

Review

Using Stress Models to Evaluate Immuno-Modulating Effects of Nutritional Intervention in Healthy Individuals

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There is clear evidence that nutritional supplementation helps to restore immune function and contributes to optimal resistance to infections in malnourished people. However, the literature is less clear on the suggested benefits of dietary supplementation for immune function in healthy, well nourished subjects. Such studies are hampered by large variability in immune function markers and clinical outcome measures, which are known to be affected by factors such as genotype, age, gender, history of infections and vaccinations, and various stressors associated with lifestyle. Therefore, there appears to be a need to employ experimental models that control and/or manipulate the factors that are responsible for this variability. Conceivably, such a model could experimentally apply various forms of stress to physiologically suppress the immune system and assess whether nutritional intervention can (partially) compensate the deleterious effects. Here we review effects of psychological stress, physical exertion, and sleep deprivation on various aspects of immune function and susceptibility to common infections. We focus on the usefulness of such stress models to evaluate the putative beneficial role of diets/nutrients on immune function in healthy individuals.

Key teaching points:

- Nutrition plays a key role in the maintenance of a healthy immune system.
- Some forms of stress are associated with impaired immune function.
- Impaired immune function increases the risk of contracting common infections.
- Models that employ chronic psychological and/or physical stress appear to be suitable for the identification of immuno-enhancing ingredients.

INTRODUCTION

There is a large variation in susceptibility to infection among healthy individuals. Genetics, age, gender, lifestyle factors (such as diet, alcohol intake, smoking, sleep, psychological stress, and habitual physical activity), and history of infections and vaccinations may contribute to this large variation in susceptibility. This has therefore prompted research to examine the effects of various stress conditions, such as psychological stress, severe physical exertion, and sleep deprivation, on immune function (Table 1). ‘Impaired immune function’ in healthy individuals probably represents a ‘sub-optimal’ level of immune functioning that should not be compared with

‘depressed’ immune status that may occur in populations suffering from malnutrition (eg, in developing and emerging countries and sub-populations of the elderly). However, even in healthy individuals a sub-optimal immune function is potentially detrimental to everyday well-being because this may increase the risk of contracting common infections such as the cold or flu. Therefore, in the modern day environment where most people experience some form of stress that may potentially impact upon their health, there is now considerable interest in the possibility of stimulating individuals’ immune systems by nutritional means in order to optimise or maintain health (Fig. 1). Nutritional intervention strategies, such as vitamin E, vitamin A, iron, and zinc supplementation are known

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Table 1. Summary of the Major Findings for Stress Induced Effects on Immune Parameters

Immune marker	Psychological stress [2]		Exercise stress		Sleep deprivation [5-7]	
	Acute	Chronic	Acute/intense [3]	Chronic/intense [4]	Acute	Chronic
Clinical endpoint	none	↑ URTI	↑ URTI	↑ URTI	↑ URTI	?
Anti-body response to vaccine	↓ (flu)	↓ (flu)	?	no effect	↓ (Hep A)	?
DTH response	↑	↓	↓	?	?	?
NKCA	↑	↓	↑	↓	↑ / ↓	↓ / no change
Neutrophil phagocytic function	?	?	↑	↓	↓	?
T lymphocyte proliferation	↓	↓	↓	↓	↑ / ↓ / no change	?
Total T-cells (CD3+)	↓	↓	?	?	↓ / no change	↓
T-helper cells (CD4+)	↓ / no change	↓	↑	?	↓ / no change	↓
Cytotoxic T cells (CD8+)	↑	↓	no change	?	↓ / no change	↓
Inflammatory monokines (eg, TNF α , IL-6)	↑	↑	↑	?	↑ / ↓ / no change	no change
Eicosanoids (eg, PGE $_2$)	?	?	?	?	?	?
Serum [Ig]	?	?	↓ / no change	no change	?	?
Salivary [IgA]	↑	↓	↓	↓	?	?
Th1 cytokine production	no change	↓	?	?	↑ / ↓	no change
Th2 cytokine production	↑	?	?	?	?	?

URTI = upper respiratory tract infection, DTH = delayed type hypersensitivity response involving an inflammatory skin reaction, NKCA = natural killer cell activity involved with recognising and killing abnormal cells, Neutrophils = involved with engulfing and destroying microbes, T-helper cells = involved with regulating responses by secretion of cytokines, Cytotoxic T cells = involved with specific lysis of infected cells, TNF α = tumor-necrosis factor- α , PGE $_2$ = prostaglandin-2 involved with initiation of inflammatory responses, Ig = immunoglobins that are antigen-specific anti-bodies secreted by B lymphocytes, Th1 = type 1 cell mediated driven response, Th2 = type 2 anti-body mediated driven responses, Th1 cytokines (e.g., IL-2, IFN γ) and Th2 cytokines (e.g., IL-4, IL-10, IL-13) act via specific receptors to regulate the behavior of cells.

to be efficacious for reducing the incidence of infection and disease in malnourished populations [1]; however, the efficacy in healthy populations is equivocal.

In a previous review the concept of employing nutritional intervention to assist with the adaptation to stress [8] was introduced. A number of dietary components were highlighted as playing a possible role in alleviating some of the systemic

effects of stress in animal and human models, although the anti-stress effects were largely focused on hypothalamic pituitary-adrenal axis (HPA) regulation. However, Kelly (1999) did not consider immune markers and clinical endpoints, and the specific stress model used. Therefore the efficacy of employing specific stress models for nutritional immunology studies in healthy individuals remains equivocal. Thus, in the present

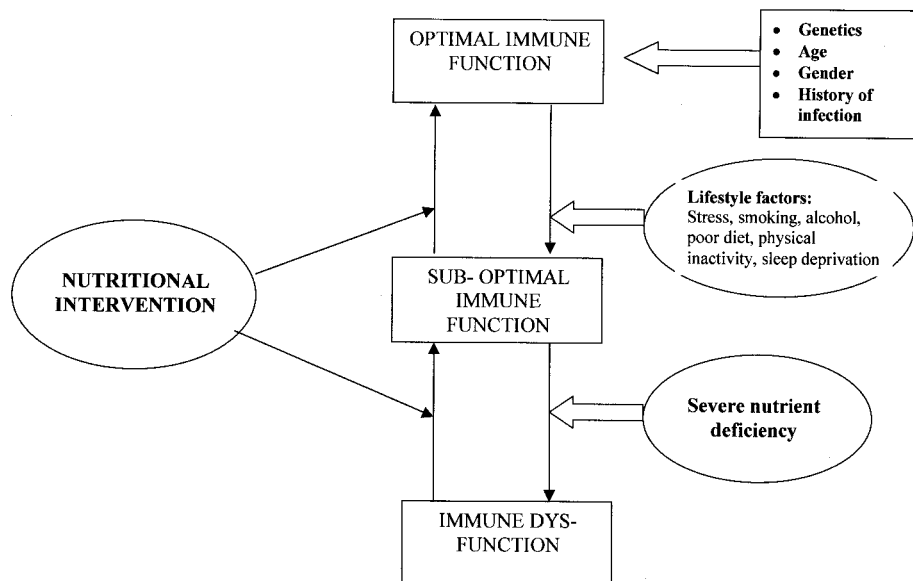


Fig. 1. Conceptual model to show factors that impact on immune function. The optimal level of immune function depends on non-modifiable factors including genotype, age, gender, and historical exposure to infections and vaccination. However, modifiable factors related to lifestyle can cause sub-optimal immune function (eg, stress, poor diet, etc). Nutrition plays a key role in the balance between optimal, sub-optimal, and immune dysfunction.

review the stress model is viewed as a tool that may be used to identify nutritional ingredients for the enhancement of a healthy immune system in normal free-living conditions. The purpose of the present review is not to comprehensively assess the efficacy of nutritional intervention for alleviating stress induced impairment of immune function, but to highlight appropriate modifiable human stress models that may be used to test the efficacy of immuno-enhancing ingredients in healthy individuals.

PSYCHOLOGICAL STRESS

Individuals undergo psychological stress when a situation is perceived to be threatening to oneself. However, psychological distress results only when the demands of the stressor are perceived to exceed one's own ability to cope. The 'fight' or 'flight' response that is often elicited in response to stress produces a number of psychophysiological changes, which include increases in cardiac output, blood pressure, skeletal muscle vasodilatation, and activation of the HPA axis and sympathetic nervous system (SNS) causing the release of a number of important stress hormones (cortisol and catecholamines). The effects of stress on the body can be differentiated into 'acute' and 'chronic.' Acute stress relates to the immediate adaptive responses to a discrete event (eg, laboratory stress task, bereavement, examinations) whereas chronic stress relates to the accumulative effects of every day hassles and life events (eg, work pressure, interpersonal conflict, bereavement).

Acute Stress

Acute stress has been generally associated with immune stimulation, with increases in delayed type hypersensitivity (DTH) response, natural killer cell activity (NKCA), CD8+ cell number, inflammatory monokines, type 2 (Th2) cytokines and mucosal immunoglobins (Ig), with the exception of reduced lymphocyte proliferation response (Table 1). Cortisol release during acute stress is thought to shift the balance away from Th1 driven cell mediated responses toward Th2 driven anti-body mediated responses, which may increase susceptibility to certain infections. The data from Cacioppo *et al.* [9] demonstrated that high cardiac and HPA reactivity in response to an acute stress task (6-min mental arithmetic/6-min speech task) predicted immuno-suppression to latent Epstein-Barr Virus (EBV). Also, Cohen *et al.* [10] demonstrated that subjects showing greater cortisol reactivity to a simulated public speaking task (2-min preparation followed by a 3-min video taped speech delivery) had increased risk of verified upper respiratory tract infection (URTI) but only when they reported high negative life events. However, cross-sectional analysis demonstrated that subjects showing smaller immune responses (NKCA and CD8+ cell reactivity) to the speech task had

increased risk of self reported URTI during high stress weeks [10]. Unfortunately, in the Cohen study [10], of the 51 subjects meeting the self reported criteria for URTI, only 30 actually went to the clinic for verification. Therefore, because of the limitations in the number of verified URTI's, the later reported analysis relied upon self-reported URTI that may reflect bias in reporting of illness. In another recently published cross-sectional study Miller *et al.* [11] demonstrated that subjects with higher levels of self-reported cumulative daily stress levels in the 10 days following influenza vaccination had significantly reduced antibody responses compared with lower-stress groups. Taken together, these studies show that individual stress reactivity may be useful to predict those individuals most susceptible to infection, although from the current correlational type of data it is not possible to infer causation.

Chronic Stress

Epidemiological studies have demonstrated that family conflict and high life event stress is correlated with an increased incidence of serologically verified URTIs [12–14]. Early virus-challenge studies, in which subjects were exposed to a cold or influenza virus and assessed for stress levels using stressful life events or emotional distress scales, provided mixed support for a relation between chronic stress and impaired immune response [15,16]. However, more recently various studies have provided evidence to indicate that chronic stress (or life events) suppresses the secondary immune response to vaccination [17–20]. It is unethical to experimentally induce severe chronic stress in human subjects, which unfortunately considerably weakens the experimental design of these studies [17–20] and therefore makes it difficult to imply causation from the observational data. Chronic stress is also associated with suppression of other immune markers such as reduced DTH response, NKCA, CD4+ and CD8+ cells, lymphocyte proliferation response, and Th1 cytokines (Table 1).

In summary, up-regulation of the immune system in response to acute stress may be viewed as a normal adaptation to psychological challenge, whereas chronic stress may be associated with more serious impairment of immune function, which may have implications for ill-health. However, it is also likely that chronic stress influences the immune responsiveness to acute stress [21,22].

Nutritional Intervention and Psychological Stress Models

Although common in animal research, psychological stress models in humans to examine the efficacy of immuno-enhancing ingredients have rarely been employed Shenkin *et al.* [23] recently presented data from a randomised placebo controlled trial to show micronutrient supplementation did not affect the immune response to examination stress in students. However, there are indications from correlational data to suggest that dietary intake may play an important moderating role in the

overall relationship between psychological stress and immune function. Firstly, there is evidence to demonstrate stress may evoke a change in diet, although taken together the data is inconsistent and suggests that the stress effects on the diet are dependent on the type of stress and individual responses. For example, an identical stressor may cause some people to increase food intake whilst others to decrease their intake. However, Oliver *et al.* [24] examined students during stress and showed that irrespective of whether eating increased or decreased, sweet foods and chocolate intake increased in both groups whilst fruit, vegetables, meat, and fish decreased in both groups. From a neurochemical viewpoint, the best coping strategies to alleviate stress and mood deterioration would result in increased activity of relevant serotonergic and opioid mediated pathways, which may be achieved by high carbohydrate/low protein consumption. High carbohydrate/low protein meals, which raise the plasma ratio of tryptophan, have been demonstrated to produce anti-depressant effects on clinical populations [25]. Furthermore, increases in brain serotonin appear to modulate adreno-cortical reactivity probably through alterations in 5-HT_{1a} and 5-HT₂ receptor sites located at the hypothalamic and pituitary brain area [26]. In two randomised placebo controlled trials, Markus *et al.* [27,28] examined the effect of altering the tryptophan/large neutral amino acid ratio on the cortisol response to stress. In the first study [27], a high carbohydrate/low protein meal, which increased plasma Try:LNAA by 42%, reduced the cortisol response to a laboratory stressor in high stress prone subjects. In the second study [28], a dietary protein enriched in tryptophan (α -lactalbumin) had a similar effect in reducing cortisol responses to a laboratory stressor.

EXERCISE STRESS

There is a general perception that athletes are under increased risk of URTI during periods of intense exercise training and after competition [29]. In many ways exercise stress provokes similar physiological responses to psychological stress and can also be viewed in terms of acute and chronic. Acute exercise relates to a single bout of activity and is often described in relation to an individual's maximum aerobic power (VO₂max) for a certain duration (e.g., 30 min at 50% VO₂max, which would be the equivalent of brisk walking). Chronic exercise refers to the accumulation of regular bouts of exercise (training).

Acute Exercise

The incidence of URTI after a major event such as the marathon is elevated between 2–6 times in runners compared with matched non-participants [30] and an impaired DTH response has been observed in triathletes following a long triathlon event [31], although these results have not been repeated

using a randomised experimental design. Also, a relationship between declines in salivary IgA concentration during acute intense exercise and the appearance of URTI in athletes has been observed [32,33]. However, when using athletes in acute exercise studies it is difficult to distinguish whether the effects are related to an acute or chronic exercise stimulus because all athletes are chronically trained leading up to a major event, such as a marathon. Other important findings include 10–21% reductions in lymphocyte proliferation response (on a per cell basis) after severe exercise [34,35]; increases in NKCA of up to 100% during or immediately after exercise (which may be a reflection of changes in circulating cell number as opposed to changes in cytotoxic function), followed by a 10–60% NKCA suppression during recovery [36]; increases in the phagocytic function of neutrophils that may remain increased for up to 24 hrs [37]; an increased inflammatory cytokine response [38]. For a further in depth review of this area readers are directed to Rowbottom and Green [3].

Chronic Exercise

The risk of URTI appears to be related to training volume in athletes. For example, Heath *et al.* [39] reported a dose-response relationship between yearly training distance and risk of URTI in runners. There is consistent data to demonstrate that chronic, intense training alters neutrophil priming, decreases NKCA and neutrophil phagocytic function, and reduces Ig levels [4]. However, competitive swimmers who were monitored for 7 months throughout their training, that exhibited Ig levels in the lowest 10th percentile relative to clinical norms, were still able to mount clinically appropriate antibody responses when immunised with a pneumococcal vaccine [40]. Furthermore, no impairment of antibody response to other vaccines (tetanus and diphtheritis toxoid) were found in triathletes compared with controls [31].

In summary, the literature suggests that in response to high intensity, acute exercise and intense training periods athletes suffer mild suppression of the immune system. It is possible that these mild immuno-suppressive effects of exercise only become detrimental to the athlete's health if combined with other factors such as psychological stress, poor nutritional status, and inadequate recovery and rest. Therefore, more complex models that also consider these additional lifestyle factors may provide a clearer picture.

Nutritional Intervention and Exercise Stress Models

The exercise stress model has been employed by a number of researchers to examine the efficacy of immuno-enhancing ingredients. An intense bout of exercise, such as marathon running, is usually adopted in these models. There is some evidence to suggest that consumption of carbohydrate drinks during intense training and competition may attenuate some of the immuno-suppressive effects of prolonged exercise. For example, prevention of exercise-induced falls in neutrophil

function [41] and reduction in the extent of diminution of PHA-stimulated T-lymphocyte proliferation following prolonged exercise [42] have been observed. The mechanism may be associated with an attenuated rise in plasma catecholamines and cortisol following carbohydrate feeding during exercise. However, Nieman *et al.* [43] recently showed that carbohydrate supplementation during a competitive marathon race did not prevent declines in salivary IgA concentration and there were no differences between the placebo and carbohydrate group for those runners reporting URTI during the 15 day post-race period. Nevertheless, Nieman *et al.* [44] have recently shown that carbohydrate supplementation during a 3 hr run on a treadmill in experienced marathon runners resulted in an attenuation of the increase in plasma IL-6, IL-10, and IL-1ra, and gene expression for IL-6 and IL-8 in comparison with placebo in a randomised trial.

Bouic *et al.* [45] using a double blind placebo controlled trial also utilised the marathon running stress model to examine the effect of a mixture of plant sterols/sterolins on red and white blood cell counts, CD3+ and CD4+ lymphocyte subsets, and neutrophils. However, unfortunately the experimenters were unable to gain access to the athletes until 3 days after the event, and given the differences in baseline between the groups for some measures (B cells, IL-6, and cortisol) the findings appear to merely reflect differences in individual variation.

That glutamine is considered to be important for lymphocyte function and macrophage phagocytic activity and is known to decline during prolonged exercise has prompted a number of intervention studies to examine the effects of glutamine supplementation on immune function following exercise. Two studies [46,47] have provided evidence to demonstrate that oral glutamine supplementation can have beneficial effects on the immune system. For example, Castell *et al.* [46] demonstrated that glutamine consumed immediately after and 2 hr after a marathon reduces the incidence of URTI in the 7 d following the race. However, other studies have not found glutamine supplementation to have beneficial effects on exercise-induced immune suppression [48,49], despite the maintenance of pre-exercise plasma glutamine level. These discrepancies in the literature may be related to exercise mode and intensity because the positive findings [46,47] were obtained during competitive races (marathon running and triathlon) whereas the studies that did not support the effects of glutamine supplementation used laboratory simulated cycle ergometry exercise [48,49]. Furthermore, Rohde *et al.* [48] used an intermittent exercise protocol consisting of bouts of 60, 45, and 30 min each separated by a 2 hr recovery that may have been less demanding than a continuous exercise bout.

Two other double blind placebo controlled randomised studies have examined the effects of ginseng and echinacea on the mucosal immune response using a "Wingate cycling test" model [50,51]. This model differs from previously discussed exercise models because it consists of three consecutive 30 sec exercise bouts of maximal intensity with passive recovery in

between echinacea [51] but not ginseng [50] supplementation eliminated the mucosal immune suppression that was apparent in the placebo group after the Wingate testing. However, given that athletes who have severely depressed IgA levels can mount clinically appropriate antibody responses [40] suggests that the 43% decline in IgA that was observed in the placebo group after the Wingate test may have little clinical relevance. Thus, the conclusions drawn from these studies regarding the immuno-modulating effects of echinacea and ginseng should be interpreted with caution because using the mucosal immune marker alone is not adequate enough to demonstrate efficacy.

SLEEP DEPRIVATION/LOSS AND INSOMNIA

Sleep is commonly considered a restorative process with supportive influences on immune functions. There is considerable circadian rhythm of the immune system, which is likely to be linked with both circadian and sleep dependent hormone release [52]. For example, cortisol is known to peak in the early morning inducing an influx of neutrophils from the bone marrow and growth hormone is secreted during sleep promoting T-cell function [53]. The experimental paradigms used to study the effect of sleep deprivation on immune function include prolonged periods of wakefulness for up to 72 hrs (total sleep deprivation: TSD) or disrupted sleep (partial sleep deprivation: PSD), which, for example, may involve sleep deprivation from 3am–7am.

In general, the sleep models have produced inconsistent findings [5]. For example, monocyte cell counts were reported to be unaffected after 48 hrs of TSD [54], but also increased after 64 hrs TSD [5]. PHA-induced lymphocyte blastogenesis has been reported to decrease [55,56] and also not change after TSD [5]. Similarly, PWM-induced lymphocyte blastogenesis has been reported to increase [56] or show no change [5]. Also, NKCA increases [5] and decreases [57] have been observed. For the cytokines, inconsistencies have also been reported. For example IL-2 was decreased after PSD [58] but elevated after a night of TSD [56]. Also, IL-6 secretion was reduced during PSD [52], not effected after TSD [5], or increased during the daytime post-sleep deprivation [59]. These inconsistencies may arise from different blood sampling protocols (eg. some studies sampled every 2 hrs whereas others may have sampled every 20–24 hrs) which may not take diurnal variation into consideration. There appears to be limited research regarding the effects of sleep deprivation on the adaptive immune response. Only recently has such a study been conducted that examined the effect of a single night of sleep deprivation on the antibody response to Hepatitis A vaccination on the previous day [60]. The results showed that subjects who had regular sleep after vaccination displayed nearly a two fold higher antibody titre ($p = 0.18$) after 4 weeks compared to sleep deprived subjects. These recent findings provide more substantial evidence to

support a relationship between sleep deprivation and immune suppression. In addition, other recent studies have implied that the quality and depth of sleep may be a more important determinant of immune function. For example, Redwine *et al.* [52] suggested that increased secretion of IL-6 during sleep was associated with stages 1–2 and REM sleep, whereas IL-6 secretion during slow wave sleep was comparable to the levels found whilst awake. Furthermore, immune alterations from sleep deprivation and the potential to impact upon one's health may be dependent on sleeping patterns over a chronic time period. For example, Savard *et al.* [6] showed that patients with chronic insomnia demonstrated reductions in CD3+, CD4+, CD8+ cell counts, although no differences in NKCA, to the acute stress caused by a sleep laboratory procedure in comparison with good sleepers. Irwin *et al.* [7] further observed reduced NKCA in patients with chronic insomnia during a laboratory sleep study. This discrepancy between the two studies may be because the insomnia patients in Irwin's study showed distinct differences in sleeping patterns (efficiency, electroencephalography (EEG) sleep continuity, and stage 2 rapid eye movement sleep) compared with controls although there were no such differences (EEG, electro-myography, electro-oculography, respiratory disturbance, and limb movement) between the groups in Savard's study. Nevertheless, taken together this data shows that chronic sleep deprivation influences some markers of immune function. The psychological distinction between sleep deprivation (a procedure shortening sleep because of reduced opportunity to sleep) and insomnia (a complaint of reduced sleep despite the opportunity to sleep) may be a key aspect in the relationship between sleep and immune function.

Nutritional Intervention and Sleep Deprivation Models

Presently, the authors are unaware of any studies that have utilized sleep deprivation models to examine the efficacy of immuno-enhancing ingredients. However, given that a reasonably large proportion of the population undertake shift work, this would appear to be an important area of future work.

CLUSTER MODELS

The relationship between stress and immune function is complex and it is unlikely that one factor such as psychological stress is causally related to sub-optimal immune function, but more likely that the relationship is mediated by a number of other lifestyle factors, plus genetics, age, gender, and previous history of infection (Fig. 1). Thus, it may be important to consider a cluster of factors when examining models of impaired immune function.

Psychophysiological Challenge and Sleep Deprivation

The army training environment provides a useful model that can be used to examine the combined effects of various stressors on immune function. Military life is characterised by extreme physical exertion, psychological stress with a loss of autonomous control, and sometimes sleep and food deprivation. A number of studies have been conducted using army cadets to examine immune function during basic training. Boyum *et al.* [61] examined immune parameters in men participating in a 5–7 day military training exercise that involved continuous light physical exercise (35% VO₂max), sleep deprivation (2–3 hrs of sleep during the whole course), and calorie restriction (with a daily intake less than 3,000 kJ). Results demonstrated significant reductions in CD4+ T cells, CD8+ T cells, B cells, and NK cells. Also, serum levels of immunoglobulins were decreased (IgG 6–7%; IgA 10–20%; IgM 20–35%). Although there was no increase in infection rate during the 5 wk follow-up period, these data suggest that certain immune parameters were suppressed during the training. That light exercise has been associated with immuno-enhancing properties suggests that when combined with the other stressors there may be an immuno-suppressive effect. Furthermore, that the cadets were all young (22–24 yrs) and healthy may also have explained why no clinical outcomes were observed. Glaser *et al.* [62] examined the effect of a six week cadet basic training period (a combination of physical and mental stress) followed by academic exams on the reactivation of three latent herpes viruses. The results showed that Epstein-Barr virus (EBV) antibody titers were significantly higher during the examination week compared with baseline and during the six week basic training. That academic stress and not basic training modulated the expression of latent EBV suggests the type of stressor is important in determining the immunological effect. An important aspect of this differential immune modulation may be activation of the HPA axis—catecholamine and cortisol patterns in response to stressors may be largely dependent on perception of distress rather than physical effort [63]. Thus the perceived social support that existed during basic training compared with the individual challenge of exams may have resulted in greater cortisol responses and therefore immuno-suppressive effects during the latter situation.

Shift workers provide a further useful model to examine the combined effects of mental stress and sleep deprivation/disturbance. Two studies have examined immune parameters of shift workers in comparison with day working controls [64,65]. Shift workers rotated among three sequential shifts that were changed on a weekly bases. Both studies provided evidence of reduced T-lymphocyte function (PHA and con A stimulation) in the shift workers. There is also further evidence for a relationship between mental stress/sleep deprivation and sub-optimal immune function, where sleep disturbance in depressed patients appears to moderate immune function [66–68]. For

example, Hall *et al.* [66] studied individuals seeking treatment for bereavement-related depression and observed that intrusive thoughts and avoidance behaviors were associated with more time spent awake during the first non-rapid eye movement period, which was associated with lower NK cell numbers.

Lifestyle

Two cross-sectional studies [69,70] have demonstrated a relationship between poor lifestyle practises and suppressed immune function. Nakano *et al.* [70] administered a questionnaire to 291 middle aged workers that was composed of 19 items to assess lifestyle, which included working habits, sleeping, exercise, smoking and alcohol consumption, health condition, and a number of stress measures. Each item was assessed as healthy or unhealthy and analysis was then performed using the total number of unhealthy practices for each individual as the independent variable. The proliferative responsiveness of the lymphocytes to PHA was significantly negatively correlated with the number of unhealthy lifestyle practices ($r = -0.37, p < .05$). However, due to the relatively weak design of the questionnaire and data analysis it is impossible to identify which lifestyle factors were responsible for the effects. Also, the questionnaire did not contain any questions relating to diet, which would be considered as an important lifestyle factor. Morimoto *et al.* [69] employed a slightly different questionnaire assessing 8 lifestyle practises (smoking, alcohol consumption, eating breakfast, sleep, work hours, exercise habits, nutrition, and mental stress) but again assessed each item as 'healthy' or 'unhealthy.' Three groups were subsequently formed comprising poor, moderate and healthy lifestyle. Subjects with healthy lifestyles showed significantly greater NKCA. Stable mental health status was also related to greater NKCA activity and smoking was related to lower lymphokine activated killer cell activity. That other studies examining the effect of chronic stress and immune response to vaccination [17,71] have not found other lifestyle factors to impact on the relationship between chronic stress and immune function suggest that psychological stress itself is the main mediating factor in lifestyle models. It is probable that stressed individuals are more likely to have unhealthy lifestyles, such as poor sleep, alcohol and drug abuse, etc., but it seems that these factors do not directly impact on immune function.

However, another important mediating factor may be age. Glaser *et al.* [72] have demonstrated that differences in the immune response between chronically stressed subjects and controls was age dependent. Age related immune dysfunction may influence the stress-immune response through an enhanced release of cytokines such as IL-6, which may effect the functioning of the endocrine system. IL-6 is known to be a potent stimulator of corticotropin-releasing hormone, which may lead to heightened HPA activity and cortisol levels during stress. Furthermore, older adults may be more vulnerable to

negative emotions and distress due to smaller social support networks and ineffective coping strategies.

CONCLUSIONS AND RECOMMENDATIONS

There is a need to employ experimental models that control and/or manipulate the factors that are responsible for variability in immune function, which can be used to more clearly identify the impact of nutrition on immune function in healthy individuals. Psychological stress and exercise appear to produce robust changes in immune function. However, only the exercise model has been extensively employed to study the efficacy of immuno-enhancing nutrients. These exercise models have mostly employed prolonged intensive exercise stress (such as marathon running) to produce changes in immune parameters, although short-term maximal bouts of exercise also seem to be an alternative strategy. Inconsistencies in the exercise/nutritional immunology literature seem to arise due to the intensity and mode of exercise, the exercise setting, the range of immune markers measured, and the experimental design. For example, running in a marathon (a competitive environment) seems to produce more consistent effects possibly because the athletes are prepared to run at higher intensities and there may be added psychological stress. However, in competitive environments it is often difficult to randomise subjects to exercise or non-exercise conditions. The chronic psychological stress and sleep deprivation models are potentially useful models for future nutritional immunology research, although further research into sleep and immune function is required to elucidate the effects of chronic sleep deprivation, psychological interactions, and the clinical relevance of sleep related immune alterations.

Although models that target clusters of stressors do not seem to produce any greater or more robust effects on immune function, these models may be also useful for future research. For example, the army training environment provides a readily available pool of healthy subjects who are exposed to a cluster of different stressors, where, due to the regimented nature of their environment, it is also relatively easy to manipulate their dietary intake through nutritional intervention. Furthermore, the nutritional status of subjects is an important factor to be considered when employing an appropriate model. For example, female athletes appear to be at risk of compromised nutritional status, which in combination with the effects of exercise stress, could increase the risk of impaired immune function. Also, students who may be at similar risk of under nutrition, combined with an examination period may provide another useful model.

The strongest evidence for the efficacy of immuno-modulating nutrients should be provided by a double blind placebo controlled randomised trial that utilises clinical outcome measures, such as the occurrence of URTI, and measures from a

challenged immune system, such as antibody response to vaccination. A number of nutritional intervention studies that have been examined in the current review have merely provided “status immune markers,” such as cell counts and concentrations, which cannot provide information on immune function *per se*. Therefore, it is important that future studies measure a range of immune markers that will allow accurate conclusions to be drawn. For example, although carbohydrate supplementation produces attenuated rises in IL-6, IL-10, and IL-8 after marathon running, it has not been shown to prevent falls in sIgA that suggests carbohydrate may act on specific immune parameters. Thus, the specific model of immune suppression should be related to the action of the active immuno-modulating component of the ingredient. For example, severe exercise appears to cause suppression of innate immune function and therefore this model should be used to test immuno-modulating ingredients that are thought to have an effect on innate immune function. In contrast, chronic psychological stress in humans has been shown to effect some aspects of the adaptive immune response. Finally, because of the inherent large variability between individual immune responses and the measures of immune function it is important that the models possess a certain degree of repeatability and reliability that may be achieved by testing with known immuno-modulating actives before being used to assess the action of unknown ingredients.

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