COST is supported by the EU Framework Programme Horizon 2020.

cost EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

SCIENTIFIC, N°6, 2018

NEWSLETTER

EUROCAROTEN

EUROPEAN NETWORK TO ADVANCE CAROTENOID RESEARCH AND APPLICATIONS IN AGRO-FOOD AND HEALTH

CAROTENOIDS AND CHRONIC DISEASE PREVENTION

Torsten Bohn
Luxembourg Institute of Health, Department of Public Health, Luxembourg

Bohn, T. (2018). Carotenoids and Chronic Disease Prevention. COST Action EUROCAROTEN (CA15136) Scientific Newsletter 6, 1-14.

Insights 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 could reduce the burden of cardiovascular disease, such as of ischemic heart disease, by approximately 30%[1]. The proposed positive effects have also resulted in the so called "*Five a day recommendation*", that is consuming five times a day 80-100 g fruits/vegetables, originally proposed by the World Cancer Research Fund[2]. 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 by Hamer and Chida (2007)[3], following close to 140.000 subjects for up to 13 years, it was shown that carotenoid, but not flavonoid and vitamin C intake, was significantly correlated with a decreased risk of developing type 2 diabetes. In another meta-analysis of case-control and prospective cohort studies targeting head and neck-cancers, it was concluded that β-carotene reduced the risk of pharyngeal cancer and lycopene, α-carotene and β-cryptoxanthin were all associated with reduced risk for oral and laryngeal cancer[4].

torsten.bohnl@gmx.ch

Similarly, in a meta-analysis of elderly people, including over 1100 subjects who were followed for up to 10 years, plasma β-carotene was associated with reduced overall mortality, by up to 30% in the highest quartile[5]. Of course, such observational studies cannot prove causality, and are prone to many confounding factors, such as other life-style factors of subjects and other dietary constituents with potential health benefits such as dietary fibre and polyphenols, also typically found in carotenoid-rich plant food items. Thus, placebo-controlled randomized intervention trials would be needed to establish causality.

However, such and similar findings have resulted in the proposition of a carotenoid health index[6], where total carotenoid plasma/serum levels below 1 µM were associated with a general increased risk of chronic diseases. Further epidemiological observational studies are shown in **Table 1**. Given that carotenoids could even contribute slightly to reduce chronic disease risk, the high prevalence of cardiovascular disease, cancer and age-related macular degeneration would mean that these mostly 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.

CAROTENOIDS AND CHRONIC DISEASE PREVENTION

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

Carotenoids investigated	Study design	Subjects	Outcome / health effect	Findings	Ref
Total plasma β-carotene	Meta-analysis of prospective cohort studies, up to 10 y follow-up	5 studies, total of 1168 elderly men & women	All-cause mortality	Reduced mortality by 30% with highest β-carotene status	5
Total plasma carotenoids	Review of 62 studies of plasma carotenoids & health outcomes, mostly prospective cohort studies or population-based 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 140000 participants	Type 2 diabetes	Reduced risk of developing type 2 diabetes by 23% with highest carotenoid intake	3
Lycopene dietary intake and plasma levels	Meta-analysis of case control and prospective cohort studies	26 studies were included with total of 17517 cases of prostate cancer reported from 563,299 participants.	Prostate cancer	Borderline sign. Effect of higher lycopene intake and reduced prostate cancer	72
Carotenoid intake and head and neck cancers	Meta-analyses of prospective cohort study (1) and case control studies (15)	16 studies: total of 5482 cases & 14130 controls. prospective cohort study: 34691 postmenopausal women	Various head and neck cancers	β-Carotene reduced the risk of pharyngeal cancer. Lycopene, α-carotene and β-cryptoxanthin were all associated with reduced risk for oral & laryngeal cancer	4
Lutein and zeaxanthin intake	Meta-analysis of prospective cohort studies	6 studies with total of 4416 cases & 41999 participants	Age related cataract	Highest & the lowest categories of dietary lutein & zeaxanthin intake: Statistically significant inverse association for nuclear cataract but not for cortical cataract or posterior subcapsular cataract	73
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 significant effect on overall odds ratio for pancreatic cancer	56
Various carotenoids in blood plasma	Meta-analysis of prospective cohort studies	8 studies with total of 3055 cases and 3956 matched controls	Breast cancer	Statistically significant inverse associations with breast cancer for α-carotene, β-carotene, lutein+zeaxanthin, lycopene and total carotenoids	74
Various carotenoid intakes	Meta-analysis and meta-regression of observational studies (case control and 6 cohort studies)	33 studies, total subject number not specified	Breast cancer	Dietary α- and β-carotene intake statistically significantly reduced breast cancer risk	54

Health detrimental findings in supplementation intervention trials with high carotenoid doses

In the pursuit of the positive health effects of dietary carotenoids proposed by the epidemiological studies, several (placebo controlled) intervention studies with 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]) trial, 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. Unfortunately, lung cancer risk rather increased rather than decreased, and studies had to be terminated, for reasons that were never entirely been revealed (see following chapter and **Figure 1**).

CAROTENOIDS AND CHRONIC DISEASE PREVENTION

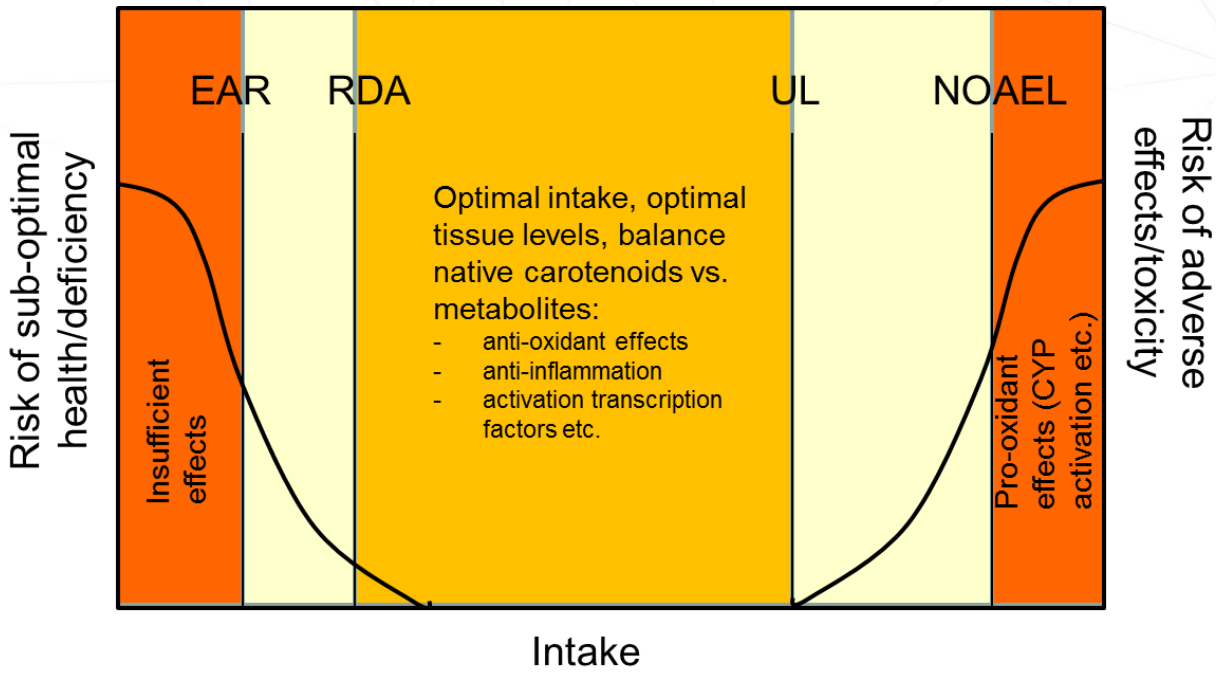

Figure 1. General dose-response relation of nutrients and health related outcomes, adapted for carotenoids. CYP: Cytochromes P450; EAR: Estimated average requirement; NOAEL: No observed adverse effect level, RDA: Recommended dietary allowance; UL: Upper tolerable intake.

Similarly, in a systematic review and meta-analyses by Bjelakovic and co-workers (2008)[10], intervention trials with β-carotene supplements, alone or in combination with other antioxidants, resulted in an increased total mortality by on average of 7 % with β-carotene intake. One lesson learnt from these studies is surely that a higher intake of β-carotene does not always help more: As for other nutrients (**Figure 2**), there is no general linear dose-response relationship, but a recommended level of intake, with higher intakes resulting in increased risk for producing adverse effects. It is also plausible that isolated carotenoids from supplements act differently than carotenoids embedded in a complex plant food matrix, where other synergistic nutrients such as vitamin E are present[11, 12]. Finally, also bioavailability and kinetics of such supplements can be quite altered compared to carotenoids in their native matrix[13, 14]. Thus, prior to giving supplements to specific population groups, it is paramount to obtain a more clear picture of how carotenoids are implicated in health promoting effects, and which dose-response relationship exists for such effects.

Mechanistic aspects – carotenoids as direct antioxidants

The controversy between observational studies and intervention trials has further fostered the elucidation of carotenoid bioactive properties. 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[15], can react with singlet oxygen[16], and also capture photons of short wavelengths (UVA and UVB but also blue light) which otherwise could harm the skin[17] or the human eye[18]. Not too amazingly, it was earlier thought that these rather direct antioxidant properties would be the main mechanisms via which carotenoids act on the human body and promote health. And indeed, it is highly plausible that carotenoids contribute to the stability of cell membranes, protecting lipids from oxidation with potential reactive oxygen species (**Figure 3**)[19]. Specifically, β-carotene appears to act complementary to nitric oxide and vitamin E in protecting

CAROTENOIDS AND CHRONIC DISEASE PREVENTION

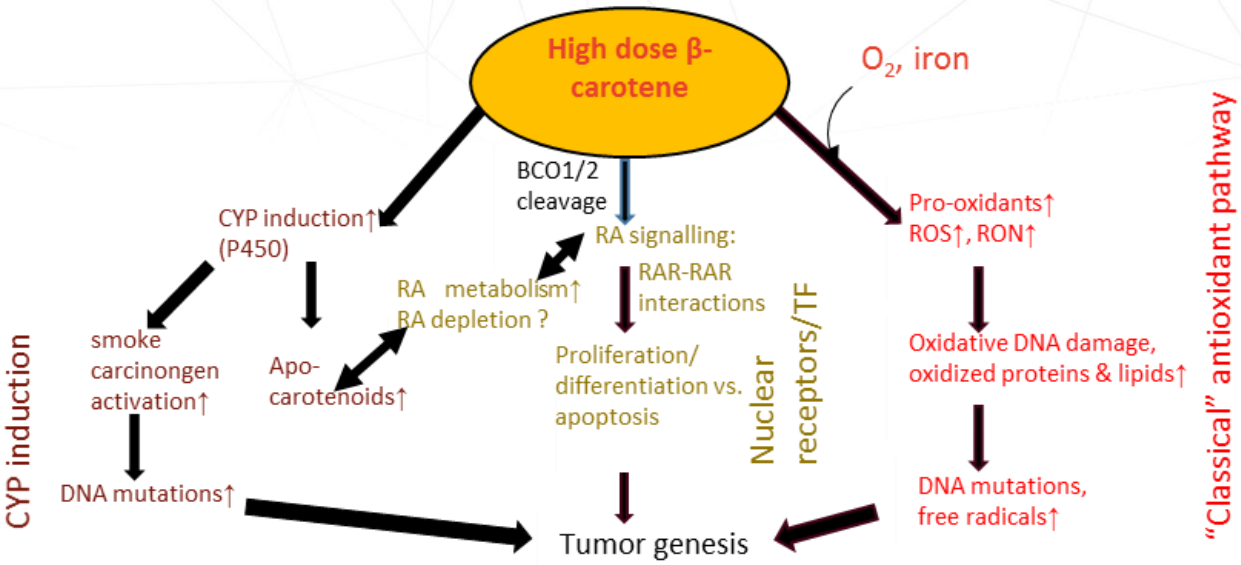

Figure 2. Hypothetical adverse relation of smoking, β-carotene supplements, and risk of cancer based on the hypothesis by Goralczyk[27]. BCO1/2: β-carotene oxygenase 1/2. CYP: cytochrome P450. RA: Retinoic acid. TF: Transcription factors.

cell membranes, especially from damage induced by singlet oxygen[20]. Furthermore, higher concentrations of carotenoids in the human skin have been shown to reduce erythema induced by UV-light. It should be noted that photo-protection took around 7-10 weeks to be effective (possibly due to the time it took to reach the outer skin areas when orally ingested), and was only recommended as an adjuvant[17].

However, the direct antioxidant hypothesis has been questioned[21, 22]. One primary reason is that the anti-oxidant balance of the human body is controlled by numerous endogenous and exogenous factors, with carotenoids possibly only playing a minor role. For instance, endogenous antioxidants such as glutathione, uric acid, albumin, endogenous enzymes such as catalase and superoxide dismutase, as well as exogenous antioxidants such as vitamin E and C appear to play a more pronounced role, also considering their much higher molar concentrations in biological tissues, e.g. 200 µM for uric acid and 20 µM for vitamin E[23]. However, it can be safely assumed that a certain concentration of carotenoids is beneficial and aids in preventing oxidative damage such as in cell membranes[24].

Which carotenoid has the strongest antioxidant potential in this respect? According to some studies, this appears to be lycopene, possibly due to its elongated conjugated double bond system[24, 25]. As lycopene is, together with β-carotene, also the most abundant carotenoid in blood and tissues, it may indeed 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 *in vitro* studies that higher concentrations of carotenoids (4-10 µM) may rather have pro-oxidant effects, resulting in DNA damage[26]. 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 may negatively interact with the already damaged lungs of smokers (**Figure 1**)[27]. Another reason is that at under high oxygen partial pressure – as perhaps more likely in lung tissue[12], and possibly the presence of pro-oxidant metal oxidation states such as Fe (III)[23], carotenoids can react to pro-oxidants, fostering e.g. tumor progression[28].

CAROTENOIDS AND CHRONIC DISEASE PREVENTION

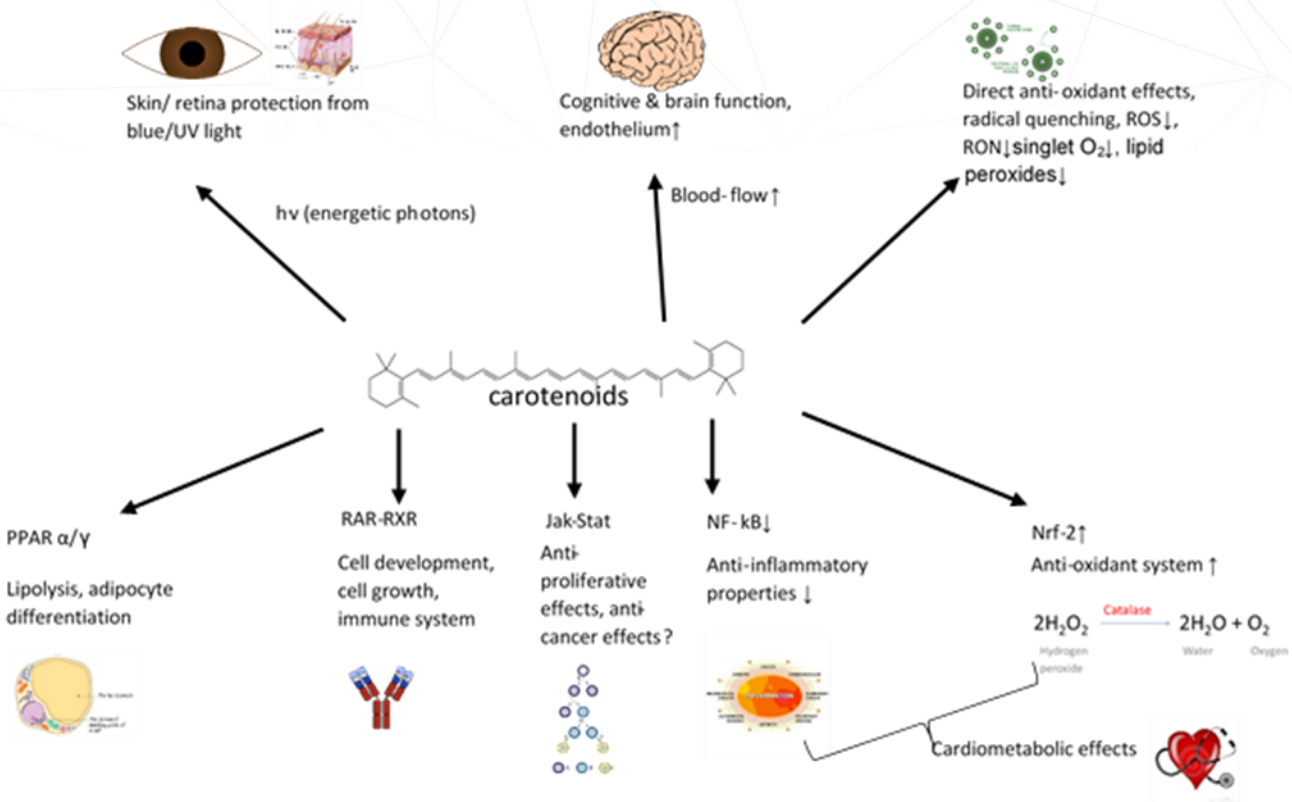

Figure 3. Pathways involved in potential carotenoid health benefits.

Mechanistic aspects – carotenoids and metabolites as inducers of cellular signalling cascades

An increasing body of evidence suggests that rather the indirect effects of carotenoids, especially altering gene expression, may be the primary health promoting routes of carotenoids. Again, it is important to note that 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*. With regard to influencing transcription factors, including the nuclear receptors RXR/RAR (retinoid X receptor; retinoic acid receptor), it is noteworthy that the more polar apocarotenoids are likely better targets for the interaction with these cellular junctions, as they a) have a higher cytosolic solubility due to their lower logP values and b) are more electrophilic and my easier bind to cysteine residues of NF-κB[29] and Nrf-2[30], causing inactivation and activation of these transcription factors, respectively. As the amount of metabolites formed appears to depend preliminary on bioavailability of the native carotenoids, in conjunction with the activity of BCO1 and 2, and single nuclear polymorphisms (SNPs) in these enzymes have shown to largely influence plasma appearance of the native carotenoids[31, 32], it can be hypothesized that the biological responses and health benefits also vary considerably between people[33]. In addition, there appear to be tissue differences in the expression of BCO1 and 2 (with e.g. BCO1 being more prominent in the liver than the brain of mice[34] and BCO2 absent in some human tissues such as the colon and the skin epidermis[35]), and also the subcellular location of BCO1 and BCO2 differs, with BCO1 being present in the cytosol, while BCO2 is present in the mitochondria. These aspects could further influence potential health benefits, resulting in more specific tissue and compartmental effects of carotenoid metabolites.

CAROTENOIDS AND CHRONIC DISEASE PREVENTION

Which cellular pathways are now influenced by carotenoids and their metabolites? An overview is given in **Figure 3**. A large number of cellular signal transduction pathways have been reported to be influenced by carotenoids and their metabolites:

1. NF-κB: This often also termed master switch of inflammation is activated in the cytosol by the dissociation of its inhibitor (IKBα), following phosphorylation by a kinase, the IkB kinase (IKK). Carotenoids 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[22]. As a result, this transcription factor 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.

2. Nrf-2. This transcription factor is likewise present in the cytosol of cells and bound to an inhibitor, Keap-1 (Kelch-like ECH associated protein 1). However, carotenoids and metabolites may bind and result in the dissociation of Keap1, resulting in liberation and translocation of the TF to the nucleus[22]. Upon binding to the antioxidant response element, genes related to the body's own antioxidant system, such as superoxide-dismutase (SOD), catalase (CAT) and heme-oxygenase 1 (HO-1), are activated.

3. Jak-Stat. Though there is less data present, a few studies have reported that certain carotenoids can also act via suppression of the Jak-Stat pathway, resulting in reduced cell proliferation, angiogenesis and invasiveness, and thus may act against the formation of cancer[36, 37].

4. RXR/RAR activation: This nuclear receptor dimer responds especially to retinoic acid[38]. However, it has been proposed that also other apocarotenoids, such as those resulting from lycopene cleavage by BCO2, may activate this nuclear receptor[39, 40]. This nuclear receptor is responsible for activating a number of immune related target genes, as well as being implicated in cell differentiation/growth control and apoptosis (**Table 2**),

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

TF factor or nuclear receptor	Downstream target protein	Major functions	Ref
NF-κB	IL-2, IL-6, IL-8, TNF-α, NO, IFN-γ, IgG, MHC, ICAM-1, VCAM-1, hepcidin, COX-2,	Pro-inflammatory	75
Nrf-2	SOD, CAT, HO-1, GPX, GST, NQO1	Anti-oxidant	76
RAR-RXR	Wnt1, Gas2, Cidea, Wnt10b	Immune system, cell growth and proliferation, apoptosis	77
PPARs	FABP1, ELOVL6, MOD1	Adipocyte differentiation, lipid metabolism	78

CAT: catalase, Cidea: cell death-inducing DFFA-like effector a; ELOVL6: elongation of very long chain fatty acids protein 6; FABP1: fatty acid binding protein 1; GPX: glutathione peroxidase, COX-2: cyclo-oxygenase 2; Gas2: growth arrest specific 2; GST: glutathione S-transferase; HO-1: heme-oxygenase; IgG: immunoglobin G, heavy chain; ICAM-1: interellular adhesion molecule 1; IL: interleukin; IFN-γ: interferon-gamma; 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; TNF-α: tumor necrosis factor alpha; VCAM-1: vascular cell adhesion molecule; Wnt1: Wnt family member 1; Wnt10b: Wnt family member 10B.

though a totality of over 500 genes have been proposed to be related to this nuclear receptor. Other nuclear receptor dimers such as RXR/PPARα may also be responsive to carotenoids and their metabolites, which may influence the development of adipocytes and thus obesity[41].

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 several cardiometabolic diseases. These include for example type 2 diabetes[3], [42], the metabolic syndrome[43] and also stroke[44].
As stated above, the problem with these observational studies – typically prospective cohort studies – is that cause and effect relation cannot be clearly established, and that potential confounding factors – despite multivariate models trying to control these – could still interfere. In this respect, 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 beneficial health effects[45], as many of these alternative constituents could also modify inflammation and oxidative stress pathways and thus interact with cardiometabolic diseases.

However, there are many *in vitro* and also animal studies, and recently also a number of growing randomized human dietary intervention trials with isolated carotenoids (in form of supplements), which indicate that indeed carotenoids at least contribute to some potential health effects as encountered in the observational studies. 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[46]. In another study, lutein (20 mg/day for 3 months) improved

Table 3. Recommendations for dietary and supplemental total carotenoid intake.

Carotenoid	Type of recommendation - Authority, body or country	Recommendation (mg/d)	Ref
Astaxanthin	ADI-EFSA	0.034 (mg/kg bw/d)	79
β-Carotene	Safe intake for smokers-EFSA	15	80
	UL-UK	7	81
	RDI-DGE	2	82
Cantaxanthin	ADI-EFSA	0.03 (mg/kg bw/d)	83
Lutein	OSL-EFSA	15.	84
	ADI-EFSA	1.0 (mg/kg bw/d)	85
	Dietary intake (for eye health)*	10	86
Lycopene	OSL-EFSA	75	84
	ADI-EFSA	0.5 (mg/kg bw/d)	87
Zeaxanthin	Safe intake-EFSA	53	88

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, Huang *et al*. (2015)[86].

CAROTENOIDS AND CHRONIC DISEASE PREVENTION

IL-6 and plasma monocyte chemoattractant protein 19 (MCP-19) in arthrosis patients[47]. Effects regarding oxidative stress and inflammation were also seen in new-borns. In 2 studies with term infants receiving 2 doses of 0.28 mg of lutein, total hydroperoxides significantly decreased in plasma, while FRAP (ferric reducing antioxidant power assay), an assay for detecting antioxidant capacity, did increase[48, 49]. Similarly, in pre-term infants, the 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[50].

It is difficult to pinpoint the most relevant mechanisms underlying the potential beneficial effects with regard to cardiovascular diseases. However, 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 nitric oxide endothelial bioavailability, may also play a role as reviewed recently[51, 52]. 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 cardiovascular diseases.

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[53]. Thus, there may be somewhat less leverage of carotenoids on cancer risk reduction compared to cardiovascular diseases.

Nevertheless, as cancer is, together with cardiovascular diseases, 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 implication of carotenoids in inflammatory, anti-oxidant, 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[54], and possibly gastric cancer[55] as well as prostate cancer, at least for lycopene[56]. However, there are also several 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 on lung cancer mentioned above, 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 selenium (30000 adults, 5 years) resulted in reduced cancer risk (especially of the stomach)[57]. However, it was critizied that subjects generally showed a poor micronutrient status and may have been deficient at baseline. In a meta-analysis summarizing randomized controlled intervention trials with β-carotene given for up to 12 years (typically 20-30 mg/day), no significant reduction effect was found on all cancers combined. However, lung and stomach cancer risk increased significantly – though β-carotene was given partly in combination with either vitamin A, vitamin E, vitamin C, selenium, and/or zinc[58]. Also, it is possible that effects may be gender dependent. In the French SU.VI.MAX study with over 13000 participants, a combination of antioxidants (120 mg vitamin C, 30 mg vitamin E, 6 mg β-carotene, 100 µg selenium, and 20 mg zinc taken on average for 7.5 years) reduced the cancer incidence in men, but not women – perhaps as men had a lower basal antioxidant intake than women. However, no conclusions for carotenoids individually could be drawn from this study[59].

Only very few short-mid-term intervention studies exist including biomarkers that may be relevant to cancer. Some investigated the effect of lycopene supplementation on prostate specific antigen (PSA) in prostate cancer subjects as reviewed by Van Patten (2008)[60], and found partially positive health effects regarding the PSA development over time when receiving 15 mg lycopene/day, though again in combination with other antioxidants[61]. This is in line with earlier observational studies suggesting an association with lycopene and α-carotene intake and lower PSA levels[62].

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, at

least when given at high doses over long time to smokers, may increase the risk of some types of cancer such as of the lung and stomach.

Carotenoids, eye health and brain

The dietary intake and plasma levels of lutein and zeaxanthin have been related to the prevention and amelioration of age related macular degeneration (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 carotenoids, especially lutein, zeaxanthin, and meso-zeaxanthin, the latter formed from lutein in the human body[63]. Due to their photoprotective properties, these macula pigments protect the eye from oxidative damage. Possibly filtering energetic blue light, in addition to direct antioxidant properties of carotenoids, do play major roles. In subjects with more poorly visual acuity as present with AMD, these pigments have often been depleted to some extent. Reciprocally, regular dietary intake of lutein and zeaxanthin, including supplements, have shown to slow the progression of AMD toward its late forms and to improve visual acuity in subjects at risk for developing AMD[64]. Due to these findings, supplementation strategies such as for lutein (10 mg/d) and zeaxanthin (2 mg/day) have been proposed for the general population[65]. Likewise, due to the apparent benefits of lutein and zeaxanthin in the elderly for AMD amelioration, these carotenoids have discussed as being of conditional essential character[66]. As at least dietary derived lutein and zeaxanthin are deposited in the retina, they also must pass the blood-brain barrier. Indeed, high concentrations of lutein have been detected in brain tissues, making lutein the most abundant carotenoid in this organ (around 170 pmol/g), as reviewed by Erdmann[67]. It has even 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[68], though similar associations were reported earlier for β-carotene[69]. However, the possible mechanism for this is uncertain. Similar as for polyphenols, carotenoids may add to improved endothelial stability and flexibility, improving blood-flow[70]. Other mechanisms include antioxidant mechanism in synergy with vitamin E, enhancing gap junction communication, modulation of synaptic membranes, and the influence on gene expression influencing inflammation and oxidative stress, as reviewed by Johnson *et al*.[71].

Intake recommendations

As of to date, no dietary reference intakes (DRI) exist for carotenoids. Several countries and authorities have issued recommendations for individual carotenoids, either based on dietary intake, supplemental intake, or, more typically, both combined (**Table 2**). Both recommended intakes but also levels comparable to the upper tolerable intake level (UL) have been recommend, especially following the negative health effects found in the ATBC and CARET trial. When comparing these recommendations, higher levels of β-carotene above 15-20 mg/day are rather discouraged, as these may result in elevated (>3 µM) circulating blood levels which have been shown to cause adverse effects in some population groups such as smokers. On the other hand, a certain intake, i.e. in the range of 2-7 mg/day of β-carotene has been recommended, also in sight of the >1 µM total carotenoid blood concentrations proposed by Donaldson[6]. This level may have to be increased when no preformed vitamin A is ingested – in this case, up to 6 mg additional β-carotene are recommended by some health authorities such as the German Nutrition Society (http://www.dge.de/wissenschaft/referenzwerte/vitamin-a-b-carotin/). For other carotenoids, safe intake recommendations are higher, e.g. up to 75 mg / day for lycopene.

Finally, intake recommendations are impeded by the following factors:

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 of carotenoids[33]
3) Different carotenoids may have different biological properties
4) Different populations may have different needs – e.g. targeting subjects with AMD and lutein recommendations vs. smokers where recommendations may require more prudence.

CAROTENOIDS AND CHRONIC DISEASE PREVENTION

Future perspectives of carotenoids in chronic disease prevention

Despite some negative health outcomes encountered for high-dosed β-carotene supplements, carotenoids appear promising microconstituents in our diet, with a variety of 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 and gene expression, resulting in anti-inflammatory and anti-oxidant properties and regulation of cellular differentiation and growth, which may also suggest anti-cancerogenous properties, though data from human trials remains contradictory. In general, attributing observed health effects to carotenoids has been a difficult task, especially in observational studies, due to numerous confounding factors such as the presence of other phytochemicals or interfering life-style aspects.

Gaps of our knowledge however surely exist and include especially the differentiation of health effects due to the native compounds vs. their apocarotenoid metabolites, which may in part be more potent regarding their influence on nuclear receptors, transcription factors, and gene expression. Several cartotenoids which are also frequently consumed are in addition somewhat understudied, such as the colourless carotenoids phytoene/phytofluene or the epoxycarotenoids violaxanthin/neoxanthin, in addition to plant derived apocarotenoids such as crocetin and bixin.

Thus, 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 eventually 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. Glade, M.J. Food, nutrition, and the prevention of cancer: a global perspective. American Institute for Cancer Research/World Cancer Research Fund, American Institute for Cancer Research, 1997. Nutrition 15, 523-526 (1999).

3.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).

4.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).

5.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).

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 14, CD007176 (2008).

11.El-Agamey, A. et al. Carotenoid radical chemistry and antioxidant/pro-oxidant properties. Arch Biochem Biophys 430, 37-48 (2004).

12.Young, A.J. & Lowe, G.M. Antioxidant and prooxidant properties of carotenoids. Arch Biochem Biophys 385, 20-27 (2001).

CAROTENOIDS AND CHRONIC DISEASE PREVENTION

13.Corte-Real, J. Carotenoids in the human body: bioavailability and bioconversion. EUROCAROTEN Newsletter, 1 (2017).

14.Bohn, T. Bioactivity of carotenoids – chasms of knowledge. Int J Vitam Nutr Res 10, 1-5 (2016).

15.Krinsky, N.I. & Yeum, K.J. Carotenoid-radical interactions. Biochem Biophys Res Commun 305, 754-760 (2003).

16.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).

17.Stahl, W. & Sies, H. beta-Carotene and other carotenoids in protection from sunlight. Am J Clin Nutr 96, 1179s-1184s (2012).

18.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, 120 (2017).

19.Gruszecki, W.I. & Strzalka, K. Carotenoids as modulators of lipid membrane physical properties. Biochim Biophys Acta 1740, 108-115 (2005).

20.Schafer, F.Q. et al. Comparing beta-carotene, vitamin E and nitric oxide as membrane antioxidants. Biol Chem 383, 671-681 (2002).

21.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).

22.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).

23.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).

24.Fiedor, J. & Burda, K. Potential role of carotenoids as antioxidants in human health and disease. Nutrients 6, 466-488 (2014).

25.Shi, J., Kakuda, Y. & Yeung, D. Antioxidative properties of lycopene and other carotenoids from tomatoes: synergistic effects. Biofactors 21, 203-210 (2004).

26.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).

27.Goralczyk, R. Beta-carotene and lung cancer in smokers: review of hypotheses and status of research. Nutr Cancer 61, 767-774 (2009).

28.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).

29.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).

30.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).

31.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).

32.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).

33.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).

34.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).

CAROTENOIDS AND CHRONIC DISEASE PREVENTION

35.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).

36.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).

37.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).

38.Huang, P., Chandra, V. & Rastinejad, F. Retinoic acid actions through mammalian nuclear receptors. Chem Rev 114, 233-254 (2014).

39.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).

40.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).

41.Bonet, M.L., Canas, J.A., Ribot, J. & Palou, A. Carotenoids in adipose tissue biology and obesity. Subcell Biochem 79, 377-414 (2016).

42.Sluijs, I. et al. Dietary intake of carotenoids and risk of type 2 diabetes. Nutr Metab Cardiovasc Dis 25, 376-381 (2015).

43.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).

44.Li, X. & Xu, J. Dietary and circulating lycopene and stroke risk: a meta-analysis of prospective studies. Sci Rep 4, 5031 (2014).

45.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).

46.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).

47.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).

48.Perrone, S. et al. Effects of lutein on oxidative stress in the term newborn: a pilot study. Neonatology 97, 36-40 (2010).

49.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).

50.Rubin, L.P. et al. Effect of carotenoid supplementation on plasma carotenoids, inflammation and visual development in preterm infants. J Perinatol 32, 418-424 (2011).

51.Di Pietro, N., Di Tomo, P. & Pandolfi, A. Carotenoids in cardiovascular disease prevention. JSM Atherosclerosis 1 (2016).

52.G, R., MA, G. & N, D.O. Carotenoids and cardiovascular prevention: an update. J Nutr Food Sci 6 (2016).

53.Leenders, M. et al. Fruit and vegetable intake and cause-specific mortality in the EPIC study. Eur J Epidemiol 29, 639-652 (2014).

54.Hu, F. et al. Carotenoids and breast cancer risk: a meta-analysis and meta-regression. Breast Cancer Res Treat 131, 239-253 (2012).

55.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).

56.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).

57.Blot, W.J. et al. The Linxian trials: mortality rates by vitamin-mineral intervention group. Am J Clin Nutr 62, 1424s-1426s (1995).

CAROTENOIDS AND CHRONIC DISEASE PREVENTION

58.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).

59.Hercberg, S. et al. "The SU.VI.MAX Study": a primary prevention trial using nutritional doses of antioxidant vitamins and minerals in cardiovascular diseases and cancers. SUpplementation on VItamines et Mineraux AntioXydants. Food Chem Toxicol 37, 925-930 (1999).

60.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).

61.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).

62.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).

63.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).

64.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).

65.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).

66.Semba, R.D. & Dagnelie, G. Are lutein and zeaxanthin conditionally essential nutrients for eye health? Med Hypotheses 61, 465-472 (2003).

67.Erdman, J.W., Jr. et al. Lutein and brain function. Foods (Basel, Switzerland) 4, 547-564 (2015).

68.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. J Aging Res 951786 (2013).

69.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).

70.Yamagata, K. Carotenoids regulate endothelial functions and reduce the risk of cardiovascular disease In: Cvetkovic, D. (ed). Carotenoids. InTech: Rijeka, 2017.

71.Johnson, E.J. A possible role for lutein and zeaxanthin in cognitive function in the elderly. Am J Clin Nutr 96, 1161S-1165S (2012).

72.Chen, P. et al. Lycopene and risk of prostate cancer: A systematic review and meta-analysis. Medicine (Baltimore) 94, e1260 (2015).

73.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).

74.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).

75.Pahl, H.L. Activators and target genes of Rel/NF-kappaB transcription factors. Oncogene 18, 6853-6866 (1999).

76.Gorrini, C., Harris, I.S. & Mak, T.W. Modulation of oxidative stress as an anticancer strategy. Nat Rev Drug Discov 12, 931-947 (2013).

77.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).

78.Rakhshandehroo, M., Knoch, B., Müller, M. & Kersten, S. Peroxisome proliferator-activated receptor alpha target genes. PPAR Research 2010, 612089 (2010).

CAROTENOIDS AND CHRONIC DISEASE PREVENTION

79.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).

80.EFSA. Scientific opinion. Statement on the safety of β-carotene use in heavy smokers. EFSA Journal 10, 2953 (2012).

81.Expert Group on Vitamins and Minerals. Safe Upper Levels for Vitamins and Minerals. Food Standards Agency, 2003.

82.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).

83.EFSA. Scientific Opinion on the re-evaluation of canthaxanthin (E 161 g) as a food additive. EFSA Journal 8, 1852 (2010).

84.Shao, A. & Hathcock, J.N. Risk assessment for the carotenoids lutein and lycopene. Regul. Toxicol Pharmacol 45, 289-298 (2006).

85.EFSA. Scientific Opinion on the re-evaluation of lutein (E 161b) as a food additive. EFSA Journal 8, 1678 (2010).

86.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).

87.EFSA. Revised exposure assessment for lycopene as a food colour. EFSA Journal 8, 1: 1444 (2010).

88.EFSA. Scientific opinion: Statement on the safety of synthetic zeaxanthin as an ingredient in food supplements. EFSA Journal 10, 2891 (2012).

When referring to this article, please use:

Bohn, T. (2018). Carotenoids and Chronic Disease Prevention. COST Action EUROCAROTEN (CA15136) Scientific Newsletter 6, 1-14.

***COST** (European Cooperation in Science and Technology) is a pan-European intergovernmental framework. Its mission is to enable break-through scientific and technological developments leading to new concepts and products and thereby contribute to strengthening Europe's research and innovation capacities. www.cost.eu*