scanlon GA (2003). Effect of prolonged exercise and carbohydrate on total neutrophil elastase content. *Med Sci Sports Exerc* 35, 1326–1332.
- Bishop NC, Walsh NP, Haines DL, Richards EE, Gleeson M (2001b). Pre-exercise carbohydrate status and immune responses to prolonged cycling: I. Effect on neutrophil degranulation. *Int J Sport Nutr Exerc Metab* 11, 490–502.
- Bishop NC, Walsh NP, Haines DL, Richards EE, Gleeson M (2001c). 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.
- Bouic PJ, Clark A, Lamprecht J, Freestone M, Pool EJ, Liebenberg RW *et al.* (1999). The effects of B-sitosterol (BSS) and B-sitosterol glucoside (BSSG) mixture on selected immune parameters of marathon runners: inhibition of post marathon immune suppression and inflammation. *Int J Sports Med* 20, 258–262.
- Castell LM, Newsholme EA (1998). Glutamine and the effects of exhaustive exercise upon the immune response. *Can J Physiol Pharmacol* 76, 524–532.
- Castell LM, Poortmans JR, Newsholme EA (1996). Does glutamine have a role in reducing infections in athletes? *Eur J Appl Physiol Occup Physiol* 73, 488–490.
- Castell LM, Poortmans JR, Leclercq R, Brasseur M, Duchateau J, Newsholme EA (1997). Some aspects of the acute phase response after a marathon race, and the effects of glutamine supplementation. *Eur J Appl Physiol Occup Physiol* 75, 47–53.
- Chan MA, Koch AJ, Benedict SH, Potteiger JA (2003). Influence of carbohydrate ingestion on cytokine responses following acute resistance exercise. *Int J Sport Nutr Exerc Metab* 13, 454–465.
- Contor L (2001). Functional Food Science in Europe. *Nutr Metab Cardiovasc Dis* 11, 20–23.
- DerSimonian R, Laird N (1986). Meta-analysis in clinical trials. *Control Clin Trials* 7, 177–188.
- Espersen GT, Elbaek A, Schmidt-Olsen S, Ejlersen E, Varming K, Grunnet N (1996). Short-term changes in the immune system of elite swimmers under competition conditions. Different immunomodulation induced by various types of sport. *Scand J Med Sci Sports* 6, 156–163.
- Fischer CP, Hiscock NJ, Penkowa M, Basu S, Vessby B, Kallner A *et al.* (2004). Supplementation with vitamins C and E inhibits the release of interleukin-6 from contracting human skeletal muscle. *J Physiol* 558, 633–645.
- Gerster H (1999). High-dose vitamin C: a risk for persons with high iron stores? *Int J Vitam Nutr Res* 69, 67–82.
- Gleeson M (2005). Assessing immune function changes in exercise and diet intervention studies. *Curr Opin Clin Nutr Metab Care* 8, 511–515.
- Gleeson M, Blannin AK, Walsh NP, Bishop NC, Clark AM (1998). Effect of low- and high-carbohydrate diets on the plasma glutamine and circulating leukocyte responses to exercise. *Int J Sport Nutr* 8, 49–59.
- Gleeson M, Lancaster GI, Bishop NC (2001). Nutritional strategies to minimise exercise-induced immunosuppression in athletes. *Can J Appl Physiol* 26 (Suppl), S23–S35.
- Gleeson M, Nieman DC, Pedersen BK (2004). Exercise, nutrition and immune function. *J Sports Sci* 22, 115–125.
- Grimble RF (2001). Nutritional modulation of immune function. *Proc Nutr Soc* 60, 389–397.
- Henson DA, Nieman DC, Blodgett AD, Butterworth DE, Utter A, Davis JM *et al.* (1999). Influence of exercise mode and carbohydrate on the immune response to prolonged exercise. *Int J Sport Nutr* 9, 213–228.
- Henson DA, Nieman DC, Parker JC, Rainwater MK, Butterworth DE, Warren BJ *et al.* (1998). Carbohydrate supplementation and the lymphocyte proliferative response to long endurance running. *Int J Sports Med* 19, 574–580.
- Himmelstein S, Robergs R, Koehler K, Lewis S, Qualls C (1998). Vitamin C supplementation and upper respiratory tract infections in marathon runners. *J Exerc Physiol* (online) 1, 1–17.
- Hiscock N, Petersen EW, Krzykowski K, Boza J, Halkjaer-Kristensen J, Pedersen BK (2003). Glutamine supplementation further enhances exercise-induced plasma IL-6. *J Appl Physiol* 95, 145–148.
- Juni P, Witschi A, Bloch R, Egger M (1999). The hazards of scoring the quality of clinical trials for meta-analysis. *JAMA* 282, 1054–1060.
- Keil D, Luebke RW, Pruett SB (2001). Quantifying the relationship between multiple immunological parameters and host resistance: probing the limits of reductionism. *J Immunol* 167, 4543–4552.
- Keil D, Luebke RW, Ensley M, Gerard PD, Pruett SB (1999). Evaluation of multivariate statistical methods for analysis and modeling of immunotoxicology data. *Toxicol Sci* 51, 245–258.
- Koch AJ, Potteiger JA, Chan MA, Benedict SH, Frey BB (2001). Minimal influence of carbohydrate ingestion on the immune response following acute resistance exercise. *Int J Sport Nutr Exerc Metab* 11, 149–161.

- Krause R, Patruta S, Daxbock F, Fladerer P, Biegelmayer C, Wenisch C (2001). Effect of vitamin C on neutrophil function after high-intensity exercise. *Eur J Clin Invest* 31, 258–263.
- Krzywkowski K, Petersen EW, Ostrowski K, Kristensen JH, Boza J, Pedersen BK (2001a). Effect of glutamine supplementation on exercise-induced changes in lymphocyte function. *Am J Physiol Cell Physiol* 281, C1259–C1265.
- Krzywkowski K, Petersen EW, Ostrowski K, Link-Amster H, Boza J, Halkjaer-Kristensen J, Pedersen BK (2001b). Effect of glutamine and protein supplementation on exercise-induced decreases in salivary IgA. *J Appl Physiol* 91, 832–838.
- Lancaster GI, Jentjens RL, Moseley L, Jeukendrup AE, Gleeson M (2003). Effect of pre-exercise carbohydrate ingestion on plasma cytokine, stress hormone, and neutrophil degranulation responses to continuous, high-intensity exercise. *Int J Sport Nutr Exerc Metab* 13, 436–453.
- Mackinnon LT, Hooper SL (1996). Plasma glutamine and upper respiratory tract infection during intensified training in swimmers. *Med Sci Sports Exerc* 28, 285–290.
- Marzatico F, Pansarasa O, Bertorelli L, Somenzini L, Della VG (1997). Blood free radical antioxidant enzymes and lipid peroxides following long-distance and lactacidemic performances in highly trained aerobic and sprint athletes. *J Sports Med Phys Fitness* 37, 235–239.
- McAnulty SR, McAnulty LS, Nieman DC, Morrow JD, Utter AC, Henson DA et al. (2003). Influence of carbohydrate ingestion on oxidative stress and plasma antioxidant potential following a 3 h run. *Free Radical Res* 37, 835–840.
- Mena P, Maynar M, Gutierrez JM, Maynar J, Timon J, Campillo JE (1991). Erythrocyte free radical scavenger enzymes in bicycle professional racers. Adaptation to training. *Int J Sports Med* 12, 563–566.
- Mero A (1997). Effects of bovine colostrum supplementation on serum IGF-I, IgG, hormone, and saliva IgA during training. *J of Appl Physiol Respir, Environ Exerc physiology* 83, 1144–1151.
- Mitchell JB, Pizza FX, Paquet A, Davis BJ, Forrest MB, Braun WA (1998). Influence of carbohydrate status on immune responses before and after endurance exercise. *J Appl Physiol* 84, 1917–1925.
- Moher D, Pham B, Jones A, Cook DJ, Jadad AR, Moher M et al. (1998). Does quality of reports of randomised trials affect estimates of intervention efficacy reported in meta-analyses? *Lancet* 352, 609–613.
- Nehlsen-Cannarella SL, Fagoaga OR, Nieman DC, Henson DA, Butterworth DE, Schmitt RL et al. (1997). Carbohydrate and the cytokine response to 2.5 h of running. *J Appl Physiol* 82, 1662–1667.
- Newsholme EA, Parry-Billings M (1990). Properties of glutamine release from muscle and its importance for the immune system. *JPN J Parenter Enteral Nutr* 14, 63S–67S.
- Nieman DC (1994). Exercise, upper respiratory tract infection, and the immune system. *Med Sci Sports Exerc* 26, 128–139.
- Nieman DC (1997). Immune response to heavy exertion. *J Appl Physiol* 82, 1385–1394.
- Nieman DC, Davis JM, Brown VA, Henson DA, Dumke CL, Utter AC et al. (2004). Influence of carbohydrate ingestion on immune changes after 2 h of intensive resistance training. *J Appl Physiol* 96, 1292–1298.
- Nieman DC, Davis JM, Henson DA, Walberg-Rankin J, Shute M, Dumke CL et al. (2003). Carbohydrate ingestion influences skeletal muscle cytokine mRNA and plasma cytokine levels after a 3-h run. *J Appl Physiol* 94, 1917–1925.
- Nieman DC, Fagoaga OR, Butterworth DE, Warren BJ, Utter A, Davis JM et al. (1997a). Carbohydrate supplementation affects blood granulocyte and monocyte trafficking but not function after 2.5 h or running. *Am J Clin Nutr* 66, 153–159.
- Nieman DC, Henson DA, Butterworth DE, Warren BJ, Davis JM, Fagoaga OR et al. (1997b). Vitamin C supplementation does not alter the immune response to 2.5 hours of running. *Int J Sport Nutr* 7, 173–184.
- Nieman DC, Henson DA, Fagoaga OR, Utter AC, Vinci DM, Davis JM et al. (2002a). Change in salivary IgA following a competitive marathon race. *Int J Sports Med* 23, 69–75.
- Nieman DC, Henson DA, Garner EB, Butterworth DE, Warren BJ, Utter A et al. (1997c). Carbohydrate affects natural killer cell redistribution but not activity after running. *Med Sci Sports Exerc* 29, 1318–1324.
- Nieman DC, Henson DA, Gusewitch G, Warren BJ, Dotson RC, Butterworth DE et al. (1993). Physical activity and immune function in elderly women. *Med Sci Sports Exerc* 25, 823–831.
- Nieman DC, Henson DA, McAnulty SR, McAnulty L, Swick NS, Utter AC et al. (2002b). Influence of vitamin C supplementation on oxidative and immune changes after an ultramarathon. *J Appl Physiol* 92, 1970–1977.
- Nieman DC, Henson DA, Smith LL, Utter AC, Vinci DM, Davis JM et al. (2001). Cytokine changes after a marathon race. *J Appl Physiol* 91, 109–114.
- Nieman DC, Johanssen LM, Lee JW, Arabatzis K (1990). Infectious episodes in runners before and after the Los Angeles Marathon. *J Sports Med Phys Fitness* 30, 316–328.
- Nieman DC, Nehlsen-Cannarella SL, Fagoaga OR, Henson DA, Shannon M, Hjertman JM et al. (2000a). Immune function in female elite rowers and non-athletes. *Br J Sports Med* 34, 181–187.
- Nieman DC, Nehlsen-Cannarella SL, Fagoaga OR, Henson DA, Utter A, Davis JM et al. (1998a). Effects of mode and carbohydrate on the granulocyte and monocyte response to intensive, prolonged exercise. *J Appl Physiol* 84, 1252–1259.
- Nieman DC, Nehlsen-Cannarella SL, Henson DA, Koch AJ, Butterworth DE, Fagoaga OR et al. (1998b). Immune response to exercise training and/or energy restriction in obese women. *Med Sci Sports Exerc* 30, 679–686.
- Nieman DC, Peters EM, Henson DA, Nevines EI, Thompson MM (2000b). Influence of vitamin C supplementation on cytokine changes following an ultramarathon. *J Interferon Cytokine Res* 20, 1029–1035.
- Nieman DC, Simandle S, Henson DA, Warren BJ, Suttles J, Davis JM et al. (1995). Lymphocyte proliferative response to 2.5 hours of running. *Int J Sports Med* 16, 404–409.
- Palmer FM, Nieman DC, Henson DA, McAnulty SR, McAnulty L, Swick NS et al. (2003). Influence of vitamin C supplementation on oxidative and salivary IgA changes following an ultramarathon. *Eur J Appl Physiol* 89, 100–107.
- Pedersen BK, Bruunsgaard H, Klokke M, Kappel M, MacLean DA, Nielsen HB et al. (1997). Exercise-induced immunomodulation: possible roles of neuroendocrine and metabolic factors. *Int J Sports Med* 18, S2–S7.
- Pedersen BK, Helge JW, Richter EA, Rohde T, Kiens B (2000). Training and natural immunity: effects of diets rich in fat or carbohydrate. *Eur J Appl Physiol* 82, 98–102.
- Peters EM, Bateman ED (1983). Ultramarathon running and upper respiratory tract infections. An epidemiological survey. *S Afr Med J* 64, 582–584.
- Peters EM, Anderson R, Nieman DC, Fickl H, Jogessar V (2001a). Vitamin C supplementation attenuates the increases in circulating cortisol, adrenaline and anti-inflammatory polypeptides following ultramarathon running. *Int J Sports Med* 22, 537–543.
- Peters EM, Anderson R, Theron AJ (2001b). Attenuation of increase in circulating cortisol and enhancement of the acute phase protein response in vitamin C-supplemented ultramarathoners. *Int J Sports Med* 22, 120–126.
- Peters EM, Goetzsche JM, Grobbelaar B, Noakes TD (1993). Vitamin C supplementation reduces the incidence of post-race symptoms of upper-respiratory-tract infection in ultramarathon runners. *Am J Clin Nutr* 57, 170–174.
- Peters EM, Goetzsche JM, Joseph MC, Noakes TD (1996). Vitamin C as effective as combinations of anti-oxidant nutrients in reducing symptoms of upper respiratory tract infection (URTI) in ultramarathon runners. *S A Sportsmedicine* 3, 23–27.

- Peters EM (1990). The effect of altitude on the incidence of upper respiratory tract infections following participation in ultramarathon events. *SA Sports Med* 5, 4–8.
- Petersen EW, Ostrowski K, Ibfelt T, Richelle M, Offord E, Halkjaer-Kristensen J et al. (2001). Effect of vitamin supplementation on cytokine response and on muscle damage after strenuous exercise. *Am J Physiol Cell Physiol* 280, C1570–C1575.
- Prasad JS (1980). Effect of vitamin E supplementation on leukocyte function. *Am J Clin Nutr* 33, 606–608.
- Radak Z, Pucsek J, Boros S, Josfai L, Taylor AW (2000). Changes in urine 8-hydroxydeoxyguanosine levels of super-marathon runners during a four-day race period. *Life Sci* 66, 1763–1767.
- Rennie MJ, Edwards RH, Krywawych S, Davies CT, Halliday D, Waterlow JC et al. (1981). Effect of exercise on protein turnover in man. *Clin Sci (Lond)* 61, 627–639.
- Robinson KA, Dickersin K (2002). Development of a highly sensitive search strategy for the retrieval of reports of controlled trials using PubMed. *Int J Epidemiol* 31, 150–153.
- Robson PJ, Bouic PJ, Myburgh KH (2003). Antioxidant supplementation enhances neutrophil oxidative burst in trained runners following prolonged exercise. *Int J Sport Nutr Exerc Metab* 13, 369–381.
- Rohde T, Asp S, MacLean DA, Pedersen BK (1998). Competitive sustained exercise in humans, lymphokine activated killer cell activity, and glutamine – an intervention study. *Eur J Appl Physiol Occup Physiol* 78, 448–453.
- Scharhag J, Meyer T, Gabriel HH, Auracher M, Kindermann W (2002). Mobilization and oxidative burst of neutrophils are influenced by carbohydrate supplementation during prolonged cycling in humans. *Eur J Appl Physiol* 87, 584–587.
- Shinkai S, Kurokawa Y, Hino S, Hirose M, Torii J, Watanabe S et al. (1993). Triathlon competition induced a transient immunosuppressive change in the peripheral blood of athletes. *J Sports Med Phys Fitness* 33, 70–78.
- Singh A, Papanicolaou DA, Lawrence LL, Howell EA, Chrousos GP, Deuster PA (1999). Neuroendocrine responses to running in women after zinc and vitamin E supplementation. *Med Sci Sports Exerc* 31, 536–542.
- Starkie RL, Angus DJ, Rolland J, Hargreaves M, Febbraio MA (2000). Effect of prolonged, submaximal exercise and carbohydrate ingestion on monocyte intracellular cytokine production in humans. *J Physiol* 528, 647–655.
- Starkie RL, Arkinstall MJ, Koukoulas I, Hawley JA, Febbraio MA (2001). Carbohydrate ingestion attenuates the increase in plasma interleukin-6, but not skeletal muscle interleukin-6 mRNA, during exercise in humans. *J Physiol* 533, 585–591.
- Sterne JA, Egger M, Smith GD (2001). Systematic reviews in health care: Investigating and dealing with publication and other biases in meta-analysis. *BMJ* 323, 101–105.
- Tauler P, Aguilo A, Fuentespina E, Tur JA, Pons A (2002). Diet supplementation with vitamin E, vitamin C and beta-carotene cocktail enhances basal neutrophil antioxidant enzymes in athletes. *Pflugers Arch* 443, 791–797.
- Tauler P, Gimeno I, Aguilo A, Guix MP, Pons A (1999). Regulation of erythrocyte antioxidant enzyme activities in athletes during competition and short-term recovery. *Pflugers Arch* 438, 782–787.
- Toft AD, Thorn M, Ostrowski K, Asp S, Moller K, Iversen S et al. (2000). N-3 polyunsaturated fatty acids do not affect cytokine response to strenuous exercise. *J Appl Physiol* 89, 2401–2406.
- Walsh NP, Blannin AK, Bishop NC, Robson PJ, Gleeson M (2000). Effect of oral glutamine supplementation on human neutrophil lipopolysaccharide-stimulated degranulation following prolonged exercise. *Int J Sport Nutr Exerc Metab* 10, 39–50.
- Whitehead A, Whitehead J (1991). A general parametric approach to the meta-analysis of randomized clinical trials. *Stat Med* 10, 1665–1677.

Appendix A: Search strategy

Database: SPORTDiscus 1830 to May 2005; EBM Reviews – Cochrane Central Register of Controlled Trials 2nd Quarter 2005; EBM Reviews – Cochrane Database of Systematic Reviews 2nd Quarter 2005; Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) 1966 to Present

Search strategy:

randomi[z]ed controlled trial.pt. (392250)
controlled clinical trial.pt. (134553)
Randomi[z]ed Controlled Trials/ (41104)
Random Allocation/ (72830)
Double-Blind Method/ (147455)
Single-Blind Method/ (14248)
or/1-6 (593956)
Animals/ not Human/ (2863905)
7 not 8 (570442)
clinical trial.pt. (657885)
exp Clinical Trials/ (201366)
(clinic\$ adj25 trial\$).tw. (149833)
((singl\$ or doubl\$ or trebl\$ or tripl\$) adj (mask\$ or blind\$)).tw. (173831)
Placebos/ (39895)

placebo\$.tw. (178494)
random\$.tw. (513086)
Research Design/ (42074)
(latin adj square).tw. (3033)
or/10-18 (1072000)
19 not 8 (1015274)
20 not 9 (455654)
or/9-20 (1082822)
(immun\$ adj5 (athl\$ or nutrit\$ or exercise)).mp. [mp = ti, ot, ab, nm, hw, sh, kw, tx, ct, da, fa, ce, fc, fd] (4336)
(nutrit\$ adj5 (athl\$ or immun\$ or exercise)).mp. [mp = ti, ot, ab, nm, hw, sh, kw, tx, ct, da, fa, ce, fc, fd] (6651)
(exercise\$ adj5 (athl\$ or immun\$ or nutrit\$)).mp. [mp = ti, ot, ab, nm, hw, sh, kw, tx, ct, da, fa, ce, fc, fd] (8929)
(exercise\$ adj5 (carbohydrat\$ or glutamine or vitam\$ or immun\$ or nutrit\$)).mp. [mp = ti, ot, ab, nm, hw, kw, sh, tx, ct, fc, da, fa, ce, fd] (7568)
or/23-26 (15829)
(athl\$ and immun\$).mp. [mp = ti, ot, ab, nm, hw, kw, sh, tx, ct, fc, da, fa, ce, fd] (1528)
and/27-28 (795)
and/22,29 (105)

Appendix B: Quality rating of included studies

Study	Total	1	2	3	4	5	6	7	8	9	10	11
Peters <i>et al.</i> (1993)	11	0.5	1	0.5	0	0	2	0	0.5	0.5	0.5	0.5
Peters <i>et al.</i> (1996)	11	0.5	1	0.5	0	0	2	0	0.5	0.5	0.5	0.5
Castell <i>et al.</i> (1996)	8.5	0.5	1	0	0	0	2	0	0.5	0.5	0.5	0.5
Nieman <i>et al.</i> (1997a)	9	1	1	0.5	0.5	0	2	0	0.5	0.5	0.5	0
Nieman <i>et al.</i> (1997b)	7.5	0	1	0.5	0	0	1	0	0.5	0.5	0.5	0.5
Nieman <i>et al.</i> (1997c)	9	1	1	0.5	0.5	0	2	0	0.5	0.5	0.5	0
Castell <i>et al.</i> (1997)	5.0	0	1	0	0	0	0	0	0.5	0.5	0.5	0
Nehlsen-Cannarella <i>et al.</i> (1997)	8	0	1	0.5	0.5	0	2	0	0.5	0.5	0.5	0
Mero (1997)	7	0	1	0	0	0	2	0	0.5	0.5	0.5	0
Rohde <i>et al.</i> (1998)	6.5	0.5	1	0.5	0.5	0	1	0	0.5	0.5	0	0
Henson <i>et al.</i> (1998)	9	1	1	0.5	0.5	0	2	0	0.5	0.5	0.5	0
Henson <i>et al.</i> (1999)	7.5	0	1	0	0.5	0	2	0	0.5	0.5	0.5	0
Bouc <i>et al.</i> (1999)	8	0.5	1	0	0	0	2	0	0.5	0.5	0.5	0
Himmelstein <i>et al.</i> (1998)	8.5	0.5	1	0.5	0	0	2	0	0.5	0.5	0.5	0.5
Bishop <i>et al.</i> (1999)	6	0	1	0	0.5	0	1	0	0.5	0.5	0.5	0
Singh <i>et al.</i> (1999)	7.5	0.5	1	0	0.5	0	2	0	0.5	0.5	0.5	0
Krzywkowski <i>et al.</i> (2001a)	6.5	0	1	0.5	0.5	0	1	0	0.5	0.5	0.5	0
Krzywkowski <i>et al.</i> (2001b)	6.5	0	1	0.5	0.5	0	1	0	0.5	0.5	0.5	0
Walsh <i>et al.</i> (2000)	6	0	1	0	0.5	0	1	0	0.5	0.5	0.5	0
Toft <i>et al.</i> (2000)	6	0.5	1	0.5	0.5	0	0	0	0.5	0.5	0.5	0
Bassit <i>et al.</i> (2000)	6	0.5	0.5	0	0	0	0	0	0.5	0.5	0.5	0.5
Nieman <i>et al.</i> (2000b)	7.5	0	1	0.5	0	0	1	0	0.5	0.5	0.5	0.5
Starkie <i>et al.</i> (2000)	7	0	1	0.5	0.5	0	1	0	0.5	0.5	0.5	0
Bishop <i>et al.</i> (2001b)	6.5	0	1	0	0.5	0	1	0	0.5	0.5	0.5	0
Starkie <i>et al.</i> (2001)	7	0	1	0.5	0.5	0	1	0	0.5	0.5	0.5	0
Nieman <i>et al.</i> (2001)	9	0.5	1	0.5	0	0	2	0	0.5	0.5	0.5	0
Koch <i>et al.</i> (2001)	7	0	1	0	0	0	2	0	0.5	0.5	0.5	0
Peters <i>et al.</i> (2001b)	7.5	0.5	0.5	0.5	0	0	1	0	0.5	0.5	0.5	0
Peters <i>et al.</i> (2001a)	7.5	0.5	0.5	0.5	0	0	1	0	0.5	0.5	0.5	0
Petersen <i>et al.</i> (2001)	8.5	0	1	0.5	0.5	0	2	0	0.5	0.5	0.5	0
Krause <i>et al.</i> (2001)	7.5	0.1	1	0.5	0	0	1	0	0.5	0.5	0.5	0
Bassit <i>et al.</i> (2002)	6	0.5	0.5	0	0	0	0	0	0.5	0.5	0.5	0.5
Bishop <i>et al.</i> (2002)	6.5	0	1	0	0.5	0	1	0	0.5	0.5	0.5	0
Tauler <i>et al.</i> (2002)	5.5	0.5	0.5	0.5	0	0	2	0	0.5	0.5	0.5	0
Bacurau <i>et al.</i> (2002)	7.5	0	1	0	0.5	0	2	0	0.5	0.5	0.5	0
Nieman <i>et al.</i> (2002b)	8.5	0.5	1	0.5	0.5	0	2	0	0.5	0.5	0.5	0
Scharhag <i>et al.</i> (2002)	8	0	1	0	0.5	0	2	0	0.5	0.5	0.5	0
Lancaster <i>et al.</i> (2003)	6	0.5	1	0.5	0.5	0	0	0	0.5	0.5	0.5	0
Palmer <i>et al.</i> (2003)	8.5	0.5	1	0.5	0.5	0	2	0	0.5	0.5	0.5	0
Robson <i>et al.</i> (2003)	7.5	0.5	1	0	0.5	0	2	0	0.5	0.5	0.5	0
Nieman <i>et al.</i> (2003)	5.5	0.5	1	0	0.5	0	0	0	0.5	0.5	0.5	0
Chan <i>et al.</i> (2003)	7	0	1	0	0	0	2	0	0.5	0.5	0.5	0
Hiscock <i>et al.</i> (2003)	7	0	1	0	0.5	0	2	0	0.5	0.5	0.5	0
Bishop <i>et al.</i> (2003)	6.5	0	1	0	0.5	0	1	0	0.5	0.5	0.5	0
Nieman <i>et al.</i> (2004)	7	0.5	1	0.5	0	0	1	0	0.5	0.5	0.5	0

1 = Athletes selection: all eligible athletes with number of, and reason for, exclusions given (1); attempt to do so, but reasons for failure and exclusion not given (0.5); selected athletes/eligible athletes not described (0); homogeneity of type-of-sports practiced: yes (1); no (0.5); not given (0). 2 = Athletes characteristics at baseline: Similar distribution between groups reported (0.5), not reported/not applicable (0); basal oxygen consumption reported (0.5), not reported (0). 3 = Randomisation: concealed randomisation (computer, centralised, etc.) (2); potentially manipulable (sealed envelope, date of admission, medical records, birth date, etc.) (1); [can't tell/unknown/unclear] (0). 4 = Blinding: double blinded (research team/athletes) (2); single blinded (1); unblinded/can't tell (0). 5 = Intention-to treat analysis: reported (1), not reported (0); 6 = Allocation concealment: adequate (2), unclear (1), inadequate (0.5), allocation concealment was not used (0). 7 = Intervention: nutritional supplement described explicitly – yes (0.5), no (0); dosing regimen (dose, frequency) reported (0.5), not reported (0); duration of treatment reported (0.5), not reported (0); placebo reported (0.5), not reported (0); dietary intake control done (1), not done (0.5) not reported (0). 8 = Explicit description of type of exercise performed: yes (0.5), no (0); exercise regimen (amount, frequency) reported (0.5), not reported (0); duration of exercise reported (0.5), not reported (0). 9 = Withdrawal: described number and reason for withdrawal (1); described one of above (0.5); described neither (0). 10 = Adherence to protocol: reported (0.5), not reported (0). 11 = Infections after exercise: reported: yes (0.5), no (0); direct medical examination (1), indirect questionnaire/interview (0.5), not done/not applicable (0).