From carotenoid intake to carotenoid blood and tissue concentrations – implications for dietary intake recommendations

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Lead Article

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This review article is dedicated in memoriam to Catherine Caris-Veyrat (+29.2.2019), a great carotenoid researcher, our EUROCAROTENE / LYCOCARD partner, a phantastic person and a good friend.

There is uncertainty regarding carotenoid intake recommendations, because positive and negative health effects have been found or are correlated with carotenoid intake and tissue levels (including blood, adipose tissue, and the macula), depending on the type of study (epidemiological vs intervention), the dose (physiological vs supraphysiological) and the matrix (foods vs supplements, isolated or used in combination). All these factors, combined with interindividual response variations (eg, depending on age, sex, disease state, genetic makeup), make the relationship between carotenoid intake and their blood/tissue concentrations often unclear and highly variable. Although blood total carotenoid concentrations <1000 nmol/L have been related to increased chronic disease risk, no dietary reference intakes (DRIs) exist. Although high total plasma/serum carotenoid concentrations of up to 7500 nmol/L are achievable after supplementation, a plateauing effect for higher doses and prolonged intake is apparent. In this review and position paper, the current knowledge on carotenoids in serum/plasma and tissues and their relationship to dietary intake and health status is summarized with the aim of proposing suggestions for a "normal," safe, and desirable range of concentrations that presumably are beneficial for health. Existing recommendations are likewise evaluated and practical dietary suggestions are included.

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INTRODUCTION

Carotenoids are a group of > 1100 tri-, tetra-, and pentaterpenoid lipophilic pigments (but mostly tetraterpenoid) produced by plants and many bacteria and fungi, but not by humans, who rely on dietary intake as the exclusive source.[1] Green leafy vegetables are a major source of these compounds in the human diet, but they are also present in other foods, such as kiwis, maize, peppers, eggs, dairy products, oils, and some types of fish.[2,3] Intake of carotenoids through diet, as well as their concentrations in various body compartments, especially in plasma/serum, has been correlated with the reduced incidence of several chronic diseases, including type 2 diabetes[4]; cardiovascular diseases,[5] including stroke[6]; and several types of cancer, such as those of the upper intestinal tract.[7,8] Moreover, meta-analyses of prospective cohort studies[9,10] have found reduced all-cause mortality among participants with increasing circulating β-carotene concentrations.

The National Health and Nutrition Examination Survey III confirmed that carotenoid concentrations in serum correlate with mortality, though relationships differed by carotenoid type.[11] Although the all-cause mortality was reduced until serum levels were ∼1000 nmol/L, little impact of higher concentrations was found. Lower concentrations of serum lycopene most strongly predicted all-cause mortality, followed by total carotenoid concentration.[11] Donaldson,[12] on the basis of 62 non-intervention studies, proposed a carotenoid health index for total plasma carotenoids, with <1000 nmol/L reflecting a high risk for developing chronic diseases, including cardiometabolic diseases and cancer, whereas participants with plasma concentrations ≥ 2500 nmol/L appeared to be generally "protected." Of note, it is possible that the observed health benefits may be due to other bioactive compounds, or overall dietary pattern, because blood carotenoid levels are useful biomarkers of fruit and vegetable intake[13] and carotenoid levels reflect, indirectly, a generally healthier lifestyle.

Carotenoid-related health benefits previously were attributed mainly to their antioxidant properties, such as radical quenching.[14,15] More recently, the relevance of this effect has been questioned at typical endogenous (ie, non-supplemented), physiological, and nutritionally relevant concentrations.[16] Alternative antioxidant and anti-inflammatory effects mediated via carotenoids and their metabolites, acting at the gene-expression level, have recently been proposed and summarized,[17] though their physiological and nutritional relevance has not been conclusively proven and additional confirmation in humans is required. In addition, some carotenoids can also act as vitamin A precursors (eg, α- and β-carotene, β-cryptoxanthin) because they are cleaved in the intestinal mucosa, as well as after uptake in various other human tissues,[18–20] by β-carotene oxygenase 1 and β-carotene oxygenase 2 into retinal and other apocarotenals, respectively.[21] Other carotenoids, especially lutein and zeaxanthin, appear to be important in protecting the macula of the retina, aiding in the prevention of age-related macular degeneration (AMD),[22] the major cause of vision loss in the elderly.

These previously reported positive health effects have inspired intervention trials. Supplementation trials with smokers were launched because of the postulation that the effects of smoking are mediated via antioxidant effects of carotenoids. The aim of the trials was to ameliorate or inhibit proradical effects induced by smoking. Two large, randomized intervention trials investigated the effect of β-carotene supplementation on lung cancer: The Alpha Tocopherol, Beta Carotene Prevention trial (ATBC) included 29,000 participants who received 20 mg of β-carotene daily for 5–8 years,[23] and the β-Carotene and Retinol Efficacy Trial (CARET),[24] which included >18,000 participants who received 30 mg of β-carotene daily for 4 years. β-Carotene was given as water-soluble beadlets, resulting in much higher blood concentrations of β-carotene (up to ∼10 times higher) compared with typical dietary intake. The average β-carotene plasma concentrations after supplementation were ∼3800 and ∼5600 nmol/L for the CARET and ATBC trials, respectively, compared with, for example, the 95th percentile concentration of 90–900 nmol/L found in the US population.[25] Both studies showed an increased lung cancer rate in the β-carotene groups (16% and 28% in the ATBC trial and the CARET study, respectively). Also, overall mortality was significantly increased in both intervention groups. The interaction of smoking and high β-carotene supplementation was later shown in animal models to increase CYP activation and nuclear hormone receptor–mediated signaling via β-carotene metabolites (ie, these interactions via retinoid-mediated effects were demonstrated in follow-up studies using ferret and mice models). These studies focused on the lung, where, after high β-carotene supplementation, reduced

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local retinoic acid concentrations and expression levels of retinoic acid receptor target genes were described, resulting in a more vulnerable local tissue status with respect to smoking.[26,27] On the other hand, in the Physician's Health Study[28] and the Heart Protection Study,[29] in which participants received 50 mg of β-carotene every other day for 13 years and 20 mg/day for 5 years, respectively, resulting in 2200 and 1220 nmol/L serum concentrations, respectively, of β-carotene, supplements did not increase lung cancer risk. Furthermore, the Chinese Linxian intervention trial[30] ($n = 30{,}000$ participants, mostly nonsmokers) reported preventive effects of a combined supplement of 15 mg β-carotene, 30 mg α-tocopherol, and 50 µg selenium (resulting in 1000 nmol/L mean plasma carotenoid concentration) for stomach cancer and total mortality. Perhaps participants were originally marginally deficient in some of these micronutrients. Also, blood concentrations of β-carotene may serve as a better health marker than intake alone.

In a systematic review and meta-analysis,[31] it was emphasized that supplementation with β-carotene (alone or in combination with other antioxidants) was associated with increased total mortality and other adverse effects in a mixed population. However, results were strongly biased by the ATBC and the CARET trials, whereas other trials did not suggest negative effects. Thus, the negative findings are possibly only relevant for high doses of carotenoids in smokers and perhaps asbestos workers, as included in CARET. Nevertheless, the results of these studies added to the controversy regarding carotenoid health and safety, emphasizing that dosing, form of preparation, combination with other nutrients, duration of intervention, and subject characteristics (nutritional status, physiological vs pathological condition) are important factors.[32] Although it was emphasized that typical dietary carotenoid intakes (ie, via fruits and vegetables) appear to show beneficial health effects, supplemental intake of >20 mg/day may result in adverse effects, including increased cancer and total mortality risks, especially in smokers.

Because of this continuing controversy, no recommendations regarding carotenoid intake or desired blood or tissue concentrations have been issued by the majority of health authorities; no dietary reference intake recommendations (DRIs) exist, to our knowledge. However, agencies in the United Kingdom have proposed safe upper intake levels for supplemental carotenoid intake (7 mg/day for β-carotene[33]), whereas the German Nutrition Society, in response to the CARET and ABTC trials, recommended 2 mg/day total dietary carotenoid intake.[34] Median intakes in European countries currently are ~2.7 mg/day and 2.9 mg/day for men and women, respectively.[35] Despite contradictory scientific results, supplemental "antioxidants" are widely used by the general population in many countries. Data from the United States indicate that 70% of surveyed adults (all age groups) used dietary supplements, mostly multivitamins and minerals, and thus not only antioxidants.[36] For Europe, values range between 4% and 58%,[37] with higher adoption in northern countries.

For individual carotenoids, there is also dispute. The importance of lutein and zeaxanthin for eye health has been acknowledged for people at risk for AMD,[38] and intakes of 10 mg/day have been advocated,[39] equivalent to ~100 g spinach/day.[40] Recommendations are impeded, however, because carotenoid bioavailability (ie, the fraction of an ingested carotenoid that can be absorbed and used for physiological function and/or stored) depends on several factors, adding to their interindividual variability after intake.[21] These include dietary and host-related factors. For example, although a diet rich in lipids can aid in solubilizing carotenoid levels, dietary fiber may reduce their bioavailability.[41,42] Furthermore, an individual's genetic makeup, through single nucleotide polymorphisms in genes coding for digestion enzymes (eg, lipase), intestinal transporters (eg, SR-BI, CD36, NPC1L1) and cleavage enzymes (ie, β-carotene oxygenase 1) influence carotenoid bioavailability.[43–46] In this review, we highlight the relationship between carotenoid intake and carotenoid blood plasma/serum and tissue levels and emphasize how the present knowledge could be used to develop dietary intake recommendations for carotenoids.

EXISTING DIETARY RECOMMENDATIONS FOR CAROTENOID INTAKE

Recommendations regarding provitamin A carotenoids

When investigating published recommendations for carotenoid intake, provitamin A carotenoids and non–provitamin A carotenoids must be discussed separately. Because vitamin A is an essential micronutrient with recommendations differing between countries, the provitamin A carotenoids, of which β-carotene is the most prominent, is discussed first. Two additional main dietary carotenoids, α-carotene and β-cryptoxanthin, can also be cleaved to vitamin A. In 1967, the Food and Drug Administration, in conjunction with the World Health Organization, defined that 6 µg of β-carotene would be of equivalent vitamin A activity as 1 µg of retinol. Other provitamin A carotenoids were defined as being half as active as β-carotene. These proposed conversion factors remained unchanged over decades.[47,48] In 1988, a joint Food and Agriculture Organization and World Health Organization Expert Consultation, on the basis of controlled depletion–repletion studies in adult men,

confirmed these conversion factors for mixed diets, considering that these were only best approximations that could under- or overestimate bioavailability, depending on various factors, such as items consumed cooked or raw, whole or pureed, with or without dietary fat.[48,49]

In 2001, the US Institute of Medicine, on the basis of various studies[50–54] resulting in bioefficacy ratios between 1:2 and 1:28, revised the bioefficacy of β-carotene in a mixed diet from 1:6 to 1:12 and to 1:24 for other provitamin A carotenoids.[55] In 2004, Food and Agriculture Organization and World Health Organization, in view of the most recently available data, proposed revised equivalency factors of 1:14 for β-carotene and 1:28 for other provitamin A carotenoids from usual vegetable diets.[49] In contrast, other countries (eg, Germany, Italy, United Kingdom) continued using the factor 1:6.[56–58] In 2012, in the Nordic Nutrition Recommendations, the bioconversion factors proposed by the Institute of Medicine in 2001[59] were adopted. In 2015, the European Food Safety Authority published its Scientific Opinion on Dietary Reference Values for Vitamin A and considered current evidence insufficient to support a change of the conversion factors proposed earlier[47,48] for the European population, confirming 1 μg of retinol equivalent being equivalent to 1 μg of retinol, 6 μg of β-carotene, and 12 μg for other carotenoids with provitamin A activity.

Vitamin A recommendations are generally expressed as retinol equivalents[49] or as retinol activity equivalents.[55] According to these definitions, the contribution of each food to the vitamin A intake (mg/d) is expressed as retinol equivalent = retinol + (β-carotene / 6) + (α-carotene / 12) + (β-cryptoxanthin / 12) or as retinol activity equivalent = retinol + (β-carotene / 12) + (α-carotene / 24) + (β-cryptoxanthin / 24). In the latter form, the contribution of provitamin A carotenoids is half compared to that using retinol equivalents. For provitamin A carotenoids, it can be calculated that persons not consuming other sources of vitamin A (due to lack of availability, as in many developing countries, or for vegetarians, especially vegans), ~10.8 mg/day β-carotene or 21.6 mg/day α-carotene (or other provitamin A carotenoids) would fulfil the dietary reference intake RDA for vitamin A (900 μg) for healthy adult men. However, no recommendations exist specifically for vegetarians or vegans, though it was recommended to increase β-carotene consumption to 7 mg/day in people with low preformed vitamin A intake.[61] Having pregnant women take β-carotene supplements has also been recommended for the development of mammalian tissues because it is considered "safer" than preformed vitamin A.[62] The mean dietary intake of β-carotene is in the range of 1.5–1.8 mg/day, and provitamin A intake is <3 mg/day in most European countries (Table 1).

Besides the carotenoid content in foods, their bioavailability is of utmost importance to establish recommendations, because there are many diet- and host-related factors that may affect vitamin A equivalency of β-carotene.[83] These include the food matrix, food-processing techniques, β-carotene dose, and amounts of dietary fat, fiber, vitamin A, and other carotenoids in the diet, as well as vitamin A status, nutrient deficiencies, gut integrity, and genetic polymorphisms associated with β-carotene metabolism.[21,43,84–88] We recently discussed these aspects influencing individual carotenoid responses in a separate review,[21] emphasizing that approximate calculations, as given in previous paragraphs, would be accurate only on average.

There is also controversy about the bioavailability between various carotenoids. For example, it is assumed that the bioavailabilities of α-carotene and β-cryptoxanthin are equal, with each of the 2 compounds having half the bioconversion factor of β-carotene, as shown in humans for α-carotene.[89] However, β-cryptoxanthin, mainly supplied by red and orange fruits, seems to be more efficiently absorbed and converted into vitamin A than is α-carotene.[90,91] This is supported by a study from Estevez-Santiago et al,[92] in which the authors indicated the bioaccessibility (ie, the fraction of a compound that is released from the matrix and available for additional uptake) of β-cryptoxanthin was greater than that of β-carotene in nearly one-half of the fruits analyzed. This has also been corroborated by human studies, as reviewed recently.[93]

Recommendations regarding non-provitamin A carotenoids

There are no generally accepted dietary recommendations for non-provitamin A carotenoid intake, because their absence from the diet does not cause specific deficiency symptoms.[94] Thus, suggestions for intake are mainly based on epidemiologic and intervention studies and beneficial health effects. Among the non-provitamin A carotenoids, the 2 xanthophylls (oxygen-carrying carotenoids) lutein and zeaxanthin have received much interest in the past decades. These are, besides the in vivo–formed meso-zeaxantin,[95] the UV- and blue light–protecting compounds in the macula lutea (the yellow spot of the human eye), discussed as protective agents against AMD. The large Age-Related Eye Disease Study 2 (AREDS2), an intervention study, showed that lutein and zeaxanthin should be used rather than β-carotene for eye health, because of safety concerns regarding β-carotene.[23,24] In general, main sources of lutein and zeaxanthin are vegetables; the contribution from oils, fats,[96] and eggs and egg products appears to be rather small.[72] Lutein and zeaxanthin in foods or in supplements increased the macular pigment

Table 1 **Average daily intake of carotenoids and characteristics of studies examining carotenoid serum/plasma concentrations and intake in women and men**

Reference	Country	Women/men, age (no.)	Method	Intake (mg/d)[a]						Serum or plasma (nmol/L)[a]					
				ACAR	BCAR	L/Z	BCRY	LYC	Total caros[b]	ACAR	BCAR	L/Z	BCRY	LYC	Total caros *
	Costa Rica														
El-Sohemy et al (2002)[63]		Women 59 ± 10 y (344)	FFQ	0.73	4.67	2.89	0.55	5.77	14.61	180	821	328	275	585	2189
		Men 56 ± 11 y (115)	FFQ	0.45	3.41	2.41	0.38	5.45	12.10	135	484	316	181	501	1617
	France														
O'Neill et al (2001)[64]; Olmedilla et al (2001)[65]		20–45 y (75)	FFQ	0.74	5.84	2.50	0.45	4.75	14.28						
		Men								120	540	330/80	200	660	1930
		Women								170	870	400/90	230	660	2420
	Ireland														
Carroll et al (1999)[66]		Women	FFQ												
		25–45 y (32);		2.43	8.80	2.62	0.73	8.05	22.63	107	462	320	296	253	1438
		>65 y (25)		1.22	5.50	2.12	0.32	4.62	13.78	166	553	223	123	111	1167
		Men	FFQ												
		25–45 y (32);		2.29	8.08	2.32	0.47	7.64	20.80	92	393	304	191	297	1277
		>65 y (29)		1.22	5.28	1.88	0.30	2.03	10.71	122	472	187	117	91	989
O'Neill et al (2001)[64]; Olmedilla et al (2001)[65]		20–45 y Northern Ireland (65)	FFQ	1.04	5.55	1.59	0.99	5.01	14.18						
		Men								70	390	140/40	140	640	1420
		Women								80	450	160/40	190	690	1610
		20–45 y Ireland, Republic (73)	FFQ	1.23	5.16	1.56	0.78	4.43	13.16						
		Men								90	460	150/50	160	730	1640
		Women								120	610	170/40	250	570	1760
	Italy														
Lucarini et al (2006)[67]		Men and women, total diet (1968)	Individual dietary survey, 7-d diaries	0.15	2.60	2.21 (F), 2.27 (M)	0.17	5.12 (F), 6.54 (M)	10.25 (M)						
Sette et al (2011)[68]		Men and women, total diet with supplements (3323)	Individual dietary survey, 3-d consecutive food records	0.18 (F), 0.15 (M)	2.37										
	Luxembourg														
Biehler et al (2012)[69]		Luxembourg population (1432)	FFQ	7.6[c]	1.5	1.4	1.8	17[d]							

(continued)

Table 1 **Continued**

Reference	Country	Women/men, age (no.)	Method	Intake (mg/d)[a]						Serum or plasma (nmol/L)[a]					
				ACAR	BCAR	L/Z	BCRY	LYC	Total caros[b]	ACAR	BCAR	L/Z	BCRY	LYC	Total caros *
	Netherlands														
O'Neill et al (2001)[64]		20–45 y (72)	FFQ[64]	0.68	4.35	2.01	0.97	4.86	12.87						
Olmedilla et al (2001)[65]		Men								70	470	180/50	240	540	1560
		Women								80	430	230/60	370	530	1700
	Spain														
O'Neill et al (2001)[64]		20–45 y (64)	FFQ[64]	0.29	2.96	3.25	1.36	1.64	9.50						
Olmedilla et al (2001)[65]		Men								70	380	270/110	400	530	1760
		Women								70	360	280/70	420	510	1710
Olmedilla-Alonso et al (2014)[70]		Men (54) Women (54) 20–35 y (54) 45–65 y (54)	3 x 24-h dietary recalls			0.96/0.08 1.19/0.11 0.90/0.09 1.24/0.10						229/51 223/49 192/48 260/53			
Beltran-de-Miguel et al (2015)[71]; Estevez-Santiago et al (2016)[72]		National Survey of Dietary Intake in Spain (2009–2010). 18–64 y (3000)	Individual dietary intake survey (24-h dietary recall and 3-d diet diary	0.27	1.49	1.24	0.32	3.06	6.38						
Beltran-de-Miguel et al (2015)[71]; Estevez-Santiago et al (2016)[72]		National Survey of Dietary Intake in Spain (2009–2010) From vegetables only. 18–64 y (3000)	Individual dietary intake survey (24-h dietary recall and 3-d diet diary)	0.25	1.26	0.78	0.02	2.64	4.95						
Beltran-de-Miguel et al (2015)[71]; Estevez-Santiago et al (2016)[72]		National Survey of Dietary Intake in Spain (2009–2010) From fruits only. 18–64 y (3000)	Individual dietary intake survey (24-h dietary recall and 3-d diet diary)	0.01	0.10	0.06	0.31	0.33	0.81						
	Sweden														
Wawrzyniak et al (2013)[73]		Women 56–75 y (159)	FFQ	1.03	3.47	2.64	0.46	2.15	9.75	80	459	293	505	611	1948
	United Kingdom														
Pezdirc et al (2016)[74]		British women 18–30 y (30)	FFQ	1.46	5.24	1.70	0.32	4.63	13.35		559	527	181	931	2198

(continued)

Table 1 **Continued**

Reference	Country	Women/men, age (no.)	Method	Intake (mg/d)[a]						Serum or plasma (nmol/L)[a]					
				ACAR	BCAR	L/Z	BCRY	LYC	Total caros[b]	ACAR	BCAR	L/Z	BCRY	LYC	Total caros *
	United States														
Young et al (1994)[75]		Women 29–39 y (98)	Diet records FFQ	0.57 0.75	2.65 3.33	1.86 2.39	0.03 0.04	3.06 3.35	8.17 9.87	110	340	460	170	580	1660
Tucker et al (1999)[76]		Women 67–93 y (346)	FFQ	0.86	4.51	3.09	0.08	7.00	15.54	117	510	560	270	610	2067
		Men 68–91 y (201)	FFQ	0.66	3.79	2.68	0.06	7.64	14.38	82	330	520	200	640	1772
Curran-Celentano et al (2001)[77]		Women and men 18–50 y (280)	FFQ		2.94	1.10		8.37	12.41		280	371		601	1252
Tangney et al (2004)[78]		Women 65–87 y (34)	24H FFQ	0.64	4.65 4.01	3.19	0.08	8.16	6.08	70	400	360	160	350	1340
		Men 66–86 y (25)	24H FFQ	0.63	4.74 4.72					70	520	350	180	510	1630
Burke et al (2005)[79]		Women 45–73 y (61)	FFQ		4.52	1.83		7.40	13.75		723	450		438	1611
		Men 45–73 y (37)	FFQ		2.85	1.47		8.14	12.46		463	401		435	1299
Talegawkar et al (2008)[80]		African American women 34–84 y (247)	24H Short FFQ Long FFQ	0.14 0.35 0.25	2.77 2.56 2.21	2.61 2.15 1.93	0.10 0.11 0.13	1.47 2.79 2.60	7.09 7.96 7.12	70	640	320	180	1240	2450
		African American men 34–84 y (155)	24H Short FFQ Long FFQ	0.18 0.39 0.33	2.93 2.80 2.21	2.94 2.26 1.85	0.09 0.11 0.11	2.22 3.54 3.16	8.36 9.10 7.66	70	510	320	170	1440	2510
George et al (2012)[81]		Women 40–69 y (217)	DHQ 24H	0.57 0.24	3.51 2.53	2.40 1.67	0.14 0.08	4.22 0.28	10.84 4.8	130	460	274	199	762	1825
		Men 40–69 y (253)	DHQ 24H	0.50 0.25	2.91 2.54	2.17 1.95	0.13 0.08	5.64 1.15	11.35 5.97	103	341	243	176	818	1681
	United States and Canada														
Fraser et al (2016)[82]		American, Canadian 50–90 y (909)	24H NBP 24H BP FFQ NBP FFQ BP	0.56 0.42 1.10 1.04	3.40 3.30 6.16 7.17	2.29 2.46 3.38 4.84	0.14 0.11 0.18 0.24	4.10 2.14 5.37 3.59	6.39 8.43 10.82 16.88	170	670	410	250	550	2050
Range				0.01-2.43	0.1-8.8	0.06-4.84	0.02-1.4	0.28-10.70	0.81-22.63	70-180	280-870	200-560	117-505	91-1440	989-2510
Mean				0.7	4.1	2.2	0.3	4.6	11.8	104	495	331	229	594	1725

(continued)

Reference	age (no.)	Intake (mg/d)[a]						Serum or plasma (nmol/L)[a]					
		ACAR	BCAR	L/Z	BCRY	LYC	Total caros[b]	ACAR	BCAR	L/Z	BCRY	LYC	Total caros *
SD		0.5	1.7	0.8	0.4	2.4	4.2	36	138	101	94	271	377
Relative contribution (%)		5.9	14.4	18.6	2.5	39.0	100	6.0	28.7	19.2	13.3	34.4	100

[a]Blank cells represent nondetermined carotenoid concentrations or no data are available.
[b]If not measured, estimates were based on individual carotenoids.
[c]Combined α- and β-carotene. In the Olmedilla et al[65] report, mean values for serum and median values were used for the intake.
[d]Including other carotenoids (eg, violaxanthin, neoxanthin, phytoene, phytofluene).
Abbreviations: 24 H, 24 h dietary recalls; ACAR, α-carotene; BCAR, β-carotene; BCRY, β-cryptoxanthin; BP, black participant; caros, carotenoids; DHQ, diet history questionnaire; FFQ, food frequency questionnaire; LUT, lutein; LYC, lycopene; L/Z, lutein/zeaxanthin; NBP, non-black participant; SD, standard deviation; ZEA, zeaxanthin.

optical density (MPOD) in several human intervention studies. Stringham and Stringham[97] showed that an intake of 7.4 mg/day of macular carotenoids (6.2 mg lutein;0.7 mg zeaxanthin;0.5 mg meso-zeaxanthin) was the most efficient dose regarding serum response, whereas MPOD most efficiently increased after intakes of 13.1 mg/day (10.9 mg lutein;1.3 mg zeaxanthin;0.9 mg meso-zeaxanthin). This intake, thus, seems to be a good basis for discussion about developing recommendations, at least for dietary lutein and zeaxanthin. However, in a study with healthy volunteers ($n = 108$), MPOD was lower in older (45–65 years) vs younger (20–35 years) participants, despite a higher dietary intake and higher serum concentrations of lutein and zeaxanthin.[70] It was concluded that age ranges should be considered when establishing normal or reference ranges for lutein and zeaxanthin in serum and that the levels of these xanthophylls should be expressed in relation to blood lipid concentration as a better predictor of MPOD, at least in persons >45 years old.[70] Thus, a recommendation for a minimum serum concentration or daily intake of lutein and zeaxanthin appear to be needed, though they may be difficult to attain[98] via dietary intake alone.

Recently, lutein by itself has been proposed for intake recommendations because of its relation to chronic disease prevention and health promotion[99]; based on the following criteria: (1) accepted definition of the compound; (2) a reliable analysis method; (3) inclusion in a food database; (4) conducted cohort studies; (5) conducted clinical trials regarding metabolic processes; (6) clinical trials regarding dose response and efficacy; (7) availability of safety data; (8) systematic reviews and/or meta-analyses; and (9) a plausible biological function. A lutein intake of 6 mg/day has been associated with a decreased risk of several chronic diseases.[98] These levels are much higher than its intake in many populations (Table 1).[72] For instance, an intake of 15 mg 3 times a week during 2 years increased serum lutein concentrations to 600–1050 nmol/L.[100] Such concentrations were associated with lower risk of AMD, cataract disease, and atherosclerosis,[100,101] but they are typically greater than the 95th percentile regarding lutein plasma concentration in persons in Westernized countries.[98] Available evidence suggests that such concentrations (600 and 1050 nmol/L) produce no obvious adverse effects, are achievable through diet, and constitute a desirable target.[98]

Potential adverse effects cannot be excluded, however, so developing carotenoid intake recommendations requires a risk assessment for the individual compounds. Such an assessment was published for lutein and lycopene[102] (Table 2) on the basis of results from human intervention studies investigating effects of different doses. None of these studies found any adverse

Table 2 **Recommendations for dietary and supplemental intakes of carotenoids**

Carotenoid	National RDIs (mg/d)	National RUL or similar (mg/kg body weight/d)	(mg/d)	Reference
Astaxanthin		0.043[a]		EFSA (2014)[103]
β-Apo-8′-carotenal		0.05[a]		EFSA (2012)[104]
β-Carotene			15[b,c]	EFSA (2012)[105]
β-Carotene			5–10[d]	EFSA (2012)[106]
			15[e]	EFSA (2012)[106]
			7[f]	Expert Group on Vitamins and Minerals (2003)[33]
	2[g]			Müller (1996)[34]
	8[g,h]			Müller (1996)[34]
Canthaxanthin		0.03[a]		EFSA (2010)[107]
Lutein			20[i]	Shao (2006)[102]
		1.0[a]		EFSA (2010)[108]
	10[j]			Huang (2015)[39]
Lycopene			75[b]	Shao (2006)[102]
		0.5[a]		EFSA (2010)[109]
Zeaxanthin			53[b]	EFSA (2012)[110]

[a]EFSA acceptable daily intake.
[b]EFSA safe intake.
[c]For smokers.
[d]From food additives and supplements alone.
[e]From all sources.
[f]United Kingdom, safe upper intake level.
[g]German Nutrition Society's recommended daily intake.
[h]Vegetarians.
[i]Council for Responsible Nutrition, observed safe level.
[j]Dietary intake for eye health.
Abbreviations: EFSA, European Food Safety Authority; RDI, recommended daily intake; RUL, recommended upper limit.

effects. The only documented adverse effect for supplementing high concentrations of carotenoids is carotenodermia, resulting in a yellow to red skin, which is reversible.[111] In the Spanish cohort of a European multicenter intervention trial with β-carotene, lycopene, or lutein (15 mg/day for 4 months), carotenodermia was reported by 95% of participants supplemented with α- and β-carotene, 40% of those in the lutein group and 25% of those taking lycopene capsules.[65] Thus, there appears to be no basis for identifying lowest observed or no-observed adverse effect levels. As a consequence, observed safe levels for humans were defined (Table 2) that are difficult to achieve by dietary means without fortified foods or supplements. Finally, the European Food Safety Authority has published acceptable daily intake recommendations for several carotenoids (Table 2), typically made on the basis of milligrams per kilogram of body weight, with doses that, likewise, appear relatively high. For example, for lycopene, this estimate is 0.5 mg/kg body weight, which is normally not reached from dietary sources, despite its variable intake from natural sources (Table 1), especially from tomatoes and tomato-derived products. High consumption of such items may result in intakes of 20 mg of lycopene per day. An average intake between 0.5 and 5 mg/day has been reported (Table 1). In southern European countries such as Italy, the main lycopene sources are raw tomatoes, cooked tomatoes, and pizza, whereas main contributors for other European regions (eg, United Kingdom, Ireland, France, the Netherlands) are canned tomatoes and soups, and, in the United States, pasta sauces.[112]

INTERRELATION OF DIETARY CAROTENOID INTAKE AND PLASMA/SERUM CONCENTRATION

Regarding the carotenoid type, the majority of the dietary intakes across a variety of studies, as well as serum/plasma measurements included only 6 carotenoids, namely, α-carotene, β-carotene, lutein, zeaxanthin, β-cryptoxanthin, and lycopene (Table 1), and significant correlations between intake and serum concentrations have been reported. In Europe, blood carotenoid concentrations have been shown to be influenced by body mass index (BMI), sex, and smoking status, though country-specific carotenoid intake patterns also were discussed.[13] At the individual level, the intake of fruits; root vegetables, including carrots; and tomato products are good predictors of β-cryptoxanthin, α-carotene, and lycopene plasma concentrations, respectively. Importantly, carotenoid serum concentrations can help discriminate population-level consumption of fruits and vegetables[113] (Table 1).

Several studies have suggested that women, on average, consume 19% to 63% higher amounts of α-carotene, β-carotene, and β-cryptoxanthin, and have higher blood concentrations of 33% to 69% than men do,[63,76]

resulting, in part, in higher correlations between intake and plasma concentrations for women than for men. Similarly, Burke et al[79] reported elevated carotenoid concentrations in blood of women vs men for β-carotene, lutein, zeaxanthin, and lycopene, but only when the intake of β-carotene was significantly higher. Also, Olmedilla et al[114–116] found higher blood concentrations in women than in men, whereas Tangney et al[78] observed higher lycopene concentrations in men (46% higher), together with a 31% higher intake, perhaps due to higher consumption of pizza and pasta-related tomato products. Of note, because women have lower body weight and plasma volume than men, it can be assumed that a similar intake results in somewhat higher plasma concentrations in women. This is in line with a study by George et al,[81] who found no significant difference for total carotenoid intake, though slightly higher serum levels for women were encountered. In women, their menstrual cycle also could influence plasma carotenoid concentrations. In studies with premenopausal women, total and individual carotenoid plasma concentrations (lycopene, β-carotene, lutein, and zeaxanthin) were lowest in the early follicular phase and significantly higher thereafter, which may affect estimating plasma carotenoid-disease relationships.[117] Taken together, the results imply that intake is the main predictor of blood carotenoid concentrations, and the overall absorption and clearance of carotenoids do not seem to significantly differ between sexes, though similar intake may result in slightly higher plasma concentrations in women.

Dosing, especially at prolonged high and non-nutritionally relevant concentrations, can result in a plateau of plasma carotenoid levels. For example, in a dose-escalating study with lycopene administered at 15, 30, 45, 60, 90, or 120 mg/day for 12 months in elderly men (mean age, 74 years), lycopene concentration plateaued after 3 months, and plasma levels for 15- and 90-mg doses were similar, resulting in 500–700 nmol/L lycopene (120 mg resulting in 1300 nmol/L).[118] Likewise, the intake of 60 g of tomato puree (containing 17 mg of lycopene) for 3 weeks increased plasma lycopene concentration by 500 nmol/L,[119] whereas 25 g of the same product for 2 weeks[120] produced an increase of 400 nmol/L, emphasizing that plasma lycopene concentrations do not respond in a linear dose-dependent manner and that low amounts of a bioavailable source suffice to improve and maintain plasma levels.

Similarly, in a dose-escalating study with lutein supplements,[121] with either 2.5, 5, or 10 mg lutein/day for 6 months, final serum concentrations were 620, 1040, 1180 nmol/L, respectively. Contrarily, no plasma plateau was reached for participants ingesting various amounts of fruits or vegetables per day during a 1-year intervention study, with <250 g/day vs 250–500 g/day, 500–750 g/day, and >750 g/day, resulting in β-carotene concentrations of 250, 420, 450, and 530 nmol/L, respectively,[122] perhaps due to a more even distribution of carotenoid intake over time and rather physiological doses.

Regarding time, volunteers receiving carotenoid supplements showed a plateau in serum concentrations of 1500, 2000, and 1200 nmol/L for lutein, β-carotene, and lycopene, respectively, after ~15 mg/day of each carotenoid, starting from 8 weeks and maintained until week 20.[65] Similarly, administration of 180 mg/day β-carotene resulted in a plateau of ~9500–18,500 nmol/L between 1.5 and 4 weeks.[123] Ranges and average concentrations of plasma β-carotene after supplementation trials have been published[25] and highlight that only easily bioavailable doses (eg, dissolved in oil) of 20 mg/day or more (such as in the CARET or ATBC study) resulted in β-carotene plasma concentrations >1900 nmol/L—doses that were related with adverse effects and clearly higher-than-typical average concentrations in populations (>500 nmol/L) (Table 1). Interestingly, when effects of dose, supplementation duration, formulation, sex, smoking status, and study design were evaluated among 57 human studies, dosing was the most prominent factor affecting β-carotene plasma response. Multiple-unit dosing was more effective than single-unit dosing and outweighed the effect of dose within the range of 2–30 mg/day.[124] It is possible that the amount of carotenoids that can be absorbed at 1 time, or those that can be additionally secreted via chylomicrons and transported in lipoproteins, is limited.

Age has also been proposed to be associated with carotenoid blood concentrations, but effects are inconsistent and may be heavily confounded by dietary intake and habits. In women >65 years old compared with women 56–65 years old, significantly lower β-carotene, lutein, zeaxanthin, β-cryptoxanthin, and lycopene concentrations were found,[73] likely due, at least in part, to lower dietary intake. Contrarily, Wu et al[125] did not confirm a trend of lycopene plasma concentrations with age and, likewise, Palli et al[126] found no such age effects for, α-, β-, and γ-carotene. Similarly, Grolier et al[127] reported no effect of age on lutein, β-cryptoxanthin, and β- and α-carotene concentrations in blood but noted significantly lower lycopene levels (50% lower) in elderly compared with young persons (<35 years vs >60 years), in agreement with Hodge et al.[128] Lower lycopene concentrations in the elderly were emphasized in several studies (reviewed by Bohn et al[21]), though it is unclear whether lower intake or less efficient absorption at older age plays the main role. Interestingly, Olmedilla-Alonso et al[70] found even higher serum

concentrations in older vs younger study participants, though this was due to higher lutein and zeaxanthin dietary intake (both crude and energy adjusted). Likewise, Anlasik et al[129] found a statistically significant effect of carotenoid intake (for lower [0–100 g/day] and higher [>350 g/day] consumption of fruits and vegetables), on plasma concentrations, independent of age and sex in elderly participants (65–102 years old). Thus, the observed relationship between age and circulating carotenoid concentrations is most likely be dominated and explained by differences in dietary intake.[130]

In some studies, a positive association between education and plasma/serum concentrations with predominant carotenoids was noted.[73] The EPIC study indicated higher fruit and vegetable intakes in the United Kingdom compared to EPIC centers in Spain and Italy, similar to the report by Buijsse et al.[9] It is likely that people with a higher education are more health conscious than people with a lower education and also can afford a healthier diet (ie, rich in fresh fruits and vegetables).[131]

Studies assessing the effect of smoking on carotenoid concentrations in serum/plasma demonstrated up to 44% reduced concentrations in smokers.[128,132–135] Furthermore, Palli et al[126] confirmed that the highest β-carotene levels were found in women who had quit smoking, whereas the lowest concentrations were found in present smokers. Inverse associations of serum carotenoids with the metabolic syndrome[136] and diabetes[137] were more evident in current smokers than in nonsmokers.[138] Although not all studies adjusted for dietary carotenoid intake, some did. Walmsley et al[139] suggested that the reduced dietary intake in observed smokers could only partly explain lower circulating plasma carotenoid concentrations. Similarly, in their small-scale study, Rust et al[132] did not find different dietary patterns, but they did report lower plasma carotenoid concentrations. Finally, in the large-scale National Health and Nutrition Examination Survey III study, which included almost 8000 apparently healthy participants, smoking, even after dietary adjustments, was associated with significantly reduced β-carotene concentrations.[133] Thus, though not all studies found lower carotenoid concentrations in smokers after adjustment for dietary intake,[140] most did, suggesting, indeed, either lower bioavailability or enhanced turnover.

The effect of alcohol consumption on plasma concentrations of several carotenoids was studied in healthy men consuming low or moderate amounts of alcohol, patients with alcohol addiction but without severe liver disease, and in a control population. Plasma concentrations of all carotenoid fractions were significantly lower in the alcohol-addiction group than in the low-drinking group. After withdrawal, plasma concentrations of all carotenoids increased.[141] On the other hand, lycopene concentrations in serum were 8% higher among drinkers compared with nondrinkers,[125,142] and higher alcohol consumption (up to 20 g or ~25 mL/day or 1.5 drinks) was associated with increased serum/plasma carotenoid levels in men but not in women. However, alcohol consumption exceeding 40 g/day (~50 mL or 3 drinks) was associated with decreased β-carotene levels in serum/plasma, and this decline was more pronounced in men. Lyle et al[143] also demonstrated lower β-carotene serum concentrations in drinkers with intakes of $\geq$ 91 g of alcohol per week, suggesting that increased alcohol intake is also co-associated with an altered dietary pattern high in certain processed food items. For example, poor diet quality, and thereby increased convenient food intake, such as pizza and ketchup, which are high in lycopene,[144] was associated in middle-aged men from France with increased alcohol intake.[145]

In conclusion, carotenoid intake and plasma/serum concentrations appear reasonably well correlated. Women tend to have higher serum/plasma carotenoid concentrations than men, and age is associated with a decline of lycopene levels,[130] though different dietary patterns of the elderly with lower consumption of processed tomato products could play a role.

INTERRELATION OF DIETARY CAROTENOID INTAKE AND TISSUE CONCENTRATION

Interestingly, a human postmortem study showed that the distribution pattern of carotenoids in serum/plasma was similar to that in the organs of the individuals, although there were significant quantitative differences in the levels of various carotenoids between organs.[146] This was corroborated by additional studies investigating tissue carotenoid concentrations[147–149] (Table 3). Thus, there is a correlation between intake and blood and tissue levels. Care should also be taken that many body compartments are assessed for carotenoid concentrations by different techniques, noting that absence of data does not necessarily mean absence of carotenoids. Furthermore, the association between intake and plasma/tissue concentrations is influenced on the individual level by genetic factors, which have been recently reviewed by our group and others, and we refer the reader to these comprehensive overviews.[21,164]

Intestine and liver

The intestine is among the first organs exposed to dietary carotenoids. Carotenoid concentrations in the small intestine are poorly documented. In the colon, β-carotene tissue levels were 60 $\pm$ 30 nmol/kg and were

Table 3 **Concentrations of carotenoids in various tissues, all data in nmol/L (nmol/kg or L)**

Tissue	BCAR	ACAR[a]	BCRY	LYC	LUT	ZEA	PHYE	PHYF	Total caros[b]	Reference
Serum/plasma	360 ± 10	120 ± 10	230 ± 10	740 ± 10	380 ± 10	90 ± 10	40 ± 20	170 ± 70	1940 ± 20	Al-Delaimy (2005)[150]
Serum/plasma	ATBC: 823 ± 277; 9CBC: 22 ± 13; 13CBC: 29 ± 22 Sum: 874			ATLYC: 190 ± 25; 5CLYC: 130 ± 20; 9CLYC: 9 ± 5; 13/15CLYC: 55 ± 25; Sum: 384						Schierle et al (1997) [151], Fröhlich (2007)[152]
Abdominal adipose tissue	1472 ± 286	280 ± 74	417 ± 462	3329 ± 448	456 ± 62[c]	–	–	–	–	Chung et al (2009)[153]
Liver	5900 ± 6300	–	–	8400 ± 11,500	2200 ± 1600[c]	–	–	–	16,500	Bohn et al (2017) [21]
Skin[d]	430 ± 45	95 ± 20	225 ± 35	695 ± 45	180 ± 35	175 ± 35	320 ± 90	46 ± 20	0-7001730	Alaluf et al (2002)[154] Ermakov et al (2013)[155,156]
Lung	350 ± 440	230 ± 270	420 ± 750	570 ± 1110	480 ± 660	–	–	–	1905 ± 2820	Schmitz et al (1991)[147]
Kidney	550 ± 730	300 ± 400	450 ± 1040	620 ± 620	1210 ± 2830	–	–	–	3050 ± 4210	Schmitz et al (1991)[147]
Brain[e]	10–30	–	<10	–	20–80	10–30	–	–	–	Vishwanathan et al (2014)[157]
Adrenals	5600[f] (680–31,830)	1220[f] (110–7520)	660[e] (10–2900)	1900[f] (190–5600)	–	–	–	–	9400 ± 7800[g]	Stahl (1992)[148]
Breast tissue									38,000–50,000	[158]
Testes	2680[f] (750–4770)	370[f] (140–610)	160[f] (10–290)	4340[f] (410–9380)	–	–	–	–	7550	Stahl (1992)[148]
Bone	745 ± 95	95 ± 35	125 ± 35	280 ± 35	175 ± 35	140 ± 60	825 ± 185	275 ± 45	–	Ermakov et al (2013)[155]
Colon tissue	60 ± 30	–	–	–	–	–	–	–	–	Pappalardo et al (1997)[159]
Breast milk	60–200	20–40	2–10	5–25	10–25	–	–	–	–	Gossage et al (2002)[160]
Uterus	503[h]	–	870	–	–	–	–	–	12,500 ± 6000	Czeczuga-Semeniuk et al (2008)[161]
Prostate	600	300	100	700	300	200	–	–	2700	Clintonet al (1996)[162]
Eye (retina)	–	–	–	–	∼3125–12,496[i]		–	–	–	Rapp et al (2000)[163]

All values represent mean ± SD, with data reported as nmol/L (nmol/kg or L). Values in brackets reflect ranges.
[a]Blank cells represent nondetermined carotenoids or no data were available.
[b]Sum of listed carotenoids, unless otherwise stated.
[c]Sum of lutein and zeaxanthin.
[d]Dermis and epidermis of back, forehead, inner forearm, and hand.
[e]Infants, prefrontal cortex, frontal cortex, hippocampus, auditory cortex, and occipital cortex.
[f]Including upper and lower level of this range.
[g]Standard error of the mean.
[h]Values given in literature as "carotenes."
[i]This concentration is based on a total amount of 0.25 nmol in a retina, which is calculated on a predicted retina weight of 10–80 mg.
Abbreviations: 5CLYC, 5-cis-lycopene; 9CBC, 9-cis-β-carotene; 9CLYC, 9-cis-lycopene; 13CBC, 13-cis-β-carotene; 13/15CLYC, 13/15-cis-lycopene; ACAR, α-carotene; ATBC, Alpha Tocopherol, Beta Carotene Prevention Trial; ATLYC, all-trans lycopene; BCAR, β-carotene; BCRY, β-cryptoxanthin; caros, carotenoids; LUT, lutein; LYC, lycopene; PHYE, phytoene; PHYF, phytofluene; ZEA, zeaxanthin.

significantly increased (2.6 times) by supplementation (30 mg/day for 43 days).[159] Patients with colon cancer displayed a lower total carotenoid content compared with healthy participants.[159] This is in line with a previous study in which researchers showed that β-carotene concentrations were lower in colon and rectum cancer samples, as well as in other cancer tissues (cervix, endometrium, ovary, breast, lung, and liver), compared with control tissues.[165] Whether this reflects altered cellular uptake, distribution, or a faster degradation is currently unknown.

The liver is acknowledged to accumulate carotenoids, especially β-carotene [148] and lycopene.[147] The liver may constitute a rather fast-exchanging carotenoid pool, compared with, for example, adipose tissue.[166] In adults, the total carotenoid concentration in liver varied from 2500 to 77,000 nmol/kg,[147,149] and significant correlations were observed between serum and liver α- and β-carotene levels.[167] Conversely, there were no correlations between liver vitamin A and individual or total carotenoids in normal livers,[149] perhaps due to the limited contribution of total carotenoids to vitamin A, emphasizing the importance of preformed vitamin A, at least for participants regularly consuming animal products. It has also been suggested that liver diseases could interfere with the uptake, excretion, or metabolism of carotenoids.[167]

Adipose tissue

It has been established that carotenoids are stored to a notable extent in adipose tissue,[153,168–171] with lycopene and β-carotene predominating.[170,172] Chung et al[153] identified lycopene as the most prevalent carotenoid in this tissue (3329 ± 448 nmol/kg; >50% of total carotenoids), followed by β-carotene (1472 ± 286 nmol/kg), lutein and zeaxanthin (456 ± 62 nmol/kg), β-cryptoxanthin (418 ± 462 nmol/kg) and α-carotene (280 ± 74 nmol/kg).[153]

Adipose tissue concentrations of carotenoids appear to be similar in men and women. The total carotenoid concentration appears to be site specific, with abdominal concentrations being higher than in the buttocks or thigh.[153] Interestingly, circulating concentrations of most carotenoids are inversely correlated to fat mass, and to both general and central adiposity in mostly normal-weight and overweight persons.[153,173] This may suggest that in people with higher BMI, carotenoids are sequestered within the adipose tissue, though lower intake or increased turnover rates may also play a role. Indeed, most studies revealed a strong inverse correlation between BMI and all measured carotenoids in plasma in normal, overweight, and obese participants,[9,73,79,125,126,128,130] except for few studies finding otherwise, such as for lycopene,[174] suggesting that the adipose tissue acts as a sink for circulating carotenoids.

Researchers have also measured carotenoids in adipose tissues. Studies[63,175] have revealed statistically significant positive correlations between individual carotenoid concentrations ($P < 0.01$ for lutein and zeaxanthin, lycopene, β-cryptoxanthin, and β-carotene) in blood and adipose tissue. Similarly, β-carotene content in adipose tissue correlated weakly ($r = 0.2$) with plasma.[176,177] In another study, total carotenoid content, except for lycopene and lutein/zeaxanthin, in adipose tissue was strongly associated with serum levels.[153] Similarly, breast adipose tissue carotenoid content was correlated with levels in plasma, except for β-cryptoxanthin.[178] It is noteworthy that, at least for β-carotene, even though its serum concentration was lower in obese people, the total body pool of β-carotene was similar in obese and nonobese people, when taking into account the total fat mass.[179] Thus, higher fat mass appears to be related with lower plasma concentrations, which, in turn, appear to correlate with lower adipose tissue concentration of carotenoids. In other words, higher fat mass seems related to lower concentration of carotenoids in adipose tissue, also.

Adipose tissue carotenoid content is correlated not only with plasma levels but also with other tissue concentrations. For example, lutein adipose tissue content was positively correlated with macular pigment density in men (though not in women).[180] Conversely, weight loss was associated with increased lutein and zeaxanthin serum concentrations.[181] Though hypercarotenemia could be expected to develop at the onset of the pronounced postoperative weight loss after bariatric surgery, a consistent and continuous drop of all serum carotenoids to levels at or below the fifth percentile of the reference ranges was observed in patients followed up for 18 months.[182]

Factors influencing carotenoid distribution in adipose tissue uptake and turnover are poorly understood. On the basis of single-dose studies such as those by Diwadkar-Navsariwalaet al[183] and Moran et al,[166] it was suggested that adipose tissue is a major component of a slow exchanging pool, as proposed for lycopene and phytoene. The uptake of carotenoids by adipose tissue was not linked to carotenoid physicochemical properties,[184] suggesting the involvement of transporters. In accordance, the involvement of CD36 in lycopene and lutein uptake by adipose tissue and adipocytes has been demonstrated.[185] Thus, adipose tissue carotenoid content may be considered a reasonable mid- to long-term indicator of dietary carotenoid intake,[176] though it has been shown to increase after supplementation. β-Carotene concentrations in adipose tissue increased

from 1470 nmol/kg to 2090 nmol/kg after 5 days of a high, single, oral dose (120 mg).[171] Lutein and zeaxanthin levels (230,000 ± 70,000 nmol/kg dry tissue) in adipose tissue significantly increased after spinach and corn consumption (10.8 mg/day lutein; 0.3 mg/day zeaxanthin) in healthy participants, with a maximum measured at 8 weeks of intervention (470,000 ± 80,000 nmol/kg dry tissue).[186] Finally, tomato-oleoresin supplementation (15 mg lycopene/day) significantly increased lycopene concentration in adipose tissue (from 230 ± 160 nmol/kg to 340 ± 230 nmol/kg).[187]

Dietary carotenoid intake also correlated strongly with abdominal adipose tissue concentration, but less so with buttock or thigh adipose tissue, for α- and β-carotene, β-cryptoxanthin, *cis*-lycopene isomers and total carotenoids (NB, we use *cis*/*trans* terminology in this article, rather than *E*/*Z*).[153] However, correlations varied largely and were strongly influenced by sex. El-Sohemy et al[63] reported mostly significant correlations in women between intake and concentrations in adipose tissues of α- and β-carotene, β-cryptoxanthin, and lutein/zeaxanthin of 0.25, 0.29, 0.44, and 0.17, respectively, but not in men ($r < 0.23$ for all). The origin of this discrepancy is unknown, but carotenoid adipose tissue concentrations may be affected by factors other than intake, such as circulating hormones.

Breast milk

Few studies have been dedicated to exploring carotenoid concentrations in human milk, which appears to be an important source of both provitamin A and non-provitamin A carotenoids during the first months of life.[160] In an American cohort, carotenoid concentrations in milk at day 4 ranged from 50 to 380 nmol/L, depending on the carotenoid (α-carotene < β-cryptoxanthin < lutein ≈ lycopene ≈ β-carotene), with a high interindividual variability. Similar concentrations were found by others. Khachik et al[188] found carotenoid concentrations between 2 and 49 nmol/L, whereas Johnson et al[189] reported higher concentrations (ie, ∼800 nmol/L for β-carotene, and 165–185 nmol/L were reported by Alien et al[190] for lycopene, though all were rather small-scale studies with < 10 participants. As observed for fat-soluble vitamins, milk carotenoid concentrations decreased during the first month to reach mature milk concentrations of ∼10–130 nmol/L. These concentrations were equivalent to 5% to 10% of plasma concentrations, except for lutein, which was present at concentrations equivalent to 30%, constituting 50% of total milk carotenoids. This suggests a specific flow of lutein into milk.[160] Some lutein in milk may be present in the form of esters, and re-esterification of lutein has been proposed.[191] Supplementation with β-carotene (30 mg/day) during the first month of lactation affected neither milk β-carotene nor other carotenoid concentrations,[160] contrary to previous work.[192] In this latter study, by Canfield et al,[192] breast milk retinol was not significantly different among the groups over the treatment period, but breast milk β-carotene concentration was greater after palm oil supplementation (90 mg over 10 days), compared with an equivalent supplementation with pure β-carotene and vs a placebo. The difference observed between the 2 trials may be due to differing efficacy of milk enrichment in β-carotene, which seems to be directly linked to milk fat content.[160,192] The accumulation of lutein in mothers' milk and the association of carotenoids in breast milk with plasma concentrations has been confirmed in recent studies.[193–196] This highlights the possible importance of neonatal exposure to carotenoids during development and may help establish dietary recommendations and in the design of human milk mimetics. It has been suggested that lutein can accumulate in various brain tissues[157] and may constitute an important microconstituent for optimal brain health during the early phases of life.

Lung, kidney, brain, and bone

Different studies have shown that total carotenoid contents of kidney and lung ranged between 200 and 12,700 (mean, 3100) nmol/kg and 100–8400 (mean, 1900) nmol/kg in tissues, respectively.[147] Interestingly, and similar to liver, lung and kidney β-carotene concentrations were positively correlated with α-carotene, lycopene, and total carotenoids.[147]

Major carotenoids identified in the brain were lutein, zeaxanthin, anhydrolutein, α-cryptoxanthin, β-cryptoxanthin, α-carotene, β-carotene, and lycopene, similar to blood, with concentrations of 10–80 nmol/kg (Table 4). Xanthophylls accounted for 66% to 77% of total carotenoids in all brain regions examined. This differs from plasma, perhaps suggesting discriminative steps in brain-tissue uptake. As for the neural retina, the ratio of zeaxanthin to lutein was high, and both xanthophylls were significantly correlated.[199] Interestingly, the frontal lobes, but not the occipital lobes, exhibited an age-related decline in total xanthophylls and total carotenoids.[199]

Carotenoids also exist in human bone and surrounding fatty tissue, both in significant and individually variable concentrations, up to almost 1000 nmol/kg for individual carotenoids (eg, phytoene). Measurements of biopsied tissue samples, determined by Raman spectrometry, revealed that all carotenoids known to exist in human skin (ie, β-carotene, lycopene, β-cryptoxanthin, lutein, and zeaxanthin) are also

Table 4 **Correlation coefficients between blood plasma and/or serum concentrations and dietary intake of carotenoids in humans**

			Blood serum or plasma (nmol/L)[a,b]						
Reference	Location; women and/ or men, age (no.)	Method	ACAR	BCAR	LUT	ZEA	L/Z	BCRY	LYC
Fraser et al (2016)[82]	American, Canadian women and men 50–90 y (909)	24-h Non-black participants 24-h Black participants FFQ Non-black participants FFQ Black participants	0.59 0.62 0.52 0.54	0.52 0.49 0.47 0.44			0.40 0.52 0.25 0.45	0.50 0.41	0.32 0.27 0.28 0.23
Olmedilla-Alonso et al (2014)[70]	Spanish men and women (108) 20–35 y (54) 45–65 y (54)	3 24-h diet recalls			0.27 0.14	0.21 0.37	0.31 0.17		
Wawrzyniak et al (2013)[73]	Swedish women 56–75 y (159)	FFQ	0.25	0.37			0.29	0.30	0.24
George et al (2012)[81]c	American women 40–69 y (217) American men 40–69 y (253)	DHQ 24-h DHQ 24-h	0.44 0.36 0.39 0.29	0.28 0.37 0.37 0.30	0.30 0.33 0.31 0.34	0.08[b] 0.21 0.13[d] 0.17		0.52 0.47 0.48 0.38	0.29–0.33 0.26–0.28 0.30 0.30
Talegawkar et al (2008)[197]	African –American women and men 34–84 y (373)	24-h Short FFQ Long FFQ	0.41 0.32 0.18	0.32 0.12 0.21			0.39 0.13 0.20	0.44 0.29 0.25	0.40 0.24 0.14
Tangney et al (2004)[78]	American women 65–87y (34) American men 66–86 y (25)	24-h FFQ 24-h FFQ	0.47 0.54	0.24[d] 0.22[d] 0.10[d] 0.37[d]			0.03[d] 0.13[d]	0.44 0.46	−0.02[d] 0.09[d]
El-Sohemy et al (2002)[63]	Costa Rica women 59± 10 y (344) Costa Rica men 56± 11 y (155)	FFQ FFQ	0.26 0.24	0.13[d] 0.22			0.22 0.20	0.55 0.44	0.19 0.35
Curran-Celentano et al (2001)[77]	American women and men, 18–50 y (280)	FFQ		0.16	0.19	0.03[d]			
Carroll et al (1999)[66]	Irish women 25–45 y (32); >65 y (25) Irish men 25–45 y (32); >65 y (29)	FFQ FFQ 	0.24[d] −0.14[d] 0.31[d] 0.70	0.11[d] 0.12[d] −0.09[d] 0.34 [d]			−0.02[d] 0.10 [d] 0.27[d] 0.39 [d]	0.53 0.65 0.68 0.54	0.50[d] 0.44[d] 0.26[d] 0.47
Tucker et al (1999)[76]	American women 67–93 y (346) American men 68–91 y (201)	FFQ FFQ	0.33 0.18	0.36 0.25			0.27 0.10[d]	0.44 0.32	0.35 0.21
Yong et al (1994)[198]	American women 29–39 y (60)	Diet records FFQ	0.59 0.52	0.52 0.44	0.29 0.29			0.49 0.30	0.41 0.28

(continued)

Reference	Location; women and/ or men, age (no.)	Method	Blood serum or plasma (nmol/L)[a,b]						
			ACAR	BCAR	LUT	ZEA	L/Z	BCRY	LYC
Range			0.14–0.70	0.09–0.52	0.14–0.34	0.03–0.37	0.02–0.52	0.25–0.68	0.02–0.50
Mean			0.39	0.29	0.27	0.17	0.24	0.45	0.29
Standard deviation			0.16	0.14	0.07	0.11	0.14	0.11	0.11

Pearson correlation coefficient is reported unless otherwise indicated.
[a]Either serum or plasma.
[b]Blank cells represent nondetermined correlations due to no data, data not determined, or missing values of measured carotenoids.
[c]Spearman coefficient reported.
[d]Not statistically significant.
Abbreviations: 24 H, 24 h dietary recalls; ACAR: α-carotene; BCAR: β-carotene; BCRY, β-cryptoxanthin; DHQ, diet history questionnaire; FFQ, food frequency questionnaire; LUT, lutein; LYC, lycopene; L/Z, lutein/zeaxanthin; ZEA, zeaxanthin.

present in human bone, but additional studies are needed to establish correlations with plasma or skin levels.[155]

Buccal mucosal cells and skin

Strong correlations (R) between plasma and buccal mucosal cell concentrations of lutein (up to 0.873), β-cryptoxanthin (up to 0.815), α-carotene (up to 0.796), and β-carotene (up to 0.775) were observed.[200] In supplementation studies, responses in β-carotene concentrations varied considerably between participants. Data suggested the existence of weak and strong responders,[201] a concept well documented in postprandial studies.[202] Conversely, although lycopene cellular content increased after supplementation (70 mg/day lycopene via oleoresin, tomato juice, or beadlets), correlations between lycopene concentrations in plasma and in buccal mucosal cells were weak and not significant for any treatment.[200] This contradicted another study, showing that both β-carotene and lycopene were incorporated into mucosal tissues within 7 days, but it was not clear whether the change in carotenoid plasma concentrations was reflected in existing buccal mucosal cells or in those produced during the elevated plasma concentrations.[203] Interestingly, buccal mucosal cell concentrations of β-carotene were correlated with (1) skin type (the darker the skin, the more β-carotene),[204] perhaps suggesting a fundamental role of epithelial cells and protection from light; and (2) smoking, with smokers having lower carotenoid concentrations in these cells vs nonsmokers,[204,205] due to lower consumption or increased turnover.

Predominant carotenoids detected in the skin are lycopene, β-carotene, α-carotene, β-cryptoxanthin, lutein, zeaxanthin, phytoene, and phytofluene, with highest amounts for lycopene.[156] Skin carotenoids contribute to skin color and photoprotection.[154,206–208] The skin carotenoid score (SCS) can be assessed by Raman spectroscopy and may well reflect carotenoid intake. Skin carotenoid concentrations of up to 650 nmol/kg for individual carotenoids have been reported (Table 3). Indeed, a trial showed that SCS increased with daily consumption of a carotenoid-rich juice (~15 mg/day), but returned to initial levels 3 days after the last intake.[209] This is in line with a previous study that showed SCS predicted plasma concentrations ($r = 0.72$; $P < 0.001$), indicating that changes in SCS closely follow changes in plasma across a broad range of intakes (from carotenoid-depleted diets to 59–65 mg mixed carotenoids per day). Moreover, at the individual level, skin carotenoids predicted plasma levels ($r = 0.70$; $P < 0.001$), confirming that SCS can be a noninvasive, objective biomarker of vegetable and fruit intake.[210]

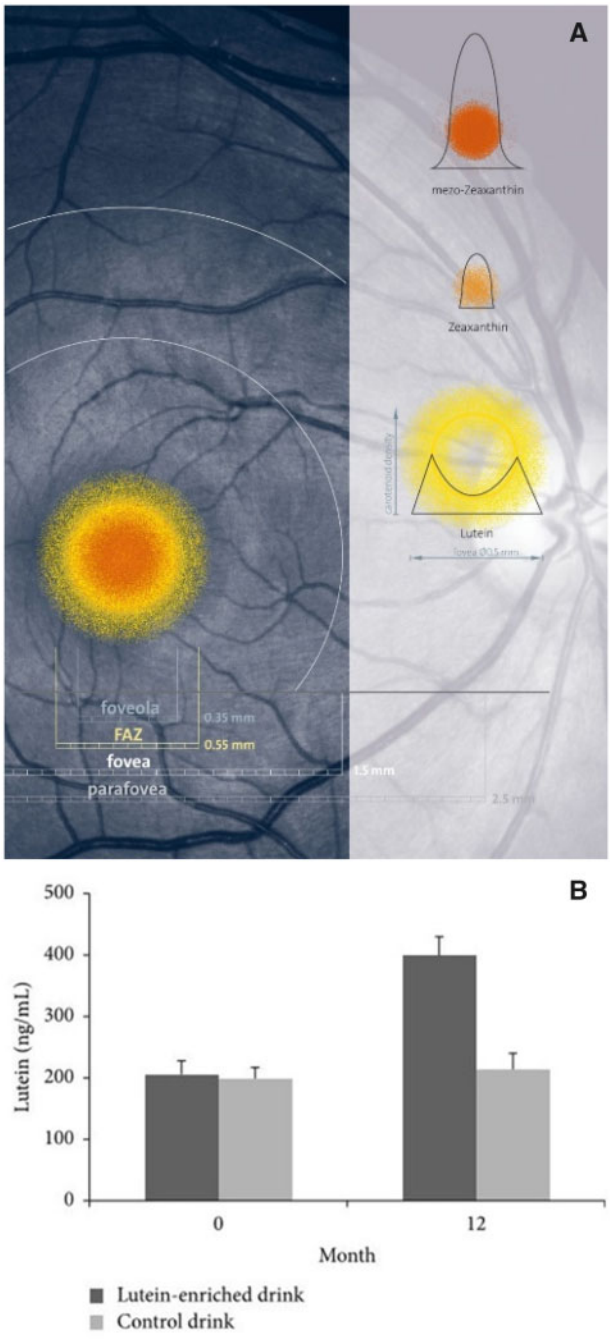

Figure 1 **(A) Distribution of macular pigments lutein, *meso*-zeaxanthin, and zeaxanthin, presented in scale in a photograph of a healthy human retina. Image from Robert Kochling, Berlin, Germany; and John Nolan, Waterford, Ireland, with permission.** (B) Mean ± standard error of plasma lutein concentration at 0 and 12 months for the lutein (dark grey) and the placebo (light grey) groups.[207] *Abbreviation:* FAZ, foveal avascular zone.

Among children aged 5–17 years, consuming 30–120 mL (2.8–11 mg carotenoids) per day of a carotenoid-rich juice significantly increased skin carotenoid status over 8-week periods.[211] However, Walfisch et al[187] showed that skin lycopene only slightly increased (1.6-fold) after 1–7 weeks of supplementation with tomato oleoresin (30 mg lycopene/day)[1]. As stated by Stahl and Sies,[212] optimal skin protection against UV-light–induced erythema may take 8–10 weeks following increased carotenoid intake, perhaps explaining limited changes found in some previous studies, though increased concentrations of carotenoids were detected after 4 weeks of intake.[213]

Breast and reproductive organs: ovary, uterus, testes, and prostate

Thirteen carotenoids were found in female breast adipose tissue around neoplastic tissue in fairly high concentrations (mean, ≈38,000–50,000 nmol/kg). Lutein-epoxide and violaxanthin were predominant in breast adipose tissue, in malignant and benign areas. Mutatoxanthin, lutein epoxide, zeaxanthin, canthaxanthin, lutein, and neoxanthin were predominant in neoplastic material. β-Carotene and lutein epoxide were found in all samples, whereas α-carotene was found only in 50% of the samples. The total carotenoid tissue content was slightly lower for cancerous tissue and the surrounding adipose compared with benign tissues, and was significantly higher in the adipose tissue surrounding the tumors, irrespective of their histological structure.[158]

Similarly, up to 14 carotenoids, including β-carotene, β-cryptoxanthin, lutein, lutein epoxide, violaxanthin, and mutatoxanthin, were identified in uterine[161] and ovarian tissue.[214] In normal uteri, the mean carotenoid concentration was highest in the follicular phase endometrium (18,000 nmol/kg), whereas the highest percentage of provitamin A carotenoids (β-carotene and β-cryptoxanthin) was found in the luteal phase (18.2%).[161] In all ovarian pathological lesions, total carotenoid concentration was relatively low (mean ~3000 nmol/kg), whereas it was higher in the ovarian endometriosis group (4000 nmol/kg).[214] High levels were also found in uterus endometrioid adenocarcinoma (20,000 nmol/kg), suggesting that certain enzymatic defects in carotenoid metabolism occur during lesion evolution.[161] Another trial showed that α-carotene and β-carotene cervical tissue concentrations were significantly correlated.[215] Thus, though a high diversity of carotenoids in the physiologic, benign, and malignant tissues of both breast and reproductive tract in women has been highlighted, differences in carotenoid patterns do not allow drawing conclusions on its relation to the pathophysiological state.[216]

Testes also accumulated significant amounts of carotenoids, with lycopene being predominant (4300 nmol/kg) in a small German cohort.[148] A study of elderly men in the United States showed that

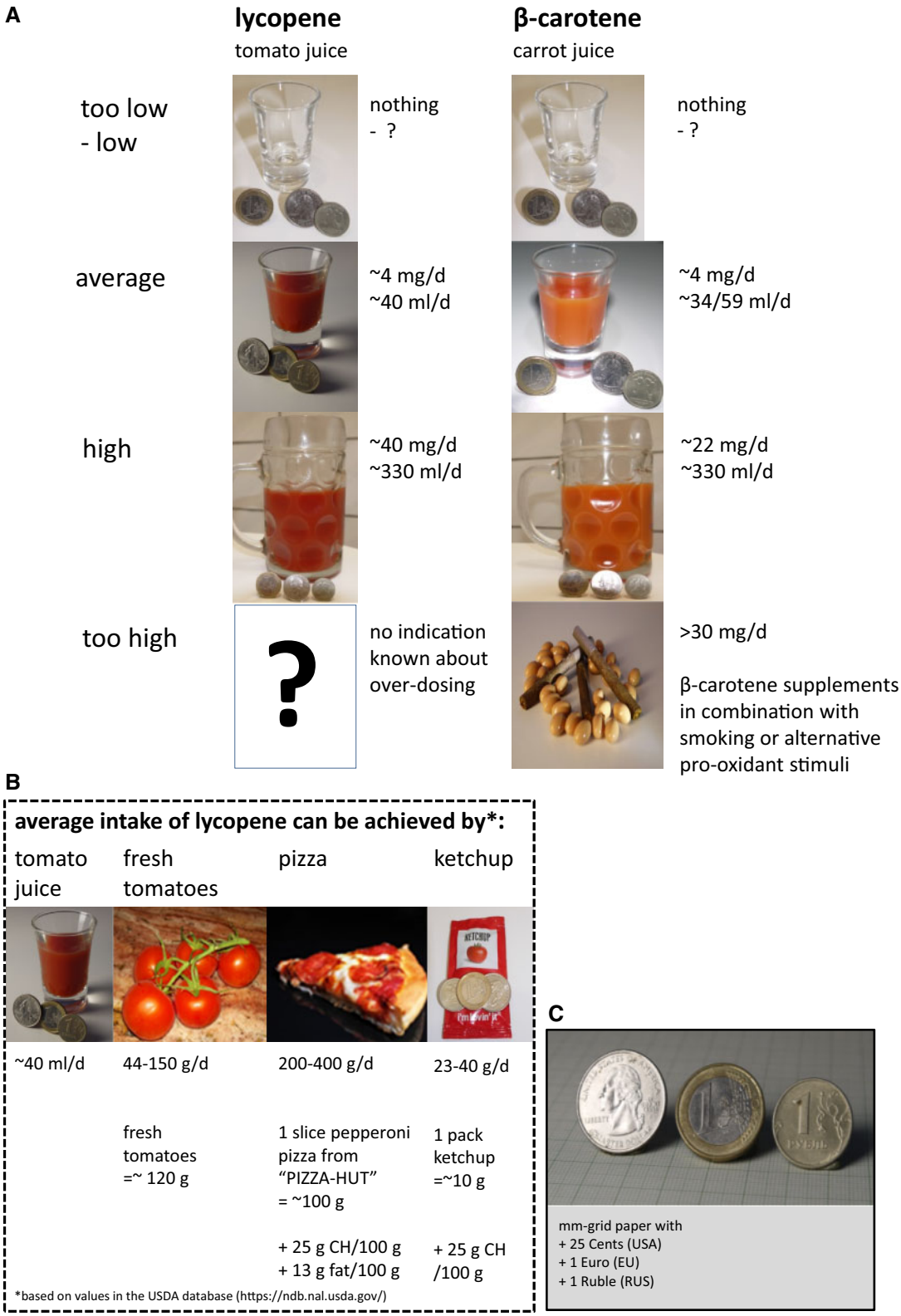

Figure 2 **(A, B) Summary of foods and amounts as examples of a balanced and recommended diet or, alternatively, a common Western diet rich in calories and fats, to fulfil dietary recommended intake of the 2 reference carotenoids: lycopene and β-carotene, as exemplified by the intake of frequently consumed standardized food items available virtually worldwide.** (C) For the tomato juice and carrot juice pictures, 3 coins of international currencies were used: 1 Euro, 25 US cents, and 1 Russian ruble, shown exemplified on millimeter-grid paper as an extra figure. A standard liquor glass (40 ml) and a half litre beer tankard (500 ml) were used as a standard measure for the liquid display. Abbreviations: CH, carbohydrates; USDA, US Department of Agriculture.

concentrations of specific carotenoids in the benign and malignant prostate tissue from the same participants were highly correlated. Lycopene and all-*trans*-β-carotene were the predominant carotenoids, with concentrations ranging from 0 to 2580 nmol/kg and 90 to 1700 nmol/kg, respectively. Also detected were 9-*cis*-β-carotene, α-carotene, lutein, zeaxanthin, and β-cryptoxanthin. Although no significant correlations between the concentration of lycopene and any other carotenoid were observed, strong correlations between prostate β-carotene and α-carotene concentrations and between several other carotenoid pairs were highlighted, possibly reflecting similar dietary origins.[162]

Macular carotenoids and macular pigment optical density

An important target tissue to consider for developing dietary recommendations for carotenoids is the macula, with MPOD constituting the most accessible marker for xanthophyll concentration in the macula. The macula (or macula lutea) is a small, specialized tissue at the center of the retina (being 4% of the retina area), mediating sharp, central, and color vision. The fovea, the center of the macula, is a small, central pit composed of closely packed cones and is responsible for almost all useful day vision.[217] Only primates accumulate the macular carotenoids at the fovea. Several carotenoids, due to their conjugated double bonds, have short-wavelength (blue) light-filtering properties.[218–220] The xanthophylls, lutein, zeaxanthin, and *meso*-zeaxanthin (MZ) accumulate in the central retina (Figure 1). Previous studies[220,221] have demonstrated that macula optical density peaks at the center of the fovea, with a concentration 3 orders of magnitude above that found in normal serum. Bone et al[221] quantified foveal total carotenoid concentrations as ranging from 0.05 ng/mm^2 in the peripheral retina to 13 ng/mm^2 at the fovea. The lutein-to-zeaxanthin ratio changes drastically from the serum of humans (4:1 to 2:1)[222,223] to the macula, where the ratio varies across the fovea. Zeaxanthin is concentrated in the central fovea (ratio of lutein to zeaxanthin, ∼1:2.4), whereas lutein is concentrated in the periphery (ratio of lutein to zeaxanthin, ∼2:1).[221]

Recent work highlighted that enrichment of macular pigment (MP) in patients with early AMD enhances visual function by improving contrast sensitivity,[224] consistent with findings that supplementing with lutein, zeaxanthin, and MZ enhances visual performance in persons with and without retinal disease.[225–227] A recent meta-analysis in humans also emphasized that the increase in MPOD was significantly greater when MZ was included in the supplement.[228] Thus, supplementing with all 3 carotenoids for 12 months, at ∼20 mg/day total, resulted in the best outcomes in terms of MPOD and visual function.[228] In formulations lacking MZ, up to 25% of study participants exhibited a nonresponse regarding MP levels.[229,230]

As for plasma carotenoid concentrations, the source and type of foods consumed have a strong impact also on MPOD. For humans, vegetables provide >7 times the concentration of macular carotenoids compared with eggs and nearly 20 times that of fruits.[231] However, carotenoids from plant sources are of limited bioavailability. In contrast, carotenoids in egg yolk are highly bioavailable because they are present in a digestible lipid matrix. Recently, a human intervention with a buttermilk drink with egg yolk proved to be a highly bioavailable source of lutein and zeaxanthin[232] (Figure 1B). MPOD also increased significantly, from 0.45 to 0.52 optical density units in the active treatment group ($P < 0.001$) but was unchanged in the placebo group (same drink without egg yolk). The Egg Xanthophyll Intervention Trial showed that carotenoid-enriched eggs (2/day for 8 weeks) significantly increased serum concentrations of lutein, zeaxanthin, and MZ compared with standard eggs. However, neither of these interventions significantly increased MPOD,[233] perhaps because study participants were healthy and middle aged at study onset.

Lutein and zeaxanthin are evenly distributed between low-density lipoprotein and high-density lipoprotein,[234] the lipoprotein profile is likely to affect MP levels.[234] Apolipoproteins can act as cofactors of enzymes involved in lipoprotein metabolism, such as lipoprotein lipase.[235] One study found that apolipoprotein E (ApoE) levels were higher in patients with AMD than in controls.[235] The ApoE lipoprotein APOE-ε4 allele is likely associated with a reduced risk of AMD,[236,237] possibly due to increased MP at the retina. It was postulated that ApoE could influence the transport, capture, and stabilization of lutein and zeaxanthin at the macula.[238] There is a specific deposition of MP within the eye (Figure 1), which suggests a biological process governing the capture, deposition, and stabilization of carotenoids at the macula. This regulation is believed to be elicited by binding proteins. Bernstein et al[239] identified tubulin as a possible locus for concentrating MP in the fovea; Li et al[240] identified pi isoform glutathione S-transferase as a binding protein for zeaxanthin and MZ, and steroidogenic acute regulatory domain was identified as a lutein binding protein.[241] However, mechanisms controlling the deposition and stabilization of MP are not fully understood.[242] An atypical spatial profile of MP exists in the fovea of some people.[229] This central dip in MP has been proposed to be due to a lack of MZ in these individuals, potentially due to an inability to convert lutein to MZ.[243,244] The clinical trial reported by Nolan et al[229]

demonstrated that supplements containing MZ could rebuild central dips in MP.

In some trials, the MPOD of participants supplemented with lutein and zeaxanthin did not increase[245–247] despite increased serum concentrations. Micozzi et al[246] observed that supplementing β-carotene reduced serum concentrations of lutein in men, possibly due to competition for absorption. Also, in AREDS2, lutein and zeaxanthin serum concentrations were lower in the group receiving additional β-carotene supplements.[248] The Lutein Nutrition Effects Measured by Autofluorescence (LUNA) study suggested that malabsorption or impaired serum transport of the macular carotenoids was not responsible for the failure of supplements to improve MPOD in retinal nonresponders (because serum concentrations of these carotenoids increased with supplementation) but that impaired capture and/or stabilization of lutein and zeaxanthin within the retina contributed to poor macular response.[230] One explanation was that the supplement was lacking MZ, because when MZ was present in the formulation, 100% of participants exhibited an increase in MP.[226]

MP determinants can be either modifiable (eg, cigarette smoking) or nonmodifiable (eg, age). A family history of AMD was associated with significantly lower MPOD despite normal serum MP concentrations.[249] A significant but modest age-related MPOD decline in study participants >50 years old was observed,[250] a finding supported by other studies.[70,249,251–253] Moran et al[254] reported that even when controlling for supplemental intake, MPOD showed age-specific correlations in older but not younger study participants.[254] Oxidative stress, being higher in older individuals, may be a contributing factor.[255,256] Therefore, any carotenoid intake recommendation targeting MPOD may need adjustment for age. Sex is also related to MPOD, with men having significantly higher MPOD than women, even after adjusting for serum lutein and zeaxanthin levels.[249,257] This difference was due to the lack of women in the highest MP range,[258] as corroborated previously.[259] Removal of the top 5% of men with the highest MP values resulted in minimal differences for MP. BMI is also inversely related to serum and MPOD levels.[249,254,257,260] An association between oxidative stress and BMI, as well as competition between adipose tissue and the retina for uptake of MP, are possible causes.[181,254,261–263] Hammond et al observed an inverse relationship between MPOD and BMI ($n = 680$, $r = -0.12$), stating that the relationship was driven by participants with higher BMI (>29), as these had 21% less MP compared to participants with a BMI < 29.[262] Also, carotenoid intake of participants with higher BMI was lower than of those with normal BMI. Similar as for plasma, Hammond et al[258] found that cigarette smokers had, on average, 25% less MP compared with nonsmokers, consistent with studies by Nolan et al[258,264] in which an inverse relationship between MPOD and smoking frequency was demonstrated.[249,265] Finally, an association between education and MPOD was found.[265,266] Education was a positive predictor of MPOD even after adjustment for confounders (eg, age, sex, diet). Heritability of MP was estimated in 1 study at 84%, indicating that genetic factors play a key role in the distribution profile of MP.[267] Furthermore, it was shown that genetic factors explain 27% of the variation in MPOD in response to supplemental lutein and zeaxanthin.[268] These factors have been reviewed elsewhere.[21,87]

In summary, it appears that carotenoid accumulation in human organs is highly variable, both between and within individuals, and tissue carotenoid concentration can be modulated by (1) host factors (eg, low vs high responders, age, smoking status); (2) dietary intake, food matrix, and processing; (3) types of carotenoid; and (4) the pathophysiological state of the organs,[42,269] though factors governing distribution between tissues in an individual are not well understood.

Although analytically challenging, compartmental approaches based on isotope administration are interesting and allow the study of fluxes between body compartments, as done for β-carotene.[270,271] Moran et al[272] developed a model for ^{13}C-labelled lycopene that was based on an earlier model.[183] These studies indicated that the major body pools are a slow turnover pool, likely representing body tissues such as adipose tissues, and a fast turnover pool, possibly including the liver. Transport rates across the pools were mentioned, with irreversible losses from the slow turnover pool, given with 2500 nmol/day (1.3 mg/day) lycopene, which eventually would need to be replenished. However, it thus is difficult to predict the amount needed to ingest to maintain certain tissue levels in individuals, though on population levels, estimates appear possible.

INSIGHTS FROM LESS FREQUENTLY CONSUMED CAROTENOIDS

In addition to β-carotene, α-carotene, lycopene, lutein, zeaxanthin, and β-cryptoxanthin, other less frequently and locally consumed, or investigated, carotenoids and apo-carotenoids are also present in the human diet. These have also been detected in human plasma. Though their nutritional relevance remains largely unclear, because they mostly cannot be converted into vitamin A active metabolites, some of these compounds are bioavailable and bioactive. For example, phytoene and phytofluene are present at high concentrations in a variety of fruits and vegetables, including carrots,

tomatoes, apricots, and oranges.[69,273] Despite occurring together with lycopene, many bioavailability studies, focusing on tomato-based foods, regularly overlook phytoene and phytofluene. Yet, they are among the predominant carotenoids found in plasma.[274–276] The bioaccessibility[273,277] and even bioavailability[166] of these colorless carotenoids appear to be even superior to that of lycopene.

Astaxanthin is a marine carotenoid bioavailable in humans[278–280] and found in aquatic animals. Synthetic asthaxanthin is used in high amounts in "blue farming" (ie, in the open ocean) and also as a food colorant (E161f). This yields the consumer-preferred orange to pink color of fish such as trout and salmon.[281] Main dietary sources of astaxanthin include salmon, trout, red seabream, shrimp, and lobster.[282] However, the role of astaxanthin as a novel food ingredient has also received increasing attention on the European market, as published in a Scientific Opinion by a European Food Safety Authority Panel.[103] Guerin et al[283] have reviewed the potential of astaxanthin in human health and nutrition and highlighted its role as an antioxidant and photoprotective agent. In fact, astaxanthin has been reported to be a more potent antioxidant than lutein or β-carotene.[218] In a double-blind supplementation trial, participants were randomly allocated to either a supplement (4 mg of astaxanthin twice per day) or to a placebo for 3 months. The authors reported a significant reduction of plasma levels of 12- and 15-hydroxy fatty acids.[278] These results highlight the potential role of astaxanthin against lipid peroxidation in vivo.

Fucoxanthin, a carotenoid commonly found in the marine environment, is present in brown seaweeds such as *Undaria pinnatifida* (wakame), *Hijikia fusiformis* (hijiki), *Laminaria japonica* (ma-kombu), and *Sargassum fulvellum*. All are popular foodstuffs in East Asia.[284] This carotenoid has an unusual allenic bond (C = C = C) and a 5,6-monoepoxide in its molecule that is responsible for its radical scavenging and singlet oxygen-quenching activity. Its antioxidant potential has been reported to be higher than that of β-carotene, lycopene, or astaxanthin.[285] Furthermore, fucoxanthin has also been shown to have anti-obesity and antidiabetic properties in vitro in a mouse-derived white adipose cell culture.[286,287] Metabolism of fucoxanthin can result in fucoxanthinol accumulating in plasma and liver of mice,[288] as well as in human plasma,[289] but their physiological and nutritional relevance is unknown.[290]

Violaxanthin and neoxanthin are frequently consumed epoxycarotenoids, especially from green leafy vegetables such as spinach and kale.[69] In a previous study, it was estimated that their intake constitutes ~10% of total carotenoid intake.[69] However, it is also known that the bioavailability of these compounds and their gastrointestinal degradation products such as neochrome, a metabolite of neoxanthin, is low[291,292] because they can undergo epoxy-furanoid transition in the acid milieu of the stomach. Nevertheless, cellular trials with Caco-2 cells have shown that they can be taken up by enterocytes.[293,294] Their fate still remains to be elucidated. It is possible they are shuffled out of the enterocyte back to the lumen or are transformed into yet-unknown compounds. However, recent studies have suggested their presence in humans, such as in breast cell tissues.[158]

Of note, other apo-carotenoids are also bioavailable and bioactive, though they are possibly taken up by the diet rather than produced in humans. However, the intake of these compounds is likely inferior to the native carotenoids This is the case, for example, of crocetin from safflower, norbixin and bixin from annatto seeds, and abscisic acid, a plant hormone present in many leafy vegetables. For example, plasma crocetin concentrations of up to 760 nmol/L were measured in humans after the consumption of a single 22.5-mg dose.[295] Likewise, abscisic acid was absorbed in humans, with cytokine-like activity in human granulocytes,[296] ameliorating experimental inflammatory bowel disease in rats.[297] Abscisic acid also improved glucose tolerance in rats and humans,[298] interacting on apoptosis via the retinoic acid receptor.[299] This may allow conclusions to be drawn also for apo-carotenoid metabolites produced in vivo. However, compared with the main native carotenoids, these apo-carotenoids remain marginally examined.

DISCUSSION AND PERSPECTIVES

Several carotenoids are implicated in health-related outcomes, from AMD (lutein and zeaxanthin) to possible effects regarding cardiometabolic diseases (predominantly β-carotene and lutein) and cancer (predominantly lycopene). However, because of the large variability of bioavailability according to carotenoid source and host factors,[164] in conjunction with possible negative effects of some carotenoids at higher doses (at least for specific target populations), it is difficult to establish dietary intake recommendations. The only possible recommendation is to consume a diet rich in a variety of fruits and vegetables and their products to provide a sufficient combination of health-maintaining and promoting bioactive compounds. In opposition to these suggestions, it has to be considered that in modern Western societies, a strong desire for convenience food exists, which is generally satisfied by large food companies. These food items are processed and "ready

Table 5 **Translation of food intake to recommendations: carotenoid concentrations in serum/plasma following dietary intervention trials**

Food source and conditions	Reference	BCAR (nmol/L)	LUT (nmol/L)	LYC (nmol/L)
Deficiency				
Carotenoid free diet for 14 d, average intake before supplementation	Watzl et al (1999)[315]	600 ± 360	350 ± 120	160 ± 80
Average dietary intake				
Unknown intake of tomato juice, carrot juice, and dried spinach	Watzl et al (1999)[315]	740 ± 440	370 ± 140	160 ± 70
β-Carotene supplements or β-carotene–rich food				
330 mL carrot juice/d for 14 d (15.7 mg ACAR/ 21.6 mg BCAR/0.5 mg LUT)	Watzl et al (1999)[315]	2050 ± 720*	360 ± 110	150 ± 50
BCAR supplements: ATBC Study and CARET (20 mg BCAR ATBC; 30 mg BCAR CARET)	The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group (1994)[23] Omenn et al (1996)[24]	3800*/5600*		
Lycopene supplements or lycopene-rich food				
330 mL tomato juice/d for 8 wk (22 mg LYC; 1.0 mg BCAR)	Bohn et al (2013)[316]	390 ± 160*		1240 ± 30*
Tomato extract (45 mg LYC/d) for 7 d	Wood et al (2008)[317]	540*	250	127*
30 mg/d LYC supplement (tomato extract) for 8 wk	Vrieling et al (2007)[318]			610 ± 220*
15 mg/d LYC supplement (extract, tomato oleorosin) for 6 mo	Schwarz et al (2008)[319]			1240 ± 310*
330 mL tomato juice/d for 14 d (40 mg LYC / 1.5 mg BCAR)	Bub et al (2000)[320]	650 ± 250	330 ± 120	380 ± 130*
Lutein/zeaxanthin supplements or lutein-rich food				
6-mo L/Z supplementation (10 mg lutein; 2 mg zeaxanthin)	Korobelnik et al (2017)[321]		590 ± 390*	
10 g dried spinach powder per day for 14 d (11.3 mg LUT / 3.1 m BCAR)	Bub et al (2000)[320]	1210 ± 510*	710 ± 170*	140 ± 60

Values are reported as mean ± standard deviation.
*Statistical significance vs control group or beginning of trial (baseline), $P < 0.05$.
Abbreviations: ACAR, α-carotene; ATBC, Alpha-Tocopherol, Beta Carotene Prevention Trial; BCAR, β-carotene; CARET, β-Carotene and Retinol Efficacy Trial; LUT, lutein; LYC, lycopene; L/Z, lutein and zeaxanthin.

to eat" and are based on economically more favorable basic ingredients rich in saturated fats, sugar, and added ingredients for preservation and coloring purposes, rather than being dense in micronutrients and secondary plant compounds.[300,301] This is in contrast to the dietary suggestion of a diet rich in fruits and vegetables promoted mainly by the scientific community. Unfortunately, these latter recommendations are mainly reaching people of higher socioeconomic status who tend to be more sensitive and aware of a healthier lifestyle, including diet,[302] whereas the problem rests rather with the less educated, often following a more unhealthy lifestyle including poor dietary choices,[303] smoking,[304] and alcohol abuse.[305] Thus, these suggestions are engrossed and partly opposite from common consumer demands and availability or affordability, and also lack strong advertising and lobbying efforts by the media and political forces.

Intake of artificial carotenoids occurs at multiple levels, either as direct vitamin or carotenoid supplements or as added food colorants. These range from the E160 (carotenoids)/E161 (xanthophylls) cluster (β-carotene [E160a], capsanthin [E160c], lycopene [E160d], apo-8′-carotenal [E160e]/-esters [E160f], lutein [E161b], violaxanthin [E161e], canthaxanthin [E161g], and zeaxanthin [E161h]) to astaxanthin [E161j].[306] In addition, indirect supplementation with often synthetically produced carotenoids, vitamins, and food-colorant mixtures occurs via livestock, farm animals, and blue farming.[307] Thus, a considerable or even the major fraction of dietary carotenoids and metabolites (retinol) may originate from direct or indirect supplementation of artificially synthesized carotenoids or retinoids,[308,309] which are regulated by governmental bodies. Dietary recommendations regarding single carotenoids are quickly adapted by the food industry, with fortification to the food chain, even adding higher amounts to ensure sufficient levels in food products lasting throughout their shelf life, to also meet governmental requirements for the quantity of listed food ingredients.

Governmental bodies, therefore, should make intake recommendations based on scientific expertise

beyond the common "more fruits and vegetables" suggestion. This should encompass direct and indirect supplementation with single or multiple carotenoids, considering age, sex, and possibly genetic background and disease status for prevention strategies. By contrast, a recommended balanced diet rich in fruits and vegetables does not appear to require upper tolerable levels of intake, due to lack of evidence for harmful effects.

Dietary intake of carotenoids has also changed over time. Although lycopene intake was uncommon in the preindustrialized human diet, especially considering the primarily European-focused worldview, it strongly increased in Western society due to a high consumption of tomatoes and tomato products.[310] Lycopene-associated anticancer effects may be expected to contribute to lower cancer incidence in Western societies,[112] though such results have not been observed, possibly due to other confounding factors also influencing cancer prevalence.[311]

Major studies using lutein and zeaxanthin supplementation are often sponsored by the industry and have been focusing on eye-related effects, promoting higher dietary intake. These studies, however, neglected the possibility that higher intake of lutein or zeaxanthin and resulting higher serum or plasma and tissue concentrations may interfere with beneficial signaling mediated by other carotenoids, as indicated by endogenously relevant β-carotene–mediated retinoid signaling.[20,312] Proposing recommendations based on single carotenoids without evaluating the entire picture of carotenoid-mediated signaling within the whole organism is preliminary and risky.

Dietary recommendations of single and multiple carotenoids should focus on end points clearly related to beneficial health effects and conclusive mechanisms of action. However, this connection between mechanistic effects and observed health outcomes has not been clearly established in humans. Carotenoids are generally considered precursor lipids (mainly for bioactive vitamin A or retinoids) in the diet, whereas their complex and multistep metabolic pathways and the relation to health beneficial effects are still poorly understood.

As a suggestion, a first aim should be to estimate (1) the "normal" concentration ranges of serum and plasma carotenoids, based on a basal healthy diet, and (2) plasma and serum concentrations associated with specific deficiencies like those related to optimal protection against AMD. On the basis of these results, supplementation strategies and recommendations should aim to identify (1) the dose and type of carotenoids needed and which carotenoid mixtures can be recommended, at least for well-defined objectives; and (2) for foods and supplements enriched in carotenoids, concentration ranges should be calculated on the basis of carotenoid intake suggestions and their amount already consumed as part of a healthy diet, to avoid unneeded and/or potential adverse oversupplementation.

CONCLUSIONS AND RECOMMENDATIONS

On the basis of current knowledge, the following statements can be made:

1. **A high intake of a variety of fruits and vegetables is advised.**
2. **Supplementation with individual carotenoids may be beneficial for specific purposes, such as for lutein- and zeaxanthin-associated eye diseases.**

Additional associations between carotenoids and health are based on observational studies and are not yet proven as causal. Because carotenoid intake via fruits and vegetables has never been associated with negative effects, dietary intake recommendations should especially focus on carotenoid intake from fortified foods or supplements, including food ingredients and additives such as colorants, to fully evaluate a risk and benefit ratio considering the bioactivity of carotenoids and their metabolites.

The adverse effects observed after individually administered carotenoid supplementation in smokers, resulting in high circulating concentrations, as found in the CARET or the ATBC study, are not generalizable to the whole population, especially non-smokers. Under such circumstances, carotenoids are not working endogenously as nutritionally relevant antioxidants by neutralizing free radicals and cannot be recommended as anti-smoking dietary supplements.[313] Pro-oxidative events, free-radical formation, and enhanced CYP activity are just some of the potential negative effects of tobacco smoking, which is clearly the major risk factor for increased lung cancer incidence found in the CARET and the ATBC study.[23,24]

As a cornerstone, benchmark concentrations for carotenoids should be suggested, including both normal and deficiency threshold ranges. These ranges should correlate with well-defined and established disease markers, including early markers of diseases and impairments of physiological important functions, also including novel "omics" markers related to diseases.

The basal benchmark concentration indicating a higher risk for chronic diseases appears to constitute a total carotenoid plasma and serum concentration $<$1.000 nmol/L. The second benchmark concentration

reflecting normal carotenoid intake are average plasma and serum concentrations of individual and total carotenoids indicating, and here defined as, a healthy, varied diet. These may be estimated from the data in Table 1, with ~1725 nmol/L total carotenoids: 100 nmol/L α-carotene, 500 nmol/L β-carotene, 600 nmol/L lycopene, 230 nmol/L β-cryptoxanthin, and 330 nmol/L lutein and zeaxanthin. On the basis of these 2 benchmark concentrations and the correlating observed carotenoid intake (Table 1), one can estimate normal daily carotenoid intakes (ie, total carotenoids [11.8 mg] and individual carotenoids (0.7 mg α-carotene; 4.1 mg β-carotene; 2.2 mg lutein/zeaxanthin; 0.3 mg β-cryptoxanthin, 4.6 mg lycopene) (Table 1). Such levels can then be translated into the intake of relevant food items rich in carotenoids (Table 5), on the basis of correlations between reported average intakes for lutein, β-carotene, and lycopene with serum concentrations (Table 1) and considering intervention with carotenoid-rich foods (Table 5). It can be calculated that a combination of ~50 mL of carrot juice, 65 mL tomato juice, and 20 g of cooked spinach per day are sufficient to achieve such average plasma and serum concentrations of the major carotenoids (summarized in Figure 2). This estimation is intended to serve as an example and this calculation can be transferred to alternative food sources and combinations.

Because carotenoids usually exhibit a large interindividual variability in their bioavailability,[164] depending on several host-related factors,[21] establishing recommended daily allowances for carotenoids on the basis of the relationship between carotenoid intake and their blood or tissue concentrations is sensible at the population level. An important aspect will be to refine our knowledge about the individual determinants of carotenoid bioavailability, further metabolism and carotenoid-metabolite and retinoid-mediated signaling and resulting omics-based disease-marker expression in target tissues to provide possibly more personalized dietary recommendations based on individual characteristics (eg, sex, age, disease state, genetics, eating behavior).

Carotenoid intake recommendations may have to consider individual carotenoids or at least groups of carotenoids with similar effects (ie, relevant chronic health problems related to the respective carotenoid, and possibly the food matrix and bioavailability aspects) in addition to lifestyle factors such as gastrointestinal diseases, age, sex, or smoking status.[21]

However, more data, especially including accepted health and disease markers and including novel omics approaches, including lipidomics, transcriptomics, and proteomics, regarding any potential adverse effects, are needed to establish a better correlation and additional metabolic links between carotenoid intake, carotenoid status, and chronic disease prevention. Instead of dozens of individual studies focusing on selected aspects of the carotenoid-signaling pathway, a highly concerted action is advised involving a critical mass of competent groups in single- or multi-centered human supplementation trial focusing on low, average, and high carotenoid supplementation and targeting multiple relevant end points.

As a final simple and general take-home message regarding carotenoid intake, the following is suggested:

Consumption of a large variety of food items is encouraged* especially combining several green-yellowish, yellow, orange, or pink-red food items, indicating a variety of carotenoids in food, with not too little or too much# of each single food component&$.**

**"food items" include naturally generated carotenoids and those present in foods, including indirect food fortification via previously supplemented animals, direct fortified human food as well as dietary supplements including fruit/vegetable extract, natural carotenoid extracts or supplements with synthetic carotenoids; **"not to less" is based on sufficient dietary carotenoids calculated to achieve a proposed 1000 nM (1 μM) total plasma carotenoid concentration and #to avoid excess intakes of single carotenoids >30 mg/d or more; &for smokers carotenoid supplements should not be recommended; $chronic inflammatory diseases are associated with higher carotenoid targeted or untargeted utilization or carotenoid excretion, these are not included in this general recommendation.*

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