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Carotenoids in human nutrition and health

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[1]Abbreviations used

[1] *Abbreviations used:* AMD, age-related macular degeneration; AREDS, Age-Related Eye Disease Study; ATBC = α-Tocopherol, β -Carotene Cancer Prevention Study; CARET = β -Carotene And Retinol Efficacy Trial; CI = confidence interval; CS = contrast sensitivity; GT = glare tolerance; HR = hazard ratio; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Linxian = Linxian General Population Trial; L = lutein; MPOD = Macular Pigment Optical Density OR = odds ratio; PHS = Physicians' Health Study; PL = placebo; PP = photophobia; PRT = photostress recovery; RR = relative risk; SD = standard deviation; TICS = Telephone Interview Cognitive Status; Z = zeaxanthin

Highlights

Carotenoids are pigments found in most fruits and vegetables.

They exert antioxidant and other effects.

Carotenoids have a range of functions in human health.

Further research on carotenoid supplementation is needed.

Abstract

Carotenoids are naturally occurring pigments found in most fruits and vegetables, plants, algae, and photosynthetic bacteria. Humans cannot synthesize carotenoids and must ingest them in food or via supplementation. Carotenoids have a range of functions in human health. They primarily exert antioxidant effects, but individual carotenoids may also act through other mechanisms; for example, β-carotene has a pro-vitamin A function, while lutein/zeaxanthin constitute macular pigment in the eye. The benefit of lutein in reducing progression of age-related macular eye disease and cataracts is strengthening; an intake recommendation would help to generate awareness in the general population to have an adequate intake of lutein rich foods. There is evidence that carotenoids, in addition to beneficial effects on eye health, also produce improvements in cognitive function and cardiovascular health, and may help to prevent some types of cancer. Despite the evidence for the health benefits of carotenoids, large population-based supplementation studies have produced mixed results for some of the carotenoids. To establish and confirm the health benefits of the different carotenoids more research, including clinical studies, is needed.

Keywords: cancer prevention, carotenoids, cognitive function, eye health, healthy ageing

Introduction

In today's world, people live longer; average life expectancy has risen globally, reaching 80.6 years in the European Union in 2013 – an increase of a decade when compared to half a century earlier. However poor dietary habits and metabolic factors are counteracting this development and are among the leading risk factors for mortality worldwide [1]. The most common causes of morbidity and premature death in developed countries are generally noncommunicable diseases, such as ischemic heart disease, stroke and cancer [2]. A substantial proportion of diabetes mellitus, ischemic heart disease and cancer can be attributed to obesity and high cholesterol levels [3]. In addition, the World Health Organization estimates that a low intake of fruit and vegetables results in 31% of ischemic heart disease, 11% of strokes and 19% of gastrointestinal cancers worldwide [3]. In developing countries, maternal and childhood undernutrition involves micronutrient deficiencies as well as a lack of protein and energy [3]. Large-scale dietary improvements are needed, and the EAT-Lancet Commission is currently evaluating whether it is feasible to implement a global food system that can deliver a healthy, sustainable diet to a world population that is expected to reach 9 billion by 2050 [4].

Fruit and vegetables help reduce the risk of cardiovascular disease as well as some cancers through various mechanisms, including the provision of antioxidants, dietary fiber and micronutrients such as carotenoids, flavonoids, vitamin C and folic acid, which can reduce oxidative damage and block the actions of carcinogens [3].

Carotenoids are pigments found in nearly all coloured fruits and green leafy vegetables. Consumption of carotenoids has been associated with various health benefits, including a reduced risk of age-related macular degeneration and cataract, some cancers and coronary heart disease [5]. There is also some evidence of a beneficial effect on cognitive function [6].

This article provides an overview of highlights in carotenoid research and their role for human health.

Carotenoids

Carotenoids are naturally occurring pigments found in plants, fungi, algae and bacteria [5]. More than 650 different types exist in nature [7], including up to 100 that are present in the food chain and human diet [5]. Humans do not synthesize carotenoids; instead, they must be ingested in food or via supplementation [8]. They have been part of the food chain for many thousands of years.

Only 30–40 carotenoids have been found in human blood samples, with lycopene, lutein, zeaxanthin, β-cryptoxanthin and β-carotene generally being the most abundant [8]. The European Prospective Investigation into Cancer and Nutrition (EPIC) study measured the plasma levels of six carotenoids in 3043 people and found the following mean levels: lycopene 0.43–1.32 µmol/L, lutein 0.26–0.70 µmol/L, β-carotene 0.21–0.68 µmol/L, β-cryptoxanthin 0.11–0.52 µmol/L, α-carotene 0.06–0.32 µmol/L, zeaxanthin 0.05–0.13 µmol/L [9].

Several carotenoids, including β-carotene, lutein, zeaxanthin, lycopene, astaxanthin and canthaxanthin are produced at an industrial scale and are available in fortified foods or as supplements. Carotenoids are also used as colorants in food, beverages and pharmaceutical applications.

Although the chemistry of carotenoids has been studied extensively, information about their bioavailability, metabolism, and particularly their biological functions, is emerging only since few years.

Potential contribution to health

The main functions of carotenoids in human health are summarized in Table 1 [10-26]. Mechanistically, the primary benefits of carotenoids can be explained by their antioxidant potential [27]. However, specific carotenoids may also act through additional mechanisms. For example, β-carotene has added benefits due to its ability to be converted to vitamin A [28], while lutein and zeaxanthin absorb specific wavelengths of light which could help protect the eyes [29]. Carotenoids may guard against certain types of cancer by limiting the abnormal growth of cells and/or by enhancing gap-junctional communication [30]. In addition, carotenoids may help prevent heart disease by blocking the formation and oxidation of low-density lipoprotein [31,32].

Pro-vitamin A function

Vitamin A deficiency is a major public health problem in developing countries; it particularly affects preschool children and pregnant women and can cause blindness, poor growth and death [3,28]. The major sources of vitamin A are animal products such as dairy, liver and fish, which contain preformed vitamin A (retinol), and plants such as yellow/orange vegetables and fruits and leafy greens, which contain carotenoids, predominantly β-carotene, which can be converted into retinol [28,33].

Various public health strategies are used to combat vitamin A deficiency. Supplementation with vitamin A or β-carotene is effective, but can be difficult to implement in poor, rural areas. Another approach is to fortify commonly used food items such as sugar, fats and cereal products by adding vitamin A; however, it can be difficult to ensure such foods are reaching the target population, and that people do not ingest an excess amount. Education programs to teach people which foods to eat, and agricultural programs to grow suitable foods are sustainable options. For example, carrots and orange-fleshed sweet potato, which are staple

foods in many countries, are rich in carotenoids, and cultivars that contain particularly high levels of β-carotene have been bred. Biofortified crops such as golden rice, which has an enhanced provitamin A content, can be produced using transgenic methods. However, there is currently no consensus on the recommended daily intake of β-carotene, and the bioavailability of carotenoids varies between different plants and according to the amount of fat ingested concurrently [28]. In addition, other factors controlling carotenoid metabolism and conversion to retinol, such as genetics and polymorphisms have not been fully elucidated [34].

Eye health

Two dietary carotenoids, lutein and zeaxanthin, and the isomer *meso*-zexanthin are found in the human retina [35]. They are concentrated in the macula and are therefore known as macular pigment. The highest concentration is found in the fovea, where the total carotenoid level has been measured at approximately 13 ng/mm^2 compared with approximately 0.05 ng/mm^2 in the periphery [36]. Macular pigment absorbs blue light (high energy, short wavelength light), protecting the retina from photochemical injury [29], and has local antioxidant properties, with the ability to neutralize singlet oxygen and reactive oxygen species (e.g. hydroxyl radical and superoxide anions), protect against UV-induced peroxidation, and reduce the formation of lipofuscin and associated oxidative-stress induced damage [35]. Thus, these carotenoids provide potential benefits for ocular function and health.

Many people have low macular pigment optical density (MPOD) levels of 0.2 or lower [13,37], and it has been shown that supplementation with lutein/zeaxanthin can increase macular pigment optical density [38-45]. A MPOD value of 0.4 to 0.6 is desirable and is associated with health benefits, especially in older adults, i.e. for protection of the retina and delaying of cognitive decline [37]. Intake of lutein and zeaxanthin differ with age, sex and

ethnicity. Among all age groups intake of lutein is higher than for zeaxanthin, independently from sex and ethnicity. Lower zeaxanthin to lutein ratios are reported for groups at risk for age-related macular degeneration (elderly, females) [46]. Studies have also demonstrated that lutein/zeaxanthin supplementation can improve visual performance, including contrast sensitivity, glare tolerance and photo stress recovery, even in healthy people (Table 2) [38-45,47,48].

Age-related macular degeneration (AMD) is an increasing problem among the elderly population worldwide [49]. Studies of the effects of lutein/zeaxanthin supplementation on AMD have produced mixed results, with some finding a protective effect and others not; however, many were inadequately powered [35]. Nonetheless, some important data were provided by secondary analyses of the large Age-Related Eye Disease Study 2 (AREDS2) [11,12].

This randomized trial investigated the effect of adding lutein 10 mg lutein/2 mg zeaxanthin, omega-3 fatty acids 1.0 g or both to the original AREDS formulation (vitamin C, vitamin E, β-carotene, zinc and copper) or to variations of this formulation (excluding β-carotene and/or with reduced zinc). Participants (n=4203) were followed for a median 5 years. The primary analysis found no beneficial or harmful effect for lutein/zeaxanthin and/or omega-3 fatty acids on progression to late AMD compared with placebo. However, a prespecified secondary analysis found a significant 26% risk reduction for progression to advanced AMD when comparing lutein/zeaxanthin supplementation with no lutein/zeaxanthin supplementation in the quintile with the lowest dietary intake of these two carotenoids (median 0.7 mg/day), as indicated by a hazard ratio of 0.74 (95% confidence interval [CI] 0.59–0.94, p=0.01). In addition, a post hoc analysis showed that the formulation containing lutein/zeaxanthin but excluding β-carotene was more effective than the original AREDS

formulation containing β-carotene but no lutein/zeaxanthin for reducing progression to advanced AMD (hazard ratio 0.82, 95% CI 0.69–0.96, p=0.02) [11].

There is also some evidence suggesting there is a relationship between lutein/zeaxanthin status and the risk of nuclear cataracts [50], and in the AREDS2 trial the addition of lutein/zeaxanthin supplementation reduced the risk of progression to cataract surgery in the quintile with the lowest dietary intake of these carotenoids (hazard ratio 0.68, 95% CI 0.48–0.96, p=0.03) [51].

It has been estimated that full utilization of the AREDS2 complex (i.e. vitamins C and E, zinc, copper, lutein/zeaxanthin and omega-3 fatty acids) by all adults aged >55 years would result in an average of 1,031,555 avoided AMD and cataract events per year in the USA (based on a risk reduction of 23.6% for AMD and 16.2% for cataracts), which would be associated with net annual cost savings of US$1.2 billion, due to reduced healthcare expenditure [52].

The China Nutrition Society has reacted to these data by implementing recommendations for a daily intake of lutein 10 mg and zeaxanthin 2 mg per day. However, despite the evidence supporting the benefits for eye health, most countries do not have dietary reference intake values for lutein, which is classified as a non-essential nutrient. Arguments can be made that new approaches to the development of nutrient reference values for non-essential nutrients are needed for bioactives that promote optimal health and/or prevent chronic diseases [53], and that lutein and zeaxanthin should be classified as conditionally essential nutrients as they fulfil the criteria necessary to be considered for intake recommendations [54,55]. The establishment of dietary guidelines for lutein would encourage the consumption of lutein-containing foods or supplements and raise public awareness about the potential health benefits. As a next step, key policy makers from the FDA, NIH and other authorities should convene and evaluate the strength of the supporting research, gaps in knowledge and barriers,

and propose actions to overcome them. Establishing intake recommendations for lutein is an important step to support and optimize visual performance and reduce risk for age-related macular eye diseases in the general population. Globally, this may be a significant contribution forwards for public health as we face an ever-increasing ageing population.

Cognitive performance

Several carotenoids appear to have effects on cognitive functioning. The underlying mechanism is not clear, but it may relate to antioxidant activity [56]. The Physicians' Health Study II found that long-term β-carotene supplementation (50 mg on alternate days) helped to maintain cognitive performance in a generally healthy population [14]. This placebo-controlled study enrolled 4052 men. After a mean duration of 18 years' treatment, significant benefits were seen in terms of verbal memory, Telephone Interview of Cognitive Status and global score (Table 3).

Placebo-controlled studies also suggest that supplementation with lutein or lutein plus zeaxanthin may have beneficial cognitive effects in older men and women. Verbal fluency score improved in women who received lutein 12 mg/day for 4 months ($p<0.03$) [15] and the complex attention ($p<0.02$), cognitive flexibility ($p<0.04$) and composite memory ($p=0.04$; men only) domains of the CNS Vital Signs test battery improved in men and women who received lutein 12 mg plus zeaxanthin 2 mg for 12 months [57]. In another recent study Walk et al. showed in preadolescent children, that a higher MPOD is correlated with a better cognitive performance and better neural efficiency. Overall this results in better and more efficient scholastic performance in children [13].

Animal studies support an effect of lutein on cognitive function. For example, mice fed with lutein demonstrated a superior learning performance compared to control mice in a study in which the animals had to learn which station among the four in a cage would deliver water.

The error rate among the lutein-fed mice was less than half that of the control mice ($p<0.0001$). In addition associative memory was also improved in the lutein fed group [58].

Cardiovascular health

Oxidative stress, inflammation, dyslipidemia and thrombosis are implicated in the development of cardiovascular disease, and there is evidence that carotenoids may have beneficial effects on some of these factors.

Astaxanthin, a carotenoid derived from marine animals, has been shown to reduce low-density lipoprotein peroxidation and improve blood lipid profiles and blood flow capacity. Increased susceptibility of low-density lipoprotein (LDL) to oxidation is known to contribute to atherosclerosis. In a study involving 24 healthy adults who took astaxanthin at doses of 1.8, 3.6, 14.4 and 21.6 mg/day for 14 days, doses of 3.6 mg and above reduced the susceptibility of LDL to oxidation, as indicated by prolongation of the LDL oxidation lag time 5.0%, 26.2%, 42.3% and 30.7%, respectively, compared with baseline [31].

Several studies have shown that astaxanthin improves lipid profiles [32,59]. For example, in a 12-week study involving 61 non-obese Japanese subjects with moderate hypertriglyceridemia (fasting serum triglyceride 120–200 mg/dL), multiple comparison testing showed that consumption of astaxanthin 6 and 12 mg/day increased serum high-density lipoprotein (HDL)-cholesterol significantly versus baseline, and doses of 12 and 18 mg/day decreased serum triglyceride levels significantly ($p<0.05$) [32].

Astaxanthin 6 mg/day for 10 days shortened mean blood transit time compared with baseline in healthy men (from 52.8 to 47.6 seconds, $p<0.01$), with a significant difference in final values compared with placebo (54.2 seconds, $p<0.05$) [60]. A faster blood transit time may benefit the microcirculation. Another study showed that astaxanthin 6 or 12 mg/day for 12 weeks reduced peroxidation of red blood cells in middle-aged/elderly people (n=30), as

indicated by a significantly lower erythrocyte phospholipid hydroperoxide concentration versus a placebo comparison group (8.0 and 9.7 versus 14.9 pmol/mL packed cells, both $p<0.05$) [61].

Another carotenoid, lycopene, shows potent antioxidant activity *in vitro* and reduces serum cholesterol in animal studies but, so far, data from epidemiological and human intervention studies on the potential role of lycopene in cardiovascular health are conflicting [62,63]. There is also some evidence that lutein may be beneficial: a meta-analysis involving mostly observational studies suggested that higher dietary intake and blood concentrations of lutein are associated with lower risk of coronary heart disease and stroke [64].

Bone health

Since the beginning of the millennium it was demonstrated that β-cryptoxanthin, an asymmetric xanthophyll, may add some benefits to bone health. *In vitro* studies in rats and epidemiological data suggest that β-cryptoxanthin regulates bone homeostasis [65]. The group of M. Yamaguchi did pioneering work and showed that β-cryptoxanthin has a stimulating effect on bone formation via osteoblast stimulation and on the other hand inhibits osteoclast activity [66]. The underlying mechanism might be a transcriptional regulation. β-Cryptoxanthin may change gene expression for proteins that are involved in bone formation and mineralization in osteoblastic cells [25]. Therefore, β-cryptoxanthin may be important for bone homeostasis, especially in postmenopausal women to support prevention of osteoporosis [67]. Also, for other carotenoids, data are emerging that show a positive association for bone mineralization; for example, in a small Korean study with 189 postmenopausal women, β-carotene was positively correlated with higher lumbar spine bone mineral density [68].

Sun protection

Many carotenoids which are consumed as part of a normal diet accumulate in the skin and very efficiently protect the skin from UV light-induced damage, sunburn and skin aging. Through their unique structure with ten or more conjugated double-bonds, carotenoids have a high potential to scavenge reactive oxygen species, such as peroxide radicals or singlet oxygen molecules [18,19]. In several studies the protective effect of β-carotene on sunburn, i.e. erythema was demonstrated in clinical settings [20,21]. The colourless carotenoids phytoene and phytofluene have also the potential to efficiently protect the skin. Nevertheless, photoprotection with individual dietary carotenoids such as β-carotene or lycopene is considerably lower than that achieved by using topical sunscreens. Carotenoids are important as basal protection of the whole skin surface against UV irradiation and contribute to maintenance of skin health, good skin hydration and appearance.

Weight management

During the last decade, evidence has accumulated from *in vitro* [69] and animal studies showing that carotenoids and their cleavage products such as retinoids and apo-carotenoids have a beneficial effect on adipocyte differentiation [70,71]. This ultimately leads to a reduction of abdominal and subcutaneous fat through a variety of underlying mechanisms. The carotenoid metabolites can regulate transcription through various members of the nuclear hormone receptor superfamily such as retinoic acid receptors. Further, carotenoids and their metabolites may regulate pathways, i.e. NF-κb or Nrf-2. Finally, the antioxidant potential of carotenoids might also reduce the overall oxidative burden or decrease its build up, thus exerting beneficial effects on weight management and obesity [70].

In a small randomized placebo controlled clinical trial involving 17 children with obesity Canas et al. reported the effectiveness of mixed carotenoid supplementation on various obesity parameters and markers of insulin resistance [72]. For example, BMI z-scores, waist-to-hip ratio, and subcutaneous adipose tissue levels, all parameters associated with weight increases and ultimately obesity, were improved after 6 months supplementation with mixed carotenoids.

Immune function

β-Carotene and other carotenoids are inversely associated with immune activation and inflammatory markers in patients with cardiovascular disease [23]. CAD patients had higher immune activity (CRP, soluble interleukin 2 receptor, CD4+/CD25+ cells) compared to healthy controls and at the same time the plasma levels of β-carotene as well as lycopene was significantly lower than in the control group.

Vitamin A supplementation and improved vitamin A status, respectively, are associated with a clinically meaningful reduction in morbidity and mortality in children. Therefore, it has been advised that the policy of universal supplementation for children aged less than five years with β-carotene or vitamin A in developing countries, especially in populations at risk of vitamin A deficiency, should be maintained [24].

Cancer prevention

Lycopene is found in relatively high concentrations within the prostate gland [73], and some (but not all) epidemiological studies have found an inverse association between lycopene intake and prostate cancer. For example, a large cohort study evaluating dietary information for 49,898 male health professionals found that men in the highest quintile of dietary

lycopene intake had a 28% lower risk of lethal prostate cancer compared with the lowest quintile (hazard ratio 0.72, 95% CI 0.56–0.94, p=0.04) [74]. A meta-analysis of 42 epidemiological studies found that dietary lycopene consumption was associated with a reduced risk of prostate cancer (RR=0.88, 95% CI 0.78–0.98; plasma concentration of lycopene was as well inversely and significantly correlated with prostate cancer risk (RR=0.88, 95% CI 0.79-0.98) [75].

Plasma lycopene levels are reduced in patients with non-alcoholic fatty liver disease [76], a disorder which is associated with hepatocellular carcinoma. There is therefore some interest in determining whether lycopene intake could affect the risk of liver disease, including cancer. Animal studies have shown that lycopene supplementation for 24 weeks increased hepatic lycopene levels and had a potential preventative effect against hepatic tumorigenesis [77].

Infant nutrition

There is evidence that carotenoids such as lutein/zeaxanthin are important for visual and cognitive development in infants [78,79]. Lutein and zeaxanthin are the major carotenoids in human milk (Figure 1) [80]. Infants fed human milk have higher blood concentrations of lutein/zeaxanthin than infants fed with formula milk [8,81,82]. In one study, at 1 month after birth, the plasma lutein/zeaxanthin level had increased from 48 µg/L at birth to 96 µg/L in breast-fed infants, whereas it had decreased from 49 µg/L to 33 µg/L in infants fed with unfortified formula [8,81]. Thus formula-fed infants may be particularly vulnerable to lutein/zeaxanthin insufficiency.

The infant brain doubles in size during the third trimester, and 75% of brain growth occurs during the first year of life. Lutein/zeaxanthin are the predominant carotenoids found in the infant brain, making up approximately 66–77% of the total carotenoid concentration in the

brain [83]. It has been shown that lutein/zeaxanthin are found in regions of the brain that are specialized for visual processing, memory, learning and language. In all brain regions studied, the level of lutein was up to 3- to 4-fold higher than the levels of other carotenoids present [83].

Large supplementation trials

Results from large supplementation trials with carotenoids have produced mixed results, as illustrated by Table 4 which summarizes data on the incidence of lung and gastric cancer and the risk of progression of AMD [11,84-91].

Among cancer prevention trials evaluating the effect of β-carotene, the Linxian General Population Trial found it had a protective effect against lung and gastric cancer [84-86], while the Physicians' Health Study found neither a beneficial nor harmful effect [87,88]. In contrast, the α-Tocopherol, β-Carotene Cancer Prevention Study (ATBC) and the β-Carotene And Retinol Efficacy Trial (CARET), both of which enrolled only smokers, former smokers and asbestos workers found that β-carotene supplementation was associated with an increased risk of lung cancer and gastric cancer [89,90].

Studies that evaluated either β-carotene [91] or lutein/zeaxanthin [11] in patients with AMD found evidence that both carotenoids had a beneficial effect by reducing progression to advanced AMD.

The variation in results between these population-based supplementation trials could be due to a number of factors, including the use of different doses and formulations, administration with different combinations of other nutrients, and the effect of differences in diet and lifestyle factors. Notably, blood concentrations of β-carotene were substantially higher in the

ATBC and CARET trials, which found harmful effects in high-risk populations (smokers and asbestos workers), than in other large population-based studies (Figure 2).

Current perspectives for carotenoids in nutrition and health

The unexpected outcomes of some of the β-carotene trials in the 90s had major consequences for carotenoid applications and research. Authorities reduced intake recommendations for β-carotene, with the UK specifying an upper limit of 7 mg for β-carotene from supplements, and the European Food Safety Authority recommending a maximum of 15 mg for the safe use of β-carotene. In addition, the results of these clinical supplementation trials, i.e. an increased lung cancer risk for smokers, generated a barrier to supplementation and fortification in developing countries, and led to funding organizations stopping research projects on carotenoids, with some scientists leaving the field of carotenoid work. The number of publications on carotenoids has gone down, from a peak of 2000 publications in 2012 to 1625 in 2016.

However, there is an established application for lutein/zeaxanthin in eye health, with emerging evidence suggesting this could be expanded to include several other areas, such as the brain. New tools and methods may provide alternative approaches for research on important health issues such as Alzheimer disease, cognitive function, diabetes and cancer. In addition, the role of genetic polymorphisms needs to be evaluated as it could have implications for setting dietary reference intake targets for carotenoids.

Organizations such as the International Carotenoid Society play a role in advancing interest in carotenoids, but a joint approach by all stakeholders is required to advocate for more research and applied science in humans and animals on the role of carotenoids in health.

Conclusion

Carotenoids have a range of functions in human health. There is evidence that they have beneficial effects on eye health, cognitive function and cardiovascular health, and may help reduce the risk of some types of cancer. However, intake of carotenoids via foods or supplements is low in many population groups; in particular in the elderly. This is especially an issue for lutein which has demonstrated health benefits in reducing progression of age-related macular eye disease and cataracts. For lutein, the health benefits and safety has been demonstrated in large intervention studies. Based on these positive outcomes an intake recommendation should be established to encourage people to maintain adequate intake of lutein rich foods or supplements. In general, carotenoids are promising bioactives of the food chain and further research is required to study the health benefits and to establish recommendations for adequate and optimal intake via foods or via supplementation.

Conflicts of interest

M. Eggersdorfer and A. Wyss are employees of DSM Nutritional Products.

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Figure legends

Figure 1: The 5 major carotenoids in human milk are depicted in nmol/L [80]. Highest are the xanthophylls Lutein/Zeaxanthin followed by lycopene and β-carotene, β-cryptoxanthin was quite low in this study. Depending on the region and season beta-cryptoxanthin concentrations might be higher.

Figure 2: Beta-carotene plasma levels from 6 large intervention studies are illustrated as concentration range and/or median concentration. In the CARET and the ATBC clinical trials large carotenoid doses were supplemented to subjects already at higher risk of developing lung cancer (smokers and asbestos workers). A second reason for the high plasma levels were the use of formulations that resulted in high bioavailability. The high plasma levels that were achieved in these two studies were associated with a higher risk for lung cancer [90]. In contrast the β-carotene plasma range from 0.28-0.4 μM (15-20 μg/dL) was associated in many prospective studies with a lower risk of various cancers. Also depicted on the left side is the plasma range (5^{th} to 95^{th} percentile) found in the NHANES III survey. The range of 0.09-0.9 μM is slightly different in the age groups and is also lower in smokers, (graph adapted from S. Mayne [10, 92]).

Abbreviations: CARET; the Beta-Carotene and Retinol Efficacy Trial (1996). PHS, Physicians' Health Study II (2007), ATBC; The α-tocopherol, beta-carotene lung cancer prevention study (1994), Linxian; General Population Trial in Linxian, China (1993), AREDS1; The Age-Related Eye Disease Study (2001), AREDS2; The Age-Related Eye Disease Study 2 (2013).

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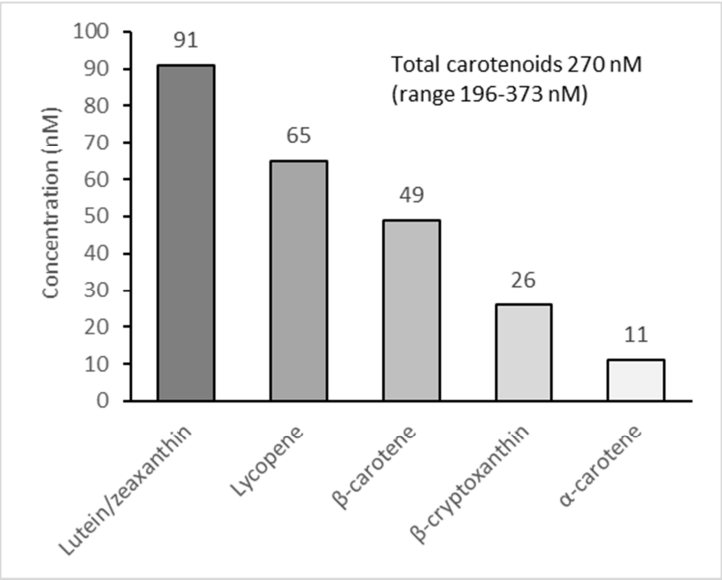

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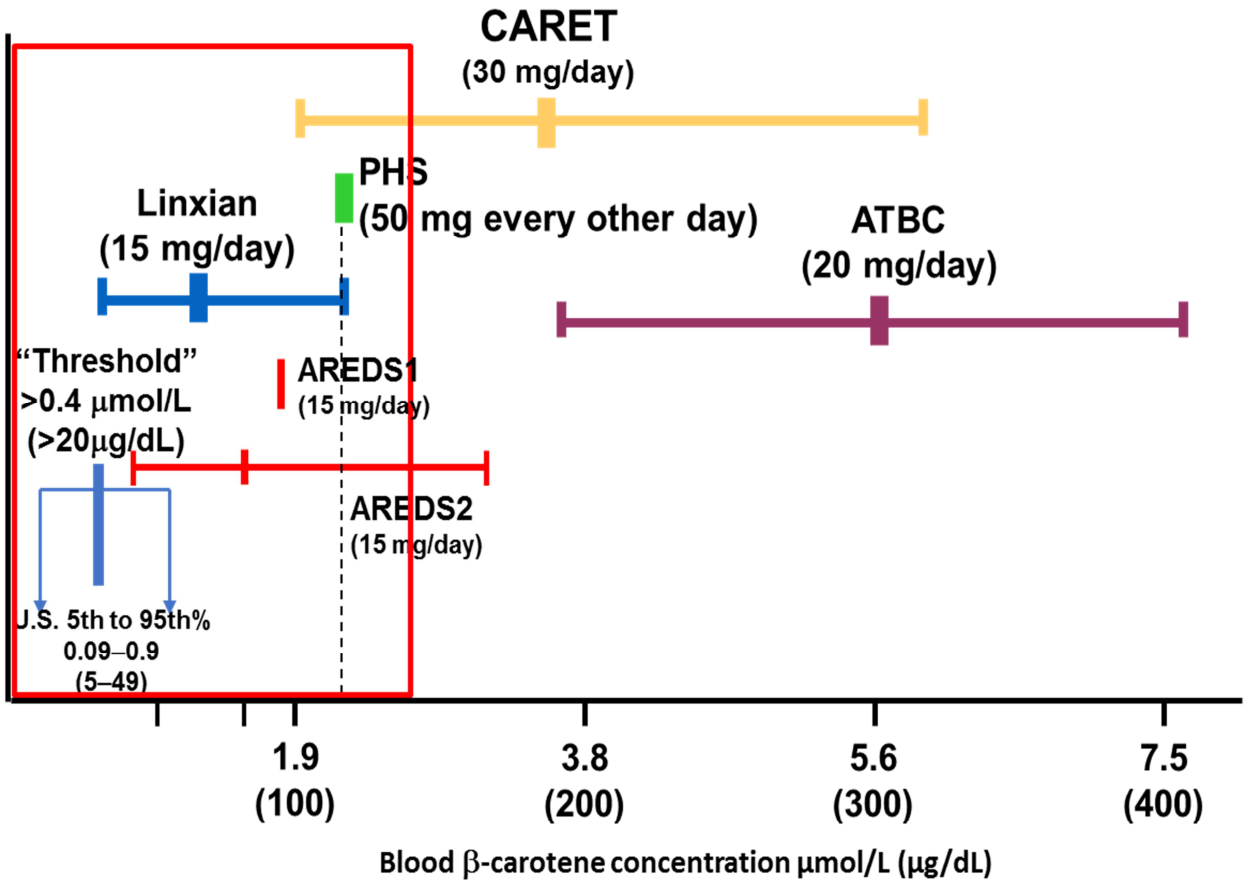

Table 1. Main functions of carotenoids in human health

Health benefit	**Carotenoid**	**Dose, recommendation/d**	**Reference**
Vision, provitamin A	α- and β-Carotene, β-cryptoxanthin	800 μg retinol, 1.6 mg β-Carotene from supplements 9.6 mg β-Carotene from dietary sources 19.2 mg other provitamin A carotenoids from dietary sources	[10]
Eye health	Lutein, Zeaxanthin	10+2mg ADREDS formulation	[11,12]
Brain – cognitive functions	Lutein, β-carotene	-	[13-15]
Heart health	Lycopene	-	[16]
Cancer prevention	Lycopene	-	[17]
Maternal and infant nutrition	Lutein	-	[10]
Skin – UV protection	Lycopene, β-carotene	-	[18-21]
Fertility	β-carotene, Lutein	-	[22]
Immune modulation/stimulation	β-carotene	-	[23,24]
Genomic effects on transcription/translation	Lycopene, β-carotene	-	[25,26]

Table 2. Studies demonstrating improved visual performance (contrast sensitivity, glare tolerance, photostress recovery) with lutein/zeaxanthin

Study (year) [Reference]	Subjects	Supplement	Duration	Main variable	Main result (p-value)
Hammond et al. (2014) [38]	100 young healthy adults	L 10 mg + Z 2 mg vs PL	1 year	CS, GT, PRT	CS (0.03) and PRT (0.013) improved vs PL
Yao et al. (2013) [39]	120 healthy adult drivers	L 20 mg vs PL	1 year	CS under glare	CS improved in L group vs baseline (<0.05) but not in PL group
Loughman et al. (2012) [40]	36 healthy adults	L 20 mg + Z 2 mg vs L 10 mg + Z 12 mg vs PL	6 months	CS under glare	CS improved in L 10 mg + Z 12 mg group with and without glare vs baseline (<0.05) but not in other groups
Nolan et al. (2011) [41]	121 healthy adults	L 12 mg + Z 1 mg	1 year	CS under glare	No significant effect on CS or GT over time; however, at 1 year (not baseline), CS under glare was better in upper vs lower macular pigment tertile (0.042)
Richer et al. (2011) [42]	60 adults with AMD	Z 8 mg vs Z 8 mg + L 9 mg vs L 9 mg	1 year	CS, glare recovery	CS improved in L group (p=0.05) and glare recovery improved in L group (0.02) and L+Z group (0.002)
Stringham et al. (2008) [43]	40 healthy adults	L+Z 12 mg	6 months	GT, PRT	GT (<0.0001) and PRT (0.0003) improved
Wenzel et al. (2006) [44]	10 healthy adults	L 30 mg + Z 2.7 mg	12 weeks	PP	Macular pigment correlated with PP ratio (0.002). PP ratio increased after L+Z (0.011)
Kvanaskul et al. (2006) [45]	34 adults	L 10–20 mg[a] vs Z 10–20 mg[a] vs L 10 mg + Z 10 mg vs PL	1 year	CS	CS improved in L group (0.001). Trend towards improvement in Z and L+Z groups
Nolan et al. (2016) [47]	100 healthy adults with low retinal concentrations	L 10 mg + Z 12 mg vs PL	1 year	CS	CS improved vs PL (0.002)
Olmedilla et al. (2003) [48]	17 adults with cataract	L 15 mg	2 years	GT	GT improved (0.005)

[a] 10 mg for 6 months followed by 20 mg for 6 months.

AMD = age-related macular degeneration; CS = contrast sensitivity; GT = glare tolerance; L = lutein; PL = placebo; PP = photophobia; PRT = photostress recovery; Z = zeaxanthin.

Table 3. Cognitive performance with long-term β-carotene supplementation [14]

Parameter	**Placebo (n=2021)**	**β-carotene (n=2031)**	**Mean difference**
Verbal memory[a], mean z score (SD)	-0.032 (0.74)	0.031(0.73)	0.063 (95% CI 0.02–0.11; p=0.007)
TICS, mean points (SD)	34.23 (2.80)	34.41 (2.73)	0.18 (95% CI 0.01–0.35; p=0.04)
Global score[b], mean z score (SD)	-0.024 (0.71)	0.023 (0.69)	0.047 (95% CI 0–0.09; p=0.03)

[a] Combination of immediate and delayed recalls of 10-word list and East Boston Memory Test.

[b] Global score (primary outcome) including verbal memory, TICS and category fluency.

CI = confidence interval; SD = standard deviation; TICS = Telephone Interview Cognitive Status

Table 4. Large carotenoid supplementation trials: effect of β-carotene on lung cancer and gastric cancer, and effect of β-carotene and lutein/zeaxanthin on age-related macular degeneration

Study	Population	Supplementation	Key results
Linxian [84-86]	29,584 men and women in China	β-carotene 15 mg + vitamin E + selenium (5 years)	Lung cancer mortality HR 0.98 (95% CI 0.71–1.35, p=0.88) Gastric cancer mortality RR 0.79 (95% CI 0.64–0.99); 10 years after stopping supplementation HR 0.89 (95% CI 0.79–1.00, p=0.043)
PHS [87,88]	22,071 US male physicians	β-carotene 50 mg every other day (12 years)	Lung cancer RR 0.9 (95% CI 0.7–1.2) Gastric cancer RR 0.9[a]
ATBC [89]	29,133 Finish male smokers	β-carotene 20 mg (5–8 years)	Lung cancer RR 1.18 (95% CI 1.03–1.36, p=0.01) Stomach cancer RR 1.25[a] (p-value not stated)
CARET [90]	18,314 US men and women smokers and asbestos workers	β-carotene 30 mg + vitamin A (4 years)	Lung cancer RR 1.28 (95% CI 1.04–1.57, p=0.02)
AREDS1 [91]	3640 US men and women	β-carotene 15 mg + vitamin C + vitamin E + zinc	Progression to advanced AMD OR 0.66 (99% CI 0.47–0.91)
AREDS2 [11]	4203 US men and women	Lutein 10 mg + zeaxanthin 2 mg	Progression to advanced AMD among people with lowest dietary lutein/zeaxanthin intake, HR 0.74 (95% CI 0.59–0.94, p=0.01)

[a] Estimated based on number of cases of cancer reported in β-carotene and placebo groups.

AMD = age-related macular degeneration; AREDS = Age-Related Eye Disease Study; ATBC = Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; CARET = Beta-Carotene And Retinol Efficacy Trial; CI = confidence interval; HR = hazard ratio; Linxian = Linxian General Population Trial; OR = odds ratio; PHS = Physicians' Health Study; RR = relative risk.