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Carotenoid metabolites, their tissue and blood concentrations in humans and further bioactivity via retinoid receptor-mediated signalling

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Abstract

Many epidemiological studies have emphasised the relation between carotenoid dietary intake and their circulating concentrations and beneficial health effects, such as lower risk of cardiometabolic diseases and cancer. However, there is dispute as to whether the attributed health benefits are due to native carotenoids or whether they are instead induced by their metabolites. Several categories of metabolites have been reported, most notably involving (a) modifications at the cyclohexenyl ring or the polyene chain, such as epoxides and geometric isomers, (b) excentric cleavage metabolites with alcohol-, aldehyde- or carboxylic acid-functional groups or (c) centric cleaved metabolites with additional hydroxyl, aldehyde or carboxyl functionalities, not counting their potential phase-II glucuronidated / sulphated derivatives. Of special interest are the apo-carotenoids, which originate in the intestine and other tissues from carotenoid cleavage by β-carotene oxygenases 1/2 in a symmetrical / non-symmetrical fashion. These are more water soluble and more electrophilic and, therefore, putative candidates for interactions with transcription factors such as NF-kB and Nrf2, as well as ligands for RAR–RXR nuclear receptor interactions. In this review, we discuss *in vivo* detected apo-carotenoids, their reported tissue concentrations, and potential associated health effects, focusing exclusively on the human situation and based on quantified / semi-quantified carotenoid metabolites proven to be present in humans.

Key words: **Apo-carotenoids: Apo-lycopenoids: Cleavage products: Tissue concentrations: Liver**

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Introduction

Carotenoids are typically colourful, mostly C-40-based pigments that are generally obtained via plant food items. Over 1100 different carotenoids have been identified[1], and additional new carotenoids are being discovered, including shorter (C-30), and longer (C-50) analogues of bacterial origin[2]. Likewise, apo-carotenoids, carotenoid breakdown products formed in plants[3] or after human ingestion[4], can be considered to belong to this group.

The interest in these secondary plant compounds has increased considerably in the past two to three decades, due to the relation of their intake and circulating plasma concentrations with chronic disease risk. A high carotenoid intake within a plant-food-rich diet and carotenoid concentrations in plasma have been related, among others, to a reduced risk of type-2 diabetes[5], age-related macular degeneration[6], some types of cancer such as those of the prostate[7], and even total mortality[8]. The underlying mechanisms for such associated health benefits are not quite clear and are the topic of controversial discussions, but potential mechanisms include direct antioxidant effects such as quenching of singlet oxygen and lipid peroxides[9], interactions with transcription factors related to inflammatory pathways (e.g. NF-kB) and oxidative stress (e.g., Nrf-2)[10], and also their interaction with the nuclear factors retinoid-X receptors (RXRs) and retinoic acid receptors (RARs) together with peroxisome proliferator-activated receptors (PPARs)[11–13].

It has also been postulated that the potential health benefits are conveyed not necessarily by the native carotenoids, following their absorption in the small intestine, but by their metabolites / cleavage products. Carotenoids as lipophilic constituents are absorbed following their micellisation into enterocytes, where they may partly undergo cleavage by carotenoid oxygenases, namely BCO1 and BCO2, resulting in the formation of symmetrical or non-symmetrical cleavage products[14].

While some of the symmetrical cleavage products have vitamin A activity (following e.g. cleavage of β-carotene or β-cryptoxanthin) by BCO1, the biological role of the other

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cleavage products remains uncertain. These cleavage products or apo-carotenoids have been proposed to be bioactive. For instance, in *in vitro* studies, lycopene derivatives were shown to have higher affinity to Nrf-2 and NF-kB, due to their higher electrophilicity, and perhaps better aqueous solubility[15–19]. Lycopene has been shown to act in part similarly to vitamin A metabolites in normalising a vitamin-A-deficient diet in rats / mice[20]. It cannot also be excluded that bacteria in the colon produce more hydrophilic metabolites of carotenoids that are bioavailable and bioactive[21].

Therefore, there has been increased interest in carotenoid metabolites and their potential connection to health benefits. A limitation of their detection in human specimens is the lack of commercial standards, in addition to their lower concentration and the lower sensitivity of UV- detection, the most common technique employed in their quantification, due to the shortened delocalised electron system in the molecule.

In this review, we strive to present the current state of knowledge of metabolites and breakdown product of carotenoids in humans, their known concentration ranges, and potential health benefits involved, as well as pointing out gaps and potential ways forward in this research domain. In this review, we focused exclusively on the human situation, owing to the proven presence of the described carotenoids and carotenoid metabolites in humans.

Carotenoid metabolites in plasma and tissues

Rationale for interest in metabolites and overview of metabolites

Carotenoids, with major human food relevance (Fig. 1 and Table 1), were investigated mainly for their metabolism in the human body, and it is uncertain whether the native compounds alone or rather their metabolites are responsible for the attributed health effects. Mainly nuclear hormone receptor-mediated effects were the focus of these studies[22,23]. These ligand-activated receptors include RARs and RXRs, which may become activated, resulting in altered gene expression of a large set of genes involved in inflammation, differentiation, proliferation and lipid metabolism / homoeostasis[24–27].

The activation of RARs and / or RXRs was shown to be related to physiologically and nutritionally relevant levels of endogenous carotenoid metabolites[28–31]. Thus, native carotenoids may not interact on their own with gene-regulatory pathways, but rather via their metabolites, the apo-carotenoids, here conclusively the apo-15-carotenoids / retinoids and potentially others, such as apo-13/14-carotenoids that might interact with the binding grooves of RARs and RXRs[32–34]. Here, a focus for activating compounds is put on apo-carotenoids with an acid functionality, while apo-carotenoids with aldehyde or alcohol functionalities might result in low affinity activators / antagonistic compounds[34]. Consequently, knowing more on their identity, concentration, metabolic pathways and homeostatic control and further RAR-RXR-mediated signalling appears critical for estimating potential health benefits of carotenoids[35–39] (Tables 1 and 2).

Individual carotenoids, listed in Table 1 along with their endogenous levels in serum / plasma as well as selected organs, may be cleaved by either BCO1 (centric cleavage) or BCO2 (excentric cleavage) to produce a variety of apo-carotenoids / retinoids (Fig. 1 and Tables 1 and 2)[40,41].

In general, there are three different types of carotenoid metabolites that occur in human plasma / serum and tissues and have been detected especially after carotenoid supplementation: (a) non-cleaved carotenoids with modifications at the cyclohexenyl ring or the polyene chain, such as epoxycarotenoids, geometric isomers and metabolites resulting from further rearrangement pathways; (b) excentrically cleaved metabolites with also alcohol, aldehyde or carboxylic acid functionalities; and (c) centrically cleaved metabolites with additional alcohol, aldehyde or carboxylic acid functionalities (Fig. 1 and Tables 1 and 2). Of note, glucuronidated products are also formed, following phase II conjugation, prior to their excretion via the kidney, as reported, for example, for retinoic acids[42–44].

The origin of selected apo-15-carotenoids / retinoid derivatives, such as retinyl esters, retinol, retinal and retinoic acids, might occur from various metabolic pathways including (a) central cleavage of individual carotenoids such as β-carotenes or β-cryptoxanthins (Fig. 1) by BCO1 cleavage[45–47]; (b) by interaction of these previously mentioned carotenoids with environmental or endogenous oxidants and following cleavage[10,48,49]; or (c) by BCO1 cleavage of individual apo-carotenoids, which might originate from food directly or from mitochondrially based BCO2 cleavage in the human organism[14,45,47].

Alternatively, these apo-15-carotenoids / retinoids might originate from food-derived apo-15-carotenoids present at high concentration in animal-derived food matrices, such as retinol and retinyl esters, or from bioactive retinoids, such as retinoic acids and retinal, which are present in low amounts in the food matrix. Unfortunately, it is not possible to quantitatively describe which derivative originated from which individual pathway, or even at which percentile amount, due to the large variety of individually consumed food sources and individual enzymatic pathways present in humans[49–51].

Interestingly, some studies have reported that blood and tissue concentrations of active vitamin A retinoids differ significantly between disease and health state (reviewed in ref.[52]). These results raise the question as to whether the differences in such levels are caused by the disease or whether low intake of carotenoids has led to the development of these conditions. It appears that, at least in inflammation-related diseases, vitamin A active compounds are often less abundant in plasma, likely as a consequence and not as a cause of the disease[52], as a potential feedback to counteract inflammation mediated by bioactive vitamin A derivatives induced by pro-inflammatory RAR- and RXR-mediated signalling[53,54].

In many countries, shortage of food and especially vitamin A deficiencies are still common[55], and supplementation with provitamin A carotenoids / vitamin A appears to be a prudent strategy. However, in our Western society, vitamin A intakes are very often quite high, while carotenoid intake is generally lower[56,57]. This has partly been associated with pathophysiological situations[58–63]. Whether increased all-*trans*-retinoic acid

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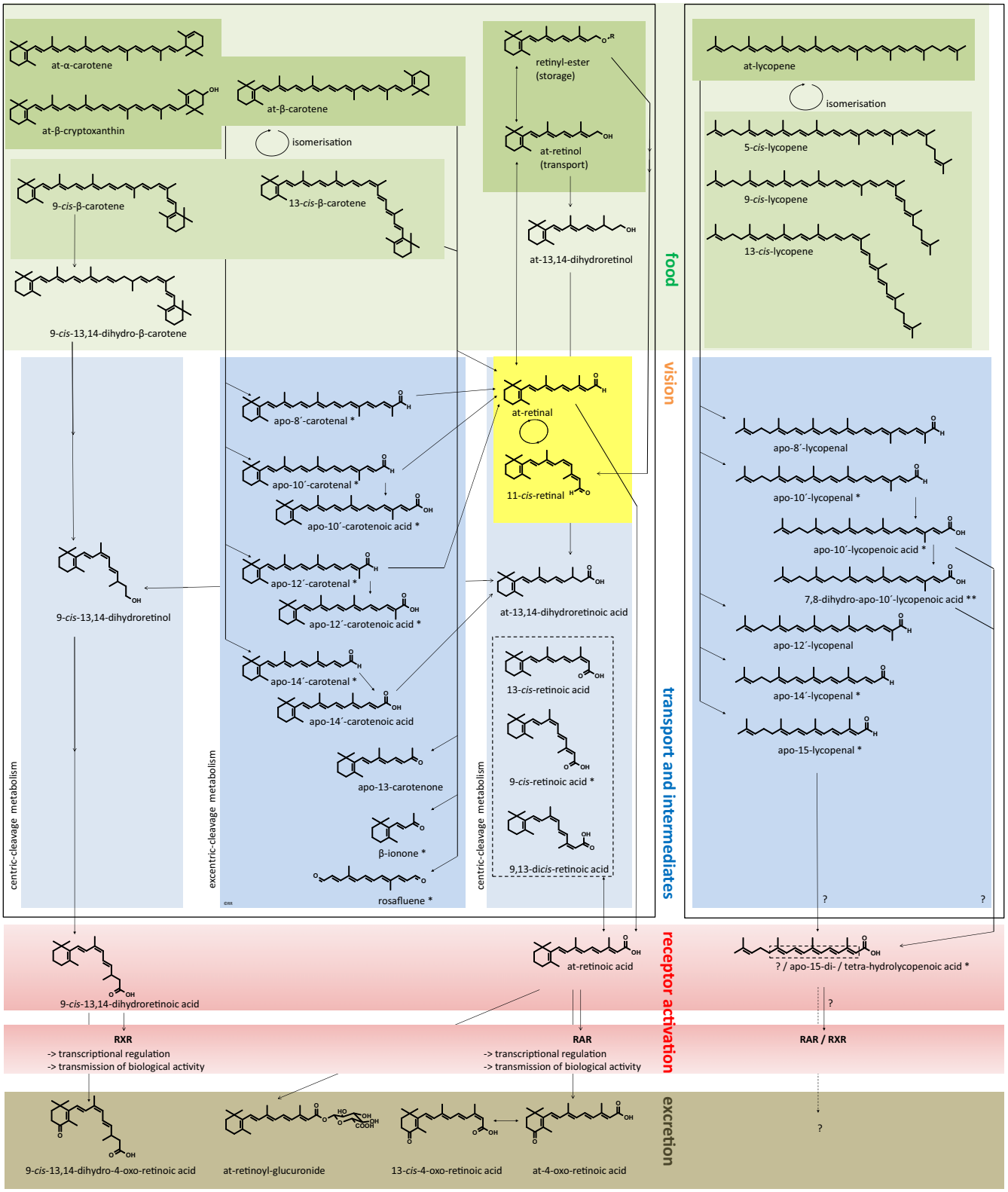

Fig. 1. Metabolic pathway starting from all-*trans*-β-carotene and all-*trans*-lycopene via (a) geometric isomerisation, (b) eccentric cleavage metabolism and (c) centric-cleavage mechanisms. Starting from food, towards transport and intermediate derivatives, nuclear hormone receptor activating ligands including further regulation of transcription and thereby major mediation of biological signalling of carotenoids and further deactivation / excretion metabolites. Arrows in the figure indicate potential and simplified metabolic pathways. Derivatives that were not conclusively identified to be present endogenously in humans were marked with a star (*) and represent derivatives which that were suggested as metabolites and identified in *in vitro* or *in vivo* experimental approaches. Additional derivatives, which were predicted based on analytical studies, were indicated by two starts (**). Abbreviations: AT: all-*trans*-, RAR: retinoic acid receptor, RXR: retinoid-X receptor.

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Table 1. Concentrations of carotenoids in various tissues, all data in nM (nmol/kg or L), adapted from ref.[97]

Tissue	BCAR	ACAR	BCRY	LYC	PHYE	PHYF	Ref.
Serum / plasma	360 ± 10	120 ± 10	230 ± 10	740 ± 10	40 ± 20	170 ± 70	(159)
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			(160,161)
Abdominal adipose tissue	1472 ± 286	280 ± 74	417 ± 462	3329 ± 448			(162)
Liver	5900 ± 6300			8400 ± 11 500			(50)
Skin[&]	430 ± 45	95 ± 20	225 ± 35	695 ± 45	320 ± 90	46 ± 20	(163–165)
Lung	350 ± 440	230 ± 270	420 ± 750	570 ± 1110			(166)
Kidney	550 ± 730	300 ± 400	450 ± 1040	620 ± 620			(166)
Brain[$]	10–30		<10				(167)
Adrenals	5600[*] (680–31 830)	1220[*] (110–7520)	660[*] (10–2900)	1900[*] (190–5600)			(168)
Testes	2680[*] (750–4770)	370[*] (140–610)	160[*] (10–290)	4340[*] (410–9380)			(168)
Bone	745 ± 95	95 ± 35	125 ± 35	280 ± 35	825 ± 185	275 ± 45	(165)
Colon tissue	60 ± 30						(169)
Breast milk	60–200	20–40	2–10	5–25			(170)
Uterus	503[£]	870					(171)
Prostate	600[**]		300	100	700		(172)

All values represent mean ± SD; 'blank' represents non-determined carotenoids or no data available.
[$], infants, prefrontal cortex, frontal cortex, hippocampus, auditory cortex and occipital cortex.
[£], values given in literature as 'carotenes'.
[&], dermis and epidermis of back, forehead, inner forearm and hand.
[*], including upper and lower level of this range.
[**], indicates a value as a potential sum of BCAR and ACAR.
ACAR, α-carotene; BCAR, β-carotene; BCRY, β-cryptoxanthin; LYC, lycopene; PHYE, phytoene; PHYF, phytofluene; CBC: *cis*-β-carotene; CLC: *cis*-lycopene; ATLYC: all-*trans*-lycopene; ATBC: all-*trans* β-carotene.

(ATRA) concentrations in plasma or tissue following carotenoid supplementation are purely beneficial has thus been the subject of controversial discussion[64–67]. The lipid hormone ATRA has been described to be associated with cell differentiation, proliferation and apoptosis with beneficial relevance mainly for cancer prevention[68,69], and various diseases related to reduced inflammatory competence[70,71]. Unfortunately, ATRA has also been associated with toxic effects, especially embryonic toxicity[72,73].

Recently, ATRA has been discussed more controversially in the context of diabetes, obesity, allergies and osteoporosis[74–76]. Especially the adverse effects of retinoids regarding inflammatory processes, related to many diseases in Western societies, and alteration of local and systemic lipid metabolism and homoeostasis are regarded as critical[73,77,78]. Therefore, it must be carefully evaluated whether retinoid / carotenoid supplementation in such countries is generally beneficial.

General properties of metabolites originating from β-carotene and β-cryptoxanthin

When focusing on β-carotene, we may obtain a large variety of known and yet unknown, although partly postulated, metabolites (Fig. 1). In this chapter, β-carotene isomers such as α- or γ-isoforms of carotene, geometric isomers of these carotenes, were included, as well as the provitamin A carotenoid β-cryptoxanthin as a relevant precursor for the carotenoid metabolites addressed later in this subchapter (Fig. 1 and Table 1).

Firstly, several chain-modified carotenoid metabolites have been identified, including in mammals and human serum, with epoxy-, oxo- and hydroxyl-containing functional groups located at the cyclohexenyl ring or at the polyene chain, as well as additional isomers[79,80]. Whether these metabolites originate from plant-based metabolism or from mammalian endogenous metabolism is not always obvious. Concentrations of these potential metabolites, which are usually lower than those of their parent direct / indirect nutritional precursor all-*trans*-β-carotene, are rarely reported. Precise quantification presents a challenge, owing to the lack of commercially available standards and also lower UV–Vis sensitivity.

Several similar compounds may also be generated during digestion or food processing[80]. For example, upon gastrointestinal exposure to oxidising agents, such as iron, a large variety of degradation products in the intestine have been reported, including several β-apo-carotenals[81], epoxides and diketones[82]. On the other hand, many reports have stated that carotenoids from plant matrices remain relatively stable upon *in vitro* digestion, as demonstrated for β-carotene[83], lutein[84] and lycopene[85]. Whether such degradation products can be absorbed, and whether they are then further metabolised *in vivo*, remains unknown[81].

Various apo-carotenoids originating from excentric cleavage of carotenoids were identified in mammals and partly in the human organism after carotenoid supplementation[86,87]. Both BCO1 and BCO2 appear able to cleave β-carotene. While

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Table 2. Levels of major carotenoid metabolites / retinoids in blood plasma / serum and tissues including molecular weight in Dalton (Da) and molecular formula of each retinoid

Carotenoid metabolite	Parent carotenoid	Serum / tissue	Metabolite concentration in ng/g (mL)	in nM	Remark	Ref.	Occurrence
all-*trans*-retinoic acid ($C_{20}H_{28}O_2$ / 300·4 Da)	BCAR	Serum	2·8 ± 0·8	9·3 ± 2·6	***	(173)	end.
			1·4 ± 2·3	4·7 ± 7·7	German children	(67)	
			1·2 ± 1·6	4·0 ± 5·3	Turkish children	(67)	
			1·4 ± 0·3	4·7 ± 1·0	***	(35)	
			0·8 ± 0·2	2·7 ± 0·7	***	(174)	
			0·9 ± 0·2	3·0 ± 0·6	Fasted adults	(175)	
			1·2 ± 0·3	4·0 ± 1·0	Before supp.	(37)	
			2·0 ± 0·3	6·7 ± 1·0	After supp. of food rich in BCAR		
		Skin	0·7 / 2·1	2·3 / 7·0	***	(173)	end.
		Pancreas	5·9 ± 2·1	19·7 ± 7·0	***	(176)	end.
		Liver	15·8 ± 8·3	52·7 ± 27·7	***	(35)	end.
9-*cis*-retinoic acid (?*)	BCAR	Serum	(?*) 0·03	(?*) 0·1	Fasted adults	(175)	end.
		Liver	(?*) 0·6 ± 0·2	(?*) 2·0 ± 0·7	***	(35)	end.
13-*cis*-retinoic acid (#)	BCAR	Serum	1·8 ± 1·0	6·0 ± 3·3	***	(35)	end.
			1·1 ± 0·2	3·7 ± 0·7	***	(174)	
			1·2 ± 0·3	3·9 ± 1·0	Fasted adults	(175)	
		Liver	1·5 ± 0·4	5·0 ± 1·3	***	(35)	end.
9,13-di*cis*-retinoic acid (#)		Serum	1·0 ± 0·3	0·3 ± 0·1	Fasted adults	(175)	end.
all-*trans*-13,14-dihydroretinoic acid ($C_{20}H_{30}O_2$ / 302·5 Da)	BCAR	Serum	ecnd	ecnd	***	(117)	end.
		Pancreas	88 ± 72	290 ± 238	***	(113)	end.
9-*cis*-13,14-dihydroretinoic acid	BCAR	Serum	4·8 ± 0·7	15·8 ± 2·3	***	(114,117)	end.
9-*cis*-4-oxo-13,14-dihydroretinoic acid ($C_{20}H_{28}O_3$ / 316·4 Da)	BCAR	Liver	10·3	32·6	***	(35)	end.
all-*trans*-4-oxo-retinoic acid ($C_{20}H_{26}O_3$ / 314·4 Da)	BCAR/$CA_{(1)}$	Serum	0·6 ± 0·3	1·9 ± 0·9	***	(174)	end.
13-*cis*-4-oxo-retinoic acid	BCAR/$CA_{(1)}$	Serum	2·4 ± 1·8	7·6 ± 5·7	***	(35)	end.
all-*trans*-apo-13′-carotenone ($C_{18}H_{27}O_1$ / 255·4 Da)	BCAR	Serum	0·8–1·3	3–5	***	(32)	end.
all-*trans*-apo-14′-carotenoic acid ($C_{22}H_{23}O_2$ / 323·5 Da)	BCAR	Serum	1·3 ± 0·6	4·0 ± 1·9	***	(177)	end.
all-*trans*-retinoyl-glucuronide ($C_{26}H_{36}O_8$ / 476·6 Da)	BCAR	Serum	3·2 ± 1·9	6·8 ± 4·0	***	(178)	end.
all-*trans*-retinol ($C_{20}H_{30}O_1$ / 286·5 Da)	BCAR	Serum	510 ± 217	1777 ± 759	***	(173)	end.
			980 ± 110	2233 ± 345	***	(35)	
			641 ± 99	3414 ± 383	***	(174)	
		Skin	207 / 253	724 / 885	***	(173)	end.
		Pancreas	96 ± 86	336 ± 301	***	(176)	end.
all-*trans*-3,4-dehydroretinol ($C_{20}H_{28}O_1$ / 288·5 Da)	BCAR	Skin	9–70	31·2–240	**, ***	(179)	end.
all-*trans*-13,14-dihydroretinol ($C_{20}H_{32}O_1$ / 284·4 Da)	BCAR	Serum	ecnd	ecnd	***	(117)	end.
9-*cis*-13,14-dihydroretinol	BCAR	Serum	0·9	3·1	***	(117)	end.
all-*trans*-retinal ($C_{20}H_{28}O_1$ / 284·4 Da)	BCAR	Eye	ecnd	ecnd	***	(103)	end.
11-*cis*-retinal	BCAR	Eye	ecnd	ecnd	***	(103)	end.
apo-6′-lycopenal ($C_{32}H_{42}O_1$ / 443·1 Da)	LYC	Plasma	<0·1 ± <0·0	0·1 ± 0·1	After tomato supp. diet	(125)	After supp.
apo-8′-lycopenal ($C_{30}H_{40}O_1$ / 416·6 Da)	LYC	Plasma	0·2 ± 0·1	0·6 ± 0·3	"	(125)	After supp.
apo-10′-lycopenal ($C_{27}H_{36}O_1$ / 376·6 Da)	LYC	Plasma	0·1 ± <0·0	0·3 ± 0·1	"	(125)	After supp.
apo-12′-lycopenal ($C_{25}H_{34}O_1$ / 350·1 Da)	LYC	Plasma	0·2 ± 0·1	0·7 ± 0·4	"	(125)	After supp.
apo-14′-lycopenal ($C_{22}H_{30}O_1$ / 310·5 Da)	LYC	Plasma	0·03 ± <0·0	0·1 ± <0·0	"	(125)	After supp.

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Table 2. (*Continued*)

Carotenoid metabolite	Parent carotenoid	Serum / tissue	Metabolite concentration in ng/g (mL)	in nM	Remark	Ref.	Occurrence
References: just in mouse							
all-*trans*-retinal ($C_{20}H_{28}O_1$ / 284·4 Da)	BCAR	Serum	0·6	~2		(180)	Just mouse
			9·1 ± 1·8	32·2 ± 6·2		(181)	
		WAT	~8·5–11·4	~30–40		(180)	Just mouse
			17·9 ± 1·4	63 ± 5		(181)	
all-*trans*-retinol	BCAR	Serum	257 ± 31	900 ± 110	****	(181)	Just mouse
			170 ± 10	595 ± 35		(35)	
9-*cis*-retinol	BCAR	Serum	8·6 ± 2·9	30 ± 10		(181)	Just mouse
13-*cis*-retinol	BCAR	Serum	11·4 ± 2·9	40 ± 10		(181)	Just mouse
dihydro-apo-10′-lycopenoic acid ($C_{27}H_{38}O_2$ / 394·6 Da)	LYC	WAT	?	?	*****	(130)	Just mouse

* , likely just an isomerisation product of ATRA during sample preparation.
** , present in different concentrations in different zones of the human skin.
*** , healthy adults.
**** , all-*trans*-retinol levels in mouse are just used as reference for comparison with 9-*cis*- and 13-*cis*-retinol levels, which were just determined in mouse serum and not in humans.
***** , derivatives which were predicted by analytical studies.
# , 9,13-di*cis*- and 13-*cis*-retinoic acid usually co-elute during HPLC separation and are not identified separately in many described studies.
(1): 4-oxo-retinoic acid was described as an *in vitro* metabolite of canthaxanthin[182].
BCAR, β-carotene; LYC, lycopene; CA, canthaxanthin; WAT, white adipose tissue; end., endogenous; supp., supplementation; ecnd, exact concentration was not determined.

BCO1 appears to favour full-length provitamin A carotenoids resulting in centric cleavage, BCO2 appears to cleave both provitamin A carotenoids and xanthophylls excentrically[40,41,88] and is induced in BCO1$^{-/-}$ mice adipose tissue, leading to β-apo-10'-carotenol accumulation[89]. It is possible that some of these metabolites are themselves substrates for BCO1/2, as indicated for β-apo-8′-carotenal, β-apo-10′-carotenal, β-apo-12′-carotenal and β-apo-14′-carotenal in chicken and rats[86,90,91]. Unfortunately, as already outlined, a clear ordination as to which individual carotenoid metabolite is created by which specific individual metabolic pathway with specific substrate / product derivatives is not possible due to the large diversity of food sources and individual human enzymatic pathways[49,92]. This large variety of food sources with individual carotenoid-metabolite precursors and endogenous enzymatic pathways is an important feature of the mammalian organism[49,92]. It entails the use of various regionally and timely restricted available food sources to create and degrade ligands for nuclear hormone receptors to enable normal healthy biological functions. This also includes auto-regulative metabolic and uptake pathways to regulate ligand creation and degradation, as exemplified and described in detail in a review relevant for β-carotene[49], outlined in Fig. 1.

Different apo-carotenals and apo-carotenoic acids of various chain lengths were found after β-carotene supplementation[86,87]. These were then synthesised *ex vivo* and further studied in molecular biological experiments, and partly identified after direct supplementation of β-carotene and food items rich in β-carotene. These described apo-carotenoids are of different chain lengths, ranging from apo-8′-, apo-10′-, apo-12′- and apo-14′-carotenals, and can further be oxidised to apo-carotenoic acids (Fig. 1). Contrarily, endogenously produced levels have rarely been described, only for selected derivatives[93]. Following the ingestion of tomato juice high in β-carotene (360 ml, 30 mg β-carotene, 35 μg apo-carotenoids per day), only apo-10′- and 12′-carotenal were reported to be detected in plasma of some individuals under the set quantification limit, though unfortunately without any added visualised analytical confirmation[94]. It could not be distinguished whether these were absorbed or formed *de novo in vivo*[81,94]. In a study by Kopec *et al.*[81], (^{13}C-10)-β-carotene was administered to healthy subjects. Though non-symmetrical β-apo-carotenals were found in the gut, none was observed in the plasma TRL fraction, suggesting a low bioavailability.

Following excentric cleavage, shorter products such as β-ionone, β-cyclocitral and related derivatives have been described *in vitro* as well as in animals[41,95]. Carotenoid metabolites, originating from two-side carotenoid cleavage, were also described as carotenedials *in vitro*, including rosafluene and crocetindial[45], but these have not yet been identified *in vivo* and thereby were also not further investigated regarding physiologically relevant nuclear hormone-mediated signalling.

Finally, and possibly most important for the biological activity of carotenoids, centric-cleavage metabolites have been described. These metabolites of β-carotene, α-carotene and β-cryptoxanthin are the apo-15-carotenoic acids, termed retinoic acids[96]. Retinoic acids are well-known endogenous derivatives, functioning as lipid hormone receptor ligands, responsible for activating two major families of nuclear hormone receptors, that is, the RARs and RXRs. These receptors can, following ligand activation, modify transcription of receptor-specific genes[22,23]. The major products are retinoic acids, mainly in the form of ATRA, the endogenous ligand of the RARs (RARα, β, γ), as reviewed previously[31]. Endogenous levels of ATRA in serum / plasma were in the range of 0·8–2·8 ng/ml (2·7–9·3 nM) and up to 6 ng/g (20 nM) in the pancreas and 16 ng/g (53 nM) in the liver (Table 2). Thus, these concentrations are at least one to two magnitudes lower than those of β-carotene in the bloodstream, with concentrations of approximately 0·1–2 μM (Table 1[97]). While these centric cleavage products are the main activators of RARs and RXRs[38,39], the excentric apo-carotenoid apo-13-carotenone is present at lower endogenous levels of

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0·8–1·3 ng/ml (3–5 nM) and has been demonstrated to act as 'antagonist' or low-affinity partial agonist or competitive antagonist, but the physiological and nutritional relevance is not yet known[32,87]. The physiological and nutritional relevance of the 'antagonism' / partial agonist activity was never convincingly determined for humans, though in *in vitro* experiments, with weak and questionable prediction potential for humans, but is deemed plausible when considering endogenous concentrations in human serum (3–5 nM, Table 2 and Fig. 1).

In addition to ATRA, other geometric isomers were identified endogenously, such as 13-*cis*-, 9,13-di*cis*- and 9-*cis*-retinoic acid[98–100], with low concentrations (Table 2). A large focus was placed on 9-*cis*-retinoic acid (9CRA), which was postulated as 'an' or even 'the' endogenous ligand of RXRs (RXRα, β, γ)[29,30]. However, this is seen as controversial by the authors / additional experts in the field of retinoid lipidomics[36,101,102] focusing on ultrasensitive retinoid-lipidomics analysis, as its endogenous presence and function as a physiologically relevant lipid hormone could not be confirmed. Alternative endogenous geometric isomers of retinoic acid, including 13-*cis*-, 9,13-di*cis*- and 11-*cis*-retinoic acid were not described to be of relevant major biological activity mediated via the activation of RARs–RXRs[29]. For retinal, the endogenous cycle between all-*trans*-retinal and 11-*cis*-retinal in the visual cycle in the eye is well established[103,104], but it is of no systemic relevance for the whole human organism.

For ATRA, increased serum levels of 1·2 to 2·0 ng/ml (4·0 to >6·7 nM) were found following supplementation of β-carotene-rich foods[37]. Whether these increased serum levels reflect also tissue levels and increased RAR-mediated signalling was and could not be identified. The physiological and nutritional relevance in humans could also not be evaluated. This intervention with food items rich in β-carotene resulted in low and non-significant alterations of interleukin (IL) secretion and immune response as indicators of RAR-mediated signalling[105,106]. Whether such β-carotene interventions are beneficial for humans is questionable. Interestingly, the strongest effects were identified in the carotenoid wash-out phase prior to intervention, resulting in reduced IL-2, natural killer (NK) cell cytotoxicity and lymphocyte proliferation, a potential consequence of β-carotene (or general carotenoid) or even vitamin A deficiency and possibly reduced RAR–RXR-mediated signalling[106]. These reductions were rapidly recovered after β-carotene or lycopene supplementations, likely as a consequence of recovered RAR–RXR-mediated signalling[106]. In animal studies, β-carotene supplementation resulted in the recovery of vitamin A deficiency indicated by visualised RARE-mediated signalling. In addition, serum, but not liver, ATRA concentrations were improved, while retinol levels recovered and even increased[20]. It can be assumed that β-carotene supplementation can reinstate basal retinol and ATRA concentrations and RAR-mediated signalling. However, no further increase in ATRA concentrations in organs and enhanced RAR-mediated signalling could be observed as a result of increased storage and transport of retinol due to a highly regulated homoeostasis of retinoid / vitamin A / RAR-mediated signalling pathways.

Nutritionally relevant β-carotene intake mainly contributes to the anti-infective properties of vitamin A, which is commonly identified as its major activity besides ocular functions[12,107]. It is suggested that provitamin A carotenoids are relevant for maintaining vitamin A activity, while being of no further physiologically or nutritionally proven relevance.

In contrast, long-term high-dose supplementation of pure synthetic all-*trans*-β-carotene, studied in tobacco-smoke-exposed ferrets, may alter RAR–RXR-mediated signalling by a negative feedback regulation[108], thereby strongly reducing RARβ- and ATRA levels in the lung, as the target organ[109,110].

In addition, it is questionable whether higher-than-basal RAR-mediated signalling is more beneficial or whether it can be considered as detrimental, while increased RXR-mediated signalling may be considered mainly beneficial[25]. On the basis of these limited studies, we conclude that β-carotene can prevent general vitamin A deficiency[37,106], reaching a plateau, while higher and pure β-carotene supplementation seems unrelated to improved health status[49]. It seems unlikely that moderate or even high dietary consumption of natural food items rich in β-carotene and additional bioactive derivatives including other carotenoids has non-beneficial effects.

Recently, dihydro-metabolites of apo-15-carotenoids were described in mice, likely originating from 13,14-dihydroretinol[111] (Fig. 1 and Table 2). In a larger cohort study, 13,14-dihydroretinol and the novel identified endogenous all-*trans*-13,14-dihydroretinoic acid[112,113] and 9-*cis*-13,14-dihydroretinoic acid[33,36,102] were analysed in human serum[114] as well as adipose tissue (Rühl *et al.* unpublished). All-*trans*-13,14-dihydroretinoic acid was described as a medium-affinity endogenous RAR ligand[38,115], and recently, 9-*cis*-13,14-dihydroretinoic acid (9CDHRA) became a focus of attention, as it appears to be 'an' or even 'the' physiologically and nutritionally relevant RXR ligand in mammals, serving as a novel endogenous lipid hormone[33,36,102]. Further nutritionally relevant precursors of 9CDHRA, such as 9-*cis*-13,14-dihydroretinol, 9-*cis*-dihydrocarotenoids and even the well-known 9-*cis*-β-carotene were recently postulated[116] and confirmed[117] as even being a new independent vitamin A signalling pathway, termed vitamin A5 (Fig. 1)[118].

Metabolites of lycopene

In addition to β-carotene, lycopene is one of the major carotenoids present in the diet, resulting in high tissue and blood concentrations (Fig. 1 and Tables 1 and 2). However, the metabolism of lycopene has been studied to a much lesser extent compared with that of β-carotene and especially when focusing on the human situation.

Oxidative metabolism of lycopene and of additional acyclic carotenoids such as phytoene and phytofluene (Table 1) has been described[119], while such metabolism was neither conclusively observed nor the focus in studies employing lutein and other carotenoids with hydroxyl- / oxo-functional groups, such as zeaxanthin, canthaxanthin, β-cryptoxanthin and astaxanthin, which would have broader relevance for the human situation. Selected xanthophylls were described to interact and block apo-carotenoid-mediated signalling[120,121], while

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no mechanism involving xanthophyll-metabolites was mentioned and outlined. Both excentric and centric metabolism was described for lycopene[40,41]. With the exception of lycopenoids, there was no further focus on the identification of potential endogenous derivatives or molecular biological examination to investigate their biological activities[122–124]. Various lycopenals were identified and predicted in the food matrix and in the human organism after a tomato product intervention. Human serum levels were reported to be low (Fig. 1 and Table 2[125]).

While many studies display a complex pattern of lycopene metabolism via various pathways[40,125–129], and potential lycopene metabolites were found after supplementing high amounts of lycopene in experimental animal models[124,130–132], a direct association of human relevance was only recently indirectly concluded[133]. Indirect evidence of lycopene activity and a further lycopene metabolite for RAR activation was revealed, using a RARE-luciferase-expressing mouse model[20,134]. Based on RARE-mediated signalling, a partial vitamin A activity following lycopene intervention was found[20]. An identification of the involved functional metabolites was only partly achieved, and apo-15-lycopenoic acids were claimed to be present endogenously, especially after lycopene supplementation[124,135].

Other lycopenoic acids might also be bioactive, as it was shown previously in a mouse study that the potential lycopene metabolite apo-10′-lycopenoic acid[131] reduced hepatic fat accumulation[136]. The physiological and nutritional relevance of apo-10′-lycopenoic acid was only shown in ferrets[131], but could not be confirmed *in vivo* in mice and *ex vivo* for humans[130]. Alternatively, due to extensive metabolism, a dihydro-apo-10′-lycopenoic acid analogue was identified and on the basis of UV and mass spectrometry characteristics predicted to be 7,8-dihydro-apo-10′-lycopenoic acid. How lycopene is metabolised to dihydro-apo-10′-lycopenoic acid and whether apo-10′-lycopenoic acid is a potential intermediate are yet unanswered questions. These dihydro-apo-10-lycopenoids are likely to be direct precursors of dihydro-apo-15-lycopenoids, which might be highly potent RAR and / or RXR ligands[124].

Summary for carotenoid metabolites

Thus, for many metabolites it remains inconclusive whether they derive from human metabolism or are ingested via animal origin as pre-formed carotenoid metabolites in the forms of retinol and mainly retinyl esters[12,137]. In addition, the biological function and the concentration-dependent activity of various carotenoid metabolites besides ATRA has generally not been studied, mostly due to the lack of available standard compounds and established sensitive and selective analytical methods. Furthermore, the direct link between carotenoid intake and RAR–RXR-mediated transcriptional signalling as a multi-step procedure has not yet been proven. However, each step of this cascade has been clearly demonstrated with experimental data: (a) higher carotenoid supplementation resulting in higher carotenoid levels in supplemented individuals[105,138], (b) higher β-carotene levels correlating and resulting in increased ATRA concentrations[37,49], (c) higher ATRA levels causing increased RAR-mediated signalling[134]; and (d) higher RAR-mediated signalling resulting in increased individual-specific immune responses[52,77,139,140] and altered lipid metabolism[141,142], with partially beneficial or detrimental effects.

Recently, a novel class of bioactive carotenoid metabolites, namely strigolactones, was described to be enzymatically synthesised in certain plants, such as carlactones[143–145] and identified as plant-relevant hormones during germination[143] and branching inhibition[146]. Whether these derivatives are of direct or indirect relevance for the human organism remains speculative.

In summary, human supplementation studies with food items rich in β-carotene / lycopene or supplemented β-carotene / lycopene, focusing on multi-targeted analyses, and identifying β-carotene / lycopene and retinoid concentrations and further RARE-mediated signalling, have not yet been performed and should be addressed. Due to the access of multi-omic techniques, serum markers or novel transcriptional markers of diseases[147,148], possibly also co-associated with vitamin A / carotenoid deficiency or reduced RAR–RXR-mediated dysfunction[25,149], should be compared with carotenoid intake and serum / plasma carotenoid / retinoid concentrations to obtain valuable correlations.

Discussion and perspectives

Several carotenoids are implicated in health-related outcomes, from AMD (lutein and zeaxanthin) to possible effects regarding cardio-metabolic diseases (predominantly, β-carotene) and diabesity / cancer (predominantly, lycopene). The dietary intake of carotenoids has also changed over time. While lycopene intake was uncommon in the pre-industrialised human diet, especially considering the primarily European-focused world view, it strongly increased in Western society, due to a high consumption of tomatoes and tomato products[150].

Additionally, it became obvious that light irradiation[151] and more practically relevant thermal food processing[152], as also reviewed by Khoo *et al.*[153], including cooking >100°C, appears to constitute important mechanisms for carotenoid isomerisation, yielding different precursor carotenoids for different functional apo-carotenoids, as well as non-endogenous human-generated apo-carotenoids, serving as easy accessible substrates for functional apo-carotenoids[154]. This highlights cooking and food processing as important cultural achievement for generating bioactive derivatives for enabling a healthy and well-functioning human organism[155].

However, carotenoids are generally considered as lipid precursors (mainly for bioactive vitamin A / retinoids) in the diet, while their complex and multi-step metabolic pathways and the relationship with health beneficial effects are still poorly understood. In this review, we summarised all available relevant information focusing on the human organism with implication of mechanistic results from further *in vitro* to *in vivo* experiments. Unfortunately, these experimental results are difficult to generalise to humans owing to the non-similar nutri-kinetics pattern of carotenoids[156] and different eating behaviour in humans compared with the pure vegetarian dietary pattern of rodents, which are frequently used as experimental animal models.

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Conclusions

As a cornerstone, we suggest that, besides benchmark concentrations for carotenoids, retinoids should also be considered, including both 'normal' and deficiency threshold ranges. These ranges should correlate with well-defined and established nuclear hormone receptor signalling cascade markers, disease markers, prognostic early markers of diseases and markers of impairments of physiologically important functions based on novel 'omics' markers such as transcriptomics, lipidomics and proteomics, which are now frequently published for various target diseases[157]. In the case of diseases and dysfunctions related to carotenoid and vitamin A deficiency, underlying molecular mechanisms such as RAR–RXR- / RXR-plus additional nuclear hormone receptor (NHR)-dysfunctional signalling[22,25,158] (i.e. signalling not associated with a healthy condition as present in various diseases of Western society) should also be considered.

On the basis of these two ranges, targeted supplementation strategies may be recommended to overcome deficiencies and reach and maintain 'normal' concentration ranges. A correlation between dietary intake, serum levels and bioactive carotenoid metabolites and further examination of RXR–RAR / RXR–NHR in an easily accessible compartment such as peripheral blood mononuclear cells (PBMCs), plus target genes of relevant diseases, are missing in carotenoid / retinoid nutritional research.

The basal benchmark concentration indicating a higher risk for chronic diseases appears to constitute a total carotenoid plasma / serum concentration <1·000 nM and should further focus on endogenous retinoids. The second benchmark concentration reflecting 'normal' carotenoid intake is average plasma / serum concentration of individual and total carotenoids indicating, and here defined as, a healthy varied diet. Such levels can then be translated into the intake of relevant food items rich in carotenoids, based on correlations between reported average intakes for β-carotene and lycopene with serum concentrations and considering intervention with carotenoid-rich foods[97].

In this review article, we summarised the current mechanisms of carotenoid metabolism including reference levels of bioactive carotenoid metabolites with relevance to the human organism. To summarise, elucidation of carotenoid-to-bioactive-metabolite metabolism is important to justify which biological-response pathway of carotenoids is enabled to elicit valuable beneficial effects. This is paramount in order to evaluate if there might be a problem in individual dietary intake of food enriched in specific carotenoids is present or if a genetic hereditary problem in metabolism of carotenoids to bioactive carotenoids based on genetic polymorphisms is the cause of disturbed occurrence of bioactive carotenoid metabolites.

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References

1. Yabuzaki J (2017) Carotenoids Database: structures, chemical fingerprints and distribution among organisms. *Database (Oxford)* **2017**, bax004.
2. Rodriguez-Concepcion M, Avalos J, Bonet ML, *et al.* (2018) A global perspective on carotenoids: metabolism, biotechnology, and benefits for nutrition and health. *Prog Lipid Res* **70**, 62–93.
3. Felemban A, Braguy J, Zurbriggen MD & Al-Babili S (2019) Apocarotenoids involved in plant development and stress response. *Front Plant Sci* **10**, 1168.
4. Harrison EH & Quadro L (2018) Apocarotenoids: emerging roles in mammals. *Annu Rev Nutr* **38**, 153–172.
5. Sluijs I, Cadier E, Beulens JW, van der AD, Spijkerman AM & van der Schouw YT (2015) Dietary intake of carotenoids and risk of type 2 diabetes. *Nutr Metab Cardiovasc Dis* **25**, 376–381.
6. Zhou H, Zhao X, Johnson EJ, *et al.* (2011) Serum carotenoids and risk of age-related macular degeneration in a Chinese population sample. *Invest Ophthalmol Visual Sci* **52**, 4338–4344.
7. Dulińska-Litewka J, Hałubiec P, Łazarczyk A, Szafrański O, Sharoni Y, McCubrey JA, Gąsiorkiewicz B & Bohn T (2021) Recent progress in discovering the role of carotenoids and metabolites in prostatic physiology and pathology – a review – part II: carotenoids in the human studies. *Antioxidants (Basel, Switzerland)* **10**, 319.
8. Zhao LG, Zhang QL, Zheng JL, Li HL, Zhang W, Tang WG & Xiang YB (2016) Dietary, circulating beta-carotene and risk of all-cause mortality: a meta-analysis from prospective studies. *Sci Rep* **6**, 26983.
9. Krinsky NI & Johnson EJ (2005) Carotenoid actions and their relation to health and disease. *Mol Aspects Med* **26**, 459–516.
10. Bohn T (2019) Carotenoids and markers of oxidative stress in human observational studies and intervention trials – implications for chronic diseases. Antioxidants 8, pii: E179.
11. Bonet ML, Canas JA, Ribot J & Palou A (2016) Carotenoids in adipose tissue biology and obesity. *Subcell Biochem* **79**, 377–414.
12. Rühl R (2007) Effects of dietary retinoids and carotenoids on immune development. *Proc Nutr Soc* **66**, 458–469.
13. Mounien L, Tourniaire F & Landrier J-F (2019) Anti-obesity effect of carotenoids: direct impact on adipose tissue and adipose tissue-driven indirect effects. *Nutrients* **11**, 1562.
14. Amengual J, Widjaja-Adhi MA, Rodriguez-Santiago S, Hessel S, Golczak M, Palczewski K & von Lintig J (2013) Two carotenoid oxygenases contribute to mammalian provitamin A metabolism. *J Biol Chem* **288**, 34081–34096.
15. Linnewiel K, Ernst H, Caris-Veyrat C, Ben-Dor A, Kampf A, Salman H, Danilenko M, Levy J & Sharoni Y (2009) Structure activity relationship of carotenoid derivatives in activation of the electrophile/antioxidant response element transcription system. *Free Radic Biol Med* **47**, 659–667.
16. Linnewiel-Hermoni K, Motro Y, Miller Y, Levy J & Sharoni Y (2014) Carotenoid derivatives inhibit nuclear factor kappa B activity in bone and cancer cells by targeting key thiol groups. *Free Radic Biol Med* **75**, 105–120.

https://doi.org/10.1017/S095442242200021X Published online by Cambridge University Press

17. Nidhi B, Sharavana G, Ramaprasad TR & Vallikannan B (2015) Lutein derived fragments exhibit higher antioxidant and anti-inflammatory properties than lutein in lipopolysaccharide induced inflammation in rats. *Food Funct* **6**, 450–460.
18. Farkhondeh T & Samarghandian S (2014) The effect of saffron (*Crocus sativus* L.) and its ingredients on the management of diabetes mellitus and dislipidemia. *Afr J Pharm Pharmacol* **8**, 541–549.
19. Bruzzone S, Ameri P, Briatore L, *et al.* (2012) The plant hormone abscisic acid increases in human plasma after hyperglycemia and stimulates glucose consumption by adipocytes and myoblasts. *FASEB J* **26**, 1251–1260.
20. Aydemir G, Kasiri Y, Bartok EM, Birta E, Frohlich K, Bohm V, Mihaly J & Rühl R (2016) Lycopene supplementation restores vitamin A deficiency in mice and possesses thereby partial pro-vitamin A activity transmitted via RAR signaling. *Mol Nutr Food Res* **60**, 2413–2420.
21. Dingeo G, Brito A, Samouda H, La Frano MR & Bohn T (2020) Phytochemicals as modifiers of gut microbial communities. *Food Function* **11**, 8444–8471.
22. Evans RM & Mangelsdorf DJ (2014) Nuclear receptors, RXR, and the big bang. *Cell* **157**, 255–266.
23. Mangelsdorf DJ, Thummel C, Beato M, *et al.* (1995) The nuclear receptor superfamily: the second decade. *Cell* **83**, 835–839.
24. Balmer JE & Blomhoff R (2002) Gene expression regulation by retinoic acid. *J Lipid Res* **43**, 1773–1808.
25. Desvergne B (2007) RXR: from partnership to leadership in metabolic regulations. *Vitam Horm* **75**, 1–32.
26. Tourniaire F, Musinovic H, Gouranton E, *et al.* (2015) All-*trans* retinoic acid induces oxidative phosphorylation and mitochondria biogenesis in adipocytes. *J Lipid Res* **56**, 1100–1109.
27. Karkeni E, Bonnet L, Astier J, Couturier C, Dalifard J, Tourniaire F & Landrier JF (2017) All-trans-retinoic acid represses chemokine expression in adipocytes and adipose tissue by inhibiting NF-κB signaling. *J Nutr Biochem* **42**, 101–107.
28. Mangelsdorf DJ, Ong ES, Dyck JA & Evans RM (1990) Nuclear receptor that identifies a novel retinoic acid response pathway. *Nature* **345**, 224–229.
29. Levin AA, Sturzenbecker LJ, Kazmer S, *et al.* (1992) 9-*cis* retinoic acid stereoisomer binds and activates the nuclear receptor RXR alpha. *Nature* **355**, 359–361.
30. Heyman RA, Mangelsdorf DJ, Dyck JA, Stein RB, Eichele G, Evans RM & Thaller C (1992) 9-cis retinoic acid is a high affinity ligand for the retinoid X receptor. Cell 68, 397–406.
31. Petkovich M, Brand NJ, Krust A & Chambon P (1987) A human retinoic acid receptor which belongs to the family of nuclear receptors. *Nature* **330**, 444–450.
32. Eroglu A, Hruszkewycz DP, dela Sena C, *et al.* (2012) Naturally occurring eccentric cleavage products of provitamin A beta-carotene function as antagonists of retinoic acid receptors. *J Biol Chem* **287**, 15886–15895.
33. Krezel W, Rühl R & de Lera AR (2019) Alternative retinoid X receptor (RXR) ligands. *Mol Cell Endocrinol* **491**, 110436.
34. Schierle S & Merk D (2019) Therapeutic modulation of retinoid X receptors – SAR and therapeutic potential of RXR ligands and recent patents. *Expert Opin Ther Pat* **29**, 605–621.
35. Schmidt CK, Brouwer A & Nau H (2003) Chromatographic analysis of endogenous retinoids in tissues and serum. *Anal Biochem* **315**, 36–48.
36. de Lera AR, Krezel W & Rühl R (2016) An endogenous mammalian retinoid X receptor ligand, at last! *ChemMedChem* **11**, 1027–1037.
37. Rühl R, Bub A & Watzl B (2008) Modulation of plasma all-trans retinoic acid concentrations by the consumption of carotenoid-rich vegetables. *Nutrition* **24**, 1224–1226.
38. Allenby G, Bocquel MT, Saunders M, *et al.* (1993) Retinoic acid receptors and retinoid X receptors: interactions with endogenous retinoic acids. *Proc Natl Acad Sci USA* **90**, 30–34.
39. Allenby G, Janocha R, Kazmer S, Speck J, Grippo JF & Levin AA (1994) Binding of 9-*cis*-retinoic acid and all-*trans*-retinoic acid to retinoic acid receptors alpha, beta, and gamma. Retinoic acid receptor gamma binds all-*trans*-retinoic acid preferentially over 9-*cis*-retinoic acid. *J Biol Chem* **269**, 16689–16695.
40. dela Sena C, Narayanasamy S, Riedl KM, Curley RW Jr, Schwartz SJ, Harrison EH (2013) Substrate specificity of purified recombinant human beta-carotene 15,15′-oxygenase (BCO1). *J Biol Chem* **288**, 37094–37103.
41. dela Sena C, Sun J, Narayanasamy S, Riedl KM, Yuan Y, Curley RW Jr, Schwartz SJ & Harrison EH (2016) Substrate specificity of purified recombinant chicken beta-carotene 9′,10′-oxygenase (BCO2). *J Biol Chem* **291**, 14609–14619.
42. Barua AB & Olson JA (1986) Retinoyl beta-glucuronide: an endogenous compound of human blood. *Am J Clin Nutr* **43**, 481–485.
43. Sass JO, Forster A, Bock KW & Nau H (1994) Glucuronidation and isomerization of all-*trans*- and 13-*cis*-retinoic acid by liver microsomes of phenobarbital- or 3-methylcholanthrene-treated rats. *Biochem Pharmacol* **47**, 485–492.
44. Radominska A, Little JM, Lehman PA, Samokyszyn V, Rios GR, King CD, Green MD & Tephly TR (1997) Glucuronidation of retinoids by rat recombinant UDP: glucuronosyltransferase 1.1 (bilirubin UGT). *Drug Metab Dispos* **25**, 889–892.
45. Amengual J, Lobo GP, Golczak M, Li HN, Klimova T, Hoppel CL, Wyss A, Palczewski K & von Lintig J (2011) A mitochondrial enzyme degrades carotenoids and protects against oxidative stress. *FASEB J* **25**, 948–959.
46. von Lintig J & Vogt K (2000) Filling the gap in vitamin A research. Molecular identification of an enzyme cleaving beta-carotene to retinal. *J Biol Chem* **275**, 11915–11920.
47. Kiefer C, Hessel S, Lampert JM, Vogt K, Lederer MO, Breithaupt DE & von Lintig J (2001) Identification and characterization of a mammalian enzyme catalyzing the asymmetric oxidative cleavage of provitamin A. *J Biol Chem* **276**, 14110–14116.
48. Caris-Veyrat C, Schmid A, Carail M & Böhm V (2003) Cleavage products of lycopene produced by in vitro oxidations: characterization and mechanisms of formation. *J Agric Food Chem* **51**, 7318–7325.
49. Bohn T, Desmarchelier C, El SN, Keijer J, van Schothorst E, Rühl R & Borel P (2019) beta-Carotene in the human body: metabolic bioactivation pathways – from digestion to tissue distribution and excretion. *Proc Nutr Soc* **78**, 68–87.
50. Bohn T, Desmarchelier C, Dragsted LO, Nielsen CS, Stahl W, Rühl R, Keijer J & Borel P (2017) Host-related factors explaining interindividual variability of carotenoid bioavailability and tissue concentrations in humans. *Mol Nutr Food Res* **61**, 1600685.
51. Bohn T, Bonet ML, Borel P, *et al.* (2021) Mechanistic aspects of carotenoid health benefits – where are we now? *Nutr Res Rev* **34**, 276–302.
52. Rubin LP, Ross AC, Stephensen CB, Bohn T & Tanumihardjo SA (2017) Metabolic effects of inflammation on vitamin A and carotenoids in humans and animal models. *Adv Nutr* **8**, 197–212.

https://doi.org/10.1017/S095442242200021X Published online by Cambridge University Press

53. Al Senaidy AM (2009) Serum vitamin A and beta-carotene levels in children with asthma. *J Asthma* **46**, 699–702.
54. Nogueira C, Borges F, Lameu E, Franca C, Rosa CL & Ramalho A (2015) Retinol, beta-carotene and oxidative stress in systemic inflammatory response syndrome. *Rev Assoc Med Bras (1992)* **61**, 116–120.
55. Akhtar S, Ahmed A, Randhawa MA, Atukorala S, Arlappa N, Ismail T & Ali Z (2013) Prevalence of vitamin A deficiency in South Asia: causes, outcomes, and possible remedies. *J Health Popul Nutr* **31**, 413–423.
56. EFSA Panel on Dietetic Products N, and Allergies (NDA), European Food Safety Authority (EFSA) (2015) Scientific opinion on detary reference values for vitamin A. *EFSA J* **13**, 4028.
57. Biehler E, Alkerwi A, Hoffmann L, Krause E, Guillaume M, Lair ML & Bohn T (2012) Contribution of violaxanthin, neoxanthin, phytoene and phytofluene to total carotenoid intake: assessment in Luxembourg. *J Food Comp Anal* **25**, 56–65.
58. Aage S, Kiraly N, Da Costa K, Byberg S, Bjerregaard-Andersen M, Fisker AB, Aaby P & Benn CS (2015) Neonatal vitamin A supplementation associated with increased atopy in girls. *Allergy* **70**, 985–994.
59. Rühl R (2013) Non-pro-vitamin A and pro-vitamin A carotenoids in atopy development. *Int Arch Allergy Immunol* **161**, 99–115.
60. Milner JD, Stein DM, McCarter R & Moon RY (2004) Early infant multivitamin supplementation is associated with increased risk for food allergy and asthma. *Pediatrics* **114**, 27–32.
61. Michaelsson K, Lithell H, Vessby B & Melhus H (2003) Serum retinol levels and the risk of fracture. *N Engl J Med* **348**, 287–294.
62. Ruiz-Castell M, Le Coroller G, Landrier JF, Kerkour D, Weber B, Fagherazzi G, Appenzeller BMR, Vaillant M & Bohn T (2020) Micronutrients and markers of oxidative stress and inflammation related to cardiometabolic health: results from the EHES-LUX study. *Nutrients* **13**, 5.
63. Crandall C (2004) Vitamin A intake and osteoporosis: a clinical review. *J Womens Health (Larchmt)* **13**, 939–953.
64. Mihaly J, Gericke J, Lucas R, de Lera AR, Alvarez S, Torocsik D & Rühl R (2016) TSLP expression in the skin is mediated via RARgamma-RXR pathways. *Immunobiology* **221**, 161–165.
65. Rühl R, Taner C, Schweigert FJ, Wahn U & Gruber C (2010) Serum carotenoids and atopy among children of different ethnic origin living in Germany. *Pediatr Allergy Immunol* **21**, 1072–1075.
66. Gericke J, Ittensohn J, Mihaly J, Dubrac S & Rühl R (2013) Allergen-induced dermatitis causes alterations in cutaneous retinoid-mediated signaling in mice. *PLoS ONE* **8**, e71244.
67. Gruber C, Taner C, Mihaly J, Matricardi PM, Wahn U & Rühl R (2012) Serum retinoic acid and atopy among children of different ethnic origin living in Germany. *J Pediatr Gastroenterol Nutr* **54**, 558–560.
68. Wei S, Kozono S, Kats L, *et al.* (2015) Active Pin1 is a key target of all-trans retinoic acid in acute promyelocytic leukemia and breast cancer. *Nat Med* **21**, 457–466.
69. Connolly RM, Nguyen NK & Sukumar S (2013) Molecular pathways: current role and future directions of the retinoic acid pathway in cancer prevention and treatment. *Clin Cancer Res* **19**, 1651–1659.
70. Tan X, Sande JL, Pufnock JS, Blattman JN & Greenberg PD (2011) Retinoic acid as a vaccine adjuvant enhances $CD8^+$ T cell response and mucosal protection from viral challenge. *J Virol* **85**, 8316–8327.
71. Saiag P, Pavlovic M, Clerici T, Feauveau V, Nicolas JC, Emile D & Chastang C (1998) Treatment of early AIDS-related Kaposi's sarcoma with oral all-*trans*-retinoic acid: results of a sequential non-randomized phase II trial. Kaposi's Sarcoma ANRS Study Group. Agence Nationale de Recherches sur le SIDA. Aids 12, 2169–2176.
72. Tzimas G & Nau H (2001) The role of metabolism and toxicokinetics in retinoid teratogenesis. *Curr Pharm* **7**(9), 803–831.
73. David M, Hodak E & Lowe NJ (1988) Adverse effects of retinoids. *Med Toxicol Adverse Drug Exp* **3**, 273–288.
74. Govind Babu K, Lokesh KN, Suresh Babu MC & Bhat GR (2016) Acute coronary syndrome manifesting as an adverse effect of all-*trans*-retinoic acid in acute promyelocytic leukemia: a case report with review of the literature and a spotlight on management. *Case Rep Oncol Med* **2016**, 9.
75. Henning P, Conaway HH & Lerner UH (2015) Retinoid receptors in bone and their role in bone remodeling. *Front Endocrinol (Lausanne)* **6**, 31.
76. Trasino SE & Gudas LJ (2015) Vitamin A: a missing link in diabetes? *Diabetes Manag (Lond)* **5**, 359–367.
77. Rühl R, Garcia A, Schweigert FJ & Worm M (2004) Modulation of cytokine production by low and high retinoid diets in ovalbumin-sensitized mice. *Int J Vitam Nutr Res* **74**, 279–284.
78. Wansley DL, Yin Y & Prussin C (2013) The retinoic acid receptor-alpha modulators ATRA and Ro415253 reciprocally regulate human IL-5^+ Th2 cell proliferation and cytokine expression. *Clin Mol Allergy* **11**, 4.
79. Khachik F, Spangler CJ, Smith JC Jr, Canfield LM, Steck A & Pfander H (1997) Identification, quantification, and relative concentrations of carotenoids and their metabolites in human milk and serum. *Anal Chem* **69**, 1873–1881.
80. Britton G, Liaaen-Jensen S & Pfander H (2008) *Carotenoids handbook*. Basel: Birkhäuser.
81. Kopec RE, Caris-Veyrat C, Nowicki M, Gleize B, Desmarchelier C & Borel P (2018) Production of asymmetric oxidative metabolites of ^{13}C β-carotene during digestion in the gastrointestinal lumen of healthy men. *Am J Clin Nutr* **108**, 803–813.
82. Sy C, Dangles O, Borel P & Caris-Veyrat C (2013) Iron-induced oxidation of (all-E)-beta-carotene under model gastric conditions: kinetics, products, and mechanism. *Free Radical Biol Med* **63**, 195–206.
83. Ferruzzi MG, Lumpkin JL, Schwartz SJ & Failla M (2006) Digestive stability, micellarization, and uptake of beta-carotene isomers by Caco-2 human intestinal cells. *J Agric Food Chem* **54**, 2780–2785.
84. Granado-Lorencio F, Olmedilla-Alonso B, Herrero-Barbudo C, Perez-Sacristan B, Blanco-Navarro I & Blazquez-Garcia S (2007) Comparative *in vitro* bioaccessibility of carotenoids from relevant contributors to carotenoid intake. *J Agric Food Chem* **55**, 6387–6394.
85. Richelle M, Sanchez B, Tavazzi I, Lambelet P, Bortlik K & Williamson G (2010) Lycopene isomerisation takes place within enterocytes during absorption in human subjects. *Br J Nutr* **103**, 1800–1807.
86. Eroglu A & Harrison EH (2013) Carotenoid metabolism in mammals, including man: formation, occurrence, and function of apocarotenoids. *J Lipid Res* **54**, 1719–1730.
87. Harrison EH, dela Sena C, Eroglu A & Fleshman MK (2012) The formation, occurrence, and function of beta-apocarotenoids: beta-carotene metabolites that may modulate nuclear receptor signaling. *Am J Clin Nutr* **96**, 1189s–1192s.
88. Bandara S, Thomas LD, Ramkumar S, Khadka N, Kiser PD, Golczak M & von Lintig J (2021) The structural and

https://doi.org/10.1017/S095442242200021X Published online by Cambridge University Press

biochemical basis of apocarotenoid processing by β-carotene oxygenase-2. *ACS Chem Biol* **16**, 480–490.
89. Amengual J, Gouranton E, van Helden YG, *et al.* (2011) Beta-carotene reduces body adiposity of mice via BCMO1. *PLoS ONE* **6**, e20644.
90. Kim YS & Oh DK (2010) Biotransformation of carotenoids to retinal by carotenoid 15,15′-oxygenase. *Appl Microbiol Biotechnol* **88**, 807–816.
91. Harrison EH (2022) Carotenoids, β-apocarotenoids, and retinoids: the long and the short of it. Nutrients 14, 1411.
92. Böhm V, Lietz G, Olmedilla-Alonso B, *et al.* (2021) From carotenoid intake to carotenoid blood and tissue concentrations – implications for dietary intake recommendations. *Nutr Rev* **79**, 544–573.
93. Ho CC, de Moura FF, Kim S-H & Clifford AJ (2007) Excentral cleavage of β-carotene in vivo in a healthy man. *Am J Clin Nutr* **85**, 770–777.
94. Cooperstone JL, Riedl KM, Cichon MJ, Francis DM, Curley RW, Schwartz SJ, Novotny JA & Harrison EH (2017) Carotenoids and apo-carotenoids in human plasma after continued consumption of high β-carotene or high lycopene tomato juice. *FASEB J* **31** (1 Supplement), 635.613.
95. Sommerburg O, Langhans CD, Arnhold J, *et al.* (2003) Beta-carotene cleavage products after oxidation mediated by hypochlorous acid – a model for neutrophil-derived degradation. *Free Radic Biol Med* **35**, 1480–1490.
96. von Lintig J & Wyss A (2001) Molecular analysis of vitamin A formation: cloning and characterization of beta-carotene 15,15′-dioxygenases. *Arch Biochem Biophys* **385**, 47–52.
97. Böhm V, Borel P, Corte-Real J, *et al.* (2021) From carotenoid intake to carotenoid blood and tissue concentrations – implications for dietary intake recommendations. *Nutr Rev* **79**, 544–573.
98. Horst RL, Reinhardt TA, Goff JP, Nonnecke BJ, Gambhir VK, Fiorella PD & Napoli JL (1995) Identification of 9-*cis*,13-*cis*-retinoic acid as a major circulating retinoid in plasma. *Biochemistry* **34**, 1203–1209.
99. Kane MA, Chen N, Sparks S & Napoli JL (2005) Quantification of endogenous retinoic acid in limited biological samples by LC/MS/MS. *Biochem J* **388**, 363–369.
100. Sass JO & Nau H (1994) Single-run analysis of isomers of retinoyl-beta-D-glucuronide and retinoic acid by reversed-phase high-performance liquid chromatography. *J Chromatogr A* **685**, 182–188.
101. Wolf G (2006) Is 9-*cis*-retinoic acid the endogenous ligand for the retinoic acid-X receptor? Nutr Rev 64, 532–538.
102. Rühl R, Krzyzosiak A, Niewiadomska-Cimicka A, *et al.* (2015) 9-cis-13,14-dihydroretinoic acid is an endogenous retinoid acting as RXR ligand in mice. *PLoS Genet* **11**, e1005213.
103. Palczewski K (2012) Chemistry and biology of vision. *J Biol Chem* **287**, 1612–1619.
104. von Lintig J, Moon J & Babino D (2021) Molecular components affecting ocular carotenoid and retinoid homeostasis. *Prog Retin Eye Res* **80**, 100864.
105. Watzl B, Bub A, Brandstetter BR & Rechkemmer G (1999) Modulation of human T-lymphocyte functions by the consumption of carotenoid-rich vegetables. *Br J Nutr* **82**, 383–389.
106. Watzl B, Bub A, Briviba K & Rechkemmer G (2003) Supplementation of a low-carotenoid diet with tomato or carrot juice modulates immune functions in healthy men. *Ann Nutr Metab* **47**, 255–261.
107. Underwood BA (1994) Was the "anti-infective" vitamin misnamed? *Nutr Rev* **52**, 140–143.
108. Lee LM, Leung CY, Tang WW, *et al.* (2012) A paradoxical teratogenic mechanism for retinoic acid. *Proc Natl Acad Sci USA* **109**, 13668–13673.
109. Wang XD, Liu C, Bronson RT, Smith DE, Krinsky NI & Russell M (1999) Retinoid signaling and activator protein-1 expression in ferrets given beta-carotene supplements and exposed to tobacco smoke. *J Natl Cancer Inst* **91**, 60–66.
110. Lotan R (1999) Lung cancer promotion by beta-carotene and tobacco smoke: relationship to suppression of retinoic acid receptor-beta and increased activator protein-1? J Natl Cancer Inst 91, 7–9.
111. Moise AR, Kuksa V, Imanishi Y & Palczewski K (2004) Identification of all-*trans*-retinol:all-*trans*-13,14-dihydroretinol saturase. *J Biol Chem* **279**, 50230–50242.
112. Moise AR, Alvarez S, Dominguez M, *et al.* (2009) Activation of retinoic acid receptors by dihydroretinoids. *Mol Pharmacol* **76**, 1228–1237.
113. Bazhin AV, Bleul T, de Lera AR, Werner J & Rühl R (2016) Relationship between all-*trans*-13,14-dihydro retinoic acid and pancreatic adenocarcinoma. *Pancreas* **45**, e29–e31.
114. Lucas R, Szklenar M, Mihály J, Szegedi A, Törőcsik D & Rühl R (2022) Plasma levels of bioactive vitamin D and A5 ligands positively correlate with clinical atopic dermatitis markers. *Dermatology* **238**, 1076–1083.
115. Sani BP, Venepally PR & Levin AA (1997) Didehydroretinoic acid: retinoid receptor-mediated transcriptional activation and binding properties. *Biochem Pharmacol* **53**, 1049–1053.
116. Rühl R, Krezel W & de Lera AR (2018) 9-*cis*-13,14-dihydroretinoic acid, a new endogenous mammalian ligand of retinoid X receptor and the active ligand of a potential new vitamin A category: vitamin A5. Nutr Rev 76, 929–941.
117. Krężel W, Rivas A, Szklenar M, Ciancia M, Alvarez R, de Lera AR & Rühl R (2021) Vitamin A5/X, a new food to lipid hormone concept for a nutritional ligand to control RXR-mediated signaling. *Nutrients* **13**, 925.
118. Bohn T, Hellmann-Regen J, de Lera Á R, Böhm V & Rühl R (2022) Human nutritional relevance and suggested nutritional guidelines for vitamin A5/X and provitamin A5/X (submitted for publication).
119. Khachik F, Carvalho L, Bernstein PS, Muir GJ, Zhao DY & Katz NB (2002) Chemistry, distribution, and metabolism of tomato carotenoids and their impact on human health. *Exp Biol Med (Maywood)* **227**, 845–851.
120. van den Berg H (1998) Effect of lutein on beta-carotene absorption and cleavage. *Int J Vitam Nutr Res* **68**, 360–365.
121. van den Berg H & van Vliet T (1998) Effect of simultaneous, single oral doses of beta-carotene with lutein or lycopene on the beta-carotene and retinyl ester responses in the triacylglycerol-rich lipoprotein fraction of men. *Am J Clin Nutr* **68**, 82–89.
122. Caris-Veyrat C, Garcia AL, Reynaud E, Lucas R, Aydemir G & Rühl R (2016) Lycopene-induced nuclear hormone receptor signalling in inflammation and lipid metabolism via still unknown endogenous apo-10′-lycopenoids. *Int J Vitam Nutr Res* **86**, 62–70.
123. Ford NA & Erdman JW Jr (2012) Are lycopene metabolites metabolically active? Acta Biochim Pol 59, 1–4.
124. Aydemir G, Kasiri Y, Birta E, Beke G, Garcia AL, Bartok EM & Rühl R (2013) Lycopene-derived bioactive retinoic acid receptors/retinoid-X receptors-activating metabolites may be relevant for lycopene's anti-cancer potential. *Mol Nutr Food Res* **57**, 739–747.
125. Kopec RE, Riedl KM, Harrison EH, Curley RW Jr, Hruszkewycz DP, Clinton SK & Schwartz SJ (2010) Identification and quantification of apo-lycopenals in fruits,

https://doi.org/10.1017/S095442242200021X Published online by Cambridge University Press

vegetables, and human plasma. *J Agric Food Chem* **58**, 3290–3296.
126. Ferreira AL, Yeum KJ, Russell RM, Krinsky NI & Tang G (2003) Enzymatic and oxidative metabolites of lycopene. *J Nutr Biochem* **14**, 531–540.
127. Reynaud E, Aydemir G, Rühl R, Dangles O & Caris-Veyrat C (2011) Organic synthesis of new putative lycopene metabolites and preliminary investigation of their cell-signaling effects. *J Agric Food Chem* **59**, 1457–1463.
128. Lindshield BL, Canene-Adams K & Erdman JW Jr (2007) Lycopenoids: are lycopene metabolites bioactive? Arch Biochem Biophys 458, 136–140.
129. Wang XD (2012) Lycopene metabolism and its biological significance. *Am J Clin Nutr* **96**, 1214s–1222s.
130. Gouranton E, Aydemir G, Reynaud E, *et al.* (2011) Apo-10'-lycopenoic acid impacts adipose tissue biology via the retinoic acid receptors. Biochim Biophys Acta 1811, 1105–1114.
131. Hu KQ, Liu C, Ernst H, Krinsky NI, Russell RM & Wang XD (2006) The biochemical characterization of ferret carotene-9′,10′-monooxygenase catalyzing cleavage of carotenoids in vitro and in vivo. *J Biol Chem* **281**, 19327–19338.
132. Gajic M, Zaripheh S, Sun F & Erdman JW Jr (2006) Apo-8′-lycopenal and apo-12′-lycopenal are metabolic products of lycopene in rat liver. J Nutr 136, 1552–1557.
133. Moran NE, Thomas-Ahner JM, Fleming JL, *et al.* (2019) Single nucleotide polymorphisms in beta-carotene oxygenase 1 are associated with plasma lycopene responses to a tomato-soy juice intervention in men with prostate cancer. *J Nutr* **149**, 381–397.
134. Aydemir G, Carlsen H, Blomhoff R & Rühl R (2012) Lycopene induces retinoic acid receptor transcriptional activation in mice. *Mol Nutr Food Res* **56**, 702–712.
135. Ben-Dor A, Nahum A, Danilenko M, *et al.* (2001) Effects of acyclo-retinoic acid and lycopene on activation of the retinoic acid receptor and proliferation of mammary cancer cells. *Arch Biochem Biophys* **391**, 295–302.
136. Ip BC, Liu C, Lichtenstein AH, von Lintig J & Wang XD (2015) Lycopene and apo-10′-lycopenoic acid have differential mechanisms of protection against hepatic steatosis in beta-carotene-9′,10′-oxygenase knockout male mice. *J Nutr* **145**, 268–276.
137. Rühl R (2009) Retinoids, vitamin A and pro-vitamin A carotenoids. Regulation of the immune system and allergies. *Pharm Unserer Zeit* **38**, 126–131.
138. Muller H, Bub A, Watzl B & Rechkemmer G (1999) Plasma concentrations of carotenoids in healthy volunteers after intervention with carotenoid-rich foods. *Eur J Nutr* **38**, 35–44.
139. Rühl R, Hanel A, Garcia AL, Dahten A, Herz U, Schweigert FJ & Worm M (2007) Role of vitamin A elimination or supplementation diets during postnatal development on the allergic sensitisation in mice. *Mol Nutr Food Res* **51**, 1173–1181.
140. Zunino SJ, Storms DH & Stephensen CB (2007) Diets rich in polyphenols and vitamin A inhibit the development of type I autoimmune diabetes in nonobese diabetic mice. *J Nutr* **137**, 1216–1221.
141. Landrier JF, Kasiri E, Karkeni E, *et al.* (2017) Reduced adiponectin expression after high-fat diet is associated with selective up-regulation of ALDH1A1 and further retinoic acid receptor signaling in adipose tissue. *FASEB J* **31**, 203–211.
142. Rühl R & Landrier JF (2016) Dietary regulation of adiponectin by direct and indirect lipid activators of nuclear hormone receptors. *Mol Nutr Food Res* **60**, 175–184.
143. Al-Babili S & Bouwmeester HJ (2015) Strigolactones, a novel carotenoid-derived plant hormone. *Annu Rev Plant Biol* **66**, 161–186.
144. Jia K-P, Baz L & Al-Babili S (2018) From carotenoids to strigolactones. *JExB* **69**, 2189–2204.
145. Alder A, Jamil M, Marzorati M, *et al.* (2012) The path from β-carotene to carlactone, a strigolactone-like plant hormone. *Science* **335**, 1348–1351.
146. Gomez-Roldan V, Fermas S, Brewer PB, *et al.* (2008) Strigolactone inhibition of shoot branching. *Nature* **455**, 189–194.
147. Casamassimi A, Federico A, Rienzo M, Esposito S & Ciccodicola A (2017) Transcriptome profiling in human diseases: new advances and perspectives. *Int J Mol Sci* **18**, 1652.
148. Pedrotty DM, Morley MP & Cappola TP (2012) Transcriptomic biomarkers of cardiovascular disease. *Prog Cardiovasc Dis* **55**, 64–69.
149. Altucci L, Leibowitz MD, Ogilvie KM, de Lera AR & Gronemeyer H (2007) RAR and RXR modulation in cancer and metabolic disease. *Nat Rev Drug Discov* **6**, 793–810.
150. Jenab M, Ferrari P, Mazuir M, *et al.* (2005) Variations in lycopene blood levels and tomato consumption across European countries based on the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *J Nutr* **135**, 2032s–2036s.
151. Xu Y & Harvey PJ (2019) Red light control of β-carotene isomerisation to 9-*cis* β-carotene and carotenoid accumulation in *Dunaliella salina*. *Antioxidants (Basel, Switzerland)* **8**, 148.
152. Schieber A & Carle R (2005) Occurrence of carotenoid *cis*-isomers in food: technological, analytical, and nutritional implications. *Trends Food Sci. Technol* **16**, 416–422.
153. Khoo HE, Prasad KN, Kong KW, Jiang Y & Ismail A (2011) Carotenoids and their isomers: color pigments in fruits and vegetables. *Molecules* **16**, 1710–1738.
154. Cooperstone JL, Novotny JA, Riedl KM, Cichon MJ, Francis DM, Curley RW Jr, Schwartz SJ & Harrison EH (2018) Limited appearance of apocarotenoids is observed in plasma after consumption of tomato juices: a randomized human clinical trial. *Am J Clin Nutr* **108**, 784–792.
155. Rosati A (2018) The dietary practice coincided with increases in brain size, evidence suggests. Sci Am. https://www.scientificamerican.com/article/food-for-thought-was-cooking-a-pivotal-step-in-human-evolution/ (assessed July 15, 2022).
156. Lee CM, Boileau AC, Boileau TW, Williams AW, Swanson KS, Heintz KA & Erdman JW Jr (1999) Review of animal models in carotenoid research. J Nutr 129, 2271–2277.
157. Olivier M, Asmis R, Hawkins GA, Howard TD & Cox LA (2019) The need for multi-omics biomarker signatures in precision medicine. *Int J Mol Sci* **20**, 4781.
158. Szanto A, Narkar V, Shen Q, Uray IP, Davies PJ & Nagy L (2004) Retinoid X receptors: X-ploring their (patho)physiological functions. *Cell Death Differ*, **11** (Suppl 2), S126–S143.
159. Al-Delaimy WK, Slimani N, Ferrari P, *et al.* (2005) Plasma carotenoids as biomarkers of intake of fruits and vegetables: ecological-level correlations in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Eur J Clin Nutr* **59**, 1397–1408.
160. Schierle J, Bretzel W, Bühler I, Faccin N, Hess D, Steiner K & Schüep W (1997) Content and isomeric ratio of lycopene in food and human blood plasma. *Food Chem* **59**, 459–465.
161. Fröhlich K (2007) Lycopin-Isomere in Lebensmitteln und Humanplasma - Strukturaufklärung, antioxidative Aktivität,

https://doi.org/10.1017/S095442242200021X Published online by Cambridge University Press

Gehalte und relative (E)-(Z)-Verhältnisse. Germany: Friedrich-Schiller-Universität Jena.
162. Chung HY, Ferreira AL, Epstein S, Paiva SA, Castaneda-Sceppa C & Johnson EJ (2009) Site-specific concentrations of carotenoids in adipose tissue: relations with dietary and serum carotenoid concentrations in healthy adults. *Am J Clin Nutr* **90**, 533–539.
163. Alaluf S, Heinrich U, Stahl W, Tronnier H & Wiseman S (2002) Dietary carotenoids contribute to normal human skin color and UV photosensitivity. *J Nutr* **132**, 399–403.
164. Ermakov IV, Sharifzadeh M, Ermakova M & Gellermann W (2005) Resonance Raman detection of carotenoid antioxidants in living human tissue. *J Biomed Opt* **10**, 064028.
165. Ermakov IV, Ermakova MR, Bernstein PS, Chan GM & Gellermann W (2013) Resonance Raman based skin carotenoid measurements in newborns and infants. *J Biophotonics* **6**, 793–802.
166. Schmitz HH, Poor CL, Wellman RB, Erdman JW Jr (1991) Concentrations of selected carotenoids and vitamin A in human liver, kidney and lung tissue. J Nutr 121, 1613–1621.
167. Vishwanathan R, Kuchan MJ, Sen S & Johnson EJ (2014) Lutein and preterm infants with decreased concentrations of brain carotenoids. *J Pediatr Gastroenterol Nutr* **59**, 659–665.
168. Stahl W, Schwarz W, Sundquist AR, & Sies H (1992) *cis-trans* isomers of lycopene and beta-carotene in human serum and tissues. *Arch Biochem Biophys* **294**, 173–177.
169. Pappalardo G, Maiani G, Mobarhan S, *et al.* (1997) Plasma (carotenoids, retinol, alpha-tocopherol) and tissue (carotenoids) levels after supplementation with beta-carotene in subjects with precancerous and cancerous lesions of sigmoid colon. *Eur J Clin Nutr* **51**, 661–666.
170. Gossage CP, Deyhim M, Yamini S, Douglass LW & Moser-Veillon PB (2002) Carotenoid composition of human milk during the first month postpartum and the response to β-carotene supplementation. *Am J Clin Nutr* **76**, 193–197.
171. Czeczuga-Semeniuk E & Wolczynski S (2008) Dietary carotenoids in normal and pathological tissues of corpus uteri. *Folia Histochem Cytobiol* **46**, 283–290.
172. Clinton SK, Emenhiser C, Schwartz SJ, Bostwick DG, Williams AW, Moore BJ & Erdman JW Jr (1996) cis-trans lycopene isomers, carotenoids, and retinol in the human prostate. Cancer Epidemiol Biomarkers Prev 5, 823–833.
173. Mihaly J, Gamlieli A, Worm M & Rühl R (2011) Decreased retinoid concentration and retinoid signalling pathways in human atopic dermatitis. *Exp Dermatol* **20**, 326–330.
174. Arnhold T, Tzimas G, Wittfoht W, Plonait S & Nau H (1996) Identification of 9-*cis*-retinoic acid, 9,13-di-*cis*-retinoic acid, and 14-hydroxy-4,14-retro-retinol in human plasma after liver consumption. *Life Sci* **59**, Pl169– Pl177.
175. Arnold SL, Amory JK, Walsh TJ & Isoherranen N (2012) A sensitive and specific method for measurement of multiple retinoids in human serum with UHPLC-MS/MS. *J Lipid Res* **53**, 587–598.
176. Bleul T, Rühl R, Bulashevska S, Karakhanova S, Werner J & Bazhin AV (2015) Reduced retinoids and retinoid receptors' expression in pancreatic cancer: a link to patient survival. *Mol Carcinog* **54**, 870–879.
177. Aydemir G, Domínguez M, de Lera AR, Mihaly J, Törőcsik D & Rühl R (2019) Apo-14′-carotenoic acid is a novel endogenous and bioactive apo-carotenoid. Nutrients 11, 2084.
178. Barua AB & Sidell N (2004) Retinoyl beta-glucuronide: a biologically active interesting retinoid. *J Nutr* **134**, 286s–289s.
179. Vahlquist A, Lee JB, Michaelsson G & Rollman O (1982) Vitamin A in human skin: II concentrations of carotene, retinol and dehydroretinol in various components of normal skin. *J Invest Dermatol* **79**, 94–97.
180. Wang J, Yoo HS, Obrochta KM, Huang P & Napoli JL (2015) Quantitation of retinaldehyde in small biological samples using ultrahigh-performance liquid chromatography tandem mass spectrometry. *Anal Biochem* **484**, 162–168.
181. Kane MA, Folias AE & Napoli JL (2008) HPLC/UV quantitation of retinal, retinol, and retinyl esters in serum and tissues. *Anal Biochem* **378**, 71–79.
182. Stahl W, Hanusch M & Sies H (1996) 4-Oxo-retinoic acid is generated from its precursor canthaxanthin and enhances gap junctional communication in 10T1/2 cells. Adv Exp Med Biol 387, 121–128.

https://doi.org/10.1017/S095442242200021X Published online by Cambridge University Press