News and Views

Carotenoids, Chronic Disease Prevention and Dietary Recommendations

Torsten Bohn

Luxembourg Institute of Health (LIH), Department of Population Health, Strassen, Luxembourg

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Abstract: Carotenoids are C-30, C-40 or C-50 terpenoids produced by a number of bacteria, fungi, and plants. In addition to acting as vitamin A precursors such as β-carotene, their dietary intake and blood plasma/serum and tissue levels have been associated in several epidemiological studies to the reduced incidence of chronic diseases, including the reduction of type 2 diabetes and other cardiometabolic diseases, as well as some types of cancer. Lutein and zeaxanthin also appear to play a role in the amelioration of age-related macular degeneration (AMD), the main cause of blindness in the elderly, and may be regarded as conditionally essential nutrients for the elderly. Furthermore, some studies have proposed that carotenoids may improve cognitive functions. Though the underlying mechanisms remain to be fully elucidated, it is perceived that direct antioxidant effects and protection from UV-light, as well as rather indirect effects, acting on transcription factors such as NF-κB, Nrf-2, and nuclear receptors such as RAR/RXR (retinoic acid receptor/retinoid X receptor), altering gene expression, all can play a role. Despite individual intervention trials suggesting negative effects of high doses of β-carotene on smokers, perhaps due to effects related to cytochrome enzyme activation, there is accumulating evidence that these colourful pigments indeed contribute to a healthy life and well-being. However, further research is warranted to better understand factors influencing variable inter-individual responses following carotenoid consumption and to establish more detailed recommendations regarding their dietary intake and toward establishing health claims.

Keywords: Xanthophylls, carotenes, cardiovascular disease, brain, eye, vitamin A, cellular signalling

Introduction and findings from observational epidemiological studies

It is well understood that a diet rich in fruits and vegetables is an important hallmark of healthy living. For example, the World Health Organization (WHO) has stated that sufficient fruit and vegetable intake can reduce the burden of cardiovascular disease (CVD), such as ischemic heart disease, by approx. 30% [1]. Though there is a large array of possibly health promoting constituents in these food items, including dietary fiber, polyphenols, antioxidants, vitamins (C, E), phytosterols, and many more, it has also been proposed that there is an independent effect of carotenoids.

For example, in a meta-analysis of prospective cohort studies [2], it was shown that carotenoid, but not flavonoid and vitamin C intake, was significantly correlated with a decreased risk of developing type 2 diabetes (Table 1). In another meta-analysis of case-control and prospective cohort studies targeting head and neck-cancers, β-carotene reduced the risk of pharyngeal cancer; while lycopene, α-carotene and β-cryptoxanthin were all associated with reduced risk for oral and laryngeal cancer [3]. In another meta-analysis comprising elderly people (Table 1), plasma β-carotene was associated with reduced all-cause mortality, by up to 30% in the highest quartile [4]. Similarly, in the US NHANES [5] study, total serum carotenoid concentration as well as individual carotenoids such as α-carotene and lycopene were significantly (inversely) associated with all-cause mortality, though only α-carotene was significantly associated with CVD mortality, and none with cancer mortality. Of course, such observational studies cannot prove causality, and are susceptible to confounding factors, such as other life-style factors of subjects and other dietary constituents with potential health benefits, including dietary fibre and polyphenols, also typically found in carotenoid-rich plant food items.

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Table 1. Selected meta-analyses and systematic reviews of epidemiological observational studies suggesting health benefits of dietary carotenoids.

Carotenoids investigated	Study design	Subjects	Outcome/ health effect	Findings	Reference
Total plasma β-carotene	Meta-analysis of prospective cohort studies, up to 10 y follow-up	5 studies, total of 1.168 elderly men & women	All-cause mortality	Reduced mortality by 30% with highest β-carotene status	4
Total plasma carotenoids	review of 62 studies of plasma carotenoids & health outcomes, mostly prospective cohort studies or case-control studies	Men & women, total number not specified	All-cause mortality	very high risk: <1 μM, high risk: 1-1.5 μM, moderate risk: 1.5-2.5 μM, low risk: 2.5-4 μM, and very low risk: >4 μM. >95% of USA population falls into moderate or high risk category	6
Total carotenoid intake	Meta-analysis of prospective cohort studies	Total of 140.000 participants	Type 2 diabetes	Reduced risk of developing type 2 diabetes by 23% with highest carotenoid intake	2
Lycopene dietary intake & plasma levels	Meta-analysis of case control & prospective cohort studies	26 studies, total of 17.517 cases and 563.299 participants	Prostate cancer	Borderline sign. effect of higher lycopene intake & reduced prostate cancer	78
Carotenoid intake & head & neck cancers	Meta-analysis of prospective cohort study (1) & case control studies (15)	16 studies: total of 5.482 cases & 14.130 controls. prospective cohort study: 3.4691 postmeno-pausal women	Various head & neck cancers	β-Carotene reduced risk of pharyngeal cancer. Lycopene, α-carotene & β-cryptoxanthin were all associated with reduced risk for oral & laryngeal cancer	3
Lutein & zeaxanthin intake	Meta-analysis of prospective cohort studies	6 studies with total of 4.416 cases & 4.1999 participants	Age related cataract	Highest & lowest categories of dietary lutein & zeaxanthin intake: Stat. sign. inverse association with nuclear cataract but not with cortical cataract or posterior subcapsular cataract	79
Various carotenoid intakes	Meta-analysis of case-control studies	13 studies, total subject number not specified	Prostate cancer	Reduced risk of aggressive prostate cancer with higher lycopene intake. No sign. effect on overall odds-ratio for pancreatic cancer	64
Various carotenoids in blood plasma	Meta-analysis of prospective cohort studies	8 studies with total of 3.055 cases & 3.956 matched controls	Breast cancer	Stat. sign of inverse associations of breast cancer with α-carotene, β-carotene, lutein+zeaxanthin, lycopene & total carotenoids	80
Various carotenoid intakes	Meta-analysis & meta-regression of observational studies (case control & 6 cohort studies)	33 studies, total subject number not specified	Breast cancer	Dietary α- & β-carotene intake stat. sign. reduced breast cancer risk	62

However, such and similar findings have resulted in the proposition of a carotenoid health index [6], where total carotenoid plasma levels <1 μM were associated with a general increased risk of chronic diseases. Even if carotenoids would only slightly contribute to reduced chronic disease risk, the high prevalence of CVD and cancer would mean that these colourful phytochemicals may considerably reduce health care costs. In a recent study in Canada, too low intake of fruits and vegetables was estimated to result in additional health care costs of 3.3 billion Canadian dollar per annum [7]though of course this cannot be linked to increased carotenoid intake only.

The aim of this brief article is to highlight major mechanisms via which carotenoids are likely implicated in observed health outcomes, to emphasize existing intake recommendations, and to point out limitations in our understanding, which are needed to be tackled prior to establishing more precise and individual dietary intake recommendations of these liposoluble phytochemicals. The article is thus targeted toward general stakeholders in the

domain of dietary counselling, food supplements, and intake recommendations.

Controversial findings in intervention trials with carotenoid supplements

Following the positive findings in epidemiological studies, several (placebo controlled) intervention studies with rather high doses of β-carotene were carried out. Two prominent examples were the ATBC (The Alpha-Tocopherol, Beta-Carotene Intervention Trial [8]) and CARET (the Beta-Carotene and Retinol Efficiency Trial [9]), both targeting smokers, due to the perceived increased oxidative stress risk in these persons. In these intervention trials, 20 mg β-carotene (with 50 mg α-tocopherol in the ATBC trial) and 30 mg β-carotene (with 25,000 IU retinyl palmitate) were given daily, for several years. Lung cancer risk rather increased than decreased, and studies had to be terminated, for reasons that are still investigated (see following chapter).

In a systematic review and meta-analysis by Bjelakovic and co-workers [10], intervention trials (>30) with β-carotene supplements, alone or in combination with other antioxidants, significantly increased total mortality by on average of 5 % with β-carotene intake. However, these results were mostly influenced by the ATBC/CARET trials in smokers. It is noteworthy that other intervention trials did not find negative results regarding CVD (or all-cause mortality), such as the Linxian Trial [11], the Physicians' study [12], or the SU.VI.MAX study [13], perhaps as a more general population was targeted, which may in addition have had rather low starting plasma carotenoid concentrations.

However, as for other nutrients, there exists no general linear dose-response relationship, but a recommended level of intake, with higher intakes beyond the tolerable upper intake level (UL) and animal derived NOAEL (no-observed-adverse-effect levels) potentially resulting in increased risk for adverse effects, depending possibly on the nutritional status and individual risk factors such as smoking, or additional aspects contributing to inter-individual variation of carotenoid metabolism [14]. Regarding β-carotene intake and smoking, ferret animal models suggested that down-regulated RARβ gene expression, related to reduced levels of retinoic acid, and overexpression of activator protein-1 [15, 16], involved in differentiation, proliferation, and apoptosis, likely contributed to the formation of cancerous lesions in the lung.

It is also plausible that isolated carotenoids from supplements act differently than carotenoids embedded in a complex plant food matrix, where other synergistically acting nutrients such as vitamin E are present [17, 18]. Finally, also bioavailability and kinetics of such supplements can be altered compared to carotenoids in their native matrix [19, 20]. Thus, prior to supplementing specific population groups, it is paramount to obtain a clear picture of how carotenoids are implicated in health promoting effects, and which dose-response relationships exist.

Mechanistic aspects

Carotenoids as direct antioxidants

It has been realized for some time that carotenoids can act as strong antioxidants. At least *in vitro* it was shown that carotenoids can quench free radicals such as lipid peroxides [21], can react with singlet oxygen [22], and also capture photons of short wavelengths (UVA/UVB, blue light) which otherwise could harm the skin [23] or the human eye [24]. Unsurprisingly, it was earlier assumed that these rather direct antioxidant properties would be the main mechanisms via which carotenoids promote health. Indeed, it is plausible that carotenoids contribute to the stability of cell membranes, protecting lipids from oxidation with potential reactive oxygen species [25]. Specifically, β-carotene appears to act complementary to nitric oxide (NO) and vitamin E in protecting cell membranes, especially from damage induced by singlet oxygen [26]. Furthermore, higher concentrations of carotenoids in the human skin have been shown to reduce erythema induced by UV-light, though photo-protection took around 7–10 weeks to be effective (to reach the outer skin areas), and was recommended as an adjuvant [23].

However, the direct antioxidant hypothesis has been questioned [27, 28]. One reason is that the antioxidant balance of the human body is controlled by numerous endogenous and exogenous factors, with carotenoids possibly only playing a minor role. Endogenous antioxidants such as glutathione, uric acid, albumin, enzymes such as catalase and superoxide dismutase, as well as exogenous antioxidants such as vitamin E/C appear to play a more pronounced role, considering their much higher concentrations in biological tissues, e.g. 200 μM for uric acid and 20 μM for vitamin E [29].

Nevertheless, it can be assumed that certain concentrations of carotenoids aid in preventing oxidative damage such as in cell membranes [30]. According to some studies, lycopene appears as the strongest carotenoid antioxi-

dant *in vitro*, possibly due to its elongated conjugated double bond system [30, 31]. As lycopene is, with β-carotene, and sometimes β-cryptoxanthin, the most abundant carotenoid in blood/tissues (at least in Westernized countries), it may be assumed that its antioxidant effect contributes to observable health benefits.

In line with the negative findings from the ATBC and CARET trial, it was also found *in vitro* that higher carotenoid concentrations (4–10 μM) may rather have pro-oxidant effects, resulting in DNA damage [32]. It is likely that high concentrations of antioxidants can act as pro-oxidants, especially when interacting with cytochrome-oxidases (e.g. P450), resulting in pro-oxidant intermediates, which, together with the effects communicated via RARβ as stated above, may negatively interact with the already damaged lungs of smokers [33]. Furthermore, under high partial oxygen pressure, as in lung tissue [18], and possibly the presence of pro-oxidant metal oxidation states such as Fe (III) [29], carotenoids can react as pro-oxidants, fostering tumor progression [34].

Carotenoids and metabolites as inducers of cellular signalling cascades

An increasing body of evidence suggests that the more indirect effects of carotenoids, altering gene expression, may be their primary health promoting routes. Notably, both carotenoid derived metabolites, i.e. plant apocarotenoids and apocarotenoids formed within the human body, such as via β-carotene oxygenase 1 (BCO1, centric cleavage) and 2 (BCO2, eccentric cleavage), may play a role *in vivo*. Regarding transcription factors (TF), including the nuclear receptors RXR/RAR, it is noteworthy that the more polar apocarotenoids are likely better targets for such interactions, due to their higher cytosolic solubility (lower logP values), and they are more electrophilic and may easier bind to cysteine residues of NF-κB [35] and Nrf-2 [36], causing inactivation and activation of these TF, respectively. As the amount of metabolites formed may depend preliminary on the bioavailability of the native carotenoids in conjunction with the activity of BCO1/2, and single nuclear polymorphisms (SNPs) in these enzymes have shown to largely influence plasma appearance of the native carotenoids [37, 38], it can be hypothesized that the biological responses and health benefits also vary considerably between people [14]. In addition, there appear to be tissue differences in the expression of BCO1/2 (with BCO1 being more prominent in the liver than the brain, at least in mice [39] and BCO2 being absent in some human tissues, such as the colon and the skin epidermis [40]), and also differing subcellular locations of BCO1/2, with BCO1 being present in the cytosol, while BCO2 is present in the mitochondria. Further SNPs, involved in the further transport and biodistribution of carotenoids such as SR-BI (scavenger receptor class B type 1) and several apolipoproteins (APOA4, APOB, APOE) do likewise influence variability between subjects [14, 41]. These differences could influence potential health benefits, resulting in specific tissue and compartmental effects of carotenoid metabolites. A large number of TFs/nuclear receptors have been reported to be influenced by carotenoids/metabolites (Table 2, Figure 1):

Table 2. Implication of transcription factors and nuclear receptors and their major downstream targets which may be activated by carotenoids and their metabolites.

TF or nuclear receptor	Downstream target protein	Major functions	References
NF-κB	IL-2, IL-6, IL-8, TNF-α, NO, IFN-γ, IgG, MHC, ICAM-1, VCAM-1, hepcidin, COX-2,	Pro-inflammatory	81
Nrf-2	SOD, CAT, HO-1, GPX, GST, NQO1	Anti-oxidant	82
RAR-RXR	Wnt1, Gas2, Cidea, Wnt10b	Immune system, cell growth & proliferation, apoptosis	83
PPARs	FABP1, ELOVL6, MOD1	Adipocyte differentiation, lipid metabolism	84

CAT: catalase, Cidea: cell death-inducing DFFA-like effector a; COX-2: cyclo-oxygenase 2; ELOVL6: elongation of very long chain fatty acids protein 6; FABP1: fatty acid binding protein 1; Gas2: growth arrest specific 2; GPX: glutathione peroxidase; GST: glutathione S-transferase; HO-1: heme-oxygenase; IgG: immunoglobin G, heavy chain; ICAM-1: intercellular adhesion molecule 1; IFN-γ: interferon-gamma; IL: interleukin; MHC: major histocompatibility complex; MOD1: alcohol oxidase or enoyl-[acyl-carrier-protein] reductase [NADH], chloroplastic 1; NF-kB: nuclear factor kappa-B; NQO1: NAD(P)H:quinone oxidoreductase 1; Nrf-2: nuclear-factor (erythroid-derived)-2 like 2, PPARs: peroxisome proliferator activated receptor; RAR-RXR: retinoic acid receptor – retinoid-X-receptor; SOD: superoxide dismutase; TF: transcription factor; TNF-α: tumor necrosis factor alpha; VCAM-1: vascular cell adhesion molecule; Wnt1: Wnt family member 1; Wnt10b: Wnt family member 10B.

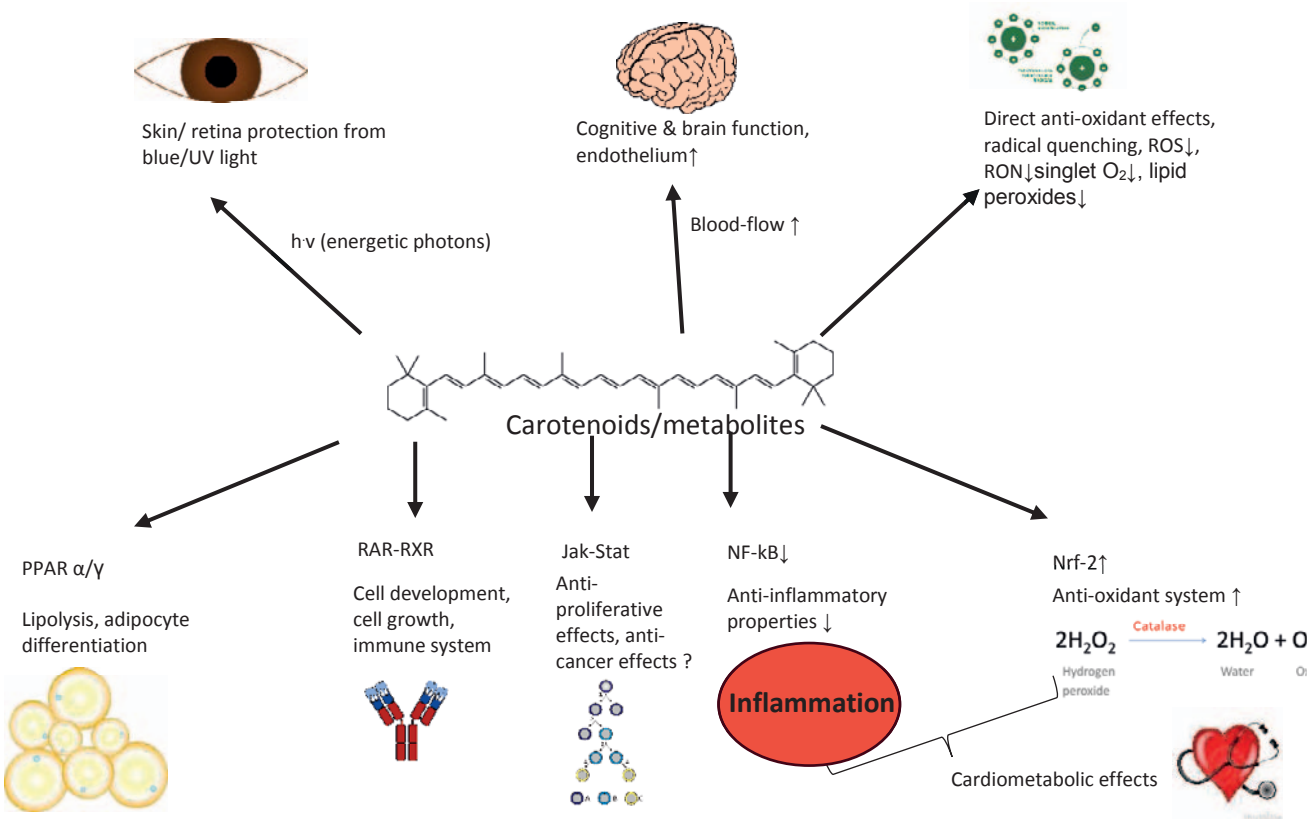

Figure 1. Pathways involved in potential carotenoid health benefits.

- NF-κB: This TF is activated in the cytosol by the dissociation of its inhibitor (IKBα), following phosphorylation by a kinase, the IkB kinase (IKK). Carotenoids/their metabolites have been reported to either bind to IKK, preventing phosphorylation and dissociation of NF-κB, or to prevent the already phosphorylated NF-κB complex from proteosomal degradation and dissociation [28]. As a result, this TF cannot dissociate and travel to the nucleus where it may activate, upon binding to response elements, further downstream genes related to the expression of various pro-inflammatory cytokines such as IL-6 and TNF-α.
- Nrf-2. Present likewise in the cytosol, this TF is bound to an inhibitor, Keap-1 (Kelch-like ECH associated protein 1). Carotenoids/their metabolites may bind and result in the dissociation of Keap1, allowing for liberation and translocation of the TF to the nucleus [28]. Upon binding to antioxidant response element (ARE), genes related to the body's own antioxidant defence system such as superoxide-dismutase (SOD), catalase (CAT) and heme-oxygenase 1 (HO-1) are activated.
- Jak-Stat. Though there is less data available, a few studies have reported that certain carotenoids can also act via suppressing the Jak-Stat pathway, reducing cell proliferation, angiogenesis and invasiveness, and thus may act against the formation of cancer [42, 43].
- RXR/RAR: This nuclear receptor dimer responds especially to retinoic acid [44]. However, it has been proposed that also other apo-carotenoids such as those resulting from lycopene cleavage by BCO2, may activate it [45, 46]. In contrast, certain eccentric cleavage metabolites of beta-carotene, e.g. β-apo-14'-carotenal, β-apo-14'-carotenoic acid, and β-apo-13-carotenone may act as antagonists [47]. This nuclear receptor is responsible for activating a number of immune related target genes (>500), and is implicated in cell differentiation/growth control and apoptosis (Table 2). Other nuclear receptor dimers such as RXR/PPARα (peroxisomeproliferator-activated receptor) may also be responsive to carotenoids/their metabolites, which may influence the development of adipocytes and thus obesity [48]. For example, it was reported that the eccentric cleavage product β-apo-14'-carotenal acted as a repressor of RXR and PPARα, thereby decreasing adipogenesis [49].

Carotenoids in the prevention of cardiometabolic diseases

Several studies have highlighted that carotenoid intake and circulating levels of carotenoids are correlated with a decreased risk of developing cardiometabolic diseases. These include type 2 diabetes [2, 50], the metabolic syndrome [51] and also stroke [52]. As stated, observational studies cannot clearly establish a cause and effect relation, and potential confounding factors – despite multivariate statistics trying to control these – could still interfere. It has been argued whether carotenoids are not merely an indicator for a healthy diet rich in fruits and vegetables, and that other factors such as dietary fiber, antioxidant vitamins (C, E), polyphenols, phytosterols, glucosinolates etc., or their combined activity, result in the observed health benefits [53], as many of these alternative constituents could also modify inflammation and oxidative stress related to cardiometabolic diseases.

Nevertheless, there are many *in vitro* and animal studies, and recently also a number of randomized human intervention trials with isolated carotenoid supplements, which indicate that carotenoids at least contribute to potential health effects. For example, lycopene given for 12 weeks (70 mg/week) to middle aged, overweight subjects resulted in reduced serum-amyloid-A, a marker of systemic and HDL-associated inflammation [54]. In another study, lutein (20 mg/d for 3 months) improved IL-6 and plasma monocyte chemoattractant protein 19 (MCP-19) in arthrosis patients [55]. Effects regarding oxidative stress and inflammation were also seen in new-borns. In 2 studies with term infants receiving 2 times 0.28 mg of lutein, total plasma hydroperoxides significantly decreased, while FRAP (ferric reducing antioxidant power assay), an assay detecting antioxidant capacity, increased [56, 57]. Similarly, in pre-term infants, a combination of lutein/β-carotene/lycopene (220, 210 and 140 µg/L formula food taken for up to 40 weeks) reduced circulating C-reactive protein (CRP) and retinopathy severity, compared to those receiving control formula [58].

It is difficult to pinpoint the most relevant mechanism underlying the potential beneficial effects regarding CVD. In addition to the possible direct antioxidant effects via stabilization of lipoproteins, alterations in inflammatory and oxidative stress pathways involving cellular transcription factors and their downstream targets seem plausible. Additional pathways such as the increase in NO endothelial bioavailability may also play a role, as reviewed recently [59, 60]. Taken together, the results of both observational studies and intervention trials with supplements have considerably added to the body of evidence of carotenoids as bioactive agents against CVD.

Carotenoids in cancer prevention

Recent epidemiological studies have suggested that the influence of fruit and vegetable intake is less strong regarding cancer prevention compared to cardiovascular diseases [61]. Thus, there may be somewhat less leverage of carotenoids on cancer risk reduction compared to CVD. Nevertheless, as cancer is, together with CVD, the predominant cause of death in developed countries, there has been much interest regarding the effects of carotenoids on cancer progression. As emphasized above, due to the interaction of carotenoids/their metabolites in inflammatory, antioxidant, and apoptotic cellular pathways, the implication of carotenoids in processes involving cancer appears plausible.

Several observational epidemiological studies proposed a relation of carotenoids and the prevention of breast cancer [62], and possibly gastric cancer [63], as well as prostate cancer, at least for lycopene [64]. However, there are also observational studies not finding significant associations. Only few long-term intervention studies with carotenoid supplements targeting cancer risk exist. In addition to the ATBC and CARET trial, only few other studies were carried out, and often carotenoids (typically β-carotene) were given in combination. In the Linxian trial in China, β-carotene given together with vitamin E and Se (30.000 adults, 5 y) resulted in reduced cancer risk (especially of the stomach) [11]. However, subjects generally showed a poor micronutrient status and may have been deficienct at baseline. In a meta-analysis summarizing randomized controlled intervention trials with β-carotene given for up to 12 y (typically 20–30 mg/d), no significant reduction effect was found on all cancers combined. However, lung and stomach cancer risk increased significantly for β-carotene doses above 20 mg/d – though β-carotene was given partly in combination with either vitamin A, vitamin E, vitamin C, Se, and/or Zn [65]. Of note, the effects were only significant in asbestos workers and smokers. Also, a gender dependent effect was suggested, as for women and lower intake of β-carotene, a reduction for stomach cancer risk was found. In the French SU.VI.MAX study with over 13.000 participants, a combination of antioxidants (120 mg vitamin C, 30 mg vitamin E, 6 mg β-carotene, 100 µg Se, and 20 mg Zn taken on average for 7.5 y) reduced the cancer incidence in men, but not women – perhaps as men had a lower basal antioxidant intake than women.

Only very few short/mid-term intervention studies exist investigating biomarkers that may be relevant to cancer. Some investigated the effect of lycopene supplementation on prostate specific antigen (PSA) in prostate cancer subjects [66], and found partially positive effects regarding the PSA development over time when receiving 15 mg lycopene/d, though again in combination with other

antioxidants [67]. This is in line with earlier observational studies suggesting an association with lycopene and α-carotene intake and lower PSA levels [68].

In summary, despite some positive findings based on epidemiological studies, long-term intervention trials have failed to show a clear relationship between carotenoid intake and reduced cancer risk. Contrarily, β-carotene, also in combination with other antioxidants, may increase the risk of some types of cancer such as of the lung and stomach at higher intake.

Carotenoids, eye health and brain

The dietary intake and plasma levels of lutein and zeaxanthin have been related to the prevention and amelioration of AMD, the major cause of blindness in the elderly. AMD involves the macula of the eye, which is part of the retina. This is an area of the eye paramount for central vision or visual acuity. This area contains a high concentration of especially lutein, zeaxanthin, and meso-zeaxanthin, the latter formed from lutein in the human body [69]. Due to their photoprotective (blue light) and antioxidant properties, these macula pigments protect from oxidative damage. In subjects with poor visual acuity as present with AMD, these pigments have often been partly depleted [70]. Reciprocally, regular dietary intake of lutein/zeaxanthin, including supplements, has shown to slow the progression of AMD toward its late forms and to improve visual acuity in subjects at risk for developing AMD [70]. Due to these findings, supplementation with lutein (10 mg/d) and zeaxanthin (2 mg/d) has been proposed in general for the elderly population at risk of developing AMD [71]. Accordingly, these carotenoids have been discussed as being of conditional essential character [72].

As at least dietary derived lutein and zeaxanthin are deposited in the retina, they must pass the blood-brain barrier. High concentrations of lutein have been detected in brain tissues, making lutein the most abundant carotenoid in this organ (around 170 pmol/g) [73]. It has been speculated whether lutein may improve cognitive performance, as positive associations between serum and brain lutein concentrations and cognitive performance were found in the elderly [74], though similar associations were reported earlier for β-carotene [75]. Similar as for polyphenols, carotenoids may add to improved endothelial stability and flexibility, improving blood-flow [76]. Other mechanisms include antioxidant activity in synergy with vitamin E, enhancing gap junction communication, modulation of synaptic membranes, and the influence on gene expression influencing inflammation and oxidative stress [77].

Dietary intake recommendations

No dietary reference intakes (DRI) exist for carotenoids. Several countries and authorities have issued recommendations for individual carotenoids, either based on dietary intake, intake via food additives, and/or supplemental intake (Table 3). Both recommended intakes but also levels

Table 3. Recommendations for dietary intake, considering also in part supplements and food additives.

Carotenoid	Type of recommendation	Authority issuing recommendation	Recommendation	Remark	Reference
Astaxanthin	ADI	EFSA	0.034 mg/(kg bw/d)	all sources	85
β-Carotene	safe intake for smokers	EFSA	15 mg/d	no ADI	86
	safe upper level	UK	7 mg/d	supplements	87
	RDI	DGE	2 mg/d	all sources[$]	88
Cantaxanthin	ADI	EFSA	0.03 mg/(kg bw/d)	as food additive	89
Lutein	OSL	Shao & Hathcock	20 mg/d	all sources	90
	ADI	EFSA	1.0 mg/(kg bw/d)	as food additive	91
	dietary intake (for eye health)*	authors	10.0 mg/d	supplements	92
Lycopene	OSL	Shao & Hathcock	75 mg/d	all sources	90
	ADI	EFSA	0.5 mg/(kg bw/d)	all sources	93
Zeaxanthin	safe intake	EFSA	53 mg/d (0.75 mg/(kg bw/d)	synthetic form	94

ADI: Acceptable daily intake; bw: body weight; DGE: German Nutrition Society; EFSA: European Food Safety Authority; OSL: Observed safe level; RDI: recommended daily intake; UK: United Kingdom; UL: tolerable upper intake level. *recommendation by authors. [$]plus 1.0 mg retinol equivalents for vitamin A requirements

comparable to the UL have been proposed. When comparing these recommendations, higher levels of β-carotene above 15–20 mg/d are rather discouraged, as these may result in elevated (>3 μM) circulating blood levels which have been related to adverse effects in some population groups such as smokers. On the other hand, intake in the range of 2–7 mg/d of β-carotene has been recommended, also in sight of the >1 μM health beneficial total carotenoid plasma/serum concentrations proposed [6]. This level may have to be increased when no preformed vitamin A is ingested, up to 6 mg additional β-carotene are recommended by some health authorities such as the German Nutrition Society (DGE) (http://www.dge.de/wissenschaft/referenzwerte/vitamin-a-b-carotin/). For other carotenoids, safe intake recommendations are higher, up to 75 mg/d for lycopene (Table 3).

Finally, intake recommendations are impeded by the following: 1) Many dietary and host factors do influence carotenoid bioavailability, and a simple intake recommendation may fall short in assuring sufficient availability; 2) there is a large inter-individual variability regarding carotenoid responses, related to individual differences in digestion, absorption, cleavage and biodistribution [14]; 3) different carotenoids may have different biological properties; 4) different populations may have different needs – e.g. targeting elderly subjects with AMD and lutein recommendations vs. smokers where recommendations may require more prudence.

Future perspectives of carotenoids in chronic disease prevention

Despite some negative health outcomes encountered for high-dosed supplements, especially for subjects prone to lung diseases (smokers or asbestos exposed), carotenoids appear promising dietary microconstituents, with several potential health benefits. These include, in addition to the role of some carotenoids as vitamin A precursors, their likely health promoting effects regarding cardiometabolic diseases and the amelioration of AMD. Prominent mechanism explaining their bioactivity include direct antioxidant effects, photo-protective properties, and their influence on nuclear receptors/TF and gene expression, resulting in anti-inflammatory and anti-oxidant effects and regulation of cellular differentiation/growth, which may also suggest anti-cancer properties, though data from human trials remains contradictory, implying also a dose-dependency. In general, attributing observed health effects to carotenoids is a difficult task, especially in observational studies, due to numerous confounding factors. Gaps of our knowledge surely exist and include the differentiation of health effects due to the native compounds vs. their apocarotenoid metabolites. Several cartotenoids which are also frequently consumed are somewhat understudied, e.g. the colourless carotenoids phytoene/phytofluene, or the epoxycarotenoids violaxanthin/neoxanthin, in addition to plant derived apocarotenoids such as crocetin and bixin. More research on both mechanistic and dose-related aspects of carotenoids and their potential effects on health and well-being are much desired, especially when aiming at dietary recommendations and health claims.

References

1. World Health Organization. Global strategy on diet, physical activity and health.: World Health Organization; 2003.
2. Hamer, M. & Chida, Y. Intake of fruit, vegetables, and antioxidants and risk of type 2 diabetes: systematic review and meta-analysis. J Hypertens 25, 2361 – 2369 (2007).
3. Leoncini, E. et al. Carotenoid intake from natural sources and head and neck cancer: A systematic review and meta-analysis of epidemiological studies. Cancer Epidemiol. Biomarkers Prev. 24, 1003 – 1011 (2015).
4. Buijsse, B. et al. Plasma carotene and alpha-tocopherol in relation to 10-y all-cause and cause-specific mortality in European elderly: the Survey in Europe on Nutrition and the Elderly, a Concerted Action (SENECA). Am. J. Clin. Nutr. 82, 879 – 886 (2005).
5. Shardell, M.D. et al. Low-serum carotenoid concentrations and carotenoid interactions predict mortality in US adults: the Third National Health and Nutrition Examination Survey. Nutr Res 31, 178 – 189 (2011).
6. Donaldson, M.S. A carotenoid health index based on plasma carotenoids and health outcomes. Nutrients 3, 1003 – 1022 (2011).
7. Ekwaru, J.P. et al. The economic burden of inadequate consumption of vegetables and fruit in Canada. Public Health Nutr 20, 515 – 523 (2017).
8. The Alpha-tocopherol Beta-carotene Cancer Prevention Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. N. Engl J Med 330, 1029 – 1035 (1994).
9. Omenn, G.S. et al. Risk factors for lung cancer and for intervention effects in CARET, the Beta-Carotene and Retinol Efficacy Trial. J Natl Cancer Inst 88, 1550 – 1559 (1996).
10. Bjelakovic, G., Nikolova, D., Gluud, L.L., Simonetti, R.G. & Gluud, C. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. Cochrane Database Syst Rev, Cd007176 (2012).
11. Blot, W.J. et al. The Linxian trials: mortality rates by vitamin-mineral intervention group. Am J Clin Nutr 62, 1424s-1426s (1995).
12. Hak, A.E. et al. Plasma carotenoids and tocopherols and risk of myocardial infarction in a low-risk population of US male physicians. Circulation 108, 802 – 807 (2003).
13. Hercberg, S. et al. The SU.VI.MAX Study: a randomized, placebo-controlled trial of the health effects of antioxidant vitamins and minerals. Arch Intern Med 164, 2335 – 2342 (2004).

14. Bohn, T. et al. Host-related factors explaining interindividual variability of carotenoid bioavailability and tissue concentrations in humans. Mol Nutr Food Res 61, doi: 10.1002/mnfr.201600685 (2017).
15. Wang, X.D. et al. Retinoid signaling and activator protein-1 expression in ferrets given beta-carotene supplements and exposed to tobacco smoke. J Natl Cancer Inst 91, 60 – 66 (1999).
16. Liu, C. et al. Effects of physiological versus pharmacological beta-carotene supplementation on cell proliferation and histopathological changes in the lungs of cigarette smoke-exposed ferrets. Carcinogenesis 21, 2245 – 2253 (2000).
17. El-Agamey, A. et al. Carotenoid radical chemistry and antioxidant/pro-oxidant properties. Arch Biochem Biophys 430, 37 – 48 (2004).
18. Young, A.J. & Lowe, G.M. Antioxidant and prooxidant properties of carotenoids. Arch Biochem Biophys 385, 20 – 27 (2001).
19. Corte-Real, J. Carotenoids in the human body: bioavailability and bioconversion. EUROCAROTEN Newsletter, 1 (2017).
20. Bohn, T. Bioactivity of carotenoids – chasms of knowledge. Int J Vitam Nutr Res 10, 1 – 5 (2016).
21. Krinsky, N.I. & Yeum, K.J. Carotenoid-radical interactions. Biochem Biophys Res Commun 305, 754 – 760 (2003).
22. Terao, J., Minami, Y. & Bando, N. Singlet molecular oxygen-quenching activity of carotenoids: relevance to protection of the skin from photoaging. J Clin Biochem Nutr 48, 57 – 62 (2011).
23. Stahl, W. & Sies, H. beta-Carotene and other carotenoids in protection from sunlight. Am J Clin Nutr 96, 1179s-1184s (2012).
24. Eisenhauer, B., Natoli, S., Liew, G. & Flood, V.M. Lutein and Zeaxanthin-Food Sources, Bioavailability and Dietary Variety in Age-Related Macular Degeneration Protection. Nutrients 9 (2017).
25. Gruszecki, W.I. & Strzalka, K. Carotenoids as modulators of lipid membrane physical properties. Biochim Biophys Acta 1740, 108 – 115 (2005).
26. Schafer, F.Q. et al. Comparing beta-carotene, vitamin E and nitric oxide as membrane antioxidants. Biol Chem 383, 671 – 681 (2002).
27. Erdman, J.W., Jr., Ford, N.A. & Lindshield, B.L. Are the health attributes of lycopene related to its antioxidant function? Arch Biochem Biophys 483, 229 – 235 (2009).
28. Kaulmann, A. & Bohn, T. Carotenoids, inflammation, and oxidative stress--implications of cellular signaling pathways and relation to chronic disease prevention. Nutr. Res. 34, 907 – 929 (2014).
29. Bouayed, J. & Bohn, T. Exogenous antioxidants - Double-edged swords in cellular redox state: Health beneficial effects at physiologic doses versus deleterious effects at high doses: Oxid Med Cell Longev 3, 228 – 237 (2010).
30. Fiedor, J. & Burda, K. Potential role of carotenoids as antioxidants in human health and disease. Nutrients 6, 466 – 488 (2014).
31. Shi, J., Kakuda, Y. & Yeung, D. Antioxidative properties of lycopene and other carotenoids from tomatoes: synergistic effects. Biofactors 21, 203 – 210 (2004).
32. Lowe, G.M., Booth, L.A., Young, A.J. & Bilton, R.F. Lycopene and beta-carotene protect against oxidative damage in HT29 cells at low concentrations but rapidly lose this capacity at higher doses. Free Radic Res 30, 141 – 151 (1999).
33. Goralczyk, R. Beta-carotene and lung cancer in smokers: review of hypotheses and status of research. Nutr Cancer 61, 767 – 774 (2009).
34. Palozza, P., Serini, S., Di Nicuolo, F., Piccioni, E. & Calviello, G. Prooxidant effects of beta-carotene in cultured cells. Mol Aspects Med 24, 353 – 362 (2003).
35. Linnewiel-Hermoni, K., Motro, Y., Miller, Y., Levy, J. & Sharoni, Y. Carotenoid derivatives inhibit nuclear factor kappa B activity in bone and cancer cells by targeting key thiol groups. Free Radic Biol Med 75, 105 – 120 (2014).
36. Linnewiel, K. et al. Structure activity relationship of carotenoid derivatives in activation of the electrophile/antioxidant response element transcription system. Free Radic Biol Med 47, 659 – 667 (2009).
37. Borel, P., Desmarchelier, C., Nowicki, M. & Bott, R. A combination of single-nucleotide polymorphisms is associated with interindividual variability in dietary beta-carotene bioavailability in healthy men. J Nutr 145, 1740 – 1747 (2015).
38. Borel, P. et al. Interindividual variability of lutein bioavailability in healthy men: characterization, genetic variants involved, and relation with fasting plasma lutein concentration. Am J Clin Nutr 100, 168 – 175 (2014).
39. Kim, Y.K., Zuccaro, M.V., Costabile, B.K., Rodas, R. & Quadro, L. Tissue- and sex-specific effects of beta-carotene 15,15‘ oxygenase (BCO1) on retinoid and lipid metabolism in adult and developing mice. Arch Biochem Biophys 572, 11 – 18 (2015).
40. Lindqvist, A., He, Y.G. & Andersson, S. Cell type-specific expression of beta-carotene 9‘,10‘-monooxygenase in human tissues. J Histochem Cytochem 53, 1403 – 1412 (2005).
41. Borel, P. et al. Human plasma levels of vitamin E and carotenoids are associated with genetic polymorphisms in genes involved in lipid metabolism. J Nutr 137, 2653 – 2659 (2007).
42. Kowshik, J. et al. Astaxanthin inhibits JAK/STAT-3 signaling to abrogate cell proliferation, invasion and angiogenesis in a hamster model of oral cancer. PLoS One 9, e109114 (2014).
43. Yu, R.X., Hu, X.M., Xu, S.Q., Jiang, Z.J. & Yang, W. Effects of fucoxanthin on proliferation and apoptosis in human gastric adenocarcinoma MGC-803 cells via JAK/STAT signal pathway. Eur J Pharmacol 657, 10 – 19 (2011).
44. Huang, P., Chandra, V. & Rastinejad, F. Retinoic acid actions through mammalian nuclear receptors. Chem Rev 114, 233 – 254 (2014).
45. Aydemir, G. et al. Lycopene supplementation restores vitamin A deficiency in mice and possesses thereby partial pro-vitamin A activity transmitted via RAR signaling. Mol Nutr Food Res 60, 2413 – 2420 (2016).
46. Aydemir, G. et al. Lycopene-derived bioactive retinoic acid receptors/retinoid-X receptors-activating metabolites may be relevant for lycopene‘s anti-cancer potential. Mol Nutr Food Res 57, 739 – 747 (2013).
47. Eroglu, A. et al. Naturally occurring eccentric cleavage products of provitamin A beta-carotene function as antagonists of retinoic acid receptors. J Biol Chem 287, 15886 – 15895 (2012).
48. Bonet, M.L., Canas, J.A., Ribot, J. & Palou, A. Carotenoids in adipose tissue biology and obesity. Subcell. Biochem 79, 377 – 414 (2016).
49. Ziouzenkova, O. et al. Asymmetric cleavage of beta-carotene yields a transcriptional repressor of retinoid X receptor and peroxisome proliferator-activated receptor responses. Mol Endocrinol 21, 77 – 88 (2007).
50. Sluijs, I. et al. Dietary intake of carotenoids and risk of type 2 diabetes. Nutr. Metab. Cardiovasc. Dis. 25, 376 – 381 (2015).
51. Leermakers, E.T. et al. The effects of lutein on cardiometabolic health across the life course: a systematic review and meta-analysis. Am J Clin Nutr 103, 481 – 494 (2016).
52. Li, X. & Xu, J. Dietary and circulating lycopene and stroke risk: a meta-analysis of prospective studies. Sci Rep 4, 5031 (2014).
53. Al-Delaimy, W.K. et al. Plasma carotenoids as biomarkers of intake of fruits and vegetables: ecological-level correlations in the European Prospective Investigation into Cancer and Nutrition (EPIC). Eur J Clin Nutr 59, 1397 – 1408 (2005).

54. McEneny, J. et al. Lycopene intervention reduces inflammation and improves HDL functionality in moderately overweight middle-aged individuals. J Nutr Biochem 24, 163 – 168 (2013).
55. Xu, X.R. et al. Effects of lutein supplement on serum inflammatory cytokines, ApoE and lipid profiles in early atherosclerosis population. J Atheroscler Thromb 20, 170 – 177 (2013).
56. Perrone, S. et al. Effects of lutein on oxidative stress in the term newborn: a pilot study. Neonatology 97, 36 – 40 (2010).
57. Perrone, S., Negro, S., Tataranno, M.L. & Buonocore, G. Oxidative stress and antioxidant strategies in newborns. J. Matern. Fetal Neonatal Med. 23 Suppl 3, 63 – 65 (2010).
58. Rubin, L.P. et al. Effect of carotenoid supplementation on plasma carotenoids, inflammation and visual development in preterm infants. J Perinatol (2011).
59. Di Pietro, N., Di Tomo, P. & Pandolfi, A. Carotenoids in cardiovascular disease prevention. JSM Atherosclerosis 1 (2016).
60. G, R., MA, G. & N, D.O. Carotenoids and cardiovascular prevention: an update. J Nutr Food Sci 6 (2016).
61. Leenders, M. et al. Fruit and vegetable intake and cause-specific mortality in the EPIC study. Eur J Epidemiol 29, 639 – 652 (2014).
62. Hu, F. et al. Carotenoids and breast cancer risk: a meta-analysis and meta-regression. Breast Cancer Res Treat 131, 239 – 253 (2012).
63. Zhou, Y., Wang, T., Meng, Q. & Zhai, S. Association of carotenoids with risk of gastric cancer: A meta-analysis. Clin Nutr 35, 109 – 116 (2016).
64. Key, T.J. et al. Carotenoids, retinol, tocopherols, and prostate cancer risk: pooled analysis of 15 studies. Am J Clin Nutr 102, 1142 – 1157 (2015).
65. Druesne-Pecollo, N. et al. Beta-carotene supplementation and cancer risk: a systematic review and metaanalysis of randomized controlled trials. Int J Cancer 127, 172 – 184 (2010).
66. Van Patten, C.L., de Boer, J.G. & Tomlinson Guns, E.S. Diet and dietary supplement intervention trials for the prevention of prostate cancer recurrence: a review of the randomized controlled trial evidence. J Urol 180, 2314 – 2322 (2008).
67. Schroder, F.H. et al. Randomized, double-blind, placebo-controlled crossover study in men with prostate cancer and rising PSA: effectiveness of a dietary supplement. Eur Urol 48, 922 – 930; discussion 930 – 921 (2005).
68. Wang, Y., Cui, R., Xiao, Y., Fang, J. & Xu, Q. Effect of carotene and lycopene on the risk of prostate cancer: A systematic review and dose-response meta-analysis of observational studies. PLoS One 10, e0137427 (2015).
69. Nolan, J.M., Meagher, K., Kashani, S. & Beatty, S. What is meso-zeaxanthin, and where does it come from? Eye (Lond) 27, 899 – 905 (2013).
70. Puell, M.C., Palomo-Alvarez, C., Barrio, A.R., Gomez-Sanz, F.J. & Perez-Carrasco, M.J. Relationship between macular pigment and visual acuity in eyes with early age-related macular degeneration. Acta Ophthalmol 91, e298 – 303 (2013).
71. Chew, E.Y. et al. Secondary analyses of the effects of lutein/zeaxanthin on age-related macular degeneration progression: AREDS2 report No. 3. JAMA ophthalmology 132, 142 – 149 (2014).
72. Semba, R.D. & Dagnelie, G. Are lutein and zeaxanthin conditionally essential nutrients for eye health? Med Hypotheses 61, 465 – 472 (2003).
73. Erdman, J.W., Jr. et al. Lutein and brain function. Foods (Basel, Switzerland) 4, 547 – 564 (2015).
74. Johnson, E.J. et al. Relationship between serum and brain carotenoids, alpha-tocopherol, and retinol concentrations and cognitive performance in the oldest old from the Georgia Centenarian Study. Journal of Aging Research 2013, 951786 (2013).
75. Perrig, W.J., Perrig, P. & Stahelin, H.B. The relation between antioxidants and memory performance in the old and very old. J. Am. Geriatr. Soc. 45, 718 – 724 (1997).
76. Yamagata, K. Carotenoids regulate endothelial functions and reduce the risk of cardiovascular disease In: Cvetkovic, D. (ed). Carotenoids. InTech: Rijeka, 2017.
77. Johnson, E.J. A possible role for lutein and zeaxanthin in cognitive function in the elderly. Am J Clin Nutr 96, 1161S-1165S (2012).
78. Chen, P. et al. Lycopene and risk of prostate cancer: A systematic review and meta-analysis. Medicine (Baltimore) 94, e1260 (2015).
79. Ma, L. et al. A dose-response meta-analysis of dietary lutein and zeaxanthin intake in relation to risk of age-related cataract. Graefes Arch Clin Exp Ophthalmol 252, 63 – 70 (2014).
80. Eliassen, A.H. et al. Circulating carotenoids and risk of breast cancer: pooled analysis of eight prospective studies. J Natl Cancer Inst 104, 1905 – 1916 (2012).
81. Pahl, H.L. Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene 18, 6853 – 6866 (1999).
82. Gorrini, C., Harris, I.S. & Mak, T.W. Modulation of oxidative stress as an anticancer strategy. Nat Rev Drug Discov 12, 931 – 947 (2013).
83. Delacroix, L. et al. Cell-specific interaction of retinoic acid receptors with target genes in mouse embryonic fibroblasts and embryonic stem cells. Mol Cell Biol 30, 231 – 244 (2010).
84. Rakhshandehroo, M., Knoch, B., Müller, M. & Kersten, S. Peroxisome proliferator-activated receptor alpha target genes. PPAR Research 2010, 612089 (2010).
85. European Food Safety Authority. Scientific opinion on the safety of astaxanthin-rich ingredients (AstaREAL A1010 and AstaREAL L10) as novel food ingredients. EFSA Journal 12, 1 – 35 (2014).
86. EFSA. Scientific opinion. Statement on the safety of β-carotene use in heavy smokers. EFSA Journal 10, 2953 (2012).
87. Expert Group on Vitamins and Minerals. Safe Upper Levels for Vitamins and Minerals. Food Standards Agency, 2003.
88. Muller, H. [Daily intake of carotenoids (carotenes and xanthophylls) from total diet and the carotenoid content of selected vegetables and fuit]. Z Ernahrungswiss 35, 45 – 50 (1996).
89. EFSA. Scientific Opinion on the re-evaluation of canthaxanthin (E 161 g) as a food additive. EFSA Journal 8, 1852 (2010).
90. Shao, A. & Hathcock, J.N. Risk assessment for the carotenoids lutein and lycopene. Regul. Toxicol Pharmacol 45, 289 – 298 (2006).
91. EFSA. Scientific Opinion on the re-evaluation of lutein (E 161b) as a food additive. EFSA Journal 8, 1678 (2010).
92. Huang, Y.M. et al. Effect of supplemental lutein and zeaxanthin on serum, macular pigmentation, and visual performance in patients with early age-related macular degeneration. Biomed Res Int 2015, 564738 (2015).
93. EFSA. Revised exposure assessment for lycopene as a food colour. EFSA Journal 8, 1: 1444 (2010).
94. EFSA. Scientific opinion: Statement on the safety of synthetic zeaxanthin as an ingredient in food supplements. EFSA Journal 10, 2891 (2012).

Torsten Bohn, PhD
Luxembourg Institute of Health (LIH)
Department of Population Health
Rue 1 A-B, Thomas Edison
1445 Strassen
Luxembourg

torsten.bohn@gmx.ch

