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Vitamin B12: A Review of Clinical Use and Efficacy I N-acetylcysteine: A Review of Clinical Use and Efficacy I Zinc: A Review of Clinical Use and Efficacy I Lactobacillus rhamnosus GG: A Review of Clinical Use and Efficacy I Migraine Headaches Opportunities for Management with Precision Nutrition

NUTRITIONAL MEDICINE JOURNAL

The Nutritional Medicine Journal (NMJ) is a specialist peer-reviewed scientific publication related to the application of personalised dietary interventions, foods, dietary factors, and nutritional supplements in clinical practice.

Topics include clinical nutrition, personalised nutrition, lifestyle medicine in the context of nutritional medicine, micronutrients, amino acids, fatty acids, phytochemicals, probiotics, prebiotics, and functional beverages and foods.

The aim of the NMJ is to provide health professionals with authoritative and scientifically accurate articles on topics in nutritional medicine.

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Pain Management and Nutritional Medicine

Benjamin I. Brown

Chronic pain is one of the most widespread health problems, with up to 43% of people in the UK reporting that they experience chronic pain, of which 10–14% report moderate to severely disabling chronic pain.[1] Chronic pain is typically defined as pain persisting longer than 3 months, and generally refers to pain that has become a disease entity of itself and distinct from pain associated with acute injury or disease.[2] As a unique disease state, chronic pain, like other chronic diseases, has unique biological features, clinical symptoms and long-term consequences.

Chronic pain can be divided into primary or secondary classifications. Secondary chronic pain is divided into several sub-categories, including cancer-related pain; postsurgical or posttraumatic pain; secondary headache or orofacial pain; secondary visceral pain; and secondary musculoskeletal pain.[3] Primary chronic pain, also termed nociplastic pain, is used to broadly classify conditions in which pain itself has become the primary disease, and includes presentations such as fibromyalgia and non-specific low-back pain.[4] Primary chronic pain syndromes frequently overlap, and patients often present with a mosaic of conditions that include temporomandibular joint disorders, fibromyalgia, irritable bowel syndrome, chronic headaches, interstitial cystitis, chronic pelvic pain, chronic tinnitus, whiplash-associated disorders and vulvar vestibulitis.[5] The consequences of chronic pain are significant and impact psychological and physical health, including an adverse impact on sleep, cognitive processes and brain function, mood, mental health, cardiovascular health, sexual

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function, appetite and nutrition, medication dependence, social connection, and overall quality of life.[6,7]

The biology and pathophysiology of chronic pain is diverse and highly individual; however, there may be important sharded mechanisms across seemingly disparate clinical conditions that could help identify mechanism-based as opposed to symptom-based treatments.[8] A key feature of chronic pain is central sensitisation, which is characterised by increased activity in neurons and circuits involved in processing pain in the central nervous system coupled with reduced inhibition and ineffective endogenous pain control.[9] A 25-item Central Sensitisation Inventory (CSI) has been used as a screening tool for chronic pain, and CSI score reliably predicts pain intensity among patients with different types of chronic pain.[10] Central sensitisation may be more important than local tissue changes and provide a more unified explanation for chronic pain shared by different disorders, in this sense pain syndromes such as fibromyalgia, irritable bowel syndrome and chronic headaches are different manifestations of the same underlying aetiology.[11]

Although by definition primary chronic pain syndromes have no apparent functional cause, there are in fact several factors implicated in the pathogenesis of central sensitisation and chronic pain that may also, in some cases, be modifiable treatment targets. Pro-inflammatory mediators, although not consistently detectable in the periphery of people with chronic pain, may be active in the central nervous system and play an important role in the development of central sensitisation.[12] Inflammatory mediators sensitise pain-sensing neurons and enhance pain transmission.[13]

Mitochondria are implicated in the development of chronic pain, with dysfunction of mitochondrial metabolism and related adenosine triphosphate (ATP) deficiency, excessive reactive oxygen species, and impaired calcium buffering amongst several potential mechanisms that may be involved in pain sensitisation.[14] Preclinical studies indicate that mitochondrially targeted treatments may improve mitochondrial function and have important analgesic effects.[15]

Oxidative stress could contribute to the development and maintenance of chronic pain. In the central nervous system, reactive oxygen species can produce central sensitisation and hyperalgesia in the absence of nerve damage or tissue inflammation.[16] Furthermore, elevated biomarkers of oxidative stress have been observed in patients with a wide range of chronic pain syndromes, including chronic and recurrent neck pain,[17] low-back pain,[18] tension-type headache,[19] migraine headache,[20] fibromyalgia,[21] irritable bowel syndrome[22] and interstitial cystitis.[23]

Nutritional neuropathies are an important but often overlooked cause of chronic pain, with deficiencies in vitamins B1, B3, B6, B12 vitamin E and copper of particular importance.[24] Due to the multifaceted roles of nutrients in regulating nervous system function, inflammation, mitochondrial energy metabolism and oxidative stress, many other nutrients can play an important role in the development and therapy of chronic pain.[25] Several nutrients have been shown to modulate pain, including amino acids (tryptophan, phenylalanine and carnitine), fatty acids (omega 3 fatty acids, resolvins and N-palmitoylethanolamide), minerals (selenium, magnesium, iron and manganese) and vitamins (vitamins B, C, D, E and K).[26] Screening patients with chronic pain for underlying contributory nutritional deficiencies could help identify personalised nutritional interventions and improve clinical management.

In a retrospective observational study of 17 834 patients receiving opioids for chronic pain, a biomarker assay that determines possible modifiable nutritional drivers of pain suggested that 86% of patients had at least one abnormal biomarker.[27] In a randomised-controlled trial of this biomarker assay in clinical practice, it was found that clinicians using that assay were more likely to identify a micronutrient deficiency (41.5%), treatable metabolic dysfunction (29.4%) and underlying oxidative stress (26.1%), and less likely to prescribe opioids, order unnecessary imaging or order an unnecessary pain referral.[28] This suggests that assessment of nutritional biomarkers relevant to pain could improve clinical management; however, patient pain outcomes were not measured in this study so more research is needed to determine if there is any impact on pain severity or medication use. Nevertheless, this work does support a role of nutrition in pain management.

Dietary therapy is a cornerstone of chronic pain management.[29] Important mechanisms for the benefit of dietary changes include modulation of inflammation,[30] reduction of oxidative stress,[31] direct analgesic effects[32] and reduced exposure to dietary factors that provoke pain.[33] A range of dietary interventions across different clinical pain syndromes have been studied. A review of 37 clinical trials including patients with generalised chronic musculoskeletal pain, low-back pain, neck pain, osteoarthritis, fibromyalgia, chronic headache or migraine, generalised chronic musculoskeletal pain and abdominal pain found that dietary interventions such as caloric restriction and fasting, enriched polyunsaturated fatty acid diets, low-fat plant-based diets, high-protein diets and elimination diets all generally revealed positive results.[34]

Dietary recommendations for patients with chronic pain can be based on anti-inflammatory and antioxidant foods and food components. Guidelines and a food pyramid have been developed, and broadly include advice to consume carbohydrates with a low glycaemic index, fruits and vegetables, yogurt and extra virgin olive oil daily; legumes and fish, white meat, eggs and fresh cheese weekly; and red or processed meats once per week, in addition to personalised nutritional supplementation.[35]

In this issue of the *Nutritional Medicine Journal,* one of the most frequent and debilitating pain disorders, migraine, is reviewed in the article *Migraine Headaches: Opportunities for Management with Precision Nutrition.*

However, opportunities for pain management with precision nutrition are clearly not limited to migraine, and represent an underappreciated clinical strategy that has potential to improve management and reduce suffering for an untold number of people living with chronic pain.

Benjamin I. Brown
Editor, *Nutritional Medicine Journal*

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Nutritional Medicine Journal

Vitamin B12: A Review of Clinical Use and Efficacy

Karin Elgar

Abstract

Vitamin B12 (B12) is an essential cofactor in cellular metabolism – adequate supplies are needed for normal blood formation and neurological function, and deficiency can therefore lead to macrocytic anaemia and neurological deficits. Deficiency can be due to inadequate dietary intake, especially in vegans, but is otherwise more likely due to problems with absorption, which is more complex than that of other vitamins. Apart from vegans and people with gastrointestinal conditions, infants and children from B12-deficient mothers and the elderly are at particular risk of B12 deficiency.

Whilst in conventional medical practice B12 deficiency is generally treated with intramuscular injections, it is generally accepted that oral (per os) administration at a high dose is as effective at improving B12 status. Diagnosis of B12 deficiency is complicated by the fact that levels of B12 in the blood are maintained even when stores are low at the expense of tissue levels. Where a deficiency is suspected but serum levels are normal, other markers can be used to confirm a deficiency.

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Introduction

Vitamin B12 (B12), or cobalamin, is a cobalt-containing compound, and exists in four forms, methylcobalamin (methyl-B12) and adenosylcobalamin (adenosyl-B12) that naturally occur in the body, and cyanocobalamin (cyano-B12) and hydroxocobalamin (hydroxo-B12) that are synthetic forms used in supplementation and food fortification.[1] Adequate supplies are essential for normal blood formation and neurological function.[2]

Methyl-B12 is an essential cofactor in the one-carbon methylation cycle converting homocysteine to methionine, and in the course converting methyl-tetrahydrofolate back to tetrahydrofolate; deficiency can therefore lead to elevated homocysteine levels and impaired synthesis of DNA.[3] The latter affects blood formation, leading to anaemia, a hallmark sign of B12 deficiency.[3]

Adenosyl-B12, on the other hand, is a cofactor for the enzyme converting methylmalonyl-CoA to succinyl-CoA, which is crucial for channelling fatty acids and amino acids into the Krebs cycle for energy production.[3] In B12 deficiency, methylmalonic acid (MMA) can accumulate, and it is thought that elevated MMA, alongside elevated homocysteine, contributes to damage of the myelin sheath (which protects nerve cells and speeds up nerve transmission), which can cause irreversible neurological problems.[3]

Vitamin B12 deficiency

The haematological effects of B12 deficiency are the same as for folate deficiency, and are characterised by macrocytic anaemia (with abnormally large red blood cells).[2] Folate supplementation can correct these haematological effects of B12 deficiency but not the neurological complications, which include neuropathies, such as tingling and numbness in the arms and legs, sensory and motor disturbances, including abnormal gait, and cognitive changes.[2] Seventy-five–90% of patients with B12 deficiency present with neurological complications, which may be the only manifestation in up to 25% of cases.[2]

B12 deficiency can be due to inadequate dietary intake, in particular in people following a strict vegan diet (B12 is only found in animal foods). As B12 is stored in the liver, it can take 3 years following a switch to a vegan diet before deficiency occurs.

More commonly though, deficiency is due to poor absorption of this large vitamin. Stomach acid and pepsin are required to free food-bound B12, which then binds to salivary R-proteins. Pancreatic proteases release B12 in the small intestine where it then binds to intrinsic factor (IF), which is secreted by the parietal cells in the stomach. The IF–B12 complex is absorbed in the ileum, the distal part of the small intestine.[2] If anything goes wrong with any of these steps, such as low stomach acid (e.g. due to ageing or through acid-lowering medication), or bariatric surgery, lack of pancreatic enzymes, small intestinal bacterial overgrowth, certain intestinal parasites or dysfunction of the small intestine (e.g. in coeliac or Crohn's disease), this can lead to B12 deficiency.[2] Metformin (an antidiabetic drug) and nitric oxide have also been shown to decrease B12.[4,5,6]

The term 'pernicious anaemia' refers to an autoimmune disease that destroys the parietal cells, leading to a lack of IF and thus B12 deficiency.[7]

Apart from vegans and people with gastrointestinal conditions, infants and children from B12-deficient mothers and the elderly are at particular risk of B12 deficiency, and pregnant and breastfeeding women have higher requirements.[4]

Diagnosis of deficiency

There is no simple definitive laboratory test to establish B12 deficiency.

After absorption, B12 is bound to transport proteins, 75–80% to haptocorrin, which transports B12 in the blood, and 20–25% to transcobalamin, which delivers B12 to the tissues, also referred to as 'active B12' or 'holo-transcobalamin'.[4] Both total B12, i.e. bound to haptocorrin and transcobalamin, can be tested in serum. However, serum levels may be maintained at normal or borderline levels at the expense of tissue concentrations.[2]

Serum levels of 300 ng/l and above are usually considered normal.[3] The UK National Institute for Health and Care Excellence (NICE) states that a cut-off of 200 ng/l identifies 97% of people with B12 deficiency, and a level below 100 ng/l is usually accompanied by metabolic or clinical evidence of B12 deficiency.[8] A full blood count, to check for macrocytic anaemia and other haematological parameters, should also be carried out.[8]

Where results are unclear, serum MMA and homocysteine levels (both of which accumulate with B12 deficiency as discussed above) can also be used to confirm a diagnosis, although homocysteine is not specific to B12 deficiency, as it may also be caused by deficiencies in other B vitamins.[2,3]

Correction of deficiency – different routes of administration

Depending on the cause of the deficiency, different strategies to correct it can be applied, although in conventional medical practice the most common treatment is intramuscular (i.m.) injection of B12.[9] Where IF is present, a deficiency can easily be overcome by oral supplementation of crystalline cobalamin, which does not need to be cleaved from food proteins.[2]

Clinical trials have shown that approximately 1% of crystalline B12 is absorbed passively, i.e. without the need for IF, and that high doses (1000 µg per day) of oral B12 can therefore be effective even in pernicious anaemia.[2,9,10] At the start of oral vitamin B12 replacement therapy, close monthly monitoring of laboratory results and symptoms is recommended, thereafter annual monitoring should suffice; however, patients with severe neurological complications or critically low B12 status should be treated with i.m. injections initially to rapidly replenish stores.[10] Compared with i.m. B12, oral therapy could not only save resources and time, but may also be preferable for patients with coagulation issues (coagulopathies or on blood-thinning medications) and those who are adverse to injections, for example, elderly patients with sarcopenia for whom i.m. injections are painful, whilst for those with poor compliance with oral intake, injections may be preferable.[10]

A number of studies have investigated sublingual administration, and all found sublingual B12 to be effective in normalising B12 status in deficient adults,[11,12,13,14] infants[15] and children.[16] Most studies included patients with various aetiologies of B12 deficiency, including pernicious anaemia, and improvements were seen across different causes. Dosing regimens have ranged from 350 µg per week (in vegetarians) to 2000 µg per day in a study including patients with pernicious anaemia, with the most common daily dose being 1000 µg per day. A number of clinical trials compared sublingual with i.m. administration and found comparable results.[16,17]

It is unclear whether B12 is absorbed via the oral mucosa or in the small intestine with sublingual administration, and research into this question is scarce.[18] A study in 2003 compared oral versus sublingual cyano-B12 (500 µg per day for 8 weeks), and found

that both increased B12 levels significantly without a significant difference between the two.[13] Another study, comparing oral and sublingual administration of a complex of B12, folate and vitamin B6 also found no significant differences in effect on homocysteine between the two modes of administration.[19]

Correction of deficiency – different formulations of B12

As mentioned above, cobalamin exists in various forms. The most commonly used form for supplementation, both injectable and oral, is cyano-B12, which contains a cyanide moiety attached to cobalamin.[7] Cyano-B12 can be converted to methyl-B12 and adenosyl-B12.[2] Other commercially available forms include the naturally occurring methyl-B12, and less commonly adenosyl-B12, and hydroxo-B12, which is used mainly in prescription products.

In 2015, Obeid *et al*. reviewed the evidence as to whether any of the available forms of B12 are superior, and concluded that all forms can be converted to the active methyl- and adenosyl-B12 forms, and that there was not sufficient evidence to suggest that any form was superior in terms of bioavailability, biochemical effects or clinical efficacy.[20]

In the same year, Thakkar and Billa also compared the benefits of the different forms, and argued that methyl-B12 alone may not address the neurological complications in B12 deficiency that arise partly from the lack of adenosyl-B12.[21] However, as all supplemented forms are converted to cobalamin, which then gets converted to either the methyl or adenosyl form,[18] this does not appear to be plausible. The authors suggest that either methyl-B12 and adenosyl-B12 should be given together, or either cyano-B12 or hydroxo-B12 on their own, both of which can be converted into both active forms. The authors also point out that in some rare genetic disorders of B12 metabolism, the active forms may be required, and that smokers, who tend to have an excess of thiocyanate in their blood, may benefit from a taking a form other than cyano-B12.[20]

In 2017, Paul and Brady came to similar conclusions that all four forms appear to be effective, although they point out research that shows that tissue retention of cyano-B12 is lower and urinary excretion higher than that of the other forms, although this statement is based on a review from 1965.[18] As Thakkar and Billa, they point out potential concerns for smokers, but again this is based on an article from 1970. Paul and Brady also note that various single-nucleotide polymorphisms (SNPs) may influence the efficacy and bioavailability, but that at this time there is neither sufficient clinical research in this area nor are tests for these SNPs commercially available.[18]

Support for the poorer bioavailability of cyano-B12 comes from a study comparing sublingual cyano- with methyl-B12 (both 1000 µg per day), which found that both forms led to a normalisation of B12 status, but B12 levels increased significantly more in the methyl-B12 form compared with the cyano-B12 form, although the formulations also had other differences – the former was a tablet, the latter a spray.[16]

The aim of this paper is to review the evidence for the use of B12 in clinical practice beyond the replacement in deficiency. Not all articles specify which form of B12 was used in the respective study; where the form is reported, it is stated in this paper.

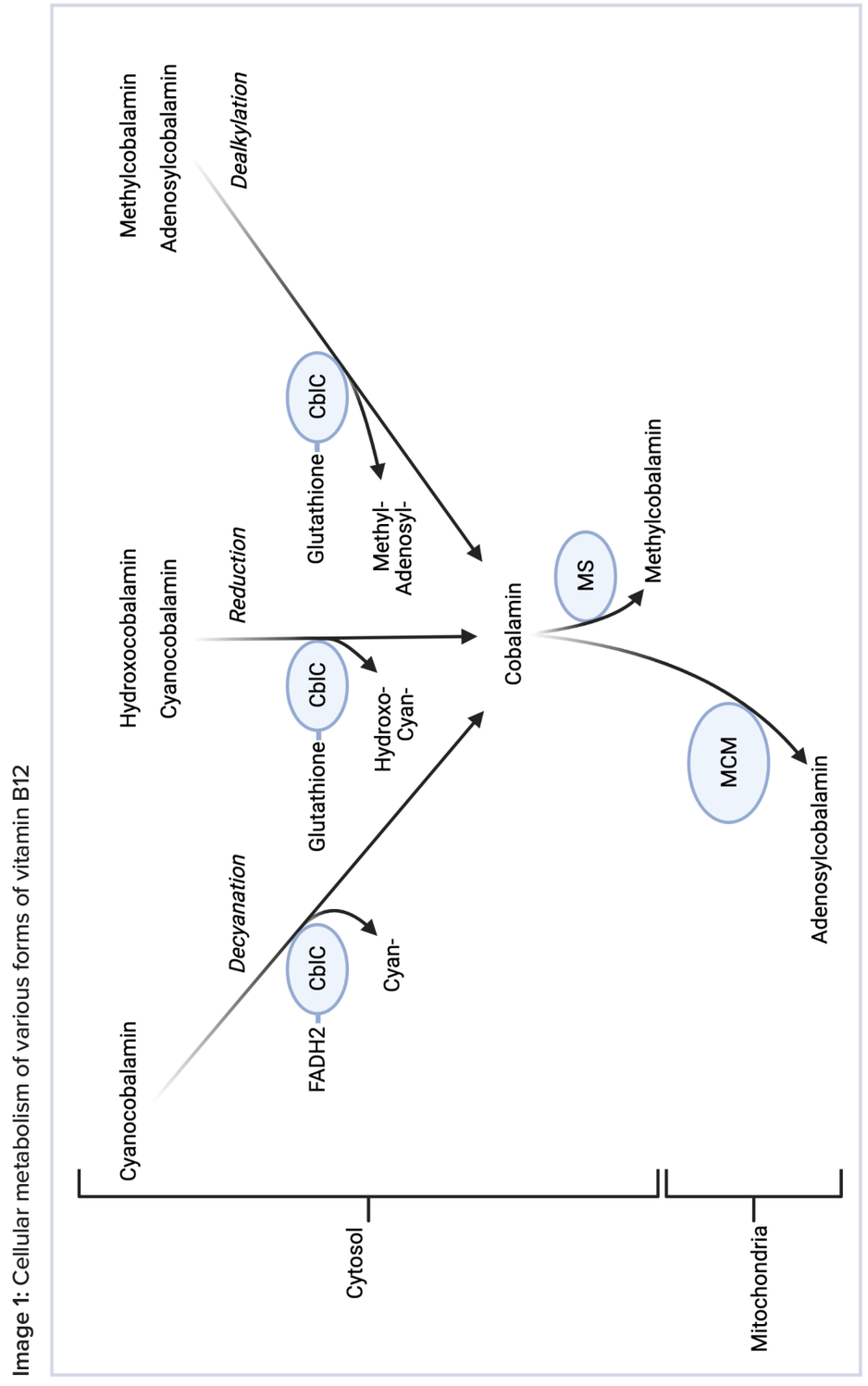

Image 1: Cellular metabolism of various forms of vitamin B12

Image: Simplified depiction of the metabolism of various forms of vitamin B12 supplements in the cytosol. Vitamin B12 is reduced to cobalamin regardless of form (cyan-, methyl-, adenosyl-, hydroxo-) then re-assembled as methylcobalamin by MS for use in the cytosol, or adenosylcobalamin by MM for use in the mitochondria. Key: CblC; cytosolic chaperon, methylmalonic aciduria, and homocystinuria type C protein FADH2; Dihydroflavine-adenine dinucleotide, MM-CoA; methylmalonyl-CoA mutase, MS; methionine synthase, MCM: methylmalonyl-CoA mutase.

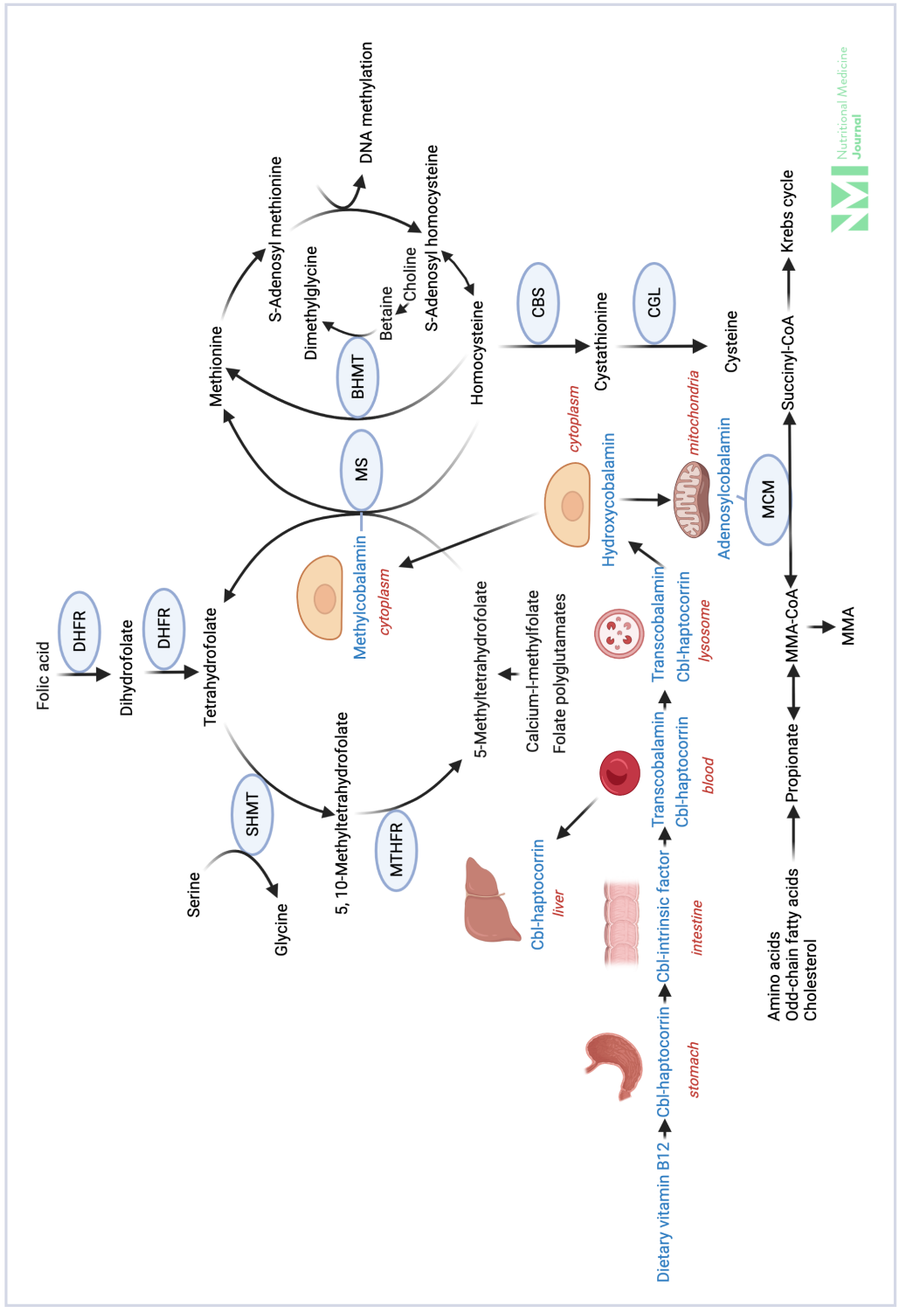

Image 2: Vitamin B12 absorption and metabolism

Image: Simplified vitamin B12 absorption and metabolism and inter-relationship with the folate cycle. Key: Cbl; cobalamin, DHFR; dihydrofolate reductase, MTHFR; methylene tetrahydrofolate reductase, SHMT; serine hydroxymethyltransferase, MS; methionine synthase, CBS; cystathionine β-synthase, CGL; cystathionine gamma lyase, NADPH; nicotinamide adenine dinucleotide phosphate, IGC-II; ntestinal glutamate carboxypeptidase II (folate hydrolase), MMA; methylmalonic acid, MM-CoA; methylmalonyl-CoA mutase.

Clinical uses

Aphthous ulcers

Aphthous (mouth) ulcers are common lesions in the oral mucosa, affecting up to 50% of the general population.[22] The causes of aphthous ulcers are unknown, and may include systemic diseases, nutritional deficiencies, food allergies, genetic predisposition, immune disorders, medications and human immunodeficiency virus infection.[23] As early as 1967, the benefits of i.m. B12 for healing of the oral mucosa were shown in a clinical study in patients following oral surgery.[24]

A study in 2008 showed that increasing B12 levels through i.m. cyano-B12 decreased the frequency of recurrent aphthous ulcers in patients with normal (defined as a B12 status of ≥ 140 ng/l) as well as with low levels of B12 at baseline.[25] The dosing schedule was 1000 µg daily for 7 days, then 1000 µg once per week for 1 month, then 1 × 1000 µg injection monthly for 6 months. In 2009, a double-blind randomised-controlled trial (RCT) found significant reductions in duration of outbreaks, the number of ulcers and the level of pain in those on B12, 1000 µg per day sublingually (form not reported), regardless of B12 level.[23] After 6 months of supplementation, 74% in the B12 group had reached "no aphthous ulcer status", compared with 32% in the placebo group. In 2015, another double-blind RCT reported significant improvements in pain associated with aphthous ulcers with a B12 ointment compared with a B12-free ointment.[22]

Whilst evidence is limited, it consistently reports a benefit of B12 for aphthous ulcers regardless of B12 status. Both sublingual and injectable forms have shown benefits.

It has been thought that B12 deficiency may play a role in the development of aphthous ulcers.[25] Volkov *et al*., who found that benefits were regardless of B12 status, discuss the possibility that patients in their study were deficient despite serum levels being within normal limits, as no other biomarker tests were carried out.[23]

Autism spectrum disorder (ASD)

Autism spectrum disorder is a common neurodevelopmental disorder, characterised by reduced social communication, and restrictive and repetitive behaviours and interests.[26] Biochemical abnormalities commonly seen in patients with ASD include impaired methylation, sulphation capacities and low glutathione (GSH) redox capacity, all of which can potentially respond to B12 supplementation.[26]

A review of 17 clinical studies of various trial designs, including pro- and retrospective, concluded that "B12, particularly subcutaneously injected mB12 [methyl-B12], improves metabolic abnormalities in ASD along with clinical symptoms".[26] Only two of the reviewed studies were RCTs of B12 on its own in children with ASD, both using subcutaneous methyl-B12. One of the RCTs found significant mean improvements in Clinical Global Impressions-Improvement (CGI-I) score and cellular methylation capacity, but not in the Aberrant Behaviour Checklist (ABC), Social Responsiveness Scale (SRS) or GSH metabolism, with those who had the lowest methionine status at baseline (suggesting impaired methylation capacity) significantly more likely to respond to supplementation.[27] The other RCT found no significant mean differences between B12 and placebo; however, it did report a significant clinical response in 30% of children on B12, which correlated with significant improvements in GSH redox status following B12 injections.[28] Dosages were 64.5 and 75 µg/kg bodyweight every 3 days for 6 and 8 weeks, respectively.

Overall, although limited, the evidence suggests a benefit of methyl-B12 for children with ASD. Certain subgroups may be more likely to respond to treatment, including those with lower methionine levels and/or lower GSH redox status.

The study by Hendren *et al*. suggests that improvements in methylation may explain

the benefits of B12 in ASD,[27] whilst the results from Bertoglio *et al*. point towards the positive effect of B12 on GSH redox capacity as a possible mechanism of action[28] that may improve brain function as well as the immune system and the gastrointestinal tract in individuals with ASD.[26] Other possible mechanisms include improvements in mitochondrial function and a reduction of glutamate, an excitatory neurotransmitter, which is also a precursor to GSH.[26] People with ASD have been shown to be more likely to have certain SNPs affecting B12 bioavailability, therefore they may require higher intakes.[26]

Cognitive function [including Alzheimer's disease (AD)]

B12 deficiency is common in the elderly, with a prevalence of 15–40%, especially in the elderly with mental disease.[29] Due to its importance in the synthesis of myelin and neurotransmitters, as well as in keeping homocysteine levels low, all of which are associated with cognitive function, B12 has been studied for a potential role in cognitive function.[30]

A 2021 meta-analysis on the effects of B12 on cognitive function found no benefit with either B12 alone (based on four RCTs) or B12 combined with other B vitamins (based on 13 studies).[30] Three of the four RCTs of B12 alone used oral B12 (1000 µg per day) and one used i.m. B12 (1000 µg per week). In 2003, a Cochrane review of three RCTs on cognitive function in patients with AD or other forms of cognitive impairment also found no evidence of benefits of B12 on cognitive function compared with placebo.[31]

Since the 2021 review, one more RCT has been published that compared oral B12 alone, 25 µg per day (form not reported), or with folate (800 µg per day) versus no supplements for 6 months in patients with mild cognitive impairment.[32] B12 with folate, but not alone, led to significant improvements in cognitive function.

In the 1990s and 2000s, further non-RCT studies in the elderly with or without dementia/AD and low B12 status have also mostly shown no benefit of B12 for cognitive function.[33,34,35,36,37]

One study suggests that length of time from diagnosis of cognitive impairment is important for response rate, in that patients who had experienced symptoms for less than 12 months improved on B12, whilst those with symptoms for more than 12 months continued to decline.[38] The dose was 1000 µg i.m. daily for 1 week, once a week for 1 month, and then monthly for at least 6 months. Similarly, another study found that hydroxo-B12 (1000 µg i.m. every other day 10 times, then once a month) was of benefit for people with mild to moderate but not for those with severe dementia.[29] A study in the elderly without cognitive impairment also found significant improvements with hydroxo-B12, 1000 µg i.m. weekly for 1 month, then monthly for 4 months, in cerebral and cognitive function, which were related to reductions in homocysteine.[39]

Most studies used B12 i.m., with slightly varying dosing regimens, commonly 1000 µg daily for 5–7 days, followed by weekly injections for another 3–4 weeks, and then monthly injections. In many of the above studies, patients were reported to have subnormal B12 levels.

Overall, there is not sufficient evidence to recommend B12 on its own for the elderly with impaired cognitive function, although those with early symptoms may benefit. This may be due to damage induced by inadequate B12 supply and affecting brain function, becoming irreversible after a certain period of time.

Depression

Major depressive disorder (MDD) affects 4.2–17% of populations worldwide, and is a common cause of disability.[40] High homocysteine is associated with poor response, and high B12 levels with a good response to anti-depressant treatment.[40]

Four RCTs have evaluated the effects of B12 in depression. One study, which also evaluated cognitive function in patients with high MMA concentrations, found no significant effects on the MDD inventory score, but only 30 of the 140 subjects had depression at baseline, which may have diluted any benefits.[41] Patients received 1000 µg cyano-B12 per week i.m. for 4 weeks or placebo injections. A study in patients with irritable bowel syndrome or inflammatory bowel disease with concomitant fatigue and depressive symptoms at baseline also found no benefits with oral B12, 1000 µg per day for 8 weeks (form not reported), on depressive symptoms despite significantly raising B12 levels, although baseline B12 levels were considered normal in all patients (> 203 ng/l).[42] Another RCT studied cyano-B12, 1500 µg three times per day for 2 weeks (route not reported), in patients with seasonal affective disorder and found no improvements in either the B12 or the placebo group.[43]

One RCT evaluated B12 alongside anti-depressants in patients with depression and moderately low B12 status (190–300 ng/l) and found that 100% of those on B12, 1000 µg per week i.m. for 6 weeks (form not reported), responded to treatment compared with 69% on anti-depressants alone, a statistically significant difference.[40]

There is insufficient evidence to suggest that B12 on its own is beneficial in depression.

Fatigue

Fatigue is a common symptom of B12 deficiency, but evidence that B12 beyond the correction of deficiency has any benefits for energy is lacking.

A double-blind, placebo-controlled trial in 95 patients with irritable bowel syndrome or inflammatory bowel disease and normal B12 levels found no significant improvements in fatigue, although B12 levels increased in the B12 group who received 1000 µg per day per os (p.o.) for 8 weeks (form not reported).[42] A double-blind crossover study from 1973 in patients complaining of tiredness found benefits of hydroxo-B12, 5000 µg twice per week i.m. for 2 weeks, on happiness and general wellbeing scores, but not on energy, sleep or appetite.[44]

An open-label, uncontrolled trial in 51 patients with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) found improvements in 34 patients after 3 months of supplementation with hydroxo-B12, 10 000 mcg twice per week as nasal drops. In this study, B12 levels were 244 pmol/l or more in all patients at baseline, but in those who improved, B12 levels almost quadrupled, whilst in non-responders B12 levels went up by only 50%.[45] Because this study had no control group it is impossible to establish whether the observed benefits were due to a placebo effect. Nasal administration is not well studied,[1] and the difference in effect on B12 levels amongst individuals may be due to the route of administration.

At this point, there is insufficient evidence to recommend B12 supplementation for fatigue beyond correcting a deficiency.

Homocysteine

Elevated homocysteine is a risk factor for cardiovascular disease, and folate, B12 and vitamin B6 have been shown to reduce homocysteine levels.[46] This section will look at the effectiveness of B12 in lowering homocysteine either on its own or the additional effect it has with other B vitamins.

There is a significant body of research, mostly from the 2000s, on the homocysteine-lowering benefits of B12 in patients with end-stage renal disease and who are on haemodialysis (HD), 80–100% of whom have elevated homocysteine levels.[47]

Most studies in patients on HD have shown benefits of B12 either on its own or extra benefit when added to other B vitamins

(mostly folate).[47,48,49,50,51,52,53,54] All these studies used injectable B12, most commonly given intravenously (i.v.) after HD two–three times per week, and the most common dose was 1000 µg per injection. The forms of B12 were not always reported, but included cyano-, hydroxo- and methyl-B12.

A couple of studies found no benefits of B12 for lowering homocysteine in HD patients, one used oral B12, 2000 µg three times per week for 6 weeks,[55] the other used methyl-B12 i.m. at a dose of 500 µg twice a week,[56] a dose lower than in most other trials. Another RCT found a decrease in homocysteine with 1000 µg hydroxo-B12 monthly for 3 months by 2 µmol/l, whilst the control group had a 2 µmol/l increase, although the difference was not statistically significant; however, the trial was powered to only detect much larger reductions.[57]

Positive effects of B12 on homocysteine levels have also been observed in other patient populations, including healthy adults,[46] vegetarian women,[58] generally healthy women,[59] older adults with elevated MMA and homocysteine,[37] and patients with type 2 diabetes mellitus.[60] Dosages ranged from 2 µg per day p.o. for 12 months in healthy women[59] to 1000 µg per day i.m. for 3 weeks in diabetics.[60] Two studies used methyl-B12,[58,60] the others did not mention the form.

Overall, the evidence supports the use of B12, on its own or in addition to other supplements, for the reduction of homocysteine levels. The dosing regimen may depend on the specific patient population, with low-dose, long-term supplementation possibly being sufficient in healthy individuals, whilst patients with end-stage renal disease probably need higher dosages and ideally in combination with folate.

Pain

Animal studies have shown beneficial effects of B12 on the regeneration of nerves and the inhibition of pain-signalling pathways, including cyclooxygenase enzymes.[61] Clinical trials in humans have evaluated the benefits of B12 in a number of painful conditions.

Back pain

Low back pain is very common, it is estimated that 84% of the general population experience low back pain at least once in adulthood, with up to 90% being non-specific and without known cause.[62]

Two double-blind RCTs investigated the effectiveness of B12 in adults who had been suffering from low back pain for more than 6 months, and both found significantly better pain reduction in the B12 compared with the placebo group. Both studies administered B12 i.m., one used methyl-B12 at a dose of 500 µg three times per week for 2 weeks,[63] the other used cyano-B12, 1000 µg daily for 2 weeks.[64]

Positive effects of B12, 2500 µg daily sublingually for 90 days (form not reported), have also been seen in musculoskeletal pain as a side-effect of aromatase inhibitors in patients with breast cancer, with a 34% reduction of pain.[65] However, as this was an open-label, uncontrolled trial, it is impossible to assess how much of the improvement was due to a placebo effect.

Although clinical trials are limited, the evidence suggests that B12 injections at a dose of at least 500 µg three times per week for 2 weeks are effective in relieving chronic low back pain.

Herpetic neuralgia

Intense neuritic pain and neuralgia are common in patients with herpes zoster (HZ, shingles), and acute herpetic neuralgia (AHN) is defined as pain within 30 days of HZ infection.[66] Post-herpetic neuralgia affects one in five patients with HZ and, whilst most patients fully recover within 1 year, for some symptoms can persist for years or may be permanent.[67]

In 2018, a meta-analysis of four RCTs found significant benefits for herpetic neuralgia of locally injected methyl-B12, 1 mg on 6 days per week for 2–4 weeks.[68] All four studies appear to have been carried out by the same group of researchers. The same research group also investigated local injection of methyl-B12 in optic herpetic neuralgia alongside local lidocaine injection versus systemic B12 administration, i.m. or p.o., alongside local lidocaine, and found the local B12 injection to be significantly more effective in pain relief than either i.m. or p.o. B12 administration.[69]

An RCT of 31 patients with HZ and AHN investigated the effectiveness of i.m. B12 on 3 consecutive days (dose reported as 1 mg/ml, form not reported) alongside valaciclovir (an antiviral drug) for 5 days and diclofenac (a non-steroidal anti-inflammatory drug) versus valaciclovir and diclofenac alone.[70] At 3 weeks, the pain scores of both groups had significantly decreased from 5.6 and 5.1, respectively, to 0.9 in both groups, with no difference between groups. Unfortunately, pain scores were not assessed at any earlier points in this study, it is therefore unknown whether pain had decreased more in the B12 group at an earlier time point.

Whilst local injection of methyl-B12 appears to be effective in relieving herpetic pain alongside lidocaine injections, there is at present no evidence that systemic B12, either i.m. or p.o., is of benefit.

Peripheral neuropathy (PN)

In PN, nerves in the arms and legs are damaged, leading to a variety of symptoms, including pain, burning, numbness, tingling, loss of balance and co-ordination, and muscle weakness. About 1 in 10 people aged 55 years and over are affected by PN, and the most common cause is diabetes. Other causes include physical injury to nerves, viral infection such as HZ, alcohol and certain medications.[71]

Most research on the use of B12 focusses on diabetic neuropathy.

In the past 8 years, four meta-analyses, including 16–26 RCTs each, have pooled the data from studies comparing prostaglandin E1 (PE1) with and without B12 and with or without additional alpha-lipoic acid. The combination of B12 plus PE1 has been shown to be more effective in improving symptoms and nerve conduction velocity (NCV) than either B12[72] or PE1[73] alone. The addition of lipoic acid further increased improvements in both clinical symptoms and NCV.[74] A meta-analysis comparing B12 alone and with lipoic acid found that the combination is more effective than B12 alone.[75]

A number of studies on the use of B12 on its own in patients with diabetic neuropathy have been carried out, and all showed benefits in symptoms and/or neurophysiological parameters.[76,77,78,79,80,81,82,83] Not all studies reported which form of B12 was used, but all that specified form had used methyl-B12. B12 was given either orally[76,78,81,82] or injectable, i.m. 2000 µg twice per week for 3 months,[79] i.v. 500 µg three times per week for 6 months,[80] or intrathecally (into the cerebrospinal fluid) 2500 µg per month for several months.[83] For oral administration, dosages were in the range of 750–1500 µg per day for 12 weeks to 1 year.

Two clinical trials investigated the benefits of B12 in non-diabetic neuropathy. An open-label RCT compared two dosing regimens, i.m. methyl-B12 500 µg three times per week versus 1500 µg once a week for 2 weeks, in 24 patients with PN and 10 healthy volunteers.[84] A significantly higher increase in B12 levels was seen in both patients with PN and healthy volunteers with the three times per week compared with once a week administration. Both dosage regimens showed significant benefits in PN symptoms compared with baseline with no significant difference between the groups.

Another open-label trial involving 14 patients with immune-mediated or hereditary neuropathy evaluated i.v. methyl-B12, 25 mg daily for 10 days followed by 25 mg monthly for 5 months. Neuropathy scores improved

in seven patients, mostly in patients with immune-mediated neuropathy, but remained unchanged or worsened in the remaining five patients (two patients dropped out due to adverse events that were considered unrelated to B12).[85]

In a post-marketing surveillance study, methyl-B12, 750 µg per day p.o. for 4 weeks, has also shown benefit alongside pregabalin (a drug commonly used for neuropathic pain), but as this was an uncontrolled trial the contribution B12 made to the improvements cannot be established.[86]

Overall, the evidence is overwhelmingly in favour of the use of methyl-B12, alone or alongside other treatments, in diabetic neuropathy, with oral and injectable B12 showing benefits. Evidence from RCTs for the use of B12 in other types of neuropathies is scarce, although non-randomised/uncontrolled studies have shown some benefits.

Sleep–wake cycle

In the 1990s, a number of trials were carried out into the effects of B12 on circadian rhythm and the sleep–wake cycle, but no more recent studies have been published on this topic.

A number of small studies under laboratory conditions showed that B12 can alter melatonin levels and affect circadian rhythm.[87,88,89]

Results from studies on whether B12 can improve sleep–wake rhythm have been mixed. Whilst some studies found beneficial effects in patients with AD[36] and dementia[90] and in shift workers,[91] others found no improvements in patients with sleep–wake rhythm disorders.[92,93] However, in the latter patient population, B12 has shown some benefits when used alongside other approaches including light therapy and drugs, although these studies had some methodological weaknesses, making it difficult to interpret the results.[94,95] All of the studies had small sample sizes, and study details are mostly not well reported.

With no further research available at the moment, there is insufficient evidence to recommend B12 for sleep rhythm disorders.

The mechanism behind the possible influence of B12 on the circadian rhythm is not clear, but there is evidence to suggest that B12 may increase the light sensitivity of the circadian clock.[96]

Safety

B12 is considered safe even at high doses, and no adverse effects have been associated with excess B12 in healthy individuals.[2,97] Studies and clinical practice of treatment of pernicious anaemia with regular high-dose injections has confirmed the safety of B12 at high dosages, and therefore neither the Institute of Medicine (IOM) in the USA nor the European Food Safety Agency (EFSA) have set an upper limit.[2,97] The EFSA also states that B12 has not been found to be carcinogenic or mutagenic in *in vitro* and *in vivo* studies.

Caution is advised in patients with Leber optic atrophy, a genetic disorder caused by chronic cyanide intoxication (such as from smoking, alcohol or some plants), against the use of cyano-B12 due to its cyanide moiety, whilst hydroxo-B12 is a cyanide antagonist and can therefore be used in these patients.[1,2]

Allergic reactions, including anaphylactic shock, are rare and happen more often with injectable forms.[1] General side-effects of hydroxo-B12 listed in the British National Formulary (BNF) are diarrhoea, dizziness, headache, hot flushes, nausea, skin reactions and urine discolouration; the frequency of these reactions is unknown.[98] More side-effects are listed for injectable forms.

Drug interactions

No interactions with medications are listed in the BNF or other reports.[1,2,98]

Pregnancy

According to the EFSA, there is no evidence for teratogenicity or adverse effects on fertility or postnatal development with B12.[97] B12 requirements are increased during pregnancy.[2,97]

Lactation

B12 requirements are increased during breastfeeding.[2,97] Human milk naturally contains B12, and levels correlate with maternal intake and B12 status.[99] Daily doses of at least 50–100 µg are needed in cases of maternal deficiency, and should improve inadequate levels in the infant without exposing the infant to excessive B12.[99] B12 deficiency in infants can cause anaemia, abnormal skin and hair development, convulsions, failure to thrive, mental developmental delay and possibly abnormal movements, and direct supplementation of the infant is recommended in such cases.[99]

Children

There are no specific safety concerns for children.

Conclusion

Whilst severe B12 deficiency has a clear clinical picture with characteristic haematological and/or neurological features, establishing mild or borderline deficiency can be problematic as serum levels are maintained at the expense of tissue concentrations, and therefore serum levels of B12 may be in the low normal or borderline range despite a deficiency. Other markers are not commonly measured either in clinical practice or in many clinical trials, making it difficult to interpret whether positive outcomes in some studies are due to correcting a deficiency or due to other pharmacological functions of B12.

There is clear evidence for the use of B12 in diabetic neuropathy, either alone or with other treatments, and in lowering homocysteine levels, again either alone or with other nutrients. Benefits have also been shown in low back pain, autistic spectrum disorders and aphthous ulcers, although this is based on small numbers of clinical trials.

There is insufficient evidence for the use of B12, beyond correction of deficiency, in cognitive impairment, depression and fatigue. However, in view of the excellent safety profile of B12, even at high doses, supplementation, ideally as part of a more comprehensive nutritional programme, may still be of value when the clinical picture and/or other factors (e.g. vegans, elderly) are suggestive of subclinical deficiency, and with borderline or low normal serum B12 when confirming a deficiency through numerous tests is not possible.

It is generally accepted that oral high-dose B12 is as effective as i.m. administration in increasing B12 levels, even with IF deficiency, although in severe deficiency i.m. administration is recommended, at least initially, to quickly normalise levels. There is no evidence that sublingual administration has any benefits over oral intake, but it may be preferable for people with swallowing problems. Cyano-B12 is the most commonly used, and cheapest, form of B12 with no substantial evidence that other forms are more effective in improving B12 status.

B12 is generally considered safe in all life stages even at high doses and there are no known interactions with any medications, but some drugs can increase the risk of B12 deficiency, including acid-lowering drugs and metformin.

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Nutritional Medicine Journal

N-acetylcysteine: A Review of Clinical Use and Efficacy

Karin Elgar

Abstract

N-acetylcysteine (NAC) is a derivative of the amino acid cysteine and a precursor to glutathione, the master antioxidant of the body, which makes it an important compound in detoxification processes, and it is well known for its use as an antidote to paracetamol poisoning. NAC also has direct antioxidant as well as various anti-inflammatory effects, making it a useful supplement in inflammatory conditions. NAC has been extensively used for its mucolytic properties, and research has also demonstrated its ability to interrupt biofilms.

There is evidence for benefits of NAC in diverse conditions, including respiratory infections, various mental health disorders, male infertility and polycystic ovary syndrome.

NAC is generally well tolerated but has a few potential drug interactions, and caution is advised in some underlying conditions including gastrointestinal ulceration, bronchial asthma, liver and kidney failure.

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Introduction

N-acetylcysteine (NAC) is a derivative of the amino acid cysteine with an acetyl group (CH3CO) attached to its nitrogen atom.[1] Clinically, NAC is probably best known as an antidote to paracetamol (acetaminophen) poisoning, but it is also used for a large variety of other indications, based on its antioxidant, anti-inflammatory and mucolytic effects.[2]

NAC is available as a dietary supplement and as a licensed medicine, and can be administered orally, intravenously (IV) and by inhalation.[2] In the UK, NAC is licensed for IV use in paracetamol poisoning, as eye drops in tear deficiency, and orally as a mucolytic in adults with respiratory conditions such as chronic obstructive pulmonary disease (COPD).[3] There has been controversy over the classification of NAC as a licensed medicine or a supplement in the USA, and the US Food and Drug Administration (FDA) excluded NAC from the dietary supplement definition in 2020.[4]

The focus of this white paper is the use of NAC as a dietary supplement and, unless otherwise stated, refers to an oral administration.

NAC is readily absorbed in the gastrointestinal tract, but there are contradictory research findings as to whether NAC can cross cell membranes and the blood–brain barrier directly or whether it crosses as cysteine after de-acetylation.[1]

General effects

Antioxidant

NAC contains a thiol (sulfhydryl, -SH) group that can interact with reactive oxygen species (ROS; highly reactive and therefore potentially damaging molecules).[5] However, its most important antioxidant effect is thought to be as a precursor for glutathione (GSH),[6] a tripeptide made from the amino acids glutamate, glycine and cysteine, and the body's major antioxidant. Of the three amino acids, cysteine has the lowest concentration within cells and can therefore be rate-limiting for GSH synthesis.[5] Many of the clinical benefits of NAC are based on its ability to correct or prevent GSH depletion.[2]

A 2020 meta-analysis pooled data from 12 randomised-controlled trials (RCTs) looking at the effect of NAC on biomarkers of oxidative stress, and found a significant decrease in malondialdehyde (a marker of oxidative stress)[7]. Dosages used in the studies ranged from 400 to 2000 mg per day.

A number of studies looking at various clinical benefits have also looked at the effect of NAC on GSH levels, and found increases in blood cell GSH following NAC administration.[8,9,10,11,12,13]

Anti-inflammatory

Oxidative stress plays an important role in inflammation, so the antioxidant effects of NAC confer anti-inflammatory benefits. But NAC also appears to modulate inflammation through other mechanisms, including inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB; a protein complex involved in inflammation) and pro-inflammatory cytokines.[5,14]

Many clinical trials have reported levels of anti-inflammatory markers that were summarised by two recent meta-analyses. Whilst one meta-analysis of 24 RCTs found a decrease in the pro-inflammatory markers C-reactive protein (CRP) and interleukin-6 (IL-6),[15] the other (based on 28 studies) found a decrease in interleukin-8 (IL-8) when pooling all data and a decrease in IL-6 in a subset of studies using a dose of 1200 mg NAC per day or less, but no difference in CRP.[7] Dosages used in the studies ranged from 400 to 2000 mg per day.

Detoxification

The effectiveness of NAC as an antidote to paracetamol poisoning is based on its ability to restore GSH levels in the liver and to act as an alternative substrate for conjugation of toxic paracetamol metabolites.[16] GSH is also essential

for detoxifying toxic substances, including xenobiotics (chemicals foreign to the body) and heavy metals and, by increasing GSH levels, NAC is thought to play an important role in detoxification in general.[2]

Mucolytic

NAC has been used for its mucolytic (mucus-thinning) properties for decades. A number of mechanisms appear to contribute to the mucolytic and mucoregulatory benefits of NAC, including directly breaking disulphide bonds between the molecules forming the mucus with its thiol groups and reducing mucus production through its antioxidant effects (oxidative stress has been shown to increase mucus production).[17]

Biofilms

NAC has attracted interest for its potential to disrupt biofilms through its mucolytic properties.[18] A biofilm is an aggregate of bacteria in an extracellular mucus-like matrix that adheres to a surface. These biofilms can play an important role in infections as they protect pathogenic bacteria from antibacterial substances.[18] NAC has also been shown to have direct antibacterial effects, adding to its biofilm-disrupting potential.[19]

Clinical uses

Alzheimer's disease/cognitive function

Oxidative stress has been implicated in neurodegenerative conditions and impaired cognitive function and, in animal models, NAC has been shown to have a beneficial effect on cognitive function.[20]

There is one early blinded, placebo-controlled trial of NAC on its own in 43 patients with Alzheimer's disease that found NAC, 50 mg per kg bodyweight (equivalent to 3750 mg for a person weighing 75 kg) per day for 6 months, to be superior to placebo in almost all outcome measures, although not all reached statistical significance.[21] Three clinical trials, one open-label and uncontrolled,[22] one open-label and placebo controlled,[23] and one double-blind and placebo controlled,[24] investigated a multi-nutrient formulation containing NAC 1200 mg per day (other nutrients: folate, vitamin B12, alpha-tocopherol, S-adenosyl methionine and acetyl-L-carnitine) for 3–12 months in patients with Alzheimer's disease, and found significant benefits in Dementia Rating Scale and clock-drawing tests as well as in assessments by caregivers in those receiving the multi-nutrient supplement versus placebo or versus baseline.

A double-blind, placebo-controlled trial in frail elderly people without cognitive impairment found significant improvements in cognition from baseline with NAC, 1800 mg per day for 6 weeks, whilst there were no improvements in the placebo group.[25] However, the difference between the NAC and the placebo group failed to reach statistical significance. Both groups also underwent the same exercise programme.

A 2020 meta-analysis evaluated the effects of NAC on cognitive function in patients with schizophrenia.[26] Three out of the five RCTs reviewed in this meta-analysis showed improvements in at least one cognitive function outcome measure, and pooled data from three trials showed a significant improvement in working memory, whilst improvements in processing speed failed to reach statistical significance. Trials that showed benefits used dosages between 1200 and 2700 mg per day for 12–24 weeks.

One double-blind, placebo-controlled trial that investigated the effects of NAC, 2000 mg per day for 6 months, on cognition in patients with bipolar disorder (BD) found no improvements in cognitive function.[27]

Whilst evidence is limited in Alzheimer's disease, due to lack of clinical trials using NAC on its own, it is overall in favour of benefits with a dose of at least 1200 mg per day, and ideally as part of a multi-nutrient approach. NAC also appears to improve some aspects of cognition in patients with schizophrenia with a dose of at least 1200 mg per day.

Exercise performance

Intensive or prolonged exercise can result in excessive production of ROS and oxidative damage in muscles.[28] The potential benefits of antioxidants, including NAC, have therefore been studied in sports nutrition.

In 2017, a meta-analysis of seven RCTs (86 participants) found no benefits of NAC supplementation on exercise performance.[29] Since then another two small double-blind, placebo-controlled studies confirmed these results.[30,31] It is important to note that all studies were very small (maximum 18 participants), and looked at acute or short-term (up to 9 days) supplementation. A study published in 2018 established baseline GSH levels, and found that those with low GSH status at baseline also had lower physical performance, increased oxidative stress and impaired redox metabolism of erythrocytes at baseline compared with those with medium or high levels.[32] The low GSH group, but not the other two groups, experienced improved exercise performance, improved GSH status and oxidative stress with NAC 1200 mg per day for 30 days compared with placebo.

Several earlier small studies investigated NAC for other aspects of exercise physiology with mixed results,[33,34] although two studies found improvements in muscle fatigue with acute or short-term (7 days) NAC supplementation.[35,36]

Overall, the evidence does not support a benefit of NAC to enhance exercise performance, although it needs to be stressed that all studies were very small and either acute or short-term. There may be benefits for people with low GSH status at baseline.

Heavy metal toxicity

In vitro and animal studies have shown the potential of NAC to chelate (bind) and detoxify heavy metals,[37] but only a few human clinical studies have investigated NAC in heavy metal toxicity.

Gold-based drugs are sometimes used in the treatment of rheumatoid arthritis, and can cause serious side-effects. An early study compared excretion of gold through urine in 40 such patients with or without NAC, a single dose of 3000 mg by IV infusion over 6 hours, and found a 54% increase in excretion in the NAC group.[38] The same article also reports two cases with severe gold-induced bone marrow suppression who received 3000–6000 mg per day IV for 7 days and made a progressive recovery over 2 weeks.

Lead binds to thiol groups, and may therefore inactivate GSH as well as other enzymes with functional thiol groups, and has been shown to increase homocysteine levels (see below for homocysteine).[39] A RCT, including 171 workers occupationally exposed to lead, evaluated the effects of three different daily dosages of NAC, 200 mg, 400 mg or 800 mg, compared with no intervention, for 12 weeks.[39] Blood lead levels significantly decreased in all three NAC groups compared with no treatment, with no apparent effect of dose. Homocysteine levels, which were elevated at baseline, and protein carbonyl groups (a marker of oxidative stress) decreased in a dose-dependent way, with a 37% reduction of homocysteine and a 23% reduction of protein carbonyl groups with 800 mg per day.

Whilst evidence from clinical trials is limited, NAC promises to be effective at increasing heavy metal excretion, with a dose of 800 mg per day sufficient to lower blood levels of lead in exposed workers.

Helicobacter pylori eradication

Helicobacter pylori (H. pylori) is a bacterium that causes 70–80% of gastric and 95% of duodenal ulcers, and is also associated with acute and chronic gastritis and gastric cancer.[40] Usual treatment is based on triple therapy with two antibiotics and a proton pump inhibitor (PPI). However, there is a 10–20% treatment failure rate, at least in part due to antibiotic resistance. Research has shown that *H. pylori* forms a biofilm, which may prevent the antibiotics

from reaching the bacteria, and NAC, with its ability to disrupt biofilms, has therefore been investigated in a number of clinical trials alongside standard therapy.[18]

A 2019 Cochrane review of eight RCTs of NAC alongside standard treatment versus standard treatment alone or standard treatment with placebo[41] found that when pooling data from all studies, the improvement in eradication rates failed to reach statistical significance, but when only data from those five RCTs that used current eradication regimens (triple therapy in four RCTs; PPI, one antibiotic and bismuth subcitrate in the remaining study) were pooled, there was a significant 29% increase in eradication rate with the addition of NAC. Most trials in this meta-analysis used NAC, 1200 mg per day, one used 600 mg per day, and one used 1800 mg per day. The study with the highest dose found no significant beneficial effect of NAC.

In 2020 another open-label RCT of triple therapy with or without NAC, 1200 mg per day, was published, and reported that addition of NAC did not improve eradication rates.[42]

Whilst the evidence from clinical trials is mixed, overall there appears to be a benefit of NAC at a dose of 1200 mg per day alongside triple therapy.

Hyperhomocysteinemia

Elevated plasma levels of homocysteine is an independent risk factor for cardiovascular disease, as well as other chronic degenerative conditions. Homocysteine is a thiol-containing compound, 75–80% of which is bound to albumin (a blood protein). This bond can be broken by NAC and the homocysteine replaced by NAC, thus increasing free homocysteine and its clearance through the kidneys.[43,44]

A couple of uncontrolled studies in healthy volunteers showed significant homocysteine-lowering effects of acute administration of NAC, 7200 mg within 24 hours,[45] and 1200–4000 mg once,[44] with average reductions of 44% and 22%, respectively.

Beneficial homocysteine-lowering effects of NAC have also been found in a number of longer-term RCTs, with mean reductions in the range of 10–45%, depending on baseline level, patient population and dosage.[39,43,46,47,48] Dosages used have ranged from 800 to 2000 mg per day in longer-term studies (2–12 weeks), with the highest dose seeing the largest reductions in homocysteine.[48] Studies comparing different dosages also found a dose-dependent increase in reduction of homocysteine levels with increasing dose.[39,44,47] In one crossover study,[47] homocysteine levels returned to baseline when NAC was stopped for the 1-month washout period, suggesting that effects are immediate and that NAC may need to be taken continuously to help maintain homocysteine levels.

As results from clinical trials have consistently shown a homocysteine-lowering effect, NAC at a dose of at least 800 mg per day could be recommended to people with elevated homocysteine levels.

Male subfertility

Infertile men have been shown to have higher levels of ROS in semen than fertile men, and ROS have been implicated in sperm dysfunction and male infertility.[49]

Three clinical trials found significant improvements in sperm quality following supplementation with NAC, 600 mg per day, for 3–6 months. A double-blind placebo-controlled trial of NAC versus selenium versus selenium plus NAC versus placebo found significant improvements in all semen parameters with both NAC and selenium trialled singly, and additive effects when both were used together.[50] An increase of testosterone, which was associated with a decrease in luteinising hormone (LH) and follicle-stimulating hormone (FSH), was also observed with NAC and selenium, individually and combined, in this study. Elevated levels of FSH and LH are associated with male infertility.[51] Whilst mean baseline levels for FSH and LH in study participants were within normal range, median levels were borderline high, suggesting that a considerable number of participants may have had elevated levels of LH and/or FSH.[50] Another RCT reported significant improvements in volume, motility and viscosity of semen, plasma total antioxidant capacity (TAC) and oxidative stress, but not sperm count or morphology with NAC compared with placebo.[52] An open-label, uncontrolled trial saw significant improvements in sperm motility, count, morphology and DNA fragmentation, testosterone, LH, FSH, TAC and oxidative stress.[49]

Based on the results from the above studies, the two mechanisms by which NAC may improve male fertility are thought to be its antioxidant effects as well as effects on the hypothalamus–pituitary–gonadal axis, normalising sex hormone levels.[50,52]

The evidence from the three clinical trials on NAC and male fertility suggests that 600 mg per day for 3–6 months is effective in improving sperm and semen quality, hormone levels and antioxidant status.

Non-alcoholic steatohepatitis

Non-alcoholic fatty liver disease (NAFLD) is characterised by excessive fat accumulation in the liver, and is associated with insulin resistance, oxidative stress and inflammation. NAFLD develops in four stages, steatosis (simply a fatty liver that is thought to be largely harmless), non-alcoholic steatohepatitis (NASH; where the liver has become inflamed), fibrosis (with the formation of scar tissue) and finally cirrhosis (permanent damage that can lead to liver failure and cancer).[53] In preclinical models of NAFLD, NAC has been shown to block fat accumulation in the liver.[54]

Four open-label clinical trials have been conducted into the potential benefits of NAC in NASH: one 4-week trial of NAC, 600 mg per day, versus no treatment, found statistically significant improvements in alanine aminotransferase (ALT; a liver enzyme and marker of liver damage) in both groups, and significant improvements in aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT) in the NAC group only.[55] However, the authors did not interpret this as evidence for therapeutic effects of NAC but of the fluctuating course of the biochemical parameters in NASH. A 3-month RCT of NAC, 1200 mg per day, versus vitamin C, 2000 mg per day, found significant improvements in ALT and spleen span, but not other liver enzymes or liver span with NAC, whilst improvements in steatosis grade failed to reach statistical significance.[56] Two longer-term studies, 48 and 52 weeks, respectively, found significant improvements in NAFLD/NASH activity score with NAC, 1200 mg per day, together with metformin.[57,58] The 52-week study also found improvements in a number of biochemical parameters, including glycaemic control and liver enzymes.[58]

The benefits of NAC in NAFLD are thought to be due to its antioxidant and anti-inflammatory properties with an attenuation of lipid peroxidation.[54]

Based on the above studies, NAC, at a dose of 1200 mg per day, appears to have benefits in patients with NASH, with treatment durations of 48 weeks or more and in combination with metformin.

Polycystic ovary syndrome

Polycystic ovary syndrome (PCOS) is a common endocrine disorder that affects about 1 in 10 women, and is characterised by enlarged ovaries that contain many fluid-filled sacs (follicles) that surround the eggs, excess testosterone and irregular periods. One of the underlying causes of PCOS is thought to be insulin resistance.[59]

Fertility is commonly affected by PCOS, due to lack of ovulation, and first-line treatment for anovulation in women with PCOS is usually

clomiphene citrate (CC), but some women do not respond to this treatment (CC-resistant).[60] Fertility in PCOS has been subject to more than 10 clinical trials on the potential benefits of NAC.

In 2015, a review and meta-analysis of eight studies, including 910 women with PCOS, found that NAC increased ovulation (four trials), pregnancy (three trials) and live birth (one trial) rate by more than three times compared with placebo.[60] There was no difference in miscarriage, menstrual regulation, acne, hirsutism, change in body mass index, testosterone and insulin levels between NAC and placebo. There was also no difference in adverse effects reported and no reports of ovarian hyper-stimulation syndrome, a potentially life-threatening condition associated with drugs stimulating ovulation.[60]

Since then, several more RCTs have found that NAC, when used alongside CC or letrozole, another ovulation-stimulating drug, increased pregnancy and ovulation rates versus placebo,[61] or was as good or better than metformin[62,63,64] or L-carnitine.[65] Regimens used in these studies were 1200–1800 mg per day on days 3–7 of the menstrual cycle, for two–three cycles.

NAC has also shown benefits in improving oocyte quality and/or sex hormone levels in candidates undergoing intracytoplasmic sperm injection (ICSI).[64,66,67] The regimen used in these trials was 1800 mg per day from day 3 of the menstrual cycle to oocyte aspiration (approximately 6 weeks).

In one trial of candidates for intrauterine insemination with NAC, 1200 mg per day on days 3–7 of the menstrual cycle, improvements in number of mature follicles and pregnancy rates failed to reach statistical significance.[68]

NAC, at a dose of 1800 mg per day, has also been shown to improve glycaemic control, lipid profiles and/or oxidative stress/TAC in women with PCOS in some of the fertility studies above,[62,64,65,67] as well as in two longer-term studies of 24 weeks continuous supplementation.[69,70]

A number of possible mechanisms for the role of NAC in PCOS have been discussed in the literature. Increased intracellular GSH levels can influence insulin receptor activity thereby increasing insulin sensitivity, which in turn balances insulin and testosterone levels with a positive effect ovulation.[62] The antioxidant effects of NAC may also be responsible for the endometrial thickening observed in some trials.[63] The mucolytic action of NAC is thought to counteract the negative effects of CC on cervical mucus.[62]

Overall, the evidence shows that NAC, at a dose of 1800 mg per day on days 3–7 of the menstrual cycle, is beneficial as an add-on for women with PCOS who are undergoing fertility treatment with an ovulation-stimulating drug, such as CC. At that dose, NAC has also been shown to improve glycaemic control, hormone levels and antioxidant status in this patient group, and longer-term supplementation may therefore be beneficial.

Psychiatric disorders

Many psychiatric disorders are associated with oxidative stress, reduced antioxidant capacity and inflammation. NAC may influence inflammation directly or via its antioxidant properties, and animal studies have shown increased GSH levels in the brain following NAC administration.[71] NAC also appears to have a balancing effect on neurotransmitter dysregulation, in particular glutamate homeostasis.[71] Glutamate is the main neurotransmitter in the central nervous system, and extracellular glutamate levels are maintained by the exchange of extracellular cysteine for intracellular glutamate.[72]

Addiction

Substance use disorders (SUDs) are a major health issue, accounting for 14.5% of disability-adjusted life years according to the Global Burden of Disease Study, 2010.[72]

A 2021 meta-analysis of 16 RCTs, involving 987 patients with SUDs, found a significant decrease in craving symptoms and a significant improvement in depressive and withdrawal symptoms with NAC compared with control, whilst lower smoking frequency failed to reach statistical significance.[72] The authors reported that there was no difference in adverse events (AEs) between the NAC and control groups. Another meta-analysis of seven double-blind, placebo-controlled trials on the effect of NAC on craving specifically also found a significant reduction in craving symptoms.[73] Most individual studies were small, less than 50 participants, but there was not much heterogeneity. Dosages used ranged from 1200 to 3000 mg per day for 4 weeks to 14 months, or from 2400 to 3600 mg for short-term use (3–4 days).

A small pilot study investigated the effect of NAC on pathological gambling.[74] There was a reduction in the Yale Brown Obsessive Compulsive Scale Modified for Pathological Gambling (PG-YBO) score from a mean of 20.3 at baseline to 11.8 at the end of an 8-week open-label phase where participants received NAC, starting with 600 mg per day and increasing to up to 1800 mg per day, depending on their response. Almost 60% of subjects responded to treatment, and 13 responders were enrolled into a double-blind, placebo-controlled extension where they received either NAC, at the highest dose they had received in the open-label part of the study, or placebo. A further improvement was seen during the extension in those receiving NAC, although this failed to reach statistical significance compared with placebo, possibly due to the small numbers or due to the initial improvement being a placebo effect.

The evidence from clinical trials shows consistently that NAC can support substance use cessation, with dosages of 1200–3000 mg per day longer term. Although based on only one study, NAC could also be trialled in pathological gambling, which has similar pathophysiological mechanisms as other addictions.

Addictive behaviours have been linked to glutamate dysregulation, and the benefits of NAC are thought to be mediated by its effect on glutamate homeostasis.[72]

Autism and attention deficit hyperactivity disorder (ADHD)

The two main symptoms of autism spectrum disorder (ASD) are a deficit in social communication and/or interaction and repetitive behaviours and/or restricted interests. Dysregulation of glutamate and GABA balance and oxidative stress with a reduction in intracellular GSH are thought to be involved in the development of ASD, making NAC a prime candidate for treatment.[75]

Five RCTs have been conducted in children with ASD, and these have been summarised and evaluated in a 2021 meta-analysis. Three of the reviewed studies found significant improvements in ASD assessment scales,[76,77,78] the other two did not.[13,79] The results of the pooled data show a significant improvement with NAC in the Aberrant Behaviour Checklist (based on four studies), whilst no improvement was seen in the Repetitive Behaviour Scale (two studies), and improvements in the Social Responsiveness Scale (two studies) failed to reach statistical significance. The dosages used in the RCTs that showed benefits ranged from 600 to 2700 mg per day for 4–12 weeks.

One of the studies evaluated GSH levels and found a significant increase in blood GSH levels in the patients receiving NAC, although this study did not find significant improvements in behavioural outcomes.[13]

Although results from the five studies are mixed, the overall evidence suggests a possible benefit of NAC in children with ASD, at a dose of at least 600 mg per day for at least 8 weeks. In most studies, NAC was used alongside medication, such as risperidone.

Only one study has investigated ADHD in patients with systemic lupus erythematosus, and found NAC, at dosages of 2400 and 4800 mg per day for 3 months, to be effective in reducing ADHD scores, with a trend to the higher dose being more effective.[80] As this was quite a specific patient group, it is not possible to extrapolate these findings to the wider ADHD patient population.

Bipolar disorder

Bipolar disorder (BD) is a common psychiatric disorder, characterised by alternating depressive and manic moods, and can be a significant cause of disability and morbidity.[81] Current pharmacological treatments tend to be less effective in the depressive than the manic phase.[81] BD is associated with a dysregulation of the glutamate neurotransmitter system and with oxidative stress, and NAC has therefore received interest as an adjunct to standard treatment of depressive symptoms in BD.[82]

There are seven RCTs for the use of NAC in BD, which have been evaluated in three meta-analyses. Whilst one meta-analysis of five RCTs found no significant improvements in any of the BD scores,[81] another meta-analysis of six studies found a significant effect of NAC on Clinical Global Impression-Severity Scale (CGI-S) score, but not any other scores.[83] The third meta-analysis of six studies combined data from different scales used to assess depressive symptoms, and found a moderate but statistically significant improvement with NAC.[82] Five out of the seven studies showed some positive results, but there was significant heterogeneity between individual study results that was not explained by NAC dose, duration of treatment or severity of depressive symptoms at baseline.[82] All studies had fairly small sample sizes, the largest including 120 patients. Dosages used in trials showing beneficial effects ranged from 1000 to 2000 mg per day for 10–24 weeks.

A small open-label pilot study investigated the effects of NAC, 2400 mg per day for 8 weeks, in 15–24 year olds with depression and an increased risk of developing BD (parent with BD).[84] There were significant improvements in depressive and anxiety symptoms and CGI-S. Proton magnetic resonance spectroscopy also showed a decrease in glutamate in the left ventrolateral prefrontal cortex, although these findings were not statistically significant.

Whilst results from clinical trials are mixed, the overall evidence suggests a possible benefit of NAC in BD at dosages of 1000–2000 mg per day for at least 10 weeks.

Depression

Four double-blind, placebo-controlled trials have looked at the potential benefits of NAC in depression, other than BD.

One evaluated the benefits of NAC, 2000 mg per day for 12 weeks, with a follow-up visit after another 4 weeks, in patients with major depressive disorder (MDD).[85] Improvements seen at 12 weeks in the NAC groups were not significantly better than in the placebo group, although some secondary outcomes were significantly better in the NAC group at 16 weeks. Inflammatory markers were also evaluated in this study, but no improvements were observed in IL-6, CRP or brain-derived neurotrophic factor.[86]

Another study in patients with BD or MDD found significant improvements in depression and anxiety scales after 12 weeks on NAC, 1800 mg per day, compared with placebo; however, data for patients with MDD only were not reported in the article.[87]

A trial in patients with trichotillomania (hair pulling, see also below under obsessive compulsive disorders) found a clinically significant improvement in depressive symptoms in 44% of patients, compared with 4% in the placebo group, a statistically significant difference.[88] Patients received NAC, 1200–2400 mg per day, or placebo for 12 weeks in this trial.

In a smoking cessation study, depressive symptoms also significantly improved by 43% in patients receiving NAC, 3000 mg per day for 12 weeks, versus 11% in the placebo group.[89]

A double-blind, placebo-controlled study in veterans with post-traumatic stress disorder (PTSD) and SUD found a 48% decrease in depressive symptoms and a 32% decrease in self-reported PTSD symptoms, results that were significantly better than placebo (15% and 3%, respectively).[90] The dose used in this study was 2400 mg per day for 8 weeks, with significant improvements noted from week 3. Another study in PTSD is currently ongoing.[91]

Evidence in patients with depression without concomitant other psychiatric disorders is limited, but NAC appears to improve depressive symptoms associated with other disorders, including BD, trichotillomania, tobacco addiction and PTSD.

As with BD, the underlying mechanisms are thought to be the antioxidant properties and regulating effect of NAC on glutamate homeostasis.[92]

Obsessive compulsive disorder

Obsessive compulsive disorder (OCD) is a common mental health disorder characterised by obsessive thoughts and compulsive behaviours. The neurotransmitter serotonin appears to play a role in the condition, but only about half of patients respond to treatment with selective serotonin reuptake inhibitors, suggesting that other mechanisms are also present.[93] Glutamatergic hyperactivity with its ensuing excitotoxicity and oxidative stress are also thought to play a role, making NAC a potential treatment option.[93]

A 2018 review and meta-analysis of five double-blind, placebo-controlled trials of NAC in OCD found that pooled data of four studies just failed to reach statistical significance for improvements in the Yale-Brown Obsessive Compulsive Scale (Y-BOCS).[93] Three of the individual studies found significant improvements in Y-BOCS whilst two did not, although the latter two studies found improvements in anxiety or compulsion subscales. NAC dosages in those studies that saw benefits in Y-BOCS score ranged from 2000 to 2400 mg per day for 10–12 weeks.

A small double-blind pilot study of 11 children with OCD, aged 8–17 years, found a significant 33% reduction in the children's Y-BOCS score compared with placebo.[94] Children received 900 mg per day in the first week, 1800 mg per day in the second week and 2700 mg per day from week 3.

NAC has also been investigated in specific OCDs, including trichotillomania (hair pulling) and skin picking disorder (SPD), which can lead to serious skin infections.

A double-blind, placebo-controlled trial in adults with trichotillomania showed a significant 56% reduction in hair pulling with NAC, 1200 mg per day for 6 weeks followed by 2400 mg per day for another 6 weeks, compared with a 16% reduction in the placebo group, with improvements noticeable from 9 weeks.[88] A study in children with trichotillomania by the same team, however, found no benefits of NAC at a dose titrated from 600 mg per day to 2400 mg per day, study duration 12 weeks.[95]

A number of case reports have also been published in the literature reporting benefits in both children[96,97] and adults with trichotillomania.[96,98,99]

A double-blind, placebo-controlled trial in adults with SPD found a 39% improvement with NAC compared with placebo (21% improvement).[100] Forty-seven percent of patients in the NAC group were "much or very much improved", compared with 19% in the placebo group. Both results were statistically significantly better in the NAC group. NAC dose was titrated from 1200 mg per day to 3000 mg per day, and duration of the trial was 12 weeks. An open-label study in adult and paediatric patients with Prader-Willi syndrome (a rare genetic disorder that causes a range of

physical symptoms, learning difficulties and behavioural problems, including obsessive compulsive symptoms) and SPD also found significant benefits of NAC, 450–1200 mg per day for 12 weeks, with 25 out of 35 patients having a complete resolution of skin lesions, and the remaining 10 participants had significant improvements.[101]

A number of case reports in both adults[102,103] and children[104] have also shown benefits of NAC in skin picking. Two of these case series suggest that dosage may be of importance, with individual differences and higher doses more effective than lower ones where dose was titrated up (dosage ranges 600–1800 mg per day).[102,104] These case series also suggest that ongoing treatment may be necessary to maintain remission.[102]

One double-blind, placebo-controlled trial evaluated NAC in children and adolescents with Tourette syndrome ("tics"), which is commonly associated with OCD, but found no benefit of 2400 mg per day for 12 weeks versus placebo.[105]

Although evidence is mixed, overall the research suggests a benefit of NAC in OCDs, including trichotillomania and SPD. Dosage may need to be established for the individual, for example, starting with 600 or 1200 mg per day and titrating up to 2400 mg per day if necessary. Results have been seen from 8 weeks of supplementation.

Schizophrenia

Schizophrenia is a severe chronic psychiatric condition with symptoms including hallucinations, delusions and cognitive impairment. A key molecular target for treatment of schizophrenia is the N-methyl-D-aspartate (NMDA) receptor, a glutamate receptor and ion channel in neurons, which appears to be modulated by NAC.[106] Schizophrenia has also been associated with oxidative stress and reduced GSH levels in the brain.[26]

A 2020 meta-analysis of seven RCTs, including 440 patients with schizophrenia, found a significant improvement in Positive and Negative Syndrome Scale (PANSS) total and negative scale with NAC supplementation, especially in trials of 24 weeks or longer duration.[26] Working memory also improved in those on NAC versus controls. Dosages used in the reviewed studies ranged from 600 to 3600 mg per day for 8 weeks to 1 year, with studies using dosages of 1000–2000 mg per day showing the best responses. An open-label, uncontrolled study using 1200 mg per day for 8 weeks also found significant improvements in negative symptoms and CGI-S.[107]

Three small double-blind trials have looked at the effects of NAC on electroencephalogram (EEG) measures in patients with schizophrenia, and found significant improvements after 8 weeks with dosages of 2000–2400 mg per day.[106,108,109] It is thought that these EEG improvements may precede clinical improvements.[108]

A couple of recent double-blind, placebo-controlled, crossover studies using magnetic resonance imaging of the brain also showed significant beneficial changes with acute, single-dose (2400 mg) administration of NAC.[110,111]

Whilst duration of NAC supplementation appears to be important, another potential cause for some of the heterogeneity observed in clinical trials may be the length of time patients have been suffering from schizophrenia, with those with a history of schizophrenia for 20 years or more responding best to NAC.[112]

Overall, the evidence is in favour of a benefit of NAC for patients with schizophrenia, with most trials using a dose of 1200–2000 mg per day, and durations of at least 24 weeks appear to be necessary to see positive results.

Respiratory conditions

Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is characterised by breathing difficulties, and includes chronic bronchitis and emphysema. It is a major cause of morbidity and mortality worldwide, especially in elderly people.[113] COPD is associated with an abnormal inflammatory response in the lungs, and with excessive mucus production and reduced mucus clearance.[113] Due to its mucolytic, anti-inflammatory and antioxidant properties, NAC is commonly used in COPD.[113]

Three meta-analyses covering 11, 12 and 13 RCTs, respectively, all found a beneficial effect of NAC in reducing the number of exacerbations by 10–25%.[113,114,115] Dosages in the included studies ranged from 260 to 1800 mg per day, with both high (> 600 mg per day) and low doses (≤ 600 mg per day) being effective. Duration of studies ranged from 3 months to 3 years, and one study found that significant benefits were only seen with a duration of at least 6 months.[113] All three meta-analyses reported that there was no increased risk of AEs with NAC compared with control.

The overall evidence is in favour of the use of NAC in COPD, and NAC is licensed in many countries as a mucolytic for patients with COPD and other airway diseases, such as cystic fibrosis.[3,116] The most commonly used dose is 600 mg per day, and long-term use is recommended.

Respiratory infections

In addition to its mucolytic, antioxidant and anti-inflammatory properties, the potential of NAC to disrupt biofilms is thought to be of benefit in acute respiratory infections.[19] Although evidence from clinical trials for its benefits is limited, NAC is commonly used in respiratory infections.

A Cochrane review from 2013 pooled data from six RCTs of NAC or carbocysteine (another mucolytic) in children with respiratory infections and found some benefit, although it was deemed to be of limited clinical relevance.[117] Only three of the studies included in this review were on NAC, but data were not divided into the separate compounds and there is no public access to the original studies that were published in 1972 and 1989, making it difficult to assess the use of NAC in this context.

A study of 120 hospitalised children with bronchopneumonia evaluated inhaled NAC versus ambroxol (another mucolytic drug), and found NAC to be superior to ambroxol, with faster recovery, shorter hospital stay and less AEs in the children receiving NAC.[118]

In a double-blind, placebo-controlled trial of 60 mechanically ventilated patients at high risk of developing ventilator-associated pneumonia (VAP), NAC significantly reduced the risk of VAP versus placebo, 26.6% versus 46.6%, and shortened intensive care unit (ICU) stay (14.4 versus 17.8 days) and hospital stay (19.2 versus 24.6 days).[119] Patients received NAC, 1200 mg per day, or placebo by nasogastric tube.

NAC also appears to be of benefit in preventing influenza. A double-blind, placebo-controlled trial of 262 subjects who were either younger than 65 years and had a chronic degenerative disease or over 65 years with or without chronic disease, but without chronic respiratory disease, received NAC, 1200 mg per day, or placebo for 6 months.[120] Participants who received NAC had a significant decrease in flu-like episodes, severity of illness and length of time confined to bed.

Although evidence from clinical trials is limited, NAC appears to be of benefit in a variety of contexts for treatment or prevention of respiratory infections, at a dose of 1200 mg per day orally (or by nasogastric tube), or as inhalation.

COVID-19

In view of the benefits of NAC in other types of respiratory illnesses and its possible ability to

stop a cytokine storm,[121] it has received much attention in the treatment of COVID-19.

In the context of the COVID-19 pandemic, a review evaluated the effectiveness of NAC in acute respiratory distress syndrome and acute lung injury, conditions similar to signs and symptoms of severe COVID-19.[122] The authors concluded that NAC may improve markers of inflammation or oxidation, systemic oxygenation, the need for/duration of ventilation, rate of patient recovery and clinical improvement score, whilst effects on length of stay, CT/x-ray images, mortality rate and pulmonary complications were inconclusive. NAC was mostly administered IV in an ICU setting.

A number of case reports and series of COVID-19 patients requiring mechanical ventilation have shown significant benefits in most patients.[121,123,124] Most of these patients received NAC IV at high doses.

However, to date, only one double-blind, placebo-controlled trial has investigated the possible benefits of NAC in severe COVID-19.[125] One-hundred and thirty-five patients were randomised to receive NAC, 21 g over 20 hours IV, or placebo. There were no significant differences in need for mechanical ventilation, time of mechanical ventilation, admission to ICU, time in ICU, or mortality between the NAC and the placebo group.

There are no clinical studies of NAC in mild to moderate COVID-19.

At present there is no evidence from clinical trials for the use of NAC in COVID-19, and more research in this area is urgently needed.

Safety

NAC is generally well tolerated. Several meta-analyses showed that there was no significantly increased risk of AEs in the NAC compared with control group in various disorders, including COPD,[113,114,115] addiction,[115] PCOS[60] and *H. pylori*.[126]

Side-effects

The following side-effects are listed for licensed oral NAC for adults:[3,116]

- Uncommon (1 in 1000 to 1 in 100): diarrhoea; fever; gastrointestinal discomfort; headache; hypotension; nausea; stomatitis; tinnitus; vomiting; allergic reactions; increased pulse rate; fever.
- Rare or very rare (less than 1 in 1000): haemorrhage.
- Frequency not known: facial oedema.

Cautions

NAC should be used with caution in patients with current or a history of gastrointestinal ulceration, bronchial asthma, liver and kidney failure.[3,116]

Drug interactions

Nitroglycerine

Concomitant administration of NAC and IV or transdermal nitroglycerine has been shown to cause severe hypotension[127] and headaches[128] in patients with angina pectoris.

Anticoagulant/antiplatelet drugs

Clinical research suggests that NAC, when administered IV, has anticoagulant and platelet-inhibiting properties,[129,130] in the context of major surgeries.

Pregnancy

NAC can cross the placenta,[131] but research into the safety of oral NAC during pregnancy is limited.

An RCT of NAC, 600 mg per day, in pregnant women with a history of preterm birth and bacterial vaginosis showed that women receiving NAC had a lower rate of preterm birth and a higher gestational age at delivery, compared with placebo.[132] No negative effects on mother or baby were reported. Another RCT evaluated the use of NAC, 2400 mg per day, in pre-eclampsia, and found no differences in maternal or neonatal outcomes between the NAC and the placebo groups.[133]

Whilst the available evidence suggests that NAC is safe during pregnancy, in view of the scarcity of data, NAC supplementation should only be recommended to pregnant women by a suitably qualified and experienced healthcare professional.

Lactation

There are no studies reporting the safety of NAC during breastfeeding.

Children

NAC has been used safely in children and adolescents at doses of 600–2400 mg per day in a number of clinical trials,[78,94,95,105] although one RCT in autistic children reported constipation (16.1%), increased appetite (16.1%), fatigue (12.9%), nervousness (12.9%) and daytime drowsiness (12.9%), which occurred significantly more commonly in the NAC than in the placebo group.[77]

A Cochrane review of the use of NAC in children with respiratory infections evaluated the safety data from 34 clinical trials, involving 2064 children, and found NAC to be safe, although the authors note that 59 cases of paradoxically increased bronchorrhoea (increased sputum production) in infants under the age of 2 years were reported to the French pharmacovigilance system.[117,134]

Overall, NAC at a dose of 600–2400 mg per day appears to be safe for children over the age of 2 years.

Conclusion

NAC has been extensively used, both as a dietary supplement and as a prescription medication, and has been found to be generally well tolerated in adults and children. Research has been in favour of using NAC in numerous indications, including respiratory infections, various mental health disorders, *H. pylori* infection, male infertility and PCOS. These clinical benefits are thought to be mediated through its antioxidant, anti-inflammatory and mucolytic properties, its importance in detoxification processes, and potentially its ability to disrupt biofilms.

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Nutritional Medicine Journal

Zinc:
A Review of Clinical Use and Efficacy

Karin Elgar

Abstract

Zinc is an essential trace element and is required for many vital functions, including protein folding, as a co-factor for enzymes, in regulating gene expression, supporting cell membrane structure and cell signalling. It also has antioxidant and anti-inflammatory properties. As such, zinc plays an important role in growth and development, immune function, neurotransmission, vision and reproduction. Zinc deficiency is common, especially in developing countries, and can be due to dietary factors, malabsorption and alcoholic liver disease. Zinc supplementation at appropriate levels is considered safe, and has shown benefits in a wide range of medical conditions, including depression, diabetes, attention deficit hyperactivity disorder, male infertility, and the common cold in children.

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Introduction

Zinc is an essential trace element, and it is needed for growth and development, immune function, neurotransmission, vision and reproduction.[1] Zinc can bind to more than 300 enzymes and more than 2000 transcription factors.[1] On the cellular and molecular levels, zinc has various functions, including a structural role in protein folding, as a co-factor for many enzymes and in regulating gene expression.[2] Zinc is also an important mediator of cellular signalling and supports cell membrane structure.[3]

Deficiency of zinc was first described in 1961 in the Middle East in men with severe growth retardation whose diets consisted mostly of bread and who also ate large amounts of clay.[4] This is thought to be due to diets high in phytates from cereal grains, which hinders the absorption of zinc.[4] It has been estimated that up to 17% of the global population may be zinc deficient, mostly in low- and middle-income countries.[5] It should be noted that these data were published in 2012, but more recent data are not available.

Whilst signs and symptoms of overt zinc deficiency are well defined, and include growth retardation, hypogonadism (reduced function of the testes in men and ovaries in women), rough skin, poor appetite, mental lethargy and frequent infections, mild zinc deficiency is less specific. However, experiments in humans have shown that an experimental low-zinc diet can lead to a number of biochemical abnormalities, including decreased serum testosterone level, decreased natural killer cell activity, decreased activity of serum thymulin (a zinc-dependent hormone produced by the thymus and important for immune function), hyperammonaemia, decreased taste, decreased visual dark adaptation and decreased lean body mass.[4]

Causes of zinc deficiency include malabsorption [e.g. in inflammatory bowel disease (IBD), unmanaged coeliac disease], alcoholic liver disease and dietary factors (e.g. malnutrition, high intake of phytates).[4] The best food sources of zinc are generally speaking protein foods, such as meat, fish, dairy, eggs, pulses, whole grains, nuts and seeds, although absorption from plant sources is lower due to their high phytate content, and it is estimated that vegetarians whose staples are grains and pulses may require up to 50% more dietary zinc (Tables 1–4).[2]

Table 1: Reference nutrient intakes, UK[6]

Age	Male (in mg per day)	Female (in mg per day)
0–6 months	4	4
7 months to 3 years	5	5
4–6 years	6.5	6.5
7–10 years	7	7
11–14 years	9	9
15 years and over	9.5	7
Pregnancy		7
Lactation 0–4 months		13
Lactation 4 months and over		9.5

Table 2: Population reference intake, EU[7]

Age	Male (in mg per day)	Female (in mg per day)
7–11 months	2.9	2.9
1–3 years	4.3	4.3
4–6 years	5.5	5.5
7–10 years	7.4	7.4
11–14 years	10.7	10.7
15–17 years	14.2	11.9
18 years and over (depending on phytate intake)	7.5-16.3	7.5-16.3
Pregnancy		Additional 1.6
Lactation		Additional 2.9

Table 3: Recommended dietary allowance, USA[2]

Age	Male (in mg per day)	Female (in mg per day)
7 months to 3 years	3	3
4-8 years	5	5
9-13 years	8	8
14-18 years	11	9
19 years and older	11	8
Pregnancy 14-18 years		12
Pregnancy 18 years and older		11
Lactation 14–18 years		13
Lactation 18 years and older		12

Table 4: Food sources of zinc[8]

Food	Zinc content in mg per 100 mg food
Calf liver, fried	15.9
Beef, lean braising steak, braised	9.5
Lamb, leg joint roasted	4.9
Chicken drumstick, roasted	2.3
Chicken breast, grilled	0.8
Oysters, raw	59.2
Crab, tinned	5.7
Cheddar cheese	4.1
Egg, boiled	1.3
Pumpkin seeds	6.6
Cashew nuts	5.9
Pecan nuts	5.3
Chickpeas, tinned, drained	1.1
Red lentils, boiled	1.0
Kidney beans, tinned	0.9
Wheat germ	14
Wholemeal bread	1.6

Testing zinc status is complex, and whilst serum or plasma levels are commonly used, these are unlikely to detect mild deficiency as levels are kept within a narrow range through homeostatic mechanisms.[2] Other factors can also affect serum or plasma zinc levels, including fasting, inflammation, diurnal rhythm and blood collection issues.[9] Depletion studies have shown a dose-dependent response of plasma zinc to zinc supplementation.[10]

A test that has been suggested for use in nutritional practice is the zinc taste test. This test uses a 0.1% zinc sulphate solution, which should produce a strong metallic taste in a person with adequate zinc levels, but is not tasted in zinc deficiency.[11] Whilst this test has been shown to correlate with some other indicators of zinc status, research validating the accuracy of this test is lacking.[12,13] Hair zinc levels are also commonly used to evaluate zinc status and have been shown to increase in response to an increase in zinc intake, but the effects of zinc depletion on hair concentrations are not conclusive.[7]

The aim of this paper is to review the evidence from human clinical trials for the efficacy of zinc supplementation in a range of conditions.

General mechanisms

Antioxidant

Oxidative stress is an important factor in all chronic degenerative conditions, including cancer, cardiovascular disease and neurodegenerative disorders, such as Alzheimer's disease.[3] Zinc has been shown to strengthen antioxidant defences through a number of mechanisms, both directly and indirectly.[1,14]

Three meta-analyses have summarised the evidence from clinical trials of zinc supplementation on antioxidant biomarkers and found significant benefits of zinc. One meta-analysis of 23 randomised-controlled trials (RCTs) reported significant increases in glutathione (GSH), superoxide dismutase (an antioxidant enzyme) and total antioxidant capacity (TAC), but not glutathione peroxidase.[14] A meta-analysis of 21 RCTs found a significant reduction in malondialdehyde (MDA; a marker of oxidative stress),[15] and another meta-analysis of 10 RCTs also showed significant reductions in MDA and increases in TAC and GSH, but no effect on nitric oxide (NO).[3] All three meta-analyses reported significant inter-trial heterogeneity, and subgroup analyses were inconsistent between biomarkers. The dosage range of zinc across all studies was 11–528 mg per day, and durations 2–48 weeks.

The evidence shows that zinc can lower levels of C-reactive protein (CRP; an inflammatory marker), suggesting an antioxidant effect, although results for other biomarkers of oxidative stress have been inconsistent. The heterogeneity observed may be due to differences in study designs and study populations.

Anti-inflammatory

Like oxidative stress, excessive/inappropriate inflammation is associated with many chronic conditions. Inflammatory processes involve the release of pro-inflammatory cytokines and mediators, such as tumour necrosis factor-alpha (TNF-α) and various interleukins (ILs).[16]

Several meta-analyses have evaluated the clinical evidence for effects of zinc on inflammatory biomarkers, and consistently showed significant reductions in CRP.[15,17,18,19] For other inflammatory markers, the results were inconsistent. Whilst some meta-analyses found reductions in TNF-α,[15] others did not find an effect.[16,17] Also, some found beneficial effects on IL-6,[15,16] others did not.[17] All meta-analyses reported heterogeneity amongst study results, but subgroup analyses were inconsistent. Dosages generally ranged between 11 and 50 mg per day, for 2–72 weeks.

These results confirm an anti-inflammatory effect of zinc. The heterogeneity observed may be due to differences in study designs and study populations.

Clinical uses

Acne

Acne is an inflammatory skin disorder affecting more than 85% of teenagers, and is characterised by inflammatory papules, pustules, comedones and sometimes cystic nodules.[20] A number of pathological processes contribute to acne, including increased sebum production, aberrant keratinisation of the sebaceous duct of the hair follicle, bacteria such as Cutibacterium acnes, hormonal influences, the skin microbiome and chronic inflammation.[20] Both topical and oral zinc have been used in the treatment of acne.

Treatment of acne with zinc was first studied in the 1970s, mostly using 135 mg zinc per day as sulphate for 3 months. Results were mixed, with some finding significant benefits over placebo[21] or benefits comparable to tetracycline antibiotics (a standard treatment for acne),[22] whilst others did not find significant benefits over placebo[23] or zinc to be inferior to antibiotics.[24] One uncontrolled, open-label study reported that 13 out of 42 patients stopped zinc sulphate (135 mg per day) due to side-effects, mostly nausea/vomiting and diarrhoea, whilst one patient experienced a perforated pre-existing gastric ulcer during treatment. Only three of the remaining 29 patients had improvements in their acne score after 4 months.[25]

In 2020, a meta-analysis of 12 observational and 13 intervention studies (which included both oral and topical zinc) found that patients with acne had significantly lower zinc levels than healthy controls.[20] Oral zinc significantly improved inflammatory papule count and rate of clinical improvement, but not number of acne pustules. Dosages ranged between 14 and 30 mg as gluconate and 72 and 138 mg as sulphate, for 6–12 weeks.

In 2021, an open-label RCT comparing zinc sulphate, 91 mg per day, versus lymecycline (an antibiotic) for 12 weeks found significant

improvements in subjective global acne grading system and acne-specific quality of life (AQOL) in both groups, with improvements in AQOL higher in the zinc group.[26]

Zinc has also shown benefits in patients with acne in combination with other nutrients: 10 mg zinc per day (as gluconate) with lactoferrin (200 mg per day) and vitamin E (22 IU per day) in one study;[27] and zinc 45 mg per day as methionine chelate combined with antioxidants and chromium (390 µg per day) in another study.[28]

Although there are some contradictory results, overall the evidence suggests a benefit of zinc in patients with acne. Most trials have been carried out with quite high dosages of zinc sulphate (commonly 135 mg per day); lower dosages of a gluconate formulation (10–30 mg per day) have also shown benefits.[27,29,30]

The mechanisms by which zinc exerts its benefits in acne are thought to be its anti-inflammatory properties, its ability to reduce sebum secretion, inhibiting the activity of androgenetic hormones and antimicrobial activity against C. acnes.[26]

Attention deficit hyperactivity disorder (ADHD)

ADHD is a common neurodevelopmental disorder characterised by hyperactivity/impulsivity and inattention, which can affect learning, cognition and personal relationships.[31]

A number of observational studies have yielded conflicting results regarding an association between blood (serum or plasma) and hair zinc levels and ADHD. Two meta-analyses of 22 and 11 studies, respectively, have found no significant difference in zinc status between children and adolescents with ADHD and controls.[31,32]

Four double-blind, placebo-controlled trials evaluated the efficacy of zinc alongside methylphenidate (Ritalin®), and found that children in the zinc group improved more than those in the placebo group,[33,34,35,36] although this was not statistically significant in one study.[35] All four studies used zinc as sulphate at dosages between 10 and 22 mg per day, for 6 weeks.

A double-blind, placebo-controlled study using zinc 15 mg or 30 mg per day (as glycinate) alongside amphetamine showed that those in the 30-mg zinc groups needed a 37% lower amphetamine dose to achieve improvements compared with control.[37]

Only one double-blind, placebo-controlled trial evaluated zinc, 40 mg per day as sulphate, on its own, and showed significant improvements in hyperactive, impulsive and impaired socialisation symptoms, but not in attention deficit symptoms, with better results in children of older age, with high body mass index score, and low zinc and free fatty acid levels.[38] Significant improvements were seen from 4 weeks of supplementation.

Two studies reported that adverse events were not significantly different between zinc and placebo groups, except for metallic taste, which was more common in those taking zinc, with 41%[36] and 53%,[38] respectively, raising the question of adequate blinding.

Overall, the evidence for the use of zinc alongside standard medication in ADHD is consistently positive, with dosages between 10 and 40 mg per day, for at least 4 weeks. Only one study evaluated zinc on its own and also showed positive results with 40 mg zinc per day.

Possible mechanisms include a positive effect of zinc on the metabolism of dopamine,[39] as well as its effects on cell membrane stability, antioxidant and hormonal performance.[33]

Age-related macular degeneration (AMD)

AMD is the leading cause of blindness in the Western world, characterised by loss of central vision resulting in an inability to read, recognise faces or discriminate colours.[40] The human retina contains the highest concentration of zinc in the body in women and the second highest in men (after the prostate), and declines with age.[41,42]

The evidence from clinical trials in reducing the progression of AMD has been mixed, with some showing benefits,[41,43,44] and some no benefits.[45]

By far the largest trial was the Age-Related Eye Disease Study (AREDS), a multi-centre, double-blind, placebo-controlled trial of 3647 participants with AMD with a mean follow-up of 6.3 years.[44] Participants were randomised to receive either antioxidants (vitamin C, 500 mg, vitamin E, 400 IU, beta-carotene, 15 mg per day), zinc with copper (80 mg and 2 mg per day, respectively, both as oxide), antioxidants plus zinc with copper, or placebo. The risk of developing advanced AMD was significantly reduced by 28% in the antioxidant plus zinc with copper group, whilst a 25% reduction in risk in the zinc with copper group was not statistically significant. In the high-risk patient group, the combination supplement reduced risk by 34% and zinc/copper alone by 29%, both statistically significant. No serious adverse events were observed.

Overall, the evidence suggests that zinc may reduce the risk of progression of AMD, especially in high-risk patients and combined with other antioxidants. Dosages of 50 mg and 80 mg longer term have shown benefits, and a combination with copper, as in the AREDS study, may be prudent to reduce the risk of a copper imbalance when taking high doses long term.

Reactive oxygen species, oxidative stress and inflammation play an important role in the development of AMD, and the antioxidant and anti-inflammatory properties of zinc are thought to explain its benefits.[41]

Asthma

Asthma is a chronic inflammatory disorder of the respiratory tract, characterised by airway constriction, inflammation and bronchial hyperresponsiveness, and symptoms including recurrent coughing, wheezing, dyspnoea (shortness of breath) and chest tightness.[46,47]

Two meta-analyses evaluated zinc levels and risk of asthma with contradictory results. Whilst a meta-analysis that looked at only children found no association,[47] another one that included children and adults found that people with asthma had lower blood levels of zinc.[46]

Two double-blind, placebo-controlled trials have shown benefits of zinc supplementation in children. In one study, severity of asthma in children admitted to hospital for an exacerbation decreased more rapidly with zinc, 15 mg (as bis-glycinate) twice a day, after 24 and 48 hours compared with placebo.[48] Fifty-seven percent of children in this study were zinc deficient at baseline. In the other study, children with low zinc status and who were on inhaled steroids were given zinc, 50 mg per day (formulation not reported), for 8 weeks, which led to significant improvements in clinical symptoms compared with placebo.[49] Zinc status was defined by serum zinc concentrations in both studies.

Zinc supplementation appears to be of short- and long-term benefit in children, at least those with low zinc levels, although data are too limited to recommend a particular dose. There are no clinical trials in adults.

The antioxidant, anti-inflammatory and immune-modulating properties of zinc are thought to mediate its benefits in asthma.[50]

Atopic dermatitis

Atopic dermatitis is a chronic inflammatory condition of the skin, characterised by itching and redness. As zinc is important for skin health and skin disorders are observed in zinc deficiency, there has been interest in zinc supplementation in atopic dermatitis.[51]

A meta-analysis of observational studies showed that atopic dermatitis is associated with lower zinc levels [serum, red blood

cells (RBCs) and hair], but two intervention trials gave contradictory results. Whilst one found significant benefits of zinc, 12 mg per day (formulation not reported), for 8 weeks alongside antihistamines and topical moisturiser,[51] another study found no benefit with 43 mg per day (as sulphate) for 8 weeks.[52] It should be noted that the former study was not placebo controlled, whilst the latter was a double-blind, placebo-controlled study.

At this point, there are insufficient data to confirm the benefits of zinc in atopic dermatitis.

Bone health

Zinc plays an important role in the growth and maintenance of healthy bones, and epidemiological studies have shown that patients with osteoporosis have lower serum zinc levels than healthy controls, and that dietary zinc intake is inversely correlated with fractures.[53]

Clinical trials have been carried out in a variety of settings, and have shown benefits of zinc supplementation for bone health, markers of bone turnover and/or bone mineral density in patients with osteoporosis and zinc deficiency,[54] patients with thalassaemia (an inherited condition leading to anaemia) and low bone mass,[55] healthy male volunteers,[56] and postmenopausal women with rheumatoid arthritis and osteoporosis,[57] but not in healthy pubertal girls[58] or patients on haemodialysis.[59] Dosages have ranged from 15 to 68 mg per day, with dosages of 25 mg per day or more showing benefits, and formulations have varied widely in these trials.

Overall, the evidence suggests a benefit of zinc for bone health, at least in some populations, at dosages of at least 25 mg per day for at least 3 months.

Cardiometabolic disorders

Cardiometabolic disorders, including heart disease, type 2 diabetes mellitus (T2DM) and metabolic syndrome, are the leading cause of deaths worldwide.[60] Zinc is known to be involved in insulin/glucose homeostasis, lipid metabolism and regulating inflammation, important underlying causes of cardiometabolic disorders.[60]

Cardiovascular risk factors

Cardiovascular risk factors include abnormal blood lipids, dysregulated glucose/insulin metabolism and hypertension. The effect of zinc supplementation on these risk factors has been assessed by a number of meta-analyses, which showed that zinc improves blood lipids and glycaemic control.[60,61,62] One meta-analysis compared low-dose (< 25 mg per day) versus high-dose (≥ 25 mg per day) and short-term (< 12 weeks) versus long-term (≥ 12 weeks) supplementation, and found low-dose, long-term supplementation to be of most benefit.[60]

A meta-analysis of epidemiological studies found patients with hypertension to have significantly lower zinc status, as determined by serum zinc level, than healthy controls.[63] The results from intervention trials, however, are conflicting, with one meta-analysis of four RCTs finding no statistically significant improvement in blood pressure,[61] whilst another meta-analysis of nine RCTs showed significant improvements with zinc supplementation in systolic, but not diastolic, blood pressure.[64] The reasons for this discrepancy are unclear, but are unlikely to be due to heterogeneity of the included RCTs, as the latter meta-analysis found significant heterogeneity for diastolic but not systolic blood pressure whilst the former found no heterogeneity for either. Baseline zinc level may play a role, three of the four studies of the former meta-analysis reported normal baseline zinc levels, whilst the latter did not report on zinc status.

Whilst the evidence for zinc supplementation in hypertension is conflicting, there appears to be a clear benefit with regards to blood lipids and glycaemic control, especially with low-dose, long-term supplementation.

Diabetes, prediabetes and metabolic syndrome

A meta-analysis of epidemiological studies showed T2DM to be associated with lower blood zinc levels compared with healthy controls, which cannot be explained by lower zinc intakes.[65] In another meta-analysis, the same researchers also found that whilst moderately high dietary zinc intake reduced the risk of developing T2DM, elevated plasma/serum levels were associated with an increased risk, although there was significant heterogeneity between the studies, with some showing an increased and others a decreased risk with higher plasma/serum levels.[66]

Four meta-analyses have been conducted over the past 2 years, and all reported benefits of zinc supplementation for glycaemic control and blood lipids in patients with diabetes or prediabetes.[17,67,68,69] Dosages have varied widely between 4 and 660 mg per day, and durations from 3 weeks to 1 year.

Improvements in blood lipids, glycaemic control and obesity indices have also been seen in Iranian children with obesity and metabolic syndrome who received zinc, 20 mg per day (formulation not reported), for 8 weeks.[70,71]

The evidence from clinical trials is in favour of zinc supplementation in T2DM, prediabetes and metabolic syndrome, with benefits for glycaemic control as well as blood lipids. A wide range of dosages have shown beneficial results, making it difficult to suggest a particular dose.

Cognition

Zinc is important for neuronal signalling and is found in high levels in the brain, in particular in areas involved in learning and memory.[72]

Studies that evaluated cognition in children are covered under children/mental development.

Two double-blind, placebo-controlled studies showed no benefit of zinc on cognition in elderly people. The abovementioned AREDS study [see section 'Age-related macular degeneration (AMD)'] found no effect of zinc plus copper supplementation (80 mg and 2 mg per day, respectively, both as oxide) on any of six cognitive tests.[73] The other trial, in healthy people over 55 years old, compared zinc, 15 mg and 30 mg per day (as gluconate), for 6 months with placebo, and found that out of eight parameters one improved and one deteriorated with zinc, but effects were significant at 3 months only.[74]

At present the evidence suggests that zinc is not effective in improving cognitive function.

Depression

Depression is a leading cause of disability globally and contributes significantly to the global burden of disease.[75] Two meta-analyses showed that people with the highest intake of zinc had a reduced risk of depression, by 28%[76] and 33%,[77] respectively.

Over the past 2 years, three meta-analyses, of three, five and eight trials, respectively, assessed zinc supplementation trials and found significant improvements in depressive symptoms.[75,76,78] Dosages ranged from 7 to 220 mg per day, with the most commonly used dose being 25 mg per day, and durations ranged from 2 to 12 weeks, with most trials lasting 12 weeks. None of the meta-analyses reported effects of dose or duration on outcomes.

The evidence suggests that zinc is beneficial in reducing depressive symptoms in depressed patients, with a dose of 25 mg per day for 12 weeks being the most commonly used regimen.

Several possible mechanisms to explain the beneficial effects of zinc in depression have been discussed, including its effects on the N-methyl-D-aspartate (NMDA) and gamma-aminobutyric acid (GABA) receptors, which are thought to be involved in the development of depression, regulation of serotonin metabolism via its anti-inflammatory effects and its involvement in regulating brain-derived

neurotrophic factor, which is important for neuroplasticity and memory.[75,77,78]

Fertility (male)

Seminal fluid is high in zinc and is thought to play an important role in sperm function.[79] Two meta-analyses of observational studies have shown seminal zinc levels to be significantly lower in infertile compared with fertile men.[79,80]

A meta-analysis of five intervention trials found significant improvements with zinc supplementation in semen volume, sperm motility and the percentage of normal sperm morphology, but not sperm viability, sperm concentration, sperm count or percentage of abnormal sperm morphology.[79] Zinc dosages ranged from 15 to 100 mg per day for 45 days to 20 weeks, with 100 mg per day (as sulphate) appearing most beneficial.

The evidence suggests that zinc supplementation is beneficial for male fertility at a dose of 100 mg for at least 45 days.

It is thought that the antioxidant properties of zinc play an important role in its beneficial effect on male fertility.[81]

Gastric ulcer

Gastric and duodenal ulcers are a disruption of the mucous layers that lead to inflammation.[82] In view of its importance in healing, zinc has been of interest and has shown promise in animal experiments.[83]

Zinc acexamate is has been marketed in Taiwan, Spain and a number of South American countries. In Spain it is licenced for the treatment and prevention of gastric and duodenal ulcers.[84] In the 1980s, 18 clinical trials were published on the effect of zinc acexamate, 12 of which were subject to a meta-analysis in 1992 that showed the zinc supplement was significantly more effective than placebo and as effective as an H2-blocker in improving gastric ulcers.[85] All but one study used 900 mg per day (elemental weight not reported), the other study used 300 mg per day, and study duration ranged from 3 to 6 weeks.

In the 1970s, a double-blind, placebo-controlled trial of 18 patients with gastric ulcer confirmed by barium meal (before and after treatment) using zinc as sulphate, 150 mg per day for 3 weeks, found a three times higher healing rate in the zinc-treated compared with the placebo-treated patients.[86] However, two more double-blind, placebo-controlled trials found no benefits with lower dosages of zinc sulphate, 50 mg per day[83] or 50 mg every other day,[82] alongside standard treatment, compared with placebo.

The evidence is in favour of zinc acexamate as an effective treatment for gastric ulcers, whilst it is unclear whether the conflicting results with zinc sulphate are due to differences in dose or other variables.

Inflammatory bowel disease (IBD)

IBD are chronic inflammatory conditions of the gastrointestinal (GI) tract, and include ulcerative colitis (UC), which affects the colon only, and Crohn's disease (CD), which can affect any part of the GI tract.[87]

Two large cohort studies of 170 776 women found that zinc intake was inversely related to the risk of developing CD, but not UC, and the association was stronger for dietary than supplemental intake.[88]

A double-blind, placebo-controlled trial of zinc, 150 mg per day (as sulphate) for 4 weeks, alongside standard treatment, in 51 patients with UC had no benefits over placebo.[89] Another study, supplementing zinc, 35 mg per day (as gluconate) for 2 months in patients with low zinc levels, found significant improvements in IL-10 and IL-2, but not other ILs, as well as improvements in the Mayo disease score, versus control group, which were patients with UC with normal zinc levels who received placebo.[87]

Three clinical trials looked at various outcome measures in patients with CD in remission, and found improvements in intestinal hyperpermeability,[90] RBC status of polyunsaturated fatty acids (linoleic acid, arachidonic acid and omega-3 fatty acids)[91] and thymulin activity.[92] All three studies used zinc sulphate at dosages of 46 mg per day for 6 weeks[91] or 3 months,[92] or 75 mg per day for 8 weeks.[90]

The evidence in UC is contradictory, whilst in CD improvements in a number of disease parameters have been observed. As all patients with CD were in remission, it is difficult to make conclusions regarding the therapeutic benefits of zinc.

Liver disease

It is estimated that, in the UK, liver disease is the third most common cause of premature death, and mortality has increased by 400% since 1970.[93] Zinc deficiency is very common in patients with liver disease, with up to 83% of patients with liver cirrhosis being zinc deficient.[93]

A review and meta-analysis of 12 RCTs found no effects of zinc supplementation (dosages not reported) on chronic hepatitis C, cirrhosis or serum albumin levels, but a significant benefit for hepatic encephalopathy (HE), based on three studies.[94] Another meta-analysis that reviewed seven RCTs also reported benefits of zinc alongside therapy with lactulose in HE, with a dosage range of 25–180 mg per day, which was administered for 6 months in most studies.[95]

A meta-analysis of four intervention trials found that zinc supplementation, dosage range 3.4–214 mg per day, had no effect on mortality from cirrhosis at 6 months.[93]

A recent double-blind, placebo-controlled trial in 56 obese/overweight patients with non-alcoholic fatty liver disease (NAFLD) found significant improvements in glycaemic control, oxidative stress and liver enzymes, but not liver steatosis or fatty liver index with zinc gluconate, 30 mg per day for 3 months, alongside a calorie-reduced diet.[96,97]

Overall, the evidence suggests that zinc supplementation is of benefit in HE and NAFLD (based on only one study), but not cirrhosis or hepatitis C.

The mechanism by which zinc improves HE is thought to be its role in ammonia metabolism, which is disrupted in patients with HE.[95] The benefits of zinc for NAFLD are thought to be mediated through its effects as an antioxidant, and by improving glycaemic control and lipid metabolism.[96]

Mucosal health

Intestinal permeability

Zinc deficiency has been shown to cause damage to the gut membrane through inflammatory cell infiltration, and patients with chronic disturbances of intestinal permeability have reduced levels of mucosal zinc.[98] In a small randomised crossover study in 10 healthy volunteers, 70 mg zinc per day as carnosine, for 5 days, prevented indomethacin-induced increases in intestinal permeability.[99]

Zinc has also been shown to have beneficial effects on intestinal permeability in patients with CD [see section 'Inflammatory bowel disease (IBD)' for details].

Whilst clinical evidence is scarce, zinc appears to be beneficial in supporting the integrity of the intestinal mucosa.

Oral mucositis (OM)

OM refers to ulcerative lesions in the oral mucosa, and is a common side-effect of chemo- and/or radiotherapy.[100]

A meta-analysis of 10 RCTs (nine with oral administration and one using a zinc mouthwash) found significant benefits in severity of OM, as well as delayed onset and faster healing, but no reduction in

incidence, in patients receiving chemo- and/or radiotherapy.[100] Zinc dosages ranged from 21 to 150 mg per day.

Since then, three more studies have shown benefits with zinc supplementation in both adults[101] and children.[102,103]

The evidence suggests that zinc is of benefit in patients receiving chemo- and/or radiotherapy in reducing severity of OM, with zinc dosages of 21 mg per day (as sulphate) in adults and 7 mg per day (as gluconate) in children showing beneficial results.

The antioxidant and anti-inflammatory properties of zinc may play a role in its effects on OM.[104]

Recurrent aphthous stomatitis (RAS)

RAS is characterised by recurrent ulcers of the oral mucosa that generally heal by themselves within 10 days, and affects about 25% of the general population. The underlying causes are unknown.[105] A meta-analysis of 19 case–control studies found that patients with RAS had significantly lower levels of zinc than healthy controls.[105]

Two studies using oral zinc sulphate have shown benefits in both treatment and prevention of RAS, with dosages of 69 mg per day for 12 weeks[106] and 50 mg per day for 1 month (both as sulphate).[107] A mucoadhesive formula of zinc sulphate, providing 5 mg three times per day, has also shown to improve lesion size from day 3 and pain from day 5 compared with placebo.[108] One study from 1982, however, showed no benefits (article not accessible, no details provided in abstract).

A study from 1977 in 32 patients with RAS found that all of those who had low zinc levels at baseline improved with zinc supplementation (up to 150 mg per day as sulphate, duration not reported), whilst only three of eight patients with normal zinc levels improved.[109]

Overall, the evidence suggests that zinc is of benefit in RAS, but this may depend on zinc status. A regimen of 50 mg per day (as sulphate) for at least 1 month has shown benefits.

Polycystic ovary syndrome (PCOS)

PCOS is a common endocrinological disorder with gynaecological, metabolic and psychological abnormalities, including high testosterone levels, hirsutism (male pattern hair growth), anovulation, infertility, menstrual disturbance, insulin resistance, obesity and mood swings.[110]

Two double-blind, placebo-controlled trials in women with PCOS found significant improvements in biochemical markers and symptoms with zinc, 50 mg per day (as sulphate) for 8 weeks: one found improvements in glycaemic control and lipid profiles,[111] whilst the other found improvements in alopecia, hirsutism and MDA, but not on hormone profiles, other oxidative stress markers or inflammatory cytokines.[112]

A study that used zinc, 8 mg per day, alongside magnesium (200 mg per day), calcium (800 mg per day) and vitamin D (400 IU per day) also found significant improvements in glycaemic control and blood lipids.[113]

Whilst the number of clinical trials is limited, they suggest a benefit of zinc supplementation for women with PCOS; 50 mg of zinc per day (as sulphate) has shown benefits within 8 weeks.

The positive effects of zinc on insulin homeostasis, lipid metabolism, improving antioxidant status and regulating inflammation[60] are likely to explain its benefits in PCOS.

Pregnancy

Due to growth taking place, the need for zinc is increased during pregnancy.[2] A 2021 Cochrane review and meta-analysis found no significant benefits for preterm birth, stillbirths, perinatal deaths or birth weight with zinc supplementation compared with controls.[114]

Gestational diabetes mellitus (GDM)

Two double-blind, placebo-controlled trials evaluated the benefits of 30 mg per day zinc (as gluconate) for 6 weeks in women with GDM, and found significant benefits in glycaemic control, high-sensitivity (hs)-CRP, TAC and some but not all blood lipids, but not in NO, GSH, MDA or pregnancy outcomes.[115,116] Similar benefits have been seen with zinc, 8 mg per day (formulation not reported), alongside magnesium (200 mg per day), calcium (800 mg per day) and vitamin D (400 IU per day) for 6 weeks.[117]

An RCT in women with gestational impaired glucose tolerance, however, found no statistically significant benefits for glycaemic control with zinc, 30 mg per day (as gluconate) for 8 weeks.

Although research is limited, it appears that zinc, either alone or with other nutrients, has beneficial effects on glycaemic control in women with GDM. A dose of 30 mg zinc per day (as gluconate) for 6 weeks has shown benefits.

Respiratory infections

Common cold

The common cold is usually a mild illness that does not progress to serious outcomes, such as pneumonia or requiring hospitalisation, but presents a significant socioeconomic burden.[118] Zinc plays an important role in immunity, and has therefore been studied in the prevention and management of the common cold.

Harri Hemilä has conducted three meta-analyses of zinc lozenges in the common cold in adults, all three finding a shorter duration of colds compared with placebo treatment.[119,120,121] The most recent meta-analysis that included five RCTs found a 33% shorter duration with zinc lozenges with dosages of 80–207 mg per day, although dosages of > 100 mg per day had no additional benefit.[121]

However, the validity of the matching between active lozenge and placebo was questioned as early as 1987, suggesting that the taste of the zinc lozenge may have unmasked the blinding.[122] In 2020, Harri Hemilä *et al*. carried out a double-blind, placebo-controlled trial of zinc lozenges (as acetate), 78 mg per day for 5 days, starting at the onset of symptoms of the common cold, versus placebo, which was matched for taste and appearance.[123] There was no significant difference in duration of cold symptoms between the zinc and the placebo lozenges. It is unclear whether this was due to the lower dose as compared with those evaluated in the above meta-analyses.

At present, the evidence is unclear as to whether zinc lozenges are beneficial in adults with the common cold or whether benefits reported in some studies are due to a placebo effect.

Zinc supplementation has also been evaluated in children with the common cold. Four studies showed a shortening of duration,[124,125,126,127] whilst two studies did not.[128,129] One of the studies that did not find a shorter duration, however, reported that symptoms were less severe from day 2 of treatment compared with placebo.[128] A reduced severity has also been reported in another study.[125] Of four studies that looked at preventive use of zinc, three reported a significantly lower frequency of colds in those treated with zinc,[125,126,127] whilst the fourth did not see a reduction in incidence.[124]

Not all studies reported dose of zinc, where it was reported it ranged from 15 mg per day for prophylaxis to 60 mg per day for treatment. A variety of formulations was used and did not affect results.

Although there are contradictory results, in children, zinc appears to be efficacious in preventing colds as well as reducing the duration, at a dose of 15 mg per day for prevention and 30–60 mg per day for treatment, started within 24–48 hours of first onset of symptoms.

Apart from its general effects on immunity, zinc has been shown to inhibit rhino-viruses,[130] which cause about 50% of common colds.[131]

Pneumonia

Pneumonia causes 15% of deaths in children under 5 years old worldwide,[132] mostly in developing countries where zinc deficiency is common.

Three meta-analyses evaluated zinc as an adjunct to treatment of pneumonia in children under 5 years old, in low- and middle-income countries, covering similar sets of studies.[133,134,135] No effect on treatment failure, change of antibiotic therapy or time to recovery was seen in any of them. A significant 57% reduction in mortality was reported in one meta-analysis,[134] whilst mortality reductions in the other two meta-analyses (36%[133] and 31%[135]) did not reach statistical significance.

A Cochrane review and meta-analysis of six RCTs concluded that zinc supplementation significantly reduced the incidence and prevalence of pneumonia in children under 5 years old.[136] All studies included in this review were from low- and middle-income countries.

Zinc supplementation appears to be beneficial for the prevention of pneumonia in young children and may reduce mortality. However, this is in the context of geographical areas where zinc deficiency is common, and can therefore not necessarily be extrapolated to children in Europe or the USA.

Sleep

A number of epidemiological studies have shown an association between zinc status and sleep, and animal studies have shown benefits of supplemental zinc for sleep.[137]

Three double-blind, placebo-controlled trials have investigated the effects of zinc supplementation on sleep. In a study of 120 healthy adults, both a zinc-rich diet and a yeast-based zinc supplement (both containing 15 mg zinc per day) for 12 weeks showed significant improvements of sleep-onset latency and sleep efficiency with zinc compared with placebo.[138] A study in 54 intensive care unit nurses also found significant improvements in sleep quality with zinc, 50 mg every 3 days (as sulphate) for 1 month.[139] A study in young women with premenstrual syndrome, on the other hand, showed no benefits for sleep parameters with 30 mg zinc per day (as gluconate) for 3 months, although a significant benefit on physical aspects of quality of life was seen.[140]

At present, the evidence for the use of zinc to improve sleep is limited and mixed. A low dose of zinc (15 mg per day) may be of benefit.

Possible mechanisms linking zinc and sleep include its effects on neurotransmitters and their receptors in the brain.[137]

Taste

As mentioned above, zinc is important for our sense of taste and smell, and zinc deficiency is associated with dysgeusia (altered taste perception).[141] Dysgeusia can also occur as a side-effect, in particular from chemo- and/or radiotherapy, affecting approximately 56% of patients on chemotherapy alone and 76% of those on combined chemo- and radiotherapy, with large variations with cancer site and type of treatment.[142]

A meta-analysis of four RCTs in patients undergoing radiotherapy for head and neck cancers found that zinc supplementation decreased the incidence of dysgeusia by 28%, but did not improve taste acuity more after radiotherapy than controls.[143] Dosages ranged from 72 to 150 mg per day.

Studies in patients receiving chemotherapy have been mixed, with two showing no significant benefits,[144,145] and one showing a reduction in the duration of dysgeusia.[146] The study that found significant benefits used a specific formulation, β-alanyl-L-histidinato zinc, 33 mg per day, whilst the others used zinc sulphate.

A number of studies also investigated the effect of zinc supplementation in dysgeusia due to other reasons. Two studies, one in adults[147] and one in children,[148] found no benefits of zinc in patients on haemodialysis. Similarly, studies in elderly people found no improvements in taste with zinc supplementation.[149,150]

Most studies in zinc-deficiency-related or idiopathic (of unknown cause) dysgeusia have found beneficial effects with zinc supplementation, especially with the β-alanyl-L-histidinato zinc formulation (34 and 68 mg per day),[151,152,153] with zinc gluconate,[154,155] an Ayurvedic zinc formulation[156] and with zinc picolinate.[157] However, another study found no benefits, using zinc sulphate.[158]

Zinc supplementation appears to be of benefit in radiotherapy-induced, idiopathic and zinc-deficiency-induced taste disorders, but evidence is mixed for chemotherapy-induced dysgeusia, and negative for taste abnormalities associated with haemodialysis and in elderly people. Whether or not zinc supplementation is beneficial also appears to depend on the formulation, rather than the dose, with all studies using β-alanyl-L-histidinato zinc and most using zinc gluconate showing benefits.

Although the exact mechanisms are unclear, it has been suggested that a zinc-dependent salivary enzyme, gustin, is crucially involved in taste sensation, and zinc is also important in the regeneration of taste bud cells.[159]

Thyroid

Zinc is involved in thyroid metabolism in various ways, including as a co-factor of the deiodinase enzymes that convert the less active thyroxine (T4) to the more active triiodothyronine (T3),[160] the binding of T3 to nuclear receptors,[161] and in the formation of thyrotropin-releasing hormone.[162]

In 2021, a systematic review evaluated both epidemiological and intervention studies, and concluded that the evidence from studies is contradictory for both an association between zinc levels and thyroid function and benefits of zinc supplementation.[163] Four of the eight intervention studies reviewed were in zinc-deficient people with Down's syndrome, with two of them showing an increase in thyroid-stimulating hormone (TSH) and two showing a decrease.[163]

Two RCTs looked at hypothyroid patients and found beneficial effects of zinc either alone or in combination with other nutrients. In one study of 68 overweight or obese hypothyroid women, zinc (30 mg per day as gluconate) was given alone and in combination with selenium (200 µg per day) for 12 weeks and compared with placebo.[160] Both the combination of zinc and selenium and zinc alone increased free T3 levels compared with placebo and selenium alone, with zinc alone being significantly more effective in raising free T3 than zinc plus selenium. Total T3, free and total T4 and TSH were not significantly different between groups after 12 weeks. In the other study, zinc was given with magnesium (250 mg per day) and vitamin A (25 000 IU twice per week) for 10 weeks, and significant improvements in free T4, weight and hs-CRP were observed compared with placebo.[164] Patients in both studies were on levothyroxine.

At present, the evidence does not suggest a benefit of zinc supplementation for thyroid function in people with Down's syndrome. In hypothyroid patients, limited research suggests a benefit both on its own and in combination with other nutrients at a dose of 30 mg per day (both studies used a gluconate formulation).

Wound healing

Zinc plays an important role in wound healing through its effects on immunity, cell proliferation, and through protein and DNA synthesis.[165] Zinc is also involved in the regulation of growth factors that are involved in

anabolic response and healing.[166] Both topical and oral zinc have been used in a number of conditions related to wound healing, including pressure and leg ulcers.

Pressure ulcers

Pressure ulcers are areas of damage to the skin and underlying tissue, and are common in elderly people, affecting up to 32% in acute and long-term settings.[141,168]

A review and meta-analysis of seven clinical trials, three on oral and four on topical zinc, found a significant benefit of zinc in the healing of pressure ulcers.[169] All three studies on oral zinc showed benefits (doses not reported).

Two more studies, using zinc (zinc 30 mg per day, formulation not specified) in combination with L-carnosine (116 mg plus 34 mg zinc),[170] and in combination with arginine (9 g) and vitamin C (500 mg),[171] reported benefits of zinc for the healing of pressure ulcers. However, a retrospective study from 2001, using 100 mg zinc per day (as sulphate), found only minor benefits but significant side-effects, with patients on zinc having 7.8 times the risk of getting an infection requiring antibiotics and 12.5 times more likely to experience nausea/vomiting.[167] Side-effects lessened when a low-dose multivitamin and mineral supplement was given concurrently, possibly by alleviating the effects high-dose zinc may have had on copper levels.

Overall, zinc appears to be beneficial for pressure ulcers, with dosages of 30–34 mg per day showing benefits.

Apart from the above-mentioned mechanisms, positive effects on Langerhans cells (macrophages in the skin) have also been observed in patients with pressure ulcers.[172]

Leg ulcers

A number of trials in the 1970s investigated the potential benefits of oral zinc in the treatment of leg ulcers, but unfortunately not all publications have been accessible. Whilst one study from 1970 showed that patients with leg ulcers had lower zinc levels than healthy controls and improved healing with zinc supplementation,[173] all other accessible early-intervention trials found no benefit of oral zinc.[174,175,176,177]

A more recent double-blind, placebo-controlled study in patients with diabetic foot ulcers found significant benefits with zinc, 50 mg per day as sulphate for 12 weeks.[178]

Whilst limited evidence suggests that zinc supplementation is of benefit for diabetic foot ulcers, the accessible evidence does not support a benefit for leg ulcers.

Children

Zinc deficiency is amongst the most common nutrient deficiencies globally, and affects children in many low- and middle-income countries where dietary patterns rely heavily on high-phytate foods.[179] Zinc is essential for cellular growth due to its direct impact on nucleic acid and protein synthesis, hormonal mediators of growth, risk of infection and appetite, and is thought to be an important factor in childhood stunting.[180] Zinc deficiency may contribute to childhood mortality and ill health.

A number of meta-analyses evaluated the effect of zinc supplementation on growth in children under 5 years old with slightly conflicting results. Whilst some found no effects,[180] others found significantly better growth indices with zinc compared with controls,[179,181] although the effect sizes were generally small. These conflicting results may be due to different study populations and baseline zinc status, as well as other factors that are known to contribute to stunting such as general malnutrition, poor maternal health/nutrition, frequent infections, especially diarrhoea, and poor feeding and care early in life.[182]

Three meta-analyses also looked at motor and mental development, and found no significant effect of zinc supplementation.[72,183,184]

In developing countries, diarrhoea is a common cause of death in young children. The World Health Organisation recommends routine use of zinc supplementation, at a dosage of 20 mg per day for children older than 6 months or 10 mg per day in those younger than 6 months, for 10–14 days, alongside oral rehydration salts.[185] A Cochrane review in 2016 concluded that "In areas where the prevalence of zinc deficiency or the prevalence of malnutrition is high, zinc may be of benefit in children aged 6 months or more. The current evidence does not support the use of zinc supplementation in children less than 6 months of age, in well-nourished children, and in settings where children are at low risk of zinc deficiency."[186]

A 2021 meta-analysis of 23 RCTs in low- and middle-income countries found that zinc supplementation in children under 5 years old significantly decreased all-cause mortality by 16%, and death from pneumonia, infection and diarrhoea by 30%, 46% and 15%, respectively.[187]

The evidence presented here for supplementation in children refers mostly to low- and middle-income countries where zinc deficiency is common, and cannot necessarily be used to inform supplementation of young children in developed countries.

Safety in children

Zinc is safe for children at appropriate dosages and with the same precautions as for adults (see below). The Tolerable Upper Intake Levels (ULs) set by the Institute of Medicine (IOM) in the USA, for children are based on age:[2]

- Birth–6 months: 4 mg per day
- 7–12 months: 5 mg per day
- 1–3 years: 7 mg per day
- 4–8 years: 12 mg per day
- 9–13 years: 23 mg per day
- 14–18 years: 34 mg per day

Safety

The main concern with longer-term excessive zinc intake is that it can reduce copper absorption and thus induce copper deficiency, which may lead to neurological disorders.[7] The European Food Safety Authority (EFSA) and the UK Expert Group on Vitamins and Minerals (EVM) set a UL/Safe Upper Level of 25 mg per day for adults from supplements,[7,188] whilst the IOM set a UL of 40 mg per day for adults on low-phytate diets.[2]

The only other side-effects noted in the British National Formulary (BNF) for zinc sulphate and acetate are digestive complaints: diarrhoea, gastritis, GI discomfort, nausea and vomiting.[189]

Caution

Caution is advised in people with renal impairment as zinc accumulation may occur.[189]

Interactions[189]

Calcium: Calcium may reduce the absorption of zinc.

Copper: Zinc hinders the absorption of copper.

Iron: Iron may reduce the absorption of zinc and vice versa.

Tetracycline and quinolone antibiotics: Zinc may reduce absorption of tetracycline and quinolone.

Penicillamine and trientine (chelating agents commonly used in Wilson's disease): Zinc may reduce the absorption of penicillamine and trientine, and vice versa.

Pregnancy/lactation

Zinc needs are increased during pregnancy and lactation, and the same ULs as mentioned above apply during pregnancy and breastfeeding.[7]

In 2021, a Cochrane review of 25 RCTs, involving more than 18 000 pregnant

women and their babies, reported no benefits for pregnancy outcomes with zinc supplementation, but also did not report any adverse effects, suggesting that zinc supplementation during pregnancy is safe.[114]

The BNF considers the risk in pregnancy and during breastfeeding: "theoretically minimal, but no information available".[189]

Conclusion

Clinical trials have shown zinc supplementation to be safe and beneficial for a wide range of conditions. It should be noted that many of the studies were carried out in countries that have a high prevalence of zinc deficiency, and benefits may be more pronounced in, or even limited to, individuals with low zinc levels at baseline.

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Nutritional Medicine Journal

Lactobacillus rhamnosus GG: A Review of Clinical Use and Efficacy

Chloe Steele

Abstract

Oral *Lactobacillus rhamnosus* GG (LGG) supplementation is generally recognised as a safe form of supplementation, which acts as an immunomodulator, an antimicrobial, and aids cell growth and proliferation. The aim of this review was to determine diseases where oral LGG supplementation has been indicated; and assess safety, colonisation, mechanisms of action and efficacy, and provide therapeutic recommendations. LGG following supplementation can successfully colonise the gut and other areas of the body owing to the expression of unique morphological features known as pili. Twenty-two disease areas were identified where LGG supplementation has been used, to determine effects. However, small study sizes, the use of multispecies probiotics and adjuvant therapies all meant that strong evidence for the use of LGG was lacking in several disease areas. Despite this, LGG was shown to be of benefit in the reduction of risk of developing attention-deficit hyperactivity disorder and gestational diabetes mellitus, in the prevention of allergies and dental caries, for improving immune reactions following vaccines, and for the management of diarrhoea associated with cancer treatments and antibiotic use.

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Introduction

Probiotics are defined as live microbes, which when administered in adequate amounts confer benefits to the host.[1] For this to occur, probiotics need to be safe, alive, of human origin and capable of surviving the pH of the gut.[2] Several different bacteria are used as probiotics, but species from the genera Lactobacillus and Bifidobacterium have been well researched and are believed to provide many health benefits.[3,4,5,6]

Amongst the most well-researched strains of probiotics is *Lactobacillus rhamnosus* GG (LGG). Its health benefits are thought to derive from its superior ability to colonise the gastrointestinal (GI) tract, outcompeting and producing antimicrobials to prevent pathogenic bacterial colonisation.[7] LGG may also promote GI barrier protection and healing through cell growth and proliferation,[8,9] and act as an immune effector both locally and systemically.[10] Based on the mechanisms of action, clinical trials in humans have been extensive, showing benefits in several disease areas.

This review paper aims to determine the disease areas where the use of LGG as an oral probiotic has been indicated, and review the clinical data on efficacy and safety with a view to making therapy recommendations. Data on the mechanistic actions of LGG will also be briefly reviewed. Randomised-controlled trials (RCTs) will dominate this review, as the beneficial effects of probiotics seem to be strain specific;[11] thus, pooling data in large meta-analyses and systematic reviews with different strains may result in misleading conclusions. Where LGG alone has been examined, these will be included.

Colonisation and adhesion

The success of probiotic supplementation relies upon the ability of the microbiota to colonise areas of the body, such as the GI tract. In comparison to other *Lactobacillus* strains, LGG has high adherence to human intestinal mucus glycoproteins[7] and, in adults, supplementation has indicated that it may survive for at least 1 week in the GI tract[12] (Figure 1). Amongst newborns and infants, a reduced intrinsic GI microbiome may ensure that LGG colonises the GI tract more readily, and it has been detected in faeces up to 2 weeks after administration, without affecting the establishment of a normal GI microbiome.[13]

Image 1: LGG has superior mucus adherence

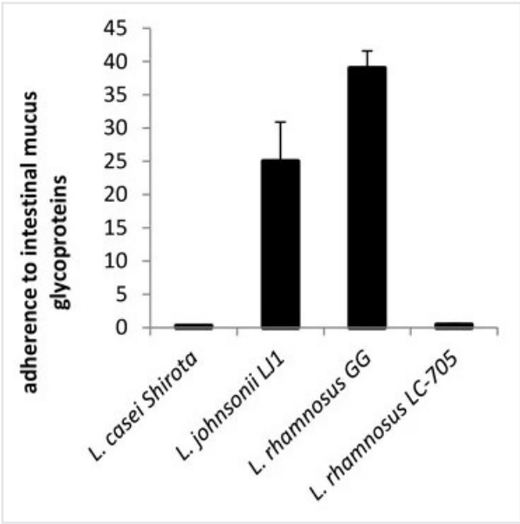

Figure 1: *Lactobacillus rhamnosus* GG (LGG) has superior adherence to mucus glycoproteins when compared with other probiotic strains, including a closely related strain of *L. rhamnosus* (LC705). In this study, radioactively labelled bacteria were allowed to adhere to isolated human intestinal mucus. The adhesion ratio (%) was determined by comparing radioactivity of bacteria added to the radioactivity of bound bacteria after washing.

Successful colonisation of LGG, compared with other *Lactobacillus* species, may be down to certain morphological features. Comparative genomic analysis with *Lactobacillus rhamnosus* LC705 has revealed the presence of a DNA sequence,

known as the *spaCBA* gene, resulting in pili-like appendages that run along the entirety of the LGG microbe, the inhibition of which lowers adhesion to the GI tract[14] (Figure 2). Differing conditions have been shown to promote or suppress the expression of the pili phenotype in other microbiota strains;[15] however, when exposed to different conditions such as low pH, the pili of LGG are still expressed.[16] Interestingly, whilst LGG still expresses pili under different conditions, when present in the oral and vaginal cavities the pili are absent,[16] which may have implications in diseases associated within these areas, such as urinary tract infections (UTIs) and dental caries.

Image 2: *Lactobacillus rhamnosus* GG-specific pili

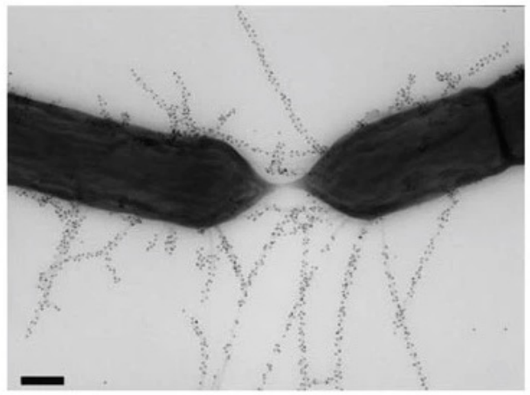

Figure 2: *Lactobacillus rhamnosus* GG (LGG)-specific pili, not present on other *Lactobacillus* spp., are involved in the mechanisms of adhesion to the intestinal mucosa. In addition, the pili facilitate a close interaction between the host and the bacteria or bacteria with each other. In this image, transmission electron microscopy reveals pili on LGG cells.

Certain probiotic strains have been shown to adhere to the GI tract, preventing mucolytic bacteria from digesting the protective layer of mucus, resulting in decreased vulnerability to intestinal permeability.[17] Although studies have shown limited effects of LGG on intestinal permeability in patients with chronic liver conditions,[18] investigations into efficacy in healthy patients is warranted.

General effects

Immunomodulation

The role of LGG in immunomodulation is controversial, with proteins isolated from it and its physico-chemical properties contributing to both inflammatory and anti-inflammatory actions. The pili on LGG have been implicated to have a role in immunomodulation. An *in vitro* study on LGG bred without the *spaCBA* gene, which encodes for the growth of the external pili, reported increased expression of the inflammatory cytokine interleukin (IL)-8, which was decreased with the wild-type strain.[19] Proteins secreted from LGG may also have an anti-inflammatory role in the immune response, and isolation of a novel soluble protein, HM0539, from LGG has been shown in colon tissue to suppress the TLR4/MyD88/NFκB inflammatory pathway.[20] However, overexpression of toll-like receptor (TLR)4 or myeloid differentiation primary response 88 (MyD88) did reverse this effect. The TLR4 signalling pathway may be responsible for upregulated inflammation in chronic and acute inflammatory disorders,[21,22] such as inflammatory bowel disease (IBD) and atherosclerosis,[23,24] indicating that LGG supplementation in highly inflammatory states may have a limited effect.

In contrast to its anti-inflammatory effects, effector substances such as lipoteichoic acid (LTA), found in the cell walls of certain gram-positive bacteria including LGG, may also be involved in the modulation of the immune response, with it displaying pro-inflammatory properties. Within the body, reporter cell lines are designed to monitor intracellular cell signalling pathways. LTA produced by a wild-type LGG strain has been shown to activate the inflammatory NF-κB signalling pathway in reporter cells, a pathway that is significantly reduced when the LTA gene is removed.[10] Removal of the LTA gene resulted in a reduced capacity to activate TLR2/6-dependent NFκB signalling in reporter cells and reduced induction of IL-8 mRNA in CACO-2 cells from the human colon, acting both locally and systemically. However, the implications of this during certain disease states and the exact role of LGG in the inflammatory and anti-inflammatory pathways still needs to be elucidated.

Supplementation of LGG in individuals with inflammatory GI diseases has shown mixed results, and is discussed later in the review.[25,26,27]

Cell growth and proliferation

Proteins produced by LGG, known as Msp1 and Msp2, have been implicated in cell homeostasis through regulation of the protein kinase B (Akt) signalling pathway and inhibition of MAP kinases.[8,9] *In vitro* studies in animal and human colon tissue have shown that Msp1 and Msp2 promoted cell growth and attenuated GI permeability in hydrogen peroxide-damaged intestinal epithelium.[8] Msp2 has also been shown in intestinal epithelial cells, *in vivo* and *in vitro*, to inhibit cytokine-induced apoptosis,[28] indicating a role for LGG in the protection and recovery from intestinal permeability and injury.

Antimicrobial

In vitro, LGG has been shown to inhibit the growth and adherence of several pathogenic bacteria belonging to the *Salmonella, Shigella*, *Escherichia* and *Streptococcus* species.[7,29,30,31,32] In rabbit models, LGG has been shown to inhibit translocation of *Escherichia coli* in a dose-dependent manner.[33] In clinical trials, a decrease in the number of children colonised with vancomycin-resistant enterococci has been reported following LGG consumption for 21 days, with increased GI *Lactobacillus* counts observed in their stead.[34]

LGG DNA contains encodes for bacteriocins, which act like antibiotics, preventing the growth of closely related bacterial strains; however, the product of these genes has not been expressed under experimental conditions. Further experiments have reported that the antimicrobial action of LGG may be due to the production of microcin-type substances, which are small bacteriocins, mediated in part by lactic acid.[32,35,36]

LGG may also communicate with other gut microbiota via a process known as quorum sensing (QS), resulting in cooperation for nutrients and cellular adhesion against pathogenic bacteria.[37] The pili-like protrusions responsible for colonisation may ensure superior competitive inhibition by LGG during QS. However, further studies are required to determine the role of QS when the GI tract is faced with pathogenic bacteria.

Clinical uses

Cancer

It has been hypothesised that gut dysbiosis may promote colorectal cancer through the colonisation of pathogenic bacteria, which drives its development.[38] Furthermore, chemotherapy treatment may alter the composition of the gut microbiota,[39] indicating areas where probiotics may be of benefit. The use of LGG in a multispecies probiotic in combination with a prebiotic has been shown to alter several colorectal cancer biomarkers after 12 weeks.[40] In this trial, *Bifidobacterium* ($P = 0.008$), *Lactobacillus* ($P = 0.021$) and interferon-gamma (IFN-γ) were all increased, with *Clostridium perfringens* ($P = 0.022$) and DNA damage decreased amongst 37 patients with colon cancer and 43 polypectomised patients. However, administration of the synbiotic also prevented a rise in IL-2 inflammatory cytokines. In contrast to IL-2 suppression, a second RCT on a multispecies synbiotic containing LGG, *Bifidobacterium*

lactis and inulin reported increased IL-2 and IFN-γ in 34 patients with colon cancer who had undergone curative resection or polypectomy.[41] IFN-γ and IL-2 were both increased at 12 weeks compared with placebo ($P \leq 0.05$ both), but no other effects on immune factors were observed. Overall, it is difficult to conclude any specific effects of LGG from these trials as, when in combination, effects may be due to other species.

Failure of cancer treatments often occurs when severe side-effects result in a reduction or cessation of treatment.[42] Side-effects, such as diarrhoea, can occur in as many as 30–87% of patients, with severe and potentially life-threatening (grade 3–4) episodes occurring in 20–40% of patients.[43] Probiotics have been shown to be a safe and effective way to prevent chemotherapy-induced diarrhoea[39] and, as monotherapy, one of the most important effects of LGG may be for its use to reduce the frequency and severity of severe diarrhoea and GI symptoms during chemotherapy treatment. In one RCT of 150 patients with colorectal cancer given LGG twice daily [1–2 × 10^{10} colony-forming units (CFU)] for 24 weeks during 5-fluorouracil chemotherapy, patients had less grade 3 or 4 diarrhoea and fewer hospitalisations due to bowel toxicity compared with a fibre supplement (22% versus 37%, $P = 0.027$; and 8% versus 22%, $P = 0.021$, respectively), resulting in decreased chemotherapy dose adjustments (21% versus 47%, $P = 0.0008$).[44]

Perioperative administration of a multispecies probiotic containing LGG plus fructo-oligosaccharide has also been associated with reduced infection rate in postoperative patients with colorectal cancer. One trial in 91 patients undergoing surgery reported decreased infections at the incision site (2% versus 21.4%, $P = 0.002$), reduced intra-abdominal abscess ($P \leq 0.001$) and reduced incidence of pneumonia ($P \leq 0.001$), indicating a beneficial effect to complications associated with cancer treatment.[3]

Helicobacter pylori may be the strongest known risk factor for gastric cancer, and eradication may be an effective therapy in its prevention.[45] However, undesirable side-effects of eradication may include diarrhoea, pain, nausea and bloating, resulting in treatment cessation.[46] During *H. pylori* eradication, the supplementation of LGG (6 × 10^9 CFU twice daily) has been reported to increase eradication tolerability ($P = 0.04$) due to decreased side-effects, such as diarrhoea, nausea and taste disturbances [relative risk (RR) = 0.1, 95% confidence interval (CI): 0.1–0.9; RR = 0.3, 95% CI: 0.1–0.9; RR = 0.5, 95% CI: 0.2–0.9].[47] Although eradication rate remained unaffected, the supplementation of LGG in individuals undergoing *H. pylori* eradication may contribute to preventing the development of gastric cancer through increased treatment tolerability.

Results have not been as positive in other cancers, with one study in 40 patients undergoing head and neck cancer surgery reporting no impact of a multispecies synbiotic on postoperative outcomes, intestinal function or GI symptoms,[48] indicating that the beneficial effects of LGG in cancer may be localised and specific.

It is apparent that LGG monotherapy (6 × 10^9 CFU twice daily) has numerous benefits, including success in symptom management of *H. pylori* treatment and reducing the risk of developing gastric cancer. For individuals undergoing chemotherapy treatment for colon cancer, LGG (1–2 × 10^{10} CFU twice daily) may be more beneficial than fibre for the reduction of diarrhoea. In combination with other probiotics and prebiotics, LGG may reduce postoperative infections and improve immune function in patients with colon cancer, which is important during a time of reduced immune function.

Irritable bowel syndrome

Irritable bowel syndrome (IBS) is characterised by abdominal pain, flatulence and irregular bowel movements, and it is estimated that 10–20% of the worldwide adult population suffers from this syndrome.[49] Treatments for IBS have limited success, and newer drugs such as 5-HT_4 agonists come with cardiovascular risks,[50] highlighting a need for treatments with limited side-effects.

In adults, it appears that several factors are involved in the pathophysiology of IBS, with mucosal large intestine low-grade inflammation and altered gut microbiota indicated.[51] As stated earlier, *in vitro* studies have highlighted that LGG may have both pro-inflammatory and anti-inflammatory properties, making its role as a supplement in inflammatory diseases uncertain.

Differences in the gut microbiota between healthy subjects and sufferers of IBS have been highlighted.[52,53] The administration of LGG may be able to promote colonisation and reinstate the composition of gut microbiota more associated with healthy individuals. Supplementation of a multispecies probiotic containing LGG (8–9 × 10^9 CFU) for 6 months in 42 patients with IBS resulted in a shift towards similar quantities of bacterial 16S rDNA to those of healthy controls; however, clinical outcomes were not reported in this study.[54] Although there was a shift in composition towards that of more healthy individuals, a gut microbiota composition that is characteristic of, or favourable for, sufferers of IBS has yet to be isolated and may be highly individual.[55] Regardless, the administration of probiotics containing LGG may be of benefit for symptom relief in individuals with IBS. In one double-blind RCT of 49 patients with IBS, the supplementation of a multispecies probiotic (5 × 10^9 CFU/day) containing LGG reported improvements to abdominal pain/discomfort and bloating after 4 weeks, which was attributed to alterations in the composition of the gut microbiota.[27] Faecal analysis highlighted that LGG, *B. lactis* and *Streptococcus thermophilus* had all significantly increased in the supplemented group, although several other strains were also included in the probiotic.

IBS can be divided into subtypes,[56] and improvements in symptoms may be highly dependent upon this, highlighting the importance of identifying the IBS type before commencing LGG supplementation. One 6-week unblinded RCT of 123 adults with IBS investigated the efficacy of LGG (6 × 10^9 CFU/day for 6 weeks) compared with a low-fermentable oligosaccharides, disaccharides, monosaccharides and polyols (FODMAP) diet on syndrome severity, assessed using the IBS symptom severity scoring system.[26] The results showed that LGG was as efficacious as the low-FODMAP diet, especially in the diarrhoeal (IBS-D) and mixed IBS (IBS-A) subtypes, with no improvement in those with the IBS constipation subtype. Quality of life (QoL) was also improved in those with IBS-D. Studies like this highlight the need to perform trials on dual therapy of diet changes and probiotics to further advance possible management strategies for IBS.

Children with IBS may spontaneously recover;[57] however, for those who have persistent symptoms, the use of LGG to effectively manage IBS may also be dependent on an accurate diagnosis. Improvements to the frequency and severity of abdominal pain when supplemented with LGG may be due to improvements in the gut barrier, and may be especially pronounced in children with IBS or functional pain.[4] In one 16-week RCT of 141 children with IBS or functional pain, LGG supplementation (6 × 10^9 CFU/day) reduced the frequency and severity of abdominal pain ($P \leq 0.01$ for both). Although it was concluded that this effect may not be unique to LGG, it is apparent that it may be of benefit. One systematic review of three RCTs with 290 children suffering from abdominal pain-related functional GI disorders concluded that a higher rate of children responded to treatment with LGG (6 × 10^9 CFU/day to 10^{10} CFU/day) compared with placebo if suffering from IBS, an effect that was not evident in children without IBS.[58] Frequency of pain was only reduced in the IBS subgroup; however, pain intensity was

reduced amongst the whole study population. This study did not perform a statistical test for publication bias, so this cannot be ruled out and conclusions should be made with caution.

A second systematic review of 11 RCTs examining various probiotic supplements in children with functional abdominal pain disorders (FAPD) highlighted that LGG reduces the frequency and severity of abdominal pain, but only in children with IBS.[25] One study of 4 weeks, which included IBS sufferers alongside those with dyspepsia and functional abdominal pain, concluded that children supplemented with 3×10^9 CFU of LGG twice daily were more likely to have improved pain frequency but not pain severity,[59] further supporting the need to identify IBS for LGG supplementation to be of benefit to symptoms.

In vitro studies have highlighted that LGG may have both pro-inflammatory and anti-inflammatory properties, and have a limited effect in inflammatory conditions. However, supplementation studies of the use of LGG to relieve symptoms of IBS in adults may be more positive, and dependent upon its use for the IBS-D and IBS-A subtypes, although this is based on data from studies using multispecies rather than LGG alone. Its use in children may be of benefit for the management of pain symptoms.

Diarrhoea

Diarrhoea is a leading cause of childhood mortality worldwide and is the second leading cause of childhood deaths.[60] The use of probiotics and LGG for the prevention and treatment of diarrhoea has been extensively researched, with varying success. One large RCT of 943 children with moderate-to-severe gastroenteritis reported that administration of a short 5-day course of LGG 1×10^{10} CFU twice daily was no more effective than placebo at decreasing the duration of all-cause diarrhoea (median LGG 49.7 hours versus 50.9 hours, $P = 0.26$).[61]

Despite negative outcomes in all-cause diarrhoea, the understanding of the mechanisms through which LGG interacts with the host may indicate specific types of diarrhoea where supplementation may have greater success. Antibiotic-associated diarrhoea (AAD) may result from dysbiosis of the host's gut bacteria.[62] LGG acts through several mechanisms to potentially prevent dysbiosis or restore normal bacterial flora resulting from antibiotic administration, such as competitive exclusion of pathogens,[63] modulation of the immune system,[64] and through outcompeting less acid-tolerant bacteria as LGG produces lactic acid.[65] The prevention of gut microbiota changes associated with antibiotic use has been shown in one RCT of 231 school-aged children.[65] In this trial, children given 10^6 CFU/ml LGG in 400 ml of milk long term reported changes in several gut bacteria, with especially increased abundance of the *Lactobacillus* spp. ($P < 0.0001$).

LGG for the reduction of risk of AAD has been extensively researched, and it may be important for the prevention of this disease and to provide new treatment options when antibiotics are prescribed. One meta-analysis of 12 RCTs and 1499 participants reported that, compared with placebo or no treatment, LGG was associated with a reduced risk of AAD (22.4% to 12.3%, RR = 0.49, 95% CI: 0.29–0.83), resulting in a number needed to treat (NNT) of 9.[66] LGG may also be one of the most effective probiotics for the prevention of AAD, and a meta-analysis of 32 RCTs has reported that LGG was superior to seven single or multispecies probiotics for the prevention of AAD (RR versus placebo = 0.30, 95% CI: 0.16–0.5).[67] Dosages of at least 2×10^9 CFU were recommended.

Although evidence exists for the use of LGG with AAD, other causes of diarrhoea have reported mixed results. As previously discussed, *in vitro* studies have indicated the ability of LGG to prevent the adherence and viability of several gut pathogens.[36,68] Amongst these, *Clostridium* spp. have been shown to be inhibited *in vitro* through the production of a bactericide that resembles microcin.[32] However, *in vivo* studies in children have not been as positive. One meta-analysis of 20 RCTs concluded that the use of LGG reduced the risk of AAD from 23% to 9.6% (RR = 0.48, 95% CI: 0.26–0.89); however, it found no effect on the risk of *Clostridium difficile*-associated diarrhoea (RR = 0.95, 95% CI: 0.06–14.85).[69] It should be noted that the results on *C. difficile* were based on only one RCT, and this warrants more research.

Studies on children with rotavirus-associated diarrhoea, which is the leading cause of vaccine-preventable diarrhoea,[60] have been more positive. One meta-analysis of 19 RCTs concluded that, compared with control, the use of high-dose LGG (10^{10} CFU/day) in children with rotavirus-positive diarrhoea reduced the duration [mean difference (MD) −31.05 hours, 95% CI: −50.31, −11.80] and frequency of diarrhoea episodes (MD −1.08, 95% CI: −1.87, −0.28).[70] This trial also looked at children with acute diarrhoea caused by a mixture of rotavirus, bacterial pathogens and norovirus and, in contrast to Schnadower *et al.* (2018),[61] high-dose LGG reduced the duration of diarrhoea episodes (MD −15.83 hours, 95% CI: −20.68, −10.98), but only in those who had suffered from diarrhoea for less than 3 days at enrolment, indicating that earlier treatment at higher doses may have more success.

Dose-dependent effects of LGG supplementation on rotavirus-associated diarrhoea are also apparent. One open-label RCT in 23 children with rotavirus-associated diarrhoea showed no changes in faecal rotavirus concentrations when supplemented with low-dose LGG 2×10^8 CFU/day (36.1×10^5 particles/ml versus 73.5×10^5 particles/ml, $P = 0.895$), but at a high dose of 6×10^8 CFU/day concentrations were reduced (64.2 × 105 particles/ml versus 9.0×10^5 particles/ml, $P = 0.012$).[71] Although rotavirus shedding and not symptoms were assessed in this trial, it is indicative of disease severity. LGG may also aid recovery from rotavirus infection, with both intestinal permeability and immunoglobulin antibodies to rotavirus improved following LGG supplementation.[72]

Large, short-term trials for the treatment of all-cause diarrhoea have shown little improvements with the administration of LGG. Understanding the type of diarrhoea may result in a more targeted and successful approach. The use of LGG at a dose of at least 6×10^8 CFU during a course of antibiotics may prevent AAD, and early high-dose treatment during rotavirus-associated diarrhoea may decrease the duration of disease, the frequency of diarrhoea episodes and aid recovery.

Inflammatory bowel disease

IBD is an umbrella term for a number of different diseases, which includes Crohn's disease and ulcerative colitis.[73] As the name suggests, inflammation plays a major role in its development, and its aetiology is thought to be a combination of both genetic and lifestyle factors.[74,75,76] The gut microbiota may also play a major role in the pathogenesis of IBD, and in individuals with Crohn's disease, gut dysbiosis has been reported with an increased growth of *E. coli* and a reduction in the bacterial phyla Firmicutes, of which LGG is a member.[77,78]

As previously discussed, LGG may have pro-inflammatory properties, and limited effects in inflammatory GI diseases such as IBD. Remission of Crohn's disease, endoscopic recurrences and relapse times have all been shown to remain unaffected by LGG supplementation. One systematic review and meta-analysis of 41 studies, two of which were in LGG, reported no difference in endoscopic recurrences when supplemented with LGG, compared with placebo (0.93; 95% CI: 0.63, 1.38).[79] In a second meta-analysis of six RCTs, four of which were in LGG, the supplementation of LGG was concluded to increase the relapse

rate of individuals with Crohn's disease.[80] In this trial of 359 individuals, placebo showed a greater benefit on clinical relapse rates in adults (RR = 1.85, 95% CI: 1.00–3.41) and children (RR = 1.68, 95% CI: 1.07–2.64) compared with LGG, with little heterogeneity between the studies (P = 0.71, I2 = 0%).

Studies on the use of LGG in children have also not been efficacious. LGG in combination with standard Crohn's disease treatment has shown marginally shorter time periods between relapses. One RCT with a 2-year follow-up in 75 children who were in a period of inactive Crohn's disease reported a non-significant shorter time between the median time to relapse in those treated with LGG compared with those on placebo (9.8 months versus 11 months, P = 0.24).[81] There is a possibility that concomitant therapies may be masking the effect of LGG; however, in combination with the previously reviewed studies that have shown no effect of monotherapy, it would suggest that this is not the case.

When LGG is combined with other gut bacteria strains, anti-inflammatory actions have been observed; however, given the previous *in vitro* and *in vivo* research, it is likely that the extent of anti-inflammatory effects is due to the gut bacteria strain it has been combined with. One open-label parallel study reported an anti-inflammatory effect with a combination of LGG GR-1 strain and *Lactobacillus reuteri* in a yoghurt supplement given to 20 participants with Crohn's disease and ulcerative colitis.[82] In this study, LGG dosage of 2×10^7 CFU/ml and 1×10^3 CFU/ml and *L. reuteri* were associated with increased levels of $CD4^+$ $CD25^{high}$ T-cells (P = 0.007), which are involved in immune regulation, and decreased inflammatory cytokines, tumour necrosis factor-alpha (TNF-α) and IL-12 compared with healthy controls. However, the anti-inflammatory effects observed may be due to the presence of *L. reuteri*.

Given the *in vitro* evidence and the supplemental studies, there is very minimal evidence for the use of LGG in inactive Crohn's disease for endoscopic recurrences, and it may even be detrimental to overall relapse rates. Individuals with Crohn's disease have been reported to have antibacterial reactivity and a loss of tolerance for their own enteric flora[83] and, given the results above showing low colonisation of LGG in the guts of those with Crohn's disease, could indicate a need to increase supplemental doses above those already tested. However, this would raise safety concerns and, given the lack of dose–response trials, this is an area that requires more research.

Body weight

The relationship between body weight, diet and gut microbiota is complex, with each component influencing the other.[84] The involvement of the gut microbiota in the development of metabolic disorders is thought to involve nutrient and lipid metabolism, and hormone and immune modulation. In animal models, diets supplemented with LGG had hypercholesterolaemic effects and caused increased satiety, with increased peptide YY production;[85] however, mechanisms are still being debated.

The role of the two major phyla, Firmicutes, to which LGG belongs, and Bacteroidetes in obesity and weight loss has been extensively researched and remains controversial. Observations in obese children and overweight/obese women with metabolic syndrome have shown an increased Firmicutes to Bacteroidetes ratio, compared with their healthy counterparts.[86,87,88] Furthermore, a reduction in the Firmicutes to Bacteroidetes ratio has been observed in obese individuals following weight loss.[89]

The controversy over the role of Firmicutes and LGG occurs when looking at supplementation. Studies would suggest that LGG has little effect on weight gain, as two trials of LGG with differing gut bacteria species have shown differing results. One RCT reported no significant effect of a combination supplement containing 6.5×10^9 CFU/day LGG and *B. lactis* on the prevention of excessive gestational weight gain in 230 obese pregnant women (probiotics versus placebo, RR = 1.14, 95% CI: 0.99–1.31).[90] However, in contrast, one RCT showed that supplementation with a multispecies probiotic of *Bifidobacterium animalis* and LGG at 1×10^9 CFU/day in 411 obese and overweight pregnant women resulted in lower maternal weight gain compared with placebo.[91] It could be concluded from these two studies that the presence of LGG is having no effect on weight loss or gain, and it is the other species of gut bacteria that may be exerting its effects.

Underlying pathophysiology during obesity may also remain unaffected by LGG supplementation. One RCT sub-study of 26 healthy adults reported that LGG supplementation of 6.2×10^7 CFU/day for 3 weeks in a milk-based fruit drink resulted in changes in serum global lipid profiles, with decreased lysophosphatidylcholines ($P \leq 0.05$), sphingomyelins ($P \leq 0.001$) and several glycophosphatidylcholines ($P \leq 0.05$), which may be involved in the pathophysiology of atherosclerosis.[92,93,94] It should also be noted that triglycerides were increased and when the trial adjusted their statistics to allow multiple hypothesis analysis, no changes were observed in global lipidomic profiles.

The effect of LGG in weight loss remains controversial. Observational studies have outlined a negative effect of the Firmicutes phyla on weight loss, but supplemental trials of specific strains are not straightforward. As effects may be species dependent, it cannot be discounted that positive trials of supplementing multispecies probiotics containing LGG were due to a symbiotic effect of the two strains or due to the *Bifidobacterium* strain present in the supplement. It should be noted that caloric intake and exercise were not measured in these trials, which could affect outcomes.

Liver disease

The gut–liver axis is now a well-recognised relationship that is thought to interact through the mesenteric portal vein.[95] The pathological progression of non-alcoholic fatty liver disease (NAFLD) development is thought to involve inflammation and lipotoxicity.[96] Thus, targeting the gut–liver axis to treat NAFLD may be promising for the treatment of this multi-factorial disease.

The use of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) as clinical markers of liver damage is well established. However, an understanding of liver disease is not limited to liver function, but also considers the presence of small intestinal bacterial overgrowth (SIBO) and intestinal permeability, as endotoxaemia may contribute to reduced life expectancy in individuals with liver cirrhosis.[97] The use of probiotics to improve SIBO is thought to occur from successful colonisation of the small intestine, which prevents microbial translocation.[98] However, in one RCT of 53 patients with chronic liver disease, successful colonisation of LGG and improved SIBO did not translate into improved intestinal permeability and liver function after 4 weeks.[18] This study used a multispecies probiotic of six different strains, including LGG, and reported increased LGG in faeces ($P \leq 0.001$) and improved SIBO ($P \leq 0.05$), but only marginally improved intestinal permeability and no changes to liver chemistry. Short treatment duration and the study population could be responsible for observations.

In comparison, longer studies on LGG monotherapy in children have reported improved liver chemistry. Compared with placebo, 8 weeks of LGG supplementation (1.2×10^{10} CFU) in 20 children with NAFLD has been associated with decreased ALT ($P \leq 0.03$) and anti-peptidoglycan-polysaccharide

antibodies (P = 0.03),[99] which are polymers from the cell wall of bacteria that may contribute to inflammation in certain chronic inflammatory diseases.[100] Differences in dosages between the studies may account for conflicting results; however, the dosage was not disclosed in the previous study. The previous study may also have been too short to observe differences, or the use of a multispecies probiotic may be masking the effects of LGG. The small number of study participants in the monotherapy study may have also been giving a false-positive.

The research surrounding the use of LGG for the improvement of liver function and SIBO is still in its infancy. The use of LGG for the improvement of liver function in paediatric liver disease is promising at a dose of at least 1.2 × 10^{10} CFU/day. In adults, more large-scale RCTs are required, as there is yet no compelling evidence for LGG efficacy in NAFLD, despite promising results for improving SIBO when given in combination with other species. More research is also required in this area, especially with regards to those with severe disease, as the use of probiotics in immunocompromised individuals has raised some concerns.[18]

Insulin resistance and type 2 diabetes

Gut dysbiosis has been linked to insulin resistance (IR) and type 2 diabetes (T2D). In contrast to studies on individuals with obesity, individuals with T2D show compositional changes of the intestinal microbiota, which include decreased Firmicutes, resulting in an increased Bacteroidetes to Firmicutes ratio within the intestinal microbiota.[101] Despite this, studies on individuals with T2D have reported increased total *Lactobacillus* anaerobes, with pronounced levels of *L. reuteri* and *Lactobacillus plantarum*;[102] however, the role of native LGG in those with T2D is unclear.

As previously discussed, gut dysbiosis and the development of intestinal permeability may lead to endotoxaemia and systemic inflammation, and this may contribute to the pathogenesis of IR and T2D. The effects of probiotics in T2D may be through the production of glutathione, decreasing inflammation and oxidative stress. Supplementation of a multispecies probiotic containing LGG for 8 weeks in 54 individuals with T2D was shown in one trial to prevent a rise in fasting plasma glucose, decrease blood markers of inflammation (−777.57 ng/ml versus +878.72 ng/ml, P = 0.02) and increase the antioxidant glutathione compared with placebo (240.63 µmol/l versus −33.46 µmol/l, P = 0.03).[103] Measures of IR were increased in both groups; however, less so in the probiotic group (+2.38 versus +0.78, P = 0.03).

As with many studies in multispecies probiotics, it is important to understand the role of LGG to rule out influences from other species. Studies on streptozocin-induced diabetic rats reported improved glucose tolerance and IR after 4 weeks consumption of LGG.[104] However, animal studies on LGG dominate, and trials in humans with T2D are lacking. Amongst 200 healthy individuals, LGG supplementation of 1 × 10^9 CFU for 90 days was shown in one trial to help maintain glycaemic control.[105] Compared with placebo, where glycated haemoglobin (HbA1c) increased over the 90 days, the LGG-supplemented group reported sustained HbA1c levels (P = 0.005 between-group comparison), indicating possible attenuation of T2D development in healthy adults.

Results from the study above indicate that supplementation of LGG may be of benefit in slowing the development of T2D, an effect that was also observed in gestational diabetes.[106] In this trial of 256 pregnant women with normal glycaemic levels, those supplemented with multispecies LGG + *B. lactis* in combination with dietary advice reported improved blood glucose control during pregnancy and a reduced risk of elevated glucose concentration compared with placebo [odds ratio (OR) 0.31, 95% CI: 0.12, 0.78, P = 0.028]. These effects were sustained 12 months post-partum; however, dosages were not stated in the trial and the role of a single-strain LGG probiotic is unclear. Moreover, a potential effect of the dietary changes cannot be discounted. In contrast, a multispecies probiotic of LGG + *B. animalis* (1 × 10^9 CFU/day) discussed previously in this review failed to prevent gestational diabetes.[91] Taken in tandem, these studies would suggest that effects on glycaemic control in pregnancy could be dependent upon dietary changes.

The use of 1 × 10^9 CFU LGG as monotherapy or as part of a multispecies probiotic to slow the pathophysiological continuum from IR to T2D in healthy individuals may be of benefit. The studies on gestational diabetes mellitus (GDM) would suggest little effect of LGG, and that dietary changes were the driving factor.

Cystic fibrosis

Intestinal inflammation is a predominant feature in adults and children with cystic fibrosis (CF), with levels similar to individuals with IBD.[107,108] Improvements to intestinal inflammation in patients with CF have been reported following probiotics[109] and LGG supplementation as a monotherapy. The restoration of disrupted intestinal microbiota and improvements to intestinal inflammation in children with CF has been reported following supplementation with LGG.[108,110] In one RCT, supplementation of 6 × 10^9 CFU/day LGG resulted in reduced faecal calprotectin (CLP), which is indicative of intestinal inflammation, in the GI tract of 22 children with CF (184 ± 146 µg/g versus 52 ± 46 µg/g, $P \leq 0.01$).[110] Correlations between reduced microbial richness and intestinal inflammation were also observed in this trial (r = 0.53, P = 0.018).

Although inflammation may be improved, the effects of LGG supplementation on pulmonary exacerbations and hospital stays remain controversial. LGG supplementation of 6 × 10^9 CFU/day reduced pulmonary exacerbations and hospital admissions in one 6-month RCT of 43 children with CF; however, the duration of stay did not differ between the groups.[111] In a more recent, larger RCT in 95 children, LGG supplementation (6 × 10^9 CFU/day) failed to show any effect on hospitalisations (OR 1.67, 95% CI: 0.75–3.72, P = 0.211) or exacerbations (OR 0.83, 95% CI: 0.38–1.82, P = 0.643).[112] In contrast to the previous trial, this trial of 95 children with CF ran for 12 months. Study design and duration may account for differences, with parallel studies enabling comparisons between treatments at the same time amongst differing individuals, whereas crossover studies negate the effects of between-patient variability. In this instance, the crossover study could eliminate differences between the participants, such as severity of disease or dietary and lifestyle differences.

The data for the use of LGG in CF mainly revolve around studies in children. Supplementation with 6 × 10^9 CFU LGG may reduce intestinal inflammation and restore gut microbiota eubiosis; however, the exact effects on pulmonary exacerbations remain unclear.

Respiratory tract infections

Removal of the adenoids has been associated with increased long-term risk for respiratory tract infections (RTIs),[113] indicating their possible involvement in the body's immune defence against respiratory diseases. Although largely considered a member of the gut microbiota, the importance of LGG for the immune system may be apparent from its presence in large amounts in the adenoids of children, which has been shown to increase following 3 weeks of supplementation.[114]

However, its role in the prevention of RTIs and its ability to reduce symptoms and duration of illness remain controversial. The use of an LGG supplement (1 × 10^9 CFU/day) for 6 weeks in 59 adults artificially infected with human rhinovirus failed to affect viral load when compared with placebo.[115] Furthermore, one study failed to show benefits to severity of cold symptoms of a live LGG supplement (1 × 10^9 CFU/day)

compared with an inactivated form and placebo in 60 adults after 6 weeks.[116] Although this study did indicate a trend towards lower occurrence and severity of cold symptoms in the active LGG group, this was not significant.

Studies in younger adults and children have shown more beneficial outcomes of LGG supplementation in RTIs. Amongst 231 healthy college students, the use of LGG (1×10^9 CFU/day) in combination with *B. animalis* for 12 weeks was shown to improve the severity and duration of upper RTIs (URTIs), with a 2-day shorter average infection, leading to fewer school days missed compared with placebo ($P = 0.002$).[117] As this study was in combination with *B. animalis*, it is difficult to determine the exact effect of LGG; however, the combination therapy was shown to be of benefit.

Over-the-counter RTI medications in children under 6 years old are often avoided and discouraged due to concerns with safety and efficacy,[118] indicating a need for alternative therapies in this cohort. Children attending day care facilities are particularly susceptible to RTIs due to factors such as increased exposure to infections[119] and cessation of breastfeeding,[120] which contributes to a number of childcare and workdays lost.[121] Therefore, the use of probiotics may be of benefit. Strain-specific effects have been highlighted, with LGG reducing the duration of RTIs in children at day care, which other strains failed to do in a systematic review and meta-analysis of 15 RCTs with 5121 children (MD 0.78 days, 95% CI: −1.46 to 0.09).[122] Interestingly, this meta-analysis reported no effect on incidence, antibiotic use or days missed from day care, which differs to an earlier systematic review and meta-analysis of four RCTs in 1805 children, which reported reduced risk of URTIs (RR = 0.62, 95% CI: 0.50–0.78, NNT = 4) and antibiotic use (RR = 0.80, 95% CI: 0.71–0.91)[123] with LGG supplementation. Differences between the results may be due to a lack of recent data supporting LGG supplementation, or could be owing to differing trial designs and outcome measures.

In high-risk children, the supplementation of prebiotics and probiotics may reduce the risk of RTIs and rhinovirus infections. In one study of 94 preterm infants, supplementation with LGG (1×10^9 CFU/day for the first 30 days, and 2×10^9 CFU/day for a further 30 days) reduced the incidence of RTIs (RR = 0.50, 95% CI: 0.28–0.90, $P = 0.022$) and rhinovirus infections (RR = 0.49, 95% CI: 0.24–1.00, $P = 0.051$).[124] In a second study, LGG supplementation (1×10^9 CFU/day) in 742 hospitalised children reduced the risk of RTIs compared with placebo, but failed to impact hospitalisation duration.[125]

The apparent lack of efficacy of LGG in adults for the prevention of RTIs may be due to suboptimal dosages, as the trials above largely used dosages like those used in the studies on children. Dosage–response studies are warranted to investigate this. Studies on children have shown positive results for the use of LGG in reducing the duration of RTIs in those attending day care. In high-risk children, LGG may reduce the occurrence of rhinovirus infections when supplemented with at least 1×10^9 CFU/day for at least 2 months.

Otitis media

Usually considered an extension of an URTI in children, otitis media is a spectrum of diseases characterised by middle ear inflammation resulting in pain, irritability and fever.[126] As previously discussed, the presence of LGG in tonsil and adenoid tissue is indicative of its role in the immune system and RTIs,[114] but there are limited data on its role in otitis media. LGG has been detected in the middle ear following supplementation, but LGG may already be present in the middle ear of children with otitis media and, compared with placebo, LGG supplementation may have limited effects on children undergoing tympanostomy, which is a procedure to prevent fluid build-up in the middle ear.[127] In one study of 309 children prone to otitis, a multispecies probiotic containing LGG supplemented ($8–9 \times 10^9$ CFU/day) for 24 weeks failed to reduce pathogenic bacteria in the nasopharynx, or reduce

frequency and occurrence of acute otitis media.[128] In contrast, a multispecies probiotic containing LGG supplemented for 6 months reduced the presence of human bocavirus (HBoV), which is the primary pathogen in otitis media, in 269 children prone to otitis (6.4% versus 19.0%, OR 0.25, 95% CI: 0.07–0.94, $P = 0.039$); however, the effects on symptoms and otitis media were not discussed.[129]

The presence of LGG in the middle ear may be indicative of its role in infection prevention; however, there are limited data regarding the use of LGG for the management of otitis media in children and adults. Although studies are positive for the use of LGG as part of a multispecies probiotic to reduce the presence of the virus, more studies are warranted on LGG in isolation.

Anxiety and depression

Anxiety and disordered gut function often coexist, with symptoms of nausea and stomach pain reported by individuals with elevated anxiety.[130] The gut microbiota may communicate in a bi-directional manner with the brain along the gut–brain axis in a number of different ways, such as signalling through metabolites, through the enteric nervous system and the neural-immune system.[131] Under physiological conditions, neurotransmitters such as γ-aminobutyric acid (GABA) may be synthesised and released from *Lactobacillus*.[132] However, under pathophysiological conditions, inflammatory cytokines produced in the gut may also have the capacity to affect the brain and stimulate the release of cortisol, dysregulating the hypothalamic–pituitary–adrenal (HPA) axis leading to initiation of the stress response.[133] Furthermore, gut dysbiosis has been implicated in mental health disorders.[134] Therefore, modification of the gut microbiota using probiotics may hypothetically provide a novel treatment target for conditions such as anxiety and depression.

Studies on probiotics in anxiety and depression are extensive; however, they may not be translatable to the use of LGG in isolation. The inclusion of LGG in a multispecies probiotic supplemented for 6 weeks in 70 petrochemical workers showed improvements from baseline in general health (16.9 ± 1.8 versus 9.8 ± 1.9, $P = 0.001$) and depression and anxiety (18.9 ± 3.2 versus 9.4 ± 4.0, $P = 0.006$), and no effects were seen in the placebo group, or in any of the groups on the HPA axis, as measured by the General Health Questionnaire and the Depression Anxiety and Stress Scale.[135] This indicates that effects may be independent of the HPA axis, or that the short study period may have been insufficient for effects on symptoms to be observed.

The use of LGG as a monotherapy may have a beneficial effect on depression in individuals following the occurrence of myocardial infarction (MI). Supplementation with LGG (1.6×10^9 CFU/day) had beneficial effects on depression, oxidative stress and inflammation in individuals post-MI who had undergone percutaneous intervention (PCI).[136] This study of 44 individuals reported that, compared with placebo, 12 weeks of supplementation with LGG decreased symptoms of depression (−5.57 versus −0.51, $P = 0.045$) and increased QoL (23.6 versus 0.44, $P = 0.023$). Biomarkers for inflammation and oxidative stress were also decreased in the supplementation group compared with placebo. Low-grade inflammation may contribute to the development of depression,[137] and the mechanism of action of LGG in depression and inflammatory diseases may be through its immunomodulatory properties.

During pregnancy, physical and psychological changes can occur leading to stress and adverse outcomes in the baby.[138] Pregnancy with obesity may increase the risk for the development of depression and anxiety compared with entering pregnancy at a normal weight,[139,140,141] and as probiotics are considered safe during pregnancy,[142] they may hypothetically provide a treatment option. However, in practice, clinical trials on

a combination of LGG + *B. lactis* failed to improve the mental health of 230 obese pregnant women at 36 weeks of gestation.[143] There were no differences between depression scores, and anxiety and physical wellbeing worsened with time.

Studies on the use of LGG in isolation in depression and anxiety are limited, and although plausible mechanisms for its use exist, further studies are warranted in this cohort of individuals. The use of LGG (1.6×10^9 CFU/day) for 12 weeks for the development of depression and anxiety in individuals who are post-MI is promising.

Attention-deficit hyperactivity disorder and Asperger's syndrome

Attention-deficit hyperactivity disorder (ADHD) is a childhood neurodevelopmental disorder that is present in as many as 3% of children and predominantly in boys.[144] Symptoms such as inattention and hyperactivity are hallmark symptoms of ADHD, which are frequently observed in children with Asperger's syndrome (AS) alongside disordered emotional behaviour.[145] As previously discussed, LGG may affect emotional behaviour via the vagus nerve and regulation of GABA in the amygdala and hippocampus areas of the brain,[146] which may also be involved in the pathophysiology of mental health disorders.[147]

Animal studies have indicated a possible benefit to learning and memory following LGG supplementation;[148] however, in humans the life stage at which LGG supplementation occurs may be important for favourable outcomes. Pre-natal and post-natal factors have been implicated as risks for ADHD and AS, and intervention with LGG at both stages may impact development later in childhood. Compared with placebo, LGG supplementation (1×10^9 CFU/day) 4 weeks prior to and 6 months after birth reduced the risk of development of ADHD and AS in 75 children, 13 years later (17.1% versus 0%, $P = 0.008$).[149]

Studies on the use of LGG in ADHD and AS are limited; however, what does exist is promising for the use of LGG (1×10^9 CFU) during late pregnancy and infancy to reduce the risk of development. With such strong mechanistic links between neurodevelopmental disorders and the use of LGG, studies are warranted to further investigate possible clinical benefits.

Urinary tract infections

UTIs are a commonly occurring condition, which are ordinarily treated with the use of antibiotics.[150] However, research would suggest that this practise may be detrimental, due to the development of multi-drug-resistant bacteria.[151] *Escherichia coli* originating from the gut microbiota is thought to be the cause in the majority of cases, and in women it may colonise the vagina, transfer to the urethral opening and ascend to the bladder.[152]

Experiments in murine models have shown that the LGG-derived effector protein, HM0539, can competitively inhibit the adhesion of *E. coli* in the GI tract.[153] However, one pilot trial of 42 post-menopausal women indicated that although the GI tract is readily colonised by LGG, vaginal swabs show poor adhesion, with only 9.5% of women having colonisation in this area.[154] Possibly owing to this, clinical trials on the use of probiotics for the prevention of recurrent UTIs have shown mixed results, with vaginal colonies recurrently transferring to the urethral opening. The use of *Lactobacillus spp.* was shown in one systematic review and meta-analysis of nine clinical trials to reduce the risk of recurrent UTIs in females (RR = 0.684, 95% CI: 0.438–0.929, $P \leq 0.001$); however, different strains showed varying efficacy and LGG was not analysed.[152] When administered as a monotherapy, the regular consumption of cranberry juice, but not LGG, was determined to prevent *E. coli*-derived recurrent UTIs in one 12-month RCT of 150 women.[155] In this trial, 39% of women in the LGG group reported recurrent UTIs compared with 16% consuming cranberry juice and 20% of control. Poor colonisation of the periurethral area and consumption only

five times per week were determined to be the possible reasons for lack of efficacy. In contrast, a multispecies probiotic containing LGG in 181 children has been reported to be effective at reducing the risk of recurrent UTIs compared with placebo ($P = 0.02$); however, in individuals who did have a recurrent event, those on probiotics had a shorter duration to recurrence (3.5 months probiotic versus 6.5 months placebo, $P = 0.04$).[156] As this study looked at multispecies probiotics, it is difficult to determine the role of LGG monotherapy in this cohort.

Amongst individuals who have had a spinal cord injury, the risk of recurrent UTIs may be higher due to physiological alterations in the urogenital system.[157,158] The daily use of LGG in combination with *Bifidobacterium* BB12 (7×10^9 CFU) in a 6-month RCT failed to show efficacy in preventing UTIs in 207 people with spinal cord injury compared with placebo.[159] It would appear that only through intravesical administration does LGG improve symptoms of UTIs.[160]

There appears to be little benefit to women in the use of LGG for the prevention of recurrent UTIs possibly due to poor colonisation in the vaginal area in the absence of the pili that aid adhesion in the GI tract, and continual transfer to the urinary tract.[16] Amongst individuals with recurrent UTIs due to spinal cord injury, the use of LGG may only be of benefit to symptoms through intravesical administration. More studies are required in children to determine the role of LGG as a monotherapy, as its inclusion in a multispecies probiotic is promising for the prevention of UTIs.

Infant health

The gut microbiome begins to develop immediately after birth, and can be determined by mode of delivery and feeding.[161,162] Infants born vaginally are typically colonised with beneficial bacteria from the mother's vaginal canal, and those born through Caesarean-section (C-section), from the mother's skin.[163] Individuals born via C-section may have a higher risk of developing several metabolic and immune disorders later in life,[164] possibly due to a lack of *Escherichia-Shigella* and *Bacteroides* species, and lower bacterial richness and diversity.[165]

Colonisation of pathogenic bacteria early in life has been shown to contribute to poorer health outcomes later in life.[166] However, despite the ability of LGG to competitively inhibit pathogenic bacteria and act as an antimicrobial in adults, results have been mixed in children, with one RCT reporting no effect of 42 weeks of LGG supplementation on the colonisation of *Staphylococci* in 60 pre-term infants, despite rapid colonisation of LGG.[167] In addition, no effects were seen on growth rate or length of hospitalisation in this trial. The analysis of only one pathogenic bacteria may not be sufficient, and other strains may need to be analysed to understand the exact effects. In contrast, benefits to height and weight of babies at 12 months have been observed following *in utero* LGG supplementation.[168] In this RCT, 208 healthy pregnant women were given LGG (7×10^8 CFU/day) in combination with *B. lactis* (7×10^8 CFU/day), resulting in increased baby weight and height at 12 months compared with placebo. Furthermore, one 6-month RCT of 120 healthy infants fed with LGG (dosage not stated) in supplemented formula have reported better LGG colonisation (91% versus 76%, $P \leq 0.05$), which led to better growth compared with formula milk without LGG. Higher than normal defecation was reported in the LGG-supplemented group; however, this was not considered to be diarrhoea or detrimental to health.[169] Differing trial durations, stages of supplementation and follow-up times could be responsible for differences between the outcomes, with at least 6 months of treatment required to show benefits.

Observational studies have indicated that maternal nutrition and the *in utero* environment may also increase the risk of offspring having poor health outcomes,[170] indicating an area where probiotic supplementation may be of benefit. Better health outcomes of mothers and babies have been reported in one 2-year

follow-up of a RCT, concluding that pre-natal multispecies probiotic use may be a safe and cost-effective way of preventing metabolic disease in offspring.[171] In this study, the use of LGG and *B. lactis* (1×10^{10} CFU/day) in combination with dietary advice in 256 pregnant women from the first trimester to cessation of breastfeeding reduced the frequency of GDM compared with dietary advice alone ($P \leq 0.003$). In those who did develop GDM, smaller birth weight of babies was observed. With birth size being a risk factor for obesity in later life, LGG supplementation may have an impact *in utero* on later development of non-communicable diseases.

Supplementing LGG *in utero* or during infancy for improved outcomes at all stages of life is apparent. Although supplementation for improved growth rates in babies is controversial, the results would suggest that 6 months of supplementation either starting *in utero* or during infancy may be required at doses of at least 7×10^8 CFU/day, and potentially in combination with *B. lactis*. Supplementing LGG (1×10^9 CFU) during pregnancy may have benefits to both the mother and child in preventing the development of GDM and non-communicable diseases in later life.

Infantile colic

Although not life threatening, the impact of a child with colic extends to parental distress, anxiety and depression, and may be associated with the development of disorders such as allergic disease, migraine and GI disorders later in life.[172] Thus, the reduction in the time a child with colic spends crying may have huge neuropsychological implications. One recent RCT of 45 colicky breastfed infants showed that a high dose of LGG (5×10^9 CFU/day) in combination with elimination of cow's milk from the mother's diet reduced crying time and GI inflammation, with no adverse events reported even at this high dose.[173] However, a lower-dose LGG supplementation (1×10^9 CFU/day) for 6 months in an RCT of 184 infants was shown not to prevent colic based on symptoms or physician's diagnosis when compared with control.[174] This finding was supported in an earlier pilot study of 17 breastfed infants given LGG (4.5×10^9 CFU/day) in combination with behavioural support and cow's milk elimination by the mother.[172] LGG supplementation did not affect crying time or GI inflammation, but crying occurrences were decreased. Differing dosages used in the trials above may account for discrepancies between the results, with a higher dosage being more successful. Elimination of cow's milk may also account for discrepancies.

LGG when included as part of a multispecies regimen has shown more consistent success. When included as part of a nine-strain multispecies synbiotic, a recent RCT of 4 weeks in 17 breastfed infants reported efficacy, with a decrease in the number of crying days and average crying duration when compared with Simethicone, which is used to relieve gas and GI discomfort.[175] Although this trial was small, these findings were also supported in a larger, earlier RCT of 50 breastfed infants with higher treatment success and higher symptom resolution when given a seven-species synbiotic containing LGG (1×10^9 CFU/day).[176]

The use of high-dose LGG supplementation as monotherapy (5×10^9 CFU/day) in combination with cow's milk elimination has been shown to be efficacious in colic to reduce crying time and GI inflammation. Furthermore, when included as part of a multispecies synbiotic regimen, LGG may be of benefit to infants with colic to improve symptoms and crying. However, there are no studies to date showing efficacy of the use of LGG as a monotherapy.

Human immunodeficiency virus

As with colorectal cancer, the success of treatments for human immunodeficiency virus (HIV) is often dependent upon their tolerability. Diarrhoea is a common side-effect of anti-retroviral treatments[177] and, in addition, patients who are immunocompromised may be at a higher risk of microbe-associated diarrhoea.[178] However, unlike patients with colorectal cancer, 17 patients infected with HIV who had suffered from diarrhoea for more than 1 month showed little improvement to diarrhoea or GI symptoms following LGG supplementation twice daily ($1–5 \times 10^{10}$ CFU) for 2 weeks compared with placebo.[179] There were no differences in faecal counts of LGG between the two treatments, indicating poor colonisation following supplementation.

Previous trials have shown lower faecal *Lactobacillus* cultures in patients infected with HIV compared with healthy individuals,[180] indicating a possible need for increased dosages. Further trials are warranted, as the use of LGG in other cohorts of patients for the prevention and treatment of diarrhoea has been of benefit.

Acne

The pathogenesis of acne involves several factors, including inflammation and alterations of insulin signalling.[181,182] Systemic supplementation of probiotics to improve the insulin signalling pathway has been discussed previously, and hypothetically LGG could be used to improve acne. Improvements to the expression of genes involved in the insulin signalling pathway of individuals with acne have been reported with supplementation of *L. rhamnosus* SP1 (3×10^9 CFU/day) for 12 weeks.[183] Subjects ($n = 10$) in the supplementary arm reported reductions in IGF-1 gene expression ($P \leq 0.001$) and increased FOXO1 gene expression ($P \leq 0.001$) from baseline, with no changes observed in the placebo arm ($n = 10$). This resulted in physician-rated improvements to skin appearance of acne in the *L. rhamnosus* SP1 group compared with the placebo group (OR 28.4, 95% CI: 2.2–411.1, $P \leq 0.05$). Although the study states that the strain used is also known as LGG, there is very little research to confirm this; however, based on the previous research on LGG and IR, it would appear it may have similar actions. It should also be noted that the study was only completed in Caucasian subjects, and translatability into the skin of other races is unknown. In addition, small sample sizes and the pilot nature of the study warrant further research.

Based on a single, small pilot study, the use of *L. rhamnosus* SP1 (3×10^9 CFU/day) for at least 12 weeks for the improvement of acne through modulation of the insulin signalling pathway is promising; however, more research is needed in larger higher-powered studies to confirm effects. Studies on the genetic sequencing of *L. rhamnosus* SP1 and its relationship to LGG or further research on LGG as the test probiotic are also warranted.

Allergy

Allergy development is thought to involve both genetic and environmental factors.[184,185] Dysbiosis and reduced diversity of the infant gut microbiome are thought to be included in the pathogenesis of allergic disease in children, due to factors such as antibiotic use *in utero* and birth by C-section.[186] However, effects may be ameliorated using probiotics. One RCT on the use of a multispecies probiotic containing LGG (5×10^9 CFU/day) during pregnancy, breastfeeding and infancy, reported altered effects of antibiotics and C-section birth on gut dysbiosis, increasing *Bifidobacteria* and reducing pathogenic *Proteobacteria* and *Clostridia*,[186] indicating that the use of probiotic supplementation during infancy may help to restore eubiosis.

Observational studies have indicated that the involvement of *Lactobacillus* species may be of particular importance in the development of allergies. The presence of LGG, *Lactobacillus casei* and *Lactobacillus paracasei* early in life is associated with lower prevalence of allergic disease in childhood, and there may be a lower presence of *Lactobacillus* in children with a genetic predisposition, due to one or more parent having allergic disease.[187,188]

The use of LGG supplementation to decrease the risk of allergy development has also been studied. Benefits to the prevalence of allergic

disease later in life were apparent in a follow-up of patients from four separate RCTs on 303 pre-term children given different strains of probiotic.[189] Children who were given LGG perinatally had a decreased prevalence of allergic disease compared with children given placebo at the 2-year follow-up (OR 0.62, 95% CI: 0.38–0.99, P = 0.047). Treatment durations from the four included trials ranged from 3 to 6 months, and dosages from 1×10^9 to 5×10^{10} CFU.

In children who have already developed an allergy such as cow's milk allergy (CMA), LGG supplementation may also be of benefit. One recent systematic review and meta-analysis of 10 studies on LGG (1.4×10^7–5×10^9 CFU/day) reported that supplementation may aid recovery from GI symptoms, promote tolerance to the allergen and improve faecal blood.[190] Evidence was rated as low-to-moderate quality, due to issues with blinding, concealment and unclear data; however, studies were RCTs. Tolerance acquisition following LGG supplementation in infants with IGE-mediated CMA may be due to its ability to influence gut microbiota structure, enabling colonisation of *Oscillospira*.[191] The modulation of epigenetic mechanisms involved in the immune system and pathogenesis of CMA may also occur following LGG supplementation, resulting in increased tolerance to cow's milk.[192]

Immunomodulation by LGG has also been observed in adults with birch pollen allergy and oral allergy syndrome.[193] This RCT of 38 patients received LGG (2×10^{10} CFU/day) for 5.5 months starting 2.5 months prior to allergy season resulting in increased allergen-specific immunoglobulin (Ig)A levels compared with baseline, effects that were not seen with placebo. This may be of benefit to symptoms, as IgA acts to prevent infections and maintain gut microbiota homeostasis, which if disrupted has been associated with an elevated risk of allergies in children.[184] A second RCT on the effects of LGG supplementation (3×10^8 CFU/day) for 3 months on allergy in 141 marathon runners reported no effect on the immune marker IgE or several other allergic inflammatory markers, compared with placebo.[194] Suboptimal dosages could be responsible for the lack of immunomodulatory effects in this trial, or the fact that the trial only looked at the inflammation-associated IgE and not the anti-inflammatory IgA. Although no symptom relief was observed in these trials, it is indicative of further immune effects in adults on IgA.

There is extensive clinical research on the efficacy and mechanisms behind the use of LGG to prevent allergic diseases and improve symptoms of CMA in children. Children at a high risk of developing allergic disease due to genetic predisposition, antibiotic use or C-section birth may benefit from at least 1×109–5×10^9 CFU/day for at least 3–6 months. Children with existing CMA may benefit from 1.4×107–5×10^9 CFU/day for at least 4 weeks and up to 3 years. Dosages of 2×10^{10} CFU LGG may be of benefit to adults with birch pollen allergy for immunomodulation and the promotion of IgA. However, further studies are warranted to determine the significance of immunomodulation, as without effects on symptoms, supplementation may be pointless.

Dermatitis and eczema

Atopic dermatitis (ADe) is the most common chronic skin condition, affecting up to 20% of children and 3% of adults worldwide.[195] Pathophysiology of ADe is not fully understood; however, dysbiosis may be involved, as individuals with ADe have lower diversity and levels of *Bifidobacterium* and *Actinobacteria*, and higher *Staphylococcus* than healthy subjects.[196,197] Furthermore, studies indicate that like other atopic diseases, gut dysbiosis may contribute to ADe development through immunomodulation.[197]

The effects of LGG supplementation on the immune system, as seen in patients with allergic disease, indicate a potential for its use in individuals with ADe. An early systematic review and meta-analysis of five RCTs with 889 subjects concluded that LGG was ineffective for the primary prevention of eczema in children, when given both prenatally and postnatally.[198] Dosages ranged from 1×10^9 to 1.8×10^9 CFU per day, and the quality of data was good.

When looking at reduction of symptoms, recent RCTs not included in the above meta-analysis have shown differing results. Intrinsic microbiota at early infancy may affect outcomes, and infants with ADe who have higher levels of *Bifidobacterium dentium* have been shown to not respond to probiotic intervention, compared with those without disease.[199] One RCT of 67 children with ADe concluded that LGG as the supplement ComProbi (350 mg) in combination with corticosteroid use was effective at decreasing symptoms of ADe after 8 weeks compared with placebo and corticosteroids (P = 0.014), based on Scoring of Atopic Dermatitis (SCORAD).[200] However, it is difficult to determine that effects were due to LGG because corticosteroids were also being used. In a second RCT in 102 infants aged 3–12 months with ADe where corticosteroids were not used as the treatment, but were not precluded during the trial if individuals wanted to use them, no therapeutic effect of LGG based on SCORAD compared with placebo after 12 weeks was reported.[201] Results from these two trials would suggest that corticosteroids and not LGG may account for the favourable outcomes.

As part of a multispecies therapy, LGG has shown more consistent results. When combined, LGG and *B. animalis* were shown in one systematic review and meta-analysis of 21 RCTs on various multispecies combinations to reduce the risk of ADe compared with placebo when administered *in utero* and during infancy.[202] Furthermore, in a recent RCT of 290 children not included in the previous meta-analysis, the administration of LGG + *B. animalis* (1×10^9 CFU/day) in late infancy for 6 months prevented the development of eczema,[203] indicating that the use of LGG as part of a multispecies regime with *B. animalis* may be of benefit for the prevention of ADe and eczema.

The use of LGG for the primary prevention of ADe and eczema may be of benefit when used as part of a multispecies regime in combination with *B. animalis*, at a dose of 1×10^9 CFU/day, for at least 6 months. While there is yet no strong evidence for LGG alone, stratification of patients with ADe according to intrinsic microbiota may be of benefit for the improvement of symptoms following LGG use; however, more studies are required. The role of LGG in combination with corticosteroids also warrants more research.

Wounds

The role of skin microbiota in wound healing is well documented, with both skin barrier function and the immune response reported to be microbially mediated.[204] Topical application of probiotics for the treatment of burns has shown positive results;[205,206] however, oral probiotic supplementation lacks research. It has been hypothesised that the gut microbiota communicates with the skin microbiota in a bi-directional manner through the gut–skin axis, evidenced by cutaneous manifestations following GI disorders.[207] Oral LGG supplementation may have the potential to help treat certain skin diseases such as ADe and acne as documented above, therefore there may be potential for it to be of benefit to wound healing. The reduction of infections at incision sites in patients with cancer, detailed previously,[3] indicates a benefit of LGG supplementation as part of a multispecies probiotic to aid postoperative healing. However, research on the effects in 20 burn victims found only a modest, non-significant improvement in the time taken to complete wound healing, and no improvements to other clinical outcomes.[208]

There is no evidence for the use of LGG in combination with other probiotic strains for improvements to postoperative wounds. Research is lacking on monotherapy, and has found little effect on healing time in burn victims.

Dental caries

The presence of *Streptococcus* and *Lactobacillus* spp. in the oral cavity has been associated with the presence and onset of dental decay.[209] However, as previously discussed, LGG may have species-specific properties and produce an inhibitory microcin-like substance, which

has the ability to inhibit bacteria such as *Streptococcus*.[32] *In vitro* studies have indicated that the consumption of an LGG probiotic may also be able to colonise the oral cavity and inhibit *Streptococcus sobrinus*.[31,210] This may translate into a reduction in the risk of the development of caries. In one RCT, 594 children aged 1–6 years were given milk containing LGG (5–10 × 10^5 CFU/ml) 5 days a week for 7 months, and showed a reduced risk of the development of dental caries (OR 0.56, 95% CI: 0.36–0.88, $P = 0.01$), based upon *Streptococcus* levels from dental plaque and saliva, and the presence of dental caries.[211]

Colonisation of the oral cavity may be affected by a lack of pili expression.[16] There is only one trial on the use of LGG for the prevention of dental caries, as detailed above,[211] and more studies are warranted given the mechanistic data. However, the trial that does exist was in many individuals over a relatively long period of time. It may therefore be of benefit to reduce the risk of dental caries in children and young adults. Dosages of at least 5–10 × 10^5 CFU may be needed in children.

Vaccine adjuvant

The recent COVID-19 pandemic and ability of SARS-CoV-2 to mutate has highlighted a need to improve immune response following vaccination. Orally ingested LGG may modulate the immune system in response to bacteria and viruses involved in the development of diseases. Research in mice given oral *Lactobacillus* has reported enhanced innate immune response following influenza virus challenge, with increased influenza-specific IgG antibodies and greater protection. RCTs have indicated that LGG may be a useful adjuvant for the immune response following influenza vaccine. One RCT in 42 healthy adults reported increased protection to the H3N2 influenza strain whilst supplementing LGG (1 × 10^{10} CFU) and inulin for 28 days following vaccination.[212] However, in the same study, no differences in seroprotection to the H1N1 or B influenza strains were observed.

Individuals with type 1 diabetes (T1D) are at increased risk of infections,[213] and influenza vaccine is recommended; however, whether influenza vaccines are truly successful in this cohort is still being debated.[214] Adjuvants to increase the immunogenicity of the influenza vaccine may be important, and use of LGG (1 × 10^9 CFU) 3 months pre- and post-influenza vaccination in 64 paediatric patients with T1D reduced the inflammatory immune response associated with T1D, decreasing IL-17, IFN-γ, IL-6 and TNF-α, without affecting the seroprotective antibodies, which are needed for effective vaccination.[215] However, although antibody-mediated immunity remained unaffected in this trial, the mediation of the inflammatory response may be important for individuals who suffer from autoimmune diseases such as T1D.

Studies on different types of vaccinations and studies on LGG as an adjuvant to the polio, rotavirus, Hib, diphtheria and tetanus vaccinations have been completed with varying success. One RCT of 66 healthy males reported that the use of LGG (1 × 10^{10} CFU), as an adjuvant to the polio vaccine, nearly doubled the increase of polio-specific IgG antibodies and significantly increased IgA antibodies, compared with placebo.[216] A second RCT of 98 pregnant women given LGG (5 × 10^9 CFU) resulted in more frequent occurrence of higher Hib antibody concentrations following vaccination with Hib, diphtheria and tetanus in the offspring; however, IgG remained unaffected. In contrast, LGG supplementation (1 × 10^{10} CFU) marginally but not significantly improved rotavirus antibodies following vaccination in 620 infants.[217] This may correspond to the findings above regarding LGG competitively inhibiting and acting as an antimicrobial against rotavirus, which could prevent the body from becoming infected and building an enhanced immune response when the body is faced with the live rotavirus as part of a vaccine.

The use of LGG supplementation (at least 1 × 10^9 CFU) as part of a vaccine adjuvant has been shown to be of benefit to the success of the response of biomarkers to vaccines, but only following influenza H3N2, polio and Hib. Further research needs to be performed with other vaccinations to determine effects.

Review
Lactobacillus rhamnosus GG: A Review of Clinical Use and Efficacy

Safety

Probiotics belonging to the genus *Lactobacillus* and *Bifidobacterium* are generally regarded as safe (GRAS) by the United States Food and Drugs Administration (FDA).[218] However, some studies on *Lactobacillus* have reported bacteraemia in specific populations, primarily amongst immunocompromised paediatric patients.[219] In adults, incidences of bacteraemia-associated endocarditis, primarily in those with a structural heart defect, have also been reported.[220] A recent systematic review has indicated that LGG may increase the risk of complications in patients who are immunocompromised, who have critical illnesses, structural heart disease or who have a central venous catheter.[69] In pregnancy and lactation, a recent meta-analysis and systematic review concluded that probiotics are safe for use during pregnancy and lactation. Data from the trials included in this review showed that adverse events in pregnancy and lactation following LGG supplementation were minor, and one systematic review and meta-analysis has concluded that probiotic use is safe during pregnancy and lactation;[142] however, it would still be recommended to consult with a doctor prior to commencement.

Drug–nutrient interactions are very few, with minor warnings whilst on anti-diabetes drugs due to potential hypoglycaemia and moderate interactions whilst taking antibiotic drugs, as LGG efficacy may be reduced.[221]

Conclusion

The unique morphological features of LGG may ensure that it has some use as an oral supplement in the reduction of risk of developing ADHD and GDM, in the prevention of allergies and dental caries, for improving immune reactions following vaccines, and for the management of diarrhoea associated with cancer treatments and antibiotic use. Three ways in which it may do this are through immunomodulation, cell growth and proliferation, and as an antimicrobial, aiding it to promote eubiosis. This results in LGG acting to prevent disease development, help manage symptoms and improve underlying pathology. The presence of pili on the exterior aid its colonisation of the GI tract, and its lack of efficacy in disease areas such as UTIs may be due to a lack of expression of these features in certain areas of the body. Effects may be systemic if there is a pathway through which LGG or its products can travel, like the gut–brain axis. However, effects may also be localised and specific, if a transmission pathway does not exist, as seen with its success only in specific cancer types, and diarrhoea treatment in colorectal cancer but not HIV.

There are, however, limitations of this study, and the inability to address genetic variation amongst LGG is apparent. Genetic variants have been found within the LGG species resulting in variations that do not have the *spaCBA* gene.[222] These variants may lack the ability to express the pili-like projections responsible for many of the physiological effects attributed to LGG. It is therefore difficult to exclude the possibility that positive or negative results were not attributable to within-strain differences. Until the research is performed, it is difficult for practitioners to determine which commercial products may have genetic variations. It may be that quality assurance legislation needs to be put in place; however, this has yet to be enacted. A second limitation is that this study could not account for individual intrinsic gut microbiota populations, which are highly personalised.[67] Therefore, although patients or disease areas may have been identified to benefit from LGG supplementation, differences between intrinsic gut microbiota may affect efficacy. Amongst the studies, issues with small sample sizes, contradictory results and the fact that the large majority of the research involved the use of multispecies supplements, and the use of other treatments and therapies amongst some of the research, means that conclusions need to be interpreted with caution.

Appendix

Cancer

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Armuzzi *et al.*[47] Italy RCT		LGG 6 × 10^9 CFU twice daily versus placebo, 14 days	60 healthy patients undergoing anti-*Helicobacter pylori* regimen	Side-effect profile Tolerability	LGG aided eradication, tolerability and overall side-effects Diarrhoea, nausea and taste disturbance all reduced by LGG (RR = 0.1, 95% CI: 0.1–0.9; RR = 0.3, 95% CI: 0.1–0.9; RR = 0.5, 95% CI: 0.2–0.9) Treatment tolerability higher in LGG (P = 0.04) No benefit to eradication rate	Not stated
Flesch *et al.*[3] Brazil RCT	To determine the effect of perioperative use of multispecies placebo + oligosaccharide in patients with colorectal cancer	LGG + *Lactobacillus acidophilus* + *Lactobacillus casei* + *Bifidobacterium* (all 1 × 10^8 –1 × 10^9 CFU/day) + fructo-oligosaccharide (6 g) versus placebo, 5 days pre-operative and 14 days postoperative	91 patients undergoing surgery for colorectal cancer	Infection occurring within 30 days of surgery	Perioperative administration of synbiotics reduced the occurrence of postoperative infections in patients with colorectal cancer Infection at incision site in one patient in synbiotic group and nine in the placebo group No infections in synbiotic group versus 7 in control group (P = 0.001)	Not stated
Lages *et al.*[48] Brazil RCT	To determine the postoperative outcomes of head and neck cancer surgical patients with multispecies probiotic + fructo-oligosaccharides versus placebo (duration not stated)	LGG + *Lactobacillus paracasei* + *Lactobacillus acidophilus* + *Bifidobacterium lactis* (all 6 × 10^9 CFU) + 6 g fructo-oligosaccharides	40 postoperative head and neck cancer patients	Intestinal function and permeability, number of total stool episodes, stool consistency and adverse GI symptoms	Synbiotics did not impact on postoperative outcomes or intestinal function of head and neck cancer surgery patients Postoperative complications similar in other groups (P > 0.05) Inflammatory markers similar in both groups (P ≥ 0.05) Total daily stools similar (P ≥ 0.05) and GI symptoms similar (P ≥ 0.05)	Method to test intestinal permeability not optimal, as antibiotic use and ageing may impact its sensitivity Small sample size
Rafter *et al.*[40] Ireland RCT	To determine whether multispecies probiotic + prebiotic can reduce the risk of colon cancer	LGG + *B. lactis* Bb12 + inulin (SYN1 brand), 12 g sachet per day, 12 weeks	37 patients with colon cancer and 43 polypectomised patients	Not stated	Probiotics may alter several colorectal cancer biomarkers Probiotic changed *Bifidobacterium, Lactobacillus* and *Clostridium perfringens* Decreased level of DNA damage in polyp patients Increased IL-2 secretion prevented in polyp patients but not cancer Increased IFN-γ in patients with cancer but not polyp group	Limited biopsies
Roller *et al.*[41] Germany RCT	To determine the effect of daily intake of multispecies probiotic + prebiotic on immune function in patients with colon cancer	LGG and *B. lactis* (1 × 10^{10} CFU/day) + 10 g inulin versus placebo, 12 weeks compared with baseline	34 patients with colon cancer who had undergone curative resection and 40 polypectomised patients	Phagocytic and respiratory burst activity of neutrophils and monocytes, lytic activity of NKCs, transforming growth factor, prostaglandin E2 and inflammatory markers	Supplementation with multispecies probiotic had modest effects on the immune system of the two study groups IL-2 significantly increased in the cancer group (P < 0.05) between 0 weeks or 6 weeks and 12 weeks IFN-γ increased at 12 weeks (P ≤ 0.05) No other immune factors affected	Limited biopsies

CFU, colony-forming units; CI, confidence interval; GI, gastrointestinal; IFN-γ, interferon-gamma; IL, interleukin; LGG, *Lactobacillus rhamnosus* GG; NKCs, natural killer cells; RCT, randomised-controlled trial; RR, relative risk.

Review
Lactobacillus rhamnosus GG: A Review of Clinical Use and Efficacy

Irritable bowel syndrome

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Francavilla *et al.*[4] Italy RCT	To determine whether LGG relieves symptoms in children with recurrent abdominal pain	16 weeks (8 weeks treatment, 8 weeks follow-up) 6 × 10^9 CFU/day	141 children with IBS or functional pain	Change in abdominal pain according to VAS score	LGG but not placebo reduced frequency and severity of abdominal pain from baseline Effects may be due to improvement of gut barrier	Effect may not be unique to LGG Did not assess gut microbiota at baseline or end Cannot exclude possibility that effect is short-lived
Horvath *et al.*[58] Poland Meta-analysis	To assess the effect of LGG for treating abdominal pain-related functional GI disorders in children compared with no treatment or placebo		3 RCTs, 290 children with abdominal pain-related functional GI disorders	Study 1: change in abdominal pain score Study 2: VAS Study 3: Faces pain scale	Beneficial effect of LGG in IBS Intensity and frequency of pain significantly reduced	Did not perform a statistical test for publication bias
Lyra *et al.*[54] Finland RCT	To determine if a multispecies probiotic can affect IBS-associated microbiota alterations	6 months LGG + *Lactobacillus rhamnosus* Lc705, *Propionibacterium freudenreichii* spp. *shermanii* JS and *Bifidobacterium* Bb99 8–9 × 10^9 CFU/day	42 patients with IBS	Changes in faecal microbial composition	Multispecies probiotic altered IBS-associated microbiota quantities of the bacterial 16S rDNA phylotypes, to those reflective of IBS-free subjects, particularly *Clostridium thermosuccinogenes*	Not stated
Pedersen *et al.*[26] Denmark Unblinded RCT	Investigate the effects of a low-FODMAP diet versus LGG in IBS	6 weeks, 6 × 10^9 CFU/day (Dicoflor 60 capsules)	123 males and females with IBS	Disease severity of IBS using IBS-SSS questionnaire	Both treatments efficacious for IBS, especially in the IBS-D and IBS-A subtypes	Lack of blinding Not placebo controlled Diet adherence not evaluated
Wegh *et al.*[25] Netherlands Systematic review	Investigate the effects of probiotics on FAPD and functional constipation in children		17 studies with 1321 children (3 on LGG)		LGG reduces frequency and severity of abdominal pain, but only in children with IBS	Majority of studies have unclear or high risk of bias Many studies did not compare the results from baseline, only between groups High heterogeneity between groups Only studies in English included Crossover studies included Studies only had a 2-week washout period
Yoon *et al.*[27] Korea RCT	Investigate the efficacy of a multispecies probiotic on IBS symptoms and gut microbiota alterations	4 weeks multispecies, 5 × 10^9 CFU/day LGG + *Bifidobacterium longum, Bifidobacterium bifidum, Bifidobacterium lactis, Lactobacillus acidophilus* and *Streptococcus thermophilus*	49 patients with IBS	Proportion of patients who experience IBS symptom relief based on answers to two questions	Multispecies probiotic supplementation is effective at relieving symptoms of abdominal pain, bloating and discomfort in individuals with IBS, and caused a change to the gut microbiota	Faecal analysis not in whole study population Faecal microflora analysis only reflects bacterial composition in the intestinal lumen Validated measurement of symptom improvement was not used Did not look at gender or IBS subtypes

CFU, colony-forming units; FAPD, functional abdominal pain disorders; FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides and polyols; GI, gastrointestinal; IBS, irritable bowel syndrome; IBS-A, IBS-mixed subtype; IBS-D, IBS-diarrhoeal subtype; IBS-SSS, IBS-Severity Scoring System; LGG, *Lactobacillus rhamnosus* GG; RCT, randomised-controlled trial; VAS, Visual Analogue Scale.

Diarrhoea

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Agamennone *et al.*[67]	To produce a guide on the use of probiotics to prevent AAD		32 RCTs		Results indicate that seven single or multispecies favouring the treatment group, with LGG being the most effective (RR = 0.30 versus placebo, 95% CI: 0.16–0.5) Dosage recommendations of at least 2×10^9 CFU may be needed	Not stated
Fang *et al.*[71] Taiwan Open-label RCT	To assess whether there is a dose-dependent effect of LGG on the reduction of faecal rotavirus shedding in children	0 CFU/day in control 2×10^8 CFU/day low dose 6×10^8 CFU/day high dose	23 children with acute rotaviral gastroenteritis	Not stated	Low-dose group had no change in faecal rotavirus concentrations (36.1×10^5 particles/ml versus 73.5×10^5 particles/ml, $P = 0.895$); however, the high-dose group did (64.2×10^5 particles/ml versus 9.0×10^5 particles/ml, $P = 0.012$) It appears there is a dose-dependent effect of LGG on faecal rotavirus shedding in children	Not stated
Korpela *et al.*[65] India RCT	To determine the effect of long-term LGG consumption on pre-school children's antibiotic use Also assessed its effect on gut microbiota	400 ml milk containing 10^6 CFU/ml LGG for 7 months	231 school-aged children	First antibiotic purchase	Long-term LGG may prevent specific bacterial infections for up to 3 years, and may prevent some of the gut microbiota changes associated with antibiotic use Increased abundance of the *Lactobacillus* spp. ($P < 0.0001$)	Not stated
Li *et al.*[70] China Systematic review and meta-analysis	Evaluate the efficacy of LGG in children with acute diarrhoea		19 RCTs	Development of persistent diarrhoea, including duration	High-dose LGG reduced duration and frequency of diarrhoea episodes Results pronounced in those who were treated early and who presented with rotavirus-positive diarrhoea Reduced duration (MD −31.05 hours, 95% CI: 50.31, −11.80) and frequency of episodes (MD −1.08, 95% CI: −1.87, −0.28)	Limitations amongst the studies included limited pathogen identification, small sample sizes, varying dosages and limited blinding
Schnadower *et al.*[61] USA RCT	Determine the effectiveness of a 5-day course of LGG compared with placebo in children with acute gastroenteritis	5 days, 1×10^{10} CFU twice daily versus placebo	943 children aged 3 months to 4 years with acute gastroenteritis	Presence of moderate-to-severe gastroenteritis	Administration of LGG to preschool children with acute gastroenteritis did not result in a smaller number of moderate-to-severe gastroenteritis cases, and did not show benefit to duration or frequency of vomiting or diarrhoea compared with children receiving placebo	Possible inaccurate recall by participants Potential for LGG preparation to be inadequately stored
Szajewska *et al.*[69] Poland Meta-analysis	To provide recommendations on the use of probiotics and prebiotics for the prevention of AAD in children		20 RCTs	Diarrhoea/AAD and *Clostridium difficile*-associated diarrhoea	Recommended using LGG or *Saccharomyces boulardii* for preventing AAD For *C. difficile*-associated diarrhoea then LGG *not* recommended AAD risk reduction (RR = 0.48, 95% CI: 0.26–0.89)	The authors question the validity of pooling different strains of probiotic, when they all have differing effects
Szajewska & Kołodziej[66] Poland Meta-analysis	To determine the efficacy of LGG to prevent AAD in children and adults		12 RCTs, 1499 participants	Incidence of diarrhoea or AAD	Treatment with LGG compared with placebo or no additional treatment reduced the risk of ADD from 22.4% to 12.3% (RR = 0.49, 95% CI: 0.29–0.83, NNT = 9)	Definition of AAD varied amongst studies Unclear risk of bias

AAD, antibiotic-associated diarrhoea; CFU, colony-forming units; CI, confidence interval; LGG, *Lactobacillus rhamnosus* GG; MD, mean difference; NNT, number needed to treat; RCT, randomised-controlled trial; RR, relative risk.

Inflammatory bowel disease

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Bousvaros *et al.*[81] USA RCT	To determine if addition of LGG to standard therapy prolonged remission in children with Crohn's disease	2-year follow-up, LGG 10^{10} CFU/day + 295 mg inulin versus placebo	75 children aged 5-21 years	Time to clinical relapse	Median time to relapse 9.8 months in LGG versus 11.0 months in placebo group ($P = 0.24$) LGG did not prolong remission in children with Crohn's disease	Concomitant therapies could be masking effects of LGG
Jonkers *et al.*[79] Netherlands Systematic review and meta-analysis	Assess the use of probiotics in IBD management		41 RCTs, two in LGG		No difference in LGG supplementation and placebo for endoscopic recurrences in inactive Crohn's disease, even though there was an OR of 0.93 (95% CI: 0.63, 1.38) High drop-out rates amongst studies	
Lorea Baroja *et al.*[82] Canada Open-labelled study	Assess whether a combination of LGG GR-1 strain and *Lactobacillus reuteri* in a yoghurt supplement was able to promote an anti-inflammatory state in individuals with Crohn's disease	125 g probiotic yoghurt per day for 30 days LGG dosage 2×10^7 CFU/ml and *L. reuteri* 1×10^3 CFU/ml	20 participants with Crohn's disease and ulcerative colitis, 20 healthy controls	Changes in the prevalence of inflammatory markers *T*reg cells ($CD4^+$ $CD25^{high}$), TNF-α and IL-12	Amongst patients with IBD, increased $CD4^+$ $CD25^{high}$ *T*-cells ($P = 0.007$) This correlated with a decrease in the percentage of TNF-α and IL-12 Probiotic yoghurt intake was associated with an anti-inflammatory effect	Not stated
Shen *et al.*[80] China Meta-analysis	Assess the effect and adverse events of *Lactobacilli* strains compared with placebo as maintenance therapy in Crohn's disease		6 RCTs, 4 trials in LGG 359 individuals	Clinical relapse rates	LGG may increase the relapse rate of those with Crohn's disease Significant benefit of placebo (RR = 1.68; 95% CI: 1.07–2.64)	Different measures of relapse rates amongst the studies Different study durations

CFU, colony-forming units; CI, confidence interval; IBD, inflammatory bowel disease; IL, interleukin; LGG, *Lactobacillus rhamnosus* GG; OR, odds ratio; RCT, randomised-controlled trial; RR, relative risk; TNF-α, tumour necrosis factor-alpha.

Body weight

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Callaway *et al.*[91] RCT Australia	To determine whether multispecies probiotic in overweight and obese women prevents GDM	Probiotic LGG + *Bifidobacterium animalis* 1×10^9 CFU/day versus placebo	411 pregnant overweight and obese women	Frequency of GDM at 28 weeks gestation Secondary outcomes: gestational weight gain, preeclampsia, hypertension, Caesarean delivery, and gestation age of delivery	Probiotics did not prevent GDM (18.4% probiotic versus 12.3% placebo, $P = 0.10$), but did prevent excessive weight gain during gestation in overweight and obese pregnant women (32.5% probiotic versus 46% placebo, $P = 0.01$)	Oral glucose tolerance test not completed at start of trial Changes to trial design meant some women only taking probiotics for 1–4 weeks
Kekkonen *et al.*[92] RCT sub-study	To determine the effect of 3-week LGG supplementation on serum lipid profiles and inflammatory markers	250 ml milk-based fruit drink with LGG 6.2×10^7 CFU/ml for 3 weeks	26 healthy adults	Not stated	LGG supplementation may lead to a change in serum global lipid profiles Decreased LysoGPCho ($P \leq 0.05$), sphingomyelins ($P \leq 0.001$) and glycerophosphatidylcholines ($P \leq 0.05$)	When allowing for multiple hypothesis testing, no changes in global lipidomic profiles
Okesene-Gafa *et al.*[90] RCT New Zealand	To determine a culturally tailored dietary intervention and/or daily probiotic in obese pregnant women reduces gestational weight gain and birthweight	Dietary intervention versus routine dietary advice + probiotic containing LGG and *Bifidobacterium lactis* BB12 6.5×10^9 CFU/day until birth	230 obese pregnant women and their babies	Proportion of women with excessive gestational weight gain Birth weight	Neither treatment had a significant effect Total maternal weight gain was lower with dietary intervention than probiotic and routine dietary advice (9.7 kg versus 11.4 kg, adjusted MD −1.76, 95% CI: 3.55–0.03)	Not stated

CFU, colony-forming units; CI, confidence interval; GDM, gestational diabetes mellitus; LGG, *Lactobacillus rhamnosus* GG; MD, mean difference; RCT, randomised-controlled trial.

Liver disease

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Kwak *et al.*[18] Korea RCT	To determine the efficacy of probiotics to improve SIBO and gut permeability in liver disease	Multispecies containing 5 × 10^9 CFU *Bifidobacterium bifidum, Bifidobacterium lactis, Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus rhamnosus* and *Streptococcus thermophilus* versus placebo, once daily for 4 weeks	53 patients with chronic liver disease	Changes in the composition of faecal bacteria, SIBO, intestinal permeability and clinical symptoms	LGG increased in faeces of probiotic group ($P \leq 0.001$) SIBO significantly disappeared in probiotic group compared with placebo ($P \leq 0.05$) Intestinal permeability improved but not significantly Liver chemistry remained unaffected Short-term probiotics effective in alleviating SIBO but not liver function in patients with chronic liver disease	Hydrogen breath test not jejunal aspiration used to test for SIBO Study participants had only mild disease as administration of probiotics in immunocompromised individuals is not recommended
Vajro *et al.*[99] Italy Double-blind, pilot study	To evaluate the effects of short-term probiotic treatment in children with NAFLD	LGG 1.2 × 10^{10} CFU/day for 8 weeks	20 children with NAFLD	Not stated	Compared with placebo, LGG was associated with a decrease in ALT ($P = 0.03$) and in anti-peptidoglycan-polysaccharide antibodies ($P = 0.03$) LGG should be considered as a therapy for children with NAFLD who do not comply with lifestyle interventions	Not stated

ALT, alanine aminotransferase; CFU, colony-forming units; LGG, *Lactobacillus rhamnosus* GG; NAFLD, non-alcoholic fatty liver disease; RCT, randomised-controlled trial; SIBO, small intestinal bacterial overgrowth.

Insulin resistance and type 2 diabetes

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Asemi *et al.*[103] Iran RCT	To determine the effect of multispecies probiotic on metabolic profiles	*Lactobacillus acidophilus* (2 × 10^9 CFU), *Lactobacillus casei* (7 × 10^9 CFU), LGG (1.5 × 10^9 CFU), *Lactobacillus bulgaricus* (2 × 10^8 CFU), *Bifidobacterium breve* (2 × 10^{10} CFU), *Bifidobacterium longum* (7 × 10^9 CFU), *Streptococcus thermophilus* (1.5 × 10^9 CFU) versus placebo, 8 weeks	54 diabetic patients	Anthropometrics Plasma glucose HbA1c levels HOMA-IR blood lipid concentrations Antioxidants	Multispecies probiotic for 8 weeks in patients with diabetes prevented a rise in fasting plasma glucose, and decreased serum hs-CRP and increased GSH Measures of IR were increased in both groups, but less so in the probiotic group ($P = 0.03$)	Not stated
Laitinen *et al.*[106] Finland RCT	To determine whether supplementation of multispecies probiotic with dietary counselling affects glucose metabolism in normoglycaemic pregnant women	LGG + *Bifidobacterium lactis* + dietary advice versus placebo during pregnancy and 12 months post-partum Dosage not stated	256 normoglycaemic pregnant women	Glucose metabolism through plasma glucose concentration and HbA1c, serum insulin and HOMA and QUICKI	In normoglycaemic pregnant women, diet + probiotics may improve blood glucose control Blood glucose at lowest in diet + probiotic group during pregnancy and 12 months post-partum ($P \leq 0.025$ for both) Better glucose tolerance in diet + probiotic group through HOMA-IR ($P = 0.028$), insulin concentration ($P = 0.032$) and QUICKI ($P = 0.028$) Reduced risk of elevated glucose concentration compared with placebo (OR 0.31, 95% CI: 0.12, 0.78, $P = 0.028$)	Not stated
Sanborn *et al.*[105] USA RCT sub-analysis	To determine whether probiotic supplementation improves glycaemic control in healthy individuals	LGG 1 × 10^{10} CFU versus placebo, 90 days	200 healthy middle-aged and older adults	HbA1c	LGG may help maintain glycaemic control in healthy adults HbA1c increased in placebo but maintained in the LGG group (between-group difference $P = 0.005$)	Not stated

CFU, colony-forming units; CI, confidence interval; GSH, glutathione; HbA1c, glycated haemoglobin; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; hs-CRP, high-sensitivity C-reactive protein; IR, insulin resistance; LGG, *Lactobacillus rhamnosus* GG; OR, odds ratio; QUICKI, Quantitative Insulin-Sensitivity Check Index; RCT, randomised-controlled trial.

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Cystic fibrosis

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Bruzzese *et al.*[110] Italy RCT	To determine the gut microbiota composition of children with CF, and whether correlations between microbial balance and inflammation exist Then to determine whether LGG restores intestinal flora and decreases inflammation	6 × 10^9 CFU versus placebo daily for 1 month	22 children with CF	Intestinal inflammation Bacterial composition	CF restored gut microbiota reducing intestinal inflammation and pulmonary exacerbations LGG reduced faecal CLP (184 ± 146 mg/g versus 52 ± 46 mg/g; $P \leq 0.01$) Correlation between reduced microbial richness and intestinal inflammation (r = 0.53; $P = 0.018$)	Not stated
Bruzzese *et al.*[112] Germany RCT	To investigate the effects of LGG on clinical outcomes of children with CF	LGG 6 × 10^9 CFU/day versus placebo, 12 months	95 children with CF	Proportion of subjects with at least one pulmonary exacerbation over the 12-month study period	LGG had no effect on respiratory and nutritional outcomes in children with CF Odds of experiencing at least one exacerbation were not significantly different from placebo (OR 0.83, 95% CI: 0.38–1.82, $P = 0.643$) The odds of hospitalisations also remained unaffected (OR 1.67, 95% CI: 0.75–3.72, $P = 0.211$)	Not stated
Bruzzese *et al.*[108] Germany Prospective study	To assess the incidence of intestinal inflammation in children with CF, and whether probiotics decrease it	LGG 5 × 10^9 CFU/day	75 children (30 with CF, 30 with IBD and 15 healthy controls)		Intestinal inflammation is a feature of CF as indicated by increased CLP (versus control, $P \leq 0.01$) similar to levels of children with IBD ($P \geq 0.05$) Intestinal microflora play a major role in this LGG reduced inflammation (210 ± 42 to 140 ± 43 mg/g, $P = 0.01$)	Not stated
Bruzzese *et al.*[111] Italy Prospective RCT crossover	To determine the effect of LGG on pulmonary exacerbations in children with CF	LGG 6 × 10^9 CFU/day for 6 months and then shifted to dissolved oral rehydration solution for 6 months Or dissolved oral rehydration solution for 6 months and then LGG for 6 months	43 children with CF	Incidence and severity of pulmonary exacerbations Number and duration of hospital admissions Route of antibiotic administration (indication of severity of episode) FEV_1 Body weight Serum immunoglobulin concentrations	LGG reduced pulmonary exacerbations and hospital admissions in children with CF Pulmonary exacerbations reduced (group A, median difference 1, CI 95%: 0.1–2, $P = 0.035$; Group B, median difference 1, 95% CI: 0–2, $P = 0.02$) Rate of hospital admissions (LGG = 16, ORS = 32) Significant differences only in period one (MD 1, 95% CI: 0.1–1, $P = 0.01$) Mean duration of hospital stay did not differ between the two groups	Not stated

CF, cystic fibrosis; CFU, colony-forming units; CI, confidence interval; CLP, calprotectin; FEV_1, forced expiratory volume; IBD, inflammatory bowel disease; LGG, *Lactobacillus rhamnosus* GG; MD, mean difference; OR, odds ratio; RCT, randomised-controlled trial.

Respiratory tract infections

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Liu *et al.*[123] China Systematic review and meta-analysis	To review the effectiveness of LGG for the prevention of respiratory infections in children		4 RCTs, 1805 children	Incidence of respiratory infections	LGG may reduce the incidence of otitis media, URTIs and antibiotic use in children LGG was associated with reduced otitis media (RR = 0.76, 95% CI: 0.64–0.91, NNT = 17), reduced risk of URTIs (RR = 0.62, 95% CI: 0.50–0.78, NNT = 4) and antibiotic use (RR = 0.80, 95% CI: 0.71–0.91) Risk of overall respiratory infections was only reduced in those older than 1 year (RR = 0.73, 95% CI: 0.57–0.92, NNT = 8) No difference in the incidence of lower respiratory infections	Not stated
Hojsak *et al.*[125] Croatia RCT	To determine the role of LGG in preventing nosocomial GI infections and RTIs in children	LGG 1 × 10^9 CFU/day in 100 ml of a fermented milk product versus placebo Duration not stated	742 hospitalised children	GI tract infections Upper and lower RTIs	LGG can decrease risk for nosocomial GI infections and RTIs in paediatric facilities Reduced risk of RTIs compared with placebo (RR = 0.38, 95% CI: 0.18–0.85, NNT = 30) No difference in hospitalisation duration	Infants prone to severe nosocomial infections were excluded The study period in most cases was short Cause of nosocomial infection was often unknown
Tapiovaara *et al.*[115] Finland Randomised control pilot study	To determine whether beneficial effects of LGG in RTIs are due to a reduced viral load	LGG 1 × 10^9 CFU/day versus placebo, 6 weeks	59 adults given human rhinovirus	Viral load	The use of LGG did not affect viral load in individuals with human rhinovirus Viral load LGG versus placebo (*P* = 0.57)	Samples collected 5 days after given human rhinovirus Validated symptom survey not used
Kumpu *et al.*[116] Finland RCT	To determine whether inactivated LGG would demonstrate similar effects to live LGG in humans with induced rhinovirus infection	LGG 1 × 10^9 CFU in 100 ml fruit juice, 6 weeks	60 individuals induced with the human rhinovirus	Occurrence, duration and severity of cold symptoms	Live LGG may be more effective in reducing rhinovirus infection than the inactivated form, but differences were not significant Occurrence and severity of cold symptoms was lowest in the LGG live group, but this was not statistically significant due to the pilot-scale of study (*P* = 0.45)	Not stated
Laursen & Hojsak[122] Denmark Systematic review and meta-analysis	To evaluate strain-specific effects of probiotics on RTIs in children at day care		15 RCTs with 5121 children in day care	Number of children with RTIs	Of the probiotics analysed, LGG significantly reduced the duration of RTIs (MD −0.78 days, 95% CI: −1.46, −0.09), but no effect on incidence, antibiotic use or days missed from day care	Studies included differed in methodological quality Only included studies in English
Laursen *et al.*[121] RCT Denmark (ComProbi study)	To determine the effects of multispecies probiotic on absence from childcare due to respiratory and GI infections in healthy infants	LGG + *Bifidobacterium animalis* 1 × 10^9 CFU, 6 months	290 infants who attend childcare	Number of days absent from childcare because of respiratory or GI infections	A multispecies probiotic for 6 months did not affect the number of days absent from childcare in healthy infants (*P* = 0.19)	Data on infant illness recorded using questionnaires
Swanljung *et al.*[114] Finland RCT	To determine whether 3-week supplementation of LGG would lead to the presence of the probiotic in adenoid tissue	8–9 × 10^9 CFU LGG in 150 ml commercial dairy product versus placebo, 3 weeks	40 children aged 1–5 years about to undergo adenotomy	Presence of LGG in adenoid tissue Secondary outcome rhinovirus and enterovirus in adenoid tissue	After 3 weeks supplementation, more LGG identified in the adenoids of children on probiotics (*P* = 0.07); however, its effect on the occurrence of rhinovirus or enterovirus was not apparent, as no significant differences between the groups (*P* = 0.67) A large amount of LGG was found in the adaenoids of the placebo group No differences in symptoms	Small study size Diaries used so reporting methods not standardised Limited diary data supplied
Luoto *et al.*[124] Finland RCT	To determine whether early prebiotic or probiotic supplementation reduced the risk of virus-associated RTIs in the first year of life in pre-term infants	LGG 1 × 10^9 CFU for first 30 days and 2 × 10^9 CFU for final 30 days versus placebo	94 preterm infants	Incidence of viral RTIs	Prebiotics and probiotics may reduce the risk of RTIs and rhinovirus infections Lower incidence of RTIs in infants receiving prebiotics (RR = 0.24, 95% CI: 0.12–0.49, *P* ≤ 0.001) and probiotics (RR = 0.50, 95% CI: 0.28–0.90, *P* = 0.022) Rhinovirus episodes also reduced in prebiotics (RR = 0.31, 95% CI: 0.14–0.66, *P* = 0.003) and probiotics (RR = 0.49, 95% CI: 0.24–1.00, *P* = 0.051) compared with placebo	Studying preterm infants may mean results are not generalisable to full-term and older infants

CFU, colony-forming units; CI, confidence interval; GI, gastrointestinal; LGG, *Lactobacillus rhamnosus* GG; MD, mean difference; NNT, number needed to treat; RCT, randomised-controlled trial; RR, relative risk; RTI, respiratory tract infection; URTI, upper respiratory tract infection.

Otitis media

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Lehtoranta *et al.*[129] Finland RCT	To determine the prevalence and persistence of HBoV, and whether multispecies probiotics reduce occurrence	LGG + *Lactobacillus rhamnosus* Lc705 + *Bifidobacterium breve* 99 and *Propionibacterium freudenreichii* JS (dosage not stated) versus placebo, 6 months	269 otitis-prone children	Not stated	Probiotic treatment may reduce the presence of HBoV in children A high load of HBoV was detected in 152 children Probiotic supplementation decreased the number of HBoV-positive samples (6.4% versus 19.0%, OR 0.25, 95% CI: 0.07–0.94, *P* = 0.039) HBoV has been associated with lower RTIs	Not stated
Tapiovaara *et al.*[127] Finland RCT	To determine the effect of 3-week oral administration of LGG on MEE in children with otitis media	8–9 × 10^9 CFU/day versus placebo, 3 weeks	40 children undergoing tympanostomy	LGG findings in MEE	LGG was detected in the middle ear of children with otitis media, but did not affect the occurrence of bacteria or viruses LGG was detectable in 4 of the children in the LGG group and 1 in the placebo group, but differences were not significant (*P* = 1.0) Pathogenic bacteria present in 12 of the samples in the LGG group and 3 of the samples in the placebo group (*P* = 0.65) The most prominent species of bacteria was *Haemophilus influenzae*	Small study size PCR-assay used may not be optimised to detect bacteria in MEE
Hatakka *et al.*[128] Finland RCT	To determine the effect of multispecies probiotic	LGG + *L. rhamnosus* Lc705, *B. breve* 99 and *P. freudenreichii* JS 8–9 × 10^9 CFU/day versus placebo, 24 weeks	309 otitis-prone children	Occurrence and duration of acute otitis media episodes	Probiotic treatment did not reduce the occurrence (probiotic versus placebo, 72% versus 65%, OR 1.48, 95% CI: 0.87–2.52, *P* = n.s.), reoccurrence (18% versus 17%, OR 1.04, 95% CI: 0.55–1.96, *P* = n.s.) or duration (5.6 versus 6.0 days, *P* = n.s.) of acute otitis media episodes	Not stated

CFU, colony-forming units; CI, confidence interval; HBoV, human bocavirus; LGG, *Lactobacillus rhamnosus* GG; MEE, middle ear effusion; OR, odds ratio; PCR, polymerase chain reaction; RCT, randomised-controlled trial; RTI, respiratory tract infection.

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Anxiety and depression

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Dawe *et al.*[143] New Zealand RCT	A secondary analysis to determine whether probiotics would improve maternal mental health	LGG + *Bifidobacterium lactis* 6.5 × 10^9 CFU/day versus placebo Duration not stated	230 women at 36 weeks of pregnancy	Depression Anxiety Functional health and wellbeing	Probiotics did not improve the mental health of pregnant women at 36 weeks gestation No difference between depression scores ($P \geq 0.05$) Anxiety and physical wellbeing worsened over time, and mental wellbeing did not differ at 36 weeks	Probiotic strain used may not be optimal Dosage may not be optimal Adherence to treatment was via self-reporting not capsule count Small sample size
Mohammadi *et al.*[135] Iran RCT	To determine the effects of multispecies probiotic and probiotic yoghurt on mental health and hypothalamic–pituitary axis	Yoghurt contained 1 × 10^7 CFU *Lactobacillus acidophilus* + *B. lactis* Multispecies probiotic contained *Lactobacillus casei* 3 × 10^3 CFU, *L. acidophilus* 3 × 10^7 CFU, LGG 7 × 10^9 CFU, *Lactobacillus bulgaricus* 5 × 10^8 CFU, *Bifidobacterium breve* 2 × 10^{10} CFU, *Bifidobacterium longum* 1 × 10^9 CFU, *Streptococcus thermophilus* 3 × 10^8 CFU/g, 6 weeks	70 petrochemical workers	GHQ DASS scores	Improvements within probiotic yoghurt group in GHQ (18.0 ± 1.5 versus 13.5 ± 1.9, $P = 0.007$) and DASS (23.3 ± 3.7 versus 13.0 ± 3.7, $P = 0.02$) Improvements within the probiotic capsule group in GHQ (16.9 ± 1.8 versus 9.8 ± 1.9, $P = 0.001$) and DASS (18.9 ± 3.2 versus 9.4 ± 4.0, $P = 0.006$) No improvements in conventional yoghurt group for GHQ ($P = 0.05$) or DASS ($P = 0.08$)	Short supplementation period Did not assess short-chain fatty acid production
Moludi *et al.*[136] Iran RCT	To determine the effects of probiotics on symptoms of depression, measures of QoL, oxidative stress and inflammation in individuals who had recently had a MI	Secondary analysis LGG 1.6 × 10^9 CFU/day versus placebo, 12 weeks	44 adults with recent MI and PCI	Depression, QoL, inflammation and oxidative stress	Probiotics had beneficial effects on depression and markers of oxidative stress and inflammation in individuals post-MI with a PCI Compared with placebo, Beck Depression Inventory score decreased (−5.57 versus −0.51, $P = 0.045$) and QoL increased (23.6 versus 0.44, $P = 0.023$) Total antioxidant capacity increased in the probiotic group (93.7 versus 27.54 mmol/l, $P = 0.009$) and malondialdehyde (−40.7 versus −4.2, $P = 0.033$) and hs-CRP (−1.74 versus 0.67 mg/l, $P = 0.04$) decreased, with levels stronger than placebo	Small sample size Short supplementation duration Sample was predominantly male

CFU, colony-forming units; DASS, Depression, Anxiety, and Stress Scale scores; GHQ, General Health Questionnaire; hs-CRP, high-sensitivity C-reactive protein; LGG, *Lactobacillus rhamnosus* GG; MI, myocardial infarction; PCI, percutaneous intervention; QoL, quality of life; RCT, randomised-controlled trial.

Attention-deficit hyperactivity disorder and Asperger's syndrome

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Partty *et al.*[149] Finland RCT	To determine the involvement of the gut–brain axis in the incidence of ADHD and AS in a cohort followed until 13 years old	LGG 1 × 10^9 CFU/day versus placebo to pregnant women 4 weeks before expected delivery, and then 6 months post-delivery to the infant Follow-up for 13 years	75 mothers and children	Clinical diagnosis of ADHD and AS	LGG supplementation in early life may reduce the risk of developing ADHD or AS By age 13 years, 6 children developed ADHD or AS or both, all of which were in the placebo group ($P = 0.008$) At 6 months old, numbers of *Bifidobacterium* were less in children with neuropsychiatric disorder than those without ($P = 0.03$) At 18 months old, *Bacteroides* and *Lactobacillus-Enterococcus* group were less in children with neuropsychiatric disorder ($P = 0.008$ and $P = 0.01$, respectively)	Not stated

ADHD, attention-deficit hyperactivity disorder; AS, Asperger's syndrome; CFU, colony-forming units; LGG, *Lactobacillus rhamnosus* GG; RCT, randomised-controlled trial.

Urinary tract infections

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Colodner *et al.*[154] Israel Pilot study	To determine the vaginal colonisation in post-menopausal women by LGG	100 ml yoghurt daily containing 1 × 10^9 CFU LGG or 200 ml yoghurt daily containing 1 × 10^9 CFU LGG, 1 month	42 post-menopausal women	Colonisation count in vaginal and rectal swabs	LGG has a low vaginal adhesion rate and is not a good probiotic for UTIs The vaginas of only 4 women (9.5%) were colonised with LGG, but 33 women (78.6%) had positive rectal swabs indicating GI colonisation	Not stated
Kontiokari *et al.*[155] Finland RCT	To determine whether recurrent UTIs can be prevented with cranberry–lingonberry juice or with LGG	50 ml cranberry–lingonberry juice daily for 6 months versus 100 ml LGG drink (4 × 10^{10} CFU) 5 times per week for 1 year versus no intervention	150 women with UTIs caused by *Escherichia coli*	First recurrence of UTI	Regular consumption of cranberry juice but not LGG prevents the recurrence of UTIs Rate of first UTI recurrence differed between the groups ($P = 0.048$) Recurrent UTIs in 16% of women in cranberry group, 39% of women in LGG group and 36% of women in control Difference between cranberry juice and control 20% reduction in absolute risk (95% CI: 3–5%, $P = 0.023$, NNT = 5.95)	Not stated
Ng *et al.*[152] Singapore Systematic review and meta-analysis	To determine whether *Lactobacillus* spp. can prevent recurrent UTIs in females		9 clinical trials with 726 patients	Prophylactic efficacy and incidence of adverse events	The use of *Lactobacillus* spp. reduced the risk of recurrent UTIs (RR = 0.684, 95% CI: 0.438–0.929, $P \leq 0.001$) However, different strains showed varying efficacy	Inter-study variability and short treatment durations
Sadeghi-Bojd *et al.*[156] Iran RCT	To determine the efficacy of multispecies probiotic for the prevention of recurrent UTIs in children	LGG 1 × 10^9 CFU + *Lactobacillus acidophilus* 15 × 10^9 CFU + *Bifidobacterium bifidum* 4 × 10^9 CFU + *Bifidobacterium lactis* 15 × 10^9 CFU	181 children with normal urinary tracts given LGG + *L. acidophilus, B. bifidum, B. lactis* versus placebo, 18 months	Composite cure at 18 months	Multispecies probiotic more effective at reducing the risk of recurrent UTIs Composite cure in probiotic 96.7% versus 83.3% placebo ($P = 0.02$) Time to first recurrent event was 3.5 months in probiotic group and 6.5 months in placebo group ($P = 0.04$)	Patients from a limited selection pool Did not include uncircumcised boys Did not test to see if supplementation reduced GI colonisation by pathogenic bacteria
Toh *et al.*[159] Australia RCT		Four arms: (i) *Lactobacillus reuteri* RC-14–*Lactobacillus rhamnosus* GR-1 (5.4 × 10^9 CFU) + LGG–*Bifidobacterium animalis* BB-12 (7 × 10^9 CFU); (ii) RC-14–GR1 (conc. as above) + placebo; (iii) LGG–BB-12 (conc. as above) + placebo; (iv) placebo + placebo, 6 months	207 individuals with spinal cord injury	Occurrence of first symptomatic UTI	No effect of either probiotic combination for preventing UTIs in people with spinal cord injury RC-14–GR-1 had a similar risk of UTI to placebo (HR 0.67, 95% CI: 0.39–1.18), and those on LGG–BB-12 also had a similar risk to those on placebo (HR 1.29, 95% CI: 0.74–2.25, $P = 0.37$)	Did not recruit the target number of 372 participants No trial follow-up
Tractenberg *et al.*[160] USA Prospective 3-stage study	To determine the efficacy of intravesical LGG on urinary symptoms in individuals with spinal cord injury	Self-administration of a catheter with LGG + saline (2 × 10^{10} CFU live organisms)	96 adults and 7 children with spinal cord injury	Change in USQNB-IC	Intravesical administration of LGG improved symptoms of UTIs compared with individuals who did not administer the probiotic ($P \leq 0.05$)	No randomisation

CFU, colony-forming units; CI, confidence interval; GI, gastrointestinal; HR, hazard ratio; LGG, *Lactobacillus rhamnosus* GG; NNT, number needed to treat; RCT, randomised-controlled trial; RR, relative risk; USQNB-IC, Urinary Symptom Questionnaire for Neurogenic Bladder-IC; UTI, urinary tract infection.

Infant health

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Chrzanowska-Liszewska *et al*.[167] Poland RCT	To determine the colonisation of LGG and its impact on growth and length of hospital stay in pre-term infants	LGG 6 × 10^9 CFU/day versus placebo, 42 days	60 pre-term infants (before 32 weeks)	Difference in the amount of Bifidogenic microflora and *Escherichia coli*	Although LGG rapidly colonised the gut of preterm formula-fed infants, this did not decrease the number of pathogenic bacteria or affect growth or hospital stay duration LGG higher in supplemented group than placebo at days 7 (P = 0.041) and 21 (P = 0.024) *Staphylococci* higher in supplemented group at days 7 (P = 0.001) and 42 (P = 0.011) No difference to weight gain (95% CI: −1.68, 305, P = 0.567) or mean hospital duration (95% CI: −13.43, 5.71, P = 0.421)	Lack of follow-up No precise CFU count for organisms analysed
Lundelin *et al*.[189] Finland Follow-up study of 4 RCTs	To determine the clinical benefit and safety of probiotics during the perinatal period Follow-up of 4 previous RCTs	Included trials were 3–6 months duration, and dosages ranged from 1 × 10^9–1 × 10^{10} CFU, 2-year follow-up	303 children pre-term or increased allergy risk	Not stated	Children given LGG had a decreased prevalence of allergic disease compared with placebo (OR 0.62, 95% CI: 0.38–0.99, P = 0.047) No difference in prevalence of asthma (OR 0.55, 95% CI: 0.24–1.25, P = 0.15), non-communicable diseases or growth	Follow-up completed unblinded
Luoto *et al*.[171] Finland Follow-up of RCT	To determine the safety and efficacy of multispecies probiotic containing LGG on pregnancy outcome, and foetal and infant growth	Diet + LGG (1 × 10^{10} CFU/day) + *Bifidobacterium lactis* (1 × 10^{10} CFU/day) versus diet + placebo from first trimester to cessation of breastfeeding	256 pregnant women 191 completed the 24-month follow-up	Pregnancy outcome and infant growth	The use of probiotics in pregnancy could be safe and cost-effective to prevent future metabolic disease Probiotics + diet reduced the frequency of gestational diabetes ($P \leq 0.003$)	Not stated
Vendt *et al*.[169] Estonia RCT	To determine the effect of LGG-enriched formula on growth and faecal microflora in the first 6 months of healthy infants	LGG dosage not stated, 6 months	120 healthy infants	Not stated	Infants fed with LGG-supplemented formula grew better than those with regular formula Length and weight higher in supplemented versus control (0.44 ± 0.37 versus 0.07 ± 0.06, $P \leq 0.01$ and 0.44 ± 0.19 versus 0.07 ± 0.06, $P \leq 0.005$) More frequent colonisation amongst supplemented formula group (91% versus 76%, $P \leq 0.05$) More frequent defecation in LGG group (9.1 ± 2.6 versus 8.0 ± 2.8, $P \leq 0.05$)	Not stated
Mantaring *et al*.[168] Philippines RCT	To determine the effect of probiotics during pregnancy and early lactation on infant diarrhoea	LGG 7 × 10^8 CFU + *B. lactis* 7 × 10^8 CFU per day versus control	208 healthy pregnant women in third trimester	Incidence of infant diarrhoea until age 12 months	Maternal supplementation showed beneficial effects on infant weight and length gain; however, did not affect incidence of infant diarrhoea Weight and height increased compared with placebo (8.97 kg versus 8.61 kg, P = 0.001 and 74.2 cm versus 73.4 cm, P = 0.031)	Limited generalisation Diet and exercise not considered

CFU, colony-forming units; CI, confidence interval; LGG, *Lactobacillus rhamnosus* GG; OR, odds ratio; RCT, randomised-controlled trial.

Infantile colic

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Cabana *et al*.[174] USA Secondary analysis of RCT	To determine whether LGG supplementation prevents infant colic	LGG 1 × 10^9 CFU/day versus control, 6 months	184 infants	Likelihood of diagnosis of colic before 4 months old	Early LGG supplementation does not prevent infant colic No difference between two groups in infants with colic based on symptoms (control 5.4% versus LGG 9.8%, P = 0.19) or physician diagnosis (control 3.2% versus LGG 7.6%, P = 0.26) or combination of both (6.5% versus LGG 13.0%, P = 0.13)	Parent report of symptoms and crying length High rate of breastfeeding in sample may mask effects of probiotic Samples were not racially or socially diverse
Kianifar *et al*.[176] Australia RCT	To determine efficacy of multispecies probiotic and prebiotic to reduce crying time	1 × 10^9 CFU *Lactobacillus casei* + LGG + *Streptococcus thermophilus* + *Bifidobacterium breve* + *Lactobacillus acidophilus* + *Bifidobacterium infantis* + *Lactobacillus bulgaricus* + fructo-oligosaccharide versus placebo, 30 days	50 breastfed infants	Treatment success	Synbiotic significantly improved colic symptoms compared with placebo At day 7 and day 30, treatment success was higher in synbiotic compared with placebo (day 7, 82.6% versus 35.7%, $P \leq 0.005$; day 30, 87% versus 46%, $P \leq 0.01$) Symptom resolution higher in synbiotic group at day 7 (39% versus 7%, $P \leq 0.03$) but not day 30 (56% versus 36%, P = 0.24)	Stool samples not evaluated at baseline or after intervention Small sample size Non-validated outcome measure, no measure of compliance
Partty *et al*.[172] Finland RCT	To determine the efficacy of LGG to reduce daily crying of infants with colic	LGG 4.5 × 10^9 CFU/day versus placebo, 4 weeks	17 healthy breastfed infants under 6 weeks old	Difference in daily average crying time between LGG and placebo	LGG in combination with behavioural support and cow's milk elimination was not efficacious for the reduction of crying time in infants with colic Daily crying time comparable between the groups (173 minutes probiotic versus 174 minutes placebo, P = 0.99) However, occurrence of crying decreased in the probiotic group compared with placebo (68% versus 49%; 95% CI: 32–66, P = 0.05)	Not stated
Savino *et al*.[173] Italy RCT	To determine the efficacy of LGG together with maternal avoidance of cow's milk in treating infantile colic	LGG 5 × 10^9 CFU/day versus placebo, 28 days	45 colicky breastfed infants	Faecal CLP, crying and fussing	LGG in combination with elimination of cow's milk from maternal diet reduced crying time (104 minutes versus 242 minutes, $P \leq 0.001$) and faecal CLP (P = 0.026), and increased total gut bacteria (P = 0.04) and Lactobacillus (P = 0.048)	Possible false-positive with the use of PCR test Small sample size

CFU, colony-forming units; CI, confidence interval; CLP, calprotectin; LGG, *Lactobacillus rhamnosus* GG; PCR, polymerase chain reaction; RCT, randomised-controlled trial.

Human immunodeficiency virus

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Salminen *et al*.[179] RCT Finland	To determine the efficacy and safety of LGG for GI symptoms in patients with HIV on anti-retroviral therapy	LGG 1–5 × 10^{10} CFU twice daily versus placebo, 2 weeks	17 HIV-infected patients with diarrhoea for more than 1 month	GI symptoms Safety parameters Faecal microbiology	LGG supplementation was well tolerated, but showed no benefits to diarrhoea or GI symptoms in HIV-infected patients No differences between faecal counts of LGG between supplemented and placebo No adverse events reported	Not stated

CFU, colony-forming units; GI, gastrointestinal; HIV, human immunodeficiency virus; LGG, *Lactobacillus rhamnosus* GG; RCT, randomised-controlled trial.

Allergy

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Tan *et al.*[190] China Systematic review and meta-analysis	To determine the effects of LGG in children with CMA	LGG dosages ranged from 1.4×10^7 CFU to 5×10^9 CFU/day, with treatment durations from 4 weeks to 3 years	10 studies 853 children		LGG may have moderate-quality evidence to promote tolerance and aid recovery from GI symptoms in children with CMA Higher tolerability rates favouring LGG over controls were observed (RR = 2.22, 95% CI: 1.86–2.66; I2 = 0.00; moderate-quality evidence) No significant differences in SCORAD values (MD 1.41, 95% CI: −4.99, 7.82, *P* = 0.67; very low-quality evidence), and LGG may have improved faecal occult blood (RR = 0.36, 95% CI: 0.14–0.92, *P* = 0.03; low-quality evidence) No adverse events reported	Limited number of studies
Korpela *et al.*[186] Finland RCT	To determine whether multispecies probiotic could ameliorate antibiotic use or Caesarean birth on infant gut microbiota	LGG (5×10^9 CFU) + *Bifidobacterium breve* Bb99 (2×10^8 CFU) + *Propionibacterium freudenreichii* spp. *shermanii* JS (2×10^9 CFU) + *Lactobacillus rhamnosus* Lc705 (5×10^9 CFU) versus placebo	199 breastfed or formula-fed infants		LGG supplementation may ameliorate changes in the gut microbiota due to antibiotic use or Caesarean birth	Not stated
Piirainen *et al.*[193] Finland RCT	To determine the effects of LGG on oral immune response of adults with birch pollen allergy	LGG (2×10^{10} CFU/day) versus placebo	38 birch pollen allergy sufferers	Not stated	rBet v1 (0.319 versus −0.136, *P* = 0.02) and Mal d1 (0.097 versus −0.117, *P* = 0.02) specific IgA levels increased compared with placebo	Not stated
Moreira *et al.*[194] Finland RCT	To determine the effect of LGG supplementation on allergic inflammatory markers in marathon runners with asthma and allergy	LGG (3×10^8 CFU/day) versus placebo	141 marathon runners with allergies	ECP, total IgE levels and Phadiatop test	Compared with placebo, LGG supplementation did not prevent an increase in allergic markers during birch pollen season	Not stated

CFU, colony-forming units; CI, confidence interval; CMA, cow's milk allergy; ECP, eosinophil cationic protein; GI, gastrointestinal; LGG, *Lactobacillus rhamnosus* GG; MD, mean difference; RCT, randomised-controlled trial; RR, relative risk; SCORAD, Scoring of Atopic Dermatitis.

Dermatitis and eczema

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Szajewska & Horvath[198] Poland Systematic review and meta-analysis	To determine the efficacy of LGG prenatally/postnatally for the primary prevention of eczema	LGG dosages ranged from 1×10^9 CFU to 1.8×10^{10} CFU	5 RCTs with 889 subjects	Eczema	LGG was ineffective in reducing eczema, and guidelines should be revised to reflect this (1 RCT: RR = 0.88, 95% CI: 0.63, 1.22, *P* = 0.69, I^2 = 0%) No reduction of risk for eczema when LGG administered during pregnancy (3 RCTs, RR = 0.93, 95% CI: 0.49, 1.76, I^2 = 72%) No reduction of risk when LGG administered to infants (1 RCT: RR = 0.93, 95% CI: 0.59, 1.45)	Different trials used different definitions of eczema

Schmidt *et al.*[203] Denmark RCT	To determine the effect of multispecies probiotic in late infancy and early childhood on the development of allergic diseases	LGG + *Bifidobacterium animalis* spp. lactis versus placebo, 6 months	290 participants starting prior to attending day care	Incidence of allergic disease	Probiotics administered in late infancy may prevent the development of eczema Incidence of eczema was 4.2% in probiotic group and 5% in eczema group (*P* = 0.036)	Study set from a previous trial of high-income families
Tan-Lim *et al.*[202] Philippines Systematic review and meta-analysis	To determine the effectiveness of multispecies probiotics in prevention of ADe in children	LGG + *B. animalis*	21 RCTs, 5406 children with ADe		Specific probiotics reduce the risk of dermatitis in children when administered *in utero,* during infancy or both Reduced risk of ADe (RR = 0.50, 95% CI: 0.27–0.94) compared with placebo LGG had less adverse events compared with placebo (RR = 0.70, 95% CI: 0.32–1.52) In infants, reduced risk of ADe (RR = −0.46, 95% CI: 0.22–0.97) All based on low-quality evidence	When ranking evidence, quality not considered
Wu *et al.*[200] Taiwan RCT	To determine the efficacy and safety of LGG in children aged 4–48 months with ADe	LGG (ComProbi brand containing 350 mg) versus control, 8 weeks	67 children aged 4–48 months with ADe[3] 15 on SCORAD	Mean change from baseline in SCORAD at 8 weeks	LGG was effective to decrease symptoms of ADe compared with placebo (*P* ≤ 0.05)	Lack of laboratory assessment Patients could use topical steroids Unethical to withhold corticosteroid treatment Lack of follow-up

Ade, atopic dermatitis; CFU, colony-forming units; CI, confidence interval; LGG, *Lactobacillus rhamnosus* GG; RCT, randomised-controlled trial; RR, relative risk; SCORAD, Scoring of Atopic Dermatitis.

Wounds

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Mayes *et al.*[208] USA RCT	To determine the efficacy and safety of LGG supplementation in acutely burned, paediatric patients	LGG 1.5×10^{10} CFU/day versus placebo within 10 days of burn until wound closure	20 acutely burned paediatric patients	Not stated	No difference between infection days, length of hospitalisation or antibiotic use Time required to complete wound healing shortened with LGG but not significant	

CFU, colony-forming units; LGG, *Lactobacillus rhamnosus* GG; RCT, randomised-controlled trial.

Dental caries

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Nase *et al.*[211] Finland RCT	To determine whether milk containing LGG had an effect on caries and caries risk in children	LGG ($5–10 \times 10^5$ CFU/ml) versus control 5 days per week for 7 months	594 children	Not stated	LGG reduced the risk of caries (OR 0.56, *P* = 0.01), an effect that was pronounced in 3–4-year-olds	Not stated

CFU, colony-forming units; LGG, *Lactobacillus rhamnosus* GG; OR, odds ratio; RCT, randomised-controlled trial.

Vaccine adjuvant

Author	Objective	Intervention period, treatment dose	Number of subjects	Main outcome measure	Findings	Limitations
Bianchini *et al.*[215] Italy RCT	To determine whether LGG can modify immune response in children and adolescents with T1D leading to an increased immune response to the influenza vaccine	LGG 1×10^9 CFU/drop, 5 drops twice per day versus placebo Three months prior and post vaccination	64 paediatric patients with T1D	Seroconversion rate	Combination of vaccine and LGG reduced the inflammatory response without dampening seroprotective antibodies IL-17 significantly lower in LGG ($P = 0.01$)	Small study size
De Vrese *et al.*[216] Germany RCT	To determine whether and how probiotics affect the immune response following polio vaccine	LGG 1×10^{10} CFU or *Lactobacillus acidophilus* CRL431 1×10^{10} CFU/serving in milk versus placebo, 5 weeks	66 healthy males	Not stated	Probiotics induce an immune response that may provide enhanced protection from viruses LGG or CRL431 nearly doubled the increase in polio-specific IgG ($P < 0.01$) IgA titre increases after vaccination ($P \leq 0.036$)	Not stated
Lazarus *et al.*[217] India RCT	To determine the effect of probiotics and/or zinc supplementation on the immune response to rotavirus vaccination	4 arms: LGG (1×10^{10} CFU) + zinc sulphate; 5 mg probiotic + placebo; zinc + placebo; placebo + placebo Duration not stated	620 infants given rotavirus at 6 and 10 weeks old	Seroconversion to rotavirus at 14 weeks old	Zinc supplementation did not improve immunogenicity of rotavirus vaccine, and probiotic supplementation only marginally increased seroconversion No changes to seroconversion in zinc arm and only modest improvement among infants receiving probiotic ($P = 0.066$)	Absence of immune correlate of protection for rotavirus vaccine
Davidson *et al.*[212] USA RCT	To determine the effects of LGG as an immune adjuvant to increase rates of seroconversion after influenza vaccine	LGG 1×10^{10} CFU + inulin twice daily versus placebo twice daily, 28 days	42 healthy adults	Protective HAI assay	LGG may be an important adjuvant to improve immunogenicity following influenza vaccine No LGG well tolerated No differences in seroprotection of H1N1 and B influenza strains Increased protective titre with LGG following H3N2 strain vaccine (OR 1.84, 95% CI: 1.04–3.22, $P = 0.048$)	Small sample size Subjects previously vaccinated were included

CFU, colony-forming units; CI, confidence interval; HAI, haemagglutinin inhibition; IL, interleukin; LGG, *Lactobacillus rhamnosus* GG; OR, odds ratio; RCT, randomised-controlled trial; T1D, type 1 diabetes.

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Nutritional Medicine Journal

Migraine Headaches: Opportunities for Management with Precision Nutrition

Benjamin I. Brown

Abstract

Chronic migraine headaches are estimated to affect between 1.4% and 2.2% of the population, and have a huge impact on wellbeing and quality of life. A diverse range of therapeutic nutritional options have been explored for migraine headaches, with varying degrees of benefit. Dietary interventions include generally healthy food plans, identification and avoidance of trigger foods, weight-loss diets, low-glycaemic-load diets, ketogenic diets, gluten-free diets, IgG-led elimination diets and a high-omega-3/low-omega-6 diet. Nutritional supplement interventions include riboflavin, niacin, homocysteine-lowering B vitamins, vitamin B12, vitamin E, vitamin D, magnesium, zinc, iron, omega-3 fatty acids, coenzyme Q10 (CoQ10), lipoic acid, soy phytoestrogens, ginger, turmeric, carnitine, 5-hydroxytryptophan (5-HTP), palmitoylethanolamide (PEA), and multi-ingredient formulas. The wide range of therapeutic options may make it challenging to approach nutritional management of migraine in a clinical setting, so a pragmatic model that helps personalise interventions from clinical signs and symptoms and reliable biomarkers would be useful, so-called 'precision nutrition'. The aim of this narrative review is to explore the clinical evidence for nutritional medicine for migraines, including diet and nutrient-based interventions, from the perspective of personalised or precision nutrition.

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Introduction

Migraine headaches are recurrent headaches characterised by painful attacks lasting 4–72 hours. Chronic migraines are estimated to affect between 1.4% and 2.2% of the population, and have a huge impact on wellbeing and quality of life.[1,2] The frequencies of migraine attacks are typically high, with 31.3% experiencing three or more attacks per month, and attacks are often severe, with 53.7% reporting severe impairment or being bedbound during the attacks.[3]

The diagnosis of migraine is based on clinical criteria, in particular recurrent headache attacks of unilateral location, pulsating quality, moderate or severe intensity, aggravation by routine physical activity, and association with nausea, vomiting, photophobia and phonophobia.[4] Although synonymous with headache attacks, migraine is more appropriately considered a symptomatically diverse neurological disorder, of which headache is just one symptom.[5] During the premonitory stage up to 3 days before headache, tiredness, mood changes, yawning, thirst, cravings, urinary frequency, light and sound sensitivity, and cranial autonomic symptoms may occur, while symptoms in the postdrome phase up to 48 hours post-headache include tiredness and difficulty concentrating.[6]

The development of chronic migraine is understood to be a gradual process, with predisposing risk factors interacting with genetic susceptibility and frequent headache pain to lower the threshold of migraine attacks and consequently increase the risk of chronic migraine.[7,8] The pathophysiology of migraine varies between individuals, but evidence does suggest features that distinguish migraineurs from non-migraineurs in the interictal phase (between headaches), including neuronal hyperexcitability,[9] systemic oxidative and nitrosative stress,[10] systemic low-grade inflammation,[11] and altered blood glucose metabolism.[12] Neurobiological changes are evident in the premonitory stage and include alterations in nociceptive signalling, while the headache phase involves activation of the trigeminovascular system and, in migraine with aura, a cortical spreading depression-like event characterised by an electrophysiological wave of excitation followed by inhibition in cortical neurons.[13] Better understanding of the complex pathophysiology and underlying pathophysiological mechanisms of migraine may improve treatment options.[14]

Over-the-counter and prescription medications for both acute relief and prevention are common treatments for migraine, but treatment response is often poor and there are important potential side-effects, thus pharmacological treatment options are limited and only result in reduction of symptoms in a portion of migraine sufferers.[15,16]

Management of migraine would benefit from an approach that extends beyond pharmacotherapy alone, and identifies exacerbating factors and relevant comorbid conditions, examines past and current medications and other therapies, and a management plan with preventive, acute and lifestyle components as well as self-monitoring with a headache diary.[17] Personalised lifestyle medicine, including sleep hygiene, stress management, aerobic exercise and dietary modification as well as nutraceuticals, should be considered in a migraine management plan.[18,19,20] The aim of this narrative review is to explore the clinical evidence for nutritional medicine for migraine, including diet and nutrient-based interventions, from the perspective of personalised or precision nutrition.

Methods

The author searched PubMed for publications describing human clinical interventions involving diet, nutrient-based supplements, plant extracts with common food use, and migraine. There was no limit on date of publication. Articles assessing the effects of diet, nutritional and nutraceutical interventions in patients with migraine were included in the final review. Phytomedicines including feverfew *(Tanacetum parthenium)* and butterbur *(Petasites hybridus)* were excluded from the review, except where they were used as components of nutrient-based interventions, and are reviewed in detail elsewhere.[21] Citations in these articles were also reviewed and located when related to additionally relevant clinical trials. Some articles not indexed on PubMed were identified and included if they were from a peer-reviewed journal. The review is based on this search and the author's collection of studies on the topic used in previous academic and continuing professional education lectures. Only studies published in English were included in the review.

Towards precision nutrition for migraine

A diverse range of therapeutic nutritional options have been explored for migraine headache, with varying degrees of benefit. Dietary interventions include generally healthy food plans, identification and avoidance of trigger foods, weight-loss diets, low-glycaemic-load diets, ketogenic diets, gluten-free diets, IgG-led elimination diets, and a high-omega-3/low-omega-6 diet. Nutritional supplement interventions include homocysteine-lowering B vitamins, niacin, vitamin B12, vitamin E, vitamin D, magnesium, zinc, iron, omega-3 fatty acids, riboflavin, coenzyme Q10 (CoQ10), lipoic acid, soy phytoestrogens, ginger, turmeric, carnitine, 5-hydroxytryptophan (5-HTP), palmitoylethanolamide (PEA), and multi-ingredient formulas. The wide range of therapeutic options may make it challenging to approach nutritional management of migraine in a clinical setting, so a pragmatic model that helps personalise interventions from clinical signs and symptoms and reliable biomarkers would be useful, so-called 'precision nutrition'.[22] Such a model may also be informative for future clinical research that attempts to explore precision nutrition approaches for migraine. The structure of this review is based on a preliminary and theoretical model that incorporates and organises nutritional interventions into specific clinical and biochemical indications; however, it should be emphasised that this model is hypothetical and requires further evaluation before being translated to practice (Figures 1 and 2).

Healthy food plans

It has been speculated that healthful dietary changes could positively influence several aspects of migraine pathophysiology, including serotoninergic dysfunction, neuronal excitability, redox stress, brain mitochondrial function, neuroinflammation and platelet aggregation.[23] Observational studies have suggested a relationship between food pattern and migraine. Higher diet quality, calculated with the Healthy Eating Index, was found to be significantly inversely associated with chronic migraine and headache attack frequency in women.[24,25] Similarly, a higher Dietary Inflammatory Index score, and thus higher dietary inflammatory potential, has been found to be associated with migraine and headache frequency.[26,27] Also a higher Dietary Diversity Score, a proxy indicator of nutritional adequacy, was inversely associated with migraine disability, pain severity and headache frequency in another study.[28] In addition to food relationships, better hydration has also been associated with lower migraine severity.[29]

Figure 1: Dietary interventions for migraine.

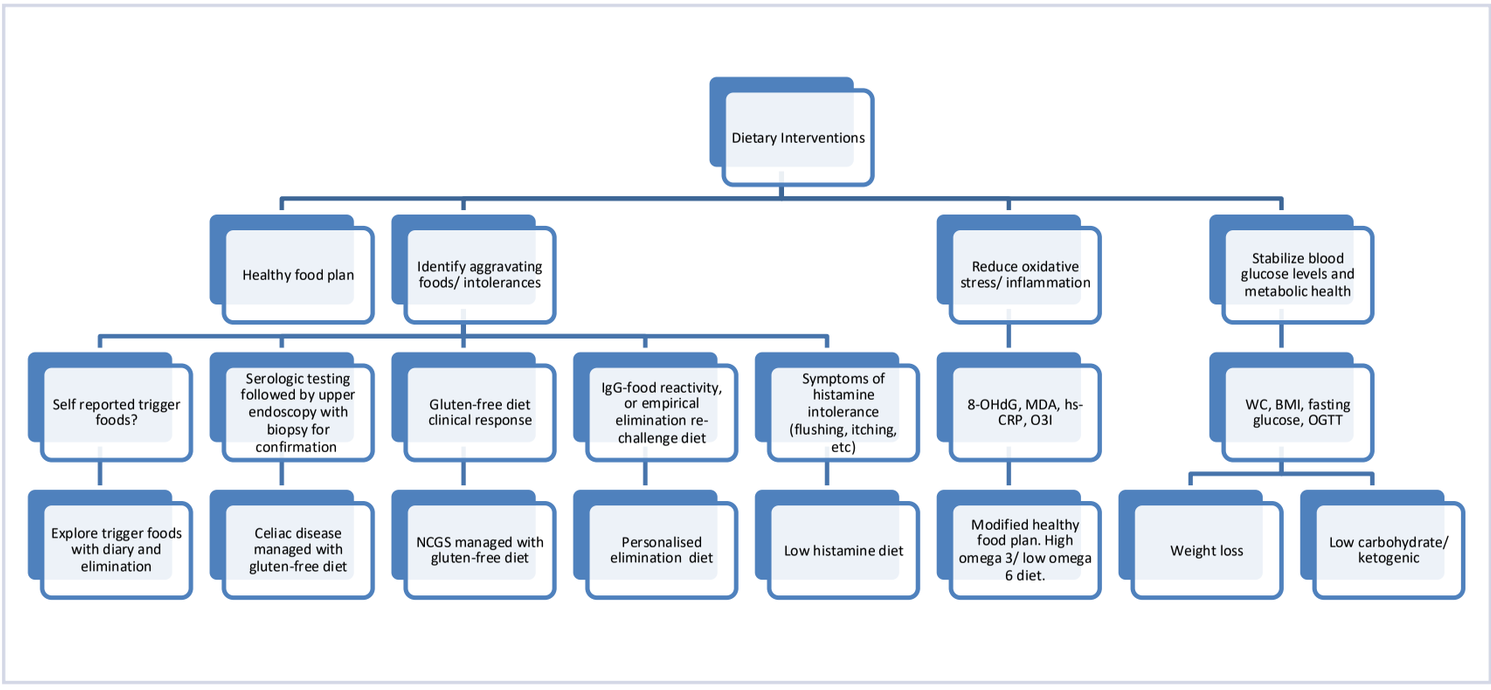

Figure 1: Dietary interventions for migraine. Dietary interventions can be broadly divided into three major categories that may also be indications for their clinical use: adopting a generally healthy diet; identifying aggravating foods or intolerances; and modifying underlying pathophysiology or dysfunction. Clinical indications, such as dietary history, and biomarkers or results of investigations, such as the positive confirmation of coeliac disease, may help identify which dietary interventions are best suited to an individual. 8-OHdG, 8-hydroxy-2′-deoxyguanosine; BMI, body mass index; hs-CRP, high-sensitivity C-reactive protein; MDA, malondialdehyde; NCGS, non-coeliac gluten sensitivity; O3I, Omega-3 Index (red blood cell omega-3 fatty acids); OGTT, oral glucose tolerance test; WC, waist circumference.

Figure 2: Individualised nutrition for migraine.

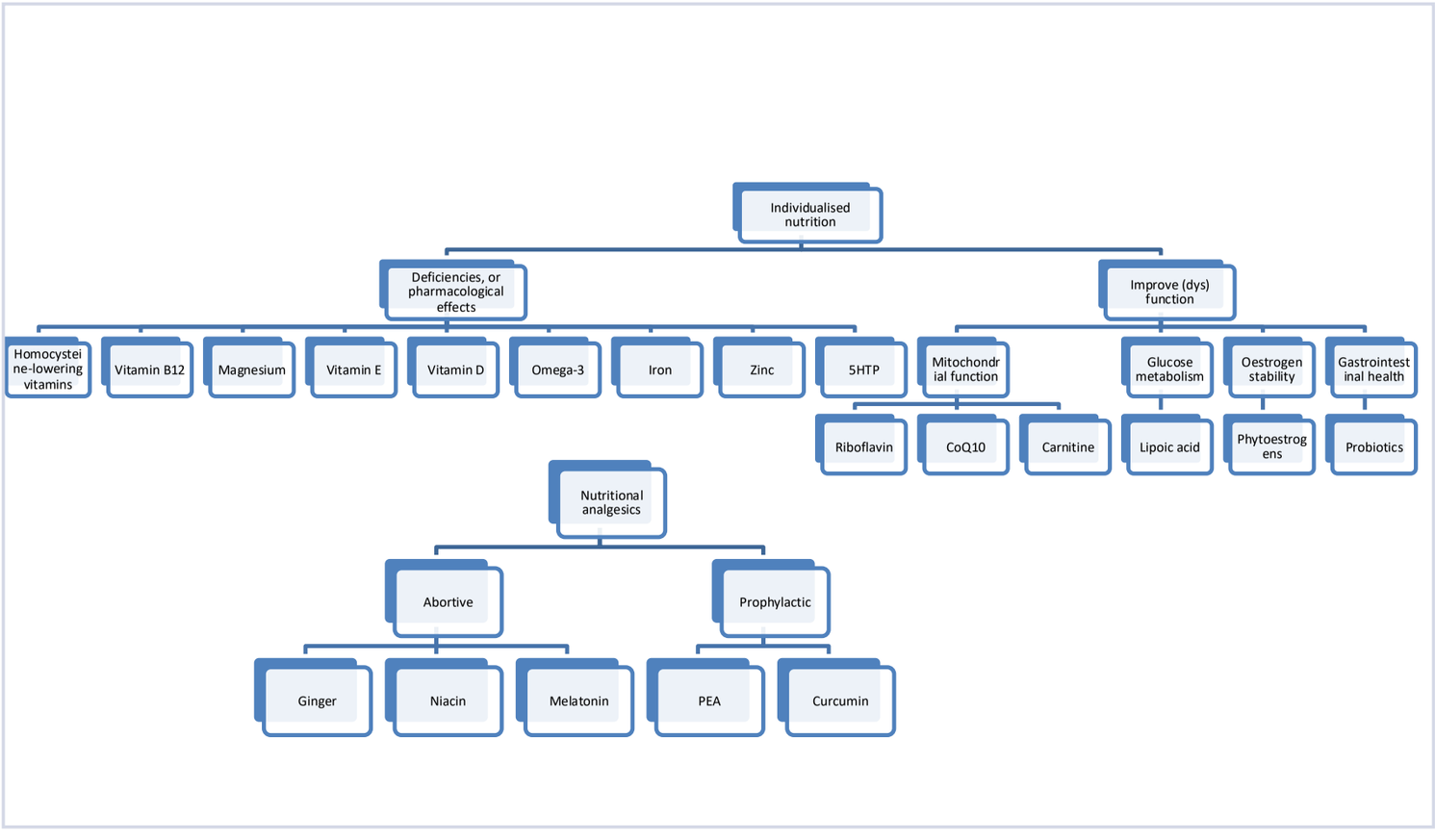

Figure 2: Individualised nutrition for migraine. A broad range of nutritional interventions is possible, so a pragmatic model that rationalises their indications and use may be helpful in clinical practice. Suboptimal intakes or deficiencies of vitamins, minerals and fatty acids can be assessed through dietary recall and/or laboratory testing, although both have limitations and may not accurately identify treatment responders, and nutrients may have pharmacological effects independent of deficiency or low dietary intake. Other nutrients have more specific indications like improving mitochondrial function, blood glucose metabolism or oestrogen stability; however, it should be emphasized that nutritional compounds have diverse mechanisms of action so this may be an oversimplification. 5-HTP, 5-hydroxytryptophan; CoQ10, coenzyme Q10; PEA, palmitoylethanolamide.

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Some dietary intervention studies have assessed the impact of improving overall diet quality in migraineurs, and those that have been performed do suggest healthier eating could result in important clinical benefits for some people. A 90-day intervention (*n* = 52) based on healthy dietary guidelines for the Brazilian population, and personalised for body weight, improved dietary habits, dietary quality, and significantly reduced migraine severity and depressive symptoms, independent of changes in body weight.[30]

A retrospective study of the impact of a 3-month dietary intervention (*n* = 30; based on the Healthy Eating Plate created by Harvard School of Public Health) on migraine found a significant improvement in headache frequency, disability scales and use of abortive drugs per month (6.07 ± 7.83 versus 9 ± 8.2 at baseline).[31] Patients statistically ate less sweets, white bread, and red or processed meat, and more whole cereals and legumes, fish, snacks (mainly with yogurt), and also water intake increased to 1.5 L a day. A second larger 12-week study (*n* = 97; also based on the Healthy Eating Plate) found that those who changed their eating behaviours had a statistically significant reduction in monthly migraine days (from 10 to less than 6) and a non-significant decrease in monthly painkiller intake (from approximately 12 to 8), with reductions in total carbohydrate, and red and processed meat consumption appearing to have the most pronounced benefit.[32]

Dietary changes characteristic of overall healthy eating patterns have individually demonstrated some benefit, including moderating fat intake and increasing plant foods. Moderating fat intake (< 20 g daily) for 12 weeks was found to reduce migraine frequency, intensity and duration, and medication intake.[33] Reducing fat intake to < 20% of total calories, but not less than 45 g daily and mostly from olive oil, for 3 months significantly reduced the frequency and severity of headache attacks.[34] A whole-food plant-based (vegan) diet was reported to result in complete remission of migraine after 3 months in one patient.[35]

Overall, there is preliminary but compelling evidence to suggest that nutrition education and counselling with subsequent improvements in dietary quality can have an important impact on migraine frequency and severity, and can reduce medication requirement.

Migraine trigger foods

A variety of foods and food ingredients can immediately (within hours) or chronically (within days or weeks) trigger migraine attacks in susceptible individuals, with anywhere from 12% to 60% of migraine sufferers reporting food as a trigger.[36] Several have been identified, including alcohol (especially red wine and beer), chocolate, coffee, caffeine, dairy products (especially aged cheese and processed products such as ice-cream), processed meats, citrus fruits, tomatoes, onions, nitrates and nitrites, biogenic amines (such as histamine, tyramine and phenylethylamine), monosodium glutamate (MSG), aspartame, sucralose, and high or low sodium intake.[37,38,39] Of these, caffeine withdrawal and MSG exposure have the strongest evidence as triggers because they are supported by positive provocation studies.[40] The mechanisms by which foods and their components may contribute to migraine are +diverse, and appear to depend on individual susceptibility, dosage and timing of exposure.[40]

Studies examining the effects of avoiding trigger foods have produced mixed results. For example, in people with self-reported chocolate-triggered migraines, chocolate triggered migraine more frequently than placebo in one study,[41] but not in others.[42,43] Chocolate does contain caffeine, so caffeine withdrawal may have been a confounder. Similarly, when compared with placebo, aspartame administration was found to increase headache frequency in some,[44,45] but not all studies.[46,47] Clinically, however, identification and avoidance of trigger foods is an important component of nutritional therapy for migraine, and can be based on observation if the food trigger is clear or a food diary if less obvious. Alternatively, suspected triggers could be included in an elimination and re-challenge diet. Identification and elimination of trigger foods has been shown to be a useful clinical strategy. In one study, a group of migraineurs (*n* = 50) who suspected food triggers was split into two groups, and embarked on dietary restriction for 2 or 4 months. At 2 months, for both groups, monthly attack frequency, attack duration and attack severity were significantly decreased, and this benefit was maintained at 4 months only in the group who continued to avoid trigger foods.[48] The same research group assessed this approach in elderly patients with migraine (*n* = 31) and found similar benefit, with significantly lower frequency of attack, attack duration, pain severity, and analgesic and triptan use at 2 months.[49] Also, an intervention (*n* = 42) based on a low-fat, plant-based diet that incorporated elimination of common trigger foods found a significant reduction in headache intensity and pain; however, issues related to study design such as the use of a vegan diet, short duration of elimination and a non-personalised approach to trigger food elimination limit the generalisability of this particular study.[50] Overall, this clinical evidence shows that identification of trigger foods with a food diary and subsequent avoidance may be a useful strategy for some people with migraine.

Gluten-free diet

Chronic and migraine headaches have been reported as a common presentation in coeliac disease, with a prevalence of up to 30%.[51,52,53] Also, gastrointestinal symptoms are more frequent in coexisting migraine and coeliac disease when compared with coeliac disease alone.[54] Importantly, gluten sensitivity may initially present with extra-intestinal symptoms in the absence of gastrointestinal symptoms or enteropathy, and has been associated with neurological disorders, including migraine.[55,56]

Gluten-free diets have been successful in reducing headaches and migraine in some studies. In one report of 10 people with episodic headache and gluten sensitivity, defined by the presence of antigliadin antibodies as well as HLA-DQ2 or HLA-DQ8 genotype, nine responded to a gluten-free diet.[57] And in a group of people with coeliac disease (*n* = 90) who were treated with a gluten-free diet, three out of four people who initially had migraine attacks had an improvement in frequency, duration and intensity, while the remaining one became migraine free.[58] A survey of people (*n* = 866) with headache and coeliac disease revealed that 24% reported headache as the main symptom that led to diagnosis of coeliac disease and, after initiation of a gluten-free diet, the frequency and intensity of migraine headaches significantly improved; symptoms relapsed on gluten exposure.[59] In contrast, one investigation found that 28% of patients with coeliac disease (*n* = 72) had migraine despite a persistent gluten-free diet.[60]

Non-coeliac gluten sensitivity (NCGS) has been suggested to play a role in migraine, but evidence is currently limited. A significantly higher incidence of migraine has been reported in people with NCGS compared with controls.[61] A preliminary study identified a subset of migraine patients with NCGS (*n* = 10) who, after commencing a gluten-free diet for 3 months, experienced a significant reduction in migraine disability.[62] Additionally, a gluten-free diet has been shown to reduce symptoms of headache in people with suspected NCGS.[63]

Because migraine may be an extra-intestinal manifestation of coeliac disease, testing for coeliac disease if indicated may help identify an underlying contributory dietary factor in some people with migraine, which in some cases could improve headache symptoms after initiation of a strict gluten-free diet.[64] If suspected, NCGS is

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determined with clinical response to a gluten-free diet for at least 6 weeks and subsequent blinded gluten challenge.[65]

IgG-based elimination diet

Diet restriction based on IgG antibodies may be a useful strategy for reducing the frequency of migraine attacks, with evidence from clinical studies generally reporting beneficial effects.[66] The first evidence came from a proof-of-concept study in which migraine patients (*n* = 39) embarked on an IgG-based elimination diet with 30–40% reporting clinical benefit.[67] In another study, 43 out of 65 patients with migraine refractory to traditional treatment had a complete remission of their migraine after 1–6 months of an IgG-based elimination diet.[68] In the first randomised-controlled clinical trial, a 6-week IgG-based elimination diet (*n* = 30) found a significant reduction in the number of headache days and number of migraine attacks in the elimination diet period.[69] However, in contrast, one study (*n* = 138) reported a significant reduction in migraine-like headaches with an IgG-based elimination diet at 4 weeks, but only a small non-significant reduction at 12 weeks of dietary therapy.[70] This study lends only modest support for an IgG-based elimination diet, but may have been limited by differences in study design such as participants having self-reported migraine and thus possibly other forms of headache, lack of dietary support for participants compared with other trials, and inability to measure dietary adherence.

People with irritable bowel syndrome (IBS) are at higher risk for migraine,[71] and gastrointestinal symptoms may respond to IgG-based elimination diets.[72] In patients with a co-diagnosis of migraine and IBS (*n* = 21), a double-blind, randomised-controlled, crossover clinical trial reported significant reductions in symptoms of both disorders, including headache frequency, severity and duration, and gastrointestinal pain and bloating after a 6-week IgG-based elimination diet.[73] In another study of a group of patients with migraine and IBS (*n* = 60), a 14-week IgG-based elimination diet with a probiotic was more effective than a control diet with a probiotic for reduction of migraine and gut symptoms.[74] Overall, it appears that an IgG-based elimination diet may be useful in cases of migraine related to suspected food sensitivities.

Empirical oligoantigenic diets, also known as elimination re-challenge diets, were examined for migraine as early as the 1980s, but since then have received little attention.[75] In a pioneering study involving migraine patients (*n* = 9) with suspected food triggers, Monro *et al.* mitigated symptomatic and immunological food reactivity with an antihistamine,[76] and then went on to report that an elimination re-challenge diet, followed by an antigen desensitisation regime, was able to reduce symptoms in people with migraine (*n* = 11).[77] Another research group reported that an elimination re-challenge diet resulted in recovery from migraine in 93% of a paediatric population (*n* = 88).[78] The same research group reported benefit of the same dietary approach in children with migraine and epilepsy or attention-deficit hyperactivity disorder.[79,80] In another study of a group of adults with migraine (*n* = 43) who were treated with an elimination re-challenge diet, 13 had a 66% or greater reduction in headache frequency, and six became headache free.[81] Elimination re-challenge diets appear to be a useful clinical approach, but would benefit from renewed interest and research.

Low-histamine diet

Elevations in histamine due to an imbalance between detoxification and dietary exposure can cause histamine receptor-mediated complications and allergic-type symptoms, including headaches and migraine. Typically, histamine intolerance (HIT) presents with symptoms such as asthma-like symptoms, rhinitis, urticaria and gastrointestinal symptoms, including diarrhoea and abdominal pain, after ingestion of foods with a high histamine content.[82] Histamine is found at a high concentration in aged foods (e.g. cheeses, alcoholic beverages, cured meats, fermented or spoiled foods), where it is produced by bacterial or yeast fermentation of the amino acid histidine to histamine. Other foods, such as citrus fruits, may have the capacity to enhance histamine release, even though they contain low levels of histamine themselves.[83]

Detoxification of dietary histamine normally occurs in intestinal epithelial cells via the enzyme diamine oxidase (DAO). If DAO fails to inactivate histamine, it is absorbed through the gut epithelium and enters systemic circulation where it may contribute to typical symptoms of HIT in some people.[84] There have been associations between genetic variants that affect DAO activity and migraine headache, suggesting increased susceptibility to HIT in genetically predisposed individuals.[85] Furthermore, blood DAO levels were found to be more frequently deficient (defined as levels below 80 HDU/ml) in migraine patients compared with controls (87% versus 44%).[86]

While there appear to be no studies of a low-histamine diet in migraine sufferers, one study found that pre-treatment with an oral anti-histamine prevented headaches after ingestion of a high-histamine food in people with HIT.[87] A potential role for a low-histamine diet is also suggested by a clinical trial of DAO enzyme supplementation in patients with migraine (*n* = 100), which reported a significantly reduced duration of migraine attacks after 1 month of treatment.[88] If clinical assessment suggests HIT, a low-histamine diet or therapeutic trial of DAO supplementation may be warranted.[89]

Weight-loss diet

Obesity can increase the risk for migraine as well as exacerbate migraine frequency and severity, a relationship that may be in part due to amplification of the inflammatory response in migraine.[90] Nutritional and behavioural weight loss is useful for people with comorbid migraine and obesity as it can reduce migraine severity and impact.[91] In one study (*n* = 135), a year-long weight-loss intervention that included diet, physical activity and cognitive behavioural training was found to significantly decrease body weight, headache frequency and intensity, use of acute medications, and disability within 6 months in obese adolescents with migraine. Furthermore, benefits persisted at least 12 months after treatment.[92] While diet and lifestyle counselling and subsequent weight loss may be beneficial, it is also possible that diet and physical activity, independent of weight change, may mitigate migraine.

Low-glycaemic-index/-load diet

Poor blood glucose regulation, especially reactive hypoglycaemia and hyperinsulinaemia, has been associated with migraine attacks, with several early studies reporting beneficial effects of low-carbohydrate diets.[93,94] In one such study, dietary therapy (a low-sugar diet and six meals daily) resulted in at least 75% reduction of frequency of attacks in all diabetic patients (100%), and most migraine sufferers (63%) who previously demonstrated reactive hypoglycaemia.[95] More recently, a clinical study of a very-low-carbohydrate ketogenic diet and a case report of a low-carbohydrate diet reported important clinical benefits in migraine sufferers, further supporting the rationale for a low-carbohydrate diet in migraine associated with dysglycaemia.[96,97] Also, a low-glycaemic-index diet significantly decreased monthly attack frequency and

migraine severity in patients (*n* = 147) after 3 months.[98] Overall, patients with migraine, and especially those with concomitant dysglycaemia, could benefit from a low-glycaemic-index/-load diet.

High-omega-3/low-omega-6 diet

An increased ratio of dietary omega-6 to omega-3 fatty acids in industrialised diets has been proposed to contribute to inflammatory disorders, including migraine.[99] In support of this, a high plasma omega-6 to omega-3 ratio was more strongly positively correlated with elevations in inflammatory mediators than levels of each fatty acid alone.[100] In a clinical study (*n* = 56), a combination of increasing dietary omega-3 with concurrent reduction in omega-6 fatty acids produced statistically clinically relevant improvements in headache hours per day, severe headache days, headache-related quality of life, and reduction in use of migraine-specific medications.[101] There was also a reduction in psychological distress and an improvement in health-related quality of life and function.[102] A subsequent study was able to demonstrate that increasing dietary omega-3 with concurrent reduction in omega-6 fatty acids is more effective than increasing dietary omega-3 alone, suggesting that the dietary omega-6 to omega-3 fatty acid ratio is indeed important.[103] The suitability of a dietary intervention that improves omega-3 to omega-6 fatty acid ratios could be based on dietary assessment or laboratory values of fatty acid status.

Ketogenic diet

Ketogenic diets have a long history of investigation in neurological diseases and have a variety of mechanisms that may explain their clinical effects, including improvement of brain energy metabolism, mitochondrial function, redox balance, reduction of neuroinflammation, pro-excitatory and inhibitory neurotransmitters, and modulation of the gut microbiome.[104,105]

The use of ketogenic diets for migraine dates back to 1928 when an investigative clinical trial found 9 out of 23 patients (39%) with migraine responded well to a ketogenic diet.[106] Then in 1930 a study reported a reduction in headache frequency of at least 50% in 75% of patients, and total remission of headache in 50% of patients with migraine.[107]

More recently the use of ketogenic diets for migraine has been once again investigated, in part due to the publication of a few case reports describing beneficial effects that led to larger scale investigations.[108,109] In a prospective observational study (*n* = 52), a ketogenic diet intervention had a 90% treatment response rate and reduction in medication use over a 1-month period while, in contrast, a standard low-calorie diet had no effect.[110] A proof-of-concept study (*n* = 45) found a significant reduction in attack frequency, number of days with headaches, and medication use intake over 1 month.[111] A randomised double-blind, crossover trial (*n* = 35) comparing the effects of a very-low-calorie ketogenic diet and a very-low-calorie non-ketogenic diet found the ketogenic diet had a 50% better response rate for days with migraine (74% versus 8%), and was superior for reducing the number of days with migraine (−3.73 days) and migraine attacks (−3.02).[112]

In a 12-week randomised-controlled crossover trial, a ketogenic diet lowered migraine duration, although the effect was not statistically significant.[113] However, there was a high dropout rate with only 11 out of 26 people completing the study, and all participants reported fatigue as a side-effect. In another study (*n* = 38), patients with drug refractory chronic migraine and medication overuse headache who were treated with a ketogenic diet for 3 months had a decrease in days with symptoms (from 30 days to 7.5 days), migraine duration (from 24 hours to 5.5 hours), migraine pain (55% response rate), and medication use (from 30 doses a month to 6 doses a month).[114] In two trials, patients with refractory migraine (total n = 48) treated with a ketogenic diet had a significant reduction in the frequency of migraine attacks, intensity of headache and medication use when compared with a control diet with a similar reduction of carbohydrate.[115]

Exogenous beta-hydroxybutyrates (ketones) have been proposed as an alternative to ketogenic diet therapy, but a clinical trial assessing supplementation with beta-hydroxybutyrate (7.4 g daily for 12 weeks) found no benefit for migraine frequency or intensity.[116] In contrast, medium-chain triglycerides (MCTs), which are sometimes used as a component of ketogenic diet therapy, were shown to have potential in one study. Treatment with MCTs (13 g total fat per serving from coconut oil) for 30 days with no other dietary changes (*n* = 14) reduced the number of migraine episodes (−39%) and migraine duration (−61%).[117]

There is promising evidence for ketogenic diets for migraine, but clinical trials to date suggest they need to be strictly ketogenic and not very-low-calorie or low-carbohydrate diets as these appear not to produce the same therapeutic effects. Furthermore, ketogenic diets for migraine may differ from ketogenic diets for other neurological disorders or those used for weight loss due to unique dietary triggers, and different ketogenic ratio (ratio between fats and carbohydrates + proteins) requirements. Di Lorenzo *et al.* have published clinical guidelines and recommendations.[118]

Riboflavin

Riboflavin has been shown to reduce migraine frequency. Several mechanisms may explain the therapeutic effect of riboflavin, including reduction of oxidative stress, improvement in mitochondrial function, amelioration of neuroinflammation and modulation of glutamate excitotoxicity.[119] A meta-analysis of 11 clinical trials in adolescents and adults concluded that riboflavin is well tolerated and that most clinical trials have shown modest reductions in migraine headache frequency after 2–3 months. However, benefits in children and adolescents were inconclusive.[120]

The optimal dose of riboflavin for migraine prevention is uncertain. Clinical studies in adolescents have typically used doses ranging from 50 mg to 400 mg daily,[121,122,123,124,125] while adult studies used doses ranging from 100 mg to 400 mg daily.[126,127,128,129] Lower doses may be as effective as higher doses but are not as well studied. In adolescents, as little as 10 mg of riboflavin daily was found to be effective for migraine prevention in one study,[130] and in adults a dose of riboflavin 25 mg daily was as effective as a combination nutritional formula providing 400 mg of riboflavin with magnesium and feverfew.[131] The maximal absorption of riboflavin after doses of up to 60 mg is 27 mg,[132] so it is plausible that there is no advantage to higher doses but this needs to be tested in dose comparison studies.

It is unlikely that overt riboflavin deficiency explains the benefit of supplementation for migraine.[133] However, it is possible that people prone to migraine have a higher requirement for riboflavin, with one study suggesting that riboflavin may be more effective in patients with certain mitochondrial DNA genetic variations, specifically those who did not have mitochondrial haplogroup H.[134] This observation is consistent with the notion that individuals have wide variations in micronutrient requirements that are genetically determined.[135]

Niacin

Niacin has been reported to prevent migraine and to be used acutely to avert migraine attacks, although research is limited. Several

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reports of intravenous (IV) and oral niacin have described important clinical benefits for migraine and tension-type headaches, including for aborting acute attacks.[136]

It has been proposed that niacin may be a useful acute treatment due to its vasodilatory effect, which may counter the cranial vasoconstriction associated with the development of migraine aura and pain.[137] In case reports, 500 mg of oral niacin taken at the onset of acute symptoms prevented migraine.[138] And in another case report, 375 mg of sustained-release niacin 1–2 times daily for 3 months prevented migraine.[139] A subsequent systematic review identified 11 clinical reports describing positive effects of IV or oral niacin for headache and migraine headache prevention and acute treatment.[140]

Vitamin B12

Vitamin B12 status has been found to be significantly associated with migraine, and correction of deficiency could resolve headaches in some people. Vitamin B12 deficiency has been associated with elevated serum tumour necrosis factor-alpha (TNF-α) and treatment with vitamin B12 lowers TNF-α, suggesting inflammation may underlie the relationship.[141] Vitamin B12 also possesses antioxidant properties, and subclinical deficiency has been associated with oxidant stress.[142]

In a study of patients with migraine (n = 140), those with serum vitamin B12 in the highest quartile had an 80% decrease in the odds of having migraine, while those in the highest quartile of methylmalonic acid (a biomarker of B12 deficiency) had a more than five times increased risk of having migraine.[143] Relevant to migraine patients using analgesics, aspirin use may contribute to vitamin B12 deficiency by damaging the gastric mucosa and interfering with vitamin B12 absorption.[144]

Several studies suggest that vitamin B12 supplementation could be useful for preventing migraine, but are limited by small sample sizes and administration of vitamin B12 with other vitamins in most studies.[145] In one study (n = 20), treatment with hydroxocobalamin (1000 mcg daily, intranasally) for 3 months was reported to reduce migraine attack frequency, duration of attacks, total number of migraine days and the number of medication doses for acute treatment used in treatment responders.[146] No relationship between cobalamin serum concentrations and efficacy was observed, suggesting a pharmacological effect independent of B12 deficiency. Identification of vitamin B12 deficiency in children and adolescents with tension-type headaches and subsequent correction with 3 months of supplementation was found to completely resolve headaches, anaemia and concomitant anxiety.[147]

Homocysteine-lowering vitamins: folate, vitamin B6 and vitamin B12

Folate, vitamin B6 and vitamin B12 supplementation for migraine is supported by several clinical studies, and could be personalised based on measurement of homocysteine, while the presence of certain 5,10-methylenetetrahydrofolate reductase (MTHFR) polymorphisms may result in a better clinical response to supplementation in some people.[148]

Elevated serum homocysteine is a frequent finding in migraine sufferers, especially migraine with aura.[149] Studies examining associations between MTHFR C677T and A1298C polymorphisms and migraine have been mixed, but a meta-analysis of 26 studies suggested that they may be a risk factor for migraine in Caucasians, but not Asian populations.[150] Folate, vitamin B6 and vitamin B12 (as well as riboflavin and choline) lower elevated homocysteine, and individuals with MTHFR polymorphisms may be uniquely susceptible to adverse effects related to low dietary intake of these vitamins.

In a review of five studies assessing supplementation with folate, vitamin B6 and/or vitamin B12, most studies reported good outcomes for migraine prevention.[151] Genotype may modify treatment response. In an open-label study (n = 16), children with migraine, hyperhomocysteinaemia and MTHFR polymorphisms responded well to folic acid (5 mg once daily) for 6 months.[152] In a subsequent clinical trial (n = 52), people with migraine and the MTHFR C677T genotype who were carriers of the C allele experienced a greater treatment response compared with TT genotypes in a 6-month clinical trial of folic acid, vitamin B6 and vitamin B12 supplementation (2 mg of folic acid, 25 mg of vitamin B6 and 400 mcg of vitamin B12).[153] Similarly, women who were C allele carriers of the MTHFR C677T variant showed a higher reduction in homocysteine levels and severity of pain in migraine in response to the same dose of folic acid, vitamin B6 and vitamin B12 supplementation in another study (n = 206).[154] Related to diet, a higher dietary intake of folate has been associated with reduced migraine frequency, particularly in individuals with the CC genotype for the MTHFR C677T.[155]

Vitamin E

Vitamin E has antioxidant activity in the central nervous system that may be relevant to the pathophysiological role of redox stress in migraine.[156] A double-blinded trial in women with menstrual migraine (n = 72) found that vitamin E supplementation at a dose of 400 IU daily for 5 days, 2 days before to 3 days after menstruation, for two cycles significantly reduced pain severity, photophobia, phonophobia, nausea and functional disability when compared with placebo.[157] In a clinical trial (n = 35), a vitamin E-containing antioxidant formula (providing N-acetylcysteine 1200 mg, vitamin C 1000 mg and vitamin E 500 IU per day) reduced the number of headaches per month (by 3 per month), migraine duration, headache pain scores, and acute headache medication use after 3 months of treatment.[158]

Vitamin D

Vitamin D deficiency or insufficiency has a higher prevalence in migraine sufferers compared with controls, and vitamin D supplementation might reduce the frequency of attacks. It has been estimated that vitamin D deficiency or insufficiency is present in 45–100% of people with migraine, and serum vitamin D level has been negatively correlated with frequency of headaches.[159] In people with migraine, vitamin D supplementation has been shown to exert a number of biological effects relevant to migraine pathophysiology, including improved Th17/Treg related cytokines balance,[160] lowering calcitonin gene-related peptide (CGRP),[161] and reducing nitric oxide synthase and interleukin (IL)-6.[162]

Several clinical studies have assessed the effect of vitamin D in people with headache or migraine, and generally showed a decrease in headache frequency after vitamin D supplementation.[163] For example, children with migraine (n = 53) who were given vitamin D (400 IU, 800 IU or 5000 IU daily if not deficient, mildly deficient, or very deficient, respectively) plus amitriptyline for 6 months had a significantly lower number of migraine attacks when compared with amitriptyline alone.[164] Adults (n = 165) with migraine who received vitamin D (4000 IU daily) for 24 weeks had significantly raised serum 25(OH)D levels and reduced frequency of migraine attacks.[165] Vitamin D may also improve medication treatment response, as noted in a report describing a positive interaction with pregabalin.[166]

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Magnesium

Magnesium deficiency adversely affects neurological function and could contribute to the development of migraine.[167] Magnesium's blockade of the glutamatergic *N*-methyl-D-aspartate receptor, a receptor known to be a contributor to pain transmission, and its role in mitochondrial functioning are some mechanisms considered particularly relevant.[168]

Low serum, saliva and brain levels of magnesium are present in people with migraine, are exaggerated during an acute migraine attack, and significantly predict migraine intensity.[169,170,171] In a population study, lower dietary intake of magnesium increased risk of migraine, while attainment of the Recommended Dietary Allowance through food and supplements was associated with reduced risk.[172]

A review of five clinical trials found that magnesium, when used as a prophylactic treatment against migraine, resulted in a favourable reduction in migraine attacks ranging between 22% and 43%.[173] Evidence of efficacy was more compelling for a dose of > 600 mg daily and for magnesium as magnesium citrate when compared with other types of magnesium.

IV magnesium sulphate has been studied as a treatment for migraine in emergency department settings and is effective, comparable to other IV medications (metoclopramide, prochlorperazine, caffeine citrate), well tolerated and has a good safety profile.[174,175,176,177]

Iron

Iron-deficiency anaemia could contribute to migraine, and there is some evidence to suggest that treatment of iron-deficiency anaemia can greatly reduce the rate of migraine attacks suggesting it may be an underlying cause of migraine in some people. Headache or migraine is not considered a typical symptom of iron-deficiency anaemia but may be overlooked. In a group of patients (n = 127) with iron-deficiency anaemia, 79.5% reported headaches and 36.2% met the criteria for migraine.[178] A case–control study found an association between iron-deficiency anaemia and migraine, especially in women and girls.[179] Iron-deficiency anaemia has also been significantly associated with menstrual migraine.[180,181]

Treatment of children (n = 98) with iron deficiency and migraine with iron (4 mg per kg per day of ferrous sulphate) for 3 months significantly reduced migraine frequency, severity, duration and disability.[182] Iron supplementation has also been reported to reduce monthly frequency and severity of migraine headache in adults (n = 183) with migraine and iron deficiency.[183] Dietary iron intake has been inversely associated with severe headache or migraine in women aged 20–50 years, suggesting optimising iron intake via diet may also be important for migraine prophylaxis.[184]

Zinc

Zinc serum levels have been observed as lower in people with migraine, [185,186] and this may have clinical relevance as zinc is essential for a wide range of neurological functions relevant to migraine.[187] Clinical studies suggest zinc may be a useful therapy for migraine. A randomised clinical trial (n = 80) of zinc sulphate (50 mg zinc daily) for 8 weeks resulted in a reduction in headache severity and migraine attack frequency, although this was not statistically significant.[188] A second study, also a randomised clinical trial (n = 60), assessed zinc gluconate (15 mg of zinc daily) over 12 weeks, and reported significantly reduced frequency and severity of migraine attacks.[189]

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Coenzyme Q10 (CoQ10)

CoQ10 deficiency, as measured by serum CoQ10, is common in patients with migraine and can be used to personalise treatment.[190] It is important to note that low serum CoQ10 is a biomarker of mitochondrial dysfunction, and that CoQ10 supplementation can improve mitochondrial function.[191] CoQ10 also has well-documented anti-inflammatory effects, and has been shown to reduce the inflammatory markers CGRP and TNF-α in people with migraine.[192]

In a meta-analysis of six clinical studies, CoQ10 reduced the duration and frequency of migraine attacks.[193] A dose–response analysis of CoQ10 in migraine suggested a non-linear association with maximal efficacy at 300 mg daily regardless of variation in bioavailability of different formulations.[194] CoQ10 may be synergistic with curcumin, as one study (n = 100) found that a combination of curcumin (80 mg) plus CoQ10 (300 mg) was significantly more effective than either therapy alone for migraine prevention.[195]

Alpha-lipoic acid

Alpha-lipoic acid (ALA) has potential for the management of migraine, in part because it improves insulin sensitivity. Disordered glucose and insulin metabolism is a key feature of migraine, although it is not clear if alterations in glucose metabolism are risk factors for or consequences of migraine.[196] Patients with migraine and insulin resistance who received ALA (400 mg twice daily) for 6 months experienced a reduction in the number of migraine attacks and days of treatment, although there was no improvement in insulin sensitivity in this study.[197]

ALA has antioxidant and anti-inflammatory effects, independent of effects on insulin metabolism, that may play a role in its therapeutic effect. In women with episodic migraine (n = 92), ALA (300 mg twice daily) for 3 months significantly decreased serum levels of malondialdehyde and C-reactive protein (CRP), and clinical symptoms of depression, anxiety and stress when compared with placebo.[198]

Improvements in mitochondrial and endothelial function have also been observed. In women with episodic migraines (n = 92), ALA (300 mg twice daily) for 12 weeks significantly reduced migraine severity and frequency, and reduced serum lactate and vascular cell adhesion molecule-1, suggesting improvement in mitochondrial and endothelial function, respectively.[199]

Other reports also suggest a therapeutic role for ALA. An open-label study (n = 54) reported a beneficial effect of ALA (600 mg daily) for migraine prophylaxis after just 1 month.[200] When ALA (300 mg daily) was added to topiramate therapy, treatment was more effective and better tolerated than topiramate alone.[201]

Carnitine

Carnitine and acetyl-L-carnitine (ALC) have well-established neurological effects relevant to migraine, including mitochondrial enhancing, antioxidant and analgesic activity, but whether these translate to clinical value is less understood.[202] The few studies assessing carnitine as a monotherapy have produced mixed results. A 12-week study (n = 72) of ALC (3 g daily) found no benefit for migraine prophylaxis in adults.[203] In contrast, paediatric patients (n = 56) with episodic migraine treated with L-carnitine (50 mg per kg per day) experienced a reduction in migraine frequency, severity and duration that was comparable to the study arm receiving propranolol.[204] Carnitine and ALC have been used as components of multi-ingredient nutritional formulations for migraine, and studies in which they

have been administered with other nutrients are discussed below. Carnitine palmitoyltransferase II (CPT2) or carnitine deficiency should be considered in treatment refractory migraine. Case reports have described cases of migraine discovered to be related to CPT2 or carnitine deficiency, and successfully managed with carnitine in adolescents[205,206] and young adults.[207] In these cases, patients had chronic and often daily headache, additional diverse symptoms (e.g. chronic constipation, abdominal pain and constant extreme fatigue) and were refractory to multiple medications.

5-Hydroxytryptophan (5-HTP)

5-HTP has been investigated for migraine based on its role as a precursor to serotonin and the potential contribution of alterations in serotonin in the pathophysiology of migraine.[208] Other potential mechanisms of 5-HTP in migraine include analgesic effects mediated by elevations in plasma beta-endorphins,[209] and reaction in cortical spreading by serotonin–oestrogen interactions.[210] However, studies of 5-HTP are few and were mostly conducted over 30 years ago with little interest in 5-HTP for migraine since this time, which coincides with the approval of the first selective serotonin reuptake inhibitor (SSRI) drugs.[211] In a clinical study (*n* = 40), 5-HTP (300 mg daily for 40 days) was reported to reduce migraine frequency and severity compared with placebo.[212] In patients with headache (*n* = 31), including migraine and other types of chronic headache, 5-HTP (400 mg daily) for 2 months showed that 48% of participants had a greater than 50% average reduction in headache symptoms.[213] A study in patients with migraine (*n* = 124) assessed 5-HTP (600 mg daily) over 6 months with a significant improvement in 71% of patients, particularly for intensity and duration of attacks.[214] In contrast, a 12-week study in a paediatric cohort with migraine found no benefit of 5-HTP (5 mg per kg of body weight daily) when compared with placebo.[215] However, it should be noted that both 5-HTP and placebo significantly reduced migraine frequency and severity.

Clinical studies suggest that 5-HTP may be a useful therapy for migraine. It should also be noted that 5-HTP has been reported to benefit other types of headaches, including paediatric headaches,[216] fibromyalgia-related migraine,[217] chronic primary headaches[218] and chronic tension-type headaches.[219]

Omega-3 fatty acids

Low dietary intake of the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) increases the risk for headaches[220] and migraine.[221] An adequate dietary supply of EPA and DHA is critical for neurological health, and low levels, as measured by the percentage of EPA/DHA in erythrocytes [the Omega-3 Index (O3I)], have been associated with impaired brain function, atrophy of key brain regions and diverse neurological diseases.[222]

A systematic review and meta-analysis of 10 randomised-controlled trials of EPA/DHA in migraine found a significant reduction of approximately 3.44 hours in the duration of migraine, but no effect on frequency or severity.[223] However, it was noted that there were several limitations that may have confounded results, including: lack of adequate placebo (olive oil was sometimes used); not controlling for omega-3:6 ratio (which may influence EPA/DHA efficacy); a masking effect by migraine medications; and lack of personalisation (e.g. not testing baseline omega-3 status or response). Subsequent to the publication of this meta-analysis, a clinical trial was published in which 66% of patients with chronic migraine who received omega-3 (1500 mg EPA/DHA daily) for 60 days in conjunction with amitriptyline had an 80.0% reduction in the number of headache days per month, compared with 33% with amitriptyline alone.[224]

In general, the evidence is suggestive for the benefits of EPA/DHA in migraine, but until better quality clinical trials are available that account for the limitations mentioned above the clinical benefit remains unclear. In practice, an exploratory therapeutic trial of 1000–2000 mg EPA/DHA daily for at least 8 weeks could be considered, and personalised based on testing of the O3I.[225]

Palmitoylethanolamide (PEA)

PEA is an endocannabinoid-like bioactive lipid mediator used as a nutritional supplement primarily for its neurological, anti-inflammatory and analgesic effects.[226] Preclinical evidence suggests that modulation of the endocannabinoid system by PEA could relieve migraine pain.[227] For pain management, case studies, clinical trials and meta-analyses of PEA have demonstrated good evidence of efficacy and excellent tolerability at typical doses of 600–1200 mg daily.[228]

In patients with migraine with aura (*n* = 20), PEA (1200 mg daily) for 90 days resulted in a statistically significant and time-dependent reduction in pain, as a composite of severity and frequency, at the 60- and 90-day time points.[229] In addition, PEA reduced non-steroidal anti-inflammatory drug (NSAID) use compared with controls. An open-label study in patients with episodic migraine (*n* = 25) assessed a PEA-containing nutraceutical (Calmux®, Bioksan, Spain; providing PEA 800 mg, *Scutellaria baicalensis* 800 mg, *Boswellia serrata* extract 500 mg and *Harpagophytum procumbens* root extract 400 mg per day) over 3 months and reported a significant reduction in pain intensity.[230] A paediatric cohort with migraine without aura (*n* = 70) who received PEA (600 mg daily) for 3 months experienced a significant reduction in pain intensity and attack frequency as well as a reduction in medication requirement.[231]

Melatonin

Melatonin, although a hormone, is available as a non-prescription over-the-counter dietary supplement in the USA, Canada and some EU countries. As a dietary supplement, melatonin is considered very well tolerated and safe at typical doses of 1–20 mg daily, and even safe when used at very high doses of up to 1000 mg daily in the short term.[232] Relevant to migraine, serum melatonin may be lower in a sub-group of patients,[233] and alterations in melatonin could play an important role in migraine pathophysiology via modulation of several neural pathways involved in the generation of migraine attacks.[234] In addition, melatonin supplementation can exert analgesic effects independent of endogenous melatonin status.[235] It is also possible that by improving sleep, melatonin may prevent migraine attacks,[236] and help resolve acute attacks by encouraging sleep, at least in children.[237]

Studies suggest melatonin may be useful for migraine prevention, and for acute management of migraine headache attacks. In a systematic review of seven studies, melatonin was generally found to be effective for migraine prevention with comparable efficacy to other preventive medications. In adults, doses of 3 mg daily appeared to be more effective than 2 mg doses, and a treatment duration of 3 months was more likely to be related to a beneficial outcome.[238]

A novel study explored the use of melatonin for acute treatment of migraine attacks.[239] Children and adolescents (*n* = 84) aged 4–17 years with episodic migraine were prescribed ‘high-dose’ or ‘low-dose’ melatonin (< 40 kg: 4 mg versus 1 mg; ≥ 40 kg: 8 mg versus 2 mg) as an edible milk-chocolate-based melatonin formulation (Good Day Chocolate, USA). Acute treatment of a migraine attack with melatonin reduced pain intensity and reduced migraine duration with a 2-hour pain-relief rate (defined as mild or no

headache) of 94% and 80% in the high- and low-dose groups, respectively. Napping occurred in 67% and 47% in the high- and low-dose groups, respectively. Both higher dose and napping after treatment predicted greater benefit.

Ginger

Ginger (*Zingiber officinale*) has well-established clinical use as an anti-inflammatory and analgesic in addition to nausea, a common feature of migraine.[240] A meta-analysis of three randomised-controlled studies (n = 227) in which ginger monotherapy (two studies) or feverfew (*T. parthenium*; one study) had been explored as an intervention for migraine prevention and acute treatment found ginger effective for reducing migraine pain, nausea and vomiting.[241] The studies attributed to positive effects were trials in which ginger was used as an acute abortive treatment while, in contrast, ginger used as a daily prophylactic over 1 month did not perform better than placebo.[242] The first study assessing ginger (with feverfew) as an abortive treatment was in fact a homeopathic remedy, and not a herbal product.[243] In the second, patients treated with ginger (a single dose of 400 mg of extract providing 5% gingerols) in addition to IV ketoprofen showed significantly better clinical response after 1 hour, 1.5 hours and 2 hours compared with medication alone.[244] Also, ginger reduced pain and improved functional status. Ginger appears to have promise as an acute abortive treatment, but this requires validation with further research. Clinically, a therapeutic trial of ginger at the first sign of headache could be considered.

Curcumin

Curcumin, an anti-inflammatory polyphenol derived from turmeric (*Curcuma longa*) rhizome, has been explored as a possible therapeutic intervention in patients with migraine, alone and in combination with other nutrients. In a clinical trial of nano-curcumin alone, patients with episodic migraine (n = 44) were randomised to nano-curcumin (80 mg daily) or placebo for 2 months.[245] Nano-curcumin was reported to significantly reduce the frequency, severity and duration of headaches. The same research group have explored mechanisms of action in study participants, demonstrating modulation of the Th2/T regulatory cell axis,[246] and reduction in gene expression and plasma levels of IL-17.[247] Another research group studied a different curcumin extract (n = 44) for a period of 8 weeks at a dose of 500 mg twice daily (providing 95% curcuminoids), administered after lunch and dinner to improve absorption.[248] In this study, participants (n = 44) receiving curcumin had a significant reduction in headache severity and duration, with a marginal reduction in frequency. There was also a significant reduction in CGRP and IL-6.

A series of studies have examined the clinical effect of a nano-curcumin and omega-3 fatty acid formulation. A clinical trial (n = 74) comparing nano-curcumin (80 mg daily), omega-3 fatty acids (1200 mg of EPA and 600 mg of DHA daily), a combination of them, or placebo found a significant reduction in migraine attack frequency in the nano-curcumin and omega-3 fatty acids combination, while curcumin or omega-3 fatty acids alone were not effective.[249] In addition, they demonstrated a significant downregulation of TNF-α gene expression with the combination only. The same research group have also reported downregulation of IL-6 gene expression, a decrease in the serum concentration of high-sensitivity (hs)-CRP,[250] reduction of serum intercellular adhesion molecule 1 (ICAM-1)[251] and serum IL-1β,[252] with the effect generally more pronounced for the combination.

A combination of nano-curcumin and CoQ10 has also been investigated. Patients with episodic migraine (n = 91) received either a combination of nano-curcumin (80 mg) plus CoQ10 (300 mg), nano-curcumin, CoQ10, or placebo for 8 weeks. The curcumin and CoQ10 combination was more effective than all the other interventions for reducing migraine attack frequency, severity and duration, suggesting a synergistic or additive effect.[195]

Overall, it appears curcumin alone or in combination with omega-3 fatty acids of CoQ10 may be useful for migraine, and both more commonly available 95% curcuminoid extracts and nano-formulations (e.g. liposomal) may be effective albeit at significantly different doses of 1000 mg (total extract) or 80 mg (curcumin) daily, respectively.[253]

Phytoestrogens

Based on the oestrogen withdrawal hypothesis of migraine aetiology, phytoestrogens have been explored as a therapeutic for migraines with some evidence of potential benefit. The oestrogen withdrawal hypothesis is based on the observation that migraine is more prevalent in women and tends to be related to fluctuations in oestrogen during menarche, menstruation, pregnancy and menopause, with migraine attacks being twice as common in the perimenstrual period when oestrogen levels decline.[254] Oestrogen directly influences several pathways involved in migraine in the central nervous system.[255] A higher rate of oestrogen decline in the late luteal phase and 2 days post-peak may represent a neuroendocrine vulnerability pattern in migraineurs versus controls.[256] Stabilising oestrogen with hormone replacement therapy can help mitigate migraine.[257] A possible alternative to hormone replacement therapy for the management of migraine is phytoestrogens; non-steroidal phytonutrients that selectively modulate oestrogen receptors.[258]

Three clinical trials have assessed the effect of phytoestrogens in menstrual migraine, including soy isoflavones with other hormone-modulating herbs,[259] soy isoflavones alone,[260] and resveratrol (Table 1).[261]

These studies suggest that soy isoflavone extract with additional herbs or the soy isoflavones genistein and daidzein alone may be useful for menstrual migraine, while resveratrol has no benefit. The reason for this difference in efficacy may be due to differences in ability to selectively modulate oestrogen receptors between soy isoflavones and resveratrol. Soy isoflavones are oestrogen receptor β-specific, while resveratrol modulates both α and β oestrogen receptors.[262] Therefore, if trialling phytoestrogens, soy isoflavones at a dose of > 76 mg daily for at least 3 months should be considered.

Table 1: Clinical trials assessing phytoestrogens for migraine prophylaxis

Study	Subjects	Intervention	Outcome
Burke *et al.* (2002)	n = 49 women with menstrual migraine	Combination of 60 mg soy isoflavones, 100 mg dong quai and 50 mg black cohosh extracts, or placebo, daily for 24 weeks	Migraine frequency during weeks 9–24; 10.3 in placebo-treated and 4.7 in patients treated with the phytoestrogen preparation
Ferrante *et al.* (2004)	n = 10 women with menstrual migraine	56 mg of genistein and 20 mg of daidzein daily, for 3 months	Average number of days with migraine decreased significantly after 3 months of therapy
Dzator *et al.* (2022)	n = 62 women with menstrual migraine	75 mg of resveratrol, or placebo, twice daily for 3 months	Resveratrol did not affect number of days with migraine, the migraine-related disability, or quality of life

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Probiotics

Digestive disorders have frequently been associated with migraine headaches, and may play a contributory or causal role through a variety of mechanisms, including alterations in the enteric immune and nervous systems by intestinal bacteria and gut-derived inflammatory and vasoactive mediators entering circulation.[262] In one study, migraine sufferers were found to have significantly higher levels of nitrate, nitrite and nitric oxide reductase bacterial genes in samples collected from the oral cavity, and slight but significant differences in faecal samples as well.[263] Modification of gastrointestinal health and ecology could be a treatment target, with a number of clinical examples that support the importance of identifying and managing gastrointestinal dysfunction in migraine sufferers. Eradication of *Helicobacter pylori,*[264,265,266] improvement of IBS with an IgG-based elimination diet, and treatment of coeliac disease with a gluten-free diet have resulted in improvement in migraine. In fact, many nutritional interventions discussed above describe dietary changes and nutritional supplements (e.g. vitamin D and omega-3 fatty acids) that may improve gastrointestinal health and ecology.[267] Interestingly, an uncontrolled study suggested possible benefit in migraine sufferers who were managed with a nutritional plan focused on improving gastrointestinal assimilation and elimination using a combination of probiotics, nutrients and herbs.[268]

Probiotics have been investigated in patients with migraine. A 12-week pilot study (n = 27) of a multi-strain probiotic (Ecologic Barrier, Winclove Probiotics, Netherlands) at a dose of 2.5 billion CFU daily reported a significant reduction in migraine frequency and intensity; however, this study lacked a control group.[269] In a subsequent randomised placebo-controlled trial (n = 63) of the same probiotic at a dose of 5 billion CFU for 12 weeks, the intervention did not reduce migraine, failing to support the previous open-label study.[270] In addition, there was no impact of the probiotic on intestinal permeability or inflammation compared with placebo. In contrast, a different probiotic was associated with clinical benefit. A 10-week randomised placebo-controlled trial (n = 79) assessed a multi-strain probiotic (Bio-Kult, ADM Protexin Limited, UK) at a dose of 4 billion CFU daily.[271] Compared with placebo, the probiotic significantly reduced migraine frequency, severity, duration and abortive drug use.

It is plausible that improvements in gastrointestinal health such as those associated with clinical management of *H. pylori*, IBS or coeliac disease may coincide with improvement in migraine through shared pathophysiological mechanisms. Dietary changes and nutritional supplements also impact microbial ecology and gastrointestinal health and function, which may, at least in part, provide one mechanism by which they exert beneficial effects. Probiotics have limited evidence in migraine, with only one study of a specific multi-strain probiotic suggesting benefit, while another was not effective. Microbiome testing, although widely available, cannot identify probiotic treatment responders so a 10-week therapeutic trial could be considered.

Nutrient combinations

In addition to the monotherapies described above, some clinical trials have studied the effects of multi-component nutrient formulations (nutrient combinations). These are summarised below (Table 2). Most of these studies have suggested clinically relevant reductions in migraine frequency and severity, but are limited by lack of placebo control (four out of seven studies). Few compared nutrient combinations with individual nutrients to determine if there is an additive or synergistic advantage to a multi-component formula over individual components. Tarighat Esfanjani *et al.*[273] did report a slightly better effect of magnesium and carnitine versus either nutrient alone, but Maizels *et al.*[272] found that a relatively low dose of riboflavin that was intended as the control was as effective as a combination of high-dose riboflavin, magnesium and feverfew. And no studies personalised interventions to pathophysiological sub-groups, such as mitochondrial dysfunction or oxidative stress, or examined biological characteristics of treatment responders, which would be informative. Despite these limitations, nutrient combinations appear to have promise for the management of migraine.

Table 2: Clinical trials of nutrient combinations for migraine

Study	Subjects	Intervention	Outcome
Maizels *et al.* (2004)[272]	n = 49 patients with migraine	Combination (riboflavin 400 mg, magnesium 300 mg and feverfew 100 mg), or riboflavin 25 mg, daily for 3 months	Both groups showed a significant reduction in number of migraines, migraine days and migraine severity
Tarighat Esfanjani *et al.* (2012)[273]	n = 133 patients with migraine	Combination (magnesium oxide 500 mg, L-carnitine 500 mg), or magnesium oxide 500 mg, or L-carnitine 500 mg for 12 weeks	All interventions significantly reduced migraine frequency and severity, but the combination was associated with a greater reduction in severity
Gaul *et al.* (2015)[274]	n = 130 patients with migraine	Combination (Migravent®, Germany; Dolovent®, USA; riboflavin 400 mg, magnesium 600 mg, CoQ10 150 mg with multivitamin and minerals) or placebo for 3 months	Significant reduction in migraine severity and non-significant reduction in frequency
Guilbot *et al.* (2017)[275]	n = 62 patients with migraine	Combination (feverfew 100 mg, CoQ10 100 mg, magnesium 112.5 mg) for 3 months	Significant reduction in days with migraine, migraine severity Reductions in depression, anxiety and improved quality of life
Hajihashemi *et al.* (2019)[276]	n = 56 patients with migraine	Combination (CoQ10 30 mg, L-carnitine 500 mg) or placebo for 8 weeks	Significant reduction in migraine severity, duration, frequency and serum lactate
Vikelis *et al.* (2020)[277]	n = 113 patients with episodic migraine	Combination (magnesium 281.25 mg, vitamin B2 4.8 mg, CoQ10 20 mg, feverfew 150 mg, *Andrographis paniculata* 100 mg) for 3 months	Significant reduction in number of migraine days, headache severity and medication use
Visser *et al.* (2020)[165]	n = 35 patients with migraine	Combination (N-acetylcysteine 1200 mg, vitamin C 1000 mg, vitamin E 500 IU) or placebo for 3 months	Significant reduction in number of headaches per month, migraine duration, headache pain scores and acute headache medication use

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Table 3: Clinical summary of nutritional supplements for migraine

Nutrient	Clinical indications	Duration	Dose
Riboflavin	Consider a routine therapeutic trial in patients with migraine	> 3 months	Adults: 400 mg daily; children: 50–200 mg daily
Niacin	Limited evidence suggests benefit as an abortive Consider a therapeutic trial	Single dose	500 mg
Vitamin B12	Screen patients for B12 deficiency	> 3 months	If deficient, 1000 mcg daily Monitor B12 status to avoid excess or toxicity
Homocysteine-lowering vitamins	Screen patients for elevated homocysteine Some MTHFR-genotypes may respond better to B vitamins	> 6 months	If indicated, B vitamin complex Monitor folate, B6 and B12 status to avoid excess or toxicity
Vitamin E	Assess dietary intake for deficiency Consider a routine therapeutic trial in patients with migraine	> 3 months	400 IU daily
Vitamin D	Screen patients for vitamin D deficiency	8 weeks	If deficient, 6000 IU/day for at least 6 weeks to achieve serum 25(OH)D > 30 ng/ml[278] Monitor vitamin D status to avoid excess or toxicity
Magnesium	Assess dietary intake and risk factors for deficiency Screen patients for magnesium deficiency Consider a routine therapeutic trial in patients with migraine	> 3 months	> 600 mg daily, in divided doses, as magnesium citrate
Iron	Screen patients for iron-deficiency anaemia	2 months	If iron-deficiency anaemia, management should be holistic and personalised[279]
Zinc	Assess dietary intake and risk factors for deficiency Screen patients for zinc deficiency	> 2 months	If deficient, > 15 mg daily
CoQ10	Assess serum CoQ10 Consider a therapeutic trial regardless of serum levels	> 6 weeks	300 mg daily, in divided doses, with food
Alpha-lipoic acid	Assess insulin resistance/blood glucose metabolism Assess redox stress Consider a therapeutic trial regardless of test results	> 3 months	300–400 mg twice daily
Carnitine	Screen for CPT2 or carnitine deficiency in treatment refractory migraine	Indefinitely	660–1650 mg daily in divided doses
5-HTP	Consider a routine therapeutic trial in patients with migraine	> 40 days	> 300 mg daily
EPA/DHA	Assess dietary intake Screen patients with the O3I test	> 60 days	> 1500 mg daily combined EPA + DHA (and depending on O3I status)
PEA	Consider a therapeutic trial in patients with migraine May be useful for reducing drug requirements	> 60 days	Adults: 1200 mg daily; children: 600 mg daily
Melatonin	May be useful as a preventative in some cases Limited evidence suggests benefit as an abortive	> 3 months (prevention) 1–2 doses (at onset of an attack)	Adults: > 3 mg daily (prevention); children and adolescents: > 8 mg in divided hourly doses (at onset of attack), possibly higher for adults
Ginger	Limited evidence suggests benefit as an abortive	> 3 months (prevention) 1 dose (at onset of an attack)	Prevention: > 400 mg of extract (5% gingerols) daily, in two divided doses Treatment: > 400 mg of extract (5% gingerols) at onset of attack
Curcumin	Consider a therapeutic trial in patients with migraine May be useful for reducing drug requirements	> 2 months	1000 mg turmeric extract providing 95% curcuminoids daily 80 mg nano-curcumin (e.g. liposomal) daily

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Phytoestrogens	May be useful for menstrual and hormone-related migraines	> 3 months	> 76 mg soy isoflavones daily
Probiotics	May be useful if gastrointestinal involvement	> 10 weeks	4 billion CFU daily of a multi-strain probiotic (Bio-Kult) daily
Nutrient combinations	Consider a therapeutic trial in patients with migraine	> 3 months	Multiple options available (see Table 2 above)

A wide range of nutritional interventions have been studied clinically and could be considered when managing migraine in clinical practice (Table 3). 'Clinical indications' include clinical history, symptoms and biomarkers that may help guide personalisation. Treatment duration and dose are based on clinical studies cited and discussed in this review, and are generally indicative of effective dose and time to see a significant clinical response. Importantly, clinical studies typically have indiscriminately tested interventions and have rarely utilised biomarkers or clinical symptoms to identify treatment responders, so the 'clinical indications' are in some cases based on the author's opinion regarding the rationalisation of different management options.

Discussion

Migraine headaches have complex relationships to food and nutrition related to pathogenesis and opportunities for clinical management. Dietary interventions can be broadly divided into three major categories that may also be indications for their clinical use: healthy diet; identify aggravating foods or intolerances; and modify underlying pathophysiology or dysfunction. Clinical indications, such as dietary history, and biomarkers or investigations, such as the positive confirmation of coeliac disease, may help identify which dietary interventions are best suited to an individual (Figure 1).

Nutritional intervention with vitamins, minerals, amino acids and other nutrients have shown considerable promise for the management of migraine. A broad range of nutritional interventions is possible, so a pragmatic model that rationalises their indications and use may be helpful in clinical practice (Figure 2). Suboptimal intakes or deficiencies of vitamins, minerals and fatty acids can be assessed through dietary recall and/or laboratory testing, although both have limitations and may not accurately identify treatment responders, and nutrients may have pharmacological effects independent of deficiency or low dietary intake. Other nutrients have more specific indications like improving mitochondrial function, blood glucose metabolism, oestrogen stability or gut microbiome ecology; however, it should be emphasized that nutritional compounds have diverse mechanisms of action so this may be an oversimplification. Additionally, currently accessible laboratory testing may not be a reliable way to direct treatment, as in the cases of mitochondrial function, oestrogen stability and gut microbial ecology, for which there are few, if any, well-established clinical assessments relevant to migraine. Until reliable assessment tools are available, a therapeutic trial with a nutritional intervention, and subsequent positive or null symptomatic response, may be the most pragmatic assessment.

There is a need for clinical guidelines, treatment algorithms and a greater number of validated biomarkers that could help clinicians match nutritional and lifestyle medicine-based interventions to patients mostly likely to benefit.[280] In the meantime, patient symptoms, health history, genetics, environment, lifestyle, diet and behaviour, amongst other considerations, can be leveraged in a clinical setting and inform personalised management plans.[281] Nutritional approaches for migraine management can be used in a personalised and pragmatic way to improve patient outcomes.[282]

Conclusion

Chronic migraine headaches are a prevalent and debilitating disease that would benefit from better management options. A diverse range of therapeutic nutritional options have been explored for migraine headache, with varying degrees of benefit. The wide range of therapeutic options may make it challenging to approach nutritional management of migraine in a clinical setting, so a pragmatic model that helps personalise interventions from clinical signs and symptoms and reliable biomarkers would be useful. There is a need for clinical guidelines, treatment algorithms and a greater number of validated biomarkers that could help clinicians match nutritional and lifestyle-medicine-based interventions to patients most likely to respond to different interventions. Although a greater understanding of the role of nutritional medicine for migraine is needed, a considerable body of clinical evidence already exists, and could inform clinical practice in a way that improves patient outcomes and reduces suffering associated with the disease.

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Notes

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