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Review

Carotenoids and Their Health Benefits as Derived via Their Interactions with Gut Microbiota

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A B S T R A C T

Carotenoids have been related to a number of health benefits. Their dietary intake and circulating levels have been associated with a reduced incidence of obesity, diabetes, certain types of cancer, and even lower total mortality. Their potential interaction with the gut microbiota (GM) has been generally overlooked but may be of relevance, as carotenoids largely bypass absorption in the small intestine and are passed on to the colon, where they appear to be in part degraded into unknown metabolites. These may include apo-carotenoids that may have biological effects because of higher aqueous solubility and higher electrophilicity that could better target transcription factors, i.e., NF-κB, PPARγ, and RAR/RXRs. If absorbed in the colon, they could have both local and systemic effects. Certain microbes that may be supplemented were also reported to produce carotenoids in the colon. Although some bactericidal aspects of carotenoids have been shown *in vitro*, a few studies have also demonstrated a prebiotic-like effect, resulting in bacterial shifts with health-associated properties. Also, stimulation of IgA could play a role in this respect. Carotenoids may further contribute to mucosal and gut barrier health, such as stabilizing tight junctions. This review highlights potential gut-related health-beneficial effects of carotenoids and emphasizes the current research gaps regarding carotenoid—GM interactions.

Keywords: Carotenes, xanthophylls, digestion, carotenoid metabolites, gut bacteria, microbiome, mucosal layer, inflammation, oxidative stress, bactericidal effects, *Bifidobacterium* spp., *Akkermansia* spp.

Abbreviations: ACE, abundance-based coverage estimator; ASC, adipose-derived stem cells; BCO1/2, beta-carotene oxygenase 1/2; CAT, catalase; CHK, choline kinase; CRTI, phytoene desaturase gene; CTP, cytidine 5′-triphosphate; DCW, dry cell weight; DMAPP, dimethylallyl disphosphate; Dxp, 1-deoxy-D-xylulose 5-phosphate; Dxs, 1-deoxy-D-xylulose 5-phosphate synthase; Fd, ferredoxin; GI, gastrointestinal; G3P, glyceraldehyde 3-phosphate; GM, gut microbiota; CMP, cytidine monophosphate; GPx, glutathione peroxidase; HMBPP, E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; HmgS, 3-hydroxy-3-methylglutaryl-CoA synthase; IBD, inflammatory bowel diseases; IDI, isopentenyl-pyrophosphate delta isomerase; Ig, immunoglobulin; IspD, 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase; IspE, 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase; IspH, 4-hydroxy-3-methylbut-2-enyl diphosphate reductase; IPI, isopentenyl diphosphate isomerase; IMO, isomalto-oligosaccharides; IPK, isopentenyl phosphate kinases; IPP, isopentenyl diphosphate; IUP, isopentenol utilization pathway; MAPK, mitogen-activated protein kinase; MK, mevalonate kinase; MVA, mevalonic acid; MEP, methylerythritol 4-phosphate; NF-κB, nuclear factor kappa B; Nrf2, nuclear factor erythroid 2-related factor 2; PPARγ, peroxisome proliferator-activated receptor gamma; OPG, obligate pathogenic; OS, oxidative stress; PPi, pyrophosphate; PSY, phytoene synthase gene; RAR, retinoic acid receptor; RNS, reactive nitrogen species; ROS, reactive oxygen species; RXR, retinoid x receptor; PMD, phosphomevalonate decarboxylase; PMK, phosphomevalonate kinase; SlgA, secretory immunoglobulin A; SlgM, secretory immunoglobulin M; SOD, superoxide dismutase; Tjp1, tight junction protein 1; TLR, toll-like receptor; UC, ulcerative colitis.

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Statement of significance

This article focuses on an important gap of carotenoid research—the interactions of carotenoids with gut microbiota in the colon and potential associated health benefits. Mechanisms including altered microbiota composition, carotenoid metabolism, barrier properties, and immune-relevance are especially highlighted.

Introduction

Carotenoids are typically C40 tetraterpenoid pigments, produced by most plants, some bacteria, and fungi, but not by humans. Although there are >1100 different carotenoids, up to 50 are found in our diets and among those only a dozen appear at measurable concentrations in the human bloodstream. These pigments are among the most abundant liposoluble phytochemicals in the bloodstream (~0.5–2 μM), despite their low daily dietary intake (ca. 5–20 mg) [1]. Some carotenoids are vitamin A precursors (i.e., provitamin A carotenoids) and following consumption they can be cleaved to produce vitamin A in the small intestine [2]. Vitamin A has well-established biological effects in eliciting cell differentiation, gap junction formation, immunity, and visual light-dark adaptation. Both provitamin A and non-provitamin A carotenoids have also received much interest because of inverse associations between the consumption of carotenoid-rich foods and cardio-metabolic diseases [3] and total mortality [4]. These health benefits have originally been ascribed to their anti-inflammatory/antioxidant properties, as carotenoids can protect against lipid peroxidation and damage caused by reactive oxygen species (ROS) [5]. In the past decade, there has been a great interest to understand how carotenoids, or their metabolites, may interact with transcription factors, including the pro-inflammatory NF-κB and the antioxidant-related Nrf-2 [6]. They could also interact via binding to nuclear receptors, including retinoic acid receptor/retinoid X receptor (RAR/RXR) and peroxisome proliferator-activated receptors (PPARs), whose downstream effects are related to cellular differentiation and the immune response [7]. Another important aspect is the association of lutein and zeaxanthin and the reduced risk of age-related macular degeneration, the main cause of vision loss in the elderly [8].

Carotenoid absorption can be relatively low and variable, depending on the carotenoid physical-chemical structure (e.g., carotenes vs. xanthophylls), food matrix effects, co-consumption with other factors (i.e., lipids, metals, etc.), and a variety of host factors [9, 10]. Thus, the bulk of a given dose of carotenoids consumed will reach the colon. It is unclear if absorption can occur in the colon [7]. A major research gap exists also regarding carotenoid metabolism in the colon and interactions with the gut microbiota (GM), i.e., the microorganisms residing in the gut [11]. Metabolized carotenoids such as apo-carotenoids have a shorter chain length and oxygen modification, which slightly increases their solubility in an aqueous environment. In addition, their higher electrophilicity would make them suitable to interact with transcription factors [12]. Recently, dose-dependent prebiotic-like effects, including anti-inflammatory ones, of lycopene were demonstrated in a clinical trial in influencing abundances of *Bifidobacterium adolescentis* and *Bifidobacterium longum* [13]. A greater abundance of *Lactobacilli* spp. was also observed when lycopene was consumed. Although the authors demonstrated that lycopene supplementation improved gut metabolism, they did not provide any mechanistic basis for the prebiotic effect. Some potential interactions of carotenoids with the GM have been highlighted recently [14], emphasizing that this may constitute an under-explored pathway via which carotenoids could exert beneficial health effects. Indeed, GM composition has been associated with many chronic health complications. For instance, in some, though not all studies, obesity was associated with lower GM diversity and a lower ratio of *Bacteroidetes* to *Firmicutes* [15], with potential negative influences on resting energy expenditure [16]. GM may also trigger inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis, and certain prebiotics may ameliorate such conditions [17]. IBD is strongly related to gut barrier functionality, and carotenoids may exert positive effects here also, either via their systemic anti-inflammatory and antioxidant properties [18], via the the GM, or impacting tight junction integrity [19]. The relation of GM to many chronic diseases is not surprising, given that the GM is required for an optimal immune function expression. The immune system is hampered in germ-free animals, as shown by underdeveloped lymphatic organs and low IgA in mice [20]. Lyu et al. recently reviewed interactions between retinoic acid (the most potent form of vitamin A), astaxanthin, and the GM [21], suggesting that astaxanthin may enhance IgA production, preventing gut dysbiosis via recognizing, and coating certain bacteria and preventing their infiltration through the epithelial barrier.

Though their precise implications in disease remain often poorly understood, the crucial role of the GM in the etiology of many diseases has been emphasized, and the involvement of the gut-brain, gut-liver, and gut-heart axes have been highlighted. Carotenoids may interact with the GM in a multitude of ways (Figure 1):

[1] modulate the abundance of bacteria that can either activate commensal pathways or suppress pathogenic pathways [14, 22];
[2] reduce oxidative stress (OS) in the gut, which could likewise alter GM composition [23];
[3] foster the production of anti-inflammatory short-chain fatty acids (SCFA) such as butyrate by altering the abundance of SCFA-producing bacteria [24], which may act on PPARγ [25], inhibiting the dysbiotic expansion of certain bacteria and being related to reduced pro-inflammatory toll-like receptor (TLR) signaling [26];
[4] maintain healthy mucosa and epithelial gut barrier and tight junction integrity [27], as proposed in a recent study

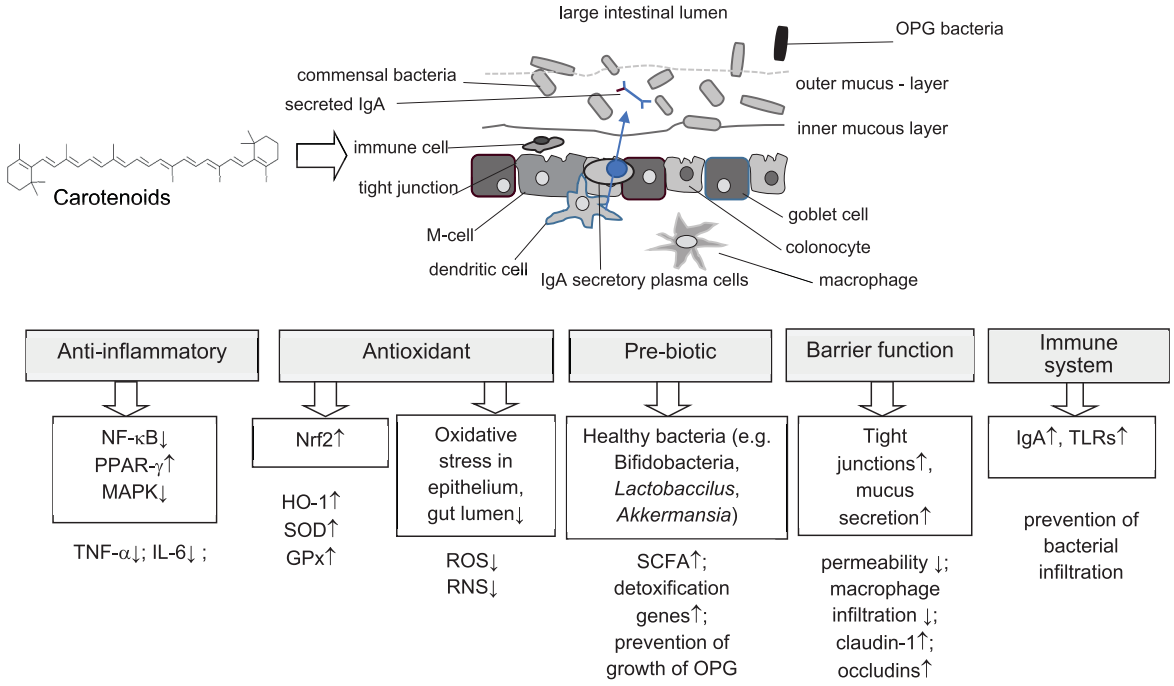

FIGURE 1. Mechanisms by which carotenoids could interact with the gut mucosa, influencing GM and intestinal barrier properties. GPx, glutathione peroxidase; HO-1, heme-oxygenase 1; IgA, immunoglobulin A; IL-6, interleukin-6; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa B; Nrf2, nuclear factor erythroid 2–related factor 2; OPG, obligate pathogenic bacteria; PPAR, peroxisome proliferator-activated receptor; ROS, reactive oxygen species; RNS, reactive nitrogen species; SCFA, short-chain fatty acids; SOD-1, superoxide dismutase 1; TLRs, toll-like receptors; TNF-α, tumor necrosis factor alpha.

in the small intestine of piglets [28]; improve mucosa integrity via favorable GM composition [27];

[5] influence gut metabolism and immune-related properties, such as IgA [21].

In this review, we highlight the present state of knowledge regarding carotenoid—GM interactions and emphasize possible pathways in the colon via which these pigments may contribute to health benefits, also pointing out gaps of knowledge and possible steps forward.

Brief overview of the processing of carotenoids in the stomach and small intestine

The processing and digestion of carotenoids in the upper digestive tract have been extensively reviewed elsewhere [9, 29–31]. Herein, we briefly recapitulate the primary factors of influence on carotenoid absorption. This evidence provides sufficient background to understand why the carotenoid proportions that remain in the digestive lumen (and are available for microbial interaction in the colon) are relatively high.

Carotenoids are very hydrophobic (pKa~10–15.6), and must be released from the food matrix and dissolved to be available for incorporation within gastric lipid droplets. The most significant factor that influences carotenoid release is the co-consumption with lipid (which provides both a medium to solubilize the carotenoid within and stimulates gastric lipase release [32, 33]). Following lipid consumption, gastric lipase cleaves free fatty acids from triglycerides, to facilitate lipid droplet emulsification [34]. Larger lipid droplets slow the rate of gastric emptying until sufficiently small droplets are achieved [35], providing additional time for both fatty acids to be cleaved from triglycerides to form smaller droplets, and for the carotenoids to be released from the matrix.

The other major factor facilitating gastric carotenoid release is the breakdown of the food matrix through preceding cooking/processing [36]. For example, Rich and colleagues [37] compared the transfer of lutein and β-carotene from raw vs. blanched and frozen spinach into lipid droplets, under gastric conditions. Both the rate of carotenoid transfer to the oil droplet, as well as the absolute concentration achieved at experiment termination at 110 min, were significantly higher (30–33x) in the blanched and frozen spinach as compared with the raw spinach. A similar trend was observed between raw and blanched carrot juice [37]. Likewise, following consumption of cooked tomato or spinach puree, a majority of the carotenoids (i.e. ~95% of the lycopene, ~75% of the lutein, respectively) remained embedded in the food matrix over 180 min of gastric digestion [38], with almost all freed carotenoids (5%–25%) being associated with the lipid droplet fraction. In the same study, 60% of the β-carotene in cooked carrot puree was transferred directly to gastric lipid droplets [38].

Because solubility and subsequent bioavailability are so limited, and because of implications in diseases where bioactivity would be most critical postabsorption, there is keen interest to improve carotenoid delivery. This goal can be attempted through providing supplemental doses (often administered as a super saturated, crystal-rich oil product) or through enhanced bioaccessibility. Recent strategies to improve bioaccessibility

have included the addition of emulsifying agents or the formation of nanoemulsions [31, 39–44]. The rationale behind these approaches is essentially to deliver smaller lipid droplets or small vesicles that do not need to be micellarized or to more rapidly produce smaller lipid droplets during the digestive process [43]. Some of these approaches have been successful, whereas others have been limited, and under some circumstances may reduce carotenoid lipid droplet incorporation [40, 45]. Additionally, the complete absence of lipids or consumption of high lipid excess (i.e., >10%), can cause crystal formation and precipitation [46, 47]. In either of these instances, a high concentration remains in the gastric lumen, which may provide a source for GM interaction in the stomach.

A number of other factors further influence the proportion of carotenoids freed in the gastric compartment from foods and have more attenuated effects, as extensively reviewed previously [30]. In brief, release is enhanced in the stomach in foods with carotenoid compartmentalization within liquid-crystalline chromoplasts of the fruit or vegetable being consumed; prolonged cooking or processing times and temperatures; co-consumption with some citrus flavanones, digestible proteins, high methyl-ester pectin (relative to low methyl-ester pectin), and encapsulation. Xanthophylls are also better released relative to carotenes, all other factors being equal, because of the presence of one or more oxygen groups and thus higher polarity [29]. In contrast, additional factors that inhibit/reduce carotenoid release into the lipid droplet include compartmentalization within solid-crystalline chromoplasts of the fruit or vegetable being consumed, co-consumption with high doses of divalent metals [48], anthocyanins [49], or with naringenin [50, 51]. It is worth noting that absence of release to the lipid droplet would still leave the carotenoid present within the gastric lumen, but sequestered in crystalline form, or in a food matrix, thus limiting GM interaction.

Following gastric emptying, the digesta is met with the release of bile salts and pancreatic lipase and colipase in the duodenum. The lipase and colipase continue to facilitate fatty acid release from triglycerides and diacylglycerols, whereas the bile salts act as a natural emulsifying agent to produce smaller lipid droplets. Collectively, the free fatty acids and bile salts produce subsequently smaller lipid droplets until mixed micelles (~4 nm in diameter) are formed [52], which also include phospholipids, monoacylglycerols, and embedded apolar compounds such as cholesterol and carotenoids.

As with the gastric compartment, lipid co-consumption is arguably the most important factor for carotenoid incorporation into micelles in the small intestine [53]. The proportion of the original carotenoid dose that ends up in the micelle is known as the "bioaccessible" fraction, which is the only proportion of the original dose thought to be available for absorption [54]. The type of lipid co-consumed strongly influences bioaccessibility, with long-chain fatty acids greatly increasing the bioaccessible (and thus bioavailable) fraction relative to medium-chain fatty acids [55–57]. In contrast, there is a limited influence of fatty acid saturation on bioaccessibility [55, 56]. Other factors that have a smaller but still meaningful impact on carotenoid bioaccessibility include competition with supplemental doses of carotenoids or fat-soluble vitamins E, D, and K for micellarization or uptake by the same transporters [29, 58], presence of divalent metals [48, 59, 60], and presence of certain types of proteins (i.e., β-lactoglobulin [61] and other whey proteins, caseinate, and soy proteins [62, 63]).

The concentration of carotenoids soluble in the duodenal and jejunal compartments remains fairly constant through the first 2 h of small intestinal digestion, before rising fourfold to fivefold over the next 2–3 h [64, 65]. This increase in concentration is at least partially driven by water reabsorption. Despite these increases, the bioaccessibility and subsequent bioavailability of carotenoids (i.e., removal from the intestinal lumen and ultimate release into the blood stream) on average remains low, but with high inter-individual variability, even under ideal conditions [10, 29]. Thus, a significant fraction of the original carotenoid dose remains available for interaction with the GM. The fact that so much of the carotenoids remain unabsorbed is perplexing, as fatty acids and bile salts are efficiently absorbed by transport proteins in the distal small intestine (i.e., 80%–95% fatty acids absorbed, depending on chain length and saturation, >90% of bile acids) [66, 67]. We could speculate that the reuptake of water and loss of fatty acid micelles would cause carotenoid crystallization to occur (if pure carotenoid supplement was fed), or at least partial association with digestible and indigestible fibers if the carotenoid dose was consumed as part of a fruit or vegetable preparation, and such carotenoid processing in the upper GI tract is likely to affect the GM. Considering that doses of 2–20 mg of carotenoids can be consumed from a single meal [68], significant carotenoid quantities are present in the large intestine and they may modulate the GM either directly or indirectly.

Human studies support these very high concentrations, using fecal carotenoid content as a proxy for what remains in the colon. For example, following 2 weeks of daily consumption of 330 mL of tomato or carrot juice, fecal concentrations of lycopene, β-carotene, and α-carotene increased 50–60-fold in healthy men [69]. Regarding the colonic carotenoid uptake, absorption of β-carotene into human exfoliated epithelial cells of the colon was demonstrated [70], and SR-B1 (transporter responsible for uptake of some carotenoids) is also expressed in the colon [71]. Transporter expression combined with the incomplete carotenoid absorption in the small intestine suggest the colon may be a place for carotenoid uptake. Studies so far have not suggested a strong correlation between carotenoid dietary intake and colon tissue concentrations [72], though in a human trial GM composition (thought to alter absorption from the epithelium) correlated with baseline serum carotenoid concentration [73]. In mice, the reduced abundance of microbial populations in the gut led to an increase in β-carotene and vitamin A storage in the liver, though no further metabolism by GM was detected [74]. The data available are too preliminary to draw conclusions.

Apo-carotenoids during digestion

Apo-carotenoids are catabolites of carotenoids (either carotenes or xanthophylls) that contain <40 carbons. They are consumed from fresh and processed fruits and vegetables at concentrations that are 100–1000× lower than the precursor carotene concentrations in the same foods [75, 76]. These proportionally low concentrations are because apo-carotenoids (most famously strigolactones and abscisic acid but including many species that have more recently been identified) serve as

potent plant hormones, whose production within the plant is regulated via carotenoid cleavage dioxygenase enzymes [77]. These enzymes produce apo-carotenoids that are ≤14 carbons in length, and serve as plant root growth regulators, aid in plant defense, provide aroma, and modulate plant stress responses [77–79]. This asymmetric or eccentric cleavage leaves a longer-chain (i.e., ≥26 carbon) apo-carotenoid as the corresponding "by-product." It is these longer-chain products for which there is the most data present to-date in mammalian systems. However, both short-chain apo-carotenoids and longer-chain apo-carotenoids may have health-relevant effects in humans. For instance, abscisic acid, a C15 carbon apo-carotenoid acting as a plant hormone, has been shown to improve insulin glucose response in a murine model of diet-induced obesity [80]. Similarly, the consumption of the C20 carotenoid crocetin improved insulin resistance in a pilot study of type 2 diabetics [81]. Further potential biological effects of some of these apo-carotenoids have been reviewed by Harrison and Quadro [82].

Interest in the production of these longer-chain apo-carotenoids during digestion has arisen as the conjugated double-bond system of carotenoids is especially labile to chemical degradation, which can also produce apo-carotenoids. The gastric compartment provides a hostile environment to carotenoids, e.g., presence of oxygen, low pH, and large lipid droplets where carotenoids and unsaturated lipids co-mingle and co-oxidation can occur. Under simulated *in vitro* gastric conditions and in the absence of enzymes (e.g., in the presence of dioxygen, with or without iron, at the proper pH, emulsified with unsaturated lipids), some parent carotenoid loss and some apo-carotenoid production has been reported [83–88]. However, both *in vitro* models using enzymes [84], as well as gastric and duodenal aspirates from human subjects during digestion [38, 64, 65], showed very limited (≤20%) carotenoid isomerization (the first step of carotenoid breakdown) during digestion. Additionally, no marked changes in apo-carotenoid concentrations have been observed. When lycopene or isotopically labeled β-carotene was digested alone in healthy men ($n = 7$), no significant change in gastric or duodenal apo-carotenoid digestate concentrations was observed, relative to the quantity of carotenoid, over 4–5 h [64, 65]. When a lycopene meal was co-digested with ferrous sulfate in healthy men ($n = 7$), the gastric and duodenal digestate concentrations of both lycopene and all but one species of lycopene-derived apo-carotenoids significantly decreased over 4 h, suggesting other lycopene degradation products were produced instead [65]. Notably, lycopene-derived apo-carotenoid absorption was observed in this study, as evidenced by the presence within the newly absorbed lipid-fraction within the blood and confirmed via follow-up Caco-2 experiments [65]. This evidence suggests that only a fraction of ingested lycopene-derived apo-carotenoids would remain in the GI tract for further interaction. In contrast, no isotopically labeled β-apo-carotenoids were absorbed in the previously cited work, suggesting that they would remain in the GI lumen, and could potentially elicit biological effects there [64].

Asymmetric apo-carotenoids may also be to be produced in the human body via one of the endogenously present carotenoid cleavage dioxygenases, i.e., β-carotene oxygenase 2 (BCO2). Indeed, BCO2 is expressed in a number of human tissues, including the small intestinal mucosa and the liver, although notably it is not expressed in the colon [89]. Its activity in model systems has been well characterized, with BCO2 demonstrating broad specificity but a clear preference for cleaving xanthophylls, specifically at the 9′–10′ double bond [90, 91]. BCO2 expression, which occurs in the mitochondria, increases in response to the knockout of the other main intestinal carotenoid cleavage enzyme (β-carotene oxygenase 1, BCO1) [92, 93]. Although the purpose of BCO2 expression is not fully understood, it has been postulated to serve an important role in preventing carotenoid concentrations from reaching levels which might expose the cell to increased oxidative damage [92]. Understanding the role BCO2 may play in humans is much more difficult, and very little work has been done. ^{13}C β-carotene (which contained a baseline concentration of ^{13}C β-apo-carotenals) was fed to healthy males, and samples of the gastric and duodenal digesta, as well as blood plasma and the newly absorbed lipid-fraction of blood were collected and isolated [64]. Notably, both ^{13}C β-carotene and ^{13}C β-apo-carotenals increased at largely the same rate over time in plasma (presumably as water was absorbed). Thus, there was no meaningful net change in the ratio of any of the β-apo-carotenals investigated/β-carotene, and no absorption of any ^{13}C β-apo-carotenoids into the blood plasma except for vitamin A isoforms. This suggest that either the ^{13}C β-apo-carotenods in the digesta were coming from the initial dose without any additional production, or it may indicate that any cleavage by BCO2 is quickly shunted toward vitamin A production, via further catabolism by BCO1.

It is also possible that apo-carotenoids could be made in other body compartments, as recently reviewed by Harrison and Kopec [94], and re-secreted into the gastrointestinal lumen with bile. Longer-chain apo-carotenoids (from dietary sources [76], or produced via BCO2 cleavage [95]) could also be cleaved further by the central, vitamin A-producing cleavage enzyme BCO1. BCO1 is expressed in some of the same tissues as BCO2, including the liver and the small intestine. BCO1 is also expressed in the stomach and the colon [89], and some evidence suggests that beyond central cleavage of β-carotene, it may also act on carotenoids such as lycopene and β-cryptoxanthin [82]. The role of intestinal BCO1 in cleavage of provitamin A, and the important effects of vitamin A on maintaining the intestinal barrier and conferring immune protection have been discussed elsewhere [91, 96–99]. The exact interplay between BCO1 and BCO2, and the relative contributions to the apo-carotenoid pool and the vitamin A pool in the human digestive lumen and within the epithelial cells themselves, remains a gap in knowledge. In addition, whether any human colonizing microbiota can synthesize apo-carotenoids remains unclear.

The biological effects of these apo-carotenoid products remain understudied; however, some apo-carotenoids have demonstrated antagonistic activity toward nuclear receptors which are stimulated by retinoic acid (the potent form of vitamin A) [7, 100]. It is also possible that apo-carotenoids produced from provitamin A carotenoids could be further cleaved or oxidized to produce vitamin A itself [101], whose functions within the GI lumen are discussed in greater detail below.

Regardless, the concentrations of apo-carotenoids within the upper gastrointestinal remain relatively low in relation to the precursor carotenoids from which they are derived, and thus very low amounts would be passed on to the colon. Thus, the remainder of this review focuses on the carotenoids themselves.

Microbial carotenoid metabolism—potential breakdown and biosynthesis of carotenoids

The GM of animals and humans are complex ecosystems that support many metabolic and biological functions within their hosts. These functions range from assisting the metabolism of

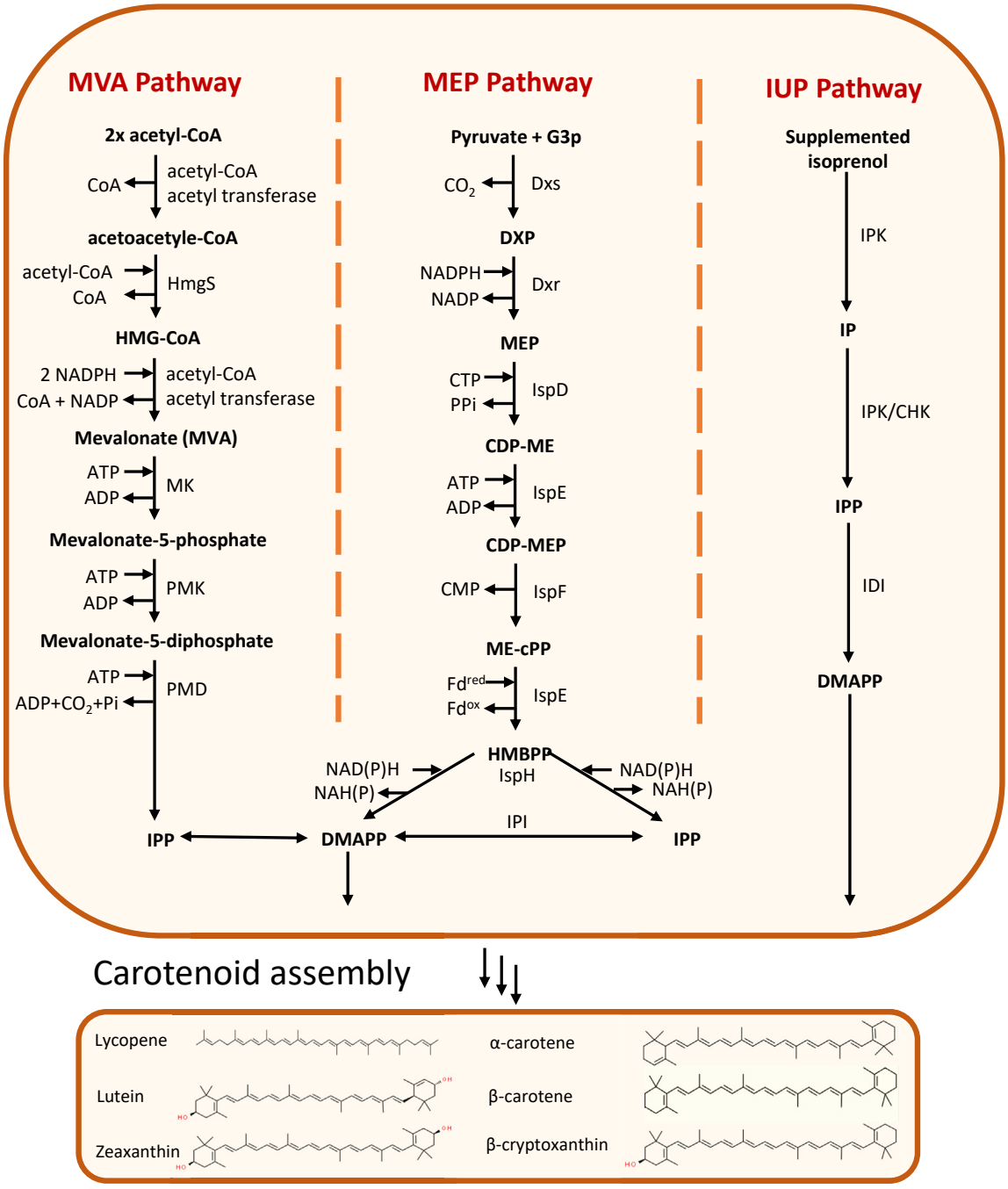

FIGURE 2. Pathways for biosynthesis of carotenoid precursors. CHK, Choline kinase; CTP, Cytidine 5'-triphosphate; DMAPP, Dimethylallyl diphosphate; Dxp, 1-Deoxy-D-xylulose 5-phosphate; Dxr, 1-Deoxy-D-xylulose 5-phosphate reductoisomerase; Dxs, 1-Deoxy-D-xylulose 5-phosphate synthase; Fd, Ferredoxin; G3P, Glyceraldehyde 3-phosphate; CMP, Cytidine monophosphate; HMBPP, E-4-Hydroxy-3-methyl-but-2-enyl pyrophosphate; HMGR, 3-Hydroxy-3-methylglutaryl-CoA reductase; HmgS, 3-Hydroxy-3-methylglutaryl-CoA synthase; IDI, Isopentenyl diphosphate isomerase; IspD, 2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase; IspE, 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase; IspH, 4-Hydroxy-3-methylbut-2-enyl diphosphate reductase; IPI, Isopentenyl diphosphate isomerase; IMO, Isomalto-oligosaccharides; IPK, Isopentenyl phosphate kinases; IPP, Isopentenyl diphosphate; IspE, CDP-methylerythritol kinase; IUP, Isopentenol utilization pathway; MK, Mevalonate kinase; MVA, Mevalonic acid; MEP, Methylerythritol 4-phosphate; PPi, Pyrophosphate; PMD, Phosphomevalonate decarboxylase; PMK, Phosphomevalonate kinase.

essential metabolites and nutrients, training the immune system to fight pathogens, and eliminating allergic stimulants, to steering the mood and behavior of its host [102]. One major symbiotic advantage of hosting microbial communities is obtaining essential amino acids and vitamins such as isoforms of all B vitamins as well as vitamin K—nutrients that the human body cannot synthesize [103]. Likewise, mammals cannot synthesize vitamin A *de novo*, meaning that it must be obtained either as preformed vitamin A from animal sources, or as provitamin A carotenoids from the diet and potentially from gut bacteria [21]. Although evidence of how microbes contribute to the bioavailability of carotenoids is still lacking, many lipid transporters that facilitate carotenoid uptake were found in the colon, where the highest microbial diversity resides [29]. For example, SR-B1 (scavenger receptor, class B type I) is expressed in colonic epithelial cells, suggesting that carotenoids can be taken up in the colon [7, 104]. The fraction of carotenoids remaining in the intestinal lumen could either be used by microbes to enhance lipid metabolism, to serve as antioxidants to reduce oxidative damage, or secreted in feces with the undigested fibers. Since carotenoids are important for the health of the human body, it would be desirable to understand how both the host and the GM assist each other to maximize their benefits from the ingested carotenoids. However, the mechanisms by which the carotenoids are made available for the colon host for absorption and are utilized by microbes are poorly understood.

Very little on colonic metabolites has been mentioned in the literature. For *E. coli*, as well as lactic acid bacteria, it has been reported that the xanthophyll fucoxanthin is metabolized into fucoxanthinol, following deacetylation [105].

Engineering carotenoid biosynthesis and the applicability to the gut

In this section, we will review the current state of the art in engineering carotenoid biosynthesis and the applicability of these strategies to the gut as a novel possibility to enhance gut health and to improve carotenoid and vitamin A status. Traditionally, food has been the main source of carotenoids. However, a variety of factors, including low crop yields or lack of access to crops with high carotenoid content [106, 107], can lead to an inability to consume a sufficient amount of provitamin A carotenoid to meet vitamin A needs. Furthermore, as carotenoids find use as food colorants, cosmetics, and pharmaceutical products, new ways to synthesize, produce, and extract carotenoids have been developed [108, 109]. Recent studies have shown that carotenoid biosynthesis in microorganisms proceeds in a highly specific fashion, whereas chemical synthesis usually yields multiple geometric carotenoid isomers [110]. Using biotechnological processes, genetically engineered microorganisms (i.e., bacteria and fungi) can be easily scaled up and are not impacted by factors such as seasonal harvest fluctuations, especially in the era of climate change [109].

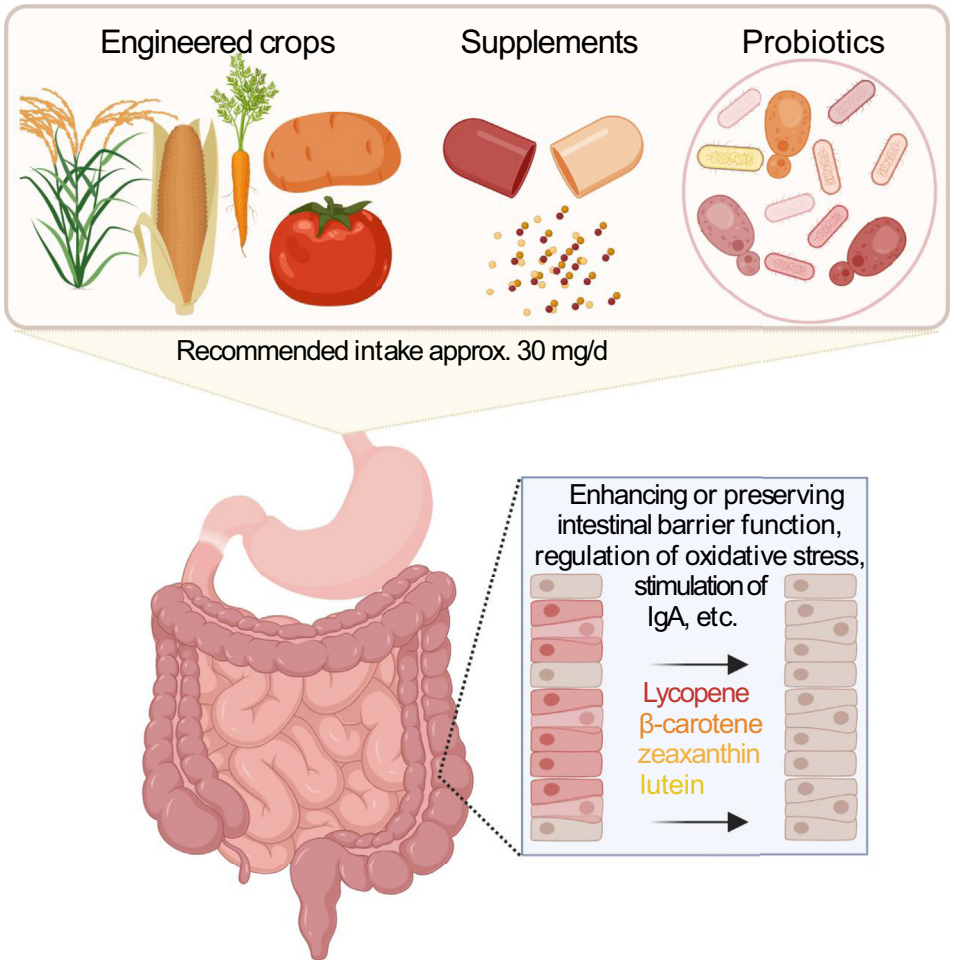

FIGURE 3. Approaches for delivering carotenoids together with probiotics to the colon. IgA, Immunoglobulin A.

Two main pathways that are responsible for the production of carotenoids in plants and microorganisms are the mevalonate (MVA) and plastidial 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway (Figure 2) [109]. Naturally, although carotenoid-producing eukaryotes and archaea mainly follow the MVA pathway, bacteria and plant plastids mainly have the MEP pathway. To create the building blocks of any carotenoids, the MVA and MEP pathways exploit molecules that are produced by the central carbon pathway, mainly pyruvate, acetyl-CoA, and glyceraldehyde-3-phosphate (G3P). Both pathways use these substrates to create iso-pentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP), which are then assembled into different carotenoid structures. For example, 7 IPP molecules and 1 DMAPP molecule are needed to create 1 molecule of lycopene. Overexpression of certain genes in those pathways, e.g., 3-hydroxy-3-methylglutaryl coenzyme A reductase (*HMGR*), phytoene synthase (*PSY*), and phytoene desaturase (*CRTI*) and introduction of homologs of these genes, can increase production of total carotenoids.

The use of microorganisms for producing relatively small molecules such as carotenoids is attractive because microbes can be cultured year-round and do not require large land areas or greenhouses [111]. With the availability and simplicity of genetic engineering tools to manipulate microbial genomes, as well as advances in fermentation strategies, the development of high carotenoid-producing strains is becoming increasingly possible [110]. Lycopene, β-carotene, zeaxanthin, lutein, and β-cryptoxanthin are the most relevant carotenoids related to human health and therefore most microbial engineering efforts have focused on optimizing their production (Figure 3) [112]. Some of the engineering approaches used to increase the production of these carotenoids in yeast and bacteria include: regulation of the biosynthesis pathways, enhancing enzyme expression, increasing membrane synthesis, optimization of the central metabolic pathways, rewiring central carbon metabolism [113], combinatorial assembly of different genetic parts and combinations of these methods (Table 1). Although these approaches and optimization of the MVA and MEP pathways have increased carotenoid production, the amount of IPP biosynthesis limits further optimization [109]. Therefore, a chemoenzymatic approach to increase IPP was explored [109]. Transfecting the isopentenol utilization pathway (IUP) into *Escherichia coli* MG1655 and *Yarrowia lipolytica* increased the biosynthesis of IPP by 15–100-fold and thus the production of carotenoids [114]. The IUP pathway uses the isopentenol isomer isoprenol or/and prenol, which can be supplemented in the growth media, as the main substrate for creating IPP and DMAPP molecules [114]. Compared with the MVA and MEP pathways, the IUP pathway only goes through 2 steps (MVA and MEP pathways contain 6 and 7 steps, respectively) to create one molecule of IPP, which consequently reduces byproducts and the energy expenditure required for creating carotenoid building blocks.

In situ production of carotenoids by probiotics in the GI tract offers a novel, potentially more sustainable approach for delivering carotenoids [115–117]. Probiotics are live microbes that are attractive vehicles for the engineered production of desired molecules and biologics. By delivering these molecules right where they are needed, they can ultimately reduce the cost of carotenoid production by bypassing purification and concentration steps [115, 116, 118]. Using probiotics for the production and delivery of carotenoids, such as β-carotene, could be most beneficial in developing countries where vitamin A deficiency is still prevalent, as probiotics are relatively easy to obtain [117].

Building on the progress of microbial engineering for the production of carotenoids, Durmusoglu et al. first demonstrated that the probiotic yeast *Saccharomyces boulardii* could produce β-carotene in the gut of gnotobiotic mice. In this study, a single dose of *S. boulardii* expressing heterologous β-carotene pathway was delivered to mice, and the fecal concentration of β-carotene was monitored over 2 weeks. The authors detected up to 37 μg/g of β-carotene (68 μM) in feces. Another study conducted by Stevens et al. focused on *Bacillus indicus* (PD01), which was isolated from the fecal matter of a healthy volunteer and found to

TABLE 1
Sources of carotenoid production by microorganisms, and their delivery mechanisms. DCW = dry cell weight.

Delivery to gut	Carotenoid	Amount	Source	Genetically modified organism	Reference
Probiotic	β-carotene	37 μg/g	*Saccharomyces boulardii*	Yes	[117]
Probiotic	lycopene	40.8 mg/mL	*Bacillus indicus*	No	[28]
Probiotic	lycopenoate	23.6 mg/mL	*Bacillus indicus*	No	[28]
Probiotic	lycopene	1.09 mg/L	*Lactococcus lactis*	Yes	[177]
Supplement	β-carotene	7 mg/L	*Corynebacterium glutamicum*	No	[178]
Supplement	β-carotene	6500 mg/L (90 mg/g)	*Saccharomyces cerevisiae*	Yes	[179]
Supplement	β-carotene	4000 mg/L	*Saccharomyces cerevisiae*	Yes	[180]
Supplement	β-carotene	2370 mg/L (73.3 mg/g)	*Saccharomyces cerevisiae*	Yes	[181]
Supplement	β-carotene	2100 mg/L	*Saccharomyces cerevisiae*	Yes	[182]
Supplement	β-carotene	704.1 mg/L	*Blakeslea trispora*	No	[183]
Supplement	β-carotene	44.2 mg/g	*Saccharomyces cerevisiae*	Yes	[184]
Supplement	lycopene	2300 mg/L	*Saccharomyces cerevisiae*	Yes	[185]
Supplement	lycopene	500 mg/g DCW	*Escherichia coli*	Yes	[186]
Supplement	lycopene	256 mg/L	*Blakeslea trispora*	No	[187]
Supplement	lycopene	220 mg/L	*Escherichia coli*	Yes	[115]
Supplement	lycopene	56 mg/g DCW	*Saccharomyces cerevisiae*	Yes	[188]
Supplement	lycopene	10 mg/g DCW	*Rhodobacter sphaeroides*	Yes	[189]
Supplement	astaxanthin	385.0 mg/L (7.0 mg/g)	*Escherichia coli*	Yes	[190]
Supplement	astaxanthin	320 mg/L	*Escherichia coli*	Yes	[191]
Supplement	astaxanthin	218 mg/L	*Saccharomyces cerevisiae*	Yes	[192]
Supplement	astaxanthin	1.7 mg/g	*Corynebacterium glutamicum*	No	[193]
Supplement	zeaxanthin	0.5 mg/g DCW	*Xanthophyllomyces dendrorhous*	Yes	[194]

naturally produce methyl-glycosyl-apo-8′-lycopenoate and glycosyl-apo-8′-lycopene [28]. The authors also used weaned piglets with impaired intestinal barrier function to examine the efficacy of PD01 to limit intestinal permeability or treat intestinal barrier dysfunction [119]. In a human cohort with healthy volunteers, the carotenoids produced by PD01 were observed in the plasma of the subjects, and PD01 was not found to impair intestinal barrier function, GI tract tolerance, and stool frequency or consistency [28].

Although only examined in a limited number of test subjects, these studies demonstrate that probiotics can deliver carotenoids to the GI tract and may provide a viable option to mitigate vitamin A deficiency and perhaps improve gut health. Future studies should focus on the effects of provitamin A-producing probiotics and testing carotenoid-producing probiotics in a greater diversity of subjects, and for a longer period of time.

Carotenoids and shift of gut microbiota—prebiotic-like and bactericidal effects against pathogens

About the same number of microorganisms exist in our gut as the number of human cells in our body (i.e., 10^{10}–10^{12}), primarily as bacteria in the colon [120, 121]. Prebiotic effects are based on preventing dysbiosis and shifting the composition of the GM toward one associated with health, by acting as a substrate for certain bacteria. In other words, prebiotics improve host health and physiology [106]. Such prebiotic effects are well recognized for several fermentable dietary fibers, such as fructooligosaccharides and galactooligosaccharides [122]. Also, some polyphenols may act similarly, since they are poorly absorbed in the small intestine and are present as glycosides that could be fermented by certain bacteria [14]. Health-beneficial bacteria include *Lactobacillus* and *Bifidobacterium*, both of which are lactic acid producers that may aid in displacing more health-detrimental bacteria, e.g., biofilm-producing *Pseudomonas* [123]. This displacement prevents an overgrowth of opportunistic bacterial pathogens (OBP), which is also an important function of prebiotics. Other fermentation products may confer protection, such as the short-chain fatty acid butyrate, which is associated with anti-inflammatory effects locally and systemically following mucosal uptake [124]. Some bacteria, such as *Faecalibacterium prausnitzii*, a dominant butyrate-producing bacteria, have been reported to be decreased under certain dysbiotic conditions, including IBD [125]. Administration of a β-carotene-rich oil (1 g· L^{-1} in tucuma oil rich in oleic acid) to the rumen resulted in a shift from acetate to propionate [126], which is considered even more anti-inflammatory than butyrate [127]. In addition, this β-carotene-rich oil also induced GM changes, though rumen physiology can be quite different from humans (Table 2).

As *Bacteroidetes* and *Firmicutes* account for >90% of the phyla in the gut [128], changes in taxa that fall under these phyla are also of interest. For example, a higher ratio of *Firmicutes to Bacteroidetes* has been associated with obesity, and a lower one with IBD, as reviewed recently [129]. It should be noted though that until recently, bacterial populations have been primarily assessed using 16S rRNA, allowing differentiation only at the genus level. Moving forward, more insightful results may be obtained on a lower taxonomic level, adding more information about functionality but requiring more elaborated metagenomics analyses [130]. Beyond composition and abundance, a better understanding of the metabolic behavior of each bacterial species, which can radically change depending on dietary intakes [131], may better elucidate the role each microbe plays in health and disease.

There is little direct evidence that carotenoids serve as prebiotics or as direct energy sources for bacteria, and certainly other dietary factors can easily confound human studies, unless pure carotenoids are supplemented. Alcoholic or lactic acid fermentation did not result in significant losses of carotenoids [132]. In contrast, β-carotene and lycopene appeared to be produced by certain bacteria, such as *Blakeslea trispora*, as reviewed recently [132]. Certain bacteria and yeast were also reported to be capable of producing carotenoids, as recently summarized [133].

Nevertheless, microbial shifts were reported following intervention with carotenoids. In animal studies, capsanthin from bell pepper (>90% purity) (dose of 200 mg· kg^{-1} body weight) was administered to obese mice for 12 weeks [134]. Notably, the abundance of *Bacteroidetes*, *Bifidobacterium,* and *Akkermansia* were increased, whereas *Ruminococcus* and *Firmicutes* were reduced. In another study [135], astaxanthin from yeast (0.04% in the diet, for 8 weeks) significantly reduced OBP (i.e., *Proteobacteria and Bacteroides)* in *β-carotene oxygenase 2* (*BCO2)* knockout mice, whereas strongly increasing *Actinobacteria* and *Bifidobacterium* in wild-type mice. In another animal experiment, pigs on a low-protein diet received carotenoid-fortified corn (20% of the diet, rich in zeaxanthin, ca. 10 μg· g^{-1} total carotenoids) or corn without carotenoids for 30 d [136]. Sequencing (16S rRNA) of feces demonstrated that, though the carotenoid-rich diet did not alter the microbiome diversity, 162 amplicon sequence variants differed in abundance in the carotenoid-treated vs. the control group, and abundance of *Epulopiscium* was higher in the carotenoid group. Another porcine study investigated changes related to intestinal inflammation induced by weaning: 24 piglets were distributed into a normal suckling group, and weaning piglets were randomized to receive somewhat supra-physiological carotenoid doses (either 40 mg· kg^{-1} body weight β-carotene or 80 mg· kg^{-1} body weight β-carotene) for 2 weeks [119]. Pigs receiving β-carotene had significantly decreased species from the *Bacteroidetes* phyla and the genus *Prevotella* and *Blautia.* The authors reasoned that reducing *Prevotella* especially could be of interest, because of its correlation with pro-inflammatory conditions. Furthermore, β-carotene increased abundance of the phyla *Firmicutes* and the genera *p-75-a5 and Parabacteroides* vs. the control group, believed to be because of changes in inflammatory cytokines. *Parabacteroides* and *Synergistes* correlated inversely with interleukin-1β (IL-1β), IL-6, and TNF-α concentrations, likewise *p-75-a5* inversely correlated with IL-6 in serum, pointing to important activations in the immune system in young piglets. Indeed, results were related to a reduction of NF-κB in the colon, also indicative of a decreased pro-inflammatory state. GM diversity indices (i.e., Chao1 and ACE) also improved with β-carotene consumption. Summarizing these animal studies, it appears that carotenoid consumption altered the abundance of various phyla and genera, and that these changes were inversely correlated with inflammatory signaling.

In terms of human studies, consumption of a mixture of blackcurrant powder, lactoferrin, and lutein (nonspecified quantities) for 2 weeks significantly increased *Bifidobacteria* and *Lactobacilli* populations but reduced levels of β-glucuronidase (associated with colon cancer) producing *Bacteroides* spp. and

TABLE 2
Studies with carotenoids involving effects on the GM

Compound	Study type	Dosing and major outcomes	Main findings	Reference
Human studies				
β-Carotene	Observational study in subjects (n = 16) with cystic fibrosis	Associations tested between fecal microbiota and corresponding micronutrient intakes.	Intake of β-carotene (and several other antioxidants) was related to lower *Bacteroides* and higher *Firmicutes*.	[139]
Methyl-glycosyl-apo-8′-lycopenoate and glycosyl-apo-8′-lycopene produced by *Bacillus indicus*	Randomized, controlled trial in overweight/obese subjects (n =67)	Consumption of carotenoid-producing bacteria vs. placebo for 6 wk, plasma carotenoid concentrations, and colonic permeability.	0.044 and 0.076 vs. 0 μM in controls as the sum of bacterial carotenoid compounds after 3 and 6 wk, respectively. No significant difference in gastro-duodenal or colonic permeability as determined by multi-sugar test (l-rhamnose; S/E : sucralose/erythritol). No difference in GI tolerance.	[28]
Lycopene	Adult subjects with obesity (n = 30), double-blinded design	7 mg or 30 mg for 30 d as supplement, GM abundances measured.	Dose-related improvement in GM profile with enhanced fractions of, e.g., *Bifidobacterium adolescentis* and *Bifidobacterium longum*. Also related to dose-dependent variation in the blood, liver metabolism, skeletal muscle, and skin measures.	[13]
Animal trials				
Fucoxanthin	Male BALB/c mice (n = 40)	For 4 wk, mice were fed NCD (normal chow diet), NCD + fucoxanthin (NCDF, 125 mg/kg), HFD + fucoxanthin (HFDF, 125 mg/kg).	No difference detected between the NCD and NCDF. *Firmicutes* and *Bacteroidetes* increased in the NCDF group (26%). In the HFDF group, it increased (13%) vs. the HFD group, suggesting a positive effect of fucoxanthin. Fucoxanthin decreased abundance of *Verrucomicrobia* phylum.	[173]
β-Carotene	Piglets (n = 24)	Suckling group, weaning group, weaning + β-carotene (40 mg/kg bw.) group, and the weaning + β-carotene (80 mg/kg bw.) group, 2 wk intervention. Serum, jejunum, colon, and feces were investigated.	β-Carotene decreased phyla *Bacteroidetes* and the genus *Prevotella*, and *Blautia*, but increased species from the phyla *Firmicutes* and *Parabacteroides* vs. weaning animals. Spearman's correlation analysis: revealed positive correlation between *Prevotella* and *Blautia*, and *Parabacteroides* and *Synergistes* were negatively correlated with concentrations of interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α). *p-75-a5* showed negative correlation with serum IL-6.	[119]
Lycopene	Mice (n = 10 per group)	Lycopene at 0.03% in the diet for 10 wk, together with a high fat diet. A number of behavioral tests and biomarkers were investigated.	Lycopene improved HFD-triggered memory loss. Lycopene also improved synaptic functioning. It also reduced insulin resistance, improved lipid metabolism dysfunction, and inflammatory responses in brain and liver. Finally, lycopene improved intestinal barrier integrity as shown by higher expressed claudin-1 and occludin as well as reduced circulating lipopolysaccharides.	[167]
Lycopene	"Cobiotic" treatment: Male Swiss albino mice, (n = 8–10 per group)	5 and 10 mg lycopene/kg bw. and isomalto-oligosaccharides (IMO), for 12 wk. A variety of systemic markers (OS, inflammation), GM, and histopathologic examinations were carried out.	Lycopene improved SCFA concentration and ileal and colonic health. This was measured by histopathology and reduced *Enterobacteriacea* and increased *Lactobacilli,* increased total SCFA as well as improving systemic markers of OS stress and inflammation (e.g., SOD and CAT and various cytokines, respectively).	[163]

(*continued on next page*)

TABLE 2 (*continued*)

Compound	Study type	Dosing and major outcomes	Main findings	Reference
			Synergistic effects were observed with IMO.	
β-Carotene	Mouse model of ulcerative colitis, number of animals nonspecified	Receiving (application mode not further specified) 5, 10, and 20 mg β-carotene/kg bw. for 28 d.	β-Carotene improved severity of UC, modulating various targets. e.g., NF-κB, cyclooxygenase- 2, interleukin 17, signal transducer and activator of transcription 3, Nrf2, matrix metalloproteinase-9, and connective tissue growth factor. Further more, β-carotene treatment maintained gut barrier integrity by increasing occludin expression. Also, β-carotene reduced plasma lipopolysaccharide levels.	[164]
Carotene (and proteins)	Duroc pigs (n = 32)	Pigs were fed 2 different diets for 1 mo.: a standard protein (SP) diet or SP + carotene-enriched (CE) diet (20% of M37W-Ph3 carotenoid-enriched corn), unspecified amount carotenes.	Proteins had a stronger modifying effect than carotenes on the pig GM patterns. 160 amplicon sequences variants differed between CE and SP.	[136]
Methyl-glycosyl-apo-8′-lycopenoate and glycosyl-apo-8′ -lycopene produced by *Bacillus indicus*	Piglets (n = 16)	Either control diet or control diet with *Bacillus indicus* for 23 d. Barrier functionality in the gut was tested.	Supplementation with *Bacillus indicus* improved expression of occludin in distal small intestine and TEER in the mid colon vs. control. No differences regarding crypt depth and villus height.	[28]
Tomato powder (TP) rich in carotenoids	Male $BCO1^{-/-}$ + $BCO2^{-/-}$ knockout mice (n = 18)	Mice were fed a HFD with or without dietary TP (42 g/kg diet) intervention for 24 wk. Carotenoid dose of 2.39 mg of lycopene, 0.11 mg of β-carotene/g TP	The fraction of Gram-positive bacteria was enhanced following TP; the fraction of Gram-negative bacteria was lowered accordingly. TP diminished the relative abundance of *Clostridium* spp. and *Mucispirillum* spp.	[174]
***In vitro* investigations**				
Lycopene	*In vitro* inhibition assay: *B. subtilis*	Extraction from tomato paste and tests on B. subtilis, 50 μg/mL lycopene.	Inhibition against *B. subtilis*.	[175]
β-Carotene and extracts of carotenoids from red paprika, apples, oranges	*In vitro* inhibition assays: HSC-2, HSG, and HTLV1	extracts of carotenoids and β-carotene from red paprika tested on infected cells (*H. pylori* and HIV-1 type IIIB) for 5 d.	Prevention of the development of *H.pylori*-associated disease, MIC_{50} of β-carotene and red paprika extract of > 200 μg/mL), extract from apples showed MIC_{50} of 36 μg/mL.	[147]
Tucuma oil rich in beta-carotene (1 g/L oil)	*In vitro* trial with rumen inoculum from donor Holstein cows	Tucuma oil at 0.5 and 1% added to diet, mostly containing oleic acid, added to rumen inocculum (n =3) during 15-d experiments.	With tucoma oil, reduced number of *Fibrobacter* and *Rikenellacea* RC9 group, and enriched *Pyramidobacter*, *Megasphaera*, *Anaerovibrio*, and *Selenomonas*. Reduced acetate and butyrate fraction of volatile fatty acids, increased fraction of propionate and valerate.	[126]
Extract carotenoids (from Shatian pummelo *C. grandis*)	*In vitro* inhibition assays: *B. subtilis, S. aureus, E. coli, A. niger, A. flavu, P. chrysogenum, R. oryzae* and *S. cerevisiae*	The extract was incubated for 24 h with the microorganisms tested.	Inhibition against *B. subtilis, S. aureus, E. coli, A. niger, A. flavus, S. cerevisiae.*	[176]
Annatto, carrot, corn, and tomato extracts	*In vitro* inhibition assays: *E.coli* and *S. aureus*	Extracts were incubated for 18–24 h with microorganisms tested.	Annatto, carrot, and tomato extracts exhibited antibacterial property for *S. aureus*. Annatto Extract, having the highest total carotenoid content, also exhibited the major MIC_{50} for *S. aureus.*	[149]

GI, gastrointestinal tract; HFD, high-fat diet; MIC, Minimally inhibitory concentration; OS, Oxidative stress; TEER, transepithelial electrical resistance.

Clostridium spp. [137] in healthy subjects. However, a similar mixture tested without lactoferrin and lutein had similar effects, suggesting that the effects may have been because of compounds in the blackcurrant extract. In a human association study, 11 taxonomic units of GM were significantly associated with serum carotenoid concentrations [73]. Specifically, serum carotenoid concentrations were negatively correlated with the abundance of *Firmicutes* and positively correlated with the abundance of *Bacteroidetes*. Similar results were found in a study with pregnant women, with plasma carotenoid concentrations positively correlating with α-diversity [138]. In cystic fibrosis patients, dietary intake of β-carotene was related to a higher *Firmicutes/Bacteroides* ratio [139]. Of course, these effects may be explained by carotenoids serving as a marker for fruit and vegetable, and thus fiber, intake. Most notably, in a 1-mo intervention trial, lycopene was tested in obese adults, and provided alone (7 mg or 10 mg/d) or incorporated into dark chocolate (7 mg/d), vs. respective controls (i.e., placebo, dark chocolate) [13]. Lycopene dose-dependently increased the relative abundance of *Bifidobacterium adolescentis* and *B. longum*, together with *Lactobacilli*. Also, dose-dependent reductions of LDL-C, LDL-peroxidase, and MDA as markers of oxidative stress (OS) were noted. Because of its low bioavailability, lycopene may reach the colon at an especially high proportion [140, 141]. The main open questions were whether lycopene directly stimulated the growth of health-beneficial bacteria or if these were indirect effects, i.e., whether the improvements in blood lipids, OS, etc., induced changes in the GM.

As carotenoids are unlikely to act as energy sources and thus as direct prebiotics for bacteria, additional modes of actions have been investigated. It is plausible that carotenoids contribute to reduced (OS) in the gut, including the colon, preventing ROS formation that may be detrimental to some bacteria [138, 142, 143]. Such effects were shown for an antioxidant peptide which decreased intracellular bacterial ROS, improving bacterial survival in the presence of antibiotics [144]. The importance of ROS for intestinal physiology, including GM and the epithelium, has been emphasized earlier. For instance, *Firmicutes* taxa were shown to be influenced by OS and the presence of antioxidants, with, e.g., higher numbers of *Lactobacilli* spp. at lower OS induced by lipoic acid [145]. Such mechanisms may be more plausible in eliciting effects on the GM, rather than through fermentation/metabolism of carotenoids, though data in this area is lacking.

Another effect by which carotenoids could alter GM composition may be because of potential bactericidal effects, though it is questionable whether sufficiently high concentrations are reached in the colon to exert such effects. Though selective suppression of pathogenic microorganisms *in vivo* has not been shown, carotenoids have demonstrated bactericidal properties ex vivo. For instance, an apple nonpolar (diethyl ether) extract (later diluted in DMSO) containing violaxanthin and zeaxanthin/lutein reduced *H. pylori* numbers at an MIC_{50} (50% minimum inhibitory concentration) of a carotenoid concentration of 36 $\mu g \cdot mL^{-1}$. Though the effects could have also been because of other apple constituents (e.g., other triterpenes), similar concentrations, though high, could be reachable *in vivo* after a carotenoid-rich meal or supplements, and the observed effects were not unlike the antibiotic metronidazole, also employed in this study [146]. In another study, citrus (*Shatian pummelo)* peel carotenoids reduced growth of especially *E. coli*, but also of potentially pathogenic bacteria including *B. subtilis, S. aureus, Aspergillus niger, A. flavus,* and *Penicillium chrysogenum* [147], with MIC_{50} of 19–140 $\mu g \cdot mL^{-1}$, concentrations that are achievable with carotenoid-rich meals. Lycopene in tomato oleoresin inhibited pathogenic *Pseudomonas aeruginosa* (MIC_{50}: 150 $\mu g \cdot mL^{-1}$), whereas MIC_{50} for other bacteria (e.g., *E. coli*) were higher [148]. Carotenoid-rich extracts (annatto, carrot, tomato, ca. 0.1–1.0 $mg \cdot g^{-1}$ carotenoids) had antibacterial activity against *S. aureus* [149]. Fucoxanthin from algae was effective against pathogenic *S. aureus* at 63 $\mu g \cdot mL^{-1}$ concentrations. Similarly, fucoxanthinol, a metabolite of fucoxanthin in the gut, was found effective (at high concentrations of 4.25 $mg \cdot mL^{-1}$) against *S. faecalis, Enterococcus* sp., as well as *S. aureus*, and *B. subtilis.* This activity was similar to the positive control chloramphenicol tested at 1 $mg \cdot mL^{-1}$ [105]. Achievable carotenoid concentrations in the colon may be around 50 $mg \cdot L^{-1}$ (50 $\mu g \cdot mL^{-1}$), as supplements with 50 mg are commercially available and a total postprandial liquid volume of digestive fluid of ~1L is common, not considering water uptake and concentration effects in the colon.

Taken together, carotenoids and carotenoid-rich extracts have been associated with a GM microbial profile that is more closely correlated with positive health attributes; however, what remains to be elucidated are 1) the underlying mechanism(s) and 2) whether changes in GM were a cause of, and not the consequence of, other favorable physiological changes observed.

Carotenoids and IgA

An important factor for gut health is also the host's immune system, and provitamin A carotenoid metabolites can interact via RAR/RXR nuclear receptors that elicit immune system responses [150]. IgA is the most abundant antibody isotype [151], expressed mainly in the mucosa and the gut. SlgA is the secreted form of IgA, which is formed by the concerted activity of plasma cells and endothelial cells. This results in secreted IgA and a free secretory component (SC) that are released into the colon by transcytosis. For further information, the reader is referred to a more detailed review [151]. SlgA has been reported to protect against enteric pathogens and toxins [152], whereas IgA can bind to specific microbiota, contributing to the gut homeostasis [153]. SC protects immunoglobulins from their proteolytic degradation by proteases. SC also contributes to activating recognition functions on SlgA and soluble IgM (SIgM), resulting in the binding and removal of bacteria [154]. IgA dysfunction has been reported to disturb GM homeostasis, reducing the abundance of *Bifidobacterium* and *Lactobacilli* and increasing *Gammaproteobacteria* [21]. De-regulated gut IgA expression has also been related to many allergies [155].

It is known that retinoids can have cell proliferative effects, and β-carotene (50 mg/kg feed) was shown in weanling mice to increase IgA antibody-secreting cells (ASC), an effect mainly attributed to all-trans retinoic acid [156]. Certainly, all-trans retinoic acid, the most potent form of vitamin A, can bind to retinoic acid receptor β (RARβ) to produce IgA in the mucosa, which may confer some protection, among other possible mechanisms [157]. Similar results were reported earlier by Nishiyama et al. [158]. In this study, pregnant mice received 50 $mg \cdot kg^{-1}$ β-carotene in the diet 6.5 d postcoitus (dpc) to 14 d postpartum (dpp). In the offspring, IgA concentrations were significantly higher in the stomach, serum, and feces of mice

receiving β-carotene vs. a control group. Of note, mice have a much higher cleavage efficiency to convert β-carotene to vitamin A than humans [159]. This further supports vitamin A as the main agonist behind IgA production in this model. Similarly, it is known that mice deficient in vitamin A have compromised IgA secretion of the colonic mucosa [160].

The interaction between carotenoids, GM, and IgA has been reviewed by Lyu et al. [21]. Both astaxanthin and retinoic acid were shown to enhance IgA production, preventing gut dysbiosis presumably via recognition and coating of pathogenic bacteria, preventing their infiltration through the epithelial barrier. However, the mechanism(s) remain unclear. For example, in weanling mice, astaxanthin-enriched yeast (120 mg/(kg· d) feed) increased IgA ASC after 7 d and enhanced small intestinal IgA mRNA expression after 14 d (colonic effects were not studied) [135]. The authors reasoned that astaxanthin could be conferring protection via ROS scavenging properties. However, it was also noted that the yeast alone could have had some IgA stimulatory effects, and this effect was not controlled for. Another study feeding mice with astaxanthin (at 200 mg/(kg· d) body weight for 10 d) or no carotenoids reported reduced bacterial loads of *H. pylori* in the stomach, reduced mucosal inflammation, and changes in T-lymphocyte response in the astaxanthin animals as compared with controls. This supports a role for astaxanthin in perhaps modulating IgA, though IgA was not measured in this study [161]. In a recent human study with young soccer players [162], astaxanthin supplementation (4 mg/d for 90 d) reduced CRP, and neutrophil levels in the blood compared with a placebo-receiving group. Salivary SlgA concentrations and secretion rate were also significantly increased by ~20%. In summary, both retinoic acid and astaxanthin consumption favorably appear to regulate IgA and SlgA expression, effects that have been associated with a more favorable GM composition in animal studies.

Carotenoids and gut barrier

Another remarkable property of carotenoids is their influence on gut barrier function, especially gut mucosa integrity and maintenance of tight gap junction functionality. Stevens et al. [28] fed the carotenoid-producing strain *Bacillus indicus* to piglets, for 23 d. Following necropsy, enhanced expression of tight junction proteins (Tjp1 and occludin) in the mid and distal small intestine, and improved colonic transepithelial electrical resistance were reported, whereas crypt depth and villus height were not influenced. The same bacterial strain fed to obese-overweight humans for 6 weeks was not shown to impact excretion of certain sugars (an objective measure of intestinal barrier function) [28], but other measures of intestinal permeability (e.g., those assessed in the piglet study) were not investigated.

Earlier studies on mice with β-carotene and lycopene likewise showed positive effects on gut barrier functionality. In one of the studies [163], Swiss albino mice received a high-fat diet with or without lycopene (5 or 10 mg/(kg· d) for 12 weeks), provided in combination with or without a prebiotic (isomalto-olig osaccharides, IMO). Lycopene alone improved ileal and colonic health. This was measured by histopathology and reduced *Enterobacteriaceae* abundance, increased *Lactobacilli* abundance, increased total SCFA concentrations, and improved systemic markers of OS and inflammation (i.e., increased concentrations of enzymes such as SOD and reduced cytokines, respectively). Additive effects were observed when lycopene + IMO was fed. In another study by Trivedi and Jena [164], β-carotene was given at 3 different doses (0, 5, 10, 20 mg/(kg· d) body weight for 21 d) to mice with induced ulcerative colitis (UC). Compared with controls, β-carotene reduced systemic markers of OS and inflammation, and increased occludin expression in the colon. This was accompanied by reduced plasma lipopolysaccharide concentrations, which also suggested that barrier integrity improved. Colon length and histology scores based on optical appearances were also improved dose-dependently with increasing β-carotene intakes. The authors hypothesized that the increase in Nrf2 expression related to OS prevention contributed to the mucosal protection. Vitamin A and β-carotene also contributed to gut-tight junction functionality in infants of HIV-positive mothers [165]. In this study, mothers received 1.5 mg retinyl palmitate and 30 mg β-carotene daily, in addition to 60 mg retinyl palmitate at delivery. Improved gut barrier function was demonstrated via mannitol and lactulose appearance in urine after an oral challenge. Because these women were at increased risk of vitamin A depletion and deficiency, the important role of vitamin A in mucus production from goblet cells was likely at least partially responsible for the effects observed [166]. In a study by Wang et al. [167], mice received 0.03% lycopene in a high-fat diet for 10 weeks. Lycopene was shown to improve intestinal barrier properties as shown by occludin and claudin-1 in the jejunum (colon was not measured), but also decreased plasma lipopolysaccharide levels, relative to a control group.

An earlier study has highlighted that gut bacteria may reduce the mucosal lining if dietary fiber is low [27], as these glycoproteins could serve as an alternative energy source. Likewise, bacterial shifts may also lead to changes in species important for the maintenance of the mucus layer, i.e., depending on the abundance and activity of mucin degraders. A reduced thickness of the mucus layer has been associated with an increased risk of allergen uptake, as well as the development of colitis and infections, as reviewed elsewhere [168]. A damaged mucus layer in the GI tract impacts microbial colonization and stability by changing the adherence and the organization of the GM and therefore changing the composition of communities [169]. Therefore, changes such as reductions in *Akkermansia muciniphila* could be important for optimal mucosa integrity and gut health [170]. Utilization of certain carotenoids has been shown to reduce the abundance of *Akkermansia,* which impacts the integrity of the gut mucosa, as reviewed previously [14]. *Lactobacillus* spp., *Lactobacillus reuteri,* and *Bifidobacterium longum* have also been reported to contribute to increased mucus layer thickness and growth [171]. Djuric et al. [73] found in a human study that carotenoids in plasma correlated with 11 operational taxonomic units, including a positive association with *Bacteroidetes* and a negative association with *Firmicutes*. It is also important to consider that carotenoids may merely serve as an indicator of a healthy diet. For example, the Mediterranean diet, which is carotenoid-rich, was also generally found to be associated with lower presence of *Firmicutes* and *Lachnospiraceae* [172]. Thus, though a direct relationship between carotenoid consumption and gut mucosa thickness is still missing, these associations merit further study to establish if such a causal relationship exists. In summary, several carotenoids may improve gut barrier functionality via increasing expression of occludin and claudin-1, increased mucus production, altered GM, and potentially antioxidant and anti-inflammatory effects.

In conclusion, there is mounting evidence that carotenoids can contribute to colonic health. Due to limited absorption in the small intestine, the majority of carotenoids are passed on to the colon, where they may act via different mechanisms. Though carotenoids are unlikely to present any significant source of energy for bacteria, potential mechanisms include alterations of the GM composition, likely via direct bactericidal effects, effects on IgA, or altering local OS levels. Additional effects such as improving tight junction integrity are also likely. Gaps in our knowledge include elucidating causal relationships between carotenoids and the mechanisms discussed herein. They also include questions on the effects of apo-carotenoids on GM, the structure and potential function of bacterially-derived metabolites, and also to what extent carotenoids or apo-carotenoid metabolites could be absorbed from the colon. A rather novel approach is the colonization of the GM with carotenoid-producing strains, which could be a promising alternative to consuming carotenoid-rich food items for carotenoid delivery. More studies on the relationship of carotenoids to the GM and colon-related health are needed.

Author disclosures

The authors report no conflict of interest.

Acknowledgments and Author Contributions

All authors were involved in the conception and the writing of the manuscript. The authors' responsibilities were as follows: AE and TB designed the outline of the manuscript and wrote large parts of it; REK wrote the chapter on carotenoid processing during digestion and on apo-carotenoids and further proofread the manuscript; ISA and NC contributed in writing several subchapters regarding carotenoid microbiota metabolism and synthesis; all authors validated the final content, have read and approved the final version.

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