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Clinical Application of Zinc and Vitamin C

Key Points at a Glance

Ascorbic Acid

- required for immune function, wound healing, production and maintenance of connective tissue
- antioxidant, anti-histamine
- clinically demonstrated to:
  - reduce the incidence of common cold in certain populations and the duration and severity of common cold in adults and children (may require high doses)
  - improve sperm parameters
  - increase the antioxidant capacity of skin
  - reduce marathon-induced increases in cortisol and inflammatory cytokines
  - attenuate the negative physiologic response to psychological stress (high dose)
  - improve tissue structure in periodontitis

Zinc

- required for normal immune system function, normal psychological and neurological development, wound healing
- essential for normal sexual function and reproduction
- antioxidant
- clinically demonstrated to:
  - reduce severity and duration of common cold in adults (>75 mg/day)
  - reduce days with upper respiratory tract infection symptoms
  - improve various aspects of immune function in adults, the elderly and children
  - improve some sperm parameters in sub-fertile men and improve pregnancy rates (may require high doses)
  - reduce hirsutism, alopecia and oxidative stress in women with polycystic ovarian syndrome
  - promote fracture healing in adults and bone formation in adults and children
  - promote healing of foot ulcers in diabetic patients
  - improve healing time in pilonidal sinus patients (high dose)
  - assist in brain recovery from head trauma (total parenteral nutrition + oral dose)
  - improve aspects of verbal and visual IQ in school-aged children and possibly support neurodevelopment in infants and school aged children
  - improve mood in adults who are taking antidepressant medications as well as those who are not
  - improve severe and inflammatory acne
  - reduce incidence of diarrhoea in children born to supplemented mothers
  - reduce urinary excretion of hydroxyhemopyrrolin 2-one (combined with vitamin B6; may require high doses)

Lysine

- required for synthesis of collagen, carnitine and elastin
- antiviral
- clinically demonstrated to:
- possibly reduce incidence of herpes simplex outbreaks when used in conjunction with a low-arginine diet and at doses higher than 1000 mg/day

Quercetin

- antioxidant, antiviral, antiallergenic, neuroprotective

Vitamin B6

- required for GABA, serotonin and dopamine production
- clinically demonstrated to reduce symptoms of premenstrual syndrome (requires up to 100 mg/day)

Manganese

- required for wound healing, superoxide dismutase production

Selenium

- required for thyroid hormone metabolism, normal male fertility and sperm production
- antioxidant, immunomodulatory
- clinically demonstrated to:
- improve some sperm parameters
- possibly reduce corticosteroid use in asthmatics (may require high dose)

β-Carotene

- pro-vitamin A, antioxidant, immune modulating
- may support immune function and provide antioxidant activity, although clinical results vary

Indications

- Common cold, immune dysfunction.
- Wound healing, acne.
- Stress support, antioxidant.
- Male infertility.
- To promote healthy cognition; depression.
- Cold sore prevention.
- Possibly as an adjunctive treatment in asthma.

Vitamin C

The link between vitamin C deficiency and infection was first identified in the early 20th century when scurvy was found to be associated with pneumonia. Animal studies have shown that vitamin C might be useful in the prevention or eradication of infections from viruses, bacteria and protozoa.[1] Maternal dietary vitamin C intake has a beneficial effect on infant immunity, and is inversely related to infant allergic disease, wheeze and food allergy.[2]

The importance of vitamin C in wound healing is seen in the case of deficiency which causes fragile granulation tissue and poor wound healing.[3]

Vitamin C is required for the formation of collagen and proteoglycans and as such is essential for the production of bone, skin, capillaries and other connective tissues.[4]

Vitamin C is a water-soluble antioxidant which scavenges free radical oxygen and nitrogen species, regenerates vitamin E, and promotes reduced glutathione.[3]

Neural and adrenal cells contain the highest levels of ascorbate in the body. In the brain, it is an important antioxidant (for example, scavenging oxygen and nitrogen radicals from rapid neuronal mitochondrial metabolism), is involved in neuronal maturation, neuronal catecholamine synthesis and acts as a cofactor for the conversion of dopamine to norepinephrine. Adrenal catecholamine synthesis also relies on vitamin C[5] and vitamin C is excreted from the adrenal glands in response to ACTH.[6]

Clinical Studies

A 2013 Cochrane review assessing regular vitamin C supplementation revealed the following:[7]

- Regular supplementation resulted in no fewer occurrences of the common cold in most populations, except for those partaking in intense physical training and/or exposed to extreme cold where the incidence was halved. Successful trials used daily doses from 250-1000 mg in adults and 1000 mg in children for 2-8 weeks.
- **Duration of common cold was reduced** by regular vitamin C supplementation in adults by 7.7% and in children by 14.2%. In these trials supplementation in adults was mostly 1 g/day, but ranged from 500 to 3000 mg/day for a duration of 2 weeks to 9 months. In children and adolescents, the dose ranged from 200 to 2000 mg/day and duration from 2 weeks to 9 months.
- A small, significant effect was found on the reduction cold severity as measured by time away from school or work with either regular vitamin C supplementation or treatment. The dose used was mostly 1 g/day for both adults and children (range 250-3000 mg/day) for 3-5 months.

The effect of vitamin C supplementation on **improving sperm parameters** has shown positive results in several studies. A small trial including 13 infertile men, showed that 1000 mg twice daily for 2 months resulted in a significant improvement in sperm count, motility and morphology.[8] Similarly, vitamin C given at 500 mg/day for 3 months after varicocelectomy resulted in a significant improvement in normal motility and morphology compared to placebo.[9] When compared with weight loss in normal, overweight and obese patients, 1000 mg vitamin C every second day for 6 months improved sperm concentration and motility in all groups, whereas exercise only improved sperm parameters in overweight and obese participants.[10]

In a 1974 study, 20 surgical patients with pressure wounds were supplemented with either placebo or 1 g ascorbic acid/day for one month. Supplemented patients had an 84% reduction in mean wound area compared with 44% in the placebo group.[11] A clinical study using vitamin C at a dose of 500 mg or 10 mg (as a control) twice daily showed no benefit in pressure ulcer healing rates over 12 weeks.[12] Other studies have mostly examined the effect of vitamin C combined with other nutrients with the exception of several trials displaying the benefit of intravenous vitamin C in recovery of severe burns patients.[13]

Supplementation with 100 or 180 mg/day vitamin C for 4 weeks has been shown to **increase the radical-scavenging activity** in the skin by 22% and 37% (100 vs 180 mg/day) with no changes found in the placebo group.[14]

In a group of 29 ultra-marathon runners, 500 mg, 1500 mg vitamin C or placebo was provided for 7 days prior to the race, the day of and 2 days after. The 1500-mg dose was found to reduce the post-race elevation in cortisol as well as interleukin-1 receptor agonist (IL-1RA) and IL-10, with a trend towards reduced IL-6 and IL-8. Researchers suggest that the attenuation of elevated cortisol and cytokines may be the mechanism by which vitamin C can reduce infection incidence in athletes.[15,16]

A 14-day, randomised, double-blind, placebo-controlled trial compared a daily dose of 3 g sustained release ascorbic acid with placebo on participant's response to psychological stress and found that the ascorbic acid group had reduced systolic and diastolic blood pressure, subjective stress rating and cortisol response, but not overall salivary cortisol levels.[17]

A 1983 study compared the effects of a daily intake of 30 mg with 3000 mg ascorbic acid for 3 weeks in police cadets who were also on a vitamin C-restricted diet prior

to completing a stress-inducing task. Cadets in the low-dose group had a higher baseline urinary excretion of adrenaline, however those in the high-dose group excreted more cortisol in response to the stressful task. The authors suggested that the increased cortisol response may be a positive reaction to stress, better preparing the individual for the task.[18]

In a small controlled trial involving 21 participants with progressive periodontitis, 70 mg/day of ascorbic acid vs no treatment was compared and was found to **improve periodontal tissue** structure by increasing cellular adhesion through increased cytoplasmic joint bridges and desmosomes.[19]

Zinc

Zinc is involved in the function of over 300 enzymes.[20] It's role in immune health can be seen in developing countries where deficiency is common, leading to growth retardation and compromised immunity, specifically:[21]

- thymic atrophy and lymphopaenia;
- compromised macrophage phagocytosis, intracellular killing and cytokine production;
- reduced neutrophil and natural killer cell defenses;
- impaired T and B cell proliferation, cytokine production and antibody production.

Although a distinct mechanism for zinc's involvement in immune function has not been identified, researchers suggest that zinc deficiency-related immune dysfunction can be attributed to a loss of fundamental cellular functions such as DNA replication, RNA transcription and cell activation, proliferation and survival. It is also thought to behave as a signalling molecule similar to cyclic AMP regulating cellular processes such as cytokine production.[21]

Healthy reproductive function in both males and females is reliant on zinc.[3] It is essential for the production of sex steroid hormones, sperm production and maturation,[22] conception, implantation, and healthy pregnancy outcomes.[23] Low seminal plasma zinc levels are associated with infertility in males.[23] In females, zinc is required for ovulation, fertilisation and the metabolism of androgens, oestrogen and progesterone.[3]

Zinc deficiency is also associated with poor wound healing. Again, researchers are unclear of the exact mechanism by which zinc affects tissue repair, but suggest it may be through binding to cell membrane repair proteins,[24] and zinc-dependant metalloproteinases which encourage autodebridement and the migration of keratinocytes that occurs during wound healing.[25]

Zinc is thought to be involved in neurogenesis, synaptogenesis and neuronal migration. Healthy neurocognitive function relies on adequate zinc levels as does neurodevelopment *in utero*, infancy and adolescence when the brain is growing rapidly.[26]

The absorption of zinc citrate was compared with that of zinc gluconate and zinc oxide in healthy individuals in a randomised, double-blind, 3-way crossover study, where each participant acted as her/his own control. Zinc citrate and zinc gluconate did not differ in their absorption, however both forms were superior to zinc oxide absorption. Three participants did not absorb zinc in the oxide form.[27]

Clinical Studies

The effects of zinc supplementation on immune function are detailed *below in Table 1*.

A meta-analysis of the effect of zinc supplementation in 563 **infertile men** showed that zinc from sulfate or gluconate at dosages ranging from 15 to 100 mg of zinc per day for between 45 days and 6 months resulted in a significant increase in the percentage of normal sperm morphology, sperm motility and semen volume.[23] A 1998 uncontrolled trial not included in the above analysis revealed a significant increase in pregnancy rate (22.5% vs 4.3%) in couples where the male partner received 113 mg/day of zinc as sulfate for 3 months.[28]

Forty-eight women with **PCOS** received either 50 mg/day of zinc as sulfate or placebo for 8 weeks. All participants also took metformin (1500 mg/day). After 8 weeks alopecia was reduced significantly in the zinc group (41.7% vs 12.5 %). Hirsutism and malondialdehyde levels were also significantly reduced in the treatment group. Levels of hs-CRP rose significantly in the placebo group but dropped (non-significantly, $p = 0.06$) in the zinc group. No effects were seen for acne, reproductive hormones, nitric oxide, glutathione, total antioxidant capacity or waist and hip circumference.[29]

A small trial conducted in Saudi Arabia showed that 50 mg of zinc as sulfate taken daily for 60 days had a positive effect on serum zinc and alkaline phosphatase levels, as well as on callus formation and fracture healing time in patients with **bone fractures**.[30]

A study of 147 premenarcheal girls found that supplementation with 9 mg/day of zinc as sulfate for 4 weeks resulted in an increase in procollagen type 1 amino-terminal propeptide, a marker of bone formation.[31]

A daily dose of 50 mg of zinc as gluconate or placebo was given to 20 healthy men for 12 weeks and found to increase total alkaline phosphatase as well as bone-specific alkaline phosphatase activity and mass concentration of bone-specific alkaline phosphatase, indicating an increase in bone formation.[32]

Trial Details	Results	Ref
Zinc and Vitamin C		
94 participants from 2 studies dose: 1000 mg/day of vitamin C plus 10 mg/day of zinc as citrate for 5 or 10 days	• quicker relief from rhinorrhoea which was significant by day 4, less nasal obstruction at day 2, and reduced overall discomfort due to sneezing and eye watering • symptoms not affected by treatment include laryngeal irritation, cough, sneezing, headache and headache	33
Zinc		
meta-analysis of 3 randomised, placebo-controlled trials involving 199 participants dose: 80-92 mg/day of zinc as acetate lozenges	• reduced duration of nasal discharge by 34%, nasal congestion by 37%, sneezing by 22%, scratchy throat by 33%, sore throat by 18%, hoarseness by 43%, muscle ache by 54%, with no change in duration of headache or fever • a secondary, two-stage meta-analysis of this same group of participants revealed the reduction in number of days with cold symptoms between 2.73 and 2.94	34,35
systematic review and meta-analysis (17 and 14 trials, respectively) – included the 3 trials analysed in the above meta-analysis[34,35] dose prescribed in the 8 trials used for the primary outcomes: • children: 15 mg as sulfate twice daily, and 10 mg as gluconate 5 to 6 times daily (depending on age) • adults: 13.3 or 23.7 mg as gluconate every 2 hours whilst awake, and 9, 12.8 or 13.3 mg as acetate every 2 hours whilst awake duration of treatment was 10 days in one trial and until symptoms subside in 7 trials (with maximums of 10 and 14 days in 2 trials)	Primary Outcomes 8 trials (468 patients: 5 trials evaluated adults, 3 trials included children less than 18 years old) • zinc treatment significantly reduced the duration of colds in adults but not in children – mean difference: -2.63 days in adults 4 of these trials (207 patients; 2 trials each for children and adults) • severity of colds was also significantly reduced in adults but not in children, however it is unclear whether the form and dose of zinc contributed to the difference* Secondary Outcomes • for the presence of symptoms at day 3 there was no difference between zinc and placebo groups, but a reduction in number of symptomatic participants at day 7 observed in the zinc group (8 trials (1252 patients) for day 3; 9 trials (1352 patients) for day 7) • those treated with zinc more frequently experienced bad taste and nausea (8 and 9 trials, respectively)	36
randomised, double-blind, placebo-controlled trial involving 40 air force cadets dose: 15 mg/day of zinc as gluconate for 7 months	• participants reported more weeks without symptoms of URI • no difference between groups in medically-reported URI	37
randomised, double-blind, placebo-controlled trial involving 100 children aged 8-13 years dose: 15 mg/day of zinc as bisglycinate for 3 months	• zinc-supplemented children experienced the same number of coughs, fevers and rhinorrhoea, however the duration of cough (1 vs 6 days), rhinorrhoea (2.0 vs 5.5 days) and the frequency of having 2 or more symptoms of the common cold (0 vs 1 days) were reduced in the zinc group	38
Cochrane review involving 6 trials and 7850 participants aged 2-59 months; the studies were conducted in low income or slum areas of Bangladesh, India, Peru, and South Africa dose: 10-21 mg of zinc as acetate, sulfate or gluconate daily for at least 3 months	• incidence of pneumonia was reduced by 21% • prevalence of pneumonia was reduced by 41%	39
randomised, placebo-controlled clinical trial involving 50 healthy adults aged between 55 and 87 years dose: 15 mg/day of zinc as gluconate for 12 months	• participants in the zinc-supplemented group had fewer infections (0.29 ± 0.46 vs 1.4 ± 0.95) • 17 participants in the zinc group had no evidence of any infection compared with three in the placebo group • *ex-vivo* generation of TNF-alpha and markers of oxidative stress decreased in the zinc group	40
randomised, double-blind, placebo-controlled trial involving 147 healthy participants aged 55-70 years dose: 15 mg/day or 30 mg/day of zinc as gluconate for 6 months	• the 15-mg group had an improvement in T helper/T-cytotoxic lymphocyte ratio, a decline in which is associated with old age and can be used as a predictor of survival in old age • B lymphocytes declined in the 30-mg group at 3 months, suggesting a possible decline in immune function at this time point	41
randomised, double blind, placebo controlled study involving 25 elderly participants residing in a nursing home with low zinc status dose: 30 mg/day of zinc as gluconate in a multivitamin (compared with the same multivitamin providing 5 mg/day of zinc as gluconate)	• serum zinc increased by 16% in the zinc group • increased T cell count resulting in increased lymphocyte proliferation	42

Zinc *cont'd*		
case-controlled, crossover study involving 21 elderly participants from 2 nursing homes dose: 4 mg/day of zinc as gluconate added to skim milk for 2 months	• improved in cytokine production indicating a healthier cell-mediated immunity • increase in anti-inflammatory cytokines and a decrease in pro-inflammatory cytokines as well as an increase in thymulin activity (involved in T cell differentiation)	43
uncontrolled trial involving 19 healthy elderly participants with moderate zinc deficiency dose: 10 mg/day of zinc as acetate for 50 days	• improved zinc status, reduced activated T helper cells indicating an improvement in immune status • no effect on Th1/Th2 ratio	44
randomised, double-blind, placebo-controlled trial involving 26 children with cystic fibrosis exhibiting mild to moderate lung disease dose: 30 mg/day of zinc as gluconate for 12 months	• children who were zinc deficient at the time of recruitment had significantly fewer days per year requiring antibiotics compared with zinc-deficient children receiving the placebo	45
randomised, double-blind, placebo-controlled trial involving 39 pregnant Bangladeshi women dose: 20 mg/day of zinc as sulfate from second trimester through to 6 months post-partum	• infants born to supplemented mothers had lower plasma zinc levels and higher (more robust) IL-7 and antibody responses to the hepatitis B vaccine	46

Table 1. Effect of zinc and vitamin C on immune function in adults and children.

Abbreviations: SMD: standard mean difference; IRR: incident rate ratio; OR: odds ratio; URI: upper respiratory infection
Notes: * both trials in children used sulfate and at a lower dose than the other child trial, the two adult trials used a dose defined as high by the authors (≥75 mg/day), and the form was acetate

The ability of oral supplementation of zinc to promote healing in pressure and arterial ulcers and wound healing in general is unclear. Cochrane reviews that analysed data to 2014, concluded there is no positive effect for healing of pressure ulcers (2 trials: 45-50 mg/day of elemental zinc as sulfate), or for healing of venous and arterial ulcers (5 full-analysis trials; 100-150 mg/day of elemental zinc as sulfate).[47,48]

There is the possibility that zinc supplementation may improve healing in patients with low serum zinc levels, however further research is needed to confirm.[49,25] A positive result however, was yielded from a randomised, double-blind, placebo-controlled study in **diabetic patients with ulcers of the foot**, receiving 50 mg of zinc as sulfate daily for 12 weeks. Treatment resulted in a reduction in ulcer size, improved markers of insulin resistance and antioxidant status, and reduced inflammatory proteins.[50] The discrepancy between this trial and the reviews cited above may be due to the beneficial effect of zinc on insulin resistance.

Zinc was shown to improve healing time in men with **pilonidal sinus wounds** in a 1967 study. A daily dose of 150 mg zinc as sulfate was given to 10 men for the healing period (mean: 46 days) and the results compared to a control group. The treated men's wounds healed 34.3 days sooner than the untreated men. Wound closure occurred at a rate of 1.25 mL/day in the treatment group compared with 0.44 mL/day in the control group.[51]

The therapeutic effect of zinc at varying doses has been shown in an Iraqi trial involving participants who were infected with Leishmania parasite. One hundred and four participants were randomised to receive either 2.5, 5 or 10 mg/kg of zinc sulfate daily or no treatment for 45 days. The cure rate was 83.9%, 93.1% and 96.9% in the 2.5-, 5- and 10-mg/kg groups respectively. None of the participants in the control group exhibited any change in disease. For a 60-kg person, these dosages equate to 33 mg, 66 mg and 132 mg per day of elemental zinc.[52]

Zinc shows some promise in neurological healing. A study of 68 patients with severe head trauma showed that the zinc-supplemented group had significantly better Glasgow Coma Scale motor scores after 15 and 21 days. Treatment and control groups received 12 mg and 2.5 mg respectively, of zinc as sulfate administered as total parenteral nutrition for the first 15 days, followed by 22 mg oral zinc as gluconate versus placebo for 2.5 months.[53]

In a small randomised, placebo-controlled clinical trial involving 17 Thai participants with type 2 diabetes (T2D), supplementation with zinc sulfate providing 30 mg of elemental zinc per day for 8 weeks resulted in an increase in transmembrane TNF-α-expressing monocytes but not lymphocytes. Patients with T2D were chosen because of their increased risk of inflammatory conditions such as cancer, and transmembrane TNF-α was measured as it is recognised as a key mechanism in the immune defence against cancer.[54]

A Brazilian study involving 36 children aged 6-9 years without zinc deficiency, showed that 5 mg/day of elemental zinc, given as sulfate for 3 months resulted in

an increase in serum zinc levels as well as an **improvement in verbal IQ scores** for factual knowledge, long-term memory and recall and performance IQ scores for alertness to detail, visual discrimination, planning, logical thinking, social knowledge, spatial analysis, abstract-visual problem solving and visual analysis and construction of objects.[55]

Zinc supplementation has shown promise in the area of neurodevelopment in infants, school-aged children and children born to supplemented mothers, however results are variable, and more research is required in this area. Preliminary results suggest poorer cognitive outcomes in children born to mothers with low zinc intake, better motor development and more playfulness in supplemented low birth-weight infants, and improved neuropsychological outcomes in some studies of supplemented school-aged children. Dosages range between 1 and 20 mg/day of zinc in supplementation trials.[26]

Zinc has been shown to have a **beneficial effect on mood and depressive symptoms** possibly via its ability to enhance production of brain-derived neurotrophic factor (BDNF). A randomised, double-blind, placebo-controlled clinical trial showed the beneficial effects of zinc supplementation (30 mg/day of zinc as gluconate) over 12 weeks in obese volunteers. Serum BDNF increased significantly in the zinc-supplemented group and depression scores were significantly lower by 12 weeks.[56]

In a pilot study involving 14 participants with depression, 25 mg/day of zinc (as acetate) supplementation alongside standard anti-depressant medication (tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs)) resulted in significantly greater reduction in depression scores compared with those treated with placebo and antidepressants.[57] A later trial used the same dose of zinc, but as sulfate, in patients already taking an SSRI. Participants taking zinc experienced a significant reduction in depressive symptoms by 12 weeks, however plasma BDNF, IL-6 and TNF-α levels did not differ from placebo.[58]

A study investigating the addition of 7 mg of zinc per day as gluconate to a multivitamin and mineral preparation for 10 weeks found zinc significantly reduced anger-hostility and depression-dejection scores in women.[59]

Acne has been successfully treated using zinc sulfate and gluconate in several clinical trials. A dose of 45 mg of zinc as sulfate given daily for 12 weeks was shown to **reduce severity of acne** according to the patient's evaluation after 4 weeks and doctor's evaluation after 12 weeks in a randomised, placebo-controlled trial.[60] This result was repeated in several trials which also showed that zinc supplementation seemed to work better in severe acne and less well in mild to moderate acne, although nausea, vomiting and diarrhoea occurred frequently.[61,62] Research with zinc gluconate showed 30 mg of zinc as gluconate daily for 2 months to be superior to placebo for reducing inflammatory scores as well as patient's and examiner's opinion with regard to severity of acne.[63] When compared with the antibiotic minocycline, the same dose of zinc gluconate was found to be effective, but inferior to the antibiotic over 3 months.[64]

Maternal supplementation of either 30 mg of zinc as sulfate, 4.5 mg of β-carotene or the combination of zinc sulfate and β-carotene taken daily from <20 weeks through to delivery showed that infants born to zinc-supplemented mothers had fewer diarrhoeal episodes, but more frequent cough episodes.[65]

A combination of zinc (25-100 mg/day) and vitamin B6 (200-800 mg/day) has been used to suppress hydroxyhemopyrrolin-2-one (HPL – also known as Mauve) excretion in patients suffering from symptoms associated with 'high Mauve'.[66] High levels of Mauve are associated with emotional stress/lability, increased oxidative stress, morning anorexia, attention deficit/hyperactivity, leukodynia as well as many other clinical signs and symptoms. A more comprehensive list can be found in McGinnis 2008.[67]

Lysine

Lysine has been shown to have antiviral activity against herpes simplex virus (HSV) *in vitro*, by competing with arginine which is required for viral replication. It is also required for calcium regulation (and may have some positive effect on bone deposition), and for collagen, carnitine and elastin synthesis.[3]

Several clinical trials have examined the effect of lysine supplementation on **recurrence and severity of herpes simplex virus** (HSV) infection. Results from these trials are inconsistent, however treatment shows promise at doses higher than 1000 mg/day in conjunction with a low-arginine/high-lysine diet. *See Table 2 for details.*

Quercetin

Animal and *in vitro* studies have shown quercetin to have antiviral activity against rhinovirus,[73] influenza virus,[74,75] hepatitis (in humans),[76] Epstein Barr virus,[77] poliovirus, HSV-1, parainfluenza virus type 3 and respiratory syncytial virus.[3]

Quercetin is also an antioxidant, protecting the lens of the eye and the renal tubules from oxidation-associated injury.[3] It is considered the most **potent antioxidant** of the flavonoid family, protecting against superoxide and nitric oxide radicals.[78]

Trial Details	Results	Ref
double-blind, crossover study in 26 participants dose: 1000 mg/day for 26 weeks, then crossed over for 26 weeks low-arginine diet throughout the study	• no significant difference in frequency of lesions • serum lysine >165 nmol/mL is associated with a significant decrease in recurrence rate	68
double-blind, placebo-controlled, crossover study in 65 participants dose: 1000 mg/day for 12 weeks then crossed over for 12 weeks	• no difference in recurrence rate • significantly more participants were recurrence free in the lysine group	69
double-blind, placebo-controlled study in 49 participants dose: 3000 mg/day for 6 months low-arginine diet throughout the study	• 2.4 fewer infections in the lysine group • days to heal reduced by 2.3 • significant improvement in symptoms	70
double-blind, placebo-controlled study in 41 participants dose: either 624 mg/day or 1248 mg/day for 24 weeks and then crossed over for 24 weeks low-arginine/high-lysine diet	• reduced recurrence rate in the higher-dose group only • no change in healing time	71
questionnaire to patients with herpes outbreaks a) using or b) not using lysine supplementation average dose: 936 mg/day	• 84% of responders said that lysine reduced the recurrence or severity of infections • 70% of non-users described their symptoms as severe or intolerable compared with 8% of those using lysine • 83% lysine users reported lesions healed within 5 days or less, 90% of non-users reported healing took 6-15 days	72

Table 2 Effect of lysine on herpes simplex outbreaks in adults.

Animal and cell line studies on quercetin have also found it to be:[78]

- cardioprotective by protecting LDL from oxidation, reducing ischaemia-reperfusion injury, protecting endothelial function and acting as an antihypertensive;
- antiallergenic and anti-inflammatory: inhibiting histamine release from mast cells and suppressing TNF-α and nitric oxide to cells exposed to lipopolysaccharide;
- antiplatelet: reducing platelet activation;
- anticataract: protecting diabetic animal's lenses through inhibiting aldose reductase activity;
- neuroprotective: reducing hyperglycaemia-induced oxidative damage to neuronal tissue by inhibiting aldose reductase activity.

Pyridoxal-5-Phosphate

Pyridoxal-5-phoshate (P5P) is the active form of vitamin B6. It is required for the production of serotonin and gamma-aminobutyric acid (GABA) and dopamine,[79] primarily inhibitory neurotransmitters, and therefore may assist in regulating the stress response.[80] One study correlates low pyridoxine levels with increased psychological stress following bereavement.[81]

A systematic review of 940 participants in 9 clinical trials found that vitamin B6 offers significant benefit over placebo in the treatment of premenstrual syndrome at doses up to 100 mg/day.[82]

Manganese

Manganese may play an important role in wound healing. *In vitro* studies show that exposure to manganese improves keratinocyte migration, an important process in re-epithelialisation of skin wounds.[83] It is also required for the enzyme prolidase which produces collagen as well as for glycosaminoglycan synthesis, both of which are involved in the healing process.[84] Manganese is required for production of the intracellular antioxidant manganese superoxide dismutase (MnSOD), which protects the mitochondria from oxidative stress.[85] Diets deficient in manganese have been associated with skeletal abnormalities, postural defects and cartilage and connective tissue disorders,[86] osteoporosis, diabetes mellitus and epilepsy.[84]

Selenomethionine

The primary functions of selenium in humans are:[3]

- antioxidant,
- immunomodulatory,
- thyroid hormone production and metabolism;
- spermatogenesis, testosterone production and normal male fertility.

Low maternal selenium levels have been associated with increased risk of wheeze[87] and asthma in children with particular genetic profiles involving glutathione peroxidase activity.[88] Zinc and selenium have both been found to be lower in the serum of adults with asthma.[89] Supplementation studies yield inconsistent results (*see below*).

Clinical Studies

Selenium given with or without other agents has been found to **improve sperm health** and male fertility. Four hundred and sixty-eight men with idiopathic oligo-asthenoteratospermia were randomised to receive either selenium 200 mcg/day, N-acetylcysteine (NAC) 600 mg/day, a combination of selenium and NAC, or placebo for 26 weeks and followed for an additional 30 weeks. Sperm count, morphology and motility all significantly increased in the treatment groups. LH and FSH decreased and testosterone increased in response to both treatments. The combination of selenium and NAC yielded the most compelling results.[90] An earlier study compared daily doses of 40 mcg selenium with 40 mcg selenium plus vitamin A (1 mg), C (10 mg) and E (15 mg) or placebo for 3 months. Both the supplemented groups achieved an improvement in sperm motility, but not in sperm density. There was no additional improvement with vitamins.[91]

A pilot trial involving 17 adults with asthma reported that supplementation with 200 mcg/day for 96 weeks resulted in a reduction in the use of inhaled and systemic corticosteroids. Results were evident after 24 weeks.[92] A later study involving 197 participants who were supplemented with 100 mcg/day of selenium for 24 weeks found a non-significant improvement in asthma-related quality of life but no change in asthma symptom scores.[93]

β-Carotene

β-Carotene is a conditionally essential nutrient in humans – it is used to produce vitamin A and only becomes essential when vitamin A intake is inadequate. Vitamin A is essential for normal immunity, maintenance of epithelial structure and function, vision and growth and development. Outside of its pro-vitamin A role, β-carotene also acts as an antioxidant, is used in intracellular communication, and may protect cells from carcinogenic changes.[3]

Clinical Studies

The effect of β-carotene on immune function is not clear – clinical trials reveal varying results.

A study investigating the effect of 15 mg of β-carotene daily for 28 days in healthy, male, non-smokers found that β-carotene was superior to placebo at increasing monocyte expression of the major histocompatablity complex class II molecule, HLA-DR which is responsible for T lymphocyte antigen-presenting function, and cellular adhesion molecules which are responsible for initiation of the immune response, as well as the *ex-vivo* stimulated TNF-α production by monocytes.[94]

Another study comparing β-carotene (8.2 mg/day), lycopene (13.3 mg/day) and placebo found no improvement in T-cell subsets or surface molecules in 58 healthy participants over 65 years of age.[95] The short- (90 mg/day for 3 weeks) and long-term (50 mg every other day for 10-12 years) effects of β-carotene supplementation on immune activity revealed no improvement in the delayed hypersensitivity skin test response, lymphocyte proliferation, IL-2 or PGE2 production, lymphocyte subsets, NK cells or activated lymphocytes due to treatment.[96]

The antioxidant activity of β-carotene has been well studied in humans – many, but not all studies, yield positive results. β-Carotene (10 mg) or placebo was provided daily for 12 weeks to 82 men who worked in zinc and lead production facilities. Blood lead, but not zinc levels decreased significantly, as did malondialdehyde and homocysteine. Thiol levels (low levels of which can be used as a reflection of oxidative stress intensity) increased in the β-carotene group, as did the antioxidant superoxide dismutase.[97] Similarly, high dose (40 mg/day) of natural β-carotene from *Dunaliella bardawil* or placebo was provided to 262 children from Chernobyl nuclear contaminated area for 3 months. Treated children had a significant reduction in oxidised conjugated dienes, which are products of lipid oxidation.[98] In contrast, 48 smokers were provided either 20 mg/day of β-carotene or placebo for 12 weeks. Supplementation failed to provide any significant LDL oxidative protection.[99]

Safety

Vitamin C has been shown to be safe in a large number of studies involving many participants. Pooled data from trials examining ≥ 1 g vitamin C per day showed 5.8% of participants experiencing adverse symptoms compared with 6% in the placebo group.[7]

Long-term vitamin C supplementation (11 years) is associated with an increased incidence of kidney stones in men, but not in women.[100]

Caution when supplementing with vitamin E. A high dietary vitamin C intake combined with supplementary dl-α-tocopherol acetate (50 mg/day) was found to increase the risk of pneumonia by 248% and 1350% in participants ≤ 60 kg or ≥ 100 kg (respectively) who started smoking at ≤ 20 years.[1]

As vitamin C increases the absorption of iron, caution is advised in patients with iron overload or haemochromatosis. It also increases aluminium absorption – take away from aluminium-containing antacids.[3]

Lysine exerts its antiviral activity on the HSV by competing for intracellular transport with arginine. Through this mechanism, lysine may inhibit other arginine-dependant

activity in the body such as nitric oxide production, collagen synthesis, wound healing and male reproductive function such as spermatogenesis and erection maintenance. A dose of 1000 mg of lysine per day taken over 12 months resulted in no change in serum arginine levels.[68] Caution in hypercalcaemia (lysine increases calcium uptake).[3] Caution is also warranted in patients with sluggish gall bladder function as lysine has been shown to reduce gall bladder motility and be associated with gallstone formation in animal studies. One case report exists of a woman developing end-stage renal disease after taking 3000 mg of lysine per day for 5 years.[101] Nephrotoxicity has also been induced in dogs when given extremely high dose lysine for a short period of time.[102]

Quercetin has been safely consumed at daily doses of 5000 mg for 28 days.[76]

Although there is a caution with supplementing *synthetic* β-carotene in smokers due to a potential increase in lung cancer risk, there is no association with naturally-sourced β-carotene.[3]

Manganese has been provided in clinical trials at doses of 30-40 mg/day for 8-16 weeks without adverse event, however there are certain populations who are more susceptible to the neurotoxic effects of manganese toxicity which can result in symptoms similar to Parkinson's disease. These patients include:[84]

- chronic liver disease patients (reduced biliary excretion);
- children (increased absorption and decreased excretion);
- iron-deficient patients (increased accumulation in the brain).

The RDI for manganese is 5 mg/day. Although toxicity from supplementation is unlikely, screen patients for liver dysfunction and iron deficiency and monitor whilst supplementing.

Supportive Formulation

These nutrients complement each other to support the following actions:

- immunostimulatory;
- antioxidant;
- healing;
- antiviral;
- adaptogenic;
- fertility enhancing;
- neuroprotective
- thymoleptic.

Indications

- Common cold, immune dysfunction.
- Wound healing, acne.
- Stress support, antioxidant.
- Male infertility.
- To promote healthy cognition; depression.
- Cold sore prevention.
- Possibly as an adjunctive treatment in asthma.

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