REVIEW

Genetic variations involved in interindividual variability in carotenoid status

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As shown in most clinical studies dedicated to carotenoids, there is a huge interindividual variability in absorption, and blood and tissue responses, of dietary carotenoids. The recent discovery that several proteins are involved in carotenoid metabolism in humans has prompted a possible explanation for this phenomenon: genetic variants in genes encoding for these proteins may affect their expression or activity, and in turn carotenoid metabolism and carotenoid status. The proteins clearly identified so far are (i) the carotene oxygenases β,β-carotene-15,15′-monooxygenase (BCMO1) and β,β-carotene-9′,10′-oxygenase (BCDO2), which are involved in carotenoid cleavage, (ii) scavenger receptor class B type I (SR-BI), cluster determinant 36 (CD36), and Niemann Pick C1-like 1 (NPC1L1), which are involved in carotenoid uptake by cells, and (iii) glutathione *S*-transferase Pi 1 (GSTP1) and human retinal lutein-binding protein (HR-LBP), which are involved in the transport of xanthophylls in the retina. Other proteins, such as ATP-binding cassette subfamily G member 5 (ABCG5) and the fatty acid-binding proteins (FABPs) are also apparently involved although firmer evidence is still required. A genome-wide association study, as well as several candidate gene association studies, has shown that groups of subjects bearing different alleles in single nucleotide polymorphisms located in or near several of the above-mentioned genes display different blood and/or tissue concentrations of carotenoids. Further studies are needed to identify all the proteins involved in carotenoid metabolism and assess whether other types of genetic variation, e.g. copy number variants and epigenetic modifications, can modulate carotenoid status. One potential application of such research could be personalized dietary guidelines for carotenoids according to individual genetic characteristics.

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1 Introduction

Carotenoids are lipid microconstituents found mainly in foods of plant origin. They can be divided into provitamin A and nonprovitamin A carotenoids. The main provitamin A carotenoids are β-carotene, α-carotene, and β-cryptoxanthin. These carotenoids possess potential vitamin A activity, i.e. they can be a source of retinol, when dietary preformed vitamin A fails to meet the body's needs. The nonprovitamin A carotenoids, the main ones being lutein, lycopene, and zeaxanthin, are not considered as vitamins because their essentiality for humans is not yet proven. However, numerous studies have suggested that they can be beneficial for human health [1, 2], and some of them may play a

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Abbreviations: ABCG5, ATP-binding cassette subfamily G member 5; **AUC,** area under the curve; **BCDO2,** β,β-carotene-9′,10′-oxygenase; **BCMO1,** β,β-carotene-15,15′-monooxygenase; **CD36,** cluster determinant 36; **FABP,** Fatty acid-binding protein; **GWAS,** genome-wide association study; **NPC1L1,** Niemann Pick C1-like 1; **SNP,** single nucleotide polymorphism; **SR-BI,** Scavenger receptor class B type I specific biological role in humans, e.g. lutein and eye biology [3–6].

Interest in the metabolism of carotenoids has increased in the last decade because of the inverse associations between several degenerative diseases, e.g. cancers, cardiovascular diseases, and eye diseases, and either the dietary intake of these molecules or their blood or tissue concentrations. In addition, clinical studies performed with carotenoids have systematically observed a huge variability in blood and, when measured, tissue response i.e. variation in concentrations of dietary derived carotenoids. This has even led some researchers to divide subjects into low and high responders to carotenoids. It was further observed that the low or high-responder phenotype was an intrinsic individual characteristic and it was hypothesized that this was due to genetic characteristics of the individuals [7]. However, when this hypothesis was advanced, only β,β-carotene-15,15′-monooxygenase (BCMO1) was known to participate in carotenoid metabolism and it was suggested that the conversion of β-carotene into vitamin A, i.e. the activity/expression of BCMO1, apparently has no major effect on chylomicron β-carotene response, i.e. β-carotene absorption [7]. In the following decade, several proteins were found to be involved in the intestinal uptake, intracellular transport and further metabolism, including cleavage, of carotenoids. A reasonable hypothesis can thus be proposed: genetic variants in genes that encode these proteins may affect expression or activity of these proteins and in turn carotenoid metabolism and carotenoid status.

This review first presents proteins involved in carotenoid metabolism in humans. It then focuses on single nucleotide polymorphisms (SNPs) that have been associated with carotenoid status and examines other types of genetic variations that might affect carotenoid status. Finally, we discuss the potential implications of these genetic variations in dietary guidelines for carotenoids.

2 Huge interindividual variability in blood and tissue responses to dietary carotenoids

A huge interindividual variability in blood and tissue concentrations of carotenoids is consistently observed in healthy subjects. Obviously, this variability is in part due to the variability in intake of these dietary microconstituents. However, most clinical studies dedicated to measuring blood and tissue responses to diets supplemented with carotenoids, or to meals enriched with carotenoids, studies that were based on standardized diets, have observed a huge interindividual variability in absorption efficiency of carotenoids and in blood and tissue responses to dietary carotenoids [7–12]. An example of the variability in postprandial chylomicron response is shown in Fig. 1. This has even led some researchers to assert that some subjects are ''nonresponders'' to carotenoids [11, 13–15]. Although the term ''nonresponder'' is inappropriate because all healthy subjects are apparently responders to dietary carotenoids, since no healthy subject without detectable carotenoids in their blood or tissue has ever been found, there is clearly a huge variability in blood and tissue response to dietary carotenoids in the healthy population. As hypothesized in Section 3, this effect is probably due to variations in genes that encode proteins involved in carotenoid absorption and metabolism.

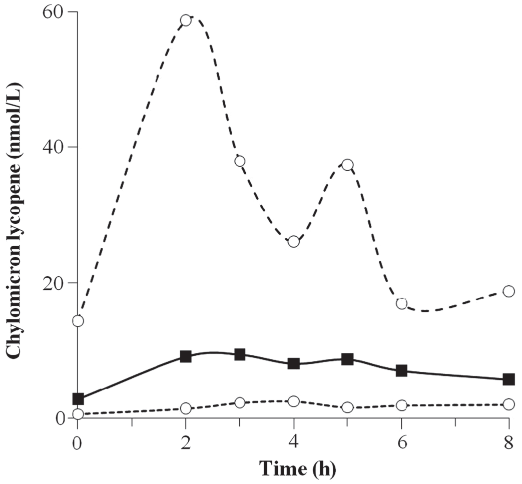

Figure 1. Interindividual variability in absorption efficiency of carotenoids – example of lycopene. Interindividual variability in absorption efficiency of lycopene as evaluated by measuring postprandial plasma chylomicron lycopene concentrations after intake of a lycopene-rich test meal. The same test meal was ingested by 39 healthy male adults. It contained 50 g fat and 100 g tomato purée as a source of lycopene (unpublished data from a clinical study funded by the FP6 Integrated European project ''Lycocard'', No. 016213). Bold curve: mean plasma chylomicron lycopene concentrations of the 39 subjects. Postprandial plasma chylomicron lycopene concentrations were not corrected from fasting plasma chylomicron lycopene concentrations. Dashed curves: curves of the lowest responder (lowest area under the curve [AUC]) and the highest responder (highest AUC). The ratio between the AUC of the highest responder and that of the lowest responder was about 20.

3 Proteins directly or indirectly involved in carotenoid metabolism

Until recently, it was assumed that carotenoids, being fat soluble, simply followed lipids from the lipid droplets present in the gastrointestinal lumen during digestion [16–18] to the intracellular lipid droplets where they are stored [19–21]. More precisely, it was assumed that they were first transferred from the food matrix, where they were embedded, in the fat phase of the meal, and then transferred to mixed micelles during triglyceride lipolysis by pancreatic lipase, before being absorbed by passive diffusion, incorporated into chylomicrons and distributed to tissues together with triglycerides and cholesterol. However, the reality is more complex because several proteins are involved, or are suspected of being involved, at different stages in this pathway. First, it is assumed that a digestive enzyme is involved in the hydrolysis of the carotenoids that are esterified. Second, it has been suggested that as observed for cholesterol, apical membrane proteins of the enterocyte are involved in the uptake of carotenoids. Finally, proteins are thought to be involved in the intracellular transport of carotenoids across the water environment, i.e. cytosol, of the cells, etc. Below we present a state-of-the-art review concerning the proteins known so far to be involved in carotenoid metabolism in humans.

3.1 Candidate proteins for hydrolysis of carotenoid esters in the intestinal lumen

Although most dietary carotenoids are nonesterified, some carotenoids, e.g. xanthophylls present in supplements, can be esterified. Like other lipids, it is assumed that only the free forms of carotenoids can be absorbed by the enterocyte. Thus, it is assumed that an enzyme in the gastrointestinal lumen and/or in the brush border of the enterocyte is responsible for the hydrolysis of carotenoid esters. Several candidates can be proposed. The first candidate, in order of appearance in the gastrointestinal tract, is gastric lipase, which can hydrolyze up to 17.5% of the dietary triacyglycerols [22]. However, no data are available on the ability of this lipase to hydrolyze carotenoid esters. A second candidate could be pancreatic lipases, pancreatic lipase and pancreatic lipase-related protein 2, which are able to hydrolyze retinyl-palmitate [23]. Unfortunately, here again there are no available data on the ability of these lipases to hydrolyze carotenoid esters. The third and principal candidate is obviously cholesterol ester hydrolase [24–27], because it has a broad substrate specificity and because it is known to be involved in the hydrolysis of zeaxanthin esters in vitro [28]. However, we note that in vivo studies using knockout mice [29, 30] to confirm the involvement of this enzyme on tocopherol ester hydrolysis in vitro [24–27] have failed to confirm its involvement in its hydrolysis in vivo. To conclude, several digestive enzymes could be involved in carotenoid ester hydrolysis in the human upper gastrointestinal tract, but no such enzyme has yet been firmly identified. Finally, we cannot exclude the possibility that a fraction of carotenoid esters can be absorbed by intestinal cells because lutein esters were found in human blood after lutein esters supplementation [31].

3.2 Proteins involved in uptake and secretion of carotenoids by cells

The first intestinal cell that makes contact with dietary carotenoids is obviously the enterocyte. Most of the studies dedicated to understanding mechanisms by which carotenoids are taken up and secreted by cells have been performed with intestinal cells or tissues. The first study was performed by the group of Hollander in the 1970s. This group, using rat everted intestinal sacs, concluded that absorption of β-carotene, and by extension absorption of all the carotenoids, was passive [32]. Although well conducted, this study was obviously limited by the technology available at that time. New studies performed during the last decade by several independent research teams [33–40] have shown that the mechanism of absorption is actually more complex than was previously assumed: passive diffusion probably occurs at high, pharmacological concentrations of carotenoids, whereas a protein-mediated transport takes place at dietary doses.

No specific protein has been involved in uptake/efflux of carotenoid by mammalian cells. Recent studies suggest that it is lipid transporters – and especially cholesterol transporters [33–39] – that are mainly involved in carotenoid uptake/efflux by cells.

3.2.1 The scavenger receptor class B type I

The first reported lipid transporter to be involved in carotenoid uptake is scavenger receptor class B type I (SR-BI). This lipid transporter is a single-chain trans-membrane glycoprotein of 80 kDa. SR-BI is found in various cell types including liver, testis, ovaries, and macrophages [41], as well as on the brush border membrane of the enterocyte from the duodenum to the colon [42]. Its main ligands are high-density lipoproteins (HDL), but also low-density lipoproteins (LDL), and very low density lipoproteins (VLDL), anionic phospholipids, and phosphatidylserine of apoptotic cells [41, 43, 44]. SR-BI is able to facilitate the selective entry into the cell compartment of esterified cholesterol recovered in HDL [45]. At the intestinal level, it was first shown that SR-BI facilitated the uptake of free cholesterol, but also of esterified cholesterol, phospholipids, and triacylglycerol hydrolysis products, thus displaying a broad ligand specificity [46, 47]. In 2005, SR-BI was identified for the first time as playing a role in the uptake of the carotenoid lutein at the intestinal level using Caco-2 cells [33], and this result was rapidly extended to other carotenoids, such as β-carotene [39], zeaxanthin [38], and lycopene [34], and to other cell types. SR-BI has also been shown to be involved in carotenoid uptake by retina pigment epithelium [38], and it has been suggested that it is involved in the preferential uptake of xanthophylls by the macula [38]. The fact that SR-BI is important for carotenoid metabolism is supported by a recent study showing that its expression is modulated by provitamin A carotenoids [48]. More precisely, by using both mouse models and human cell lines, it has been shown that carotenoid absorption by SR-BI is subject to control by retinoid signaling. Retinoic acid induces the expression of the intestinal transcription factor ISX, which represses the expression of both SR-BI and BCMO1. BCMO1 acts downstream of SR-BI and converts absorbed β-carotene into the retinoic acid precursor, retinaldehyde [48]. Thus, there is a diet-responsive regulatory network that controls SR-BI-mediated β-carotene absorption, and perhaps absorption of other dietary lipids absorbed via SR-BI [48], by a negative feedback regulation.

The role of SR-BI is not limited to the uptake of lipid molecules. It has been observed to function in both directions, i.e. uptake and efflux from cells, in the intestine [49] and in other tissues [45]. Concerning lipid micronutrients, it has been shown that SR-BI is able to efflux both vitamin E [50] and vitamin D [51] from Caco-2 cells. It may therefore be involved in the efflux of carotenoids from the enterocyte to the intestinal lumen, which may explain in part the very low absorption efficiency of these compounds, as also in the efflux of carotenoids from other tissues. In that case, it can be suggested that carotenoids are incorporated in lipoproteins that bind to SR-BI, i.e. HDL, LDL, and VLDL [41]. Unfortunately, no data are available on the possible involvement of SR-BI in carotenoid efflux in any cell type.

3.2.2 Cluster determinant 36

Another scavenger receptor apparently involved in carotenoid uptake by cells is fatty acid translocase (FAT), also known as cluster determinant 36 (CD36) in humans. This trans-membrane glycoprotein of 90 kDa is mainly expressed in epithelial cells, adipocytes, platelets, and macrophages [41], but it is also expressed in the duodenum and the jejunum [42]. As SR-BI, it can interact with a broad variety of ligands [41]. CD36 was first shown to be involved in β-carotene uptake by cells using transfected COS-7 cells and mouse brush border membrane vesicles [39]. This involvement in carotenoid uptake by cells was confirmed recently in mouse 3T3-L1 adipocytes and in mouse adipose tissue cultures [35].

3.2.3 Niemann Pick C1-like 1

Niemann Pick C1-like 1 (NPC1L1) is a 135 kDa protein widely expressed in human tissues, especially in the liver and the plasma membrane of the intestinal cell [52–56]. It is the main cholesterol and phytosterol transporter in the intestine [57]. NPC1L1 has been shown to be involved in both α-tocopherol [58] and γ-tocotrienol [59] intestinal absorption using Caco-2 cells and in situ perfusions in rats. Results are less clearcut regarding carotenoids: in a first study [37], ezetimibe, a drug used to diminish dietary cholesterol absorption and that inhibits NPC1L1, significantly inhibited the uptake of several carotenoids, i.e. α- and β-carotene (50% inhibition), β-cryptoxanthin and lycopene (20%), and lutein:zeaxanthin (7%), in Caco-2 cells. Conversely, in a second study that focused on lycopene [34], neither ezetimibe nor blocking antibody directed against NPC1L1 impaired its absorption in Caco-2. Further experiments are thus required to thoroughly assess the role of NPC1L1 in carotenoid metabolism.

3.2.4 The ABC transporters

The ABC transporters are a superfamily of transmembrane proteins able to transport a wide variety of substrates including drugs, lipids, bile salts, amino acids, peptides, proteins, and carbohydrates. Most of these transporters are efflux proteins [60] that need ATP hydrolysis by two sites of ligation to ATP [61, 62]. In particular, the (ATP-binding cassette subfamily G member 5) ABCG5/G8 heterodimer is critical in sterol homeostasis. The complex ABCG5/G8 acts as an efflux pump in the enterocyte, where it limits mainly phytosterol absorption. Interestingly, one study suggested a role of ABCG5 in lutein metabolism [63]. However, it was an association study in human that showed only an association (near-significant) between a genetic variant in ABCG5 and plasma lutein concentration. Dedicated studies are therefore required to assess the possible involvement of ABC transporters in carotenoid metabolism and the mechanism involved (uptake or efflux).

In conclusion, it appears that several lipid transporters are involved in uptake/efflux of carotenoids by the intestinal cell (Fig. 2) as well as by other cells (Table 1). Most of these transporters, SR-BI, CD36, and NPC1L1, facilitate the transport, i.e. they are not energy dependent. However, the relative contribution of these transporters to the net uptake/efflux of each carotenoid species in different tissues is not known. In addition, it is likely that not all the transporters involved, peculiarly the ABC transporters, have yet been identified.

3.3 Proteins involved in intracellular transport of carotenoids

The intracellular transport of carotenoids is not well known. It is assumed that being insoluble in water, these compounds require either intracellular proteins to cross the water compartments of the cells or incorporation into intracellular membranes, to be transported within the cells.

3.3.1 Carotenoid-binding proteins

Two xanthophyll-binding proteins have been described in human retina: glutathione *S*-transferase Pi 1 (GSTP1) [64], which binds zeaxanthin and (3R,3′S-meso)-zeaxanthin, and human retinal lutein-binding protein (HR-LBP), which binds lutein [65]. Apparently, no one has tested whether these proteins are able to bind other carotenoids, e.g.

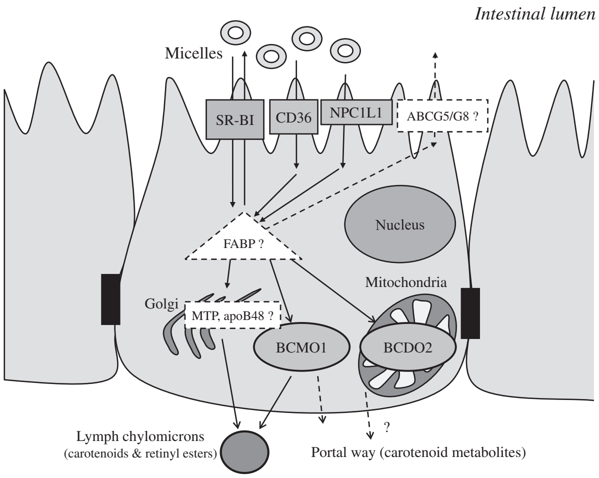

Figure 2. Proteins involved in carotenoid transport across the human enterocyte. Carotenoids are captured from mixed micelles by apical membrane transporters (SR-BI, CD36, and NPC1L1). A fraction of carotenoids might then be effluxed back to the intestinal lumen via apical membrane transporters (SR-BI and possibly ABCG5/G8 (ATP-binding cassette G1 and G8). Another fraction of carotenoids is transported to the site where they are incorporated in chylomicrons. A fraction of provitamin A carotenoids, e.g. β-carotene, α-carotene, and β-cryptoxanthin, is cleaved into retinal by BCMO1. A fraction of nonprovitamin A carotenoids, e.g. lutein, zeaxanthin, and lycopene, is possibly transported to mitochondria, where they are cleaved into apocarotenals by BCDO2. It is hypothesized that one or more proteins are involved in intracellular transport of carotenoids although they have not yet been identified. Good candidates are the FABPs. Nonmetabolized carotenoids and retinyl esters, which originate from carotenoid cleavage by BCMO1 and subsequent metabolism by vitamin A enzymes, are secreted in the lymph in chylomicrons. It could be suggested that proteins involved in chylomicron assembly, e.g. microsomal TG transfer protein, apoB48, could indirectly participate in carotenoid metabolism in the enterocyte. Finally, it is hypothesized that polar carotenoid metabolites are secreted in the portal route [107–109].

β-carotene and lycopene, but these proteins, or proteins of the same family, are good candidates for intracellular transport of carotenoids in other tissues [66].

3.3.2 Fatty acid-binding proteins

The hypothesis that Fatty acid-binding proteins (FABPs) can participate in the intracellular transport of carotenoids comes from Hollander's group. This team suggested, from results showing interactions between a fat-soluble vitamin (phylloquinone) and linoleic acid, that a FABP might also be a carrier for fat-soluble micronutrients [67]. The fact that FABPs are able to bind different hydrophobic molecules [68, 69] supports this hypothesis. Two main FABP are found in human tissues: intestinal FABP, mainly recovered in the intestine, and liver FABP, recovered in the liver but also in other tissues. These proteins belong to the FABP superfamily that is constituted by 14–15 kDa soluble proteins [70]. FABPs exhibit a similar tertiary structure that consists of two α-helices (αI, αII) and ten anti-parallel β-strands (βA-βJ) organized in two almost orthogonal β-sheets forming an hydrophobic pocket.

It is hypothesized that FABPs allow a specific intracellular trafficking of their ligands to their respective metabolic fates. Although dedicated studies need to be performed to verify whether intestinal and/or liver FABPs are involved in intracellular transport of carotenoids, the fact that in a genetic association study, a genetic variant in intestinal FABP was associated with fasting plasma lycopene concentrations [71] can be considered as a first argument that FABPs may be involved in the intracellular transport of some carotenoids.

3.4 Proteins involved in carotenoid cleavage

The requirements for vitamin A can be satisfied either by animal foods containing preformed vitamin A, i.e. retinyl esters, or by plant foods containing provitamin A carotenoids. In humans, two different carotene oxygenases are involved in carotenoid cleavage: BCMO1 and β,β-carotene-9′,10′-oxygenase (BCDO2) [72]. BCMO1 is responsible for the symmetric cleavage [73, 74], whereas BCDO2 is responsible for the asymmetric cleavage. Immunohistochemical analysis revealed that BCMO1 was expressed in epithelial cells and in a variety of human tissues [75]. It is now assumed that this cytoplasmic enzyme [76] is the main enzyme responsible for vitamin A synthesis both in the intestine and in the liver, and also for vitamin A synthesis in different tissues when local concentrations in vitamin A are insufficient [77]. The biological role of BCDO2 has been unravelled recently [78]: it was shown in BCDO2-deficient mice and in human cell cultures that BCDO2 was located in the mitochondria and that its main role was apparently to degrade carotenoids to protect mitochondria from carotenoid-induced dysfunctions. Interestingly, carotenoid homeostasis was impaired in BCDO2-deficient mice, leading to the accumulation of carotenoids in several tissues.

3.5 Proteins involved in incorporation of carotenoids into intestinal lipoproteins

As carotenoids are recovered into blood chylomicron-remnants after ingestion of meals containing carotenoids [7, 79, 80], it is assumed that carotenoids that are not cleaved in the enterocyte are incorporated into chylomicrons. Thus, proteins involved in the packaging of triacylglycerols within chylomicrons, e.g. microsomal TG transfer protein (MTP), apoB48, apoAIV, Sar1b [81], might be indirectly involved in carotenoid metabolism. Furthermore, since enterocytes also secrete HDL [82, 83], and because the origin of carotenoids

Table 1. Proteins involved in carotenoid metabolism (from the results obtained in cell studies)[a)]

	Intestine	Liver	Other tissues
β-Carotene	SR-BI [39] CD36 [39] NPC1L1 [37] BCMO1 BCDO2	BCMO1 BCDO2[b)]	SR-BI (retinal pigment epithelial cells) [38] BCDO2
Lutein	SR-BI [33] NPC1L1 [37] BCDO2	BCDO2	CD36 (adipose tissue) [35] BCDO2
Lycopene	SR-BI [34] NPC1L1[c)] [37] BCDO2	BCDO2	CD36 (adipose tissue) [35] BCDO2
Other carotenoids	Carboxyl ester lipase for zeaxanthin esters [28] NPC1L1[d)] [37] BCDO2	BCDO2	SR-BI and zeaxanthin (retinal pigment epithelial cells) [38] GSTP1 and zeaxanthin (macula) [64] HR-LBP and lutein (retina) [65] BCDO2

GSTP1, glutathione *S*-transferase Pi 1; HR-LBP, human retinal lutein-binding protein.

a) Proteins shown to be involved in carotenoid metabolism in cell studies. This table does not show proteins that have been associated with blood or tissue concentrations of carotenoids without additional evidence in cell studies. It also does not show proteins involved in metabolism of metabolites produced by cleavage of carotenoids, e.g. CRBP and CRABP that bind retinol and retinoic acid, respectively.

b) BCDO2 has been recently described as a mitochondrial enzyme with a broad substrate specificity [78]. It therefore appears throughout the table, although direct evidence of its involvement for carotenoid cleavage in each tissue is not available.

c) Not confirmed in another study [34].

d) Involvement found for α-carotene, β-cryptoxanthin, and zeaxanthin.

within these lipoparticles is still unknown, it is possible that proteins involved in the secretion of intestinal-HDL, e.g. ABCA1, are also involved in carotenoid metabolism.

3.6 Proteins involved in lipoprotein metabolism

As carotenoids are transported by blood lipoproteins [84], it has been hypothesized that genes involved in lipoprotein metabolism, e.g. genes that encode for apolipoproteins or for endothelial lipases, may indirectly affect blood concentrations of carotenoids by modulating the number, and/or the lipid composition, of the vehicles that transport carotenoids in the blood. This hypothesis is supported by several association studies, showing that blood carotenoid concentrations were significantly different in groups of subjects who bore different genetic variants in apolipoprotein A-IV [85], apolipoprotein B [85], apolipoprotein E [86], hepatic lipase [71], and lipoprotein lipase [87].

In conclusion, it appears that several proteins are involved at numerous steps in carotenoid metabolism (Table 1). Some are specific, e.g. BCMO1, others are nonspecific, e.g. cholesterol transporters, and still others are indirectly involved, e.g. apolipoproteins. The metabolism of carotenoids within the human body is therefore more complex than previously thought, i.e. a simple distribution of carotenoids within the lipid compartments of the body depending on the solubility of carotenoids in the different lipid phases. Several proteins are clearly involved in blood and tissue concentrations of carotenoids.

4 Hypothesis concerning the modulation of carotenoid status via genetic modulation of expression or activity of proteins involved in carotenoid metabolism

The fact that proteins are involved in cellular uptake, intracellular transport, and cleavage of carotenoids raises the question of the consequences of modulations in the expression or activity of these proteins on blood and tissue concentrations of these compounds. The expression and activity of proteins can be modulated by several factors, including genetic ones. Genetic variations in or near genes that encode proteins may affect protein expression, e.g. a genetic variation that affects the binding of a transcription factor in the promoter region of a gene [88], or protein activity, e.g. a genetic variation that leads to a functional modification in the amino acid sequence of the protein, and in turn the ability of these proteins to accurately perform their function in the metabolism [89]. Concerning carotenoids, it can be hypothesized that genetic variations in genes that encode proteins involved in uptake, transport, and

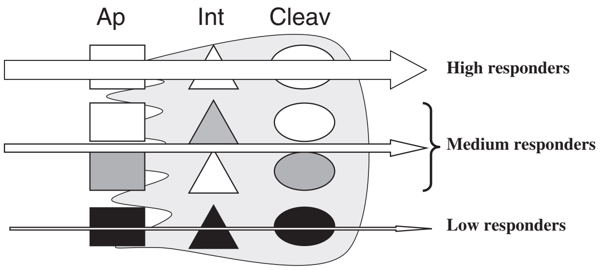

Figure 3. Interindividual variability of absorption efficiency of carotenoids as explained by genetic variation in proteins involved in transport and metabolism of carotenoids in the enterocyte. Ap: apical membrane transporter of carotenoids (e.g. SR-BI). Int: intracellular transporter of carotenoids (e.g. FABP. Cleav: enzyme involved in cleavage of carotenoids (e.g. BCMO1). A white symbol indicates that the protein works with maximal efficiency. A black symbol indicates that a genetic variation (e.g. an SNP) leads to an impaired expression or activity of the protein. A grey transporter indicates that a genetic variation leads to an intermediate expression or activity of the protein. The hypothesis is as follows: when most proteins involved in the metabolism of a carotenoid are efficient (white line), subjects absorb the carotenoid very well: these subjects are called ''high responders''. When most of the proteins involved in the metabolism of a carotenoid are inefficient (black line), subjects absorb the carotenoid very poorly: these are called ''low responders.'' When some proteins are efficient and some are inefficient, the subjects absorb carotenoids with an intermediate efficiency; these are called ''medium responders.'' This hypothesis explains the Gaussian response to β-carotene described in a clinical study [7].

cleavage of these compounds will affect their absorption efficiency (Fig. 3) as well as their blood and tissue concentrations. This hypothesis is supported by the studies showing associations between genetic variants in genes involved, or suspected to be involved, in carotenoid metabolism and blood and tissue concentrations of carotenoids (Sections 6 and 7).

5 Mutations affecting carotenoid status

Very high skin concentrations of carotenoids cause carotenodermia. Carotenodermia is usually due to very high intake of carotenoids, such as in people, e.g. infants, eating large amounts of carrots or carrot-derived products. Yet cases of carotenodermia and/or hypercarotenemia have also been reported in which subjects had normal carotenoid intakes. It was hypothesized that these subjects had an impairment in the conversion of carotenoids to retinal by BCMO1 [90–93]. In support of this hypothesis, a mutation that explains a case of metabolic carotenodermia has been identified recently [89]. The mutation led to the replacement of a threonine with a methionine, resulting in a 90% reduction of BCMO1 activity. The hypercarotenemia was explained by the fact that BCMO1, which is saturated during normal dietary conditions, cleaved less dietary carotenoids than usual, leading to higher blood concentrations of carotenoids. BCMO1 is a key enzyme in provitamin A carotenoid metabolism [72, 77]. Its efficiency is very variable among individuals [7] and recent results on the regulation of its expression by vitamin A status [94, 95] suggest that persons with very low vitamin A status convert much more efficiently than those with high vitamin A status.

6 Main SNPs affecting carotenoid status

Genome-wide association study (GWAS) is today the most popular approach for identifying genetic variants and thus genes associated with phenotypes. In this approach, the frequency of genetic variants (often involving hundreds of thousands of SNPs studied simultaneously) is investigated in large numbers of subjects (again often using DNA from thousands of subjects). There is only one GWAS study dedicated to identifying the main genetic variants associated with blood concentration of carotenoids [96]. This study, which was performed in a cohort of Italian and in two US cohorts, showed that an SNP near the BCMO1 gene, rs6564851, was significantly associated with plasma concentration of several carotenoids. More precisely, the G allele at this SNP was simultaneously associated with higher concentrations of α- and β-carotene and with lower concentrations of lycopene, lutein, and zeaxanthin. This result confirms the key role of this enzyme in the metabolism of provitamin A carotenoids and shows that it is also involved, by an unknown mechanism, in the blood status of nonprovitamin A carotenoids. We note that the association between lutein and BCMO1 genetic variants was also found in another study [97]. Although closely associated, it was calculated that rs6564851 explained 1.9% of the variance in plasma β-carotene concentration [96]. Thus other genetic variations, which could be the SNPs identified in candidate gene association studies (Section 7), are probably involved. It is likely that the genetic variance involves several genetic variants with very small effects that cannot be detected in GWAS studies. The problem with GWAS is that to avoid false-positive associations, it requires a dramatically low *p*-value threshold ($p < 5 \times 10^{-7}$) and so weaker associations have not yet been reported.

7 Other SNPs affecting carotenoid status identified by candidate gene association studies

Several candidate gene association studies have been performed in the past 5 years to assess whether SNPs in genes suspected to modulate carotenoid metabolism directly or indirectly can affect blood or tissue concentrations of carotenoids. The results of these studies are summarized in Table 2. In summary, these studies have confirmed the key

Table 2. Genetic variants (SNPs) that have been associated with carotenoid status in candidate gene association studies[a)]

Genes	Genetic variants associated[b)]	Genotypes or alleles associated with lower concentrations of carotenoids[c)]	Carotenoids associated	References
ABCG5	Q640E	G allele[d)]	Lutein	[63]
apo B	apo B-516	C allele	Lycopene	[85]
	apo B-516	C allele[e)]	β-Carotene	[85]
apo A-IV	apo A-IV-Ser347	TT	Lycopene	[85]
apoE	apo E2, E3, E4	E3/2 (versus E3/3)	Lycopene	[86]
		E3/2 (versus E3/3)	α-Carotene	[86]
BCDO2	W80X	G allele	β-Carotene	[104]
	c.196C>T	T allele	Likely β-carotene[f)]	[105]
BCMO1	rs6564851[g)]	G allele[h)]	Lycopene, lutein, and zeaxanthin	[96]
	R267S+A379V	379A	β-Carotene	[106]
	rs7501331	TT	Lutein	[97]
	rs7501331	CT	Lutein[i)]	[97]
CD36	rs1761667	A allele	Lutein[i)]	[97]
	rs13230419	CC	Lutein	[97]
HL	HL C-480T	TT[j)]	α-Carotene	[71]
		C allele[e)]	β-Carotene	[71]
FABP2 (intestinal FABP)	IFABP-Thr	GG[j)]	Lycopene	[71]
LPL	S447X	X447/X447 versus S447/S447	β-Carotene, α-carotene, and β-cryptoxanthin	[87]
SCARB1	SR-BI intron 5	C allele	α-Carotene	[85]
	SR-BI intron 5	C allele	β-Carotene	[85]
	SR-BI exon 1	GG	β-Cryptoxanthin	[85]

a) All data were obtained in humans except those of BCDO2 that were obtained in cattle and in sheep.
b) Annotations as found in the publications.
c) Associated with lower blood (plasma or serum) concentration of carotenoids when not specified.
d) Near-significant ($p = 0.08$).
e) Significant only in men.
f) Associated with yellow fat phenotype, assumed to be related to β-carotene concentration.
g) Near the BCMO1 gene (identified in a GWAS study).
h) Allele simultaneously associated with higher β-carotene and α-carotene concentrations.
i) Associated with macular pigment optical density (assumed to reflect concentration of lutein/zeaxanthin in the macula).
j) Significant only in women.

role of genetic variants in BCMO1 on blood and tissue carotenoid levels that was observed in the GWAS study on carotenoid status [96] (Section 6). They have also found that, as expected, genetic variants in proteins shown to be involved in carotenoid metabolism in cell studies, e.g. SR-BI and CD36, apparently affect carotenoid status. Finally, they have potentially identified other genes/proteins involved in carotenoid status, e.g. apolipoproteins, ABCG5, liver FABP, although the role of these genes/proteins and the effect of their genetic variants on carotenoid metabolism need to be confirmed in dedicated molecular/functional studies.

8 Other types of genetic variation that might affect carotenoid status

It is likely that the genetic component of interindividual variability in carotenoid status is due to the sum of effects due to variants in numerous genes. Other genetic and epigenetic variations may also contribute to interindividual variability in carotenoid status. Another genetic variation that occurs widely in the human genome is copy number variants. To date, there are no published studies of copy number variants and carotenoid status, but it is reasonable to expect such variants to be responsible for interindividual variation in the activities of proteins involved in carotenoid metabolism. It is now assumed that epigenetic modifications of DNA, i.e. DNA methylation patterns (CpG sites), acetylation ubiquitination of histones, and very small (noncoding) RNAs (miRNA), play a major role in controlling gene expression. Given that miRNA regulate the expression of about one-third of all protein-encoding genes across all cellular processes investigated [98], there is a high likelihood that they can play a role in carotenoid metabolism and thus in carotenoid status. In common with other regions of the genome, the domains encoding miRNA also contain SNPs [99], which adds a further layer of genetic complexity.

9 Potential implications of genetic variations in dietary guidelines for carotenoids

The fact that genetic variations can affect absorption efficiency of carotenoids and the conversion of provitamin A carotenoids into vitamin A raises two opposing questions: ''Do subjects with low-responder genotypes need a higher dietary intake of carotenoids to enjoy the beneficial effects of some carotenoids on health?'', and conversely, ''Do subjects with high-responder genotypes need to avoid high intake of some carotenoids because of the potential toxic effects of some carotenoids? [100–102]''. These questions may also be very relevant with regard to the fight against vitamin A deficiency in developing countries. If genetic variants do affect either absorption efficiency of provitamin A carotenoids, or their conversion into vitamin A, knowing that the conversion factor for provitamin A carotenoids is very low (12:1–26:1 depending on whether β-carotene is in fruits or vegetables [103]), then it may be important to know whether a large proportion of persons in populations at risk of vitamin A deficiency, e.g. populations in some developing countries, bear genetic variants that lead to lower provitamin A activity of carotenoids. In that case, it would be better to supplement these populations with preformed vitamin A rather than with provitamin A carotenoids.

10 Conclusions

Available data support the hypothesis that, together with factors that affect bioaccessibility of carotenoids within the gastrointestinal lumen [16], genetic variation is a key factor explaining the huge interindividual variability in carotenoid status. However, much work is still needed to identify all the proteins and genetic variants involved. In addition, such research will probably need to encompass both copy number variants and epigenetic variants. Because a key step of carotenoid status is their absorption efficiency, and because this first step is especially variable, it is suggested that dedicated studies should be performed to identify genetic variations explaining this variability. The effect of genetic variants on plasma and tissue concentrations of carotenoids may affect the ability of carotenoids to prevent some diseases (e.g. cardiovascular diseases and cancers). Finally, because several carotenoids are apparently beneficial for health at low, dietary doses and can be hazardous at higher, pharmacological doses, we can envisage adapting recommended dietary intake of carotenoids, or doses of carotenoids incorporated in functional foods/supplements, to population groups carrying genetic variants known to affect carotenoid status.

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