Bioactivity of Carotenoids – Chasms of Knowledge

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Abstract: Carotenoid dietary intake, especially within fruits/vegetables and their plasma levels have been associated in many epidemiological studies with a reduced risk of several chronic diseases, including type-2 diabetes, cardiovascular diseases, several types of cancer, and age-related macular degeneration. However, intervention trials with isolated carotenoids (as supplements) have fallen short of fulfilling the hopes that were placed in these lipophilic pigments, often producing no positive or even adverse effects, such as increased lung cancer rate or total mortality. More recent studies have suggested that certain metabolites, and not necessarily the native compounds may be (the most) biologically active ones, such as certain apocarotenals (originating following enzymatic cleavage) and other more polar compounds, acting as more suitable electrophiles to react with transcription factors such as nuclear factor kappa-B (NF-KB) and nuclear factor (erythroid-derived 2)-like 2 (Nrf2). In addition, it appears that questions of dosing are likewise crucial, as may be interactions of non-provitamin A carotenoids and their derivatives with retinoid acid receptors (RAR) or retinoid X receptors (RXR). Furthermore, our picture on carotenoid metabolism may be incomplete, as our knowledge on e.g. the interaction with the microbiota is virtually nil. In this position article, it is aimed to highlight some of the discrepancies that appear to trouble carotenoid-related research, and point out some of the existing gaps in our knowledge.

Keywords: Xanthophylls, carotenes, metabolites, NF-κB, Nrf-2, RAR/RXR, colon.

Epidemiological studies and carotenoids

Apart from representing essential precursors for vitamin A [1; 2; 3], several large and prospective epidemiological studies have shown a positive correlation between the dietary intake of carotenoids and reduced risk of developing several chronic diseases. For example, in a meta-analysis with over 135,000 participants, Hamer and Chida [4] have shown that the consumption of total carotenoids was associated with a reduced risk (almost 30 %) of type 2 diabetes (T2D). Similarly, in a study with over 70,000 female participants, dietary intake of α- and β-carotene was significantly associated with a reduced risk (25 % and 20 %, respectively) of coronary artery disease, when comparing quintiles of highest vs. lowest intake [5]. A comparable result was found by Buijsse et al. [6] in a meta-analysis of prospective cohort studies (with ca. 4500 participants), showing that plasma β-carotene in elderly men was associated with an overall reduced total mortality (of almost 30 %). Though the mechanisms of action remained unclear at the time, it was speculated that carotenoid antioxidant capacity (involving quenching of free radicals such as of lipid peroxides) was likely to be associated with the observed effects [7].

Intervention trials with carotenoid supplements – hard endpoints

These positive findings and related antioxidant activity seen in many in vitro trials [8] sparked a number of large-scale supplementation trials, especially with β-carotene. Most recognized for their adverse effects on smokers, both the ATBC [9] and the CARET trial [10], in which β-carotene was (co-) administered at high daily doses of 20 mg with 50 mg α-tocopherol and 30 mg with 25,000 IU vitamin A, respectively, for several years, had to be discontinued due to increased lung-cancer mortality. Similar findings were encountered in a meta-analysis by Bjeslavic et al. (also including healthy subjects), suggesting that β-carotene supplements resulted in enhanced mortality when given alone or together with other antioxidants [11]. Supplementation trials finding a positive (or at least, no negative) effect do also exist, such as the Linxian trial [12], though it may be speculated that effects can be, at least in part, explained by a reduced status of certain micronutrients in these populations, and that supplementation rather ameliorated these deficiencies.
Short-term dietary intervention trials – surrogate markers

Several intervention trials with whole foods, notably with tomato products rich in carotenoids (lycopene, β-carotene) have been conducted, which generally suggested some positive health effects, as measured by surrogate markers, such as those related to inflammation, e.g. interleukin-6 (IL–6), interleukin-8 (IL–8), interleukin-1-beta (IL–1β), tumor necrosis factor alpha (TNF-α), C-reactive protein (CRP), or oxidative stress, e.g. improving superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT), or heme-oxygenase 1 (HO–1) [13; 14]). The same appears to be true to some extent for non-healthy subjects, in which the intervention with supplements, such as with lycopene or lutein, has shown some benefits, as measured e.g. by improved serum amyloid A (a marker of inflammation), and IL–6 [14; 15].

These endpoints were chosen, as mechanistic, i.e. in vitro/cellular trials, had suggested that not the direct anti-oxidant effects (e.g. free radical quenching) may be responsible for the proposed health effects, but also (and perhaps especially) effects on gene expressions, such as via altering cellular transcription factors linked to inflammation and oxidative stress, as reviewed by Kaulmann and Bohn [16]. It appears that certain carotenoids and their derivatives can bind to cysteine residues of nuclear factor kappa-B (NF-KB) or nuclear factor (erythroid-derived) 2-like 2 (Nrf2). This can prevent the degradation of the inhibitor of NF-KB and thus the liberation of free NF-KB (and subsequent translocation to the nucleus which up-regulates pro-inflammatory gene-expression, figure 1); and for Nrf2 fostering dissociation from the kelch-like ECH-associated protein 1 (keap-1) repressor, with Nrf2 then translocating to the nucleus, up-regulating expression of anti-oxidant enzymes [17; 18].

Are we looking at the right compounds?

How to explain the conundrum of discrepancies between epidemiological findings and intervention trials? Are we targeting the wrong molecules? Are other compounds, such as dietary fibre, anti-oxidant vitamins (C/E), or other bioactive secondary plant compounds (e.g. phytosterols, glucosinates etc.), and not carotenoids, responsible for the observed health effects?

This extreme position may also fall short of the reality.

There are several aspects to consider prior to “ripping off”

Figure 1. Potential interrelation between carotenoid exposure, oxidative stress, inflammation, and toxicological relevant pathways (pattern fill).

Higher carotenoid concentrations (depending on carotenoid type, organism, bioavailability etc.) may increase the risk, at least intermittently, of reactive oxygen species (ROS) production, activating nuclear factor (erythroid derived) 2-like 2 (Nrf2) translocation and expression of anti-oxidant enzymes. Likewise, nuclear factor-kappa B (NF-κB) translocation may be inhibited, limiting pro-inflammatory responses. Lower concentrations, possibly covering the lower/physiological range, may even reduce Nrf2 translocation, effects for NF-κB are less clear. Certain derivatives of β-carotene, but also of lycopene, can alter retinoic acid receptor (RAR) and retinoid x receptor (RXR) activity, effecting apoptosis, with lower concentrations of retinoic acid/other derivatives possibly favouring cell proliferation [22]. Higher concentrations of native carotenoids may reduce the proportion of carotenoid derivatives (non-filled arrows, possibly involving β-carotene oxygenases 1/2 (BCO1/2). High concentrations of native compounds have further been suggested to trigger cytochrome P450 enzymes (CYP) activation, producing pro-carcinogenic compounds [38], *effects for higher/lowest doses (on NF-κB and Nrf2) shown in vitro and in vivo especially for astaxanthin, β-carotene, lutein, lycopene [16]. + Data for β-carotene and derivatives. $ Data for β-carotene derivatives and indications for apo-15-lycopenoids. CAT: catalase; GPx: glutathione peroxidase; HO-1: heme-oxygenase 1; IL-1β: interleukin-1-beta; IL-6: interleukin-6; NO: nitric oxide; SOD: superoxide dismutase; TNF-α: tumor necrosis factor alpha.
carotenoids the status of any health benefits. It rather appears that our understanding of carotenoid bioavailability and bioactivity, especially regarding the active compounds and possibly dose-related aspects, is incomplete: It can be hypothesized that carotenoids, administered at high doses (supplements), may override the body’s metabolism capacity, increasing the ratio of native compounds to metabolites, resulting in more pro-oxidant and pro-inflammatory conditions.

Indeed, several studies have suggested that β-carotene oxygenase 1/2 (BCO1/2) cleavage metabolites, due to their enhanced electrophilic properties (with improved binding ability to cysteine residues of NF-KB and Nrf2), and higher solubility in the cytosol, are better alternators of these pathways, resulting in anti-inflammatory effects, and stimulating the body’s own antioxidant system [17].

Highest bioactivity regarding these pathways was associated with apocarotenals with 12 C-atoms, and having a methyl-group 3 C-atoms distant from the terminal aldehyde function [17]. In addition, several studies in vitro [18] and even in vivo in rats [19] have shown that polar carotenoid breakdown products of lycopene and lutein (following UV-Vis irradiation), respectively, are more bioactive with respect to anti-inflammatory/antioxidant targets (related to transcription factors), supporting this hypothesis.

Higher, i.e. supra-physiological doses on the other hand (1-10 μM in cellular trials, or doses exceeding the daily intake of ca. 10-20 mg [20]), have in part been related to pro-oxidative effects in some, though not all studies, as reviewed earlier [16]. For example, in several cellular trials, concentrations of >1 μM of all-trans retinoic acid (ATRA), a potential metabolite of β-carotene, have been associated with pro-oxidative effects [21], as opposed to lower, nutritionally plausible concentrations (<1 μM) [16]. Earlier results in smoke-exposed ferrets [22] have likewise suggested arbitrary effects at low vs. high concentrations of β-carotene (0.4 vs. 2.4 mg/kg bw., equal to 6 and 30 mg/kg bw. for humans), in line with the ATBC/CARET trial, resulting in lower retinoic acid concentrations and reduced retinoic acid receptor (RAR)-β expression, hampering apoptosis but increasing cytochrome P450 activation, possibly resulting in the formation of harmful metabolites.

Such concentrations (>30 mg/kg bw.), taken for several weeks, are likely to considerably increase the typical β-carotene plasma concentration from 0.3-1.0 μM to 3-5 μM or higher [23; 24; 25], which thus may not be desirable. Also higher doses of lycopene (3.3 mg/kg bw. in rats) have shown arbitrary effects, interestingly especially when ethanol reduced BCO2 activity [26], also pointing out to the importance of the balance between metabolites and native carotenoids.

Are we overlooking something?

Other pathways, which so far have been mostly overlooked, may also play a role, which likewise may involve carotenoid metabolites. Caris-Veyrat et al. [27] have suggested that lycopene metabolites such as apo-15-lycopenoids show vitamin A – like behaviour, as they may activate retinoid X receptor (RXR) and retinoic acid receptor (RAR) [28; 29; 30]. Also effects together with other bioactive compounds, such as with docosahexaenoic acid (DHA) and ATRA, on RAR/RXR mediated apoptosis have been reported [31], highlighting potential additive/synergistic interactions with other micronutrients. Also in this respect, more is known for other phytochemicals such as for polyphenols [32], but little on carotenoids.

When highlighting potential dose effects, it is also important to stress out inter-individual differences in dose-responses, possibly related to genetic differences such as single nucleotide polymorphisms (SNPs), altering carotenoid metabolism and bioavailability [33; 34]. Possibly also epigenetic differences do play a role, however, no information on this is available.

Finally, several aspects of potential carotenoid metabolism have never been investigated. A good example is the human colon and its microbiota. As only 10-40 % of carotenoids are absorbed (presumably, in the small intestine), the majority of carotenoids can reach the large intestine. In vitro studies have further suggested that carotenoids are not completely recovered, only 10 % [35] – 50 % [36]. Obviously, they are fermented – but into what? From polyphenols, we know that these may be converted into numerous metabolites, following ring fission, deglycosylation, hydrolysis, deglucuronidation, and demethylation [37]. However, nothing is known regarding carotenoids. It is not impossible that bioactive, more polar degradation products are formed. Though admittedly this is speculation, it is remarkable that nothing on colonic metabolism is known.

Conclusions and Perspectives

Our current view on potential bioactive properties on carotenoids appears to be incomplete. Missing aspects include the following:

1. Which metabolites and breakdown products are formed in the human body?
2. Does the colon and especially microbiota play any role in carotenoid metabolism?
3. Are the (more polar) metabolites the (more) bioactive molecules – rather than the native compounds? If so, which exactly?
4. To what extent do various (epi-) genetic differences (SNPs, copy number variations etc.) alter inter-individual differences regarding bioavailability and bioactivity?
5. Which are the predominant mechanisms of action – which nuclear receptors are important?
6. How much of a dose- (i.e. concentration related) effect is there for the various carotenoids and derivatives or what is the “therapeutic” (and nutrition/physiological relevant) window, if any?

These merely constitute some of the most pressing questions that should be addressed in order to lift the veil of the unresolved bioactivity that carotenoids may exert, and should be addressed prior to future large-scale supplemental experiments. It can be hoped for that improved \textit{in vitro} (e.g. 3-D cell culture) and \textit{in vivo} (e.g. knock-out) models, higher availability of (metabolite) standards, and improved analytical capabilities will contribute to solve some of these persistent puzzles.

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Conflict of interest

The author is also Editor-in-Chief of this journal.

References


