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1 **Carotenoid Metabolites, their Tissue and Blood Concentrations in Humans and further Bioactivity**
2 **via Retinoid Receptor-Mediated Signalling**

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22 **Short title:** Carotenoid metabolites human tissue levels

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Abstract:

Many epidemiological studies have emphasised the relation between carotenoid dietary intake and their circulating concentrations and beneficial health effect, 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 they are rather induced by their metabolites. Several categories of metabolites have been reported, most notably due to a) modifications at the cyclohexenyl-ring or the polyene chain, such as epoxides and geometric isomers, b) excentric cleavage metabolites with also 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 carotenoids cleavage by beta-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, focussing **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.

1. Introduction

Carotenoids are typically colourful, mostly C-40 based pigments, which are generally obtained via plant food items. Over 1100 different carotenoids have been recognized [1], and additional new carotenoids are being discovered, including shorter (C-30), and longer (C-50) analogues of bacterial origin [2]. Likewise, the apo-carotenoids, their 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 been thoroughly increased in the last 2-3 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 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 the topic of controversial discussions, but have included 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 micellization into the 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 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 normalizing 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 delocalized 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 exclusively focussed on the human situation, based on the proven presence of the described carotenoids and carotenoid-metabolites in humans.**

2. Carotenoid metabolites in plasma and tissues

2.1. Rationale for interest on metabolites and overview on metabolites

Carotenoids, with major human food relevance (Figure 1 and Table 1), were mainly investigated 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 in the focus of these studies [22, 23]. These ligand-activated receptors include especially 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 / **homeostasis [24-27].**

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

109

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

113

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

122

123 The origin of selected apo-15-carotenoids / retinoid-derivatives, such as retinyl esters, retinol, retinal and
 124 retinoic acids, might occur from various metabolic pathways including a) central cleavage of individual

carotenoids such as β -carotenes or β -cryptoxanthins (Figure 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 by mitochondrial-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 bio-active retinoids, for instance 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 reported that blood and tissue concentrations of active vitamin A retinoids differ significantly between disease and health state [reviewed in 52]. These results raise the question as to whether the differences in such levels are caused by the disease or the 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 (ATRA) concentrations in plasma or tissue following carotenoid supplementation are purely beneficial has thus been discussed controversially [64-67]. The lipid hormone

152 ATRA has been described to be associated to cell differentiation, proliferation and apoptosis with
153 beneficial relevance mainly for cancer prevention [68, 69], and various diseases related to reduced
154 inflammatory competence [70, 71]. Unfortunately, ATRA has also been associated with toxic effects,
155 especially embryonic toxicity [72, 73].

156
157 Recently, ATRA has been discussed more controversially in the context of diabetes, obesity, allergies
158 and osteoporosis [74-76]. Especially the adverse effects of retinoids regarding inflammatory processes,
159 related to many diseases in Western societies, and altering local and systemic lipid metabolism and
160 homeostasis are regarded as critical [73, 77, 78]. If supplementation in such countries with retinoids /
161 carotenoids is in general beneficial must therefore be carefully evaluated.

162
163 2.2. General properties of metabolites originating from β -carotene and β -cryptoxanthin

164 When focusing on β -carotene, we may obtain a large variety of known and yet unknown, although partly
165 postulated, metabolites (Figure 1). In this chapter β -carotene isomers such as α - or γ -isoforms of
166 carotene, geometric isomers of these carotenes were included in addition we add also the provitamin A
167 carotenoid β -cryptoxanthin as a relevant precursor for the later mentioned carotenoid-metabolites under
168 this sub-chapter (Figure 1, Table 1).

169
170 Firstly, several chain-modified carotenoid metabolites have been identified, also in mammals and human
171 serum, with epoxy-, oxo- and hydroxyl-containing functional groups located at the cyclohexenyl ring or
172 at the polyene chain, as well as additional isomers [79, 80]. Whether these metabolites originate from
173 plant-based metabolism or from mammalian endogenous metabolism is not always obvious.
174 Concentrations of these potential metabolites, which are usually lower than those of its parent direct /
175 indirect nutritional precursor all-*trans*- β -carotene, are rarely reported. Problematic is their precise
176 quantification, i.e. lack of commercially available standards and also lower UV-Vis sensitivity.

Several similar compounds may also be generated during digestion or during food processing [80]. For example, upon gastrointestinal exposure to oxidizing 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 metabolized *in vivo*, remains unknown [81].

Various apo-carotenoids originating from excentric cleavage of carotenoids were identified in the mammalian and partly in the human organism after carotenoid-supplementation [86, 87]. Both BCO1 and BCO2 appear able to cleave β -carotene. While 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 *Bcmo1*^{-/-} mice adipose tissue, leading to a 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'-carotenol, β -apo-10'-carotenol, β -apo-12'-carotenol and β -apo-14'-carotenol in chicken and rats [86, 90, 91]. Unfortunately, as already outlined, a clear ordination 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 regional 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 particular detail in a review relevant for β -carotene [49], outlined in Figure 1.

Different apo-carotenals and apo-carotenoic acids of various chain lengths were found after β -carotene supplementation [86, 87]. These were then synthesized *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 oxidized to apo-carotenoic acids (Figure 1). Contrarily, endogenously produced levels have rarely been described, only for selected derivatives [93]. Following the ingestion of a high β -carotene tomato juice (360 mL, 30 mg β -carotene, 35 μ g apo-carotenoids / day), only apo-10'- and 12'-carotenal were claimed to be detected in plasma of some individuals under the set quantification limit, though unfortunately without any added visualized 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 were observed in the plasma TRL fraction, suggesting a low bioavailability.

Following excentric cleavage, the shorter products such as β -ionone, β -cyclocitral and related derivatives, have also 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, i.e. 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 (RAR α , β , γ), as reviewed earlier [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 1-2 magnitudes lower than those of β -carotene in the bloodstream, with concentrations around 0.1 - 2 μ M (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 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 Figure 1).

In addition to ATRA, other geometric isomers were identified endogenously, such as 13-*cis*-, 9,13-*dicis*-, 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 (RXR α , 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-*dicis*- 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 towards 2.0 ng/ml (4.0 \rightarrow 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 visualized 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 homeostasis of retinoid / vitamin A / RAR-mediated signalling pathways.

Nutritionally-relevant β -carotene intake is mainly contributing 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- and 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 RAR β - 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]. Based on 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]. That moderate or even high dietary consumption of natural food items rich in β -carotene and additional bioactive derivatives including other carotenoids transmits non-beneficial effects seems unlikely.

Recently, dihydro-metabolites of apo-15-carotenoids were described in mice, likely originating from 13,14-dihydroretinol [111] (Figure 1; 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 (Figure 1) [118].

2.3. 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 (Figure 1 and Table 1, 2). However, the metabolism of lycopene has been studied to a much lesser extent compared to 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) were 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 no mechanism involving xanthophyll-metabolites was mentioned and outlined.** Both excentric and centric metabolism was described for lycopene [40, 41]. Except for lycopeneoids, 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 (Figure 1 / 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]. An 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 earlier 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* in humans [130]. Alternatively, due to extensive metabolism, a dihydro-apo-10'-lycopenoic acid analogue was identified and based on UV and MS-characteristics predicted to be 7,8-dihydro-apo-10'-lycopenoic acid. How lycopene is metabolized to

dihydro-apo-10'-lycopenoic acid and whether apo-10'-lycopenoic acid is a potential intermediate are yet unanswered questions. Likely these dihydro-apo-10-lycopenoids are direct precursors of dihydro-apo-15-lycopenoids, which might be highly potent RAR- and / or RXR-ligands, as postulated in [124].

2.4. 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 synthesized in certain plants, such as carlactones [143-145] and identified as plant-relevant hormones during germination [143] and branching-inhibition [146]. If these derivatives are also of direct or indirect relevance for the human organisms 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 to carotenoid intake and serum / plasma carotenoid / retinoid concentrations to obtain valuable correlations.

3. 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 diabetes / cancer (predominantly, lycopene). The dietary intake of carotenoids has also changed over time. While lycopene intake was uncommon in the pre-industrialized human diet, especially considering the primarily European-focused world sight, it strongly increased in the 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 $>100^{\circ}\text{C}$ appears to constitute an important mechanisms for carotenoid isomerisation, yielding different precursor carotenoids for different functional apo-carotenoids, as well as a 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 to health beneficial effects are still poorly understood. In this review we summarised all available relevant information focussing on the human organism with implication of mechanistic results from further *in vitro* to *in vivo* experiments. Unfortunately, these experimental results are difficult to compare with the human because carotenoids obtain a non-similar nutri-kinetics pattern [156] and different eating

behaviour in contrast to the pure vegetarian dietary pattern of rodents, which are frequently used as experimental animal models .

4. Conclusions

As a cornerstone, we suggest that besides benchmark concentrations for carotenoids, also retinoids should be suggested, 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 as well as 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 the Western society), should also be considered.

Based on these two ranges, targeted supplementation strategies may be recommended to overcome deficiencies and towards reaching and maintaining “normal” concentration ranges. A correlation between dietary intake, serum levels and bioactive carotenoid metabolites and further examination of RXR-RAR / RXR-NHR in an easy accessible compartment such as peripheral blood mononuclear cells (PBMCs), plus target genes of relevant diseases are desperately 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 are average plasma / serum concentrations 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 pure relevance on the human organism. To summarise, carotenoid to bioactive metabolite metabolism is an important knowledge to justify which biological-response pathway of carotenoids is enabled to elicit valuable beneficial effects. This is important 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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443 **Table 1.** Concentrations of carotenoids in various tissues, all data in nM (nmol/kg or L), adapted
444 from [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]

445
446 All values represent mean ± SD; “blank” represents non determined carotenoids or no data available; [§]:
447 infants, prefrontal cortex, frontal cortex, hippocampus, auditory cortex and occipital cortex; [£]: values given in
448 literature as “carotenes”; [&]: dermis and epidermis of back, forehead, inner forearm and hand; *** : including
449 upper and lower level of this range; ACAR: α-carotene; BCAR: β-carotene; BCRY: β-cryptoxanthin; LYC:
450 lycopene; PHYE: phytoene; PHYF: phytofluene.
451

452 **Table 2.** Levels of major carotenoid metabolites / retinoids in blood plasma / serum and
453 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 ng/ml 1.4 ± 2.3 ng/ml 1.2 ± 1.6 ng/ml 1.4 ± 0.3 ng/ml 0.8 ± 0.2 ng/ml 0.9 ± 0.2 ng/ml 1.2 ± 0.3 ng/ml 2.0 ± 0.3 ng/ml	9.3 ± 2.6 nM 4.7 ± 7.7 nM 4.0 ± 5.3 nM 4.7 ± 1.0 nM 2.7 ± 0.7 nM 3.0 ± 0.6 nM 4.0 ± 1.0 nM 6.7 ± 1.0 nM	*** German children Turkish children *** *** fasted adults -before, -after suppl. of food rich in BCAR	[173] [67] [67] [35] [174] [175] [37]	end.
		skin	0.7 / 2.1 ng/g	2.3 / 7.0 nM	***	[173]	end.
		pancreas	5.9 ± 2.1 ng/g	19.7 ± 7.0 nM	***	[176]	end.
		liver	15.8 ± 8.3 ng/g	52.7 ± 27.7 nM	***	[35]	end.
9-cis-retinoic acid (?)	BCAR	serum	(?) 0.03 ng/ml	(?) 0.1 nM	fasted adults	[175]	end.
		liver	(?) 0.6 ± 0.2 ng/g	(?) 2.0 ± 0.7 nM	***	[35]	end.
13-cis-retinoic acid (#)	BCAR	serum	1.8 ± 1.0 ng/ml 1.1 ± 0.2 ng/ml 1.2 ± 0.3 ng/ml	6.0 ± 3.3 nM 3.7 ± 0.7 nM 3.9 ± 1.0 nM	*** *** fasted adults	[35] [174] [175]	end.
		liver	1.5 ± 0.4 ng/g	5.0 ± 1.3 nM	***	[35]	end.
9,13-dicis-retinoic acid (#)		serum	1.0 ± 0.3 ng/ml	0.3 ± 0.1 nM	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 ng/g	290 ± 238 nM	***	[113]	end.
9-cis-13,14-dihydroretinoic acid	BCAR	serum	4.8 ± 0.7 ng/ml	15.8 ± 2.3 nM	***	[114, 117]	end.
9-cis-4-oxo-13,14-dihydroretinoic acid (C ₂₀ H ₂₈ O ₃ / 316.4 Da)	BCAR	liver	10.3 ng/g	32.6 nM	***	[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 ng/ml	1.9 ± 0.9 nM	***	[174]	end.
13-cis-4-oxo-retinoic acid	BCAR/CA ₍₁₎	serum	2.4 ± 1.8 ng/ml	7.6 ± 5.7 nM	***	[35]	end.
all-trans-apo-13'-carotenone (C ₁₈ H ₂₇ O ₁ / 255.4 Da)	BCAR	serum	0.8 – 1.3 ng/ml	3 - 5 nM	***	[32]	end.
all-trans-apo-14'-carotenonic acid (C ₂₂ H ₂₃ O ₂ / 323.5 Da)	BCAR	serum	1,3 ± 0,6 ng/ml	4,0 ± 1,9 nM	***	[177]	end.
all-trans-retinoyl-glucuronide (C ₂₆ H ₃₆ O ₈ / 476.6 Da)	BCAR	serum	3.2 ± 1.9 ng/ml	6.8 ± 4.0 nM	***	[178]	end.
all-trans-retinol (C ₂₀ H ₃₀ O ₁ / 286.5 Da)	BCAR	serum	510 ± 217 ng/ml 980 ± 110 ng/ml 641 ± 99 ng/ml	1777 ± 759 nM 3414 ± 383 nM 2233 ± 345 nM	*** *** ***	[173] [35] [174]	end.
		skin	207 / 253 ng/g	724 / 885 nM	***	[173]	end.
		pancreas	96 ± 86 ng/g	336 ± 301 nM	***	[176]	end.
all-trans-3,4-dehydroretinol (C ₂₀ H ₂₈ O ₁ / 288.5 Da)	BCAR	skin	9 - 70 ng/g	31.2 – 240 nM	**, ***	[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 ng/ml	3.1 nM	***	[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 ng/ml	0.1 ± 0.1 nM	after tomato suppl. diet	[125]	after supp.
apo-8'-lycopenal (C ₃₀ H ₄₀ O ₁ / 416.6 Da)	LYC	plasma	0.2 ± 0.1 ng/ml	0.6 ± 0.3 nM	"	[125]	after supp.
apo-10'-lycopenal (C ₂₇ H ₃₆ O ₁ / 376.6 Da)	LYC	plasma	0.1 ± <0.0 ng/ml	0.3 ± 0.1 nM	"	[125]	after supp.
apo-12'-lycopenal (C ₂₅ H ₃₄ O ₁ / 350.1 Da)	LYC	plasma	0.2 ± 0.1 ng/ml	0.7 ± 0.4 nM	"	[125]	after supp.
apo-14'-lycopenal (C ₂₂ H ₃₀ O ₁ / 310.5 Da)	LYC	plasma	0.03 ± <0.0 ng/ml	0.1 ± <0.0 nM	"	[125]	after supp.

References:
just in mouse

all-trans-retinal (C ₂₀ H ₂₈ O ₁ / 284.4 Da)	BCAR	serum (m)	0.6 ng/ml 9.1 ± 1.8 ng/ml	~2 nM 32.2 ± 6.2 nM		[180] [181]	just mouse
		WAT (m)	~8.5 – 11.4 ng/g 17.9 ± 1.4 ng/g	~30 - 40 nM 63 ± 5 nM		[180] [181]	just mouse
all-trans-retinol	BCAR	serum (m)	257 ± 31 ng/ml 170 ± 10 ng/ml	900 ± 110 nM 595 ± 35 nM	****	[181] [35]	
9-cis-retinol	BCAR	serum (m)	8.6 ± 2.9 ng/ml	30 ± 10 nM		[181]	just mouse
13-cis-retinol	BCAR	serum (m)	11.4 ± 2.9 ng/ml	40 ± 10 nM		[181]	just mouse
dihydro-apo-10'- lycopenoic acid (C ₂₇ H ₃₈ O ₂ / 394.6 Da)	LYC	WAT (m)	?	?	*****	[130]	just mouse

454
455 *: likely just an isomerisation product of ATRA during sample preparation; **: present in different concentrations
456 in different zones of the human skin; ***: healthy adults; ****: all-*trans*-retinol levels in mouse are just used as
457 reference for comparison to 9-*cis*- and 13-*cis*-retinol levels, which were just determined in mouse serum and not in
458 humans; *****: derivatives which were predicted by analytical studies ; #: 9,13-*dicis*- and 13-*cis*-retinoic acid
459 usually co-elute during HPLC-separation and are not identified separately in many described studies; ##: this
460 concentration is based on a total amount of 0.1 ng in a retina, which is calculated on a predicted retina weight of 10-
461 80 mg; (1): 4-oxo-retinoic acid was described as an *in vitro* metabolite of canthaxanthin [182]; BCAR: β-carotene;
462 LYC: lycopene; CA: canthaxanthin; WAT: white adipose tissue; end.: endogenous; supp.: supplementation, ecnd –
463 exact concentration was not determined.

464

Figure headings

Figure 1: Metabolic pathway starting from all-*trans*- β -carotene and all-*trans*-lycopene via a) geometric isomerisation, b) excentric 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 which were not conclusively identified to be present endogenously in humans were marked with a star (*) and represent derivatives which 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 stars (**). Abbreviations: AT: all-*trans*-, RAR: retinoic acid receptor, RXR: retinoid-X receptor.

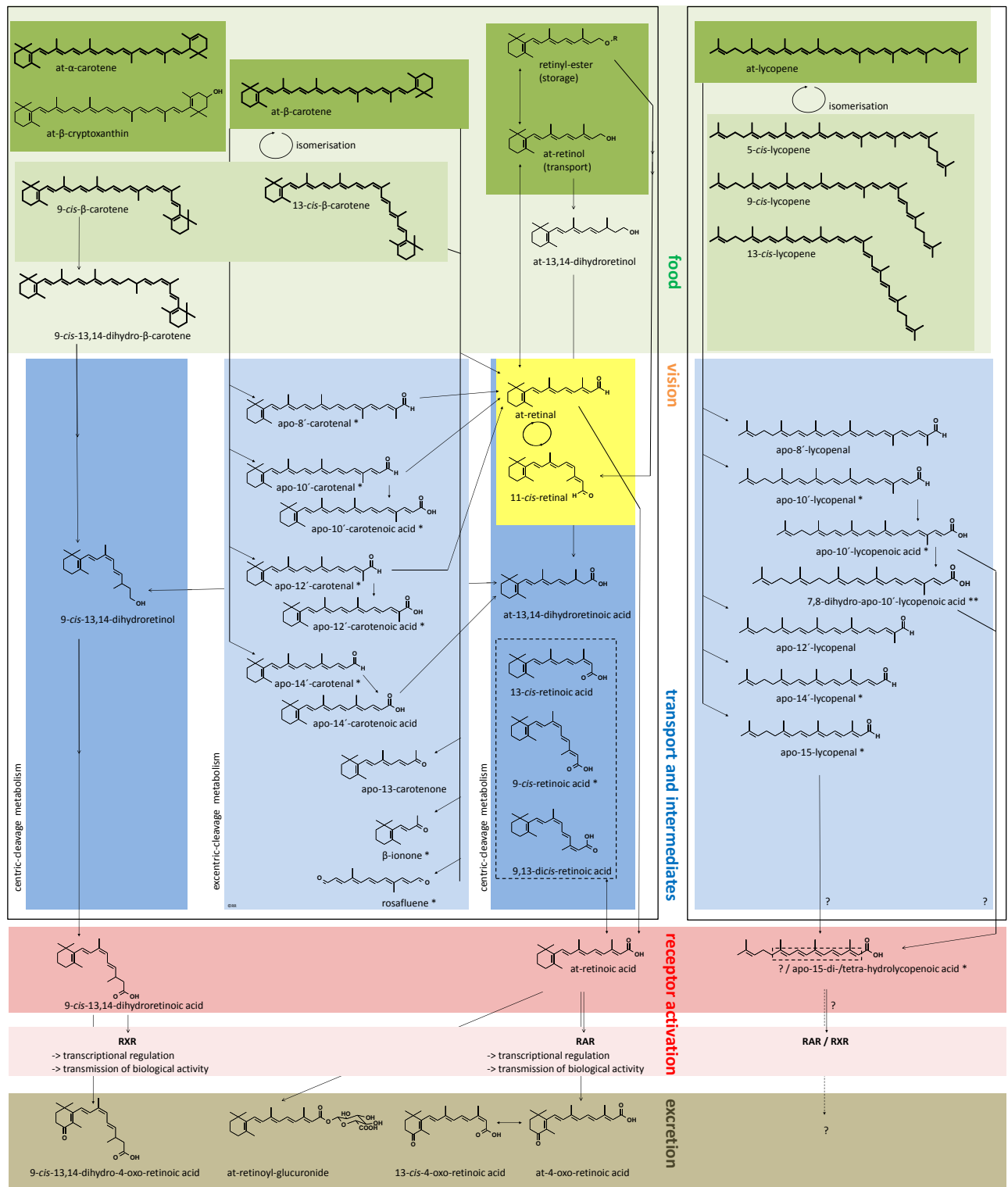


Figure 1

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