Exercise and immune function: effect of ageing and nutrition

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Strenuous exercise is followed by lymphopenia, neutrophilia, impaired natural immunity, decreased lymphocyte proliferative responses to mitogens, a low level of secretory immunoglobulin A in saliva, but high circulating levels of pro- and anti-inflammatory cytokines. These exercise-induced immune changes may provide the physiological basis of altered resistance to infections. The mechanisms underlying exercise-induced immune changes are multifactorial and include neuroendocrinological and metabolic mechanisms. Nutritional supplementation with glutamine abolishes the exercise-induced decline in plasma glutamine, but does not influence post-exercise immune impairment. However, carbohydrate loading diminishes most exercise effects of cytokines, lymphocyte and neutrophils. The diminished neutrophilia and elastase (*EC* 3.4.21.37) responses to eccentric exercise in elderly subjects were enhanced to levels comparable with those of young subjects by fish oil or vitamin E supplements. However, although vitamin C supplementation may diminish the risk of contracting an infection after strenuous exercise, it is not obvious that this effect is linked to an effect of vitamin C on exercise-induced immune changes. In conclusion, it is premature to make recommendations regarding nutritional supplementation to avoid post-exercise impairment of the immune system.

Exercise: Training: Immune function: Lymphocytes

A bout of exercise induces mobilization of immunocompetent cells to the circulation. Strenuous exercise, but not moderate exercise, is followed by a decreased concentration of lymphocytes, impaired natural immunity, and low levels of secretory immunoglobulin (Ig)A in saliva (Hoffman-Goetz & Pedersen, 1994*b*). Regular exercise may increase resistance to infections such as the common cold (Nash, 1987), whereas hard training is associated with increased respiratory tract infections (Fitzgerald, 1988). The physiological basis of altered resistance to infections is not well understood, but exercise-induced changes in the cellular immune system are among the possible explanations.

The relationship between nutritional factors and resistance to infections has generated considerable interest over the last several decades. In industrialized countries the relationship between nutrition and immunity is of special interest in elderly subjects, since it is known that age-related immunodeficiency is aggravated in malnourished subjects (Chandra, 1989). In theory, it is possible that the exercise-induced immunological changes can be modulated by nutritional factors, and that dietary factors influence resting levels of the immune system in athletes.

Exercise and immune function

Acute exercise effects

The neutrophil concentration increases during exercise and continues to increase after exercise. The lymphocyte concentration increases during exercise and falls below pre-exercise values following intense long duration exercise, but is not suppressed after moderate exercise (McCarthy & Dale, 1988).

Several reports describe exercise-induced changes in subsets of peripheral-blood mononuclear cells (PBMNC; Pedersen, 1997). Increased lymphocyte concentration is probably due to the recruitment of all lymphocyte subpopulations to the vascular compartment: CD4+ T-cells, CD8+ T-cells, CD19+ B-cells, CD16+ natural killer (NK) cells, CD56+ NK cells. During exercise, CD4 : CD8

Abbreviations: Ig, immunoglobulin; IL, interleukin; NK, natural killer; PBMNC, peripheral blood mononuclear cells; PG, prostaglandin; TNF, tumour necrosis factor; URTI, upper-respiratory-tract infections.

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decreases, reflecting the greater increase in CD8+ lymphocytes than in CD4+ lymphocytes. CD4+ and CD8+ cells contain both CD45RO+ memory and CD45RA+ virgin or naive cells, and 'true' naive cells are identified by the absence of 45RO and the presence of CD62L (Bell *et al*. 1998). Data from Gabriel *et al*. (1993) show that the recruitment is primarily of CD45RO+. We have recently found that CD45RO+ and CD45RO-CD62L− cells are mobilized to the circulation, suggesting that memory lymphocytes, but not naive lymphocytes, are rapidly mobilized to the blood in response to acute physical exercise (H Bruunsgaard, unpublished results).

To obtain information about lymphocyte turnover in cells recruited during exercise, we recently analysed telomeric terminal restriction fragment length. Telomeres are the extreme ends of chromosomes that consist of TTAGGG repeats. After each round of cell division telomeric sequence is lost because of the inability of DNA polymerase to fully replicate the 5′ end of the chromosome. Telomere lengths have been used as a marker for replication history and the proliferation potential of the cells. Cell cultures of CD8+ T-cells which have reached replicate senescence after multiple rounds of cell division lack expression of the CD28 co-stimulatory molecule and have short telomere lengths (Effros *et al*. 1996). In response to exercise, lymphocytes lacking the CD28 molecule are mobilized to the circulation and telomere lengths in CD4+ and CD8+ lymphocytes were significantly shorter compared with cells isolated at rest (H Bruunsgaard, unpublished results).

Thus, the initial increase in CD4+ and CD8+ cells after exercise is not due to repopulation by newly-generated cells but may be a redistribution of activated cells, in agreement with kinetics of CD4+ repopulation after anti-human immunodeficiency virus treatment (Kelleher *et al*. 1996) and chemotherapy (Hakim *et al*. 1997) and CD4+ and CD8+ repopulation after bone-marrow transplantation (Bengtsson *et al*. 1989). Although the number of all lymphocyte subpopulations increases, the proportion of CD4+ cells declines primarily due to the fact that NK cell numbers increase more than those of any other lymphocyte subpopulations. Accordingly, the relative proportion of lymphocyte subpopulations changes and this change contributes to the exercise-induced alterations in *in vitro* immune assays in which a fixed number of PBMNC is studied.

Following intense long-duration exercise, the function of NK and B-cells is suppressed. Thus, the NK and lymphokine-activated killer cell activity (the ability of cytotoxic cells to lyse a certain number of tumour target cells) is inhibited (Hoffman-Goetz & Pedersen, 1994*a*; Brines *et al*. 1996; Pedersen *et al*. 1997). Furthermore, the B-cell function is inhibited (Tvede *et al*. 1989) and the local production of secretory IgA in saliva decreases in response to exercise (Mackinnon & Hooper, 1994). Although the results are heterogeneous, most studies find that exercise induces decreased proliferative responses to mitogens (Nielsen & Pedersen, 1997).

There are few studies which document immune system responses *in vivo*, in relation to exercise. Bruunsgaard *et al*. (1997*b*) investigated whether an *in vivo* impairment of cell-mediated immunity and specific antibody production could be demonstrated after intense exercise of long duration (triathlon race). The cellular immune system was evaluated by means of a skin test response to seven recall antigens, whereas the humoral immune system was evaluated by means of the antibody response to pneumococcal polysaccharide vaccine (this vaccine is generally considered to be T-cell independent) and tetanus and diphtheria toxoids (both of which are T-cell dependent). The skin test response was significantly lower in the group who performed a triathlon race compared with triathlete controls and untrained controls who did not participate in the triathlon. No differences in specific antibody titres were found between the groups.

Thus, *in vivo* cell-mediated immunity was impaired in the first days after prolonged high-intensity exercise, whereas there was no impairment of the *in vivo* antibody production measured 2 weeks after vaccination.

Strenuous exercise also induces increased circulating levels of several cytokines. Initial studies described increased levels of interleukin (IL)-1 in plasma obtained after exercise (Cannon & Kluger, 1983; Cannon *et al*. 1986; Evans *et al*. 1986), but the possibility exists that the assays were not specific and that other cytokines were being measured. The latter studies were conducted before the availability of recombinant IL-1 proteins, and there have been a number of studies which failed to detect elevated levels of IL-1 in plasma (Cannon *et al*. 1991; Northoff & Berg, 1991; Sprenger *et al*. 1992; Ullum *et al*. 1994). However, IL-6 has been found to be enhanced in several studies (Northoff & Berg, 1991; Sprenger *et al*. 1992; Ullum *et al*. 1994; Bruunsgaard *et al*. 1997*a*; Rohde *et al*. 1997; Ostrowski *et al*. 1998*c*) and is followed by an increase in the concentration of the IL-1 receptor antagonist, a naturally-occurring inhibitor of IL-1 (Ostrowski *et al*. 1998*c*). Recent findings from our group show that the tumour-necrosis factor (TNF) α receptors 1 and 2 and the chemokines IL-8 and macrophage inflammatory protein 1β are also increased in response to strenuous exercise (Ostrowski *et al*. 1998*a*,*b*). Thus, exercise induces a strong anti-inflammatory response. Bruunsgaard *et al*. (1997*a*) compared concentric and eccentric exercise, and found an association between increased IL-6 level and muscle damage, as visualized by the increase in creatine kinase (*EC* 2.7.3.2). Thus, post-exercise cytokine production may be related to skeletal muscle damage, but a causal relationship between muscle damage and cytokine production has not been shown. IL-6 mRNA was present in skeletal muscle biopsies obtained from runners after, but not before, a marathon run (Ostrowski *et al*. 1998*c*). The latter finding may indicate that IL-6 is produced locally in response to strenuous exercise or exercise-induced muscle damage. IL-1 receptor antagonist mRNA was not present in the skeletal muscle, but was expressed by PBMNC obtained after, but not before, the marathon, indicating that locally produced IL-6 induces a systemic anti-inflammatory response. The cytokine cascade in response to exercise resembles that seen in response to trauma, and exercise thus may be considered a model of trauma (Ostrowski *et al*. 1998*a*,*b*).

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Chronic exercise effects

The immune function (resting levels) in athletes *v*. non-athletes has more similarities than disparities (for review, see Nieman, 1996). Natural immunity may be slightly increased, whereas neutrophil function has been reported to be slightly suppressed. The adaptive immune system (resting state) in general seems to be largely unaffected by intensive and prolonged exercise training (Tvede *et al*. 1991; Baj *et al*. 1994; Nieman *et al*. 1995). The innate immune system appears to respond differentially to the chronic stress of intensive exercise, with NK cell activity tending to be enhanced while neutrophil function is suppressed (Pedersen *et al*. 1989; Nieman *et al*. 1993; Hack *et al*. 1994; Pyne *et al*. 1995).

Based on epidemiological studies (Fitzgerald, 1991; Nieman, 1994*a*), a relationship between exercise and upper-respiratory-tract infections (URTI) has been modelled in the form of a 'J' curve. This model suggests that while the risk of URTI may decrease below that of the sedentary individual, when an individual engages in moderate exercise training, risk may rise above average during periods of excessive high-intensity exercise (Nieman, 1994*b*). The link between exercise-associated immune changes and sensitivity to infections may be explained by the so-called 'open window' of altered immunity. We (see Pedersen, 1997) have previously suggested that viruses and bacteria may gain a foothold, increasing the risk of subclinical and clinical infection. However, it remains to be demonstrated whether athletes showing the most extreme immuno-suppression following heavy exertion are those who contract an infection within the following 1–2 weeks.

Ageing, immune function and exercise

Ageing and immune function

Ageing is characterized by a decline in the ability of individuals to adapt to environmental stress (Makinodan & Marguerite, 1980; Miller, 1989, 1996). Ageing is associated with a functional decline in several components of the immune system. As a result, the elderly are more vulnerable, and are at increased risk of contracting infectious diseases, of tumorigenesis and of autoimmune disorders (Armstrong, 1990). One of the most striking features of immuno-senescence is thymus involution. In human subjects, the thymus begins to involute at an early stage of life, and the process is completed by mid-life. Many age-related changes in the immune system can be related to the loss of thymic and T-cell function. The involuted thymus has only a limited capacity to provide the circulation with naive T-cells. There is thus a significant decrease in naive T-cells relative to memory T-cells. Positive and negative intrathymic T-cell selection is compromised in the elderly, and consequently leads to the appearance of anti-self-reactive T-cells and T-cells that do not express self-major histocompatibility complex-restricted antigen recognition (Shinkai *et al*. 1996).

The elderly have been shown to have a low total lymphocyte count. Within the T-cell subsets, both CD4+ and CD8+ cells decrease with age, but the effect is more pronounced with respect to CD8+ T-cells, which show an age-related increase in the CD4 : CD8 ratio (Utsuyama *et al*. 1992). Moreover, ageing leads to an increase in memory cells CD45RO+ T-cells at the expense of naive CD45RA+ (i.e. previously unstimulated) T-cells. This change has been documented in circulating CD4+ and CD8+ T-cells (Shinkai *et al*. 1996).

The age-associated shift in T-cell phenotype could be largely responsible for the age-related decline in responsiveness to mitogen *in vitro* and to new antigens *in vivo* (Miller, 1996). The T-cells show a decreased proliferation when stimulated with mitogens (Miller, 1996). The production of IL-2 and the expression of the high-affinity receptor decreases with age, and as a consequence the T-cells show a decreased ability to proliferate. The activity of cytotoxic T lymphocytes declines with age. This process cannot be attributed entirely to the reduced number and proliferative capability of CD8+ T-cells (Shinkai *et al*. 1996). The data on human blood B-cell numbers are not consistent (Paganelli *et al*. 1992; Utsuyama *et al*. 1992; Hoffkes *et al*. 1996). It is generally reported that B-cell function is only marginally affected by the ageing process (Ennist *et al*. 1986; Ben-Yehuda & Weksler, 1992). As T-cell function and activity are adversely affected with age, regulation of B-cell function by dysfunctional T-cell mechanisms probably contribute to this observation. When looking at NK cell number and activity in the spleen from both mice and rats, there is a clear and consistent age-associated decline in NK cell number and activity (Weindruch *et al*. 1983; Saxena *et al*. 1984; Nasrullah & Mazzeo, 1992). However, when examining peripheral-blood NK cell activity in human subjects, it is frequently reported that no age-associated change is found (Ligthart *et al*. 1986; Murasko *et al*. 1986; Ferguson *et al*. 1995), and in some cases increases in NK cell number and activity have been observed (Batory *et al*. 1981; Krishnaraj & Blandford, 1987, 1988; Sansoni *et al*. 1993; Xu *et al*. 1993). The findings from one study (Facchini *et al*. 1987) indicate that the activity of NK cells (expressed per cell basis) decreases with age. Ageing is associated with immune activation and changes in the balance of cytokine secretion patterns (Fagiolo *et al*. 1992; Hobbs *et al*. 1993; Miller, 1996). Recently, we reported increased plasma levels of TNF-α, IL-6 and TNF-α receptor in a large cohort of centenarians (Bruunsgaard *et al*. 1999). Other workers have found increased circulating levels of IL-6 with age (Wei *et al*. 1992; Ershler *et al*. 1993; Hager *et al*. 1994; Kania *et al*. 1995; James *et al*. 1997), whereas other studies have found unaltered or undetectable levels of IL-6 (Peterson *et al*. 1994), TNF-α (Mooradian *et al*. 1990; Fagiolo *et al*. 1992; Peterson *et al*. 1994; Catania *et al*. 1997) and IL-1 (Mooradian *et al*. 1990; Catania *et al*. 1997).

Given that a number of age-related changes occur in many systems (e.g. neuroendocrine) known to alter immune function both at rest and during exercise, it would be of value to learn the extent to which both acute and chronic exercise influence immune function in the elderly (Mazzeo, 1994).

Acute exercise effects in the elderly

While few studies have been performed to date, recent evidence suggests that the ability of the immune system in older individuals to respond to the stress imposed by a single

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bout of exercise is maintained with age. Fiatarone *et al*. (1989) examined the effect of age on the responsiveness of NK cells to *in vivo* stimulation with exercise in eight young (30 (SE 1) years) and nine elderly (71 (SE 1) years) subjects. The elderly subjects were not found to have NK cell numbers and function that were significantly different from those of the young subjects at baseline. In response to maximal bicycle ergometry exercise both groups showed an increase in the NK activity.

Crist *et al*. (1989) have also examined the effect of a single bout of treadmill exercise in elderly women on NK cells. One group of elderly subjects had participated in 16 weeks of aerobic training, while the other group of elderly subjects acted as age-matched sedentary controls. Women participating in the training showed an increase in NK activity at rest when compared with the sedentary controls. An acute bout of treadmill exercise produced an increase in NK activity in both groups; however, the increase was more significant in the trained group compared with the controls.

A number of studies have examined the effect of dietary modulations on the acute-phase response to eccentric exercise in elderly *v*. young subjects (Cannon *et al*. 1990, 1991, 1995). These studies will be discussed later.

Chronic exercise effects in the elderly

A limited number of studies have addressed the effect of endurance training adaptations on immune function in older individuals. Nieman *et al*. (1993) examined the effect of 12 weeks of walking (5 d/week at 60 % heart rate reserve) and found no effect on basal NK activity and T-cell function in previously-sedentary elderly women (73 (SE 1) years). T-cell function and NK activity was significantly greater in a group of highly-conditioned female endurance competitors (73 (SE 2) years) when compared with age-matched sedentary controls, but remained below the level for the young sedentary women. These findings show a difference in immune function between moderately-trained and highly-conditioned women. The mechanism responsible for this difference is uncertain but may be related to differences in the training intensity, duration and frequency. Alternatively, the age at which the training is initiated should be an important factor, because the highly-conditioned women had begun exercising at a younger age (Mazzeo, 1996).

Shinkai *et al*. (1996) have examined the effect of exercise on immunosenescence in men. A cross-sectional survey examined whether subjects who had habitually participated in endurance exercise retained a higher level of T-cell function than sedentary individuals in old age. Compared with the young subjects both groups of elderly subjects had lower circulating CD3+ and CD8+ cell counts, higher CD4 : CD8 and higher proportions of activated CD3+ and memory CD4+ and CD8+ cells. Moreover, comparison between the active and sedentary elderly groups showed no differences in circulating counts of immunocompetent cells. However, the active elderly subjects demonstrated significantly greater proliferative responses to phytohaemagglutinin and to pokeweed (*Phytolacca americana*) mitogen. These results indicate that endurance training in later life is associated with a smaller age-related decline in certain aspects of circulating T-cell function.

Rall *et al*. (1996) found no changes in PBMNC subsets, production of IL-1β, TNF-α, IL-6, IL-2 or prostaglandin $(PG)E_2$ production, lymphocyte proliferation or delayed-type hypersensitivity response after 12 weeks of high-intensity progressive-resistance training in eight healthy young subjects (22–30 years) and eight healthy elderly subjects (65–80 years).

Rincon *et al*. (1996) investigated the effect of an exercise intervention programme of 60 min three times weekly for 3 months in six frail men aged 70 years or more who were at risk of a fall but not suffering from serious medical problems compared with seven controls having no intervention. Cytotoxic activity of NK cells significantly decreased during the course of the study, suggesting that exercise was having an adverse effect on NK activity in the very frail elderly.

Exercise, nutrition and immune function

The mechanisms underlying exercise-associated immune changes are multifactorial and include neuroendocrinological factors such as adrenaline, noradrenaline, growth hormone, cortisol and β-endorphin (Pedersen *et al*. 1997). Physiological factors such as increased body temperature during the exercise (Kappel *et al*. 1991) or O_2 desaturation may also have an impact (Klokker *et al*. 1995). Altered protein metabolism such as reduced plasma glutamine concentrations as a result of muscular activity has been suggested to influence lymphocyte function (Newsholme & Parry Billings, 1990), and reduced plasma glucose has been suggested to increase stress hormone levels and thereby immune function (Nieman & Pedersen, 1998). Furthermore, as a consequence of the catecholamine- and growth hormone-induced immediate changes in leucocyte subsets, the relative proportion of these subsets change, and activated leucocyte subpopulations may be mobilized to the blood. Free oxygen radicals and PG released by the elevated number of neutrophils and monocytes may influence the function of lymphocytes and contribute to the impaired function of the latter cells. Thus, nutritional supplementation with glutamine, carbohydrate, antioxidants or PG inhibitors may in principle influence exercise-associated immune function.

Glutamine

Skeletal muscle is the major tissue involved in glutamine production and is known to release glutamine into the bloodstream at a high rate. It has been suggested that skeletal muscle plays a vital role in the maintenance of the key process of glutamine utilization in the immune cells. Consequently, the activity of the skeletal muscle may directly influence the immune system. It has been suggested (the so-called ‘glutamine hypothesis’) that under intense physical exercise, or in relation to surgery, trauma, burn and sepsis, the demands on muscle and other organs for glutamine is such that the lymphoid system may be forced into a glutamine debt, which temporarily affects its function. Thus, factors that directly or indirectly influence glutamine synthesis or release could theoretically influence the function of lymphocytes and monocytes (Newsholme, 1990,

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1994). Following intense long-term exercise and other physical stress disorders, the glutamine concentration in plasma declines (Essen *et al*. 1992; Parry Billings *et al*. 1992; Keast *et al*. 1995; Lehmann *et al*. 1995).

Glutamine enhances lymphocyte proliferation and lymphokine-activated killer cell activity *in vitro*, but has no effect on NK cell activity (Rohde *et al*. 1995). Furthermore, in *in vitro* experiments glutamine was found to stimulate IL-2 and interferon γ production, without influencing the production of IL-1β, IL-6 or TNF-α (Rohde *et al*. 1996). Glutamine added to *in vitro* assays did not abolish the post-exercise decline in proliferative responses and did not normalize the low lymphocyte proliferation in human immunodeficiency virus-seropositive patients (Rohde *et al*. 1995).

A study by Castell *et al*. (1996) found that glutamine supplementation decreased the incidence of URTI after a marathon. In contrast, Mackinnon & Hooper (1996) found no differences in plasma glutamine levels between individuals who did or did not develop URTI following intensive swimming training. In two recent placebo-controlled glutamine intervention studies (Rohde *et al*. 1998*a*,*b*) it was found that glutamine abolished the post-exercise decline in plasma glutamine without influencing post-exercise impairment of NK and lymphokine-activated killer cell function, or mitogen-induced proliferative responses. Thus, the latter studies did not support the hypothesis that the post-exercise decline in immune function is caused by a decrease in the plasma glutamine concentration.

Carbohydrate

Earlier research had established that a reduction in blood glucose levels is linked to hypothalamic–pituitary–adrenal activation, an increased release of adrenocorticotrophic hormone and cortisol, increased plasma growth hormone, decreased insulin, and a variable effect on blood adrenaline level (Nieman & Pedersen, 1998). Given the link between stress hormones and immune responses to prolonged and intensive exercise (Pedersen *et al*. 1997), ingestion of carbohydrate compared with a placebo should maintain plasma glucose concentrations, attenuate increases in stress hormones, and thereby diminish changes in immunity. This hypothesis has been tested in a number of studies by Nieman and coworkers (Nehlsen-Canarella *et al*. 1997; Nieman *et al*. 1997*a*,*c*) using double-blind placebo-controlled randomized designs. Ingestion of a carbohydrate beverage before, during (about 1 litre/h), and after 2·5 h of exercise was associated with higher plasma glucose levels, an attenuated cortisol and growth hormone response, fewer perturbations in blood immune cell counts, lower granulocyte and monocyte phagocytosis and oxidative burst activity, and a diminished pro- and anti-inflammatory cytokine response. Overall, the hormonal and immune responses were diminished following ingestion of carbohydrate compared with a placebo. Some immune variables were affected slightly by carbohydrate ingestion (e.g. granulocyte and monocyte function), while other variables were strongly influenced (e.g. plasma cytokine concentrations and blood cell counts).

The clinical significance of these carbohydrate-induced effects on the endocrine and immune systems awaits further research. At this point, the data indicate that athletes ingesting carbohydrate beverages before, during and after prolonged and intensive exercise should experience lowered physiological stress. Research to determine whether carbohydrate ingestion will improve host protection against viruses in endurance athletes during periods of intensified training or following competitive endurance events is warranted.

Lipids

There are two principal classes of polyunsaturated fatty acids: the *n*-6 and the *n*-3 families (Calder, 1998). The precursor of the *n*-6 family is linoleic acid which is converted to arachidonic acid, the precursor of PG and leukotrienes which have potent pro-inflammatory and immunoregulatory properties. The precursor of the *n*-3 family of polyunsaturated fatty acids is α-linolenic acid. If the *n*-6 : *n*-3 value decreases as the result of administration of a diet rich in *n*-3 fatty acids, the PGE_2-mediated immunosuppression may be abolished.

The possible interaction between intense acute exercise, immune function and polyunsaturated fatty acids was examined in inbred female C57BI/6 mice (Benquet *et al*. 1994). The animals received either a natural-ingredient diet or a diet supplemented with various oils such as beef tallow, safflower, fish oil or linseed oil for an 8-week period. In the group receiving 18:3 (*n*-3) in linseed oil it was shown that post-exercise immunosuppression of the IgM plaque-forming cell response was abolished.

Thus, the effect of linseed oil may be ascribed to a link between a diet rich in *n*-3 polyunsaturated fatty acids and abolition of PG-related immunosuppression. In support of this hypothesis, it has been shown that when the PGE_2 production was inhibited by the PG-inhibitor indomethacin, exercise-induced suppression of the NK cell activity and B-cell function was partly abolished (Tvede *et al*. 1989; Pedersen *et al*. 1990).

Cannon *et al*. (1995) attempted to determine whether modification of dietary fatty acids influenced neutrophil and monocyte secretion after *in vivo* inflammatory stress in older human subjects. *In vivo* neutrophil degranulation was assessed by plasma elastase (*EC* 3.4.21.37) concentrations, and monocyte function was assessed by IL-1β secretion *in vitro*. In response to eccentric exercise, older subjects (> 60 years) taking a placebo showed no apparent elastase response, whereas those taking fish oil supplements responded with a significant increase (142 %) in plasma elastase, similar to responses of younger reference subjects. There was no effect of fish oil on IL-1β secretion.

The possibility that *n*-3 fatty acids may diminish the increased concentration of pro-inflammatory cytokines induced by exercise has not been investigated. However, in animal experiments it was shown that the increase in IL-1 and TNF following application of endotoxin was reduced when the animals were pre-treated with *n*-3 fatty acids (fish oil; Johnson *et al*. 1993).

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Antioxidants

During exercise, the enhanced O_2 utilization leads to production of reactive oxygen species, as indicated by the blood GSH redox status. Antioxidants may in theory neutralize the reactive species which are produced by neutrophilic leucocytes during phagocytosis (Babior, 1984; Hemila, 1992).

In the early 1970s Pauling (1971) concluded from previously-published studies that vitamin C supplementation decreases the incidence of the common cold. However, the majority of studies carried out thereafter have not found that regular vitamin C supplementation (> 1 g/d) has any marked effect on the incidence of the common cold (for review, see Hemila, 1992). The results of three placebo-controlled studies that examined the effect of vitamin C supplementation on the incidence of the common cold in subjects under heavy physical stress were analysed (Hemila, 1996). In one study the subjects were schoolchildren at a skiing camp in the Swiss Alps, in another study the subjects were military troops training in Northern Canada, and in the third study the subjects were participants in a 90 km running race. In each of the three studies a considerable reduction in the incidence of the common cold was found in the group supplemented with vitamin C. These studies indicate that vitamin C may reduce the risk of contracting an infection after extreme exercise. In the third study mentioned previously, Peters *et al*. (1993) evaluated the effect of vitamin C on the incidence of URTI during the 2-week period following the 90 km Comrades Ultramarathon. The incidence of URTI was 68 % in the placebo group, which was significantly more than that in the vitamin C supplementation group, where only 33 % reported URTI when taking a 600 mg vitamin C supplementation daily for 3 weeks before the race. In another study Peters *et al*. (1992) found that vitamin A supplementation had a non-significant effect on the incidence of URTI in marathon runners.

Only one study (Nieman *et al*. 1997*b*) has evaluated the effect of vitamin C on lymphocyte function and stress hormone levels after exercise. Supplementation with vitamin C did not influence leucocyte subsets, NK cell activity, lymphocyte proliferative responses, granulocyte phagocytosis and activated burst, catecholamines or cortisol. In a recent double-blind placebo-controlled study by Nielsen *et al*. (1998) N-acetylcysteine (6 g daily for 3 d) had no effect on exercise-induced suppression of lymphocyte proliferation or NK cell activity.

In a study of the age-related response of neutrophils and muscle damage to eccentric exercise, Cannon *et al*. (1990) examined subjects < 30 years old and subjects > 55 years old. The subject groups were further divided in a double-blind placebo-controlled protocol which examined the influence of 48 d of dietary vitamin E supplementation before the exercise. All subjects were monitored for 12 d after exercise. Dietary supplementation with vitamin E tended to eliminate the differences between the two age-groups, primarily by increasing the responses of the older subjects.

The same group investigated the influence of damaging eccentric exercise on *in vitro* production and plasma concentrations of cytokines and their relationship to muscle breakdown. In a double-blind placebo-controlled study they examined the effect of vitamin E supplementation for 48 h on the exercise-induced acute-phase response. The volunteers were either young (average age 25 years) or elderly (average age 65 years) sedentary men. They performed 45 min of eccentric exercise (downhill treadmill). At 24 h after this single session of eccentric exercise endotoxin-induced secretion of IL-1β was augmented in cells obtained from the placebo subjects, but no significant effect was observed in cells from the vitamin E-supplemented subjects (Cannon *et al*. 1991).

The finding by Cannon *et al*. (1991) that IL-1β and TNF-α secretion were increased the morning after exercise without any current changes in mononuclear cell numbers indicates that the monocytes are activated in relation to eccentric exercise. The effect of vitamin E on IL-1β and IL-6 could not be ascribed to changes in PGE_2 (Cannon *et al*. 1991). Oxygen radicals enhance endotoxin-induced IL-1 production (Kasama *et al*. 1989). Furthermore, the concentration of these reactants increases with exercise (Davies *et al*. 1982). Thus, the effects of vitamin E on the secretion of IL-1β are consistent with a mechanism involving oxygen radicals. No studies have measured the influence of antioxidants on plasma cytokines, which may be a better reflection of the *in vivo* situation.

Conclusion

Immunocompetent cells are mobilized to the circulation during an acute bout of exercise. The ability of the immune system to respond to a single bout of exercise seems to be maintained in the elderly. Following strenuous exercise the lymphocyte count declines, natural immunity and T-cell proliferation are impaired, and the level of secretory IgA in saliva is low. Few studies have addressed the potential protective role of dietary supplementation in exercise-induced immunosuppression. Exercise-related immunosuppression in animals was restored by a diet rich in *n*-3 fatty acids, but there is a lack of information from human studies. Antioxidant supplementation has no effect on exercise-induced changes in NK and T-cell functions, but vitamin E may inhibit the acute-phase response to eccentric exercise in the elderly, and vitamin C supplementation may decrease the incidence of post-race URTI symptoms. Glutamine supplementation has not been effective in abolishing post-exercise suppression of the immune system, whereas carbohydrate loading diminished the hormonal and immune responses to exercise. In our view it is premature to make recommendations regarding nutritional supplementation to avoid exercise-induced immune changes.

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