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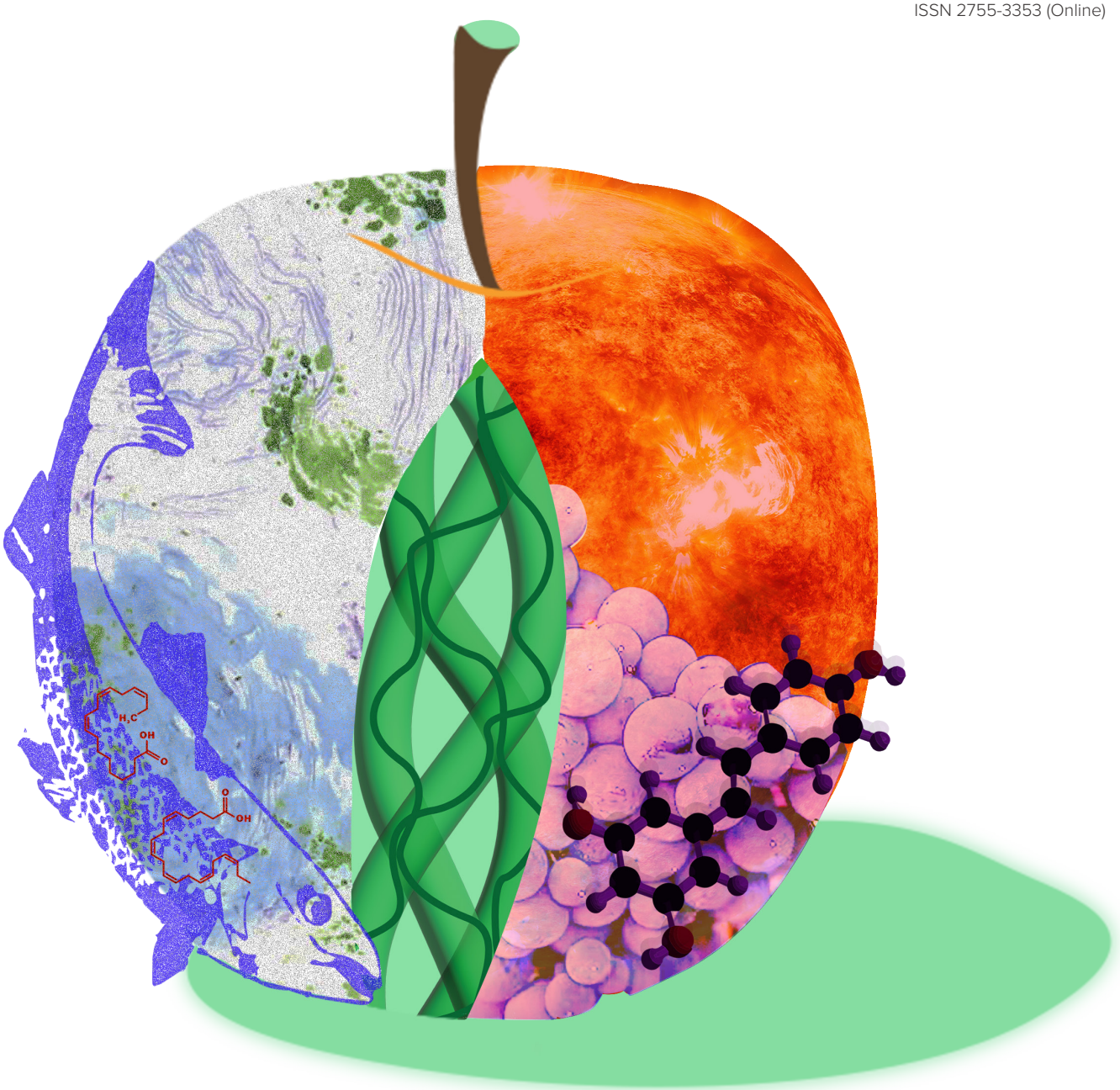

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Nutritional Medicine Journal

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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.

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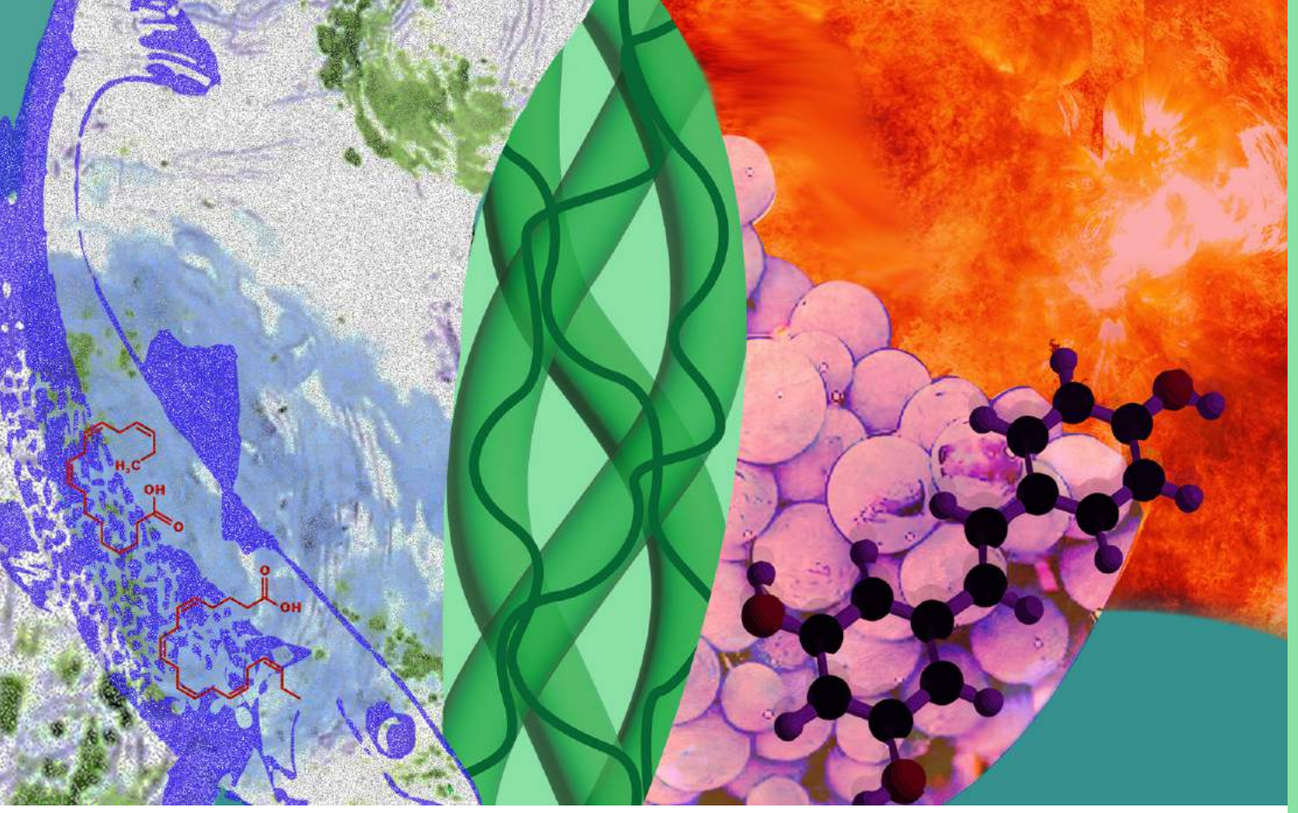

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Cover
Microscopic image of biopsy obtained during colonoscopy from a patient with painful bloody diarrhea showing chronic ulcerative colitis, a type of inflammatory bowel disease (IBD). Turmeric or curcumin longa powder. Coenzyme Q10 ubiquinone CoQ10 molecule 3D render chemical structure. Ashwagandha plant. Raw Magnesite rock. Butyrate acid flat structure.

NUTRITIONAL MEDICINE JOURNAL

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Editor
Benjamin I. Brown

Publisher
Nutritional Medicine Institute

Design
Alex Walton
Hannah Waskett

Proofing
Zara Watt

Editorial enquiries
support@nmi.health

Advertising enquiries
support@nmi.health

Publisher
Nutritional Medicine Institute
Postal address: 71-75 Shelton Street,
Covent Garden,
London, England, WC2H 9JQ
Web: www.nmi.health
Contact: support@nmi.health

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Nutritional Medicine Journal

Bioavailability, Food Supplements, and Clinical Efficacy

Benjamin I. Brown

Enhanced bioavailability of food supplements is often cited as a reason for product superiority, but in some cases this may not be substantiated with evidence. It may be that, contrary to logic, improved bioavailability does not inevitably translate to improved clinical benefit, or that poor bioavailability precludes effectiveness. This issue of the Nutritional Medicine Journal features a series of review papers on several commonly prescribed food supplements; so, with this theme in mind we explore how to navigate claims of bioavailability as they relate to clinical use. Using examples of commonly prescribed food supplements, we explore some of the myths, truths and controversies around bioavailability.

Bioavailability of dietary substances

The 'bioavailability' of a dietary substance has no strict definition, but in nutritional science it typically refers to the relative amount of a substance present in circulation from one source compared with another source.[1] Some of the main factors affecting bioavailability from a pharmacological perspective are solubility, extensive pre-systemic metabolism, poor membrane permeability, and active efflux transportation.[2]

Bioavailability of dietary substances is, however, complex, and can be further divided into extrinsic diet-related and intrinsic host-related factors.[3]

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Affiliation: B. Brown is with the Nutritional Medicine Institute, London, UK, and the British College of Nutrition and Health (BCNH), London, UK.
Corresponding author: Benjamin I. Brown (email: **ben@nmi.health**)
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Diet-related factors: the chemical form of the nutrient, the nature of the dietary matrix, interactions between nutrients and/or organic components, and food preparation and processing practices.

Host-related factors: secretion of hydrochloric acid, gastric acid and/or intrinsic factor, alterations in the permeability of the intestinal mucosa, nutrient status of the individual, age, sex, ethnicity and physiological state (e.g. pregnancy or lactation), and chronic and acute infectious disease states.

The bioavailability of nutrients from food is also strongly influenced by individual variations in gut bacteria and single-nucleotide polymorphisms (SNPs), which means there is a large inherent and natural inter-individual variability.[4,5]

Determining if enhanced bioavailability is clinically relevant

Clinically, the relevance of bioavailability is concerned with two major questions.

1. Is this product more bioavailable than another comparable product.

2. Does enhanced bioavailability result in a better clinical outcome?

The first question can be answered with a pharmacokinetic study assessing the bioavailability of the substance in question. The second question can be answered with a study comparing the clinical effectiveness of the enhanced-bioavailability substance against a comparable substance without enhanced bioavailability. For clinicians, however, pharmacokinetic studies are difficult to assess and may not be truly representative of the commercial product they supposedly substantiate. Also, comparisons between enhanced-bioavailability products and those without enhanced bioavailability on clinical endpoints such as disease biomarkers or symptoms are almost non-existent, further limiting the ability to truly determine differences in efficacy.

This makes assessing the clinical relevance of enhanced-bioavailability food supplements challenging; however, focusing on the above two simple questions may help navigate this, as we explore in the following examples.

The example of magnesium

Magnesium is a popular food supplement with claims of bioavailability tending to focus on one type of magnesium versus another. There are several different types of supplemental magnesium available (e.g. citrate, glycinate, orotate, malate, oxide, sulphate and threonate).

Several bioavailability studies exist, but they mostly suffer from a major problem in that very few use well-established long-term markers of magnesium absorption such as serum or red blood cell magnesium. Instead they are mostly based on urinary output after a single dose. Table 1 summarises human bioavailability studies of magnesium to date.

In addition to the types of magnesium described above, it is noteworthy that magnesium L-threonate has been commercialised (not y*et a*llowed for sale in the UK or EU as it has not been approved as a novel food). Currently, there is no published evidence comparing bioavailability or clinical efficacy of magnesium L-threonate with other types of magnesium in humans.[15] In addition, transdermal magnesium (e.g. magnesium-containing sprays, magnesium flakes and magnesium salt baths) are commercially available, but lack evidence to show they significantly increase serum magnesium.[16,17]

Overall, the studies in Table 1 suggest that single-dose administration of magnesium citrate results in higher urinary levels within 24 hours, but the relationship of this to long-term magnesium status or clinical outcomes

is unclear. In addition, both magnesium oxide and citrate raise magnesium status long term, but it is not clear if one is better than another for improving magnesium status or influencing clinical outcomes.

Bioavailability may become irrelevant when we consider which types of magnesium have demonstrated efficacy clinically. Comparisons between types of magnesium on clinical outcomes are non-existent, and clinical studies have generally found a variety of forms (in particular aspartate, carbonate, chloride, citrate, glycinate, orotate and oxide) are able to correct deficiency and are clinically effective for a wide range of illnesses.[18] It appears that any difference in absorption between various types of magnesium may not be meaningful in terms of actual clinical benefits.

Table 1: Human bioavailability studies of magnesium

Comparison	Duration	Assessment	Conclusion	Reference
Magnesium citrate versus oxide	Single dose	Urinary magnesium	Citrate superior to oxide	Lindberg *et al.* (1990)[6]
Magnesium aspartate versus oxide	Single dose	Urinary magnesium	Aspartate superior to oxide	Muhlbauer *et al.* (1991)[7]
Magnesium glycinate versus oxide	Single dose	Urinary magnesium	No difference	Schuette *et al.* (1993, 1994)[8,9]
Magnesium oxide versus chloride versus lactate versus aspartate	Single dose	Urinary magnesium	Chloride, lactate and aspartate superior to oxide	Firoz & Graber (2001)[10]
Magnesium citrate versus glycinate versus oxide*	60 days	Urinary, serum and red blood cell magnesium	Citrate superior to glycinate and oxide	Walker *et al.* (2003)[11]
Magnesium oxide (capsules) versus oxide (effervescent tablets)	Single dose	Urinary magnesium	Effervescent tablets superior to tablets	Siener *et al.* (2011)[12]
Magnesium oxide versus citrate	4 weeks	Intracellular magnesium levels, platelet function	Oxide superior to citrate	Shechter *et al.* (2012)[13]
Magnesium citrate versus oxide	Single dose	Urinary magnesium, intracellular magnesium	Citrate superior to oxide (urinary, not intracellular)	Kappeler *et al.* (2017)[14]

*Walker *et al*. (2003) claimed to provide a dose of magnesium glycinate in two tablets that is physically unachievable and likely provided 30% less than claimed. The author contacted the study authors to query this but had no response (personal communication).

Editorial
Bioavailability, Food Supplements, and Clinical Efficacy

The example of turmeric

Curcumin or high curcuminoid extracts of turmeric (Curcuma longa) rhizome are frequently sold as enhanced-bioavailability formulations to overcome the poor absorption of curcuminoids. Indeed, curcuminoids are poorly absorbed, but they are not incompletely absorbed, and extracts of turmeric may still be effective despite poor absorption. This apparent clinical effectiveness despite poor absorption has been coined the ‘curcumin paradox’.

Research has generally shown that curcumin is poorly absorbed with relatively low blood levels after people ingest high curcuminoid extracts of turmeric.[19] Paradoxically, clinical studies of turmeric extracts have found that it may be effective despite low absorption. For example, human clinical trials of curcuminoid-rich turmeric extracts have demonstrated preliminary evidence of efficacy in the absence of bioavailability enhancers in patients with ulcerative colitis,[20] osteoarthritis,[21] prevention of type-2 diabetes,[22] premenstrual symptoms,[23] depression,[24] and Alzheimer’s disease.[25]

There have been several proposed reasons for why high curcuminoid extracts of turmeric may be effective despite low absorption. Curcumin is metabolised into a variety of other compounds after digestion and absorption, and these metabolites may actually be more effective than curcumin itself.[26] Also, local gastrointestinal effects could also mediate gut–systemic interactions, in other words, curcuminoids may not need to be absorbed to be effective.[27]

But it is plausible that, at least in some cases, enhanced-bioavailability turmeric products could be more effective than equivalent counterparts. Although there are a large amount of pharmacokinetic studies assessing the bioavailability enhancers on serum levels, none compares such products on clinical outcomes, so it is hard to determine whether enhanced products are actually more effective clinically. Looking at two bioavailability enhancers, phosphatidylcholine and polysorbate 80, it appears translation to better clinical efficacy may depend on the formulation.

A well-studied curcuminoid–phosphatidylcholine complex (Meriva®; Indena, Italy) has demonstrated enhanced absorption and bioavailability, and over 30 clinical studies support the efficacy of a dose providing > 200 mg curcuminoids daily.[28] Although no head-to-head studies related to clinical efficacy exist, we can contrast two clinical trials in patients with psoriasis. In the first, 4500 mg curcuminoids daily for 16 weeks resulted in a low treatment response, which could have been a placebo effect.[29] In contrast, 400 mg daily of the curcuminoid–phosphatidylcholine complex for 12 weeks significantly reduced symptom scores and a serum inflammatory biomarker (IL-22) when compared with placebo.[30]

The artificial emulsifier polysorbate 80 has also been used as a bioavailability enhancer, but does not appear to improve clinical efficacy. In a pharmacokinetic study, a single dose of polysorbate 80-complexed turmeric extract providing 500 mg curcuminoids resulted in a 185-fold higher plasma concentration (area under-time curve) than a reference product, although there was less pronounced absorption in male study participants.[31] It is worth noting that although commercial products often claim 185 times higher absorption, they typically provide 10 times less of the active dose used in this study and so are not exactly representative of the study used to substantiate bioavailability claims. A subsequent clinical study of polysorbate 80-complexed turmeric extract (NovaSOL®, Aquanova AG, Germany) found that a dose of 98 mg of curcuminoids per day did not alter blood lipids, inflammation or glucose, so it is currently unclear if it is more, or less, effective than a comparable product without polysorbate 80, despite enhanced absorption.[32]

It appears that turmeric extracts may be effective in the absence of absorption enhancers, but that, in some cases, such as in the example of psoriasis above, absorption

enhancers may enhance clinical effectiveness. An important consideration is that, contrary to logic, better absorption may not always equate to better efficacy, and some formulations may lack evidence of improved clinical efficacy despite a compelling pharmacokinetic study.

A cautionary tale

The above examples provide a cautionary tale that encourages critical assessment of claims of enhanced bioavailability. Although such claims are made, they may not be well substantiated, and even when there is a pharmacokinetic study demonstrating enhanced bioavailability it may not be representative of the product composition or have been demonstrated to translate to better clinical efficacy in a head-to-head comparative study assessing disease endpoints. Further, a potential concern is that a spurious claim could be used to justify a lower dose and therefore result in an ineffective prescription. In the practice of nutritional medicine, being able to scrutinise bioavailability claims is therefore important when our aim is to optimise health through the judicious use of food supplements as a complement to patient care, while providing the most appropriate, cost-effective and evidence-based management approaches.

Benjamin I. Brown
Editor, *Nutritional Medicine Journal*

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Editorial
Bioavailability, Food Supplements, and Clinical Efficacy

Nutritional Medicine Journal

Collagen: A Review of Clinical Use and Efficacy

Chloe Steele

Abstract

Orally administered collagen in its many different forms is recognised as a highly biocompatible, safe form of supplementation, which has the potential to act on the body as an anti-inflammatory and antioxidant, and through structural remodelling and reduced lipotoxicity. The aim of this systematic review was to determine diseases where collagen has been indicated; assess safety, bioavailability and efficacy; and to provide therapeutic recommendations. It was concluded that collagen supplementation is strongly indicated for its positive therapeutic effect on pain management of osteoarthritis, balancing blood sugars in type II diabetes, wound healing, skin ageing, and post-exercise body composition and strength. Promising results were also seen for the use of collagen supplementation in osteoporosis, hypertension, rheumatoid arthritis, tendinopathy, cellulite, atopic dermatitis, sarcopenia and brittle nail syndrome. Although therapeutic recommendations were indicated in most of these diseases, owing in the large part to the use of these supplements as part of dual therapy or the uncertainty over translatability of branded products it was concluded that more studies are required to make definitive recommendations. There was a lack of clinical evidence to support the use of collagen for weight loss in obesity, gut health and in fibromyalgia.

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Affiliation: C. Steele is with the Nutritional Medicine Institute, London, England, and the Centre for Nutrition Education & Lifestyle Management (CNELM), Wokingham, England.
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Introduction

Collagen is the major constituent of connective and conjunctive tissues in the body, and is the most abundant protein in the human body.[1] Twenty-eight different types of collagen have been found, and each is unique in its distribution, structure and function throughout the body.[2] Identified using roman nomenclature, collagen type I is the main constituent of bone, skin, teeth, tendons, ligaments, vascular ligature and organs; type II is found in the cartilage; and type III is found in the skin, muscle and blood vessels.[3] The remaining types have various functions throughout the body.

Orally administered collagen peptides (CPs) in supplements or food have been demonstrated to reach the bloodstream[4–6] and tissues,[2] indicating the potential for collagen to influence the body from within. Supplement types differ depending on the parent tissue and the extraction technique performed. Derived from gelatin, a mixture of thermal treatment and enzymatic hydrolysis results in collagen hydrolysate (CH) or specific collagen peptides (SCPs), further purification of which can result in collagen tripeptide (CTP) or octapeptide,[7,8] all of which mainly contain types I and III and occasionally type II collagen.[9] Unhydrolysed or undenatured collagen usually contains type II.[3]

Supplemental collagen is traditionally bovine, porcine or ovine sourced, but marine and synthetic vegan-derived products are now more common due to increased social and religious acceptance, and heightened awareness of recent health worries related to bovine spongiform encephalopathy and foot-and-mouth disease.[10,11]

The aim of this systematic review was to determine the disease areas where collagen has been indicated, and review the human clinical literature on safety, bioavailability and efficacy with a view to making specific therapy recommendations. Although topical formulations exist, this white paper focuses on orally supplemented collagen, and unless stated otherwise constitutes the research reviewed. Many forms of collagen supplement were included in this review, including CH, unhydrolysed collagen-type II (UC-II), CTP, octapeptide and SCPs.

Bioavailability

Although it is thought that orally ingested collagen is hydrolysed in the intestinal tract prior to absorption,[9] native collagen and gelatin may not be efficiently absorbed compared with lower molecular weight forms that are often found in supplements,[12] and there is evidence that differing forms may have differing post-prandial absorption rates, bioavailability and bioactivity.

One randomised-controlled trial (RCT) of 10 healthy males reported higher absorption rates and bioavailability of several bioactive amino acids in orally administered CH compared with non-hydrolysed collagen.[13] Furthermore, CTPs containing differing amounts of the bioactive peptides glycylprolylhydroxyproline (GLY-PRO-HYP) and prolylhydroxyproline (PRO-HYP) were reported to be effectively absorbed within 1 hour in 12 human subjects, only when in the tripeptide form.[14] Dose-dependent excretion of the tripeptide and dipeptide forms was also observed, indicating relative stability within the body. This is in contrast to one study that reported no differences between the bioavailability of CH and its unhydrolysed form.[15] This study did acknowledge that processing methods may account for differences with other research, as differing processing techniques can influence digestibility.[16]

The form of CH may also dictate absorption by differing tissues within the body, indicating that unique peptide configurations may have implications for certain diseases. One animal study of CH reported absorption within 12 hours, and a higher degree of detection in cartilage if in its higher molecular weight form compared

with lower molecular weight forms, indicating better bioavailability,[2] which could have potential implications for cartilage-related conditions, such as osteoarthritis (OA).

Marine collagen sources have also been reported to have differing biological properties than the more commonly used land animal collagen, owing to the temperature and salinity of the water where they originate.[17,18]

These studies indicate that differing forms of collagen may have specific actions and efficacy within the body, and that results may not be generalisable when certain forms, brands or types of collagen are used in clinical trials.

Food sources

Vegan collagen

Collagen is a part of all animal tissues, so animal-based foods would appear to be the best sources; however, plant-based collagen products have increased on the market. Vegan collagen products are extensive in their formulations, and mainly focus around stimulating collagen production rather than supplying the original form.

In vitro studies have shown that aloe sterol, which is a plant-derived sterol resembling animal cholesterol, and andiroba oil may have the ability to stimulate collagen production; however, collagen type was not specified.[19,20] Clinical research on aloe sterol focused around skin health.[20,21] One *in vitro* study in human fibroblasts treated with aloe sterols showed increased collagen production compared with control.[21] The type of collagen produced was not specified; however, it was reported that expression of two key enzymes, COL1A1 and COL3A1, which are responsible for collagen production, was increased. In the same study, a double-blind placebo-controlled trial consisted of 56 women with dry skin who were supplemented with five tablets of aloe vera gel powder per day for 8 weeks (dosage not disclosed). No differences in skin hydration were observed between the two groups; however, skin wrinkling was improved in those supplemented, and it worsened in those on placebo, but differences were not significant. A sub-analysis of subjects ≥ 40 years old showed significantly improved skin wrinkling with supplementation compared with placebo, indicating that aloe sterol may promote synthesis in those when collagen production is reduced.

Panax ginseng extract has also been shown to promote collagen synthesis in human dermal fibroblasts; however, as with aloe sterol, the type of collagen was not specified.[22] *In vitro* studies have shown inhibited collagen type II degradation in osteoarthritic osteocytes following application of panax ginseng saponins.[23] Increased collagen type I in NIH/3T3 cells, which have a potential role in wound healing,[24] and diabetic fibroblasts[25] has also been reported, indicating a potential for use in several different diseases. Although there is potential for panax ginseng to promote collagen I production and prevent collagen II degradation, data in humans and clinical outcomes were not evident.

Further studies in Centella asiatica, a medicinal plant known as Gotu Kola, have also indicated that collagen production may be stimulated in human skin fibroblasts when in combination with other vitamins.[26] However, clinical trials in humans are lacking.

Most companies focus on boosting collagen production as previously discussed, but genetically engineering yeast to produce collagen is also possible.[27] Collagen type I, type II[28] and type III[29] have all been produced using this method. Type I collagen was also produced in one *in vitro* study, which had a structure indistinguishable from animal-derived gelatin,[30] but there was no evidence of its clinical use. The existence of vegan collagen on the market is very rare, but one notable form that is in production is PrimaColl™, a type 21 collagen, which is branded as the world's first true vegan collagen.[31] However, clinical trials are still in progress.

Collagen boosters make up the majority of the vegan collagen research. Although two studies were evident on supplementation of vegan collagen boosters in skin health, its application in other disease areas is lacking. As these studies are concerning collagen boosters, results from animal collagen may not be generalisable and applied to vegan collagen. No comparisons with animal-derived collagen were evident in the literature and, as products vary enormously among the industry, comparisons are difficult.

Animal-derived collagen

In recent years, bone broth as a functional food has seen a resurgence in popularity and, although it may contain nutrients that have health benefits, differences in preparation and source could result in variation in absorption rate, amino acid content, and bioavailable and bioactive peptide chains. One quantitative study of several different bone broth preparations reported lower levels of the amino acid precursors for collagen production in the commercially prepared varieties than supplemented CH powders and liquids, and home-prepared varieties and those from cafes had the highest amounts among all of the samples; reasons for this were left unexplained.[32]

Although bone broths may have higher amino acid content than collagen supplements, absorption rate and bioavailability are important factors to consider, and one observational study of 15 male subjects showed that although bone broth had the highest levels of collagen precursor amino acids (with the liquid collagen supplement having the lowest levels), these were more slowly absorbed than hydrolysed and non-hydrolysed collagen sources, which was attributed to the fat content of bone broth slowing digestion.[15]

In combination, these studies indicate that although bone broths, and in particular homemade versions, may have superior levels of collagen-promoting amino acids, the absorption rate and potentially the bioavailability of bone broth may be less than collagen supplements, especially if in the form of CH.

General effects

As previously reported in the section *'Bioavailability'*, the general effects of collagen may be specific to the type of collagen or bioactive peptide used.

Autoantigen/anti-inflammatory

Collagen has been shown in animal models to act as an autoantigen,[33] with an ability to modulate inflammatory pathways. However, the exact effects of collagen on the immune system are yet to be elucidated. Reports of collagen increasing inhibitory inflammatory cytokines and decreasing proinflammatory cytokines have been found.[34] UC-II is believed to activate immune cells, promoting the production of T regulatory cells, which migrate to cartilage and promote expression of anti-inflammatory cytokines interleukin (IL)-4 and IL-10.[35,36] In human trials, significantly decreased inflammatory C-reactive protein but increased levels of the inflammatory molecule bradykinin following collagen administration have been reported,[37] indicating a complex interaction with the immune system.

Antioxidant

The molecular weight of the collagen molecule can influence its ability to act as an electron donor and ultimately its antioxidant capacity, with enzymatic hydrolysis improving its antioxidant capacity.[38] CH also contains hydrophobic amino acids, such as aromatic amino acids and histidine, which are known to have antioxidant properties; however, the exact mechanisms are still unknown.[39]

Structural remodelling

The main action of collagen supplementation is its ability to promote endogenous collagen production,[40] which is the main structural

protein in the body. Clinical trials have reported that collagen can influence structural remodelling through promotion of the cartilage matrix,[41] and an increase in bone remodelling through stimulation of amino-terminal propeptide of type I collagen (P1NP), whilst maintaining the bone degradation protein C-telopeptide of type I collagen (CTX 1).[42,43]

In addition, the presence of two peptides PRO-HYP and hydroxyprolyl-glycine (HYP-GLY) in collagen has been shown in *in vitro* studies to enhance cell proliferation and enhance the production of hyaluronic acid in dermal fibroblasts.[44,45]

Lipid lowering

Lipotoxicity can result from excess fat accumulation, and can impair numerous metabolic pathways within multiple organ systems. The most prominent of these are the insulin signalling pathways such as the novel protein kinase C pathway and the JNK-1 in adipose and muscular tissues, which if impeded can result in insulin resistance.[46] Animal models have reported reduced total cholesterol, low-density lipoprotein cholesterol (LDL-c) and triglycerides following administration of hydrolysed marine collagen peptides (HMCPs),[47] which may have an impact on several areas of the body.

Clinical uses (Figure 1)

Osteoarthritis (OA)

Osteoarthritis is the most common joint disease globally[48] and, in the absence of disease-altering medications, pain management is the only option for individuals with this disease.[49] As a result, increasing interest has developed for the potential of nutraceuticals to help manage symptoms.

Osteoarthritis is not an autoimmune disease, so mechanisms for the action of collagen in this disease have been of interest, and three have been highlighted from *in vitro,* animal studies and clinical trials. Firstly, the peptides that collagen contains may stimulate the building blocks for the cartilage matrix,[41] secondly it may influence bone metabolism,[50,51] and finally it may work via the vascular system to improve symptoms.[52–54]

Inhibition of angiotensin I-converting enzyme (ACE) and regulation of nitric oxide (NO) and soluble intercellular adhesion molecule-I (ICAM-I) have been shown in animal models following treatment with chicken CH.[52] OA is often associated with venous insufficiency[55] and low-grade inflammation,[56] which can affect the vascular system.

Twelve studies were found on the use of collagen in OA, and the majority of these concentrated on CH, although a small amount of research has been conducted using UC-II. Diagnosis and the criteria measured in the majority of the literature appear to centre around self-reported symptom severity based on the Western Ontario and McMaster Universities Arthritis Index (WOMAC) and Visual Analogue Scale (VAS), which are both universally recognised.[57]

Mixed outcomes were reported among the systematic reviews and meta-analyses. One of the meta-analyses that included five studies on collagen in OA reported improvements to pain in the short, medium and long term; however, physical function was not improved compared with placebo with either CH or UC-II.[58] A second meta-analysis of five RCTs supported this finding in only one of the pain parameters measured, concluding that OA is effective in improving symptoms of pain.[59] Two of the studies that were included had a treatment period of less than 14 weeks, which may be insufficient to observe symptom relief and could account for an improvement in only one of the assessments for pain. A third systematic review of 48 human and animal studies concluded that collagen and its derivatives had beneficial effects to patients suffering from OA in pain and physical function; however, no meta-analysis was performed.[60]

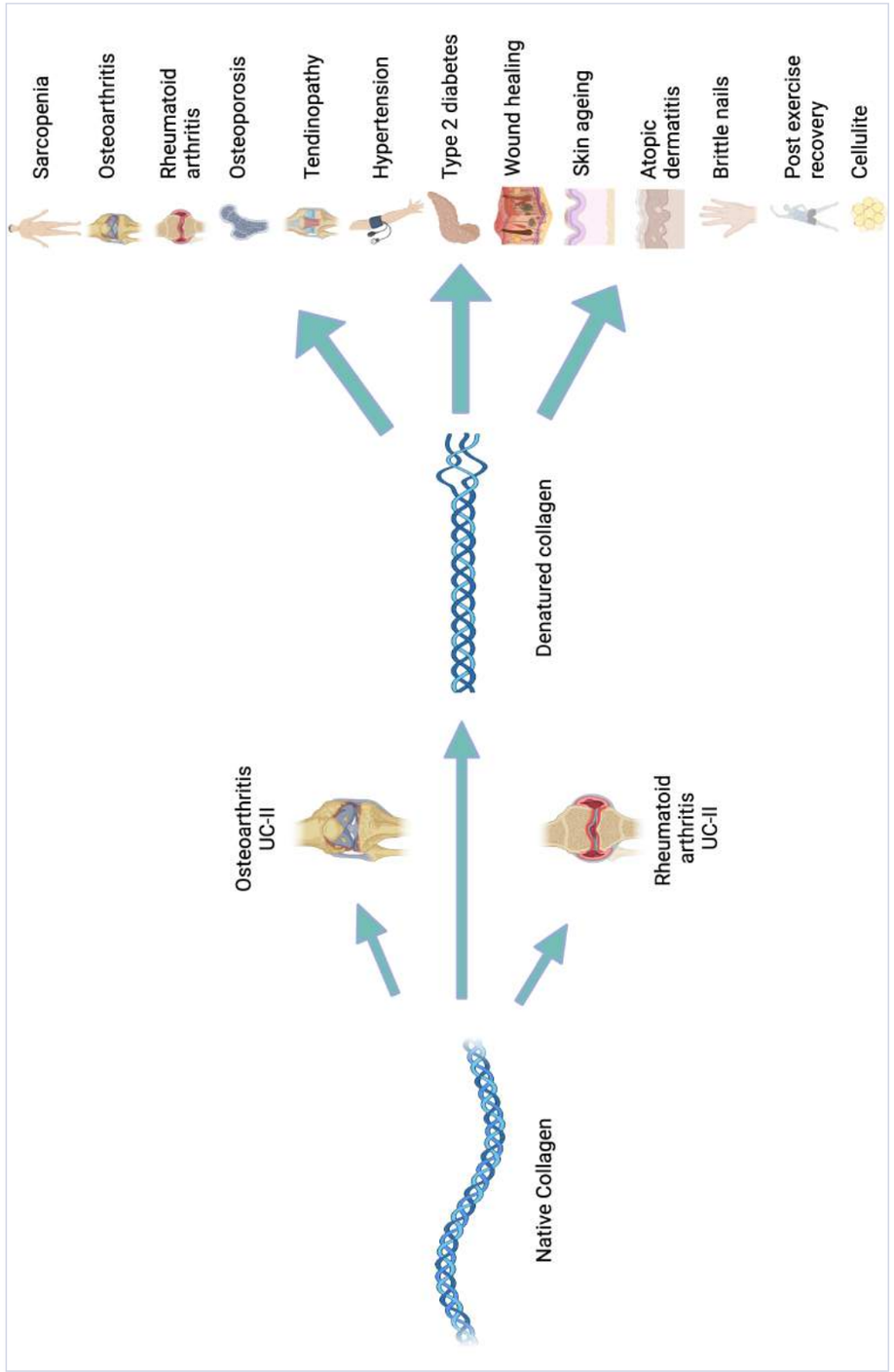

Figure 1: Areas where collagen supplementation is clinically indicated.

Native collagen in the form of undenatured collagen type-II (UC-II) has been indicated for use in both osteoarthritis (OA) and rheumatoid arthritis (RA). When chemically denatured, to produce collagen hydrolysate (CH), specific collagen peptides (SCPs), bioactive collagen peptides (BCPs) or hydrolysed marine collagen peptides (HMCPs), its use has been indicated in several disease areas.

These trials supported the use of collagen and its derivatives for the relief of OA symptoms; however, one meta-analysis of eight studies concluded that there was insufficient evidence to support the use of collagen in OA.[61] However, it should be noted that gelatin was included in this analysis, which is the unhydrolysed form of collagen, and it is thought that dietary collagen that has not been hydrolysed is poorly absorbed,[62] as previously mentioned.

Supplemental doses of CH used in the literature were mainly 10 g/day, and one RCT of 250 patients with knee OA reported significantly decreased pain and improved functioning following supplementation of 10 g CH daily over 6 months compared with placebo, results that were seen at all stages of OA.[63]

Contrary to the trial above, disease severity may dictate outcomes. One pilot RCT of 30 individuals with mild knee OA over 24 weeks reported no symptom relief with 10 g/day CH, but magnetic resonance imaging (MRI) changes were detected,[64] indicating that although symptoms may not have been relieved, internal improvements were affected. A second RCT of 389 patients supplemented with 10 g/day CH for 24 weeks reported no significant differences with placebo on measures of pain and physical function.[2] However, a subset of patients with extreme disease did report significantly improved symptoms, indicating that differences between mild and extreme disease may influence success of supplementation. Interestingly, in patients with extreme disease, symptom relief perpetuated after supplementation had ceased, suggesting long-term benefits for symptom relief.

Collagen derivatives are not the only nutraceuticals used in the relief of OA symptoms, and one RCT showed significantly reduced symptom relief with 10 g/day CH compared with glucosamine sulphate (GS) in a 13-week trial of 100 patients with knee OA, effects that were seen as early as 2 weeks after therapy commenced.[65] These results were supported in another RCT using 40 mg/day UC-II versus GS[66] and 10 mg/day UC-II versus GS plus chondroitin,[67] suggesting that collagen derivatives may have benefits above the most commonly used nutraceuticals for OA.[68]

Collagen formulations have also been investigated in relation to OA symptoms. One RCT that looked at a combination of CH (type II), chondroitin sulphate and hyaluronic acid (BioCell® collagen) containing 300 mg CH/day for 70 days was shown to improve VAS score but not WOMAC; short therapy duration was implicated in this discrepancy,[62] but this may also be related to suboptimal dosing based on the use of 10 mg/day in previous studies.

Several studies were found that focused on individuals without OA but relating to joint health. The research supported supplemental doses of CH of 1.2 g/day for improvements in joint pain but only if taken for at least 6 months,[69–71] as one study showed no symptom improvement over 12 weeks, even if 10 g/day was taken.[72]

There is a large amount of quality literature supporting the use of 5–10 mg/day CH for the long-term relief of OA symptoms especially in those with extreme disease, and these benefits may be superior to the more commonly used GS and chondroitin. Positive results for the use of 40–300 mg/day UC-II in symptom relief of OA was also evident. Research in the use of BioCell® may only be translatable when using this specific brand of supplement.

Rheumatoid arthritis (RA)

Rheumatoid arthritis is considered to be an autoimmune disorder characterised by infiltration of the joints by immune cells, attacking the collagen and resulting in chronic pain and inflammation.[33] Collagen has been shown in animal models to act as an autoantigen, supressing arthritis.[33]

When compared with standard therapy (methotrexate), collagen may not be as effective, but has less adverse events associated with it. One double-blind RCT of 211 patients with RA supplemented with 0.1 mg/day UC-II for 24 weeks reported that although improvements to pain and function were observed from the start of the trial, these were less than with standard therapy.[73] Regardless, given the improvements from the start of the trial, it was concluded that UC-II is still effective for the treatment of RA. These results, dosages and patient types were replicated in a second study of 503 patients.[74]

Stopping methotrexate supplementation in favour of collagen may result in reversal of symptom benefits. Among 92 patients with RA in a double-blind RCT, those who were first given methotrexate and then switched to UC-II 0.5 mg/day resulted in worsening of swollen joints. Those who remained on standard therapy reported no change in disease activity, indicating an inability to sustain the results of standard therapy.[75] Unfortunately no comparisons were made with a placebo group.

The previous trials indicate that UC-II supplementation may not be as effective as standard therapy; however, it could be used to enhance standard therapy. In one early RCT of 60 patients with severe RA, UC-II was reported to improve symptoms of RA after 3 months when compared with placebo, and four of the collagen participants reported complete resolution of disease.[76] Sixty-four percent of the collagen-treated patients were on standard therapies, usually methotrexate, and further improvement occurred with collagen, indicating an additive effect of collagen, which was not investigated in the previously reviewed methotrexate studies. Unfortunately, collagen dosages were not stated in the trial.

When looking at differing doses of UC-II, one double-blind placebo-controlled RCT of 274 patients reported improvements in one measure of RA symptoms, the Paulus criteria, which is a measure of joint soreness, swelling and morning stiffness, with the lowest dose of 20 µg but not at the higher doses of 100, 500 and 2500 µg.[34] However, other measures of disease improvement were not observed. The dosage used in this trial was very different to other trials.

One observational study looked at the use of low-dose UC-II in juvenile RA; however, the sample size of 13 individuals was too low to show any definitive treatment effect, but six patients who were given 60 µg UC-II and six patients who were given 540 µg reported improvements in disease symptoms. However, it was concluded that UC-II efficacy in juvenile RA may be difficult to prove.

Studies are mixed for the use of collagen in RA, maybe owing to studies focusing on higher dosages, as it has been reported that the immunosuppressive action of autoantigens is usually observed at lower doses.[77] RA did improve in certain patients, and efforts to identify individuals who may see improvements in disease are needed. Benefits were apparent for the management of RA at 20–100 µg/day, and dual therapy of UC-II and methotrexate may improve standard therapy outcomes but should not replace it in adults. If side-effects from methotrexate do preclude treatment, UC-II may be a good alternative, as it was still shown to be effective.

Osteoporosis

The complex aetiology of osteoporosis involves both modifiable and non-modifiable risk factors, such as malnutrition, lack of exercise, age and gender.[42] Current non-pharmacological strategies to maintain bone health involve supplementation with vitamin D and calcium.[78] However, as type I collagen is a component of bone,[79,80] supplementation may have some benefit.

In combination with vitamin D and calcium, 5 g/day CH was shown in an RCT of post-menopausal women to slow decreases in body bone mass density (BMD) over 12 months.[81] In a second RCT where 5 g/day SCPs was combined with calcium and vitamin D in post-menopausal women over 3 months, enhanced benefits in bone formation compared with calcium and vitamin D dual supplementation were observed; however, bone degradation remained unchanged.[43] Furthermore, in one RCT with 5 g SCPs/day, BMD was increased compared with decreased levels with placebo, and this was attributable to increased bone stimulation markers in the blood; however, bone degradation markers remained unchanged.[42]

In combination, these trials indicate the possible complementary effects of collagen, vitamin D and calcium, with collagen acting on bone formation[82] and not degradation. Increased bone formation may have implications for osteoporosis reversal; however, this needs to be better researched in the long term and in relation to bone resorption.

In contrast, an RCT that focused on supplementation of 10 g/day CH in 82 post-menopausal women did not show any differences in bone formation after 24 weeks compared with placebo, but it should be noted that this study looked at differing measures of bone formation and degradation compared with the other studies reviewed, and collagen may be specific to certain biomarkers.

There were mixed results among the few studies on the use of collagen in osteoporosis, possibly due to different measures of bone turnover throughout the studies. Research for the use of 5 g CH and SCPs in osteoporosis is promising; however, as most of the research is in combination with vitamin D and calcium, recommendations should involve dual supplementation. Also, CHs may have the ability to bind calcium ions, which improves bioavailability,[83,84] and could also account for the synergistic actions observed.

Tendinopathy

Treatments for tendinopathy focus on exercise programmes but results are inconsistent, with a large majority of sufferers failing to see results.[85] As collagen-derived peptides have been reported to modulate collagen synthesis[41] and joint-related pain,[70,71] the use of collagen in tendinopathy is promising; however, it appears that research in this area is limited.

One pilot crossover RCT on 20 individuals suffering from Achilles tendinopathy reported that 5 g/day CH for 6 months in the branded supplement TENDOFORTE® in combination with exercise improved self-reported assessments of pain and improved tendon health as indicated by ultrasound. However, tendon health also improved in the placebo group, making it difficult to determine whether improvements were because of CH or due to exercise, which is the current recognised strategy for treatment of tendinopathy.

The results above are promising for the use of 5 g CH in tendinopathy; however, it cannot be definitively concluded that collagen was solely responsible for the effects observed. Based on the current research, the recommendation of 5 g/day CH in combination with strength exercises may improve symptoms of tendinopathy.

Obesity-associated weight loss

The aetiology of obesity is complex, involving the interrelation of many physiological, psychological, genetic, sociological and economic factors contributing to development.[86] The management of weight loss involves lifestyle management, in some cases pharmacotherapy and in extreme cases bariatric surgery.[87] Diet-induced weight loss has been associated with a reduction in bone health and BMD,[88] which is where collagen supplementation may be of interest; however, no studies were found investigating this. CPs in animal studies have demonstrated the ability to regulate lipid metabolism and adipokines involved in energy intake and weight maintenance.[89]

High-protein diets have been reported to aid and help maintain weight loss in obese individuals,[90] and collagen supplements could be a nutritional strategy to increase protein intake. One RCT of 2000 mg/day skate collagen peptides in 90 overweight adults for 12 weeks reported that CPs improved body composition compared with placebo, with changes observed from 6 weeks of supplementation.[91] It should be noted that the study size was relatively small, and although the authors state that exercise levels and dietary intake were assessed at baseline, these were not stated at the end of the study and so effects may be influenced by this.

In an 8-week study of 37 overweight women, 38 g/day CH as a protein supplement compared with 40 g/day whey protein reported an increased body mass index. Furthermore, body composition in the whey protein group was significantly improved compared with CH. It was concluded that observed increased branched-chain amino acids that are involved in satiety may have been responsible for the effects observed in the whey protein group, and that collagen may not be an effective protein weight loss aid for overweight women.[92]

It cannot be ruled out that exercise levels and dietary intake were responsible for the positive results shown in the research above. Differences in the types of collagens used, the treatment cohort and the duration of study may also be responsible for the controversial results reported in the use of collagen for weight loss in overweight individuals. The main aim of the above weight loss diets was to use collagen as a high source of protein, yet collagen and its derivatives may be of low nutritional value due to deficiency or absence of some essential amino acids.[93] As a result, it is concluded that there is a lack of research for the use of collagen for weight loss.

Hypertension

Current reports suggest that 1.13 billion people suffer from hypertension worldwide,[94] indicating a need for better prevention and management strategies. Animal studies and clinical trials have indicated that CH may activate endothelial progenitor cells, modulate the renin-angiotensin aldosterone system[95] and promote vascular relaxation,[52] resulting in improved blood pressure.

One observational study showed that supplementation with 5.2 g of chicken collagen octapeptides in 15 individuals with mild hypertension resulted in significantly reduced systolic (SBP) and diastolic blood pressure (DBP) after 2 and 4 weeks.[95] In support of this, an RCT on collagen in osteoporotic post-menopausal women showed that both SBP and DBP were decreased following 5 g SCPs for 12 months; however, this was not the primary outcome measure and this cohort of patients was not diagnosed with hypertension at the start of the trial.[42] Furthermore, long treatment duration may be responsible for the resultant effects.

In contrast, an RCT of 58 people with mild hypertension receiving 2.9 g CH reported significantly decreased SBP in comparison to placebo, but no significant differences in DBP after 18 weeks.[96] Furthermore, an RCT of 13 g/day HMCPs for 3 months in 150 adults diagnosed with primary hypertension reported significantly reduced DBP; however, no differences in SBP were observed between the groups.[97] NO levels were decreased, suggesting that NO-regulated vasodilation was not responsible for this change.

Research is promising for the use of collagen for hypertension, but robust long-term RCTs with benefits to SBP and DBP are lacking. Treatment durations and type of collagens

used could account for conflicting results among the studies. The research reviewed indicates the use of at least 5 g/day CH and 13 g/day HMCPs for hypertension; however, more studies are needed in larger subject groups and those looking at dose–response in various stages of hypertension to make definitive recommendations.

Type 2 diabetes (T2D)

Type 2 diabetes is being described in the literature as a disease of epidemic proportions,[98] with an estimated 1.5 million deaths worldwide in 2019 directly due to diabetes.[99] Previous animal studies have shown promising results in the use of collagen for the reduction of hyperlipidaemia[47] and, as lipotoxicity in the pancreatic and muscle cells may inhibit insulin receptor signalling contributing to insulin resistance,[100] collagen may improve glucose metabolism through its actions on a key pathophysiological process of T2D.

In a prospective RCT of 100 patients with T2D and hypertension, 13 g/day HMCPs for 3 months resulted in improved blood sugar control compared with placebo, and these results were seen as soon as 1.5 months into the study,[101] indicating that disease progression was not simply slowed, but improved. Insulin sensitivity was increased with HMCPs; however, this remained unchanged in the patient control group. Treatment improved the lipid profile of the participants and, although causal relationships were not investigated, it could be speculated that lipid regulation improved insulin sensitivity resulting in improved blood glucose control.

A second 3-month RCT of HMCPs by the same authors of the previous study looked at 13 g HMCPs in 100 patients with non-hypertensive T2D and, in support of the findings above, blood glucose control and insulin sensitivity were improved compared with the placebo group.[37] In this study, the modulation of inflammation-related metabolic regulators was observed in HMCPs compared with control, and may contribute to the regulation of blood sugar.

The research is limited for the use of collagen in T2D; however, the literature that does exist is positive and the quality is high. What is apparent is that 13 g HMCPs may be of benefit to hyperglycaemia for a duration of at least 6 weeks.

Fibromyalgia

Fibromyalgia is characterised by widespread musculoskeletal pain, resulting from neuroendocrine dysfunction.[102]

One study was found on the use of collagen in fibromyalgia; however, this observational study over a 90-day period with 20 fibromyalgia sufferers failed to state dosages and collagen type, and although it concluded improvements to symptoms, there were no statistical analyses and the study was not blinded.[103] Therefore, it can be concluded that there is a lack of research for the use of collagen for the relief of fibromyalgia symptoms.

Wound healing

Although wounds caused by pressure ulcers or burns have very different aetiologies, both appear to include nutritional support and dietary supplements as part of their management plans.[9,104] As collagen is a major component of skin and studies involving collagen-based dressings in diabetes-related ulcers have shown efficacy on wound healing,[105] oral supplementation may be of interest.

One double-blind RCT of 16 weeks in 120 individuals suffering from pressure ulcers reported that 10 g/day CH, rich in PRO-HYP and HYP-GLY, showed improved wound healing, measures of pain and wound area compared with placebo after 16 weeks. Interestingly, this study had a third group allocated a PRO-HYP, HYP-GLY-deficient CH supplement and although no differences were found between the two CH groups, this arm did report improvements in wound healing compared with placebo,[9] indicating that improvements were regardless of amino acid content.

This was supported in a second RCT where 89 patients with pressure ulcers were given 15 g/day CH and showed improved ulcer healing compared with placebo. The cumulative improvement in ulcer healing was 96% greater in the treatment group than in the control.[106]

Different wound types were also investigated, and one double-blind RCT in 31 men with burns covering 20–30% of their body reported improved wound healing 3.7 times higher than placebo when taking 36 g/day CH and, although hospitalisation duration was not significantly lower with CH, it was deemed clinically significant. Results were attributed to better improvements in serum albumin levels observed in the CH cohort.[104]

Although not extensive, the research in the use of CH for wound healing is of high quality, and in pressure ulcers supports the use of 10–15 g CH/day for at least 16 weeks if taking the lower dose and 8 weeks if taking the higher dose. In burns, the research is also of very good quality, and supports the use of 36 g/day CH to improve wound healing and to clinically improve duration of hospital stay.

Skin ageing

The skin is the body's largest organ, with its main role being protection. It is comprised of two layers; the dermis is the deepest and collagen is its main constituent. Damage to the skin can be caused in a number of ways: UV radiation, nutrition, pollution and cigarette smoke can all result in collagen damage and subsequent extrinsic ageing.[107] Intrinsic changes involve an age-related decrease of collagen production by approximately 1% per year.[108] In tandem, this can result in the breakdown of the bonds holding the dermis to the epidermis resulting in wrinkling and decreased elasticity.[109]

Studies have shown that creams and lotions may be unable to reach the dermis to causally affect collagen production.[110,111] The aim of oral collagen supplementation is to reach the dermis from within, to restore collagen synthesis and influence the skin ageing process, and may be an alternative or additive to topical treatments. PRO-HYP derived from dietary collagen may enhance the growth of fibroblasts and promote synthesis of hyaluronic acid.[110]

Fourteen studies were found on the use of collagen and its derivatives for skin ageing during the searches, comprising one systematic review and meta-analysis, ten RCT's and three observational studies. Improvements were seen in measures of skin ageing across all dosages in one systematic review.[112] The systematic review looked at 10 publications, and reported that all of the studies on CH resulted in improved skin health looking at parameters such as moisture, elasticity, wrinkle number and dryness. Studies ranged in duration from 8 weeks to 12 months, and used doses from 500 µg to 10 g/day. No analysis was made on possible dosage effects, so it is difficult to ascertain if these exist. Data quality was assessed in this study; however, it was not commented upon and so it is difficult to ascertain the overall validity of the findings.

One triple-blind RCT of 50 women supplemented with 10 g marine-derived Vinh Wellness Collagen® reported 35% reduction in wrinkles after 12 weeks, and improved skin elasticity, hydration, radiance, firmness and reduction in wrinkles compared with placebo.[113] It was concluded that fish-derived CH may improve skin health in an ageing population. This assessment was based on a validated, subjective measure for skin appearance and clinic-trained staff questionnaires.

One observational study of 29 women supplemented with 1 g BioCell® collagen CH-II plus hyaluronic acid and chondroitin sulphate over 12 weeks reported a 76% decrease in skin dryness and a 13.2% decrease in wrinkles

from the start of the study using visual/tactile analysis.[40] Using quantifiable methods, skin collagen was shown to increase in the first 6 weeks of the study, a level that failed to be maintained to the end of the study, indicating a short-term improvement in collagen content. It was concluded that CH may affect the development of age-associated skin health.

Although the majority of the studies looked at collagen in combination or at branded collagen products, which may have implications for translatability, one double-blind RCT looked at unbranded 1 g/day CH in 64 individuals.[114] CH rich in GLY-PRO-HYP and GLY-X-Y derived from fish sources reported improved skin hydration, elasticity and wrinkling compared with placebo after 12 weeks. Improvements in skin hydration were seen as early as 6 weeks, indicating a relatively short time for results. This along with a lot of the trials was based on questionnaires, which may be subject to reporting bias.

Results from the previous trial suggest that differing peptide contents may have differing actions, and one double-blind placebo-controlled RCT of 85 individuals assessed the use of CH with differing peptides derived from fish sources.[115] Skin moisture, skin elasticity, the number of wrinkles and skin roughness were significantly improved in individuals taking a supplement high (2 g/kg) in PRO-HYP and HYP-GLY peptides compared with those taking a low (0.1 g/kg) peptide content and placebo after 8 weeks. In those taking a low-peptide-content supplement, improvements were only observed in skin moisture compared with placebo. This indicates that differing peptide contents may have differing bioactivity and benefits to skin health. PRO-HYP and HYP-GLY have been reported to be precursors for collagen production.[116]

Many of the studies reviewed used CH from fish sources; however, one placebo-controlled parallel group study of 33 individuals over 8 weeks compared porcine CH with fish, reporting no differences in improvement of skin hydration.[117] This is in contrast to other studies where the individual properties of fish-based collagen may have advantages over land-based mammals for skin health.[17,18,118]

One double-blind placebo-controlled RCT looked at the effects of topical CH application compared with 9 g/day oral supplementation with added vitamins A, C and E and zinc over a 90-day period.[119] The oral supplement acted on skin elasticity and showed more pronounced effects on pore reduction and skin hydration than the topical application, but this was not significant. It was hypothesised in the conclusion that the two treatments may work synergistically due to differing mechanisms of action.

One placebo-controlled RCT of 2.5 g/day bioactive collagen peptides (BCPs) in the branded supplement VERISOL® reported a reduction in eye wrinkles in 114 people compared with placebo after 8 weeks.[120] There was a 65% increase in skin procollagen type I and 18% increase in elastin. Improvements to eye wrinkles were seen after 4 weeks, but were even more pronounced after 8 weeks. Moreover, these effects perpetuated 4 weeks after the trial ended with an 11.5% decrease in eye wrinkle volume in the supplement group, indicating the possible long-lasting benefits of this formula.

A second RCT of VERISOL® using 2.5 g/day and 5 g/day reported improved skin elasticity and skin moisture after 8 weeks in both treatment groups; however, measurements of skin function did not achieve statistical significance in comparison to placebo,[121] indicating that although physical appearance may be improved, functional aspects of the skin were not.

Several studies were found using branded and combination formulas[122–125] and, although results were promising with regards to collagen use in skin ageing, it cannot be ruled out that the results were due to synergistic effects of the ingredients in the formula. One of the combination studies reported that although all the parameters measured were significantly improved from baseline, only two of the eight parameters measured were significantly improved compared with placebo, and this was when looking at brightness and hydration.[125]

Specific recommendations are difficult on the use of collagen for skin health due to the number of trials using branded or combination formulas. Branded formulas do not always clarify preparation and processing methods of the collagen or other ingredients that they use, which may be problematic based on the previously reviewed bioavailability data. What is apparent is that universally these trials point to the benefits of collagen and its derivatives for the appearance of skin health and functionality whether in combination with topical products or other additives, or as monotherapy. Benefits were seen with CH doses as low as 500 µg to 10 g per day, with results apparent as soon as 6 weeks, and which may perpetuate long term. The majority of the research surrounds fish collagen, and its individual environmentally driven biocharacteristics may be responsible for the benefits observed.[17,18] CH products rich in PRO-HYP and HYP-GLY may infer additional skin improvements compared with those with differing peptide characteristics. Mechanisms may include the stimulation of fibroblasts by CH bioactive peptides, free amino acids supporting the formation of collagen fibres and the production of new collagen, elastin and hyaluronic acid.[124]

Atopic dermatitis (AD)

Atopic dermatitis is characterised by decreased skin barrier function resulting in dryness and increased sensitisation to various allergens. Subsequent itching can induce inflammation, which perpetuates the condition. Given that the above studies demonstrated potential benefits to skin hydration and water loss, and its anti-inflammatory properties, dermatitis may benefit from collagen supplementation.

In a double-blind RCT of 17 patients with AD, 3.9 g/day CTP rich in GLY-PRO-HYP was shown to improve symptoms over 12 weeks, whereas regular CPs showed no symptom improvement throughout the study.[7] Analyses were not performed between the groups; however, the results highlight that differing collagen composition of the supplement may have varying benefits.

The literature points towards the use of 3.9 g/day collagen rich in GLY-PRO-HYP to improve dermatitis symptoms. However, additional research in larger sample sizes and in varying degrees of AD would be of benefit.

Gut health

The gut microbiome is an ecosystem comprising of bacteria, viruses and eukaryotes, which can modulate behaviour, energy homeostasis and nutrient processing.[126] Gut dysbiosis occurs when this highly personalised system becomes altered, resulting in reduced diversity.[126] Chronic inflammation associated with many non-communicable diseases can alter the gut microbiota and contribute to dysbiosis.[127] This may exacerbate inflammation further through the overgrowth of certain species of microbiota and the production of inflammatory signalling molecules.

Many collagen products on the market are claiming improvements to gut dysbiosis and, whilst collagen may be able to modulate inflammation, the mechanisms behind gut microbiota modulation are under-researched. One study in animal models reported that administration of CPs from fish skin to high-fat diet-fed mice resulted in a decreased abundance of the inflammatory gut bacteria *Erysipelatoclostridium* and *Alistipes*.[128] *Lactobacillus, Akkermansia, Parabacteroides* and *Odoribacter* species were all increased, which are associated with health benefits.[129–132]

Research on the use of collagen in human gut health was not evident, and so continues to be based on studies in animal models. The mechanism through which collagen could improve gut health is not evident; however, it could be hypothesised to involve modulation of inflammation and the bidirectional relationship with the gut microbiota.

Cellulite

Often seen in the thighs and buttocks, cellulite is regarded as a natural process of ageing,[133] with a complex aetiology, often resulting in negative psychological implications for those who suffer from it.

In a double-blind RCT of 105 healthy females with moderate cellulite, 2.5 g/day BCPs as the brand VERISOL® was shown to improve cellulite in women of normal weight after 3 months by 5.3% and after 6 months by 9%. This was also reflected in overweight women; however, the effect was less pronounced with a 4% improvement in cellulite after 6 months.

The research in cellulite may only be translatable if using the VERISOL® branded supplement, and supports the use of long-term therapy of 2.5 g/day BCPs to improve moderate cellulite. The effects may be more pronounced and seen earlier in women of normal weight; however, overweight women may still see an improvement with longer therapy duration.

Brittle nails

Recognised as a disorder, brittle nail syndrome is caused by impaired water binding resulting in soft, dry nails that are incapable of growth.[134] Although largely comprised of keratin, animal trials on collagen have demonstrated improvements to the epidermal barrier and nail moisture.[135] A small amount of literature was found on the use of collagen in nail disorders.

One observational study reported that supplementation with 2.5 g/day BCPs in the form of the branded supplement VERISOL® once daily for 24 weeks resulted in a 12% increase in nail growth and a decrease in the frequency of broken nails by 42%.[136] These results were further improved 4 weeks post-treatment. Assessment was conducted by a physician; however, results were observational.

Although promising, there is only a small amount of literature on the use of collagen to improve nail health, and what is available may not be translatable due to the use of a branded formulation.

Post-exercise muscle recovery and strength

Strenuous exercise can result in structural damage to skeletal muscles in the extracellular matrix, resulting in pain, swelling and reduced function.[137,138] As the extracellular matrix is predominantly collagen, supplementation may provide an interesting avenue for the improvement of muscle performance and shortened recovery times following exercise.

One double-blind RCT of 24 males supplementing 20 g/day CPs reported faster exercise recovery compared with placebo over a 9-day period; however, measures of muscle soreness and indicators of muscle damage remained comparable to placebo.[139] In support of this finding, a second double-blind RCT on 57 men supplementing 15 g/day CPs for 12 weeks during an exercise programme also reported no improvements to muscle pain following exercise compared with placebo; however, more pronounced improvements to strength were observed, but this remained insignificant.[140] Significant body composition improvements were observed with CPs, which was attributed to passive connective tissue adaptations.

Improvements to body composition were also observed in a third double-blind RCT on 77 women supplementing 15 g/day collagen during a 12-week exercise programme, with

significantly enhanced improvements to body composition compared with placebo, and improvements to hand grip strength and leg strength, although this was not significant.[141]

Myofibrillar muscle protein synthesis has been shown to be significantly increased in a parallel group study of 30 g/day CPs combined with exercise over 6 days,[142] which could account for the improvements to body composition and muscle strength observed above. However, this study compared CPs with whey protein, which reported superior muscle protein synthesis in favour of the whey protein group.

Although a small amount of research is available, it is of high quality, and indicates that the use of 15–30 g/day CPs may enhance improvements to recovery times and body composition in those undergoing an exercise programme, but not necessarily in muscle soreness. However, results may not be as pronounced as with whey protein, although this is not definitive, as only one study made direct comparisons.

Sarcopenia

Supplementation of collagen to improve the extracellular matrix could have benefits in other conditions. Sarcopenia is an age-associated decline in muscle mass and its associated functionality,[143] the onset and progression of which may be slowed by resistance exercise training,[144] but this has the potential to damage what muscle remains.

One double-blind placebo-controlled RCT was found on the supplementation of collagen in sarcopenia. This study reported that 15 g/day CPs in combination with resistance exercise for 3 months resulted in lower fat mass, and increased muscle strength, bone mass and muscular control, and this effect was more pronounced than in those who were taking placebo.[145] The placebo group also undertook an exercise regime. It was concluded that CP supplementation in combination with resistance exercise improved body composition in sarcopenic males. Based on previously reviewed trials, results could be due to improvements to OA and joint-related symptoms, allowing increased exercise.

Limited but promising research points to the use of 15 g/day collagen for 3 months to enhance the beneficial effects of exercise alone on muscular body composition in those with sarcopenia. However, as the research is currently only in men, the results may not be translatable into women, and further studies are warranted in other cohorts to make recommendations.

Safety

Collagen has been used for many years in the food and cosmetic industries due to its reported biological benefits, high biocompatibility and low side-effect profile.[93] It has been declared by the European Food Safety Authority as safe,[146] and is generally regarded as safe by the US Food and Drug Administration.[147]

Among the studies reviewed, there was a strong recommendation that collagen and its derivatives are safe to use in the conditions listed. No serious adverse events were recorded in the studies, and only one trial reported mild nausea, which was attributed to the treatment.[113]

There appear to be no contraindications for its use, other than hypersensitivity to any of the ingredients or the collagen source, and there are no known drug–nutrient interactions.[148]

Research is lacking on the effects of collagen supplementation during pregnancy, and so it is advised that it is not used during pregnancy and breastfeeding.

Table 1: Summary of findings on the clinical uses of collagen supplementation

Clinical use	Proposed mechanism	Human clinical research	Collagen type	Indicated dosage	Summary
OA	Stimulate collagen matrix,41 bone metabolism,[50,51] vascular improvements[52]	Yes	CH UC-II	5–10 mg/day 40–300 mg/day	Benefits to joint health and OA symptoms of pain
RA	Autoantigen[33]	Yes	UC-II	20–100 µg/day	Improved symptoms as adjunct to methotrexate
Osteoporosis	Bone formation[81]	Yes	CH SCPs	5 g/day 5 g/day	Improved bone formation. Majority of research in combination with vitamin D and calcium
Tendinopathy	Modulates collagen synthesis[41] and joint pain[70,71]	Yes	CH	5 g/day	Evidence when combined with strength exercises for improved pain ` and tendon health. More RCTs would be of benefit
Obesity-associated weight loss	Regulates lipid metabolism and adipokines involved in energy balance[89]	Yes	CH		Lack of evidence for its use
Hypertension	Activation of endothelial progenitor cells,[95] modulation of renin-angiotensin aldosterone system[95] and vascular relaxation[52]	Yes	CH HMCPs	5 g/day 13 g/day	Mixed results, with studies showing benefits to SBP, DBP or both. More RCT studies would be of benefit
T2D	Reduction of hyperlipidaemia[47]	Yes	HMCPs	13 g/day	Benefits to insulin sensitivity and hyperglycaemia
Fibromyalgia	None	Yes	Not stated	Not stated	Lack of clinical evidence for the use of collagen
Wound healing	Stabilisation of keratinocytes and fibroblasts in the skin[105]	Yes	CH	10–15 g/day	Improved healing in pressure ulcers and burns
Skin ageing	Restoration of fibroblasts and promotion of hyaluronic acid synthesis	Yes	CH CH rich in PRO-HYP and HYP-GLY	500 µg–10 g/day	Improved skin health, elasticity and hydration. Most research in branded formulations, which may not be translatable May be some benefits of use with topical collagen
AD	Restoration of skin moisture[113] and anti-inflammation[34]	Yes	CTP GLY-PRO-HYP	3.9 g/day	Improved dermatitis symptoms. More research would be of benefit
Gut health	Anti-inflammation[34]	No	None	None	Lack of clinical research
Cellulite	None	Yes	BCPs	2.5 g/day	Improved visible appearance. Research only in the use of VERISOL® and results may not be translatable
Brittle nails	Improved epidermal barrier and nail moisture[134]	Yes	BCPs	2.5 g/day	Increased growth and decreased frequency of broken nails Research only in the use of VERISOL® and results may not be translatable
Post-exercise recovery	Myofibrillar muscle protein synthesis[142]	Yes	CPs	15–30 g/day	Improved recovery time and body composition
Sarcopenia	Extracellular matrix support	Yes	CPs	15 g/day	Improved muscular composition Evidence to support its use to enhance the effects of resistance exercise. Research only in men

AD, atopic dermatitis; BCPs, bioactive collagen peptides; CH, collagen hydrolysate; CPs, collagen peptides; CTP, collagen tripeptide; DBP, diastolic blood pressure; HMCPs, hydrolysed marine collagen peptides; OA, osteoarthritis; RA, rheumatoid arthritis; RCT, randomised-controlled trial; SBP, systolic blood pressure; SCPs, specific collagen peptides; T2D, type 2 diabetes; UC-II, unhydrolysed collagen-type II.

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Conclusion

Collagen supplementation has been studied in multiple disease areas, and there is strong evidence for its therapeutic role in OA pain and function management, T2D, wound healing, skin ageing, and post-exercise body composition and strength. Mechanisms of action include structural remodelling in the skin and bones, acting as an anti-inflammatory, an antioxidant, and reducing lipotoxicity. Its high safety profile and biocompatibility make it an attractive therapy for these diseases and, although supplementary forms have been reported to have fewer amino acids than food sources, there is evidence that it is more bioavailable making it a more valuable source of collagen. Promising results have been seen for its use in osteoporosis, hypertension, sarcopenia, RA, tendinopathy, cellulite, AD and brittle nail syndrome but, due to these largely showing efficacy in combination with other therapies or due to branded forms used in the trials, it was concluded that more studies are needed to realise significant therapeutic potential. There was no evidence for the use of collagen in weight loss associated with obesity, gut health and fibromyalgia. It could be hypothesised that collagen may have a role in gut health through the modulation of inflammation; however, clinical trials were not evident and this indicates an avenue for new research (Table 1).

Difficulties arose in the study due to most of the research failing to comment on the type of collagen used or the preparation method, simply using the term hydrolysed collagen. As was previously reviewed, differing types and molecular weights of collagen and its peptides may have different bioactive properties. As a result it is recommended that healthcare professionals seek advice on the ingredients and preparation methods when recommending a product, and review the available data from human trials.

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Nutritional Medicine Journal

Resveratrol: A Review of Clinical Use and Efficacy

Karin Elgar

Abstract

Resveratrol is a polyphenol found in many plant foods, and in particularly high concentrations in red wine. Epidemiological studies have shown significant reductions in all-cause mortality with dietary patterns high in resveratrol, and preclinical research has identified a number of anti-inflammatory, antioxidant, anti-cancer and cardioprotective mechanisms of resveratrol.

Resveratrol has been studied in a number of conditions, including Alzheimer's disease, cognitive function, cardiometabolic conditions, osteoporosis, autoimmunity and for cancer prevention. For most clinical uses, studies are limited and conflicting results have been observed. The range of dosages used in clinical trials has also varied widely and there are some suggestions of a non-linear dose–response relationship, with high dosages potentially harmful. More clinical research is needed for practitioners to base their recommendations on.

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Affiliation: K. Elgar is with the Nutritional Medicine Institute, London, UK.
Corresponding author: Karin Elgar (email info@karinelgar.com)
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Introduction

Resveratrol is a polyphenol found in a number of plant foods, including red grapes and its products, blackberries, cacao, cranberries, peanuts and pistachios.[1] The high resveratrol content of red wine is thought to, in part, explain the 'French paradox', that is people in the South of France having a low incidence of coronary heart disease despite high intakes of saturated fat, but also of red wine.[2]

Since an article in the journal *Science* in 1997 reported anti-cancer properties of resveratrol,[3] a lot of research has focussed on its health effects and relevant mechanisms. Epidemiological studies have shown that dietary patterns high in resveratrol are associated with a significant reduction in all-cause mortality; however, findings from observational studies regarding specific conditions are often inconsistent.[4] Preclinical research has identified a large number of mechanisms by which resveratrol exerts anti-inflammatory, antioxidant, anti-cancer and cardioprotective effects.[1,4,5]

Resveratrol is poorly absorbed, and bioavailability is generally poor due to its rapid conjugation to glucuronic acid and sulphates.[2] It is thought that red wine is the most efficient way to ingest resveratrol, although resveratrol content in wine can vary widely.[1] Dose–response effects with resveratrol tend to be non-linear, indicating a complex nature of low dietary doses versus high pharmacological doses,[2] with higher dosages potentially increasing the risk of adverse effects in patients with cardiovascular disease (CVD).[6]

The aim of this paper is to review the evidence from clinical trials for the use of resveratrol in clinical practice.

General effects

Antioxidant effects

Many preclinical studies have shown that resveratrol has strong antioxidant effects through a number of pathways, including reducing reactive oxygen and nitrogen species, directly scavenging free radicals, increasing gene expression of the antioxidant glutathione, the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD), nuclear factor erythroid-2-related factor 2 (Nrf2, which regulates antioxidant pathways) and phase II detoxification enzymes (see image 1).

Anti-inflammatory effects

Resveratrol activates sirtuin 1 (SIRT1), a protein that plays an important role in the regulation of metabolism and inflammation.[2] A clinical trial has shown that over 30 days, resveratrol, 500 mg per day, can increase SIRT1 as much as caloric restriction (1000 kcal per day), which is known for its beneficial effects on inflammation and metabolism.[7] However, another clinical trial comparing calorie reduction with resveratrol did not confirm any increase in SIRT1, so more research is needed to explore this effect.[8]

Resveratrol also regulates the production of pro- and anti-inflammatory cytokines, such as tumour necrosis factor alpha (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6), and affects nuclear factor kappa B (NF-κB), which regulates inflammation and immune responses amongst other inflammatory mechanisms (see image 2).

Like other phytonutrients, resveratrol protects the plant from pathogens, in particular fungi, and ultraviolet radiation.[1] In preclinical experiments, resveratrol has also been shown to have immune-modulating properties, including modulation of both cellular and humoral immunity in the defence against pathogens.[4]

Figure 1: Activation of Nrf2 Signaling by Resveratrol

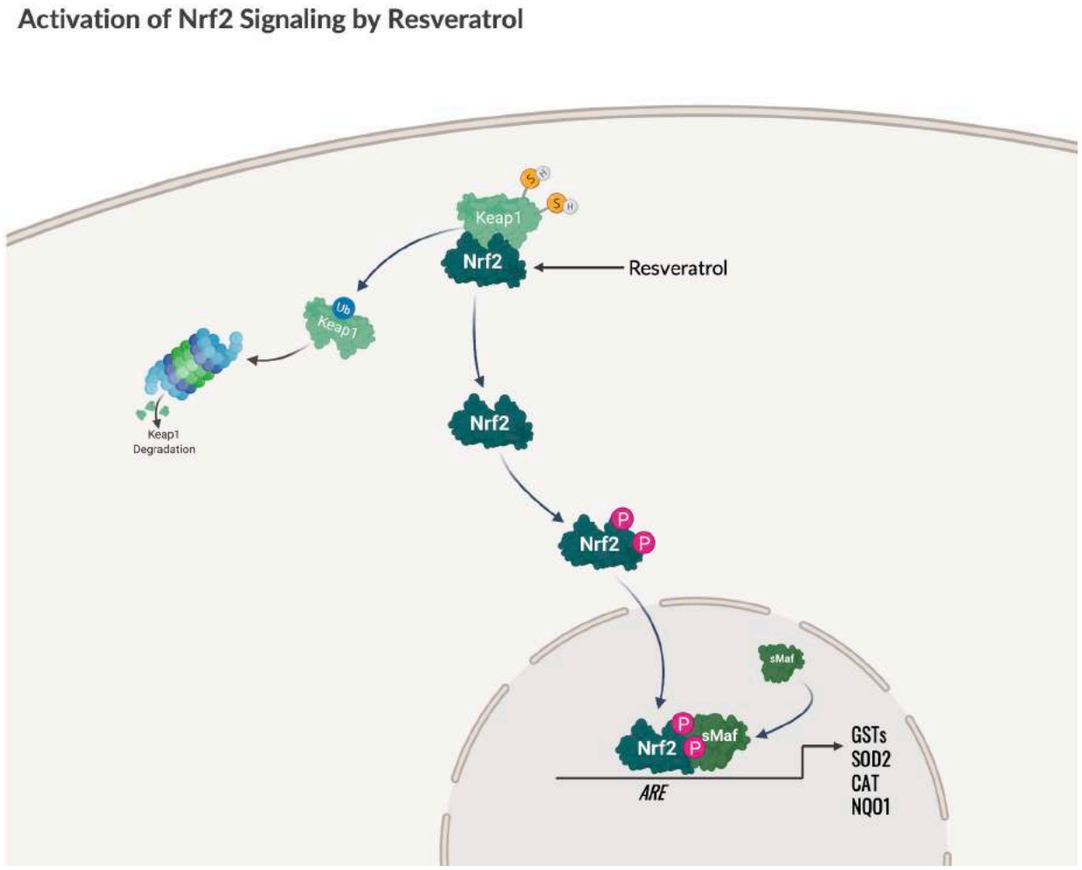

Figure 1; Resveratrol promotes the expression and function of nuclear factor-erythroid factor 2-related factor 2 (Nrf2), a redox-sensing transcriptional activator of antioxidant genes and detoxification enzymes. Under homeostatic conditions, the Nrf2 transcription factor is restrained in the cytosol by Kelch-like ECH-associating protein 1 (Keap1). In the presence of oxidative, chemical, or electrophilic stress, Nrf2 translocates to the nucleus and heterodimerizes with Maf proteins to bind antioxidant response elements (AREs) in the promoters of genes encoding antioxidant proteins, phase II detoxification enzymes and glutathione biosynthetic enzymes. Target genes involved in cellular defenses include glutathione sulfotransferases (GSTs), catalase (CAT), superoxide dismutase 2 (SOD2) and NAD(P)H quinone dehydrogenase 1 (NQO1).
Image credit: Kelly Heim, Ph.D. Created with BioRender.com.

Figure 2: Inhibition of NFκB Signaling by Resveratrol

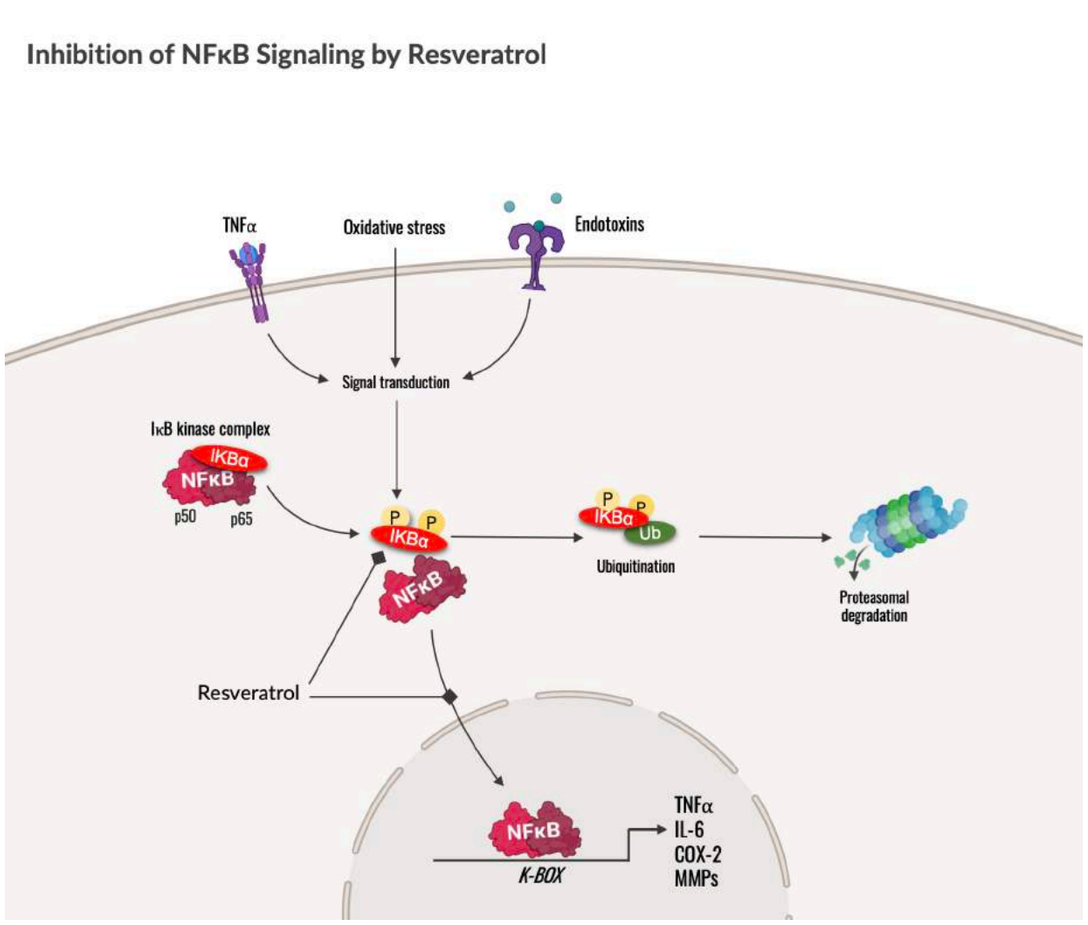

Figure 2; Resveratrol enhances cellular defenses against oxidative stress and inflammation by inhibiting nuclear factor kappa B (NFκB), a dimeric transcription factor that drives pro-inflammatory gene expression. Under basal circumstances, inactive NFκB is sequestered in the cytosol as part of the IκB kinase complex. Oxidative stress and immunological stimuli, such as pro-inflammatory cytokines (e.g., TNFα) and bacterial toxins, are perceived at the membrane via receptors that engage signal transduction and IκBα phosphorylation (yellow circles). Phosphorylated IκBα is then tagged with ubiquitin (Ub) for proteasomal degradation. This liberates NFκB to translocate to the nucleus, where it binds to DNA response elements (K-boxes) located in the promoters of target genes. DNA binding recruits the transcriptional machinery (not shown) and subsequent transcription of pro-inflammatory target genes, which include TNFα, interleukin (IL)-6, cyclooxygenase 2 (COX-2), and matrix metalloproteinases (MMPs). Resveratrol inhibits the phosphorylation of IκBα and nuclear translocation of NFκB.
Image credit: Kelly Heim, Ph.D. Created with BioRender.com.

Clinical uses

Alzheimer's disease

Worldwide, it is estimated that 50 million people live with dementia, of which 60–70% have Alzheimer's disease (AD), the most common form of dementia.[9] It is characterised by memory loss and cognitive impairment, and the main histopathological features of AD are amyloid-β (Aβ) plaques.[10] Caloric restriction is thought to prevent or delay onset of AD, possibly through activation of SIRT1.[11] As resveratrol also activates SIRT1, it has been studied in animal models of AD with promising results.[12]

One double-blind, placebo-controlled study of 104 patients with mild-to-moderate AD found statistically significant favourable effects on the biomarker Aβ40 in both blood and cerebrospinal fluid, but not on tau protein (another AD biomarker).[13] The study was too small to detect significant clinical improvements. Interestingly, brain volume decreased more in patients on resveratrol than placebo, which the authors hypothesised may be due to reduced inflammation-related swelling. A retrospective follow-up study, using frozen samples from a subset of patients with AD confirmed by biomarkers at baseline (19 in the resveratrol and 19 in the placebo group), found significant changes in a number of cytokines and other biomarkers, suggesting resveratrol modulates neuro-inflammation and may improve the integrity of the blood–brain barrier.[12] Clinical improvements were also seen in this subset of patients. The dose of resveratrol was increased gradually from 500 mg to 2000 mg per day, and the study duration was 1 year.

Another small randomised-controlled trial (RCT), looking at clinical outcomes, found that patients with AD on resveratrol deteriorated less than those on placebo over the course of the 1-year study, but this failed to reach statistical significance.[10] This study used a much lower dose, 10 mg per day.

Clinical evidence for the use of resveratrol in AD is limited and, whilst some benefits on biomarkers have been observed, data on effects on clinical symptoms are too limited to reach a firm conclusion regarding the use of resveratrol in AD. It is important to bear in mind that AD is a very complex condition and that resveratrol may work best as part of a multi-faceted approach,[14] although there is no evidence for this from clinical trials.

Autoimmune diseases

Autoimmune diseases are a leading cause of morbidity and mortality, and have been increasing over the past decades.[15] Treatment options are often limited and associated with severe side-effects. The underlying cause of autoimmunity is an imbalanced immune system that has lost the ability to distinguish self from non-self, leading to local or systemic immune responses and inflammation.[16] Whilst *in vitro* and animal studies have shown promise in a number of autoimmune diseases, human clinical trials are scarce.

One double-blind, placebo-controlled trial looked at resveratrol, 500 mg per day for 6 weeks, in patients with active mild to moderate ulcerative colitis (UC), and found significant improvements in disease activity, quality of life, oxidative stress and inflammatory markers compared with placebo.[16,17] Another study in UC is currently ongoing.[18]

Another RCT compared resveratrol, 1000 mg per day for 3 months, alongside conventional treatment with conventional treatment alone in patients with rheumatoid arthritis (RA).[19] Patients receiving resveratrol had a significant reduction in joint swelling and tenderness, disease activity score and inflammatory markers compared with those on standard treatment alone. Out of the 50 patients who received resveratrol, 41 showed a significant improvement, whilst nine were considered non-responders whose improvement in disease activity score did not reach statistical significance.

Whilst these two studies are promising for the use of resveratrol in patients with UC and RA, as well as potentially other autoimmune conditions, due to the scarcity of data it is difficult to make recommendations regarding dose or duration of supplementation.

Both RA and UC are associated with inflammation and increased oxidative stress, and the above studies suggest that the antioxidant and anti-inflammatory effects of resveratrol mediate its benefits at least in part. A small randomised pilot study in healthy volunteers also showed that resveratrol, 500 mg per day for 4 weeks, not only decreased anti-inflammatory markers and increased antioxidant status compared with controls, but also shifted the balance of T-cells (which are out of balance in autoimmunity) in a favourable way.[20]

Bone health

Osteoporosis is defined as low bone density (adjusted by age) and is an important risk factor for fractures, which can cause significant morbidity and mortality, especially in the elderly. In the USA, almost a quarter of women and over 5% of men over the age of 65 years have osteoporosis.[21] As there are no symptoms until fractures occur, it is often called a 'silent epidemic'. Resveratrol has shown promising results in preclinical research, and an emerging body of human clinical research corroborates such findings.[22]

A 2019 meta-analysis of six RCTs on bone biomarkers found that resveratrol significantly increased serum and bone alkaline phosphatase (biomarkers for bone formation).[23] Except for one study in healthy elderly people, all trials were run in patients with metabolic syndrome (metS) or type 2 diabetes mellitus (T2DM). Dosages ranged from 150 mg to 1500 mg resveratrol per day for 4–24 weeks, with dosages of 1000 mg or above showing better results.

Another study in 193 patients with T2DM showed improvements in whole-body bone mineral density (BMD), bone mineral content and T-score (a measure of bone density relative to a 30-year-old person) after 6 months in those on 500 mg resveratrol per day.[24] A study, involving 194 postmenopausal women, also found significant improvements of BMD in the lumbar spine and neck of the femur as well as a reduction in C-terminal telopeptide type-1 collagen levels (a bone resorption marker), but not in whole-body BMD, after 12 months of supplementation with resveratrol, 150 mg per day.[22] In both studies, improvements were greater in those patients whose baseline status was worse.

In view of the wide range of dosages used in clinical trials, an optimal dose is uncertain and may to some extent depend on the patient population, for example, patients with T2DM or postmenopausal women.

A number of mechanisms are thought to play a role in the bone-protective effects of resveratrol. Resveratrol is thought to reduce the activity of osteoclasts (cells that break down bone) through its oestrogenic effects and possibly also through NF-κB-mediated pathways, whilst also inhibiting adipogenesis (formation of fat cells) to favour the formation of osteoblasts (the cells that build new bone) through SIRT1-related pathways.[22,24]

Cancer

By modulating various cancer-related pathways and gene expression, resveratrol exerts a number of anti-cancer effects, including inhibition of cancer cell growth and proliferation, induction of apoptosis (programmed cell death that is abnormal in cancerous cells), promotion of anti-tumour immune responses, and prevention of migration and invasion of cancer cells.[4] Whilst preclinical research is promising, data from human trials are scarce.

One RCT in 112 men with prostate cancer, using pulverised muscadine grape skin, 500 mg or 4000 mg per day (providing 4 µg resveratrol per 500 mg) versus placebo for 4 months, found no overall effect of the supplement on doubling time of prostate-specific antigen (PSA; a prostate cancer marker).[25] However, a subgroup of patients with a particular genetic polymorphism in the gene for SOD appeared to benefit with a reduction in PSA doubling time. Another study looked at the effect of resveratrol supplementation on hormone and PSA levels in men with metS, and found a lowering of testosterone precursors, but not PSA, testosterone, free testosterone and dihydrotestosterone.[26] The dose used was 1000 mg resveratrol per day for 4 months. The authors therefore concluded that resveratrol was of no benefit in benign prostate hyperplasia (enlarged prostate, which is not thought to increase the risk of developing prostate cancer).

Three small pilot, pharmacokinetic and safety studies on resveratrol in colorectal cancer found improvements in some biomarkers: cleaved caspase-3 (a marker of apoptosis) in metastases,[27] Wnt pathways (signal transduction pathways involved in carcinogenesis) in healthy but not cancerous colon cells,[28] and reduced tumour cell proliferation.[29] Dosages ranged from 80 mg to 5000 mg resveratrol per day for 8–21 days. Two studies observed no adverse events (AEs),[28,29] whilst in the third several mild AEs were seen, mostly of a digestive nature.[27]

One RCT in 39 women at increased risk of breast cancer suggested a preventative effect of resveratrol, 5 mg or 50 mg per day for 12 weeks.[30] There was no overall improvement in biomarkers in the resveratrol versus the placebo group. However, serum levels of resveratrol and its metabolites varied significantly between individuals, and there was an association between higher serum levels and decreased methylation of a cancer-related gene, RASSF-1a, which correlated with a decrease in prostaglandin E2, an inflammatory mediator that promotes cancer. These results, and those of the Paller *et al*. study in prostate cancer mentioned above,[25] suggest that there are significant inter-individual differences in the response to resveratrol, possibly due to gene polymorphisms.

One small study with 24 patients with multiple myeloma was terminated early as five patients went into renal failure.[31] The dose of resveratrol was 5000 mg per day. The authors state that this AE appears to be specific to patients with multiple myeloma, who are at particular risk, with renal impairment occurring in 50% of patients.

Overall, there is promising research in the field of cancer, but at present insufficient evidence to make any particular recommendations.

Heart disease and cardiovascular risk factors

Cardiovascular disease is a general term for diseases that affect the heart and blood vessels, and is the leading cause of death in the USA, being responsible for one in four deaths.[32] Risk factors include hypertension, abnormal blood lipids, smoking and poor diet. Inflammation and oxidative stress play important roles in the development of CVD, so the antioxidant and anti-inflammatory effects of resveratrol should be of benefit in the prevention and/or management of CVD. As mentioned above, attention to the potential cardioprotective effects of resveratrol came through the observation of the 'French paradox', the low rates of heart disease in areas with high consumption of red wine, a rich dietary source of resveratrol.[2]

More than 20 clinical studies have evaluated the effectiveness of resveratrol on a number of cardiovascular risk factors, with inconsistent results. A recent meta-analysis of 17 studies involving 651 subjects found statistically significant benefits of resveratrol in improving the homeostatic model assessment for insulin resistance (HOMA-IR), LDL- and total cholesterol, but not any other cardiometabolic biomarkers, including blood pressure and other blood lipids and markers of glycaemic control.[33]

A meta-analysis of 17 studies evaluating hypertension found no overall benefit of resveratrol, but a subgroup of patients with diabetes receiving dosages over 300 mg per day showed a significant reduction in blood pressure.[34] Another meta-analysis of 20 studies focussed on blood lipids and found no benefit of resveratrol.[35] Similarly, a 2015 meta-analysis of 10 studies found no effect on C-reactive protein (CRP; a marker of inflammation), blood lipids, glucose or blood pressure.[36]

Looking at the individual studies used in the meta-analyses shows that there is significant inconsistency between trial outcomes, with some showing benefits, some nil effects and some even a worsening.[35,37]

A few more recent trials, not included in the above meta-analyses, on the whole showed promising results, with improvements in clinical features in patients with heart failure,[38] glycaemic control and high-density lipoprotein (HDL) cholesterol in patients with T2DM,[39,40] oxidative stress and antioxidant status in patients with T2DM,[39] atherosclerosis biomarkers in patients with T2DM,[40] and echocardiography and 24-h electrocardiogram outcomes in patients with coronary heart disease.[40] Studies used between 100 mg and 1000 mg resveratrol per day for 1–3 months.

One study found a non-significant improvement in a number of biomarkers with 300 mg resveratrol per day, but a worsening with 1000 mg per day over 3 months.[6]

The reasons for these contradictory results are unclear and appear to be unrelated to dosage. Dosages used in clinical trials have varied widely, from 10 mg per day to 1000 mg per day, and durations from 14 days to 1 year.

Overall, there appears to be a benefit of resveratrol in improving CVD risk factors, although evidence from clinical trials remains inconclusive with regards to dosages and duration as well as which populations may benefit most and for which risk factors.

Cognitive function

In animal experiments, resveratrol has been found to be neuroprotective, and to improve cognition and brain function.[41]

However, two recent reviews and meta-analyses of nine and 11 human RCTs, respectively, have only found small improvements that were not statistically significant, except for one outcome measure, delayed recognition, for which there was a statistically significant improvement.[41,42] Looking at the studies individually, five found some benefits in terms of improved memory and psychomotor speed. Different assessments and outcome measures were used across the studies. Dosages ranged from 75 mg to 1000 mg resveratrol per day or grape powder/juice, whilst duration ranged from 2 weeks to 6 months, and two studies evaluated acute effects in 1-day trials. There did not appear to be a connection between dose or duration of treatment and results.

In 2020, the results of a 24-month double-blind, placebo-controlled crossover trial were published.[43,44] One-hundred and twenty-nine postmenopausal women took either 75 mg resveratrol or placebo twice a day for 12 months, and then crossed over to the other treatment. A statistically significant 33% improvement in overall cognitive function was observed, with women over 65 years benefitting more in verbal memory, a decline of which can be an early sign of AD. Significant improvements were also seen in cerebrovascular function and insulin sensitivity, and no apparent side-effects were noted. The authors of this study conclude that the benefits may be partly mediated through endothelium-dependent cerebrovascular function, which declines with oestrogen deficiency and age. Resveratrol may act through various pathways including SIRT1 and oestrogen receptors to increase endothelial nitric oxide.[44]

Although results from clinical trials are mixed, longer-term supplementation of 12 months, with relatively low-dose resveratrol, for example 150 mg per day, may provide benefits in terms of cognitive and cerebrovascular function.

Diabetes and metabolic syndrome

There has been a significant increase in T2DM over the past decades, and it was estimated that in 2018, 13% of all US adults had diabetes, 90–95% of these having T2DM, and over a fifth not being aware that they had the disease. Based on measurements of glycosylated haemoglobin (HbA1c; a longer-term measure of glycaemic control), it was estimated that a further third of all US adults were prediabetic.[45,46] Diabetes significantly increases the risk of CVD and can also lead to a number of complications, including kidney disease, eye-related problems, neuropathy and leg ulcers.

Metabolic syndrome (metS) is not a disease as such but a cluster of cardiovascular risk factors and is closely related to T2DM. It is defined as having at least three out of the five following markers: elevated waist circumference (WC); elevated triglycerides; low HDL-cholesterol; elevated blood pressure; and elevated fasting glucose.[47]

Oxidative stress and inflammation play important roles in both the development of T2DM as well as its complications, therefore reducing oxidative stress and inflammation can be beneficial.

There are over 30 human clinical trials looking at resveratrol in T2DM or metS and related disorders (which would include T2DM), with inconsistent results.

Four meta-analyses looked specifically at T2DM. One meta-analysis found that resveratrol improved fasting glucose and insulin, HOMA-IR and blood pressure, but not HbA1c, LDL- and HDL-cholesterol.[48] Another meta-analysis in T2DM found that resveratrol improved triglyceride levels, but also led to an increase in LDL- and total cholesterol in some patient groups.[49] One meta-analysis looked specifically at levels of CRP (a marker of inflammation) in type 2 diabetics and found benefits after at least 12 weeks of resveratrol supplementation.[50] A Cochrane review and meta-analysis in 2020 came to the conclusion that: “The limited available research does not provide sufficient evidence to support any effect, beneficial or adverse, of 4–5 weeks of 10 mg to 1000 mg of resveratrol in adults with T2DM”.[51] However, this review only included three studies, compared with six–10 RCTs included in the other meta-analyses.

Benefits of resveratrol in T2DM have been reported with regards to improved bone health with 500 mg per day for 6 months,[24] and healing of diabetic ulcers with 100 mg per day for 60 days.[52] Acute supplementation of a single dose of 75 mg resveratrol has been shown to improve cerebrovascular function, neurovascular coupling (the neuronal regulation of blood flow to the brain) and cognitive function in patients with T2DM, although longer-term studies in this area are lacking.[53,54]

Only one small pilot study looked into benefits for type 1 diabetes mellitus (T1DM).[55] In this open, uncontrolled trial, 13 patients with T1DM received 1000 mg resveratrol for 8 weeks. Significant improvements in fasting glucose and HbA1c, but not other markers of glycaemic control, kidney or liver function, were seen. There was also a significant improvement in markers of oxidative stress but not inflammation.

More reviews and meta-analyses looked into the effects of resveratrol in patients with metS and related disorders, which include T2DM. A 2021 meta-analysis of 30 studies, involving 1651 participants, found significant improvements in glucose and insulin, whilst results for HOMA-IR failed to reach statistical significance.[56] Significant improvements in HbA1c were seen only with supplementation for more than 3 months in this meta-analysis,

which would be expected in view of the fact that HbA1c reflects glycaemic control over the previous 2–3 months. Subgroup analysis showed that these improvements were limited to patients with T2DM.

A meta-analysis of 31 studies, with 1722 participants, looked at blood lipids and liver enzymes in patients with metS and related disorders.[57] Whilst there was a significant decrease in total cholesterol, there were no changes in LDL- or HDL-cholesterol and, whilst two out of three liver enzymes remained unchanged, one actually increased. A meta-analysis of 10 studies involving 296 patients with metS found significant favourable effects on glucose and WC with more than 500 mg resveratrol per day taken for longer than 10 weeks, but no effect on blood lipids or weight.[58] Another meta-analysis looked at blood pressure (28 studies, 1748 subjects) and endothelial function (six studies only), and found no changes in blood pressure but significant improvements in endothelial function.[59] A meta-analysis of 24 studies (1112 patients) looking at oxidative stress and inflammatory markers in patients with metS and related disorders found significant decreases in CRP and TNF-α, but not SOD or IL-6.[60]

Overall, whilst not all individual studies report benefits, the results are promising for the use of resveratrol in T2DM and metS. Dosages in the clinical trials ranged from 8 mg to 3000 mg per day, for durations of 1 week to 1 year. Whilst longer duration appears to be more beneficial, the heterogeneity of the results does not allow at this point to suggest a dose.

The reason for the differing results is unclear and may be due to so far unknown genetic factors. One study looked at a single-nucleotide polymorphism (SNP; a genetic variation of one gene) for SIRT1 in diabetics and found that, whilst the SNP resulted in a lowered level of SIRT1 expression following resveratrol supplementation, it did not have any significant effect on any biochemical markers.[61]

One small double-blind pilot study of 28 obese men with metS found that resveratrol, 2000 mg per day for 30 days, led to a significant improvement in insulin sensitivity and glucose homeostasis in Caucasians, whereas no effects were seen in non-Caucasians, an observation that was not explained by plasma levels of resveratrol or its metabolites.[62] This study also evaluated changes in the microbiome and found that resveratrol led to a significant change in microbiome balance that differed between Caucasians and non-Caucasians, with the former having an increase in *Akkermansia muciniphila*, a bacterium that has been associated with a lower risk of obesity, diabetes and low-grade inflammation.[62] These results suggest that the microbiome composition and/or racial background may affect whether or not resveratrol exerts benefits in the individual.

The antioxidant and anti-inflammatory effects of resveratrol are thought to mediate its potential benefits in T2DM.[50] Improvements in insulin signalling (through a signalling pathway called Akt) have been observed.[63] Another study found that resveratrol upregulated SIRT1 and AMP-activated protein kinase (AMPK), an enzyme that improves insulin sensitivity by upregulating glucose transporter type 4 (GLUT4; which transports glucose into cells (see figure 3). This study also observed that whilst activity levels went down in the resveratrol group, their energy expenditure/basal metabolic rate went up, compared with the placebo group, although there were no differences in markers of glycaemic control, suggesting that resveratrol may have an exercise-mimetic effect in patients with T2DM. Finally, the study by Walker *et al*. looking at the microbiome, mentioned above, suggests that resveratrol may exert beneficial effects through affecting favourable changes of the microbiome.[62]

Figure 3: Resveratrol enhances insulin-mediated glucose disposal via the AMPK/SIRT1 Axis

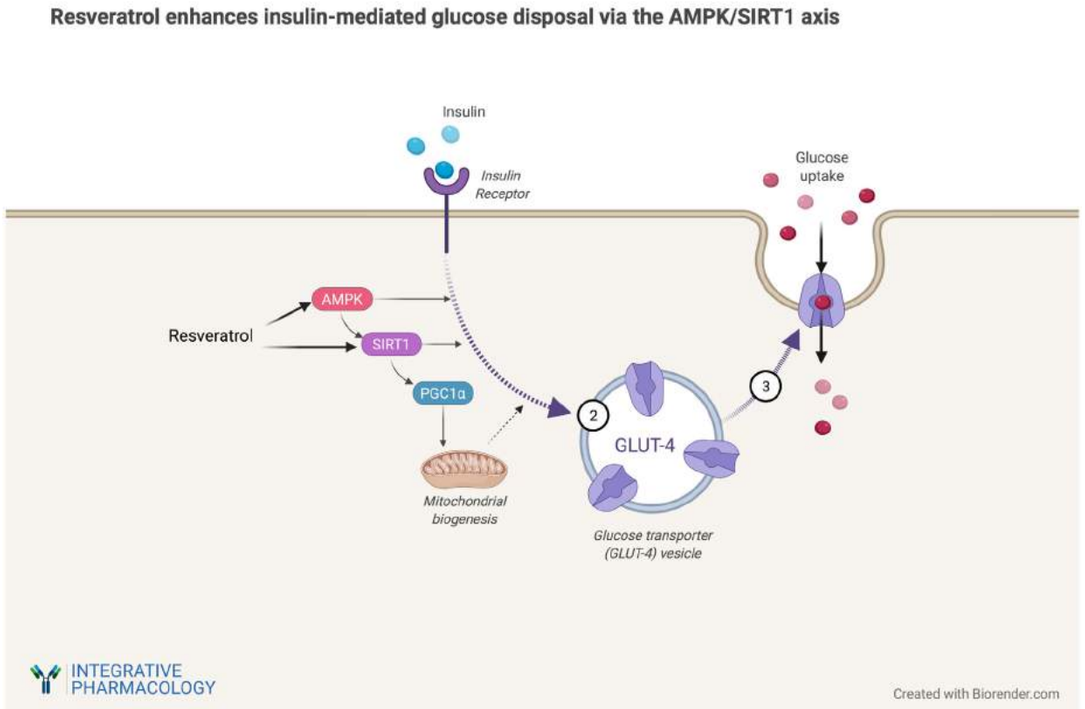

Figure 3; Resveratrol enhances insulin-stimulated glucose transporter (GLUT4) localization to the cell membrane by activating AMP kinase (AMPK), sirtuin 1 (SIRT1) and PPAR-gamma coactivator 1 alpha (PGC-1α). This pathway induces mitochondrial biogenesis, which improves in insulin sensitivity in skeletal muscle. **Image credit: Kelly Heim, Ph.D. Created with BioRender.com.**

Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is characterised by a build-up of fat within liver cells, and risk factors include obesity/overweight, metS and T2DM. NAFLD is the most common liver disease in the USA, affecting an estimated 24% of adults, and 1.5–6.5% of adults are thought to have non-alcoholic steatohepatitis (NASH).[65] The equivalent numbers in the UK are 20–30% for NAFLD and 2–3% for NASH.[66]

A number of clinical trials have evaluated the potential benefits of resveratrol in NAFLD. A meta-analysis including six studies and 266 patients with NAFLD found significant decreases in the inflammatory markers TNF-α and high-sensitivity CRP (hsCRP), but no significant changes in blood lipids, anthropometric data, glycaemic control or liver function tests.[67] Another meta-analysis that looked only at the two liver enzymes alanine transaminase (ALT) and aspartate aminotransferase (AST), involving five studies and 216 patients, found benefits in specific subgroups, people under the age of 45 years and those who are not obese.[68] This study also found favourable effects with dosages under 1000 mg resveratrol per day for longer than 12 weeks. In both meta-analyses, dosages used ranged between 300 mg and 3000 mg resveratrol per day for 8–24 weeks.

Looking at the individual studies, it appears that those using a daily dose of 300 mg or 500 mg for 3 months found more benefits, in particular with regards to ALT and inflammatory markers,[69,70] than those using higher dosages, where there were no consistent improvements.[8,71,72,73]

Overall, the evidence suggests that lower dosages of resveratrol (300–500 mg per day) may be of benefit for patients with NAFLD with regards to reducing inflammation and the liver enzyme ALT, although other biomarkers, including those for glycaemic control, blood lipids and anthropometric measures, do not appear to respond to resveratrol supplementation in this patient group.

Proposed mechanisms by which resveratrol may ameliorate NAFLD are its ability to upregulate SIRT1, which has been shown to increase insulin sensitivity and decrease inflammation, and AMPK, which is thought to have a positive effect on lipid metabolism.[64]

Obesity

Obesity is an important risk factor for a number of conditions, including CVD, T2DM and some cancers, and as such is a major public health issue.[74] In 2017–2018, the prevalence of obesity, defined as a body mass index (BMI) of 30 kg m^{-2} and over, in adults in the USA was 42.4%, and 9.2% were severely obese (BMI > 40 kg m^{-2}).[75] Obesity is associated with chronic low-grade inflammation, and adipose (fat) tissue secretes adipokines, compounds such as adiponectin and leptin, which are involved in the regulation of many metabolic processes as well as eating behaviour.[76] Resveratrol has been studied for metabolic health in overweight and obese patients.

A 2021 meta-analysis of 19 studies showed no benefit of resveratrol on weight or BMI, but a small decrease in WC.[77] Looking at the individual studies, only three of them showed benefits either on their own or together with the weight loss drug Orlistat. A 2019 meta-analysis of 28 trials found statistically significant reductions in BMI, weight and WC, although effect sizes were small, −0.17 kg m^{-2}, −0.51 kg and 0.79 cm, respectively.[78] Yet another meta-analysis of 36 studies also found statistically significant reductions in weight, BMI and WC, but effect sizes were even smaller.[79] Across all the studies, resveratrol dose ranged from 8 mg to 3000 mg per day, for 1 week to 1 year. Smaller dosages (less than 200 mg[79] or 500 mg[78]) appeared to be more effective, duration had to be at least 12 weeks to see effects, and people with obesity and/or T2DM were more likely to see benefits.[78,79]

Whilst one of the above meta-analyses found no effect of resveratrol on adiponectin or leptin,[79] another meta-analysis looking specifically at adipokines found a statistically significant effect on adiponectin but not leptin.[76]

Whilst results from clinical trials are mixed, resveratrol does not appear to have a clinically significant effect on weight loss.

As for possible mechanisms for resveratrol in weight loss, one study found that resveratrol may increase basic metabolic rate/energy expenditure.[64] This has also been observed in animal studies.[79] One small study looked at the effect of resveratrol on growth hormones (GHs) in obese men, but found no effect on circulating levels of insulin-like growth hormone-1 (IGH-1) or GH signalling in human muscle and fat cells.[80] Another small mechanistic study looked at adipose tissue morphology and gene expression in obese men, and saw a significantly decreased size of fat cells and an upregulation of various genes, including some involved in cellular lipid metabolism.[81]

Safety

A 2014 review on metabolic, biological and potential toxic effects of resveratrol reported in clinical trials concluded that resveratrol is generally well tolerated, and that with a daily dose of 500 mg or with durations of less than 1 month no AEs have been observed.[82] With higher dosages some AEs were reported, which were generally mild and transient, and most commonly affected the digestive system, including diarrhoea, abdominal pain, nausea and flatulence. At a very high dose (5000 mg per day for 2 weeks), chills, lethargy, rash, skin irritation and vascular flushing have been observed, in addition to gastrointestinal side-effects, in patients with metastatic colorectal cancer.[27] In patients undergoing peritoneal dialysis, receiving either 150 mg or 450 mg resveratrol per day for 12 weeks, constipation, headache, muscle cramps, fatigue and memory loss have been reported, which led to discontinuation of resveratrol in four patients, although the authors did not state whether they considered these AEs as related to resveratrol or which dose they appeared with.[83]

One serious AE of fever and low white blood cell count occurred in a patient with NAFLD after taking 1500 mg per day for 10 days and recurred on rechallenge.[71] As mentioned above under cancer, five cases of kidney failure have been seen in patients with multiple myeloma following the use of resveratrol, 5000 mg per day, but this serious side-effect is thought to be specific to this patient population.[31]

Shaito *et al*. discuss possible harmful effects in their 2020 review, and suggest that resveratrol may have a biphasic effect on cellular redox status (the balance between pro- and antioxidants), exerting antioxidant effects at low and pro-oxidant effects with high dosages, at least in *in vitro* and animal experiments.[84] This has in fact been observed in some studies, where low dosages had better outcomes than higher ones, and can be explained with the concept of hormesis, where a beneficial effect is caused by exposure to low doses of an agent known to be toxic at higher doses.

Drug and disease interactions

At a dose of 1000 mg per day or more, resveratrol was reported to inhibit some cytochrome P450 isoenzymes, including CYP3A4, CYP2C9 and CYP2D6, while activating CYP1A, which may lead to interactions with many drugs that are metabolised through these enzymes.[84,85]

Resveratrol is thought to have anti-platelet effects, and should therefore be used with caution in people with bleeding disorders or on blood-thinning medication.[85]

Pregnancy, breastfeeding and children

Resveratrol is considered to be safe in children, and pregnant and breastfeeding women in amounts typically found in foods.[85] There are no clinical trials investigating oral supplementation with resveratrol in these populations.

Conclusions

At present, the evidence base for the use of resveratrol in clinical practice is limited, and more research is needed to inform the choice of doses and patient populations that may benefit most. Human clinical research suggests good potential for resveratrol for bone health, limited but promising evidence for its use in AD, autoimmune disease (UC, RA) and cancer prevention (breast, colorectal). Evidence is mixed/contradictory, but overall promising for CVD, cognitive function, NAFLD and T2DM/metS. Generally, resveratrol is considered well tolerated and safe for clinical use.

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Nutritional Medicine Journal

Vitamin D: A Review of Clinical Use and Efficacy

Karin Elgar

Abstract

Vitamin D_3 is produced in the skin on exposure to ultraviolet B radiation, and is metabolised in the liver and kidneys to the biologically active form of vitamin D that binds to vitamin D receptors. Vitamin D was first recognised for its importance in calcium metabolism and therefore bone health, with the classic deficiency disease being rickets in children and osteomalacia in adults, but is now also known for its importance in modulating immunity.

Epidemiological studies have linked vitamin D deficiency to many conditions, including heart disease, cancer, allergies and autoimmunity. Vitamin D supplementation trials have confirmed benefits for some conditions, including atopic dermatitis, chronic urticaria, colorectal cancer, depression, polycystic ovary syndrome and type 2 diabetes mellitus, but not others, such as multiple sclerosis, prevention of allergic sensitisation in infants and psoriasis. Whilst there is some evidence of benefits for cardiovascular risk factors, this does not translate to a reduction in cardiovascular events in clinical trials.

Vitamin D is generally considered safe, and the upper limit set by the National Institute of Health is 4000 IU per day. The main safety concern with vitamin D is hypercalcaemia, based on its role in calcium metabolism, and caution is therefore advised in conditions and with medications that can also affect calcium metabolism.

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Affiliation: K. Elgar is with the Nutritional Medicine Institute, London, UK.
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Introduction

Vitamin D_3, or cholecalciferol, is the natural form of vitamin D that is produced in the skin on exposure to ultraviolet B radiation and is also found in some foods, in particular oily fish.[1] Other food sources include eggs and milk products, but levels in these foods are low. Vitamin D_2, or ergocalciferol, is of plant or fungal origin.[2] Although we can get vitamin D through our diet, it is strictly speaking not a vitamin but a steroid hormone.[3] Vitamin D_3 is metabolised in the liver to 25-hydroxyvitamin D_3, also called calcifediol or calcidiol, which is converted further in the kidneys to 1α,25-dihydroxyvitamin D_3, the biologically active form of vitamin D that binds to vitamin D receptors (VDRs).[4]

Vitamin D was first recognised for its role in calcium and phosphorous metabolism, and thus playing an important role in bone mineralisation, with the classic vitamin D deficiency (VDD) diseases being rickets in children and osteomalacia in adults. Over the past decades, however, it has become clear that VDRs are expressed in almost all cells,[2] and that vitamin D has many important physiological roles, in particular in terms of modulating the immune system.[5]

Michael Holick, a well-known expert on vitamin D, defines VDD as a vitamin D level of below 20 ng/ml (50 nmol/l),[a] insufficiency as 20–29.9 ng/ml (50–74.9 nmol/l) and sufficiency as 30 ng/ml (75 nmol/l) or more, with levels of 150 ng/ml or higher potentially toxic (Table 1).[1] These cut-off values are commonly used in vitamin D research and, unless otherwise noted, have been used in the research quoted in this paper. Some authorities, however, set lower cut-off points, for example the National Institute of Health (NIH) in the USA considers levels of 20 ng/ml and higher as adequate for healthy people, and a level of over 50 ng/ml as potentially harmful,[6] whilst the National Institute for Health and Care Excellence (NICE) in the UK defines VDD as a level of less than 10 ng/ml.[7]

Table 1: Serum 25(OH)D reference ranges and lab equivalents[2,10]

Range	SI Units	Conventional
Deficiency	< 50 nmol/l	< 20 ng/ml
Insufficiency	52–72 nmol/l	21–29 ng/ml
Sufficient	> 75–150 nmol/l	> 30–60 ng/ml
Toxicity	> 374 nmol/l	> 150 ng/ml

Recommended ranges vary. In the UK, NICE recommends > 25 ng/ml, while others including The Endocrine Society, National Osteoporosis Foundation, International Osteoporosis Foundation, American Association for Clinical Endocrinologists, and the American Geriatric Society recommend > 30 ng/ml.

Epidemiological studies have linked VDD to many conditions, including heart disease, cancer, allergies and autoimmunity,[5] but evidence from vitamin D supplementation trials is contradictory and does not confirm a protective effect for various conditions.[8] Whilst epidemiological studies have established associations between vitamin D levels and disease, they cannot prove cause and effect. Vitamin D levels may be a proxy for other factors, such as exposure to sunshine, which has also been shown to exert other biological effects, including nitric oxide, serotonin and melatonin production and regulating circadian rhythm.[9,10]

Many intervention studies have therefore tried to establish whether vitamin D supplementation can help prevent or treat various conditions, which will be discussed in the section *'Clinical Uses'*. For many conditions, results have been contradictory. Such inconsistencies may be explained by a number of limitations with regards to study designs.

[a] 1 ng/ml is equivalent to 2.5 nmol/l.

Limitations in clinical trials on vitamin D supplementation

Some, but not all, studies have established vitamin D levels at baseline and after supplementation. Without this information it is difficult to know whether or not supplementation was effective in raising vitamin D levels appropriately.

Even where this was done, measuring vitamin D levels to establish deficiency or sufficiency has been shown to be fraught with problems.[4] The activated forms of the vitamins have short half-lives (4–6 hours) and concentrations in blood are very low, making them difficult to measure. 25(OH)$D_{2/3}$ have much longer half-lives (25 days) and are therefore usually measured, although there is great variability in results, which is to some extent based on different methodologies being used and also due to the fact that vitamin D is bound to carrier proteins in blood.[4]

Vitamin D dosing regimens have also varied widely from daily dosages of 200 IU to bolus doses of up to 500 000 IU every 3 months or just once. Most studies have used oral vitamin D_3, but some have used vitamin D_2, active metabolites or analogues, either orally or in injectable forms. Unless discussed specifically under the respective condition, there was no clear trend as to whether certain dosing regimens were superior to others. Also, unless otherwise noted, throughout this paper the term 'vitamin D' refers to vitamin D_3.

Finally, patient populations have varied and there are known differences amongst certain patient groups, for example, it has been shown that obese people tend to respond less well to supplementation.[11] There are also significant inter-individual differences in response to vitamin D supplementation and vitamin D metabolism.[5] There is a growing body of evidence that certain genetic single-nucleotide polymorphisms (SNPs; spontaneous alterations of particular genes) are involved in vitamin D metabolism, leading to a much higher requirement for people with specific SNPs to see benefits.[5] The discussion of this topic is outside the scope of this white paper.

The aim of this paper is to review the evidence from clinical intervention trials on the effectiveness of vitamin D in either preventing or reversing conditions commonly associated with vitamin D.

General effects and mechanisms of action of vitamin D

Vitamin D exerts its effects mostly on the genetic level in that it binds to a VDR, which is a transcription factor. That is, on activation through vitamin D, it binds to specific sites in the DNA, regulating the expression of hundreds of genes in a tissue/cell-specific fashion.[2]

Calcium metabolism

Together with parathyroid hormone (PTH), vitamin D is essential for the maintenance of serum calcium concentrations that have to be within a narrow range for normal cellular function, in particular of the nervous system. When serum calcium levels fall, vitamin D triggers the expression of genes that increase absorption of calcium in the intestine and reabsorption through the kidneys as well as releasing calcium from the bones when dietary intake is low.[12] In a similar way, vitamin D and PTH are also involved in phosphorous metabolism.[13] As such, vitamin D plays an important role in bone mineralisation and with that in bone health.

Immune modulation

Vitamin D receptors are expressed on almost all immune cells, and vitamin D is essential for the proper functioning of both the innate and the adaptive immune systems. Whilst it promotes innate immunity, the first line of defence against pathogens that is non-specific, it appears to have a more inhibitory or regulatory effect on the adaptive, acquired immune system, mostly through regulating gene expression.[2] Some evidence also suggests that vitamin D may modulate immunity directly, i.e. without regulating gene expression, through stabilising endothelial membranes.[5]

Vitamin D appears to have an immune-regulatory effect by moving the immune system from a pro-inflammatory to an anti-inflammatory milieu.[14] It shows benefit in infectious conditions as well as in conditions with excessive inflammation, such as allergies and autoimmunity.

Clinical uses

Allergy/atopy

Due to its known immune modulatory properties and epidemiological research showing that vitamin D levels are lower in patients with allergic conditions, vitamin D supplementation trials have looked into its effects on both prevention and treatment of allergic and atopic conditions.

Prevention of allergic sensitisation in infants

Two trials investigating vitamin D supplementation of infants in the first year of life found no protective effect on the development of allergies, with either 400 IU or 1200 IU vitamin D per day.[15,16] In fact, infants in the high-dose supplementation group had a higher risk of milk allergy compared with the low-dose group, and high vitamin D levels in cord blood at birth was associated with an increased risk of allergic sensitisation at 12 months.[16]

A review from 2018 also found no evidence for beneficial effects of vitamin D supplementation for either the pregnant or breastfeeding mother or the infant, although the number and quality of studies reported here were considered to be low.[17]

Allergic rhinitis

There appears to be an inverse relationship between vitamin D levels and allergic rhinitis, although study results are inconsistent.[18,19]

One vitamin D supplementation randomised-controlled trial (RCT) found benefits in terms of a significantly reduced allergic rhinitis symptom score in adults with VDD at baseline with a dose of 50 000 IU per week for 8 weeks alongside the antihistamine cetirizine.[20] Another RCT found benefits of reduced symptoms and medication use in children aged 5–12 years with allergic rhinitis with 1000 IU per day for 5 months.[21] Interestingly, in this study none of the children was VDD at baseline, and vitamin D levels at the end of study were similar in both the vitamin D and placebo groups. However, there was a much greater increase in vitamin D level from a lower baseline in the vitamin D group.

Whilst evidence is limited, vitamin D alongside standard treatment appears to have additional benefits for adult and paediatric patients with allergic rhinitis. The best levels of supplementation may depend on whether or not patients are VDD at the outset.

Asthma

Asthma is a chronic inflammatory condition of the airways characterised by symptoms of attacks of shortness of breath, chest tightness, wheezing and coughing.[22] There is evidence for a link between asthma and vitamin D from both epidemiological and animal research, which is thought to be mediated through its effect on innate and adaptive immunity.[23]

A Cochrane review in 2016 pooled data from nine double-blind RCTs, seven of those in children and two in adults, and found significant reductions in exacerbations requiring either systemic corticosteroids, visits to the emergency department or hospitalisation.[24] No effects were seen on forced expiratory volume in 1 second (FEV1; a measure of respiratory function) or Asthma Control Test scores. The evidence was

judged to be of high quality. Several more meta-analyses, using largely the same set of trials, have come to the same conclusions.[23,25,26]

Effects appear to be limited to patients with low levels of vitamin D (< 25 nmol/l) at baseline.[25,26] Dosing regimens, on the other hand, did not appear to affect results, although they have varied widely, from 600 IU daily to 60 000 IU monthly, or combinations of a large bolus dose followed by low-dose daily supplementation.[23,25,26]

Since these meta-analyses were done, a number of additional RCTs have been published with inconsistent results. Two studies in children failed to show significant improvements in number of exacerbations, although numbers of children with exacerbations were small in both studies, which may have affected statistical power,[27,28] whilst another trial in children was terminated early due to futility.[29] One study in children compared a bolus vitamin D_2 injection followed by low-dose (400 IU vitamin D_3 per day) oral supplement with oral supplementation of 400 IU vitamin D_3 per day alone, and found that the bolus improved outcomes at 3 months more in severely deficient children, but there were no long-term differences in outcomes.[30] Another study, looking at dosing regimens in asthmatic children, found that oral bolus supplementation with 100 000 IU once in fall and once in winter only achieved sufficiency in just over 50% of children with insufficient levels at baseline (none of the children was classed as deficient).[31] Asthma-related outcomes were no different to the placebo group in this study.

On the other hand, two RCTs have found benefits in adults with asthma and vitamin D insufficiency/deficiency with regards to Asthma Control Test, quality of life, number of attacks and oral steroid use,[32] and respiratory function and inflammatory markers,[22] respectively. Patients received calcifediol 16 000 IU per week for 6 months and a single dose of vitamin D, 300 000 IU intramuscularly, respectively.

Overall, whilst evidence is inconsistent, there appears to be a benefit at least for those patients, both adults and children, who have insufficient vitamin D levels. A variety of dosing regimens appears to be beneficial.

Eczema/atopic dermatitis (AD)

Eczema or AD is an inflammatory condition of the skin that can cause itching, and severity of disease is associated with quality of life. It is thought to be due to a dysfunction of both the innate and adaptive immune systems, and issues with the barrier function of the skin.[33] Observational studies have shown that low serum levels of vitamin D are associated with AD.[34]

Three meta-analyses including three to four clinical trials all came to the conclusion that vitamin D supplementation can reduce the severity of AD.[33,34,35] Dosages used across the studies varied from 1000 IU per day to 6000 IU per day for up to 3 months.

Since then, three RCTs in children have been published that were not included in the meta-analyses. Two of them found significant improvements in severity scores of AD,[36,37] whilst one found no significant improvement over placebo.[38] Children in the latter study, however, had a mean vitamin D level of 47.1 nmol/l at baseline, with no difference in baseline vitamin D levels between the vitamin D and the placebo groups. The former two studies on the other hand included only children who were vitamin D deficient or insufficient. An RCT including children and adults with AD found that patients who had vitamin D levels of > 20 ng/ml had lower severity scores than those with lower vitamin D levels, whilst there was no difference between those who had levels of 20–30 ng/ml versus those with levels above 30 ng/ml.[39] These findings were regardless of whether or not that vitamin D level was achieved through supplementation, although all of the participants receiving vitamin D had levels > 30 ng/ml, whilst none of the participants on placebo did, but 41% of the latter had levels of 20–30 ng/ml.

Overall, the evidence, mostly in children, suggests that vitamin D is of benefit to patients with AD, at least for those with VDD. Dosages ranged from 1000 to 6000 IU per day, and study duration was mostly 3 months.

Autoimmunity

The NIH in the USA estimates that up to 23.5 million Americans have autoimmune diseases, making it one of the most prevalent group of diseases in the USA.[40] Worldwide, incidence and prevalence of autoimmunity have increased by 19.1% and 12.5% per year, respectively, over the past 30 years.[41] Epidemiological research has associated vitamin D levels with many autoimmune conditions.[42] However, only a few autoimmune disorders have a significant body of intervention trials to establish whether vitamin D supplementation confers a benefit.

Chronic urticaria (CU)

Chronic urticaria is characterised by continuous wheals, with or without angioedema (a swelling underneath the skin), lasting for more than 6 weeks. Whilst its exact causes are unknown, it is thought to be an autoimmune condition.[43]

Several studies, including both double-blind randomised, controlled and uncontrolled studies, have evaluated vitamin D supplementation in patients with CU and all of them found significant improvements in urticaria severity and/or quality of life, either against placebo or baseline (in uncontrolled studies).[14,43,44,45,46] The severity of symptoms was reduced by 50% or more in some studies.[44,45] Most studies included patients with VDD, but benefits have also been seen in patients without VDD.[46]

Two studies also found improvements in inflammatory markers,[14,44] although this only reached statistical significance in one of them.[14]

Dosing regimens have varied widely in these studies, from 600 IU daily to 300 000 IU per month (equivalent to about 10 000 IU per day), and positive effects were observed with all regimens. Study durations ranged from 8 to 12 weeks. Where low versus high doses of vitamin D were compared, the higher dose achieved better results.[43,46]

Based on current research, a dose of 4000 IU per day orally, either taken daily or at greater intervals, could be suggested for people with CU, in particular for patients with low levels of vitamin D, whilst monitoring vitamin D status regularly.

Inflammatory bowel disease (IBD)

Inflammatory bowel disease is an auto-inflammatory condition characterised by inflammation of the gastrointestinal tract (GIT), and encompasses mainly Crohn's disease (CD; which can affect any part of the GIT) and ulcerative colitis (UC; which only affects the colon). VDD is common in patients with IBD, although it is unclear whether this is a cause or a consequence of the disease.[47]

Two recent reviews and meta-analyses, one reviewing 12 and one 18 RCTs, evaluated the benefits of vitamin D supplementation on vitamin D levels and clinical outcomes.[47,48] Although not all studies could be included into the meta-analyses, both reviews concluded that vitamin D supplementation led to significant improvements in disease severity and inflammatory markers,[47] and in relapse rate,[48] respectively, but in the latter study improvements in high-sensitivity C-reactive protein (hsCRP) failed to reach statistical significance and no improvements were seen in erythrocyte sedimentation rate (ESR; a non-specific measure of inflammation).

An RCT in both UC and CD patients found that low-dose vitamin D supplementation (500 IU per day) for 6 months significantly reduced the incidence of respiratory tract infections (RTIs) but not influenza in those with vitamin D levels below 20 ng/ml.[49] Interestingly, this study found a worsening of UC symptoms in those patients with vitamin D levels of over 20 ng/ml at baseline, whilst no change in disease severity was observed in any other subgroup.

Several more recent studies found significant improvements in UC disease activity and severity, quality of life, oxidative stress, markers of angiogenesis (the formation of blood vessels, which is involved in the disease process) and some, but not all, inflammatory markers with vitamin D supplementation.[50,51,52,53,54] Effective dosages have ranged from 2000 IU per day to a single dose of 300 000 IU intramuscularly and a daily dose of 60 000 IU for 8 days, with duration of follow-up being mostly 3 months.

A double-blind, placebo-controlled trial found no benefits of vitamin D, 25 000 IU per week for 26 weeks, on recurrence rate in patients with CD who had undergone a resection of part of their GIT.[55] Mean vitamin D levels at baseline were in the insufficient range, 42 nmol/l and 43 nmol/l in the vitamin D and placebo groups, respectively, and almost doubled in the vitamin D group upon supplementation.

Overall, the evidence suggests a benefit of vitamin D supplementation in IBD, especially UC, with a range of dosage regimens showing good results. One should consider at least 2000 IU per day whilst monitoring vitamin D levels to ensure patients achieve adequate levels.

IBD in children

In children, IBD can lead to malnutrition and impaired bone formation.[56]

Several RCTs in children with IBD have found benefits of vitamin D supplementation in terms of disease severity, inflammatory markers and bone mineral density (BMD).[57,58,59,60] A number of studies have evaluated the efficacy of 'stoss' therapy (single high dose) in this population, and have found this regimen to be safe and effective in raising vitamin D levels, and improved clinical outcomes were reported.[58,61,62] One study compared 50 000 IU per week for 6 weeks with a single dose 300 000 IU vitamin D and found significantly higher levels of vitamin D at 12 weeks with the weekly regimen, 40.4 ng/ml versus 29.8 ng/ml.[61] Another study found vitamin D levels exceeded the safe level of 250 nmol/l in four out of 23 children who received stoss therapy (dose dependent on age) at 1 month, but no symptoms of vitamin D toxicity were observed in any of the children, including one of 20 children whose calcium level was determined and who developed elevated calcium levels at 2 weeks, which normalised again 10 days later.[58] Two studies found significant improvements with 2000 IU per day in children with or without VDD,[57,59,60] despite the fact that in one of them less than 10% of children reached vitamin D levels of > 32 ng/ml.[60]

Overall, vitamin D supplementation at a dose of at least 2000 IU per day in children with IBD appears to be warranted, especially in children who are VDD. Weekly dosing or greater dosing intervals also appear to be beneficial and safe.

Multiple sclerosis (MS)

Multiple sclerosis is an autoimmune disease affecting the central nervous system through a reaction against the myelin sheaths that protect nerve cells and are important for signal transmission along neurons. Symptoms include fatigue, visual disturbances (through affecting the optic nerve), paraesthesia (abnormal sensation of the skin), muscle spasms, weakness and stiffness, pain and mobility problems, and can lead to severe disability. Epidemiological studies have shown an inverse relationship between vitamin D status and disability in patients with MS, i.e. the higher the vitamin D status the lower the disability.[63]

Three recent meta-analyses of six to 12 RCTs have evaluated the effectiveness of vitamin D supplementation in patients with MS, and have found no benefits on disability score, relapse rates or radiological signs.[64,65,66] In fact, one meta-analysis found a worsening of relapse rate in the vitamin D compared with the control group.[65] Dosages in most trials were 4000 IU per day or higher, up to 40 000 IU per day, for durations of 6 months or more.

A recent double-blind study not included in the above meta-analyses also found no improvements in clinical or radiographical measures with either high- or low-dose supplementation (20 400 IU every other day versus 400 IU every other day), despite the fact that vitamin D levels were raised to three times the level in the high-dose group (65 ng/ml versus 22 ng/ml).[67]

The RCTs looking at biomarkers of inflammation or MS-specific markers on the whole have also not found any consistent benefits of vitamin D supplementation.[68,69,70,71]

At this point the evidence suggests that vitamin D supplementation, especially of high doses, is of no benefit in MS, a surprising finding in view of the epidemiological data and popularity of vitamin D supplementation within parts of the MS community.

Psoriasis

Psoriasis is an autoimmune condition of the skin characterised by excessive proliferation of cells in the epidermis, which leads to red, flaky patches of skin covered with silvery scales. Topical vitamin D or vitamin D analogues have been used in the treatment of psoriasis since the 1990s, and this is backed up by a significant body of clinical research.[72] For oral vitamin D, on the other hand, there are only a handful of clinical trials.

A meta-analysis of four double-blind, placebo-controlled trials found no benefit of oral vitamin D on psoriasis severity.[73] Only one of the individual studies saw significant benefits over placebo. This trial used 60 000 IU every other week for 6 months,[74] whilst two of the other studies used a monthly regimen of 100 000 IU and one a very low dose (40 IU per day).

Overall, the current evidence does not support the use of oral vitamin D for the management of psoriasis, although different dosing regimens, for example daily supplementation, have not been explored in clinical trials.

Rheumatoid arthritis (RA)

Rheumatoid arthritis is characterised by an autoimmune attack against the synovial cells, the cells lining the joints, causing painful, swollen and stiff joints, and can over time lead to joint damage. Epidemiological research has shown an inverse relationship between vitamin D levels and disease activity, and that vitamin D levels are lower in patients with RA than in healthy controls.[75] High-dose vitamin D, up to 600 000 IU per day, has been used to treat RA as early as the 1930s, although toxicities have often been observed.[76]

A 2020 meta-analysis of six trials involving 438 patients with RA found significant improvements in Disease Activity Score 28 (DAS28), tender joint count and ESR, whilst improvements in pain visual analogue scale (VAS), swollen joint count and CRP (an inflammatory marker) failed to reach statistical significance.[77] Another meta-analysis of five RCTs found a reduction of recurrence (borderline significance), whilst improvements in DAS failed to reach statistical significance and no improvement was seen in VAS.[78] Most studies used weekly or monthly bolus dosages of 50 000–100 000 IU for durations of 12 or 24 weeks.

Two open-label RCTs have been published since, one showing no difference in disease activity or BMD in patients with idiopathic juvenile arthritis receiving 2000 IU per day for 24 weeks,[79] whilst the other found a significant improvement in pain relief with 60 000 IU per week alongside calcium, 1000 mg per day, over calcium on its own in treatment-naïve patients with RA.[80]

Although research results are inconsistent, overall vitamin D supplementation appears to be beneficial for the management of RA.

Systemic lupus erythematosus (SLE)

Systemic lupus erythematosus is a systemic autoimmune condition that is characterised by inflammation in several tissues and organs,

especially joints, skin, kidneys and blood vessels, potentially causing significant damage in these organs. Epidemiological and animal research suggests that vitamin D plays an important role in SLE,[81,82] and the use of vitamin D for the treatment of lupus was first reported in the 1940s.[83]

A 2019 review and meta-analysis of five RCTs found no effects of vitamin D supplementation on disease activity (four trials) or anti-dsDNA (a marker of SLE, three trials), whilst two trials showed a decrease in fatigue.[82]

Two RCTs not included in the review found benefits of vitamin D either alone[84] or with calcium[85] on bone health, which is commonly negatively affected in patients with SLE. The former study also looked at disease activity and immune markers, but found no statistically significant changes. In both studies, all or most patients were vitamin D insufficient at baseline. Interestingly, two studies looked at the effect of different dosages on vitamin D levels in these patient populations and found that even with 50 000 IU per week only 75% of patients reached sufficiency levels, and less with lower dosages, suggesting that patients with SLE may require higher dosages, or different regimens, for full benefit.

Although evidence is limited, it would be advisable to recommend vitamin D supplementation to patients with SLE who are deficient, to ensure adequate levels for bone health. Vitamin D levels should be monitored to ensure supplements sufficiently raise vitamin D levels.

Autoimmune thyroid disease

There are two main forms of autoimmune thyroiditis (AIT): Hashimoto's thyroiditis (HT) and Grave's disease (GD). They are characterised by elevated levels of thyroid peroxidase antibodies (TPO-Ab) and/or thyroglobulin antibodies (Tg-Ab), which lead to dysfunction or destruction of the thyroid gland. Epidemiological research has shown that people with AIT have lower levels of vitamin D than healthy controls.[86]

A 2018 meta-analysis of six RCTs on the use of vitamin D in AIT, including HT and GD, found a significant lowering of thyroid antibodies, TPO-Ab and Tg-Ab, after 6 but not after 3 months.[86] Dosages used in the included studies ranged from 1000 IU daily to 60 000 IU weekly, and durations were 1–6 months. Two of the studies used calcitriol, the activated form of vitamin D at 0.25 µg per day.

One more recent open-label RCT of 23 patients with HT found that vitamin D at 60 000 IU weekly for 8 weeks followed by the same dose monthly for another 4 months actually increased TPO-Ab, although it improved markers of thyroid function, with a decrease in thyroid-stimulating hormone (TSH) and an increase in free thyroxine (fT4).[87] The authors of this study state that the cause for the increase of TPO-Ab in this study is unknown.

Overall, the evidence suggests that vitamin D supplementation is of benefit in patients with autoimmune thyroid disease, at least in those patients with insufficient vitamin D levels, with a dose of at least 2000 IU per day for 6 months having shown benefits.[86]

Bone health

As discussed in the section *'Introduction',* vitamin D was first recognised for its importance in bone health, with the classic VDD diseases being rickets in children and osteomalacia in adults. A lot of research has therefore focussed on the role of vitamin D in bone health, in particular in the prevention and treatment of osteoporosis and fractures.

Osteoporosis, BMD and risk of fractures

Osteoporosis is characterised by low bone mass and structural deterioration of bone tissue, leading to bone fragility and an increased risk for fractures, commonly of the

wrists, spine or hip, although any bone can be affected. The World Health Organization (WHO) defines osteoporosis as a BMD of 2.5 standard deviations below the mean peak mass (average of young healthy adults) as measured by a dual-energy X-ray absorptiometry (DEXA) scan. DEXA scans, however, only measure BMD and do not assess the structural micro-architecture of the bone tissue, and therefore may not accurately reflect risk of fractures.[88] There are well over 50 RCTs looking into the role of vitamin D either alone or with calcium in osteoporosis.

In 2014, a Cochrane review of 53 RCTs involving 91 791 older adults concluded that there is high-quality evidence for the benefits of vitamin D and calcium, but not vitamin D on its own, for the prevention of non-vertebral or any type of fracture.[89]

Several meta-analyses into the benefits of vitamin D with or without calcium have been published since with conflicting results. Whilst some find a benefit of supplementation with vitamin D plus calcium,[90,91,92] others did not find any benefits of either vitamin D, calcium or a combination of the two.[93,94,95]

In most studies investigating a combination of vitamin D and calcium, dosages ranged from 400 to 1000 IU and 500 to 1200 mg per day, respectively, whilst trials investigating vitamin D on its own commonly used intermittent high doses.[90]

Some of the meta-analyses carried out subgroup analyses looking at high and low dosages of both vitamin D and calcium, but found no effects,[93,95] whilst others concluded that the minimum effective doses are 1200 mg calcium and 800 IU vitamin D per day.[91]

A couple of meta-analyses specifically looked at BMD and found improvements with combinations of vitamin D and calcium,[94,96] although this was judged to be clinically irrelevant in one of the papers.[94] As with studies on fractures, dosages were mostly in the ranges of 500–1200 mg calcium and 400–1000 IU vitamin D per day.

Overall, although the evidence from clinical trials is contradictory, in view of the excellent safety profile of vitamin D and calcium, supplementing a combination of the two, at dosages of 1000–1200 mg calcium and 400–1000 IU vitamin D per day, appears to be prudent for people at risk of fractures.

Cancer

A recent review of six meta-analyses concluded that "observational evidence indicates that low vitamin D status is associated with a higher risk of cancer outcomes, randomised trials show that vitamin D supplementation reduces total cancer mortality, but not cancer incidence".[97]

Breast cancer

Epidemiological studies have shown that women with breast cancer are more likely to have low vitamin D levels at time of diagnosis compared with healthy controls.[98]

A 2020 meta-analysis of eight RCTs, involving 72 275 women, found no effect of vitamin D, either alone or with calcium, on the incidence of breast cancer.[99] None of the included RCTs individually found a statistically significant reduction either, with dosages varying widely from 400 IU per day to 200 000 IU monthly and follow-up periods of 1–12 years.

A number of studies have also looked at a variety of biomarkers in women with breast cancer. Whilst improvements have been seen in some markers, including biomarkers for angiogenesis,[100] total antioxidant capacity[101] and 27-hydroxycholesterol (27HC; which is involved in the development of oestrogen-positive breast cancer),[102] no significant changes were seen in others, including tumour proliferation or apoptosis biomarkers[103] and inflammatory markers.[101]

At present the evidence does not support the use of vitamin D for prevention of breast cancer, which is surprising in view of the epidemiological data.[98] And whilst there is evidence that vitamin D exerts positive effects on some breast cancer biomarkers, there is insufficient research to make recommendations for patients with breast cancer.

Colorectal cancer (CRC)

Colorectal cancer is the third most common cancer worldwide, and is significantly more common in developed countries.[104] The role of vitamin D in the development of CRC has been controversial because of conflicting findings from supplementation and epidemiological studies.[105]

A review and meta-analysis including 166 studies and 854 195 participants evaluated the associations between vitamin D and CRC.[104] Both vitamin D level and vitamin D intake (from diet and supplements) were associated with a significantly decreased risk of getting CRC, and higher vitamin D levels significantly decreased CRC and overall mortality. The data suggested that each 200 IU per day increase in total vitamin D intake was associated with a 10% decrease in the risk of colorectal adenoma (a pre-cancerous condition) and a 5% decrease in the risk of CRC. Similarly, a meta-analysis just focussing on vitamin D intervention trials found that vitamin D significantly improved progression-free survival and reduced adverse CRC outcomes by 30%,[105] with the best results seen in a study using 4000 IU per day for 23 months.[106] In the same publication, a reduction in CRC incidence failed to reach statistical significance. Another phase 3 clinical trial is currently underway to confirm these results.[107]

The evidence suggests that supplementation with vitamin D is beneficial both in the prevention of CRC and in improving outcomes in patients with CRC. Whilst dosages and regimens have varied between studies, 4000 IU per day has been shown to be beneficial.[106]

Prostate cancer

Prostate cancer is the second most common cancer in men. Vitamin D has been found to be involved in regulating hormone function in clinical and *in vitro* studies.[108]

A 2019 meta-analysis of six RCTs showed no effects of vitamin D on prostate-specific antigen (PSA; a marker of prostate cancer) or prostate cancer mortality.[108] However, five of these studies did not use vitamin D_3, but instead used vitamin D analogues or vitamin D_2. The study that used vitamin D_3 found a significant PSA response with 10 000 IU per day for 3–8 weeks.[109] The 2019 meta-analysis also reviewed 16 uncontrolled trials that showed a modest benefit of vitamin D on PSA response rate, with 19% of patients having a reduction of at least 50%.[108] Most of these studies used the activated calcitriol form of vitamin D.

Whilst research on vitamin D_3 itself in prostate cancer is limited, there is some evidence that a high dose may be beneficial.

Skin cancer

Sun exposure is an important risk factor for skin cancers. Seeing that sun exposure is also our main natural source of vitamin D, it is not surprising that epidemiological studies have shown an increased risk of melanoma, keratinocyte and basal cell carcinoma, although not with squamous cell carcinoma, with higher vitamin D levels.[110] Vitamin D intake through diet and/or supplements, on the other hand, is not associated with increased risks of skin cancers, except for basal cell carcinoma.[110]

Evidence for any effect of supplemental vitamin D from RCTs is currently lacking.

Chronic obstructive pulmonary disease (COPD)

Chronic obstructive pulmonary disease is a group of conditions, including emphysema and chronic bronchitis, which cause difficulties breathing. They tend to gradually get worse, can impair daily living and are amongst the top 10 causes of death globally.[111]

In 2020, a comprehensive review and meta-analysis of 25 RCTs involving 2670 patients found that supplementation with vitamin D or vitamin D analogues led to significant improvements in COPD assessment test score, lung function, sputum and 6-minute walk distance, and a halving in numbers of exacerbations.[111] Looking at the results of the individual trials suggests that vitamin D analogues and vitamin D alongside vitamin A were more effective than vitamin D on its own.

Two much smaller meta-analyses found conflicting results. One involving eight studies and 687 patients found no significant improvements in lung function, but showed significant heterogeneity.[112] The other one reviewed three trials with 469 patients and found a halving of moderate to severe exacerbations in patients with COPD with prior VDD, although no statistically significant overall improvement.[113]

Whilst the evidence is conflicting for vitamin D in patients with COPD, it seems prudent to ensure patients are not VDD, but there are insufficient data to suggest specific dosing regimens.

Cardiovascular disease (CVD) and risk factors

Cardiovascular disease is a general term for diseases affecting the heart and blood vessels, and is one of the main causes of death globally.[114] CVD usually develops over many years, and risk factors include high blood pressure, abnormal blood lipids, smoking and poor diet.[114]

A meta-regression analysis of 22 RCTs involving 83 200 participants found no statistically significant reduction of non-fatal myocardial infarction, cardiac death, coronary heart disease events or stroke in vitamin D supplementation trials, with no heterogeneity and none of the individual trials showed significant effects.[115]

By far the biggest intervention trial, the VITAL trial in the USA, randomised 25 871 participants, men 50 years of age or older and women 55 years of age or older, to either vitamin D, 2000 IU per day, or placebo with a median follow-up of 5.3 years, and found no reduction in cardiovascular events.[116]

A very comprehensive meta-analysis of 81 studies overall looked at different cardiovascular risk factors, and found significant benefits of vitamin D supplementation for systolic and diastolic blood pressure, hsCRP, total, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, and triglycerides.[117] Subgroup analysis showed better outcomes when vitamin D levels of ≥ 86 nmol/l were achieved, with doses ≥ 4000 IU per day, trial durations of less than 6 months, and in obese people, although the impact of these factors had varied effects on different risk factors.

Blood pressure

A number of meta-analyses have looked at the effects of vitamin D on blood pressure with contradictory findings. Whilst one review found no benefits,[118] others saw improvements in specific subgroups: in patients with hypertension and VDD at baseline,[119,120,121] or together with calcium in younger adults.[122] Most studies showed significant heterogeneity, and only one found dosage to be a significant factor, with dosages of 5000 IU per day or more being more effective.

Overall, the evidence for effects of vitamin D on blood pressure is mixed, but supplementation may decrease blood pressure in patients with hypertension and VDD, although the available data do not suggest an effective dosing regimen.

Cardiometabolic risk factors

A 2019 meta-analysis of 41 RCTs involving 3434 subjects looked at blood lipids and found significant improvements in total and LDL cholesterol and triglycerides, which were more pronounced in those with VDD at baseline.[123]

Another 2019 meta-analysis of eight trials and 305 subjects with CVD found significant improvements in HDL cholesterol, fasting glucose, insulin and Homeostatic Model Assessment of Insulin Resistance [HOMA-IR; a measure of insulin resistance (IR)], CRP, but not LDL, total cholesterol or triglycerides.[124]

Although results are mixed, vitamin D supplementation may help reduce cardiometabolic risk factors, especially in people with VDD.

Endothelial function

Three recent meta-analyses looked at the effect of vitamin D on endothelial function and found contradictory results. One meta-analysis of 26 RCTs involving 2808 subjects found no improvements in flow-mediated dilation, pulse wave velocity or central augmentation index (three markers of endothelial or arterial function).[125] The authors carried out a subgroup analysis, but did not find any effects of dosing regimen or vitamin D status at baseline (overall, 42% of subjects were VDD or insufficient). Looking at the individual studies though, it appears that those that used monthly bolus doses of 100 000 IU or more found significant improvements.

The two other meta-analyses,[126,127] on the other hand, found significant improvements, although in one improvements were limited to diabetics.[127] Again, dosing was reported as not affecting the outcomes.[127]

Overall, evidence for the use of vitamin D for reduction of cardiovascular risk factors is contradictory, but maintaining adequate serum levels of vitamin D may confer some benefit, although this does not seem to translate into a reduction in risk for cardiovascular events.

Depression

Three recent meta-analyses looked at the benefits of vitamin D in patients with depression. One of them reviewed 25 trials with a total of 7534 participants and found a positive effect on negative emotions, with patients with a diagnosis of major depressive disorder and VDD benefiting most.[128] Another meta-analysis also reported benefits for depression (based on nine RCTs) and sleep (two RCTs), with all individual studies included showing some benefit.[129] In the third meta-analysis (10 RCTs involving 1393 participants),[130] improvements failed to reach statistical significance but there was significant heterogeneity within the results, which did not appear to be related to dosing regimen.

Since then more clinical trials have been published and have confirmed the benefits of vitamin D supplementation on depressive symptoms and/or anxiety.[131,132,133]

Overall, the evidence suggests that vitamin D supplementation is of benefit for patients with depression, in particular those with VDD. Dosing regimens that have shown benefits varied from 1000 IU per day to 100 000 IU weekly for 8 weeks, and a single bolus dose of 300 000 IU with 12-week follow-up.

Type 2 diabetes mellitus (T2DM)/ glycaemic control

Epidemiological studies suggest a role of vitamin D in glycaemic control of patients with T2DM.[134]

Although there is some heterogeneity amongst studies, over the past 5 years a number of meta-analyses, covering over 20 RCTs overall, have reported benefits of vitamin D supplementation on glycosylated haemoglobin (HbA1c; a long-term measure of blood glucose control), fasting blood glucose (FBG) and/or HOMA-IR, especially in patients with VDD.[135,136,137,138] Only one meta-analysis found no statistically significant improvements in HbA1c (no other variables were assessed).[134]

When it comes to glycaemic control in non-diabetics at risk of IR, results are more contradictory, with one meta-analysis (12 RCTs) finding significant reductions in FBG, HOMA-IR and circulating levels of insulin,[139] whilst another found no benefits on IR,[140] and yet another benefits for FBG and HbA1c but not HOMA-IR.[141] One meta-analysis found no overall effect on FBG, IR or preventing T2DM, but noted that results differed significantly between different subgroups.[142] One meta-analysis found that vitamin D reduced the risk of developing T2DM in non-obese but not obese people with prediabetes.[143] Overall, these studies suggested that dosages of 2000 IU or more per day were more beneficial than lower dosages.

Overall, the evidence suggests that vitamin D may improve glycaemic control in people with both T2DM and prediabetes, especially those with VDD. Benefits have been seen with a range of dosing regimens, and there is some evidence that dosages of 2000 IU per day or more give better benefits.

It is thought that the ability of vitamin D to reduce inflammation and oxidative stress may mediate, at least in part, its benefits in T2DM and prediabetes.[144] Intervention studies have confirmed that vitamin D supplementation decreases inflammatory and oxidative stress markers in patients with T2DM.[144,145,146]

Muscle strength

Vitamin D deficiency has been associated with muscle aches and weakness.[147]

Vitamin D supplementation has therefore been trialled for increasing strength in various populations. Whilst benefits of vitamin D have been observed in healthy adults,[148] no benefits have been seen in athletes[147,149] or postmenopausal women.[150,151] Findings in elderly people are mixed, with some studies showing small improvements in some strength tests,[152] whilst others show no additional benefit of vitamin D in addition to that of exercise.[153]

Non-alcoholic fatty liver disease (NAFLD)

Non-alcoholic fatty liver disease is caused by a build-up of fat within liver cells, and is closely associated with abnormal glucose and lipid metabolism, and increased inflammation and oxidative stress.[154] People with NAFLD tend to have lower levels of vitamin D.[155]

Four recent meta-analyses have evaluated the potential benefits of vitamin D in patients with NAFLD, reviewing six to 10 studies each, with some overlap of included studies. Two found no improvements in liver enzymes (markers of liver damage), lipid or glucose metabolism.[154,155] One review found significant improvements in alkaline phosphatase, but not in other liver enzymes.[156] The fourth one found significant improvements in glucose metabolism, alanine aminotransferase (a liver enzyme) and triglycerides, but not cholesterol (total, LDL or HDL) or aspartate aminotransferase (another liver enzyme).[157] One of the studies carried out a subgroup analysis, but found no effects of dosing regimen, vitamin D status at baseline or other factors.[154] Looking at the individual study characteristics and results also does not show any obvious associations.

Two more RCTs have been published since, and both found significant improvements in blood markers[158,159] and transient elastography (a specific ultrasound scan).[158] Dosages used in these trials were 1000 IU per day for 12 months and 50 000 IU per week for 3 months.

Whilst findings from clinical trials are inconsistent, there may be a benefit of vitamin D in patients with NAFLD, but the research to date does not allow to make any specific dose recommendations.

Obesity

Obesity has become a major health concern, and in 2017–2018 the prevalence of obesity in adults in the USA was 42.4%, with 9.2% being severely obese.[160] Epidemiological research has shown that people with obesity have lower levels of vitamin D, and that there is a dose–response relationship between the two.[161] However, it is unclear which is cause and which is effect.

A number of clinical trials have looked at the effect of vitamin D on weight and body composition. A meta-analysis in 2019 found that vitamin D, when included in weight loss programmes, had small, but statistically significant additive effects on body mass index (BMI; average decrease −0.3) and waist circumference (average decrease −1.4cm), but not weight.[162] Two other meta-analyses found no effect of vitamin D supplementation on body fat[163] or BMI, weight or fat mass.[164]

Adipokines, including leptin and adiponectin, are cell-signalling proteins secreted by fat cells, and they play an important role in obesity. A recent meta-analysis reviewed studies looking at vitamin D supplementation and adipokines, but found no effects.[165]

Overall, the evidence suggests that vitamin D does not help with weight or fat loss as such. However, because people with obesity are at increased risk for many conditions associated with VDD, supplementation should be considered, especially in those with VDD. Vitamin D levels should be monitored and vitamin D dose adjusted accordingly, as studies have shown that obesity decreases the effect of vitamin D supplementation.[11]

Pain

Epidemiological studies have shown that patients with arthritis, muscle pain and chronic widespread pain have lower vitamin D levels than people without these painful conditions.[166]

Two meta-analyses looking at low back pain[167] and non-specific musculoskeletal pain[168] found no benefit of vitamin D supplementation, although the authors of one of the reviews noted that this is based on poor-quality evidence.[167] On the other hand, a meta-analysis of RCTs on chronic widespread pain, such as in fibromyalgia syndrome, found a halving of pain scores with vitamin D supplementation.[169] Benefits were also reported in a meta-analysis of different types of chronic pain, although benefits were only seen in clinical trials carried out in a hospital (as opposed to a community) setting.[170] The authors of the latter review hypothesise that a possible explanation for that discrepancy may be that higher dosages were used in hospital-based trials.

Overall, the evidence regarding the potential benefits of vitamin D for pain-related conditions is mixed and may depend on the type of pain.

Polycystic ovary syndrome (PCOS)

Polycystic ovary syndrome is a common condition that affects about one in 10 women, and is characterised by enlarged ovaries that contain many fluid-filled sacs (follicles) that surround the eggs, excess androgens ('male' hormones) and irregular periods.[171] Whilst generally considered a gynaecological condition, the underlying cause of PCOS is thought to be IR. PCOS has been associated with low vitamin D levels.[172]

There is a significant body of research into various markers of PCOS, which have been summarised in several recent meta-analyses. Vitamin D supplementation has been shown to improve blood sugar and lipid metabolism,[173,174,175] androgen levels,[172,174] inflammatory and oxidative stress markers,[176] and follicular development and menstrual cycle regulation.[177]

Fertility problems are common in women with PCOS. Three recent clinical trials also showed improvements in a number of fertility-related markers[178,179,180] and pregnancy rates.[180]

Overall, the evidence shows benefits for women with PCOS with regards to metabolic parameters, hormone balance and fertility. The three fertility-related studies used approximately 3000 IU per day, either as a daily or weekly supplement, dosages in other studies varied widely with both daily and bolus regimens.

Pregnancy

Pregnant women and new-borns are at increased risk of VDD,[181] raising the question whether vitamin D supplementation during pregnancy offers clinical benefits.

Gestational diabetes mellitus (GDM)

Gestational diabetes mellitus carries significant risks to both mother and baby, and is increasing worldwide.[182]

Five meta-analyses, including over 20 RCTs, have evaluated the potential benefits of vitamin D for pregnant women with GDM, and all have found significant improvements in glycaemic control and/or reduced adverse maternal or neonatal outcomes.[182,183,184,185,186,187] A wide range of dosing regimens have been used, from 400 IU daily to single bolus doses of 300 000 IU. Looking at the individual study results of the most comprehensive meta-analysis that included 19 RCTs,[183] positive results, although not always statistically significant, appeared to be consistent over a wide range of dosage regimens, with the exception of two studies that used 300 000 IU bolus dosages and showed no effects.

The evidence shows a clear benefit of vitamin D supplementation for both mother and baby in women with gestational diabetes. A wide range of dosages appeared to be beneficial, except large bolus doses.

Pre-eclampsia

Pre-eclampsia is a pregnancy-related condition, characterised by hypertension, proteinuria (protein in urine) and oedema, which is potentially life-threatening to both mother and baby.

Three recent meta-analyses, covering over 25 RCTs, all concluded that vitamin D supplementation reduced the risk of pre-eclampsia, with estimated risk reductions of 37–63%.[188,189,190]

Daily dosages have ranged from 200 IU to 5000 IU, but intermittent dosages have also been used, commonly 50 000 IU every 2 weeks. One meta-analysis suggests a dose–response relationship, with higher dosages being more protective.[189]

Overall, the evidence is in favour of vitamin D supplementation for reducing the risk of pre-eclampsia, with higher dosage being more effective.

Pregnancy outcomes

Three recent meta-analyses, covering over 20 RCTs, have shown vitamin D supplementation being of benefit in terms of birth weight.[181,191,192] One of these studies also looked at mortality, and found significantly reduced mortality with vitamin D dosages of 2000 IU per day or lower, whilst reduction of mortality lost statistical significance with higher dosages.[192] This study also showed that there was no association of vitamin D supplementation with congenital abnormalities.

Overall, supplementing 2000 IU per day of vitamin D during pregnancy appears to be beneficial for both maternal and neonatal outcomes.

Respiratory tract infections

Respiratory tract infections are a major cause of morbidity and mortality globally. Low levels of vitamin D have been associated with an increased susceptibility to RTIs.[193]

Two recent meta-analyses, covering more than 40 RCTs, have shown that daily and weekly, but not bolus, administration of vitamin D reduces the risk of RTIs.[193,194] Whilst one study found that these effects were stronger in patients with baseline vitamin D levels of below 25 ng/ml,[193] the other found better effects in children aged 1–15 years, and dosages between 400 and 1000 IU per day.[194]

COVID-19

In view of the benefits of vitamin D in reducing the risk of other RTIs, Vitamin D has received much attention as a possible factor in the incidence and severity of COVID-19. Epidemiological studies looking at vitamin D levels and the risk of getting COVID-19 have mostly shown that VDD is associated with a higher risk of getting COVID-19, with an up to 80% increased risk reported,[195,196,197,198] although Pereira *et al.* found no association.[199] Three meta-analyses looked into the risk of severe COVID-19,

and found an increased risk of up to 260% in those who were VDD.[197,198,199]

A number of RCTs have also been conducted. One RCT in asymptomatic or mildly symptomatic patients found that those who received vitamin D, 60 000 IU per day for 7 days, were more likely to have recovered within 21 days [defined as a negative polymerase chain reaction (PCR) test] and had lower biomarkers associated with severe disease than those on placebo.[200] One RCT in hospitalised patients found that a single dose of 200 000 IU did not reduce mortality, risk of admission to the intensive care unit (ICU), mechanical ventilation or length of hospital stay compared with placebo.[201]

On the other hand, a pilot RCT from Spain compared 76 patients hospitalised with COVID-19, some of whom received oral calcifediol (0.532 mg) on the day of admission, 0.266 mg on days 3 and 7, and then weekly until discharge or ICU admission in addition to standard care, whilst the controls received standard care only. Only 2% of patients on calcifediol needed transfer to the ICU and none of them died, compared with two deaths (8%) and 50% of patients needing ICU treatment in the control group.[202] This raises the question, whether the use of an activated form of vitamin D may provide more benefit in acutely and severely ill patients in whom activation of vitamin D_3 may be impaired or take too long.

A number of case series, prospective unrandomised and/or uncontrolled studies have also shown benefits of vitamin D supplementation in COVID-19 patients.[203,204,205,206]

Overall, whilst evidence is still emerging, ensuring adequate vitamin D levels appears to be a prudent approach for prevention of severe COVID-19 as well as RTIs in general, whilst administration of high-dose calcifediol should be considered in patients hospitalised with severe COVID-19. Griffin *et al.* recommend 4000 IU vitamin D per day for 1 month, followed by 800–1000 IU per day for maintenance in people at risk of VDD for prevention of COVID-19.[207]

This level of supplementation should also help prevent other RTIs.

Table 2: Guidelines for vitamin D intake in VDD[210]

Age group	For individuals at risk of deficiency		Treatment for deficiency
	Daily requirement	Upper limit	
0–1 years	400–1000 IU	2000 IU	2000 IU/day for at least 6 weeks to achieve serum 25(OH)D > 30 ng/ml
1–18 years	600–1000 IU	4000 IU	2000 IU/day for at least 6 weeks to achieve serum 25(OH)D > 30 ng/ml
> 18 years	1500–2000 IU	10 000 IU	6000 IU/day for at least 6 weeks to achieve serum 25(OH)D > 30 ng/ml

Safety

Based on its role in calcium metabolism, the main safety concern with excessive vitamin D levels is hypercalcaemia. Hypercalcaemia can lead to nausea, vomiting, muscle weakness, neuropsychiatric disturbances, pain, loss of appetite, dehydration, polyuria, excessive thirst, kidney stones and, in extreme cases, renal failure, calcification of soft tissues, cardiac arrhythmias and even death.[6]

Vitamin D has been used at a wide range of dosages, and up to 10 000 IU per day is considered to be safe[1,6] but the NIH sets the upper tolerable intake for children aged 9 years and older and adults as 4000 IU per day.[6]

A meta-analysis of 62 clinical trials with 19 389 participants reported that there was no increased risk of any or non-hypercalcaemic adverse events (AEs).[208] The authors also reported that although gastrointestinal and skin AEs have been reported, these were not more common in the vitamin D as compared with the control groups. More

participants withdrew from studies, which may indicate AEs, where calcium was taken by both the vitamin D and the control groups, but not in studies without calcium. The authors concluded that "vitamin D, by itself, does not increase the risk of non-calcemic adverse effects". The authors carried out various subgroup analyses, and found no increase in AEs regardless of whether vitamin D was given with or without calcium, length of supplementation, baseline vitamin D levels or whether vitamin D_2 or D_3 were used, a subgroup analysis by dose of vitamin D was not reported.[208]

A Cochrane review of 53 RCTs on fracture risk involving 91 791 older adults found no increased mortality in patients receiving vitamin D with or without calcium.[89] They found a small (4%) but statistically significant increased risk of gastrointestinal symptoms, especially when combined with calcium, and a 17% increased risk of kidney disease when taken with calcium, but a 41% reduced risk of kidney disease when taken without calcium. The overall risk increase for kidney disease was 16%, with an absolute risk increase from 1.69 to 1.98 in 1000. The risk of hypercalcaemia, which was usually mild (2.6–2.8 mmol/L), was more than doubled in people receiving vitamin D or an analogue compared with controls, with a more than four times higher risk in people receiving calcitriol.[89]

Interaction with medications[6]

Orlistat may decrease vitamin D absorption.

Statins suppress cholesterol synthesis and vitamin D is made from cholesterol, statins may therefore reduce vitamin D levels. Vitamin D may reduce the potency of statins.

Corticosteroids can impair vitamin D metabolism, and people on oral steroids have an increased risk of VDD.

Thiazide diuretics decrease urinary calcium excretion, which in combination with vitamin D supplements might lead to hypercalcaemia. Additional monitoring of vitamin D and calcium levels and possibly renal function should be instigated by the prescribing physician.

Cautions in specific conditions[209]

Due to its effect on calcium metabolism, vitamin D should be used with caution in patients with arteriosclerosis, histoplasmosis, hypercalcaemia, hyperparathyroidism, lymphoma, kidney disease, tuberculosis and sarcoidosis.

Pregnancy and lactation

Pregnant and lactating women are at particular risk of VDD, and therefore often advised to supplement vitamin D. The NIH sets the same upper tolerable limit (UTL) of 4000 IU per day for these groups.[6]

See also under the section *'Pregnancy'* for studies reporting reduced mortality of infants and no risk of congenital abnormalities associated with vitamin D supplementation.

Children

Vitamin D supplementation in children is generally safe, and has shown benefits for a number of conditions, including IBD, allergies and prevention of respiratory infections (see above). The NIH set UTLs according to age groups:[6]

- Up to 6 months: 1000 IU per day
- 6–12 months: 1500 IU per day
- 1–3 years: 2500 IU per day
- 4–8 years: 3000 IU per day
- From 9 years: 4000 IU per day

Conclusion

Whilst epidemiological research has linked vitamin D with many conditions, it is important to remember that a statistical association does not necessarily reflect a causal relationship. Clinical supplementation trials have shown benefits of vitamin D in a number of conditions, including AD, CU, CRC, depression, PCOS, T2DM and pregnancy-related disorders. Some benefits have also been seen for cardiovascular risk factors, but studies looking

at cardiovascular events found no reduction in risk. The reason for these contradictory findings is unknown. Interestingly, although epidemiological as well as preclinical research points strongly to an important role of vitamin D in autoimmunity, results from supplementation studies have either shown no benefits (MS, psoriasis) or have been contradictory (RA, autoimmune thyroid disease, SLE).

It is possible that vitamin D status is a proxy for other factors, in particular exposure to sunlight, which has also been shown to have other benefits. Contradictory results can also be due to differences in baseline vitamin D status and methodological problems with establishing vitamin D status, as well as to the large range of dosing regimens used in clinical trials. Further well-designed studies may find optimal dosing regimens as well as those populations who may benefit most.

From a clinical perspective, the best practice is to establish vitamin D status at the start of a programme and monitor regularly to achieve and maintain optimal levels, through appropriate sun exposure and/or supplementation.

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Nutritional Medicine Journal

Sulforaphane, 3,3'-Diindolylmethane and Indole-3-Carbinol: A Review of Clinical Use and Efficacy

Karin Elgar

Abstract

Sulforaphane (SFN), 3,3'-diindolylmethane (DIM) and indole-3-carbinol (I3C, a precursor of DIM) are compounds that are obtained through eating cruciferous vegetables, which have been shown in epidemiological studies to have health benefits. SFN and DIM have been shown to have antioxidant, anti-inflammatory and anti-cancer effects, as well as playing important roles in cellular detoxification of xenobiotics through their effects on nuclear factor-kappa B (NF-kB) and nuclear factor erythroid 2-related factor 2 (Nrf2). DIM and I3C have also been shown to affect oestrogen metabolism. In view of these properties, SFN, DIM and I3C have been studied in clinical trials and, although clinical research is still limited, promising results have been seen in a number of health conditions.

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Affiliation: K. Elgar is with the Nutritional Medicine Institute, London, UK.
Corresponding author: Karin Elgar (email info@karinelgar.com)
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Introduction

Epidemiological studies have shown that cruciferous vegetables, such as broccoli, kale, cabbage, bok choy, cauliflower, collards, kohlrabi and Brussels sprouts, may have a protective effect, for example, with regards to certain cancers and cardiovascular disease (CVD), although associations are weak and not always consistent.[1,2] These vegetables contain glucosinolates, including glucoraphanin (GPN). Glucosinolates are broken down by the enzyme myrosinase during storage, food preparation and/or chewing, yielding a variety of isothiocyanates, such as sulforaphane (SFN), and indoles, such as indole-3-carbinol (I3C).[1,3] I3C is unstable and is converted to other compounds, including 3,3′-diindolylmethane (DIM; about 60% of I3C is converted to DIM).[1]

The enzyme myrosinase is present in cruciferous vegetables, which also include watercress, radishes, rocket and mustard seeds, and starts acting on glucosinolates during chopping or chewing the vegetables. Whilst humans do not produce the enzyme, some of our gut bacteria do; therefore, the composition of the microbiome and exposure to antibiotics can influence bioavailability.[2,3] Conversion of I3C to DIM is also dependent on the acidic environment of the stomach, so increasing the gastric pH, for example through acid-lowering medication, may also affect bioavailability.[1]

After ingestion, the highest concentration of DIM is found in the liver, with lower concentrations in the kidneys, lungs, heart, plasma and brain. Metabolites of DIM can be found in both serum and urine, but there is significant clearance within 24 hours.[1] Similarly, SFN reaches peak concentrations within 1–3 hours after ingestion, with levels reverting to baseline after 72 hours.[3]

The amounts of SFN, I3C and DIM available from foods vary depending on cultivation of vegetables and food preparation, with broccoli sprouts having the highest content. All three compounds have been used in both preclinical and clinical trials in a variety of conditions and, below, human clinical studies will be explored by condition rather than compound used.

General effects

Anti-inflammatory and antioxidant effects

Nuclear factor-kappa B (NF-kB) is a protein complex regulating the expression of genes involved in various inflammatory processes. By supressing NF-kB signalling pathways, SFN can attenuate a number of inflammatory mediators and enzymes.[2] DIM has also been shown to reduce inflammation by reducing inflammatory enzymes and signalling.[1] Both SFN and DIM can also induce various antioxidant enzymes via nuclear factor erythroid 2-related factor 2 (Nrf2), an important regulator of expression of antioxidant enzymes.[1,2]

Preclinical findings are backed up by human intervention trials. For example, a study in 40 overweight but otherwise healthy individuals showed that 30 g of broccoli sprouts, a source rich in glucosinolates, per day for 10 weeks significantly reduced the inflammatory markers interleukin-6 (IL-6) and C-reactive protein (CRP). Whilst CRP reverted to baseline value in the 10-week follow-up phase, IL-6 remained lower than baseline.[4]

Further studies evaluating anti-inflammatory and antioxidant biomarkers with varying results are discussed under several conditions, see below.

Effects on detoxification

Nrf2 is not only a major regulator of antioxidant enzymes, but also of cellular detoxification through regulating gene expression. SFN can affect Nrf2 activity and thus induce enzymes of Phase II detoxification. Detoxification, or better biotransformation, of toxins, as well as metabolic end-products, excess or old hormones, happens in a two-step process. In Phase I, the usually fat-soluble compound is converted into a more reactive compound, which can then be conjugated to one of a number of amino acids by specific enzymes (Phase II) to make them water soluble and ready for excretion via bile or urine. The intermediates created in Phase I are generally more reactive and dangerous than the initial toxin.[2] Like SFN, DIM has been shown to stimulate cellular detoxification by affecting Nrf2 signalling, as well as by promoting the expression of the cytochrome P450 (CYP) family of enzymes (Phase I).[1]

In addition to a large body of preclinical evidence, a number of small, human studies have found that consuming broccoli in large amounts or broccoli high in glucosinolates can induce a number of enzymes involved in the detoxification of xenobiotics (substances that are not naturally present in our bodies).[5,6]

During detoxification, free radicals and reactive oxygen and nitrogen species are produced, and these can cause oxidative damage. As discussed above, SFN and DIM can induce antioxidant enzymes, thus also supporting this aspect of detoxification.

Detoxification of air pollutants

Four randomised-controlled trials (RCTs) from Qidong, China, a region with high levels of air pollution and a high incidence of liver cancer due to a high level of aflatoxin consumption, have evaluated the potential of SFN and GPN to increase detoxification of air pollutants.[7,8,9,10] They found that broccoli sprout drinks significantly increase urinary excretion of benzene and acrolein, two air pollutants.[8] Drinks with different levels of SFN and GPN were used, and both SFN and GPN were found to be effective on their own.[9] A combination of 600 µmol GPN and 40 µmol SFN increased benzene excretion by 63%, whilst drinks with lower levels of active ingredients failed to increase benzene excretion statistically significantly more than placebo.[7]

Effects on oestrogen metabolism

Uncharacteristically for a hormone, oestrogen is not just one specific hormone, but actually a group of interchangeable hormones, oestrone (E1), oestradiol (E2) and oestriol (E3), with varying biochemical and physiological characteristics. Oestrogens need to be metabolised for excretion and, therefore, biotransformation (or detoxification) of oestrogen plays an important role in regulating hormone metabolism.

DIM can affect the CYP enzymes, including CYP1A1, CYP1A2 and CYP3A4, involved in this biotransformation and therefore the balance of certain oestrogen metabolites, in particular 2-hydroxyestrone (2OHE1), 4-hydroxyestrone (4OHE1) and 16-hydroxyesterone (16αOHE1) and the 2OHE1:16αOHE1 ratio, which have been implicated in hormone-related cancers, including breast and ovarian, as well as other hormone-related disorders. Whilst 4OHE1 and 16αOHE1 have been associated with an increased risk of breast cancer and 2OHE1 has been suggested to have protective effects against breast cancer, it is important to note that they need to be in balance for good health.[1] Due to the fact that DIM can strongly affect that balance, it would be prudent in clinical practice to check levels before initiating supplementation.

These metabolites have been used as biomarkers for breast cancer risk and other conditions, which are discussed below.

Anti-cancer effects

The initiation and progression of cancer involves both genetic and epigenetic changes, which lead to a dysregulation of gene expression. Whilst genetic mutations are irreversible, epigenetic alterations can be affected by diet, nutraceuticals and lifestyle.[3]

All of the factors mentioned above, antioxidant and anti-inflammatory effects, detoxification of carcinogenic substances and regulation of hormone balance, contribute to the anti-cancer

effects of SFN and related compounds.[2] Further anti-cancer effects of SFN and/or DIM include their promotion of apoptosis (programmed cell death, which is aberrant in cancer cells), inducing cell cycle arrest (stopping cancer cells replicating), inhibiting angiogenesis (the formation of blood vessels to feed the tumour), and reducing invasion of tissues and formation of metastases.[1,3] A number of studies have shown large amounts of broccoli to protect cells of smokers from DNA damage.[11,12]

Human clinical studies looking at the impact of SFN, DIM and I3C on cancer biomarkers are discussed below in the section 'Clinical uses' under the respective cancer.

Clinical uses

Asthma

Oxidative stress appears to play an important role in asthma, and SFN has been investigated in asthma and airway inflammation for its potential to alleviate oxidative stress through its ability to induce Phase II antioxidant enzymes.[13]

Most studies have looked at either induction of Phase II enzymes or markers of inflammation in the airways after short-term supplementation (3–4 days) in either healthy individuals or people with asthma or other allergies. Two studies found no effects of SFN (100 g or 200 g broccoli sprouts) on Phase II enzymes or other antioxidant and/or anti-inflammatory markers.[14,15] Two other studies, on the other hand, found benefits in terms of reduction of inflammatory response[16] and induction of Phase II enzymes.[17] The latter study was a dose-escalation study and found a dose-dependent response with dosages of 100 g or more of a broccoli sprout homogenate necessary to see benefits. These four studies involved healthy volunteers rather than people with asthma.

One study, involving 44 people with moderate asthma, supplemented 100 µmol SFN for 14 days.[13] Interestingly, the results showed a significant inter-individual heterogeneity amongst the response to SFN, with 60% of patients experiencing an improvement with SFN in a challenge experiment, whilst 20% had a worsening, and the remaining 20% no change.

Whilst some of these results are promising, at this point there is not enough evidence to recommend SFN to people with asthma, as clinical trials evaluating potential benefits have given contradictory results and studies on longer-term supplementation are lacking.

Autism

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterised by impaired social communication and function, and affected individuals often display stereotypical interests and restrictive and repetitive behaviours. In the USA in 2018, the rate of ASD was thought to be 1 in 40, with boys four times more likely to be affected than girls.[18]

Abnormalities in glutathione (GSH) metabolism are thought to play an important role in ASD as GSH affects redox metabolism, oxidative stress, mitochondrial function, immune function, neuroinflammation and cell signalling.[18,19]

A 2020 review reported that all five RCTs included found benefits in a variety of outcome measures of broccoli sprouts or seeds in children with ASD.[18] Dosages ranged from about 1 to 2.5 µmol per kg body weight (µmol/kg) SFN, and duration of the studies ranged from 12 to 36 weeks. A follow-up 3 or more years after completion of one of the studies discussed in the review[20] found that out of 16 families who responded, nine were still supplementing their child with SFN with sustained improvements, one had discontinued SFN with lasting improvements, three felt that their child had not responded to the initial treatment, two were unsure as to whether to continue supplementation and one was planning on continuing, suggesting that for a substantial proportion of children, benefits were lasting for several years.[21]

A more recent double-blind, placebo-controlled trial of SFN versus placebo alongside risperidone (an anti-psychotic medication used in autism) in 60 children with ASD, aged 4–12 years, found that patients receiving SFN improved significantly more in terms of irritability and hyperactivity/non-compliance, but not other scores, than children in the placebo group.[22] Dosages were 50 µmol and 100 µmol for children weighing up to 45 kg or 45–90 kg, respectively, and the duration of intervention was 10 weeks.

Based on these study results, SFN appears to be a promising, safe support for children with ASD, with the most commonly used doses being 1–2.5 µmol/kg per day. Duration should be at least 10 weeks, with longer-term supplementation potentially maintaining benefits for years.

It is thought that the benefits of SFN are due to its ability to increase GSH levels, and as such protecting cells from oxidative damage and/or mitochondrial dysfunction and/or supporting excretion of toxic metals.[18]

Cancer

Breast cancer prevention

A number of genes have been shown to increase breast cancer risk. One of these is BRCA1, a tumour suppressor gene responsible for repairing DNA, which, when mutated, confers a very high risk of getting breast cancer (80% lifetime chance) and ovarian cancer (40%).[23] A number of studies have looked at the effect of DIM on biomarkers or other predictive factors for developing breast cancer in healthy women with this mutation. One study in 23 women taking 100 mg DIM per day for 1 year found a reduction in breast tissue density, a recognised predictive factor of breast cancer risk in seven of the women, whilst one had an increase and the remaining 15 no change.[23] Levels of oestrogen and testosterone also reduced in this study, whilst other hormones remained unchanged. Another study in 15 BRCA1 mutation carriers found that 300 mg DIM per day for 4–6 weeks increased BRCA1 gene expression by 34%, although this did not reach statistical significance possibly due to the small numbers involved.[24] There was substantial heterogeneity between individuals, with an increase in 10 participants, a decrease in two and no change in one. The same study also looked at the 2OHE1:16αOHE1 ratio and found that there was no average change in the 10 pre-menopausal women, whilst the ratio approximately tripled in the three post-menopausal women.[25] Again this failed to reach statistical significance possibly due to the small numbers. Several other trials looked at the effects of either DIM or I3C on the 2OHE1:16αOHE1 ratio, which has been associated with breast cancer risk,[26] in women at high risk of developing breast cancer but not specifically carrying the BRCA1 mutation.

An RCT of 60 women comparing 400 mg I3C per day versus a fibre supplement versus placebo for 3 months found that there was a significant mean increase of the 2OHE1:16αOHE1 ratio from 0.72 to 1.17 in the I3C group after 1 month, which was maintained over the 3-month study period.[27] No change was observed in three of the 20 women receiving I3C, suggesting inter-individual differences in response. Similar results were seen in five obese but otherwise healthy women with 300 mg I3C per day for 5 months,[28] and with 400 mg I3C per day for 4 weeks in 17 healthy high-risk women.[29] The latter study also found corresponding increases in CYP1A2 and no further benefits from taking 800 mg I3C per day. An early study looked at the best dose of I3C for improving the 2OHE1:16αOHE1 ratio in at-risk women, and found 300 mg per day to be the minimum effective dose.[30]

Two RCTs evaluated the effects of DIM on oestrogen metabolism, and found increases in the 2OHE1:16αOHE1 ratio and sex hormone binding globulin with 300 mg per day for 12 months alongside tamoxifen, and a significant increase in 2OHE1 and a non-significant increase in 2OHE1:16αOHE1 ratio with 108 mg per day (the equivalent of 400 mg I3C) for 30 days, respectively.[26,31] Benefits have also been observed in peripheral blood mononuclear cell histone deacetylase (HDAC) activity, but not in other biomarkers (H3K18ac, H3K9ac, HDAC3, HDAC6, Ki-67, p21), with a broccoli seed extract providing 180 mg GPN per day for 2–8 weeks.[32]

It is important to note that none of the studies used breast cancer incidence as an outcome and that numbers of study participants were small. Whilst the evidence suggests that I3C and DIM can have positive effects on oestrogen metabolism and other breast cancer risk markers in women at high risk of developing breast cancer, at this point there is no evidence that DIM reduces breast cancer incidence in women. It also needs to be pointed out that not all breast cancers are oestrogen sensitive. There appears to be significant inter-individual variability in response to I3C and DIM. Functional testing, for example of the 2OHE1:16αOHE1 ratio, before and after 1 month of supplementation may therefore be a good strategy to advise whether these supplements are likely to be of benefit to the individual.

Cervical and other female cancers

Cervical intraepithelial neoplasia (CIN) is an abnormal, precancerous growth of cells on the cervix that can develop into cervical cancer and is usually associated with human papillomavirus (HPV). Surgical treatment of CIN, which requires local destruction of the cervix, can affect fertility and, as CIN commonly affects women of reproductive age, alternative therapies that do not affect fertility are being sought.[33]

Three studies looked at the potential benefits of DIM for CIN. In a study of 78 women with CIN, using DIM intravaginally, 100% of patients on 200 mg DIM and 90.5% of women on 100 mg DIM had complete regression of CIN after 180 days (baseline CIN I–II), which was significantly superior to placebo (61.1%).[34] However, two other studies looking at oral supplementation did not find DIM to be more effective than placebo in improving CIN or HPV status at dosages of 150 mg per day for 6 months and 2 mg/kg body weight for 12 weeks, respectively.[33,35]

A small trial of 27 patients with CIN II–III found that four of eight patients on 200 mg I3C per day and four of nine on 400 mg I3C per day had complete regression after 12 weeks, compared with none of the 10 patients on placebo.[36] The mean change in CIN grade showed a linear dose–response relationship. The 2OHE1:16αOHE1 ratio increased in both I3C groups and decreased in the placebo group, whilst there was no difference between I3C and placebo in HPV status, suggesting that the benefits are independent of HPV.

Beneficial effects of I3C have also been seen in a small uncontrolled trial in 12 women with high-grade vulvar intraepithelial neoplasia (VIN; a precancerous condition), with both 200 mg and 400 mg resulting in a significant improvement in symptoms (pain, itch), severity and size of lesions, although tissue biopsy from the worst-affected vulval areas revealed no improvement.[37] Results did not differ between the 200-mg and 400-mg groups.

One long-term study on I3C, 400 mg per day, in women with stage III–IV ovarian cancer showed significant benefits alongside standard therapy with and without additional epigallocatechin-3-gallate (EGCG; 400 mg per day). Overall survival was 60 months in the I3C and I3C + EGCG groups, compared with 44 months on standard therapy alone, and progression-free intervals were 39.5 months, 42.5 months and 22 months, respectively.[38]

Limited evidence suggests that oral DIM does not improve outcomes of CIN; however, there is limited evidence for the benefits of I3C in CIN, VIN and ovarian cancer at a dose of 200–400 mg per day for at least 12 weeks.

Prostate cancer

Prostate cancer is the second most common cause of cancer-related deaths in men in the USA, and is associated with deregulated androgen receptor (AR) signalling.[39] Prostate-specific antigen (PSA) is a protein produced in the prostate and, although not specific for prostate cancer, is used as a screening and monitoring tool in prostate cancer, with increasing PSA levels after radical prostatectomy being indicative of cancer progression.[40] Apart from the various anti-cancer effects of DIM observed in preclinical research, DIM is also thought to act as an AR antagonist leading to a decline in PSA.[41]

Two studies looked at the effects of DIM on prostate cancer biomarkers prior to prostatectomy. One study found that in 96% of patients there was a favourable effect on AR activity, and a decline in PSA was seen in 70% of the 28 patients on DIM, 450 mg per day, for 14–72 days.[41] The other study found a significant increase in the 2OHE1:16αOHE1 ratio with 400 mg DIM per day versus placebo for 21–28 days, but changes in other biomarkers failed to reach statistical significance.[42] DIM has also been investigated in prostatic intraepithelial neoplasia (PIN; a precancerous condition), and it was found that 900 mg DIM for 1 year improved the morphological index whilst this deteriorated in the placebo group, and led to a complete remission in 45% of patients whilst no complete regressions were seen in the placebo group.[43] Changes in a variety of clinical symptoms failed to reach statistical significance in this study.

Two studies evaluated the safety and tolerability of DIM in patients with prostate cancer or PIN. One dose-escalation study established the maximal tolerated dose to be 600 mg per day for up to over 1 year,[44] whilst the other study found 900 mg per day for 3 months to be well tolerated.[45] The former study also found a decrease or stabilisation of PSA in two of the 12 participants, whilst the other 10 experienced a slowing in PSA increase during supplementation.

A number of studies looked at SFN in the form of SFN-rich broccoli foods or extracts. Beneficial effects of SFN were seen on PSA in patients following prostatectomy[40] or with recurrent prostate cancer.[46] Dosages were 60 mg (339 µmol) SFN per day for 6 months and 200 µmol SFN per day for 20 weeks, respectively, with benefits seen from 3 months of supplementation. Another study found no benefit in various genetic/epigenetic biomarkers with SFN, 200 µmol per day; however, the duration of supplementation was only 4–8 weeks so may have been too short to find statistically significant improvements.[47]

One study found benefits of GPN-rich broccoli soups in patients at risk of prostate cancer on active surveillance, with changes in gene expression and associated oncogenic pathways improving in a dose-dependent manner.[48]

Overall, the evidence suggests that DIM, SFN and GPN can have a beneficial effect on biomarkers for prostate cancer, with dosages of at least 450 mg DIM or 200 µmol SFN per day, and durations of at least 4 weeks for DIM and 3 months for SFN.

Other cancers

SFN, DIM and I3C have also been tested in a number of other cancer types, with results warranting further research.

Two studies have investigated long-term supplementation with I3C in recurrent respiratory papillomatosis (RRP), a benign disease associated with HPV but with the potential to become malignant and cause serious problems.[49,50] Standard treatment

of this condition is surgical removal of papillomas as necessary. Both studies found that about one-third of patients achieved a full response, i.e. did not need any surgery during supplementation, about one-third had a partial response, i.e. needed surgery less frequently than before, and the remaining patients showed no response. No participant had a worsening of disease. The studies involved both adults and children from the age of 2 years. The adult dose was 400 mg I3C per day, whilst the dose was weight-adjusted for children. In one study the minimum length of supplementation was 8 months, the mean duration in the other was 4.8 years.

An RCT in patients with melanoma showed a dose-dependent increase in SFN in both plasma and skin, with dosages ranging from 50 to 200 µmol per day for 4 weeks, and significant decreases in pro-inflammatory cytokines and an increase in the tumour suppressor compound decorin.[51]

In a study in patients with pancreatic cancer who received palliative chemotherapy, the mean death rate was lower during the first 6 months of intake in the group receiving additional pulverised broccoli sprouts containing 508 µmol SFN and 411 µmol GPN per day, compared with those receiving placebo.[52]

Thyroid proliferative disease (TPD), which comprises thyroid cancer and goitre, is four–five times more common in women than men, and oestrogen is thought to play a role in its development. In a small, pilot study, seven patients with TPD were given 300 mg DIM per day for 2 weeks prior to total or partial thyroidectomy.[53] DIM was detected in thyroid tissue, blood and urine, and the ratio of 2OHE1:16αOHE1 increased, suggesting a positive effect on oestrogen metabolism in these patients, but no other outcomes were evaluated.

Cognitive function

Two human studies have looked at cognitive function. One double-blind, placebo-controlled trial found that GPN, 30 mg per day for 12 weeks, led to a significant improvement in cognitive performance in older adults.[54] A small open-label pilot study with seven patients with schizophrenia found limited evidence for the benefits of SFN, 30 mg per day for 8 weeks.[55] Whilst there was a statistically significant improvement in one cognitive test parameter, all other measures remained the same. In animal models, SFN has had benefits in traumatic brain injury, but human trials are outstanding.[56]

Overall, evidence for the benefits of SFN/GPN for cognitive function is limited but promising.

The mechanisms by which SFN exerts its benefits with regards to cognitive function are thought to be due to its antioxidant and anti-inflammatory effects.[54]

Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) is associated with pulmonary and systemic oxidative stress, which correlate with severity of disease. Due to its antioxidant and anti-inflammatory benefits, SFN or its metabolites could be thought of as a potential supportive supplement.[57]

However, one double-blind, placebo-controlled trial of 85 patients with COPD found no significant change in Nrf2 expression, other inflammatory or antioxidant markers or pulmonary function with 25 µmol or 150 µmol SFN per day for 4 weeks.[57] Further research is needed to investigate higher dosages and/or longer durations of supplementation.

Cardiovascular risk factors

CVD is one of the main causes of death in Western countries and usually develops over many years. Risk factors include abnormal blood lipids, diabetes, hypertension and poor diet.

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Low-density lipoprotein cholesterol

Elevated low-density lipoprotein cholesterol (LDL-C) levels are considered an important risk factor for CVD, and it has been estimated that, at a population level, a 1% reduction in LDL-C is associated with a 1–2% reduction in risk of coronary artery disease.[58]

Two independent studies conducted by the same team showed that consuming 400 g of broccoli high in GPN (approximately 3.5 times as much as standard broccoli) for 4 weeks decreased LDL-C significantly more than eating the same amount of standard broccoli, with a decrease of 5.8% in LDL-C averaged over both trials (improvements in the individual trials failed to reach statistical significance, probably due to small numbers).[58]

A small, pilot study in 12 healthy volunteers also showed a significant beneficial effect on total, LDL-C and high-density lipoprotein cholesterol (HDL-C) after just 1 week of consumption of 100 g fresh broccoli sprouts per day.[59]

The benefit of SFN is thought to be mediated through expression of Nrf2, which is associated with modulation of lipid synthesis and mitochondrial fatty acid oxidation.[58]

Diabetes

Low-grade inflammation is associated with both the development of type 2 diabetes mellitus (T2DM) and with its complications.[60]

One double-blind, placebo-controlled study looked at the effects of SFN on a large number of biochemical parameters in 63 patients with T2DM, comparing two dosages of a broccoli sprout powder, providing either 225 µmol or 112.5 µmol SFN per day for 4 weeks, with placebo. Statistically significant benefits of the high-dose supplement compared with placebo were seen in serum insulin, HOMA-IR (homeostasis model assessment of insulin resistance), the inflammatory markers CRP and IL-6, triglycerides, oxidated LDL-C, HDL-C and atherogenic index of plasma.[60,61,62] Oxidative stress markers improved in both SFN groups compared with placebo.[63] The authors of the study considered the antioxidant effects of SFN to mediate the benefits seen in diabetic patients.

Endothelial function and hypertension

A small RCT evaluated the effects of 10 g dried broccoli sprouts per day (equivalent to 100 g fresh sprouts, providing approximately 259 µmol GPN) for 4 weeks on blood pressure and endothelial function in 40 hypertensive individuals without diabetes and with normal levels of cholesterol.[64] Changes in endothelial function and blood pressure failed to reach statistical significance compared with control patients who did not receive the preparation.

Whilst evidence is limited to a few studies, it appears that people with diabetes and elevated cholesterol levels may benefit from supplementation with SFN/GPN. Due to the fact that CVD is strongly associated with inflammation and oxidative stress, supplementing SFN or related compounds may offer benefits for people with or at increased risk of CVD, although there are insufficient data to suggest a particular dosing regimen.

Endometriosis

Endometriosis is a common condition where endometrial tissue starts to grow in places outside the uterus, such as the ovaries and fallopian tubes, causing symptoms including pelvic pain, heavy periods and subfertility. It has been associated with higher levels of 4OHE1, which is thought to have pro-proliferative effects.[65]

One small RCT in eight women with endometriosis found that DIM, 300 mg per day for 3 months, alongside standard treatment with Dienogest (a progesterone analogue) significantly improved pelvic pain and bleeding patterns in the four women receiving

DIM compared with those who only took Dienogest.[66] An accompanying tissue study showed that viability and oestrogen secretion of endometriotic but not normal endometrial cells was reduced through the use of DIM, suggesting that the effects of DIM on oestrogen metabolism and its antiproliferative properties may explain the clinical benefits.[66]

Whilst the evidence is very limited, theoretical considerations regarding the effect of DIM on oestrogen metabolism and these clinical results suggest that DIM may have potential, but requires more research.

Helicobacter pylori and gastric inflammation

Helicobacter pylori (H. pylori) is a common cause of gastritis, gastric and duodenal ulcers, and gastric cancer.[67] In preclinical studies, SFN has shown antimicrobial effects against *H. pylori*.

Three RCTs have evaluated the effects of SFN on *H. pylori*. Whilst two of these studies found some short-term benefit with regards to *H. pylori*,[68,69] the third one did not.[67] However, two of these studies also looked at markers for gastric inflammation and found significant reductions in those markers.[67,68] The studies that saw an improvement in gastric inflammation used broccoli sprouts or broccoli sprout extracts at dosages of 2 mg SFN (equivalent to 11 µmol) for 4 weeks and 420 µmol of SFN precursor for 8 weeks, respectively.

Whilst the evidence is limited, SFN appears to reduce gastric inflammation. This is unlikely to be due to any antimicrobial effects against *H. pylori* as such, but SFN is thought to protect the gastric mucosa against oxidative stress induced by *H. pylori*.[68] Due to the difference in preparations and dosages used in the trials it is difficult to suggest a dosage, but a duration of at least 4 weeks seems reasonable.

Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is a common disorder caused by fat deposits within liver cells, and is associated with obesity/overweight, metabolic syndrome and T2DM.

One double-blind, placebo-controlled study investigated the effects of a broccoli sprout extract (providing 69 µmol GPN per day) for 2 months in 52 men with NAFLD, and found a significant lowering of liver enzymes (a marker of liver health) and a marker of oxidative stress in the GPN but not the placebo group.[70] GPN is a precursor to SFN, which has cell-protective effects, including induction of detoxification and antioxidant enzymes, and is thought to mediate its benefits in NAFLD.[70]

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is an autoimmune condition that causes inflammation in various tissues, commonly affecting skin and joints, but may also involve internal organs such as the heart and kidneys. SLE is more common in women than men, and oestrogen imbalances are thought to promote disease activity.[71]

One open-label study comprising a 1-week metabolic study (17 patients) and a 3-month disease activity observation (12 patients) found a statistically significant increase of 2OHE1:16αOHE1 ratio from 1.84 to 3.15 after 1 week of supplementation with I3C, 375 mg per day.[71] SLE Disease Activity Index scores were 10.0 at baseline (scale 1–105), 6.25 following 3 months on I3C, and 8.8 at 3 months after I3C cessation, although these changes were not statistically significant. The authors concluded that whilst I3C can improve oestrogen metabolism in women with SLE, no substantial effects on disease activity during 3 months of supplementation were observed.[71]

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Pre-eclampsia

Pre-eclampsia is a pregnancy-related condition, characterised by hypertension, proteinuria (protein in urine) and oedema, and is potentially life-threatening to both mother and baby. The condition is associated with autoimmune conditions such as SLE and antiphospholipid syndrome, diabetes type 1 as well as a history of high blood pressure, and oxidative stress is considered to be one of the underlying mechanisms.

One small trial evaluating the pharmacokinetics of SFN compared a single dose of a myrosinase-activated with a non-activated broccoli sprout extract containing 32 mg SFN in six non-pregnant women, and found the activated extract to have higher bioavailability.[72] The investigators then evaluated a single dose of the activated extract, either 32 mg or 64 mg, in six women each with pregnancy-related hypertension, and found blood levels of SFN and metabolites to be significantly lower compared with the non-pregnant women. A statistically significant 10% reduction in diastolic but not systolic blood pressure was seen, with a greater reduction with the higher dose, although the difference between the dosages was not statistically significant. A significant decrease in soluble fms-like tyrosine kinase 1 (sFlt-1; an anti-angiogenic protein implicated in the development of pre-eclampsia) was also seen, irrespective of dose.

Whilst this study is promising, with no further longer-term studies there is no human clinical evidence for the benefits of SFN in preventing or managing pre-eclampsia.

Sickle cell disease

Sickle cell disease is an inherited disorder that affects red blood cells, causing their deformation and breakdown. The red blood cells have a reduced oxidative stress capacity, which is thought to be caused by decreased expression of Nrf2. Preclinical evidence suggests that SFN can activate Nrf2, which in turn increases levels of foetal haemoglobin, which reduces disease severity.[73] A small open-label, dose-escalation study found that SFN significantly increased the expression of heme oxygenase-1 (HMOX1), an antioxidant enzyme, but increases in gene expression for foetal haemoglobin failed to reach statistical significance.[73]

Safety

SFN, DIM and I3C are all generally well tolerated.[74,75,76]

Some clinical trials with SFN have reported digestive complaints, including bowel discomfort, gastrointestinal upset, heartburn and vomiting.[74] Two patients with autism and a history of seizures experienced seizures after intake of SFN, although it is unclear whether SFN increases the risk of seizure in susceptible individuals.[20]

Adverse events (AEs) reported in clinical trials with DIM include nausea,[31,33,77] rash,[31] arthralgia[31] and headaches,[77] although causality was not established. In a dose-escalation study, asymptomatic hyponatraemia was observed at 600 mg DIM per day for 28 days.[44] Hyperglycaemia, digestive complaints, fatigue, pruritus, anaemia, increased creatinine, urinary frequency and incontinence were also reported in this trial (causality unknown). One study of 600 women (400 on DIM, 200 on placebo) provided a detailed analysis of AEs, including some serious ones (requiring hospitalisation; 1% in DIM group, 3% in placebo group).[35] The only AE that was more common in women on DIM compared with placebo was a darkening of urine.[35]

AEs reported in clinical trials with I3C include rash,[29,71] musculoskeletal complaints,[29] headaches,[29] gastrointestinal complaints[29] and upper respiratory infections.[29] I3C has also been associated with unsteadiness and imbalance at higher doses (800 mg per day), which resolved on return to a lower dose.[50]

There have been concerns about SFN and thyroid function and thyroid autoimmune disease that were addressed in a recent RCT,[78] which is a subset of another study discussed under the section 'Detoxification of air pollutants'.[8] Thyroid hormones and antibodies were evaluated for 45 women before and after 84 days of consumption of a daily broccoli sprout drink containing 600 µmol GPN and 40 µmol SFN or placebo. There were no significant differences in thyroid hormone levels between the broccoli sprout and the placebo groups, and a non-significant decrease in numbers of participants with subclinical hypothyroidism and positive thyroid antibodies in the broccoli sprout group compared with the placebo group, suggesting that GPN and SFN are not associated with an increased risk of thyroid disease, at least in the short term.

Drug interactions/cautions

Due to the fact that SFN, DIM and I3C can affect the cytochrome P450 pathways, care should be taken with concomitant medications that are metabolised through these pathways, especially CYP1A2.[74,75,76]

In view of the potential of DIM to cause hyponatraemia, care should be taken with diuretic drugs.

DIM and I3C affect oestrogen metabolism, which needs to be taken into account when being used alongside oestrogen treatments, such as the contraceptive pill or hormone replacement therapy.

In animal models, I3C has been shown to have anti-thrombotic activity,[79] and may therefore potentially interact with anticoagulant and antiplatelet medications.

Pregnancy and breastfeeding

Whilst SFN, DIM and I3C are considered safe in amounts typically present in the diet, there is no information on safety at higher doses.[74,75,76]

Age limits/minimum age

I3C has been used in children with RRP from the age of 2 years long term, with a weight-adjusted dose, and no immediate or long-term side-effects were noted.[49,50] Two children who accidentally took triple their usual dose experienced unsteadiness, which resolved completely on return to their normal dose.[50]

In children with autism, SFN has been used from the age of 3 years. A number of side-effects were reported, including insomnia, flatulence, constipation, weight gain, vomiting, diarrhoea, increased aggression and exacerbation of seasonal allergies, with incidences ranging from 12% to 19%.[18] As mentioned above, two children with a history of seizures had seizures following SFN supplementation, although it is unclear whether SFN may increase seizure risk in at-risk children.[18]

Conclusion

There are promising results from clinical trials in a wide range of conditions, including cognition, autism, gastric inflammation, cardiometabolic and cancerous conditions, although the latter are mostly based on biomarker studies. Dosages have varied widely, making it difficult to suggest specific dosages. A number of studies have shown wide inter-individual variability of response with regards to effects on oestrogen metabolism, it would therefore be well advised to test oestrogen/oestrogen metabolite levels prior to supplementation.

Review Sulforaphane, 3,3'-Diindolylmethane and Indole-3-Carbinol: A Review of Clinical Use and Efficacy

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Nutritional Medicine Journal

EPA / DHA: A Review of Clinical Use and Efficacy

Karin Elgar

Abstract

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are omega-3 polyunsaturated fatty acids found in oily fish. Humans can also synthesise them from α-linolenic acid (ALA), which is considered an essential fatty acid as humans cannot synthesise it. However, conversion of ALA to EPA and DHA tends to be low, with significant inter-individual variation, making them conditionally essential nutrients. The benefits of EPA and DHA for cardiovascular health were first recognised in the 1970s. Since then, research has shown benefits in many other conditions, including metabolic, inflammatory and neuropsychiatric disorders, based on their anti-inflammatory and antioxidant effects, as well as their roles in cell membrane structure and function and in regulating gene expression. Fish oil supplements are generally well tolerated, but increased risk of atrial fibrillation and bleeding have been found in several meta-analyses.

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Introduction

Since the finding in the early 1970s that Eskimos from Greenland had low rates of heart disease despite a high-fat diet, but a diet high in marine omega-3 polyunsaturated fatty acids (PUFAs),[1] there has been significant interest in the potential benefits of fish and fish oils for health, in particular cardiovascular (CV) health.

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are omega-3 (n-3) PUFAs, fatty acids with more than one cis double-bond.[2] n-3 indicates that the first double-bond is located between the third and fourth carbon atoms counting from the methyl end of the molecule, whilst omega-6 (n-6) PUFAs have the first double-bond between the sixth and seventh carbon atoms.

The n-3 PUFA α-linolenic acid (ALA) and the n-6 PUFA linoleic acid (LA) are considered essential fatty acids, as humans cannot synthesise them, and food sources include green vegetables, flax and chia seeds and nuts, and vegetable oils, respectively. These parent PUFAs can be converted to EPA and from there to DHA and the n-6 long-chain fatty acids gamma-linolenic acid (GLA) and arachidonic acid (AA) (see figure 1).

However, conversion rates tend to be low, with up to 8% conversion of ALA to EPA in men and up to 21% in women, making them conditionally essential nutrients.[3] Apart from these gender differences, conversion can also vary significantly between individuals based on genetic variations in the enzymes involved,[4] and is known to be decreased (leading to low EPA levels) in certain chronic conditions, including diabetes.[5] The enzymes responsible for conversion also require vitamins and minerals, including magnesium, zinc, B vitamins and vitamin C, as co-factors, therefore nutrient deficiencies may also affect conversion.[6]

The n-3 PUFA status is often expressed as the Omega-3 Index (O3I), which is a measure of the percentage of EPA and DHA of all fatty acids in the membranes of red blood cells (RBCs).[7] This measure was first established to assess risk of death from coronary heart disease (CHD), and the optimal O3I is thought to be 8–12%, with < 4% conferring a significantly increased risk.[8] Levels below 2% have not been found, suggesting that this is the minimum level to support life.[9] Low O3I has also been associated with many other conditions, as discussed under the section 'Clinical uses'.

Not all clinical trials established O3I at baseline, which may explain the sometimes contradictory results. A 2019 study set out to establish a model that would allow to estimate the change in O3I depending on supplemental dose.[7] The authors took data from 14 studies (1422 individuals) and found that after dose, baseline O3I and type of supplement were the most important determinants of an increase in O3I, with triglyceride forms raising O3I more than ethyl esters (EEs). The model suggests that to increase O3I from below 4% to above 8%, 95% of people would need about 2000 mg EPA and DHA combined per day, which is significantly more than the amount found in the one–two portions of seafood per week recommended by the American Heart Association. The authors note that whilst this model would be useful on a population basis, in clinical practice direct testing of O3I would be preferable due to the significant inter-individual variability in response to supplementation.[7] In one study of 40 participants with an O3I < 5%, the change in O3I after 8 weeks of consuming a drink containing EPA 200 mg and DHA 300 mg per day ranged from −0.03% to +7.16%.[10]

Just as important as absolute intakes and levels of n-3 PUFAs is the ratio of n-6 versus n-3 PUFAs, as the same enzymes are responsible for conversion of the

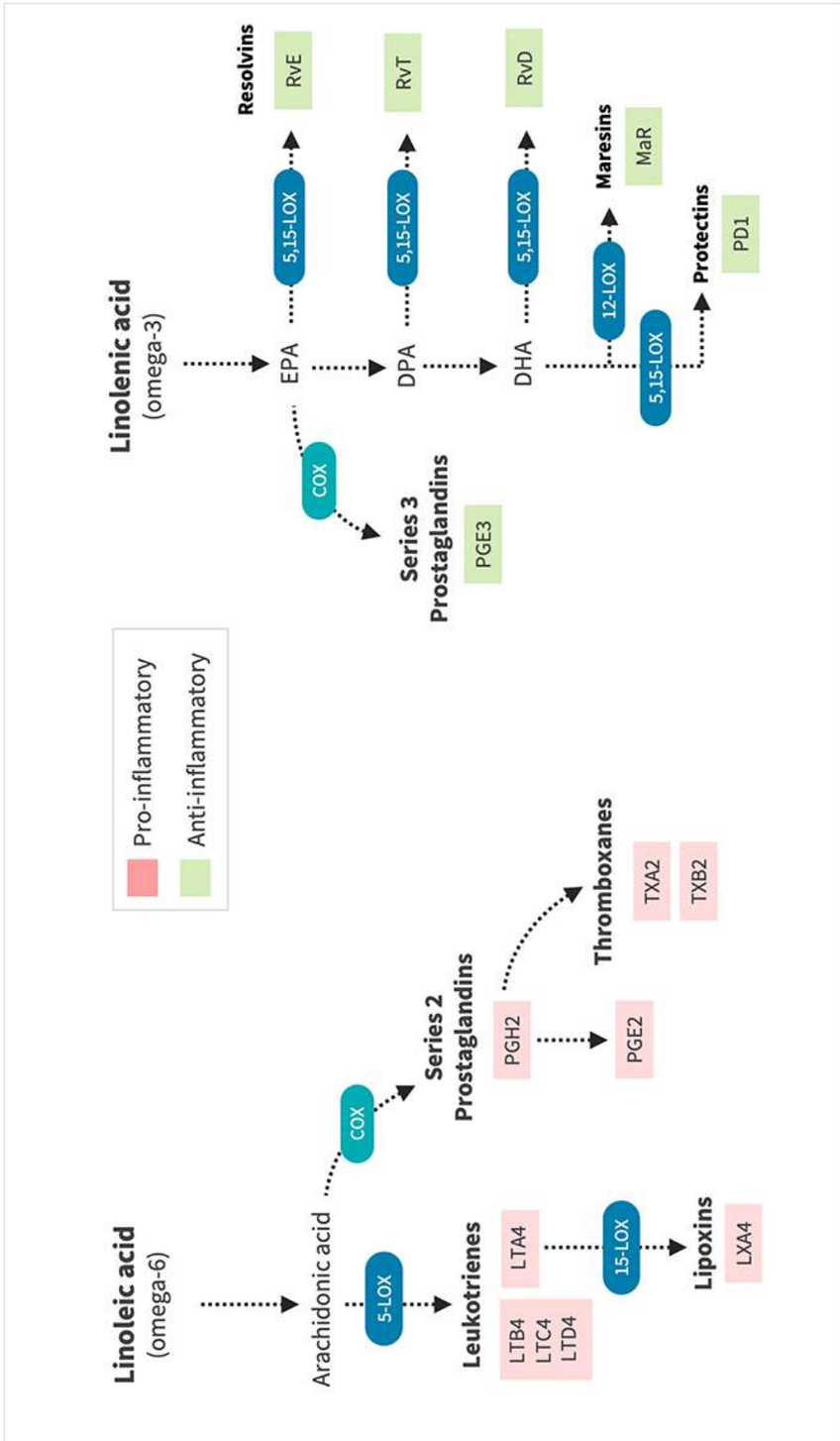

Figure 1: Synthesis of Eicosanoids and Lipid Mediators from Essential Fatty Acids

Figure 1: Dietary omega-6 fatty acids are precursors of arachidonic acid (left), which gives rise to pro-inflammatory leukotrienes, series 2 prostaglandins and thromboxanes. Conversely, omega-3 fatty acids (EPA, DPA and DHA) are precursors of various anti-inflammatory series 3 prostaglandins and specialized pro-resolving mediators (resolvins, maresins and protectins), which participate in the resolution of inflammation. Abbreviations: LOX, lipoxygenase; COX, cyclooxygenase; Rv, resolvins; PD1, protectin D1; MaR, maresins).
Illustration by Kelly Heim, Ph.D., IntegrativePharmacology.com.

parent PUFAs to EPA, DHA, GLA and AA, respectively. In a typical Western diet, the n-6:n-3 ratio tends to be very high, in the range of 10:1 to 25:1, whilst it is believed that our ancestor hunter–gatherers consumed a diet with a ratio nearer to 1:1, a change that is thought to be implicated in the high prevalence of inflammatory conditions, including cardiovascular disease (CVD).[11,12] It has also been suggested that lowering dietary n-6 may have a positive effect on n-3 status.[13]

Food sources

Oily fish, such as salmon, mackerel and sardines, is the richest dietary source of EPA and DHA.[14] Eggs from hens fed a high n-3 diet are a good source of DHA,[15] and meat from grass-fed (pastured) cattle and lamb also provide EPA and DHA[16,17,18] (see Table 1 for contents of EPA and DHA in foods).

The Scientific Advisory Committee on Nutrition (SCAN) recommends a daily intake of 450 mg EPA and DHA combined.[20] From the data in Table 1, it appears that a well-planned omnivorous or pescatarian diet, including oily fish 1–2 times per week, can provide this level of EPA and DHA. However, in 2001/2002, the average consumption of EPA and DHA in the UK was 244 mg per day in adults, 199 mg of which came from fish.[20] SCAN concludes that "The majority of the UK population does not consume enough fish, particularly oily fish, … the COMA population guideline recommendation that people should eat at least two portions of fish a week of which one should be oily. Consumption of this amount would probably confer significant public health benefits to the UK population in terms of reducing CVD risk. There may also be beneficial effects on fetal development".[21]

Table 1: Food sources of EPA and DHA

Food (literature source)	EPA (mg/100 g)	DHA (mg/100 g)
Fish, white[19]		
Cod (baked)	30	100
Coley (raw)	40	190
Haddock (poached/ steamed)	50	130
Fish, oily[19]		
Mackerel (grilled)	1170	1850
Sardines (canned in tomato sauce, whole contents)	1540	900
Salmon (baked)	730	1400
Trout (baked)	570	740
Eggs[15]		
Normal eggs, yolk only*	10	380
Omega-3-rich eggs, yolk only*	30	650
Grass-fed meat		
Grass-fed beef[16]	25	4
Grass-fed lamb[18]	25	7
Grass-fed lamb's liver[16]	75	173

*An egg contains approximately 18 g yolk.

Reasons for the low consumption of fish may include taste and dietary preferences, cultural/religious reasons, cost and availability, as well as concerns over sustainability, and contamination with heavy metals and other toxins. Most red meat consumed in the UK comes from grain-fed animals, which has much lower levels of EPA and DHA and higher levels of AA.[22]

A cross-sectional study in the USA and Canada showed that among people not taking fish oil supplements, only 3% of those consuming 1 fish serving per week and 17% consuming ≥ 2 servings achieved an optimal O3I of ≥ 8.0%.[23] The study also suggested that non-fish eaters would need to supplement 1300 mg per day of EPA and DHA to achieve an optimal O3I. The large variability in n-3 metabolism and response to intake needs to be taken into account though; in a clinical setting RBC O3I should ideally be determined at baseline to establish need, and then used to monitor response to dietary/supplement intervention.

Overall, supplementation may be necessary to make up for dietary shortfalls, and will also be necessary where high dosages are desirable for therapeutic reasons (see the section 'Clinical uses').

The DHA can be retro-converted to EPA,[3] making DHA supplements of algal origin a viable option for n-3 supplementation for vegetarians and vegans. It is important to note that both dietary intake and individual metabolism of PUFAs determine a person's PUFA status.[4]

Bioavailability of supplements

Apart from differing ratios of EPA and DHA in supplements, which are discussed in the section 'Clinical uses' where relevant, fish oils also come in various different formulations, mostly EEs, free fatty acids (FFAs) and triglycerides/triacyl glycerides (TGs). A number of studies have looked at the bioavailability of these formulations.

Whilst some studies found TG formulations to have better bioavailability than EE,[24,25,26] others have found no difference between the two.[27,28,29,30] Studies that compared FFAs with EE formulations found significantly better bioavailability of the FFA supplements, especially when administered with a low-fat meal.[26,31,32,33] This is thought to be due to the fact that lipase is needed for digestion of the EE form.[32] FFA fish oils have also been shown to be better absorbed than TG formulations.[26] The absorption of FFAs appears to be similar to that of naturally occurring fish oils, as in fish body or cod liver oil.[31]

Overall, the evidence suggests that fish oils in the FFA form have the best bioavailability, especially when taken with a low-fat meal. It should be noted that much of the research into bioavailability of fish oils was carried out in the 1990s.

The aim of this paper is to review the evidence for benefits of EPA and DHA supplements from clinical intervention studies.

General mechanisms

Membrane structure and function

The n-3 and n-6 PUFAs are essential structural components of cell membranes. The double-bonds in the carbon-chain of fatty acids introduce 'kinks', which affect the fluidity of the membrane's phospholipid-bilayer and as such its permeability, as well as the structure and function of membrane-bound proteins (including enzymes and ion channels) and cell signalling pathways.[34,35,36] Dietary intake of PUFAs can therefore play an important role in membrane structure and function.

The predominant PUFA in retinal and neuronal postsynaptic cell membranes is DHA, and as such is thought to play an important role in eye and nervous system function and development of the foetus.[35,36]

Anti-inflammatory effects

EPA and DHA are probably most used for their anti-inflammatory properties, which are mediated through a number of mechanisms.

In response to hormones, cytokines (inflammatory mediators) and other compounds, PUFAs can be released from cell membranes and converted to a range of eicosanoids that can have either pro- or anti-

inflammatory properties, as well as regulate vascular permeability, vasoconstriction and bronchoconstriction.[36] The interplay of these inflammatory mediators is complex but, generally speaking, eicosanoids derived from n-3 PUFAs are considered to be more anti-inflammatory.[36]

EPA and DHA are also precursors to specialised pro-resolving mediators, compounds that ensure that inflammation, which is an essential protective and repair mechanism, is switched off after the acute inflammation following an insult, such as injury or infection, and does not turn into chronic inflammation, which is the main underlying cause of most chronic diseases.[37]

Another anti-inflammatory mechanism of EPA and DHA is via regulation of gene expression (see below).

Over 20 randomised-controlled trials (RCTs) of fish oil have collected data on inflammatory markers, and several meta-analyses have concluded that EPA and DHA can reduce C-reactive protein (CRP; a marker of inflammation) in people with dyslipidaemia and/or high CRP levels at baseline,[38] in patients with HIV,[39] and in patients on haemodialysis,[40] whilst they found no effects on tumour necrosis factor-alpha (TNF-α) or interleukin-6 (IL-6),[39,40] two other commonly measured inflammatory markers.

Antioxidant effects

The n-3 fatty acids are also believed to have antioxidant properties, although some studies in the 1990s have actually shown pro-oxidant effects at high dosages. This is thought to be due to the high degree of unsaturation (i.e. double-bonds between the carbon atoms) that makes them unstable and prone to oxidation. Studies have shown benefits of combinations of omega-3 PUFAs with vitamin E, which acts as an antioxidant and thus protects the n-3 fats.[41]

For example, malondialdehyde (MDA; a marker of oxidative stress) and lipid peroxides (oxidised lipids) increased in a double-blind, placebo-controlled trial of a high-dose fish oil supplement, EPA 3062 mg and DHA 2262 mg per day, with tert-butylhydroquinone (a synthetic phenol used as a preservative).[42] This increase was seen both with and without additional vitamin E (900 IU per day as DL-α-tocopheryl acetate). A study using 10 000 mg fish oils per day for 4 weeks also found increases in oxidised plasma lipoproteins both in smokers and non-smokers, which was ameliorated when vitamin E (600 IU per day) was added.[43]

However, whilst there is some conflicting research on this topic, two recent meta-analyses do not confirm a pro-oxidant effect of EPA and DHA.[41,44] A meta-analysis of 39 studies found that EPA and DHA supplements can significantly increase total antioxidant capacity (TAC) and activity of glutathione peroxidase (an antioxidant enzyme), and decrease MDA, whilst it found no significant effects on reduced glutathione, nitric oxide (NO), or the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT).[44] Similarly, a meta-analysis of nine RCTs looking at co-supplementation of vitamin E and n-3 fatty acids (from plant or animal origin) found significant improvements of MDA, TAC as well as NO, but not glutathione, SOD or CAT.[41] Neither study found a worsening of any oxidative stress markers.

Whilst the evidence from clinical trials supports an antioxidant, rather than pro-oxidant, effect of EPA and DHA, it should be borne in mind that these fats are vulnerable to oxidation, and the antioxidant status of the person taking fish oils needs to be considered.

Regulation of gene expression

The n-3 PUFAs have been shown to regulate gene expression for proteins involved in lipid and carbohydrate metabolism, thermogenesis and inflammatory processes by binding to transcription factors (proteins that control the transcription of genes by binding to specific DNA sequences), including sterol regulatory element-binding protein, peroxisome proliferator-activated receptors, carbohydrate response element-binding protein and nuclear factor-kappa B.[36,45]

Clinical uses

Attention deficit hyperactivity disorder (ADHD)

Attention deficit hyperactivity disorder is characterised by a variety of symptoms, including hyperactivity, restlessness, inability to stay focused, mood swings, temper tantrums, problems completing tasks and impulsivity, and is commonly associated with other disorders including anxiety, depression and learning difficulties.[46]

A review of 15 observational studies looking at n-3 levels in people with ADHD has found lower O3I compared with healthy controls, which could not consistently be explained with dietary intakes, suggesting metabolic differences to be responsible for this finding.[46] The authors suggest that measuring baseline O3I may help identify those patients who may benefit most from fish oil supplementation. A more recent observational study from Italy confirms these findings, reporting a lower O3I in children with ADHD than in healthy controls.[47]

A 2018 meta-analysis of n-3 monotherapy RCTs in children with ADHD found significant improvements in ADHD clinical symptom scores (based on five trials) and cognitive measures associated with attention (based on three trials).[48] Dosages used in the trials included in this meta-analysis ranged from 126 to 1290 mg combined EPA and DHA per day. Except for two studies that only used EPA, all studies used combinations of EPA and DHA, at various ratios. Durations were not reported. Another meta-analysis including 10 RCTs in children or adults found no benefits of fish oils when pooling data from all studies, but significant improvements in emotional liability and oppositional behaviour when limiting the meta-analysis to high-quality studies.[49] Dosages and durations were not reported.

Significant improvements have also been seen with EPA 294 mg and DHA 206 mg per day for 12 weeks, alongside a Korean red ginseng extract.[50,51]

Overall, the clinical evidence suggests a benefit of EPA and DHA for children with ADHD, with a range of dosages showing benefits.

Asthma

Asthma is a chronic inflammatory disease of the airways, and is associated with airway hyper-responsiveness, airway smooth muscle contraction and excess mucous production.[52]

A cross-sectional study in Korean pre-school children showed that children with atopic conditions (asthma, allergic rhinitis and/or atopic dermatitis) had lower O3I than children without atopy, 7.8% versus 9.1%.[53] They also had lower overall n-3 PUFAs, and higher n-6 PUFAs and n-6:n-3 ratio. An Australian cross-sectional study on the other hand found no difference in O3I in adults with and without asthma, 6.2% versus 6.1%, and in fact showed higher n-3 PUFAs and a lower n-6:n-3 ratio in asthmatics compared with healthy controls.[52] They did, however, find that those asthmatics with the higher O3I had better controlled asthma.

A 2002 Cochrane review found no consistent effects of fish oils on markers of asthma severity, including forced expiratory volume (FEV1), peak flow rate, asthma symptoms, asthma medication use or bronchial hyperreactivity.[54] Adult dosages ranged from 1000 to 5400 mg combined EPA and DHA, at varying ratios, and durations ranged from 8 weeks to 12 months.

Since then, several more studies have evaluated the use of fish oils in asthma with contradictory results.

A double-blind, placebo-controlled trial in 12–25 year olds with overweight/obesity and uncontrolled asthma found no benefit of fish oils (EPA 3180 mg and DHA 822 mg per day) for 24 weeks.[55] A double-blind, placebo-controlled

study investigating a combination of fish oils (EPA 230 mg, DHA 125 mg per day), probiotics, and a fruit and vegetable extract in children aged 10–12 years, on the other hand, found significant improvements in pulmonary function and reduced medication use compared with placebo, although there was no difference in Paediatric Asthma Quality of Life Questionnaire score and the Childhood Asthma Control Test.[56] Because this was a multi-nutrient intervention, the effect of the fish oils cannot be determined.

In adults, a double-blind, crossover study found significant benefits for exercise-induced bronchoconstriction at a dose of EPA 3200 mg and DHA 2000 mg per day for 3 weeks.[57] On the other hand, another double-blind, crossover study, using slightly higher doses (EPA 4000 mg and DHA 2000 mg per day, for 3 weeks), found no benefits despite an observed change in blood fatty acids confirming compliance of study subjects.[58]

A couple of uncontrolled/unblinded studies found benefits of fish oils, EPA 180 mg and DHA 120 mg per day for 6 and 3 months, respectively, in adults[59] and children.[60]

Overall, the results from clinical research are contradictory. Long-term supplementation (3–6 months) with a low dose, for example EPA 180 mg and DHA 120 mg per day, appears to be of more benefit than high-dose supplementation, although the current evidence is insufficient to make clear recommendations on supplementation of fish oils in asthmatics.

Atopic dermatitis

Atopic dermatitis/eczema is a chronic inflammatory condition of the skin.

A couple of small double-blind, placebo-controlled trials showed significant benefits in patients with eczema. One study with 23 participants used EPA 1800 mg and DHA 1200 mg per day for 12 weeks, and found significant improvements of patient-assessed itch and scale and total score with fish oil versus placebo, whilst physician-assessed scores improved by 30%, but this failed to reach statistical significance.[61] Another study in 53 patients found that DHA 5350 mg and EPA 370 mg per day for 8 weeks led to a statistically significant decrease in severity scoring of atopic dermatitis (SCORAD) from 37 to 28.5, compared with a non-significant reduction with placebo from 35.4 to 33.[62]

Whilst research into the benefits of fish oils in atopic dermatitis is very limited, there are some promising results. Both studies used high doses of fish oils but very different EPA:DHA ratios, making it difficult to make any particular recommendations.

Autism

Autism spectrum disorder (ASD) is characterised by difficulties in social communication and interaction, and restricted and/or repetitive interests, activities and/or patterns of behaviour.[63] PUFAs, in particular EPA and DHA, have received much interest in the treatment of ASD due to their roles in brain functioning through their anti-inflammatory properties and importance for brain cell membrane integrity.[63] Restricted dietary behaviours in individuals with ASD may lead to nutrient deficiencies, including EPA and DHA.

One study looked at O3I of children with ASD and found a low O3I of 4.8% at baseline, which increased by 4.2% in those who received DHA.[64]

Three meta-analyses have reviewed the clinical evidence for the use of n-3 PUFAs in ASD over the past 5 years, including basically the same studies and coming to similar conclusions.[63,65,66] The most comprehensive and recent article reviewed 13 RCTs, and included nine trials, involving 372 children, in the meta-analysis.[65] It found a significant improvement with n-3 supplementation in the overall Aberrant Behaviour Checklist (ABC) score, but not in the ABC subscores or the Social Responsiveness Scale. Dosages ranged from 200 mg (DHA only) to 1500 mg of EPA and DHA combined, with various ratios of EPA and DHA. Some formulations also included n-6 fatty acids and/or vitamins and minerals. The durations of the studies ranged from 6 to 24 weeks. Eight out of the 13 studies found significant improvements in some outcome measures, but there was no obvious correlation with either dose or EPA:DHA ratio.

Two more double-blind RCTs have been published since. One RCT, using EPA 180 mg and DHA 120 mg per day for 8 weeks, found small but statistically significant improvements in stereotyped behaviour, social communication and Gilliam Autism Rating Scale, but no change in social interaction.[67] The other study used DHA (722 mg per day) for 12 months, and reported significant improvements in inappropriate speech, stereotypy, lethargy and irritability compared with placebo.[64]

Overall, there appears to be a benefit of EPA and DHA in ASD, but the data are too inconsistent to make recommendations regarding dosage or EPA:DHA ratio.

Autoimmune conditions

Inflammatory bowel disease (IBD)

Inflammatory bowel disease is an auto-inflammatory condition characterised by inflammation of the gastrointestinal tract (GIT), and includes Crohn's disease (CD; which can affect any part of the GIT) and ulcerative colitis (UC; which only affects the colon). Due to the inflammatory nature of these conditions, fish oil supplements have received much interest because of their anti-inflammatory effects.

A study in patients with CD has shown that n-3 levels in serum were half those of healthy controls.[68] This study also showed that supplementation with EPA 510 mg and DHA 344 mg per day alongside vitamin D (1000 IU per day; vitamin D is commonly low in patients with IBD) for 4–6 weeks increased n-3 status, although this did not have any effect on CRP or calprotectin (a marker of intestinal inflammation).[68]

By far the largest clinical trials into the potential benefits of fish oils in preventing relapse in CD are the Epanova Program in Crohn's (EPIC) Studies 1 and 2.[69] These double-blind, placebo-controlled trials of 363 and 375 patients, respectively, investigated the effects of EPA 2000 mg and DHA 600 mg for up to 58 weeks, and found no significant reduction in relapse rate compared with placebo. An RCT in children with CD, using EPA 400 mg and DHA 200 mg per day, on the other hand found a significant reduction of relapses over a 1-year period, 95% in the placebo group compared with 61% in the fish oil group.[70]

One RCT compared n-3 (3000 mg per day, dose of EPA and DHA not reported) versus n-6 supplementation (7600 mg per day), and found a decrease in CD Activity Index and CRP in both groups (all patients also received other medications).[71] Whilst the n-3 group had stable pro- and anti-inflammatory markers over the 2 months of supplementation, despite a lower than usual dose of steroids, the n-6 groups had a significant increase in pro- and decrease in anti-inflammatory cytokines.

Although evidence is somewhat mixed, at present the clinical research does not support a recommendation of fish oil supplements for patients with CD.

Findings from clinical trials in UC, mostly from the 1990s, are also mixed. A number of double-blind, placebo-controlled trials have found benefits for patients with UC with regards to disease markers such as calprotectin[72] and leukotriene B4 (an inflammatory marker),[73,74] as well as clinical outcomes, such as disease activity index and/or histological findings,[72,73] and reduction in medication use.[73,74] However, other double-blind/placebo-controlled trials found no significant benefits,[75,76] sometimes despite an improvement in n-3 status.[76]

A decrease in inflammatory and/or oxidative stress markers in patients with UC has also been seen in a number of unblinded and/or uncontrolled trials,[77,78,79,80,81] although these were not always associated with significant clinical improvements.[77,78,79,80]

Most of these trials used high dosages of EPA and DHA, combined dose 4100–6400 mg per day or EPA alone 2000–4500 mg per day, and durations ranged from 3 months to 2 years. There was no obvious trend of either a combination of EPA and DHA or EPA alone, or dose or duration to affect results.

An open-label pilot study, using EPA 2000 mg per day, looked at changes in the microbiome, which is commonly abnormal in patients with UC, and found improvements after 3 months, which were accompanied by clinical improvements.[82]

Although results are mixed, there appears to be some benefit of EPA and DHA in UC but, at this point, there are insufficient data to recommend a particular dose. Assessing O3I at baseline may help identify patients who may benefit from fish oil supplementation.

Multiple sclerosis (MS)

Multiple sclerosis is a chronic inflammatory disease characterised by demyelination of nerve cells and injury to the central nervous system.[83]

A 2019 meta-analysis of four double-blind RCTs found no significant effect of EPA and DHA on the Expanded Disability Status Scale (EDSS).[84] This meta-analysis also looked at inflammatory markers; both studies that investigated IL-1b found significant improvements, whilst only one of two studies that investigated TNF-α and IL-6 found significant decreases. Dosages of combined EPA and DHA used in the studies included in this meta-analysis ranged from 300 to 4000 mg per day for 12–24 weeks, with various EPA:DHA ratios. Overall higher dosages appeared to give better results.

Two double-blind, placebo-controlled studies not included in the above meta-analysis have been published. One using low levels of EPA and DHA, 180 mg and 120 mg per day, respectively, for 12 months found no effect on inflammatory markers or EDSS.[85] The other supplemented EPA 800 mg and DHA 1600 mg per day for 12 months, and found significant improvements in inflammatory markers, but not in the glutathione redox system, EDSS or relapse rate, despite a more than doubling in O3I (from 3.7% to 8.0%) and a concomitant decrease in the AA:EPA ratio.[83,86]

A small open-label study using EPA 2900 mg and DHA 1900 mg per day investigated a particular marker, matrix metalloproteinase-9 (MMP-9), which is thought to play an important role in MS by disrupting the blood–brain barrier, aiding the migration of inflammatory cells into the central nervous system.[87] The fish oil supplement decreased MMP-9 after 3 months of supplementation, but did not lead to an improvement in quality of life. A 6.3-fold increase in EPA and a 1.7-fold increase in DHA in RBC membranes was also seen in this study.

Overall, the evidence suggests that EPA and DHA have a positive effect on a variety of biomarkers but not on clinical outcomes in patients with MS.

Psoriasis

Psoriasis is an autoimmune condition of the skin that is characterised by excessive proliferation of cells in the epidermis, which leads to red, flaky patches of skin covered with silvery scales.

Two meta-analyses on the use of fish oils in psoriasis were published in 2019, with differing conclusions. One meta-analysis of 10 RCTs including 560 patients found significant improvements in Psoriasis Area and Severity Index (PASI), erythema (redness) and scaling, whilst improvements in other outcome measures, itching, desquamation, infiltration and percent total body surface area, failed to reach statistical significance.[88] Overall, eight out of the 10 studies reported some benefits. Dosages used in most of the included studies ranged from 352 to 6000 mg per day combined EPA and DHA, at various ratios, with durations of 4–36 weeks.

The other article reviewed 13 RCTs, but only included three in the actual meta-analysis and found no improvement in PASI.[89] Of the studies not included in the meta-analysis of this review article, one used intravenous EPA and DHA, and one used a supplement mostly containing n-6 (GLA). Six of the remaining eight studies did show some positive results. Dosages and durations were in similar ranges as in the above meta-analysis.

Overall, the evidence suggests that fish oils are of benefit in psoriasis, with a wide range of dosages and EPA:DHA ratios showing positive results.

Rheumatoid arthritis (RA)

Rheumatoid arthritis is a systemic autoimmune inflammatory condition affecting the joints, but also increasing the risk of CVD in patients. RA affects 0.5–1% of the population worldwide, with women more commonly affected than men.[90]

A 2018 meta-analysis of 20 RCTs involving 1252 patients with RA found that fish oil supplementation significantly improved disease-related markers: early morning stiffness; tender joint count; erythrocyte sedimentation rate (a marker of inflammation); pain; Health Assessment Questionnaire; Ritchie articular index; and grip strength.[90] Of five inflammatory markers evaluated, only one, leukotriene B4, was significantly reduced compared with placebo, whilst there was no significant improvement in CRP, IL-6, TNF-α and IL-1. Dosages ranged from 1670 to 5400 mg per day, one study used EPA and one ALA only, all other studies used various combinations of EPA and DHA, for 12–72 weeks. Notably, the study on ALA only did not find any benefits for RA outcomes.[91]

A number of RCTs not included in the above meta-analysis, many from the 1990s, have also found benefits of EPA and DHA in patients with RA[92,93,94,95,96,97,98,99] and juvenile idiopathic arthritis.[100]

A couple of studies compared high-dose versus low-dose fish oil supplements, and found improvements in clinical outcomes with the high dose but not the low dose. Doses in these studies were EPA 728 mg and DHA 156 mg versus EPA 364 mg and DHA 78 mg per day,[99] and combined EPA and DHA 5500 mg per day versus 400 mg per day.[93]

With an increase in veganism/vegetarianism, plant-based n-3 supplements have also received interest, and whilst ALA does not appear to have beneficial effects in RA,[91] DHA from algae, 2100 mg per day for 10 weeks, has been shown in a double-blind, placebo-controlled crossover study to improve clinical outcomes and decrease both RBC AA:EPA and AA:DHA ratios, resulting in a positive shift in eicosanoids.[101]

Most trials used dosages of combined EPA and DHA of 3000 mg per day and more, with a range of 1300–5800 mg per day for 3–12 months. EPA:DHA ratio varied between studies.

The evidence from clinical trials is overwhelmingly in favour of a therapeutic effect of EPA and DHA in RA, with higher dosages showing better results. A daily dose of 3000 mg per day could be suggested, whilst the ratio of EPA:DHA does not appear to make a difference.

Systemic lupus erythematosus (SLE)

Systemic lupus erythematosus is a systemic autoimmune condition, affecting mostly women, which is characterised by inflammation in several tissues and organs, including joints, skin, kidneys and blood vessels, potentially causing significant damage to these organs and increasing the risk of CVD.[102]

A cross-sectional pilot study in 33 women with SLE, with or without concomitant CVD, and 20 healthy controls found that women with SLE had a significantly lower O3I and higher AA:EPA ratio than healthy controls, irrespective of their CVD status.[103]

A 2020 meta-analysis of five RCTs involving 274 patients with SLE found a significant reduction in SLE Disease Activity Index with fish oils.[104] Dosages of combined EPA and DHA used in the studies ranged from 1280 to 4500 mg per day, for 12–26 weeks, with varying EPA:DHA ratios. Studies with dosages of 3000 mg per day or more appeared to show more benefits.

Three double-blind, placebo-controlled crossover studies from the 1990s, not included in the above meta-analysis, gave conflicting results. One study showed an improvement after 12 weeks in 14 out of 17 patients on the fish oil supplement (20 000 mg n-3 fats, EPA and DHA amounts and ratio not reported), whilst 13 out of 17 patients stayed the same or worsened on the placebo, a statistically significant difference.[105] All patients in this study followed a low-fat diet, and also received vitamin A (4000 IU) and vitamin D (400 IU). The second study found improvements in the fish oil group (200 mg per kg body weight, DHA:EPA not reported) compared with the placebo group after 3 but not after 6 months of supplementation.[106] The third study focussed on patients with stable lupus nephritis, and found no difference between fish oil, EPA 2700 mg and DHA 1700 mg per day, and placebo after 1 year of supplementation in either disease activity or renal function.[107]

There appears to be a benefit of EPA and DHA in SLE, at a dose of 3000 mg per day combined or more.

Cardiovascular disease

Cardiovascular disease is a general term for diseases affecting the heart and blood vessels, and includes CHD, which can lead to angina, myocardial infarction (MI) and heart failure, strokes and transient ischaemic attacks (TIAs), and peripheral artery disease. CVD is the leading cause of death globally, and it presents a significant healthcare burden.[108]

The n-3 PUFAs have been extensively studied in the prevention and treatment of CVD, both in clinical trials and in observational studies. There have been contradictory findings leading to controversy over the use of fish oils in patients with or at risk of CVD.

Low O3I has been associated with increased risk of CVD, and cut-off points of low (4%), intermediate (4–8%) and a desirable high level of > 8%, at which CVD risk is lowest, have been suggested in the early 2000s.[109] These findings were confirmed in a 2017 meta-analysis of 10 observational studies, which also found a 15% decrease in risk of CHD for a 2.1% increase in O3I.[110] Similar reductions were also found in the Framingham offspring cohort (2500 participants) where those in the highest O3I quintile (> 6.8%) had a 39% lower risk of CVD events compared with the lowest quintile (< 4.2%), and a 34% lower risk of all-cause death, although there was no statistically significant reduction in risk of CVD death.[111] This study found a stronger association for DHA than EPA.

In 2018, a meta-analysis of 10 RCTs, which included at least 500 participants and lasted for at least 1 year (47 803 participants overall), made headlines as it concluded that there is no statistically significant effect of EPA and DHA on either CHD event, CHD deaths, non-fatal MI or any major vascular event.[112] Dosages used in the included trials ranged from 376 to 2550 mg per day.

Since then, seven more meta-analyses of RCTs have been published, all of which show benefits of fish oils in CVD.[108,113,114,115,116,117,118] The most recent and most comprehensive one reviewed 83 RCTs and cohort studies for 11 CV outcomes.[113] The findings are as follows.

- Total mortality from CV causes:
  – 26 RCTs with 82 696 participants: statistically significant 8% reduction in risk, low heterogeneity;
  –15 cohorts with 558 826 participants: statistically significant 24% reduction, high heterogeneity.
- CV deaths:
  – 22 RCTs with 76 407 participants: statistically significant 7% reduction, low heterogeneity.
- Cardiac death:
  – 20 RCTs with more than 79 410 participants: statistically significant 10% reduction, low heterogeneity.
- Post-operative atrial fibrillation (AF):
  – 21 RCTs with 4201 participants: statistically significant 35% reduction, high heterogeneity.
- Coronary events:
  – 10 RCTs with 77 917 participants: no statistically significant effects, no heterogeneity.
  – 14 cohorts with 344 722 participants: statistically significant 11% reduction, low heterogeneity.
- Coronary deaths:
  – 10 RCTs with 77 917 participants: no statistically significant effect, no heterogeneity.
  – 10 cohorts with 357 621 participants: statistically significant 15% reduction, no large heterogeneity.
- Arrhythmias or sudden death:
  – 12 RCTs with 43 987 participants: no statistically significant effect, low heterogeneity.
  – 5 cohorts with 201 205 participants: statistically significant 47% reduction, no heterogeneity.
- CV events:
  – 9 RCTs with > 72 179 participants: no statistically significant effect, low heterogeneity;
  – 6 cohort studies with 68 954 participants, no statistically significant effect, large heterogeneity.
- MI:
  – 20 RCTs with 86 411 participants: no statistically significant effect, low heterogeneity;
  – 7 cohort studies with 274 083 participants: no statistically significant effect, large heterogeneity.
- Recurrent AF:
  – 8 RCTs with 1990 participants: no statistically significant effect, high heterogeneity.
- Stroke/TIA:
  – 11 RCTs with 32 026 participants: no statistically significant effect, low heterogeneity.

The other meta-analyses listed above come to similar risk reductions, although there is some inconsistency as to which CV outcomes have statistically significant results, which is most likely due to the differing inclusion criteria for RCTs, for example, some only included larger studies (more than 500 participants),[108,118] whilst some only included trials that used larger doses of fish oils[116] or durations of supplementation of at least 1 year.[108,115,116]

Dose in particular appears to be an important factor. Two meta-analyses found significant positive dose–response relationships for MI and total CVD,[114] and total CVD and major vascular events,[118] respectively.

A number of meta-analyses also compared lower versus higher dosages. A meta-analysis of 17 RCTs of at least 1 year duration found no significant benefits with dosages (all EPA and DHA combined) below 840 mg per day, a significantly decreased risk of cardiac death with at least 1680 mg per day (based on six RCTs), and significantly reduced risk of sudden death (based on one RCT) and stroke (based on two RCTs) with at least 2520 mg per day.[115] This study found no significant benefit for all-cause mortality and non-fatal MI at any dose.

Another meta-analysis (16 RCTs with 81 073 participants) also found no significant risk reduction with a dose of 1000 mg per day,

but significant reductions in cardiac mortality (9%), major CV events (10%) and MI (17%) with higher dosages.[116] This study also addressed the question of whether EPA alone or in combination with DHA was more effective, with inconsistent results. Whilst the risk of cardiac death was reduced with EPA plus DHA but not EPA alone, the risk of MI and major vascular events was reduced only with EPA alone and not with the combination. It should be noted that only three of the 16 studies included in this meta-analysis actually only used EPA, all others used combinations of EPA and DHA.[116]

A 2020 meta-analysis of 14 RCTs (125 763 subjects, inclusion criteria: at least 500 participants, at least 1 year duration) compared low dose (≤ 1000 mg per day) versus high dose (> 1000 mg per day), and found significant risk reductions with high dose compared with control and low dose in cardiac death (21%, all versus control), MI (29%), coronary revascularisation (26%), unstable angina (27%) and major vascular events (22%).[108] Risk reductions with the low dose versus control were seen in cardiac death (8%) and MI (9%), whilst no significant effects were found for all-cause mortality, stroke or sudden cardiac death.

This study made headlines as it also found a 35% increased risk of AF in the high dose compared with controls, as well as of bleeding events (49%), but not gastrointestinal disturbances.[108]

The issue of AF has been subject to three further meta-analyses specifically on this topic, published in 2021. One was by the same authors as the above meta-analysis, which initially reported the increased risk, and included five RCTs (the initial meta-analysis only included three RCTs for AF) and found similar results, a 37% increased risk.[119] The two other meta-analyses, based on six[120] and eight RCTs,[121] also found statistically significantly increased risks of 31% and 51% with a high dose (more than 1000 mg per day), and 12% with a low dose (1000 mg per day or less). Except for one trial, all trials included in these meta-analyses were performed in patients with established CVD or at high risk of CVD.

A mechanism by which fish oils may increase the risk of AF is unclear,[119,120] raising the question whether the association is causal.

The evidence from clinical trials suggests that EPA and DHA decrease the risk of various CV outcomes, including cardiac death and overall CV mortality, with dosages over 1000 mg per day combined EPA and DHA being more beneficial than lower dosages. At present there are insufficient data to compare EPA alone versus EPA plus DHA.

These benefits need to be weighed against the potentially increased risk of AF. It needs to be borne in mind that, whilst the relative increased risk of AF is relatively high, the absolute risk of AF is actually fairly small. For example, the REDUCE-IT trial showed significant risk reductions in various CV outcomes with a highly purified EPA product (4000 mg per day), but also found an increased risk of AF. The absolute risk of AF with EPA was 5.3% versus 3.9% with placebo, whereas the absolute risks of the primary outcomes, CV death, non-fatal MI, non-fatal stroke, coronary revascularisation and unstable angina combined were 17.2% with and 22.0% without EPA.[120,122]

CV risk factors

Cardiovascular disease usually develops over many years, and risk factors include high blood pressure, abnormal blood lipids, smoking and poor diet. Dyslipidaemia, in particular, has been studied extensively for benefits from fish oils.

A 2018 meta-analysis of 171 RCTs looked at the effect of fish oils on a number of CV risk factors, and found significant reductions in triglycerides (−0.368 mmol/l), systolic blood pressure (−2.195 mmHg), diastolic blood pressure (−1.08 mmHg), heart rate (−1.37 beats per minute) and CRP (−0.343 mg/l), whilst both low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol increased (0.15 mmol/l and 0.039 mmol/l, respectively).[123] No significant changes were found for total

cholesterol, TNF-α, fibrinogen, platelet count, soluble intercellular adhesion molecule-1, soluble vascular cell adhesion molecule-1 or flow mediated dilation (a marker of endothelial function). Dosages in the reviewed studies ranged from 180 to 15 000 mg per day, and durations from 4 to 240 weeks.

A 2020 meta-analysis of 64 RCTs compared the efficacy of krill oil versus fish oil in lowering triglycerides, and found that there was no statistically significant difference between krill and fish oils.[124]

In some countries, including the UK, high-dose EPA EEs are licenced drugs for the treatment of hypertriglyceridaemia.[125]

Children: effect of maternal supplementation during pregnancy

Adequate supplies of n-3 PUFAs, especially DHA, during pregnancy are crucial for the developing foetus, and research on the effects of supplementation during pregnancy has not only focussed on maternal and neonatal outcomes (see the section 'Pregnancy'), but also on the longer-term health of the offspring, in particular with regards to allergy and asthma, body composition and cognitive development.

Allergy/asthma

Results from observational studies suggest that intake of fish during pregnancy reduced the risk of asthma and eczema in the offspring; however, evidence from RCTs of n-3 supplementation during pregnancy give conflicting results.[126]

A 2019 meta-analysis of 10 RCTs including 3637 children found no significant effect of n-3 supplementation during pregnancy alone, or pregnancy and lactation/neonatal supplementation on any allergic outcomes (any allergy, eczema, asthma/wheeze, allergic rhinitis, food allergy) except sensitisation to eggs and peanuts.[127] Dosages ranged from 400 to 2400 mg per day with various EPA:DHA ratios. One study used two portions of salmon and one blackcurrant seed oil rather than fish oils. There were no obvious trends with regards to particular dosages or different sources being more or less effective.

In a 2020 meta-analysis of seven RCTs including 2047 children, with follow-up periods of 6 months to 16 years, focussing on fish oil supplementation during pregnancy and asthma in the offspring only, a reduction in overall risk of asthma failed to reach statistical significance.[128] However, subgroup analysis found significant reductions with a daily dose of 2000 mg combined EPA and DHA per day in those with an atopic family history and in European countries.

Overall, there is conflicting evidence for a benefit of maternal fish oils supplementation on the risk of developing childhood allergies, although those with a family history may benefit from supplementation of at least 2000 mg combined EPA and DHA per day.

Body composition/weight

Two meta-analyses investigated the potential benefits of maternal fish oil supplementation on obesity measures in the offspring. One study of 11 RCTs, including 3644 children, found no significant effects on any obesity outcomes. Dosage range was typically 400 to 1500 mg per day.[129] The other included 26 RCTs with 10 970 participants, and included fish oil supplementation during pregnancy and/or lactation, with a follow up of up to 19 years.[130] This study found significantly higher birth weight and postnatal waist circumference in those whose mother took fish oils compared with controls, but no significant effects on any obesity-related outcomes. The dosage range was 200–3200 mg per day, with various EPA:DHA ratios.

The current evidence suggests that maternal fish oils supplementation does not influence obesity-related outcomes in the offspring.

Cognitive development

Two meta-analyses evaluated cognitive function in the offspring of mothers who took part in fish oil supplementation trials during pregnancy.[131,132] The studies included five and seven RCTs, respectively, with dosages ranging from 200 to 3300 mg per day, and neither found any association with cognitive outcomes.

Cognition

The n-3 PUFAs, and DHA in particular, are essential for the integrity and function of neuronal tissues.[133] Neuronal membranes make up about 30% of brain matter and are high in DHA, which has been shown to be low in people with cognitive decline.[134] Lower O3I has been associated with a more rapid cognitive decline and an earlier onset of dementia compared with a higher O3I, as reviewed by von Schacky in 2021.[135]

A 2020 cross-sectional study also found that higher plasma EPA and DHA levels were consistently correlated with a lower risk of dementia and cognitive decline.[136] The Multidomain Alzheimer's Preventive Trial found an O3I cut-off of 5%, below which dementia-free subjects aged 70 years or older with subjective memory complaints had increased risk of cognitive decline.[137]

In 2020, a meta-analysis evaluated the results of 10 clinical trials, nine of which were RCTs, and found no significant effects of DHA in preventing age-related cognitive decline.[133] The DHA dose range was 190–900 mg per day, either with or without EPA, for 4–36 months. Another meta-analysis of 18 RCTs in adults without dementia found no effect on global cognitive function, but small, statistically significant improvements in memory.[134] Dosage ranges were DHA 200–1550 mg per day, EPA 60–1740 mg per day, and combined 300–2800 mg per day, with durations of 4 weeks to 5 years. The heterogeneity observed in the results could not be explained by either dose or duration of supplementation.

The effect of fish oils on cognition has also been investigated in young people. A meta-analysis of 29 RCTs including 4247 people up to 25 years old found no overall effect of fish oil supplementation on cognitive function (based on standardised cognitive function tests).[138] However, subgroup analysis showed significant benefits of EPA-rich, but not DHA-rich, formulations with more pronounced effects in populations with psychiatric disorders. The EPA dosage range was 0–720 mg per day, DHA dosage range 0–1200 mg per day, and combined dosage range 96–1200 mg per day, and duration was 4–48 weeks.

Overall, clinical trials do not support a role of fish oils in preventing or treating impaired cognitive function, except in young people with psychiatric disorders.

Alzheimer's disease (AD)

There are a number of intervention studies on fish oils in AD specifically.

In 2016, a Cochrane review and meta-analysis of three RCTs, including 632 patients, concluded that EPA and DHA had no significant benefit in mild to moderate AD.[139] A meta-analysis of five RCTs published in 2020 also found no effects of fish oils on cognitive function in patients with AD (no more detail available as the full article is in Spanish).[140]

Three studies, not included in the Cochrane review, also failed to find significant benefits for fish oils in AD, with dosages of EPA 600 mg and DHA 625 mg per day for 4 months,[141] EPA 1080 mg and DHA 720 mg per day for 24 weeks,[142] and EPA 1000 mg per day for 12 weeks.[143]

Those studies that evaluated RBC n-3 PUFAs found significant increases with supplementation.[141,142,143,144] One study also found increases in DHA and EPA in the cerebrospinal fluid (CSF) following supplementation, with DHA levels in CSF being inversely related to total and phosphorylated tau protein (a marker of AD).[145]

Overall, at present, clinical trial data suggest that EPA and DHA are of no clinical benefit in AD.

Diabetes

Global rates of diabetes have reached epidemic proportions, and according to the World Health Organisation the number of diabetics has risen from 108 million in 1980 to 422 million in 2014.[146] As diabetes is an important risk factor for CVD, the role of EPA and DHA on glycaemic control and other CV risk factors has been intensively studied.

Eight meta-analyses, including over 45 RCTs, have evaluated the potential benefits of fish oils in diabetics over the past 3 years, with most showing statistically significant improvements in some but not all markers of glycaemic control, inflammation and blood lipids, with inconsistency as to which markers were improved. Improvements have been seen in fasting blood glucose (FBG),[147,148] HbA1c (glycated haemoglobin),[149] fasting insulin,[147] triglycerides,[148,149,150] apolipoprotein A2,[148] LDL cholesterol,[149] TNF-α[149] and IL-6.[149] However, two meta-analyses did not find any benefits of n-3 PUFAs for glycaemic control in diabetics.[150,151] One meta-analysis found significant benefits, in terms of reduced proteinuria, in patients with diabetic nephropathy.[152]

The dosage range in adults was 400–10 000 mg per day with various EPA:DHA ratios. One earlier meta-analysis carried out a subgroup analysis of EPA:DHA ratio and found ratios of > 1 to be more effective, although this failed to reach statistical significance,[153] whilst none of the above studies reported any effects of dose.

One meta-analysis focussed on insulin sensitivity in children and found significant improvements. Subgroup analysis showed the best benefits with a dosage of 1500 mg per day or less, EPA:DHA higher than 1, and duration 6 months or less.[154]

Overall, fish oils supplementation appears to be of benefit to patients with diabetes, but there is heterogeneity between studies with regards to an effective dose, with a higher EPA:DHA ratio possibly more beneficial.

Male infertility

Infertility affects about 15% of couples worldwide, and in about half of these male infertility is present.[155] Asthenozoospermia is a specific cause of male infertility characterised by reduced or absent sperm motility.[156] A link between sperm motility and the DHA concentration in semen and sperm has been reported, in particular in asthenozoospermic men.[156]

A 2019 meta-analysis of three RCTs involving 290 infertile men reported significant increases in sperm motility and sperm DHA concentration, but no effects on sperm concentration or sperm DHA.[157] Two studies used DHA only (400–800 mg per day), and the third used EPA 1120 mg and DHA 720 mg per day, with no apparent difference in results. Studies lasted for 12–32 weeks.

Three further double-blind, placebo-controlled trials have investigated the effect of DHA-rich supplements, either alone[155,158] or with vitamin E,[156] in infertile men, and have confirmed the positive effects on sperm motility, especially in men with asthenozoospermia. No effects on other traditional sperm or semen parameters were detected in any of the trials, except for improvements in oxidative stress in semen[155] and in antioxidant status, which was associated with decreased DNA damage in the spermatozoa.[158] The DHA dosage range was 465–2000 mg per day, for 10 weeks to 3 months. One study compared daily dosages of 500 mg, 1000 mg and 2000 mg of DHA, and found that improvements were seen after 1 month with 1000 mg and 2000 mg, whilst improvements with 500 mg were only seen after 3 months.[155]

Although there are no data on pregnancy rates, the overall evidence from clinical research suggests a benefit of DHA,

either alone or with EPA, on sperm motility of infertile men, especially in men with asthenozoospermia. A dose of at least 500 mg DHA per day could be recommended.

Mental health

Depression

A review of 15 observational studies looking at n-3 levels in patients with depressive disorders found lower n-3 PUFAs, in particular DHA, compared with healthy controls, with RBC levels for DHA ranging from 0.8 to 4.0% in people with and 1.6 to 5.4% in those without depression.[46] Since then, a number of further studies on O3I and depression have been published.

A study in menopausal women, taking or not taking hormone replacement therapy (HRT), evaluated the association between O3I and depression and found a small inverse association in women taking HRT, but not in those not taking HRT.[159] However, all groups, with or without depression and with or without HRT, had O3I levels of > 8% on average, i.e. in the optimal range. An Australian study of 116 patients with depression and schizophrenia found average O3I levels to be 3.9%, compared with an O3I of 5% in the general Australian population, with more than half the patients having an O3I of ≤ 4% and none an O3I of ≥ 8%.[160] A case–control study in patients with or without major depressive disorder (MDD) reported a mean O3I of 3.9% in those with MDD versus 5.1% in those without.[161]

The O3I has been shown to be lower in adolescents with or at risk of bipolar disorder (BP) compared with healthy controls, with 97% of those who had an episode of BP having an O3I ≤ 4%, compared with 61% of healthy controls.[162] A case–control study in adolescents with or without depression showed DHA to be decreased in those with depression.[163]

In view of the overwhelming evidence that low levels of EPA and DHA are associated with depression, dozens of RCTs have been carried out evaluating the effects of EPA and/or DHA in depression.

Nine meta-analyses have been published in the last 5 years. A 2021 meta-analysis of 31 RCTs, including 41 470 participants, found no effects of n-3 PUFAs on depression or anxiety, and concluded that "Long-chain n-3 supplementation probably has little or no effect in preventing depression or anxiety symptoms".[164] However, this meta-analysis included studies with participants with and without a diagnosis of depression or anxiety, which may have weakened any effects observed in depressed patients. Another meta-analysis of four RCTs published in 2021, which looked at EPA alone or with DHA (range 1000–2000 mg combined) alongside the antidepressant drug sertraline, also found no significant effects.[165]

Dose appears to be an important factor. A meta-analysis of 10 RCTs comparing high (≥ 2000 mg per day combined EPA and DHA) and low dose (< 2000 mg per day) supplementation found both high and low dose to be significantly better than placebo in relieving depression in patients with MDD, and high dose to be better than low dose.[166] This meta-analysis only included studies of 9 weeks or less duration, and with patients who had a diagnosis of MDD. A meta-analysis of 13 RCTs including 1233 patients with MDD also found significant benefits of fish oils in MDD.[167] A subgroup analysis showed better results with higher EPA amounts (no cut-off dose reported). A 2019 meta-analysis of 26 RCTs with 2160 participants also showed significant benefits in patients with depression, especially with high-EPA formulations.[168] This study showed that DHA alone or formulations with higher DHA content had no effect, whilst supplements with EPA alone or making up at least 60% and with an EPA dose of at least 1000 mg per day showed significant benefit.

Another meta-analysis of 25 RCTs involving 1373 patients with MDD also found beneficial effects of EPA and/or DHA, although the authors note that the evidence overall is of poor quality and that there was considerable heterogeneity that was not explained by subgroup or sensitivity analyses.[169]

Two meta-analyses looked at the potential benefits of fish oils for depression in the elderly, with conflicting results. One meta-analysis of nine RCTs found no benefit and no significant moderating effects of comorbidity, baseline depression, intervention duration and EPA:DHA ratio, potentially due to limited statistical power.[170] Only three of the included RCTs were in elderly people with depression at baseline. The other meta-analysis, which included six RCTs, found benefits in people with depression (based on four studies) but not in those without depression (based on two studies).[171]

One meta-analysis of four RCTs looked at the potential benefits of EPA and DHA in 153 children with depression and no drug treatment, and found no significant improvements.[172] Dosages ranged from 1000 mg to 3400 mg per day (combined EPA and DHA) with various EPA:DHA ratios, for 10–16 weeks.

Overall, the evidence suggests that fish oils are of benefit in adults with depression, whilst there is no effect on depressive symptoms in people without diagnosed depression. EPA appears to be of more benefit than DHA, so a pure EPA or high-EPA formula should be chosen, providing at least 1000 mg EPA.

Schizophrenia

Schizophrenia is a chronic, severe mental disorder affecting 1% of the population worldwide.[173] People at high risk of schizophrenia have been found to have low O3I, with a mean of 3.0% in one study.[174]

A 2021 meta-analysis of 20 double-blind RCTs, involving 1494 patients with schizophrenia, found overall statistically significant improvements in psychopathology, particularly general psychopathology, and positive symptoms but not negative symptoms with fish oil supplementation.[175] When EPA dose was greater than 1000 mg, there was a significant beneficial effect on general psychopathology, positive and negative symptoms, whilst with dosages of 1000 mg or less, only general psychopathology improved. Subgroup analysis showed also that benefits were greater in those with more severe disease at baseline.

Since then, two more RCTs have been published, both of which did not show any improvement in schizophrenia outcomes, but EPA dosages were below 1000 mg per day, EPA 540 mg with DHA 360 mg per day for 8 weeks,[176] and EPA 360 mg with DHA 240 mg per day for 12 weeks,[173] respectively, so may have been too low for positive effects. The latter study found significant improvements in brain-derived neurotrophic factor, as well as reductions in TNF-α, CRP and IL-6.[173]

Overall, the evidence from clinical trials suggests that fish oils with EPA dose of over 1000 mg per day are beneficial in schizophrenia.

Non-alcoholic fatty liver disease (NAFLD)

Non-alcoholic fatty liver disease is characterised by excess fat accumulation in liver cells, and is estimated to affect 20–30% of the populations in Western countries.[177] Whilst low dietary n-3 PUFA intakes have been reported in patients with NAFLD, there are no epidemiological studies evaluating the O3I in this condition.[178]

Five meta-analyses, including over 20 RCTs altogether, have been published in the past 3 years assessing the benefits of n-3 PUFAs in NAFLD. One meta-analysis of 13 RCTs including 268 subjects looked at liver enzymes only, and found a significant decrease in alanine aminotransferase, but not other

liver enzymes.[179] The other four looked at a number of outcomes, one in children only,[180] and all found significant decreases in liver fat content.[177,178,180,181] Results for other outcomes were inconsistent; positive effects have been reported for liver enzymes,[177,180,181] blood lipids[177,178,181] and glycaemic control,[177,181] although n-3 fats did not affect all markers (other than liver fat) in any of these studies.

Not all meta-analyses reported dosages, durations and types of n-3 fats included in their meta-analyses, and none reported any effect of dosage on outcomes through subgroup analysis.

Fish oils appear to have a number of benefits in patients with NAFLD, but there is insufficient information to make dosage recommendations.

Obesity/weight loss

In 2018, it was estimated that 41% of men in the UK were classified as overweight and 26% as obese, in women the numbers were 30% and 29%, respectively, and 1 in 5 children is classified as obese.[182] Some animal models have suggested that n-3 PUFAs may play a role in weight management, but evidence in humans has been contradictory.[183]

A cross-sectional study from Australia found that healthy young women with obesity had a significantly lower O3I than their non-obese counterparts, 5.9% versus 6.7%, despite similar intakes.[183] Another study suggests that there may be gender differences with regards to the association of O3I and obesity, with a significant inverse correlation between EPA and DHA and body mass index (BMI), waist circumference and body fat, in women but not men.[184] A correlation between O3I and BMI has also been found in children, with 33% of obese children having an O3I of < 4% compared with 17% of non-obese children.[185]

Two meta-analyses, including 11 and 21 RCTs, respectively, have evaluated the effect of fish oils on weight loss in obesity, and both found that n-3 PUFAs can lead to a reduction in waist circumference,[186,187] although in one this was only when combined with lifestyle modification (diet and/or exercise).[187] Neither study found a significant effect on weight loss or BMI. Across the two meta-analyses, total n-3 dose ranged from 540 mg to 11 300 mg per day, with varying EPA:DHA ratios (EPA/DHA dosages are not reported). One of the meta-analyses reported that neither dose nor EPA:DHA ratio affected outcomes.[187]

One meta-analysis of six RCTs including 342 children looked at the effect of EPA and/or DHA on weight loss in children, and found no effects on either BMI, weight loss or waist circumference.[188]

A more recent RCT in women with depression and obesity found a beneficial effect of EPA and DHA, 1080 mg and 720 mg per day, respectively, for 12 weeks, alongside a calorie-reduced diet, with a significantly greater weight loss in the fish oil group compared with the placebo group, 3.07 kg versus 1.16 kg.[189] However, at follow-up 1 month after the end of the study, women in the fish oil group had regained 2.8 kg, whilst the placebo group reduced weight by a further 0.21 kg.

Overall, the evidence suggests that fish oils may be of benefit for reducing waist circumference in adults with obesity, especially when used alongside other diet or lifestyle interventions. There are insufficient data to recommend a particular dose.

Osteoarthritis (OA)

Osteoarthritis is a common inflammatory condition of the joints, and is often considered to be due to 'wear and tear'. Considering the high prevalence of OA (it is estimated that 9 million people in the UK have OA)[190] and the popularity of fish oil supplements for this indication, clinical research on this topic is limited.

An early pilot study of 26 patients with OA found that EPA (dose reported as 10 ml) for 6 months decreased pain score by 37% and interference with activities by 30%, compared with 21% and 11%, respectively, with placebo, although the difference was not statistically significant, probably due to the small sample size.[191]

Since then, four more RCTs have reported benefits of fish oils, either on their own or in combination with other nutrients. A double-blind, placebo-controlled trial using EPA 400 mg and DHA 2000 mg per day for 16 weeks found significant improvements in OA-specific pain and OA burden in the fish oil group with and without curcumin, compared with curcumin alone or placebo.[192] A study of glucosamine, 1500 mg per day, with or without fish oils found treatment with n-3 PUFAs, 600 mg per day (no further details) for 26 weeks, more efficacious than glucosamine alone.[193]

Two trials compared low- with high-dose fish oils. One of these tested EPA 400 mg and DHA 200 mg per day versus EPA 800 mg and DHA 400 mg per day versus no supplement for 8 weeks, and found significant improvements in all outcome measures with both fish oil supplements compared with control, and the low dose more beneficial than the high dose, although this was not statistically significant for all parameters.[194] The other high- versus low-dose trial tested EPA and DHA 4500 mg per day (ratio 3:2) versus 450 mg per day for 2 years.[195] The investigators found the low dose to lead to significantly greater improvements in pain and function than the higher dose, despite the finding that the latter increased RBC DHA and EPA more than the lower dose.

The evidence suggests that fish oils supplements are of benefit in arthritis, with lower doses possibly more effective than higher dosages. Dosages of 450–600 mg combined EPA and DHA have been shown to be effective.

Pain

Pain is closely associated with inflammation, and EPA and DHA are therefore commonly used for painful conditions other than arthritis.

Several double-blind, placebo-controlled studies have evaluated the effects of fish oils in non-arthritic musculoskeletal pain with conflicting results. A subgroup (1398 patients) of a larger trial found no benefit of EPA and DHA (combined dose 840 mg per day) on chronic knee pain over a median follow-up of 5.3 years.[196] A trial of EPA 1530 mg and DHA 1040 mg per day for 8 weeks in 65 patients with rotator-cuff-related shoulder pain found significant improvements in Shoulder Pain and Disability Index after 3 months, but not in Oxford Shoulder Score.[197] A study in 27 young adults found that EPA 324 mg and DHA 216 mg per day for 30 days reduced delayed-onset muscle soreness at 48 hours after eccentric training.[198] And a study combining EPA 149 mg per day with serine 596 mg per day for 8 weeks has shown significant improvements in 120 patients with knee or low-back pain.[199]

Aromatase inhibitors (AIs) are a commonly used treatment for breast cancer, and can cause musculoskeletal pain. Two double-blind, placebo-controlled trials found no benefit of fish oils compared with placebo in AI-induced pain.[200,201] The combined dosage of EPA and DHA was 4300 mg and 3300 mg per day for 24 weeks, respectively.

Randomised-controlled trials have shown promise in reducing post-operative pain in patients after weight loss surgery with EPA 3000 mg and DHA 1260 mg per day for 10 days before surgery,[202] and in neonates after major heart surgery with DHA 75 mg per kg body weight per day.[203]

An RCT in 120 women with dysmenorrhoea (painful periods) showed that 1000 mg fish oil (no further details reported) daily for 2 months was superior to ibuprofen (taken only when needed) for pain relief.[204]

Another RCT compared amitriptyline alone or with DHA 250 mg and EPA 16.67 mg per day plus alpha-lipoic acid (600 mg per day), for up to 12 weeks, in 84 women with vestibulodynia

(chronic pain in the vulvar area) associated with painful bladder syndrome, and found a significantly greater reduction in pain in those with the supplement compared with those on amitriptyline only.[205]

Overall, EPA and DHA are promising in a number of painful conditions. As both pain conditions and dosage varied widely, it is not possible to make specific recommendations, but durations of 8–12 weeks have been sufficient for more chronic types of pain, whilst in acute post-operative pain shorter durations with higher dosages were beneficial.

Polycystic ovary syndrome (PCOS)

Polycystic ovary syndrome is a common condition 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 androgens ('male' hormones) and irregular periods.[206] Whilst generally considered an endocrine disorder, the underlying cause of PCOS is thought to be insulin resistance (IR).

Two meta-analyses have evaluated the potential benefits of fish oils in PCOS, with contradictory results. A meta-analysis of three RCTs in 2017 found no benefits with regards to IR or Homeostatic Model Assessment of Insulin Resistance (HOMA-IR).[207] Dosages used in the included studies ranged from 1200 to 3600 mg n-3 PUFAs (not further described) for 6–8 weeks. A meta-analysis of nine RCTs published in 2018, on the other hand, found significant improvements in HOMA-IR (based on six studies), total and LDL cholesterol, triglycerides and adiponectin, but not sex hormone levels, BMI, fasting glucose and insulin.[208] The included studies used a dosage range of 900–4000 mg total n-3 PUFAs (no further details reported) for 6–24 weeks. Neither meta-analysis included a subgroup analysis, but the positive results of the Yang *et al*. study were based on studies that did not appear to have much heterogeneity.

Although the evidence from the two meta-analyses is conflicting, in view of the fact that the more recent one included more studies, the overall evidence appears to be in favour of a benefit of fish oils on metabolic parameters in women with PCOS. There is insufficient information to make specific dosage recommendations.

Pregnancy

The placenta actively transports fatty acids, maintaining optimal DHA levels in the foetus of 9–10%, and an optimal O3I of 8–11% has been suggested for pregnant women.[209]

Two cohort studies have found a positive association between O3I and gestational length.[210,211] A low O3I in late pregnancy has also been associated with an increased risk of postnatal depression.[212]

Gestational diabetes mellitus (GDM)

Gestational diabetes mellitus is diagnosed in about 5% of all pregnancies worldwide, and is associated with maternal and foetal complications, including macrosomia (birth weight over 4000 g) and pre-eclampsia.[213,214]

Three meta-analyses have evaluated the benefits of n-3 PUFAs in gestational diabetes or prediabetes, and all came to the conclusion that n-3 PUFAs can significantly improve fasting glucose, HOMA-IR and, where evaluated, high-sensitivity CRP, triglycerides and TAC, but not macrosomia, preterm delivery, neonatal hyperbilirubinaemia, NO, pre-eclampsia, gestational age, birth weight, total or LDL cholesterol.[213,214,215]

One meta-analysis looked at co-supplementation of n-3 PUFAs with vitamin E or vitamin D only,[213] one looked at fish oils only,[214] and one included studies using fish oils and flaxseed oils.[215] Despite the wide range of types of supplementation, there was little heterogeneity amongst the positive results, suggesting that both marine and plant n-3 PUFAs with and without co-administration of vitamin E or vitamin D can have a benefit. Most studies used EPA 180 mg and DHA 120 mg per day for 6–8 weeks.

The clinical research suggests that in women with GDM, EPA and DHA are beneficial for glycaemic control and inflammatory status at a modest dose of EPA 180 mg and DHA 120 mg per day.

Peri-/postnatal depression

The clinical research evidence for the use of EPA and DHA in depression in general has been reviewed above. It is thought that 10–20% of pregnant women are affected by perinatal MDD.[216] A meta-analysis of 12 case–control and cohort studies showed that lower serum levels of EPA, DHA and total n-3 PUFAs, and higher n-6 to n-3 ratio were associated with an increased risk of perinatal depression.[217]

There is a significant body of clinical research in peri- or postnatal depression, with three meta-analyses covering over 20 studies published in the last 2 years. Whilst one meta-analysis of 11 RCTs found no benefits either overall or in subgroups,[218] the other two showed some benefits, although their subgroup analyses gave contradictory results. One, including 18 RCTs, found significant benefits in depressed but not non-depressed women, and in post-partum women but not during pregnancy, with the greatest effects in studies looking specifically at postnatal depression.[219] The third meta-analysis found significant benefits in women with mild to moderate but not severe depression, durations of less than 8 weeks, and with EPA:DHA ratios of > 1.5.[220] Dosages in all the included studies ranged from 200 to 3348 mg combined DHA and EPA or DHA only.

There appears to be a benefit of fish oil supplementation with regards to perinatal depression, although there is conflicting evidence with regards to the group most likely to benefit and the best dosages.

Preterm birth

As mentioned above, higher O3I has been associated with longer gestational period; however, evidence from intervention trials has been mixed.

A Cochrane review and meta-analysis in 2018 concluded that there was a reduced risk of preterm birth < 37 weeks (based on 26 RCTs) and early preterm birth < 34 weeks (nine RCTs) in women taking EPA and/or DHA compared with controls, based on high-quality evidence.[221] This study also found that fish oils increased the risk of prolonged gestation > 42 weeks from 1.6% to 2.6%, based on six RCTs of moderate quality. The authors mention that, at the time of conducting their review, 23 clinical trials, including over 5000 women, were still ongoing.

Since then, two more meta-analyses have been published with conflicting results. A meta-analysis of 26 RCTs in 2020 found no overall decreased risk of preterm birth, but subgroup analysis showed that trials using a dose of > 1000 mg EPA plus DHA per day and a mix of EPA and DHA but not DHA alone showed benefits. The dosage range used in the included studies was 200–3120 mg per day. A meta-analysis of 37 RCTs published in 2021 found an 11% reduction in preterm birth and a 27% reduction in early preterm birth, although this lost statistical significance after sensitivity analysis.[222] This meta-analysis included studies using high-omega eggs and ALA-enriched margarine as sources of n-3 PUFAs, and supplemental dosages as low as 28 mg per day (combined EPA and DHA), raising the possibility that this may have affected statistical power. No subgroup analysis was carried out.

Whilst there is conflicting evidence, EPA and DHA appear to lengthen gestational period, offering a benefit in reducing the risk of preterm birth but possibly also increasing the risk of prolonged gestation. EPA and DHA combinations appear to be more beneficial than DHA alone, and a combined dose of at least 1000 mg per day has shown benefits.

Post-traumatic stress disorder (PTSD)

Post-traumatic stress disorder is an anxiety disorder caused by very stressful, frightening or distressing events, and can have a significant impact on the person's quality of life. It has been shown to be associated with psychophysiological symptoms, including heart rate and skin conductance, both at rest and in response to stimuli.[223]

A 12-week double-blind, placebo-controlled trial found that a DHA-rich fish oil supplement (DHA 1470 mg, 147 EPA mg per day) significantly reduced heart rate at rest and after a stimulus in those on the fish oil supplement, compared with the placebo group, in serious accident survivors.[223] Supplementation was started within 10 days after the traumatic event, and most participants did not develop serious psychophysiological symptoms. The mean O3I at baseline was 7.59% in the fish oil group and 7.38% in the placebo group, which increased by 3.08% in the former group.[223]

A small pilot study in patients with PTSD, however, was terminated early due to negative effects.[224] Six patients with PTSD (trauma being 2–31 years prior to start of study) were given a high-EPA fish oil (2000 mg per day) for up to 3 months. Two patients dropped out within the first month. Of the remaining four patients, one remained unchanged and the other three had a trend towards a worsening of most symptoms and a significant worsening of the avoidance subscale of the Impact of Event Scale, raising concerns of a possible negative impact of fish oil.

The contradictory results could be due to a number of factors, including differing effects of DHA and EPA, or different treatment goals and therefore patient population (prevention versus treatment of PTSD). It is also important to note that the study reporting worsening was based on only four patients.

Until further research is carried out on the effects of fish oils on PTSD, no recommendation can be made.

Prostate cancer

A study published in 2013 raised concerns that fish oils may increase the risk of prostate cancer.[225] Data from 834 men with prostate cancer were taken from a larger cohort study on the effects of vitamin E and selenium on cancer (Selenium and Vitamin E Cancer Prevention Trial, SELECT) and plasma long chain n-3 PUFAs compared with 1393 men without prostate cancer. Those in the highest quartile of plasma n-3 levels had a 43% increased risk of prostate cancer compared with those in the lowest quartile. This study has been criticised for a number of methodological problems.[226]

Since then, a number of meta-analyses have concluded that overall there is no association between prostate cancer and either dietary intake or biomarkers (i.e. blood levels) of n-3 PUFAs,[227,228,229] except for one that showed a marginal positive association between blood DHA (but not EPA or ALA) level and prostate cancer (2% increased risk).[230] One of the meta-analyses carried out a number of subgroup analyses, and found no effect of stage of prostate cancer, study design, exposure measurement or intake level on the outcomes.[228]

The data of these meta-analyses came mostly from epidemiological studies, over 40 altogether. Only one also looked at intervention trials, three RCTs and one uncontrolled trial.[227] All four intervention trials showed no effect of supplementation on prostate-specific antigen (a marker of prostate cancer), and two trials showed a decrease in inflammatory and other cancer markers. Dosages used in the intervention trials ranged from 1600 mg to 2400 mg per day combined EPA and DHA, with three of the trials lasting for 3 months or less, and one lasting for 2 years.

Overall, the concerns raised by the results of the SELECT trial regarding an increased risk of prostate cancer with high blood levels of n-3 PUFAs have not been confirmed by a number of subsequent meta-analyses that included the SELECT trial data. At present, the evidence suggests that EPA and DHA have neither a positive nor a negative effect on prostate cancer risk.

Sleep

In animal models, n-3 PUFAs have been shown to improve sleep through a number of potential mechanisms, but evidence in humans is conflicting.[231]

A 2020 review and meta-analysis of 12 clinical trials and eight longitudinal studies found benefits of n-3 PUFAs on sleep architecture in infants (based on two RCTs and three cohort studies) and children with clinical sleep problems, but not in adults (seven RCTs and three cohort studies) or healthy children.[231] Dosages used in the adult RCTs ranged from 220 to 2500 mg per day combined EPA and DHA, with varying ratios, including two studies that used DHA only, and one study that used salmon.

A double-blind, placebo-controlled trial in healthy adults published in 2021 found some interesting results, comparing a high-DHA (DHA 900 mg, EPA 270 mg per day) versus a high-EPA formula (EPA 900 mg, DHA 360 mg per day) versus placebo for 26 weeks.[232] Participants on both the high-DHA and high-EPA formulas had improved sleep efficiency and latency, although the former only reached statistical significance in the high-DHA group. Whilst the high-EPA formula also led to decreased total sleep time and total time in bed, these increased in the DHA group compared with placebo, with the difference between the fish oil groups being statistically significant. Most interestingly though, scores for feeling rested, energetic and ready to perform decreased with DHA and increased with EPA, although only some of these differences were statistically significant.[232]

A double-blind, placebo-controlled trial in patients with MDD found significant effects of n-3 PUFAs (1000 mg per day, no further details) for 12 weeks on Insomnia Severity Index, with a reduction from 20.3 to 3.5 compared with 17.6 to 10.4 with placebo.[233]

Overall, the evidence on fish oils and sleep is conflicting, and DHA and EPA may have different effects, with EPA-rich supplements potentially being more beneficial.

Safety

A 2018 meta-analysis of 21 RCTs (24 460 participants) evaluated the safety and tolerability of prescription fish oils preparations, and found them to be generally well tolerated with no evidence of serious adverse events.[234] Non-serious adverse events that were more common in the fish oil group, compared with the control group, were fishy taste and skin abnormalities (eruption, itching, exanthema or eczema), as well as abnormal laboratory values (elevated FBG, alanine transaminase and blood urea nitrogen; decreased haemoglobin and haematocrit). EPA and DHA combinations (but not EPA-only products) were also associated with belching, nausea and elevated LDL cholesterol.[234]

For prescription fish oil products (EPA EEs, which are licensed for hypertriglyceridaemia), the following side-effects are listed in the British National Formulary (BNF):[125]

Common or very common (more than 1 in 100): burping; constipation; diarrhoea; gastrointestinal discomfort; gastrointestinal disorders; nausea; vomiting.

Uncommon (1 in 1000 to 1 in 100): dizziness; gout; haemorrhage; headache; hyperglycaemia; hypotension; skin reactions; taste altered.

Rare or very rare (less than 1 in 1000): liver disorder.

As mentioned in the section 'Cardiovascular disease' under the heading 'Clinical uses', an increased risk of AF has been shown in several meta-analyses.[108,119,120,121] There is also evidence for an increased risk in bleeding,[108] possibly

due to the inhibitory effect of fish oils on platelet aggregation. However, a meta-analysis focussing on bleeding found a reduced platelet aggregation in healthy subjects (based on 32 studies) but no increased risk of perioperative bleeding in patients undergoing surgery (based on 20 studies), leading the authors to conclude that fish oil supplements do not need to be discontinued prior to surgery.[235]

Animal models as well as some human studies have suggested that EPA and DHA may suppress immunity, although an increase in infections has not been seen in human studies. However, this may be relevant for people with compromised immune systems.[236]

The Institute of Medicine in the USA has not set an upper limit for EPA or DHA, but cautions that levels of EPA 900 mg plus DHA 600 mg per day or more for several weeks may suppress immunity, and doses of 2000–15 000 mg per day EPA and/or DHA may increase bleeding time.[2] The European Food Safety Authority (EFSA) considers long-term consumption of EPA and DHA supplements at combined doses of up to about 5000 mg/day to be safe.[237]

Possible contamination

It is known that fish can be contaminated with dioxins, polychlorinated biphenyls (PCBs) and methylmercury.[21] SCAN therefore advises pregnant and lactating women to limit their intake of fish known to be particularly high in mercury (marlin, swordfish, shark and tuna). With regards to not exceeding levels of dioxins and PCBs, women of reproductive age and girls are advised to consume 1–2 portions of oily fish per week, and women past reproductive age, boys and men 1–4 portions of oily fish per week.[21]

Some fish oil supplements have also been found to contain toxin levels above safe limits, including dioxins, PCBs[238,239] and mercury.[240] It is therefore important to choose a product from a reputable company.

Drug interactions

Anti-coagulants

Due to the effect of EPA and DHA on platelet aggregation, fish oils should only be used in combination with blood-thinning drugs when extra monitoring of coagulation status is in place.

Caution should also be exercised with any other drug that may affect bleeding.

Children

EPA and DHA supplements have been used safely in children. A meta-analysis of four RCTs, including 153 children, on the use in depression reported only one case of increased defecation, but no other adverse events from the included studies in which doses of up to 3400 mg per day EPA and DHA were used.[172] A meta-analysis of 13 RCTs including 372 children with ASD reported that all adverse events were classified as mild, and were equally distributed between the fish oil and placebo groups with dosages of up to 1500 mg per day.[65] Another meta-analysis of RCTs in children with ASD also reported that none of the studies found a significant difference in side-effects between placebo and omega 3 groups, and none reported serious side-effects with dosages of up to 1500 mg.[63]

Pregnancy/lactation

The Institute of Medicine considers intakes of 1400 mg per day n-3 PUFAs during pregnancy and 1300 mg per day during lactation as adequate.[2] As for adults in general, the EFSA considers up to 5000 mg of EPA and DHA to be safe in pregnant and breastfeeding women,[237] and in intervention trials dosages up to 2700 mg have been safely used during pregnancy and breastfeeding[209] (see also under the section ‘Pregnancy’).

Conclusion

Although there have been some conflicting data, overall, EPA and DHA supplements have been shown to be of benefit in a wide range of disorders. Dosages and EPA:DHA ratios have varied widely in clinical trials, making it impossible to give robust dosage recommendations for most clinical uses. Not all trials have evaluated O3I at baseline, which may account for some of the discrepancies seen. Establishing O3I in clinical practice at baseline and for monitoring therapy is therefore advisable, as there is significant inter-individual variation in PUFA metabolism.

Whilst epidemiological studies have shown benefits of dietary fish intake, official recommendations of fish intake, which balance the potential benefits with the potential harm from pollutants, may be insufficient for many to achieve optimal O3I, making fish oil supplementation a valuable tool for prevention and clinical practice. Populations at risk of low O3I, such as strict vegans and vegetarians, may want to consider vegan DHA supplements.

Whilst fish oils are generally well tolerated, an increased risk of AF and bleeding has been observed in some meta-analyses, which should be borne in mind in at-risk populations.

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