Proteins involved in uptake, intracellular transport and basolateral secretion of fat-soluble vitamins and carotenoids by mammalian enterocytes

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PROTEINS INVOLVED IN UPTAKE, INTRACELLULAR TRANSPORT AND BASOLATERAL SECRETION OF FAT- SOLUBLE VITAMINS AND CAROTENOIDS BY MAMMALIAN ENTEROCYTES

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Comments for the authors

The present review provides an interesting and timely summary on the implication of active multifunctional transport systems in the uptake and cellular distribution of lipophilic vitamins and carotenoids.
The topic addresses a rapidly developing field of research with strong impact on nutrition, nutrition-related diseases and nutrient deficiency. The paper summarizes modern molecular biological approaches in lipid/nutrition research to resolve long known phenomena (inter-individual differences in absorption of lipids, interaction of lipids in the absorption process) and critically discuss the state of the research.

A list of abbreviations would be helpful: done

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Some comments the authors may consider are included in the text: all the comments have been addressed

Abstract

Our understanding of the molecular mechanisms responsible for fat-soluble vitamin uptake and transport at the intestinal level has advanced considerably over the past decade. On one hand, it has long been considered that vitamin D and E as well as β-carotene (the main provitamin A carotenoid in human diet) were absorbed by a passive diffusion process, although this could not explain the broad inter-individual variability in the absorption efficiency of these molecules. On the other hand, it was assumed that preformed vitamin A (retinol) and vitamin K1 (phylloquinone) absorption occurred via energy-dependent processes, but the transporters involved have not yet been identified. The recent discovery of intestinal proteins able to facilitate vitamin E and carotenoid uptake and secretion by the enterocyte has spurred renewed interest in studying the fundamental mechanisms involved in the absorption of these micronutrients. The proteins identified so far are cholesterol transporters such as SR-BI (scavenger receptor class B type I), CD36 (cluster determinant 36), NPC1L1 (Niemann Pick C1-like 1) or ABCA1 (ATP-binding cassette A1) displaying a broad substrate specificity, but it is likely that other membrane proteins are also involved. After overviewing the metabolism of fat-soluble vitamins and carotenoids in the human upper gastrointestinal lumen, we will focus on the putative or identified proteins participating in the intestinal uptake, intracellular transport and basolateral secretion of these fat-soluble vitamins and carotenoids, and outline the uncertainties that need to be explored in the future. Identifying the proteins involved in intestinal uptake and transport of fat-soluble vitamins and carotenoids across the enterocyte is of great importance, especially as some of them are already targets for the development of drugs able to slow cholesterol absorption. Indeed, these drugs may also interfere with lipid vitamin uptake. A better understanding of the molecular mechanisms involved in fat-soluble vitamin and carotenoid absorption is a priority to better optimize their bioavailability.

Key words: bioavailability, brush border membrane, transporters, uptake, absorption, intestine.

List of abbreviations:

ABCA1: ATP Binding Cassette A1

ABCG1: ATP Binding Cassette G1

ABCG5: ATP Binding Cassette G5

ABCG8: ATP Binding Cassette G8

BBM: Brush Border Membrane

CBP: Carotenoid-Binding protein

CD36: Cluster Determinant 36

CDBP: Cytosol vitamin D-Binding Protein

CRBPII: Cellular Retinol Binding Protein II

FSV&C: Fat Soluble Vitamins & Carotenoids

GI: Gastrointestinal

GSTP1: Glutathione S-Transferase Pi 1

HR-LBP: Human Retinal Lutein-Binding Protein

IDBP: Intracellular vitamin D Binding Protein

Isx: Intestine-Specific Homebox

L-FABP: Liver Fatty-Acid-Binding Protein

LRAT: Lecithin Retinol Acyl Transferase

NPC1L1: Niemann-Pick C1-Like 1

RBP: Retinol Binding Protein

SR-BI: Scavenger Receptor class B type 1

STRA6: STimulated by Retinoic Acid 6

TAP: tocopherol-associated protein

α-TTP: α-tocopherol Transfer Protein

INTRODUCTION

Vitamins are organic compounds with a relatively low molecular weight and no energetic value that are found in the diet in small quantities (< 1 g *per* d) yet are essential to ensure body growth, reproduction and functioning. As the body cannot synthesize its own vitamins (or at least not in sufficient amounts), adequate amounts of vitamins have to be provided by diet. Historically, the 13 vitamins have been divided into two classes based on their solubility: water-soluble vitamins (the Bs and C) and fat-soluble vitamins. Fat-soluble vitamins are further classed into 4 groups of compounds: vitamin A (preformed vitamin A and provitamin A carotenoids), D (cholecalciferol), E (tocopherols and tocotrienols) and K (phylloquinone and menaquinones) (see Table 1).

Nonprovitamin A carotenoids, such as lutein and lycopene (Table 2), are dietary lipid compounds. They cannot be considered true vitamins as their essentiality for humans has not yet been proven. However, they are assumed to have beneficial effects on human health [1, 2], which in some cases may prove to be specific biological effects in humans, such as lutein and eye biology [3-6]. We will thus include in this review the proteins involved in carotenoid uptake and metabolism in the enterocyte.

The fundamental mechanisms involved in the absorption of fat-soluble vitamins and carotenoids (FSV&C) were almost exclusively studied by Hollander's teams in the seventies [7-18]. Thus, most of the assumptions regarding the absorption mechanisms of FSV&C come from these studies that, for the most part, were performed in rat everted intestinal sacs. Although well performed, these studies were obviously limited by the technologies available at the time. Nevertheless, they have suggested that some FSV&C) were absorbed by passive diffusion while others (preformed A and K) were absorbed via carrier-dependent proteins. Recent studies performed by several independent research groups over the past decade have revisited these assumptions, and have shown that these

mechanisms of absorption are actually more complex than previously assumed: passive diffusion probably occurs at high, non-physiological concentrations of these compounds, while protein-mediated transport occurs at dietary doses.

Because the absorption mechanisms of these lipid molecules probably depend on the vehicle(s) in which they are provided to the apical membrane of the enterocyte, our first aim was to review current state-of-the-art on the metabolism of FSV&C in the human upper gastrointestinal (GI) lumen. Surprisingly, very little in known about the distribution of FSV&C between the different vehicles able to solubilize them in the human GI lumen, which include micelles, vesicles and, perhaps, proteins.

Our review of the literature also highlights that virtually nothing is known on the intracellular traffic of these molecules across the enterocyte, despite that fact that numerous candidate proteins are proposed with regard to the transport of these molecules in other cell types or tissues.

Finally, it is clear that numerous proteins assumed to transport cholesterol and other major lipids (fatty acids and phospholipids) are also involved in the transport of FSV&C.

This review raises numerous questions, for which we propose numerous studies highlighting key points on the metabolism in the human enterocyte of these very important molecules.

1. STATE OF THE ART ON THE FATE OF FAT-SOLUBLE VITAMINS AND CAROTENOIDS IN THE HUMAN GI TRACT

FSV&C are assumed to follow the fate of lipids in the upper gastrointestinal tract [19]. However, some key issues on the fate of FSV&C in the GI lumen still need further investigation. For example, the main intestinal site of absorption of most of these compounds is not precisely localized, and their absorption efficiency remains a rough estimate given the wide range of data obtained in different studies. Finally, although it is assumed that these compounds are relatively labile, no significant chemical modification of carotenoids or vitamins A and E has been reported at the stomach level [20, 21], and vitamin D and K are thought to follow the same pattern, although there is no clear data on this topic.

1.1 HYDROLYSIS OF FAT-SOLUBLE VITAMIN AND CAROTENOID ESTERS

It is assumed that only the free forms of the FSV&C can be absorbed, which suggests that hydrolysis should occur in the GI lumen when FSV&C esters are ingested. The main studies dedicated to this topic concern retinol, cholecalciferol and tocopherol esters. The hydrolysis may begin in the stomach, where gastric lipase can hydrolyze up to 17.5% of the triacyglycerols [22]. However, this lipase has been shown to be relatively inefficient on retinyl palmitate hydrolysis [21]. The hydrolysis of FSV&C esters thus occurs mainly in the duodenum. Contrary to previous assumptions, this hydrolysis is mainly performed by pancreatic lipase and, at least for retinyl-palmitate, by pancreatic lipase-related protein 2 [23]. Indeed, the initial involvement of cholesterol ester hydrolase [24-27] was not confirmed *in vivo* using knock-out mice [28, 29]. Other candidates could be a brush border esterase and a mucosal esterase localized to the endoplasmic reticulum of the enterocyte. Indeed, analytical subcellular fractionation studies on homogenates of isolated jejunal rat

enterocytes showed an esterase activity against tocopheryl acetate localized to this organelle [30]. The exact proportion of FSV&C hydrolyzed by these enzymes is unknown, but it is assumed that the bulk of FSV&C esters are hydrolyzed in the intestinal lumen by pancreatic lipases. The remaining esters may be hydrolyzed intraluminally by mucosal enzymes or taken up intact by the intestinal cell and hydrolyzed intracellularly. However, as stated above, the consensus is that vitamin esters cannot be absorbed by the enterocyte.

1.2 DISTRIBUTION OF FAT-SOLUBLE VITAMINS AND CAROTENOIDS BETWEEN VEHICLES ABLE TO SOLUBILIZE THEM IN THE GI LUMEN DURING DIGESTION, i.e. MICELLES, VESICLES AND PROTEINS

It is assumed that during digestion, FSV&C are transferred from the food matrix in which they are embedded to the mixed micelles generated by lipolysis of dietary triacylglycerols [19]. Mixed micelles are a mixture of phospholipids, cholesterol, lipid digestion products (such as free fatty acids, monoacylglycerols and lysophospholipids) and bile salts. It has been shown that a fraction of some FSV&C first transfers to the fat phase of the meal in the gastric environment [20, 31]. This transfer may only happen in the stomach, as mixed micelles present in the intestine apparently inhibit the carotenoid transfer from the food matrix to the fat phase [32]. FSV&C are then assumed to transfer to mixed micelles during dietary lipid lipolysis by the gut lipases, a phenomenon affected by several factors, including pH and bile lipid concentration [33, 34]. However, it should be underlined that direct transfer from the food matrix to the mixed micelles within the intestinal lumen may also occur [32]. The solubilization efficiency of FSV&C in micelles depends on a wide array of factors, including FSV&C species [19]. Indeed, the micellar solubility of different carotenoids, and even the solubility of different isomers of the same carotenoid, can be quite different [35]. It is assumed that the higher the percentage of FSV&C incorporation in micelles (a percentage called “bioaccessibility”), the higher their absorption efficiency.

Although an important step, FSV&C solubility in micelles is not the only factor to affect FSV&C absorption. Indeed, the nature of the lipids that constitute these micelles also plays a key role in this process. As expected, the amount of a liposoluble vitamin (phylloquinone) solubilized in mixed micelles dramatically increased with increasing amounts of phospholipids [36]. However, it has been clearly shown by several teams that phospholipids can impair the absorption of cholesterol [37], carotenoids [38] and vitamin E [39]. The hypothesis to explain this inhibition is that because of their high hydrophobicity, cholesterol and FSV&C are associated with the long-chain acyl moieties of phospholipids in mixed micelles, resulting in reduced uptake by intestinal cells. The inhibitory effect of phospholipids on the absorption of cholesterol, α- tocopherol and carotenoids is abolished when they are substituted by lysophospholipids [37-40]. As lysophospholipid micelles are smaller than phospholipid micelles, micelle size may be one of the factors that determine the absorption of micellar lipophilic components. However, a comparison of cholesterol uptake from lysophospholipid and phospholipid micelles, which were similar in size, showed that uptake was still lower in phospholipid micelles [41]. Given that lysophospholipid uptake by intestinal cells is much greater than phospholipid uptake [37, 39], it has been suggested that the increased cellular level of lipids they induce may shift the balance of FSV&C distribution from the mixed micelles to the cells [38].

Although it is assumed that, in the GI lumen, the FSV&C recovered in the mixed micelle-rich aqueous fraction are localized into the mixed micelles, it cannot be excluded that a part of these molecules is incorporated in other lipid structures present in this aqueous fraction. Indeed, different lipid structures do coexist in the GI lumen during digestion: lipid droplets, vesicles (monolamellar and multilamellar liposomes), micelles and, if ingested, milk fat globules. Conversely to mixed micelles which are a mixture of lipids and bile salts, vesicles are assumed to be liposome-like structures composed of either a single bilayer (unilamellar vesicles) or multiple bilayers (multilamellar vesicles) of phospholipids. The hypothesis that

FSV&C can be solubilized in vesicles during absorption is supported by the observation that some FSV&C (vitamin A, E and D) are incorporated in phospholipid bilayers [42, 43]. Moreover, some FSV&C are even able to promote vesicle formation. Indeed, α-tocopherol promoted the assembly of bilayer structures and stabilized these structures in systems composed of phospholipids, whereas in the absence of α-tocopherol, phospholipids produced micellar structures [44]. Moreover, vesicle stability to a bile salt (sodium deoxycholate) was enhanced by the presence of FSV&C (vitamin A, E and D) [42]. Finally, note that FSV&C can also transfer between unilamellar vesicles [45].

The distribution of FSV&C between the lipid structures, i.e. emulsified lipid droplets, vesicles and micelles, and their localization within these structures, i.e. in the core or the surface of lipid droplets [46] or inside or across phospholipid bilayers, depends on their solubility and their ability to interact with the different lipid classes that constitute these structures [46-50]. For example, Woodall et al. [51] found a significant difference in orientation of β-carotene (an apolar carotenoid) compared to zeaxanthin (a dihydroxy carotenoid) with respect to phospholipid bilayers. More precisely, the long axis of zeaxanthin formed a narrow angle of about 45° with the axis normal to the plane of the bilayer, while β-carotene was distributed homogeneously within the bilayer without any preferred well-defined orientation. Another example is the very different distribution of β-carotene and zeaxanthin between core and surface of triacylglycerol droplets stabilized with phospholipids [46].

The presence of FSV&C in a mixed micelle-rich aqueous phase does not rule out that a fraction of FSV&C may be associated with proteins solubilised in this aqueous phase either. Indeed, β-lactoglobulin, a lipocalin recovered in cow milk, is able to bind retinol and β-carotene [52-55]. It is therefore possible that proteins present in the gut and originating either from diet or from pancreatic/biliary secretions can bind a fraction of FSV&C and transport them to the enterocyte.

In conclusion, it is reasonable to suggest that FSV&C are not only recovered in mixed micelles but might also be distributed between different vehicles (micelles, vesicles and perhaps proteins) prior to their absorption by the intestinal cell. It is also reasonable to suggest that the mechanisms of absorption depend on the vehicles with which the FSV&C are associated. Indeed, mixed micelles may interact with some membrane proteins while vesicles or proteins may have totally distinct specific transport pathways. Thus, better knowledge of the distribution of FSV&C between the different vehicles able to solubilise them in the intestinal lumen will likely allow a better understanding of the mechanisms governing their absorption.

1.3 MAIN SITE OF FAT-SOLUBLE VITAMIN & CAROTENOID ABSORPTION

The main site of absorption of FSV&C is assumed to be in the mid sections of the GI tract. However, the exact localization of this site in humans remains unknown, as the only data on this topic have been obtained in rodents. More precisely, by using everted small bowel sacs, Hollander et al. identified the sites where maximal absorption of vitamin E, D and K takes place in rats. Surprisingly, there is no such data for vitamin A, whether for retinol or for provitamin A carotenoids. Concerning vitamin E, Hollander's group found that the highest rate of absorption takes place in the medial portion, assumed to be the jejunum, of the small bowel [9]. They also found that maximal vitamin D uptake occurs in both jejunum and ileum [15], which is in agreement with our recent data obtained in mice [56]. Finally, concerning vitamin K, Hollander's team found a maximal uptake of phylloquinone (vitamin K1) in the proximal gut segments, assumed to be duodenum and jejunum, and a relatively poor uptake in distal segments, assumed to be ileum and colon [7], while bacterial menaquinones are assumed to be preferentially absorbed in the colon.

An initial hypothesis to explain why FSV&C absorption efficiency is not identical along the gut and why different FSV&C share different main sites of absorption is that intestinal

proteins involved in FSV&C absorption are not homogeneously localized along the gut. Indeed, it is likely that the absorption efficiency of a given FSV&C is maximal where the main intestinal transporters of this FSV&C are expressed. The in-gut distribution of transporters involved in the absorption of cholesterol and long-chain fatty acids - transporters also involved in absorption of some FSV&C (see paragraph 3.1) - has recently been studied in post-mortem intestinal samples from 11 subjects. Data showed bell-shape patterns, with the highest levels in ileum for ABCA1, ABCG8, NPC1L1 and CD36 [57]. Another important point that can affect FSV&C absorption is the distribution of membrane transporters between the apical and the basolateral membrane of the enterocyte. Indeed, this distribution can vary along the gut. SR-BI, which is involved in the absorption of several FSV&C, was present on the apical surface of the proximal intestine, while the presence of SR-BI on the basolateral surface of the distal intestine suggested its possible involvement in intestinal lipoprotein uptake or secretion [58]. Moreover, SR-BI mRNA expression appears to be regulated by the intestine-specific transcription factor Isx (Intestine Specific Homebox) [59, 60][61] It is thus possible that the preferred site of absorption of FSV&C vary within individuals depending on genetics and diet. Better knowledge of the distribution of proteins involved in FSV&C absorption along the intestinal tract, and within the enterocyte, would significantly enhance our understanding of FSV&C absorption.

A second hypothesis to explain the occurrence of major absorption sites for some FSV&C is that these sites may be those where the availability of FSV&C is highest, i.e. where FSV&C are recovered at their maximal concentrations in vehicles that allow their maximal absorption. These sites would therefore depend on the ability of each FSV&C class to be extracted from its food matrix and solubilized in its physiological vehicles (micelles, vesicles, and proteins that facilitate its absorption) [53]. Nevertheless, these two hypotheses are probably linked, and we suggest that transporters are mostly expressed in the regions of the gut where FSV&C are mostly recovered in their optimal vehicles for absorption.

1.4 ABSORPTION EFFICIENCY (% OF A GIVEN DOSE)

Data on FSV&C absorption efficiency are scarce and have been obtained with very different models (animals, healthy humans or patients with intestinal malabsorption, ileostomy patients). They have also been obtained with very variable doses of FSV&C, which were incorporated into different matrices and ingested in different meals. Knowing that i) FSV&C absorption is affected by numerous factors, including FSV&C dose, food matrix and meal nutrients [19], ii) absorption is probably mediated by intestinal proteins at dietary doses but is probably passive at pharmacological doses, and iii) there is a huge inter-individual variability in the absorption efficiency of most FSV&C [62, 63], all these factors likely explains the huge variability in published data.

Concerning vitamin A, it has been reported that for preformed vitamin A (retinol), absorption efficiency ranges between 75% [64] and about 100% [65]. This range is less variable than the range reported for absorption efficiency of provitamin A carotenoids, i.e. from about 3% to 90% for β-carotene [66-71]. Furthermore, it is assumed that preformed vitamin A has a higher absorption efficiency than provitamin A carotenoids — an assumption confirmed by data obtained in our laboratory using Caco-2 cells (unpublished data). This could be explained by the presence of an efficient specific transporter for retinol, while provitamin A carotenoids are absorbed via non-specific transporters (see paragraph 3.1).

Concerning vitamin E, different studies report absorption efficiency in ranges of 10-95% [72-74]. However when deuterium-labeled vitamin E was used to estimate absorption, this range was reduced to 10-33% [75]. These nevertheless huge ranges were probably due to the different methods used to estimate absorption, to the effect of food matrix, and to broad inter-individual variability in absorption efficiency.

The absorption efficiency of vitamin D3 given in peanut oil was found to vary between 55%

and 99% (mean 78%) in patients with intestinal malabsorption and control subjects [76]. Animal experiments have reported similar rates, percentages ranging between 66% and 75%.

There is little data available on the absorption efficiency of vitamin K. The absorption efficiency of vitamin K1 supplied in its natural matrix, i.e. vegetables, is assumed to be relatively low, because it was found that only about 13% was absorbed from spinach [77]. Absorption efficiency is assumed to be higher when vitamin K is given as a supplement. Indeed, one study found that healthy subjects excreted less than 20% of a 1 mg dose of radiolabeled phylloquinone, suggesting that 80% was absorbed.

On the whole, this review of the literature shows that it is difficult to provide a single narrow range for the absorption efficiency of each FSV&C in humans. This is likely because absorption of FSV&C is affected by numerous factors [19], but also because there is a huge inter-individual variability in the absorption efficiency of these molecules [62, 63].

2. MECHANISMS OF UPTAKE BY THE INTESTINAL CELLS: PASSIVE DIFFUSION AND/OR PROTEIN-MEDIATED TRANSPORT

2.1 ARGUMENTS FOR PASSIVE DIFFUSION

It has long been assumed that intestinal absorption of vitamin D, E and carotenoids occurs by passive diffusion. The first vitamin to be studied was vitamin E. The intestinal transport of α-tocopherol was described as non-ATP-dependent, as the addition of 2,4-dinitrophenol, sodium azide or potassium cyanide, all of which inhibit ATP synthesis, to the incubation medium of everted rat bowel sacs did not impair its absorption. Moreover, the fact that vitamin E uptake was linear up to 1.2 mM was another argument in favour of passive diffusion [9]. A few years later, the same team used living unanaesthetized rats to conclude that vitamin D absorption was also passive. Indeed, cholecalciferol uptake was increased by H+ ions, which are supposed to decrease cell membrane resistance to micelle diffusion, and

increased by perfusate flow rate, which diminishes the thickness of the unstirred water layer [15]. Similar experiments performed with β-carotene led to comparable conclusions, i.e. that carotenoids were absorbed by passive diffusion through the brush border membrane (BBM) [17].

Finally, retinol uptake by Caco-2 cells was shown to occur by passive diffusion at pharmacological doses [78], which confirmed the early data obtained in rat intestinal segments for retinol concentrations above 450 nM [13, 18].

2.2 ARGUMENTS FOR PROTEIN-MEDIATED TRANSPORT

The hypothesis of a passive diffusion mechanism for carotenoid and vitamin E and D uptake cannot explain the higher absorption of vitamin E in the mid-intestine compared to other parts of the intestine [9]. It also cannot explain the huge inter-individual variability in absorption observed during postprandial studies [62, 63]. Further, it cannot explain the isomer selectivity and competition for absorption between carotenoids in Caco-2 cells [79], nor the competition between vitamin E and the carotenoid canthaxanthin observed in rats [80]. Moreover, a close look at the data obtained for β-carotene shows that a saturation of absorption in the distal part of the intestine cannot be ruled out [17]. Finally, the identification of the Drosophila gene ninaD encoding a class B Scavenger receptor essential for carotenoid distribution into cells is another pioneering finding in favour of a protein-mediated membrane transport of carotenoids [81].

Thus, all these arguments are in favour of a protein-mediated transport, which is not totally contradictory with Hollander et al's results, as i) some membrane proteins involved in FSV&C uptake are not ATP-dependent (see paragraph 3.1), and ii) both protein-mediated transport and passive diffusion are observed depending on the FSV&C dose used in experiments [56].

Besides these observations, retinol uptake at physiological doses by Caco-2 cells has been

shown to occur by a saturable carrier-mediated process [78]. This concurs with experiments performed in rats showing a facilitated uptake at a concentration of 150 nM [13, 18]. However, despite more recent *in vivo* investigations being performed [82], no protein has yet been identified as involved in retinol transport BBM level.

Similarly to retinol, phylloquinone is also thought to be transported by a protein in the intestine [14]. Indeed, the use of a nitrogen atmosphere (to inhibit mitochondrial respiration) as well as the addition of 2,4-dinitrophenol (to inhibit ATP synthesis) decreased phylloquinone absorption in rat intestine. However, again, no membrane transporter has yet been identified.

As a final point, it is equally important to underline that the phenomena of protein-mediated transport and passive diffusion may be complementary, with protein-mediated transport occurring at dietary concentrations and passive diffusion taking over at pharmacological doses.

3. MEMBRANE PROTEINS INVOLVED IN FAT-SOLUBLE VITAMIN & CAROTENOID APICAL UPTAKE AND EFFLUX

The fact that membrane proteins are involved in the uptake of FSV&C by the enterocyte, at least at dietary concentrations, raises two main questions: “are these proteins specific for some FSV&C?”, and “can different membrane proteins be involved in the uptake of the same FSV&C?”. Furthermore, as some membrane proteins involved in cholesterol uptake demonstrate broad substrate specificity, “do they play a role in FSV&C uptake?”.

The answer to the first question is probably “yes” for preformed vitamin A, i.e. retinol, because 1) a study in isolated rat intestinal segments suggested that retinol absorption is mediated by a saturable carrier-mediated process [13], 2) it has a much higher absorption efficiency than any other FSV&C, suggesting a specific transporter (personal, non published data), and 3) its absorption does not involve a membrane transporter with broad

substrate specificity identified as playing a role in other FSV&C (see paragraph 3.1.1), which again points to a very specific transporter. A good candidate as a protein specific for retinol uptake by intestinal cells would be STRA6 (STimulated by Retinoic Acid 6), which has been identified as a specific membrane receptor for RBP (Retinol-Binding Protein) [83]. This protein is found in many - but not all - tissues, and is expressed in the intestine [84]. Interestingly, there is a synergy between STRA6 and Lecithin Retinol Acyl Transferase (LRAT) expression [85].

Cells expressing both these proteins – which is the case of intestinal cells - can uptake more retinol than cells expressing each protein individually. This indicates that the conversion of retinol into retinyl ester by LRAT within the cell maintains the driving force for STRA6-mediated retinol uptake. It has been shown that STRA6 acts as a bidirectional transporter of retinol, with intracellular retinol concentrations determining the polarity of transport [85]. It is possible that STRA6 functions at a basal level to import retinol into the cell in order to maintain intracellular retinoid homeostasis. In times of surplus retinol within the cell, it can be upregulated and thus act to export retinol, protecting the cell from a potentially cytotoxic build-up of retinoid. STRA6 expression control is likely tightly regulated at both gene and protein levels, and would depend on the cellular context and retinoid status of the animal.

There is also probably a specific transporter for vitamin K, or at least for vitamin K1 (phylloquinone), which is the other fat-soluble vitamin that apparently required an energy-saturable transport mechanism for its absorption according to Hollander's studies [7, 10, 14, 16, 18]. The fact that there is a specific transporter for vitamin K, which is supported by only one study [14], should be confirmed with more recent methodologies. Moreover, it should be kept in mind that other forms of vitamin K, i.e. menaquinones, are also recovered in the diet. Menaquinones are assumed to be absorbed by passive diffusion [10], but further studies are required to assess whether the lipid transporters described below are also involved, at least in part, in phylloquinone and menaquinone uptake by intestinal cells.

The answers to the other two questions are given in the text that follows.

3.1 LIPID TRANSPORTERS INVOLVED IN FAT-SOLUBLE VITAMIN & CAROTENOID UPTAKE

In recent years, lipid transporters - and especially cholesterol transporters - have been identified as playing a role in FSV&C uptake by the intestinal cell.

3.1.1 Scavenger receptor class B type I (SR-BI)

SR-BI (also known as CLA1 for *CD36 and LIMPII Analogous-1* in humans) is a single-chain transmembrane glycoprotein of 80 kDa. SR-BI is found in various cell types including liver, testis, ovaries and macrophages [86], as well as on the BBM of enterocytes from the duodenum to the colon [87]. Its role in lipoprotein metabolism is very important because it is able to bind not just HDL (high density lipoprotein) but also LDL (low-density lipoproteins) and VLDL (very-low-density lipoproteins) [86]. SR-BI protein has the ability to facilitate the selective entry in the cell compartment of esterified cholesterol recovered in HDL [88]. At the intestinal level, it has first been shown that SR-BI facilitates the uptake of free cholesterol but also esterified cholesterol, phospholipids and triacylglycerol hydrolysis products, thus displaying broad ligand specificity [89, 90]. However, the effective role of SR-BI compared to another transporter – the Niemann Pick C1-like 1 (NPC1L1) – in terms of cholesterol transport is still a matter of debate [91]. We hypothesize that the main role of SR-BI in the intestine is to facilitate the uptake of other lipid molecules. Indeed, in 2005, a study using Caco-2 cells identified SR-BI as playing a role in the intestinal uptake of the carotenoid lutein [92], and this involvement has since been extended to other carotenoids such as β-carotene [93], zeaxanthin [94] and lycopene [95] in various other tissues. Moreover, after being shown to be involved in vitamin E uptake in the liver [96] and in the brain [97], SR-BI was then shown to mediate tocopherol uptake across the BBM in both Caco-2 and mice models [98]. Finally, SR-BI was recently shown to be involved in vitamin

D uptake via to a combined approach using Caco-2 cells, HEK cells transfected with SR-BI, mouse intestinal explants, and mice overexpressing SR-BI in the intestine [56]. SR-BI is thus involved in the uptake of a broad variety of molecules (cholesterol ester, carotenoids, vitamins E and D, among others), but not in the uptake of micellar retinol [99]. This raises questions on the mechanisms of interaction between the transporter and its ligands. We hypothesize that SR-BI interacts with the mixed micelles and facilitates the specific uptake of certain lipid molecules solubilized within them. Obviously, dedicated experiments should be performed to verify this hypothesis. Using both mouse models and human cell lines, it has recently been shown that intestinal lipid absorption by SR-BI is subject to control by retinoid signaling [59-61]. Indeed, retinoic acid induces the expression of the intestinal transcription factor ISX which represses the expression of both SR-BI and BCMO1 (carotenoid-15,15'-oxygenase). BCMO1 acts downstream of SR-BI and converts absorbed β-carotene to the retinoic acid precursor, retinaldehyde. Thus, there is a diet-responsive regulatory network that potentially controls SR-BI-mediated FSV&C absorption via a negative feedback regulation. We say "potentially" because it has been shown that ISX null mice have normal blood cholesterol levels despite intestinal overexpression of SR-BI [60].

3.1.2 Cluster Determinant 36 (CD36)

Another scavenger receptor of interest is FAT (Fatty Acid Translocase, also known as CD36 in humans). This trans-membrane glycoprotein of 90 kDa is mainly expressed in epithelial cells, adipocytes, platelets and macrophages [86]. CD36 is also expressed at the BBM level of the duodenum and the jejunum [82]. Like SR-BI, it can interact with a broad variety of ligands such as HDL, LDL, VLDL, anionic phospholipids, fatty acids, free cholesterol, thrombospondin, collagen, erythrocytes infected by *P falciparum*, and apoptotic cells [86]. CD36 appears to be involved in numerous cellular functions such as cellular adhesion [100], angiogenesis [101], lipoprotein ligation/endocytosis [102] or fat sensing in lingual papilla [103]. It is assumed to play a key role in fatty acid uptake in muscle and

adipose tissue [104], as well as in the intestine [87]. The first result on knock-out mice suggested that CD36 did not play a role in intestinal lipid absorption after a lipid load [105]. Nevertheless, a following study showed that lipid secretion in the lymph was decreased in CD36-deficient mice, but this phenomenon was actually hidden by the fact that CD36 can also regulate chylomicron catabolism [106]. It was shown that in the enterocyte, CD36 probably allows the routing of the long-chain fatty acids to the endoplasmic reticulum for chylomicron assembly. The underlying mechanism is unknown, but it may be linked to the intracellular traffic of the protein between the plasma membrane and the organites. Interestingly, CD36 colocalizes with other proteins involved in cholesterol transport, such as caveolin-1 in lipid rafts [107] which has been identified as playing a role in lipid endocytosis [108]. It is thus possible that the two proteins cooperate for lipid transport.

Regarding FSV&C, CD36 was first shown to be involved in β-carotene uptake using transfected COS-7 cells and mouse BBM vesicles [93]. This result was further supported by the fact that a CD36-related protein is involved in selective carotenoid transport in *Bombyx mori* [109]. Moreover, CD36 has been shown to be involved in both lycopene and lutein uptake in mouse 3T3-L1 adipocytes and in mouse adipose tissue cultures [110]. Recently, CD36 was also shown to mediate cholecalciferol uptake in human transfected HEK cells as well as in mouse internal explants [56].

As discussed for SR-BI, the fact that CD36 is involved in the uptake of very different substrates raises the question of the mechanism involved. Because CD36 colocalizes with other proteins, such as caveolin-1, in lipid rafts, it is possible that the modulation of its activity/expression indirectly modulates the activity of these proteins. If these proteins are involved in FSV&C uptake, FSV&C absorption may subsequently be affected.

3.1.3 Niemann-Pick C1-like 1 (NPC1L1)

Among the cholesterol membrane transporters, one of the last to be identified was NPC1L1. NPC1L1 is a 135 kDa protein widely expressed in human tissues, especially in the liver and

at the plasma membrane of the intestinal cell [111-114], as well as in Caco-2 cells [115]. NPC1L1 has been described as the main cholesterol and phytosterol transporter in the intestine [116]. Indeed, it is thought to transport about 60% of dietary cholesterol according to results obtained in knock-out mice [112, 113, 116]. The phenotype of these mice corresponds to the phenotype of mice treated with ezetimibe – an inhibitor of cholesterol absorption [112, 116]. It was first suggested that the target of ezetimibe was the annexin-2/caveolin-1 complex [117] or CD13 [118]. However, caveolin-1-deficient mice display no special phenotype for cholesterol absorption [119], and there have been no further investigations on CD13. Another study elegantly showed that ezetimibe binds specifically to NPC1L1 [114]. Recently, it has been demonstrated that NPC1L1 was recycled between the plasma membrane and the intracellular compartments via an endocytotic pathway regulated by cellular cholesterol. Cholesterol depletion would induce a relocalization of the protein at the cell surface and thus increase cholesterol uptake [120]. NPC1L1 was further shown to be involved in both α-tocopherol [121] and γ-tocotrienol [122] intestinal absorption using Caco-2 cells and *in situ* perfusions in rats. Results were less clear on carotenoids [95, 123]. In the first study, ezetimibe was shown to inhibit α- and β-carotene uptake by 50%, β-cryptoxanthin and lycopene uptake by 20%, and lutein and zeaxanthin uptake by 7% in Caco-2 cells. In the second study focusing on lycopene, neither ezetimibe nor a blocking antibody raised against NPC1L1 impaired its absorption in these cells. Additional experiments are thus needed to clarify these discrepancies. Finally, it was recently shown using *in vitro*, *ex vivo* and *in vivo* mouse models that NPC1L1 is also involved, in a moderate way, in cholecalciferol intestinal uptake [56].

3.2 LIPID TRANSPORTERS AND FAT-SOLUBLE VITAMIN & CAROTENOID APICAL EFFLUX

The enterocyte is not a simple entrance for nutrients into the body. Numerous proteins

located at the apical side of the cells can efflux molecules back to the lumen to regulate or inhibit their absorption. For example, SR-BI's role is not limited to uptake, as it can function in both directions in the intestine [124] and other tissues [88]. In particular, it was shown to be involved in both vitamin D [56] and E [98] efflux across the BBM using Caco-2 cells. Pre-charged FSV&C cells were able to efflux the vitamin back to the apical medium, especially when mixed micelles were added as acceptors, and this efflux was significantly inhibited in presence of SR-BI inhibitors (the chemical inhibitor Block-Lipid Transport 1 and/or blocking antibody).

It is very likely that other transporters can act as efflux pumps of FSV&C at the BBM level. For example, the ATP-Binding Cassette (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 [125] requiring energy in the form of ATP to translocate their substrates [126, 127]. ABC transporters are classified into 7 subfamilies (A to G) depending on their structure and homology, and on whether they are "complete" transporters such as ABCA1 or half-transporters associated in homo- or heterodimers, such as ABCG1 or ABCG5/G8 [125].

In particular, the ABCG5/G8 heterodimer is critical to sterol homeostasis. Mutations in the genes encoding these proteins cause β-sitosterolemia [128], a disease characterized by abnormally high levels of phytosterols in blood and tissues due to increased intestinal absorption and decreased secretion through the bile. The ABCG5/G8 complex acts like an efflux pump in the enterocyte where it limits mainly phytosterol absorption. Interestingly, the ABCG5/G8 complex can also transport cholesterol [129]. However, there is only one study suggesting a role of these transporters in the absorption of a FSV&C: lutein [130]. This study suggested that ABCG5 plays a role in the plasma response to dietary carotenoids.

Finally, it has been suggested that ABCA1 is expressed at the apical surface of the

enterocyte [131] and plays a role in cholesterol and phytosterol apical efflux [132, 133]. However, there is no data on the potential involvement of this transporter in apical efflux of FSV&C, and numerous studies describe ABCA1 as expressed at the basolateral side of the intestinal cell [134] where it is implicated in lipid secretion via the portal route [134-136] (see section 6.1).

In conclusion, it appears that several lipid transporters are involved in FSV&C uptake/efflux at the apical membrane of the enterocyte. As these lipid transporters are involved in the uptake of both cholesterol and different FSV&C, this likely explains the competition between these molecules for apical uptake (e.g. between lutein and carotenoids or vitamin E, and *vice versa* [137, 138], between α- and γ-tocopherol or cholesterol [98], or between vitamin D and vitamin E or cholesterol [56]). The relative contribution of the lipid transporters to the net uptake of each FSV&C is impossible to clarify at the present time due to the incomplete data available. Furthermore, it is likely that not all the transporters involved have yet been identified.

4. TRANSPORT OF FAT-SOLUBLE VITAMINS & CAROTENOIDS ACROSS THE ENTEROCYTE

FSV&C are metabolized differently by the enterocyte. Indeed, while tocopherols and cholecalciferol are theoretically incorporated into the chylomicrons without any chemical modification [72, 139], retinol is first esterified in retinyl esters by LRAT and ARAT [140] and carotenoids are partly cleaved in retinal and apocarotenoids by BCMO1 and BCDO2 [141], respectively. Finally, concerning phylloquinone, some authors have suggested that menadione could be produced by the intestine after an oral dose of phylloquinone [142, 143]. However, this hypothesis remains to be further investigated. The intracellular transport of most of these molecules in the enterocyte is a black box. It is assumed that, being non-soluble in water, these compounds require either intracellular proteins to cross the water

compartments of the cells or incorporation in intracellular cell membranes to be transferred to the basolateral side of the enterocyte. The first candidates for this intracellular transport are the apical membrane transporters displaying an intracellular cycle between the apical membrane and the cellular organelles. For example, NPC1L1, which is implicated in vitamin E and D absorption, has been observed in endosomes, perinuclear regions, lysosomes and mitochondria of the human intestinal cell [120, 144]. Another candidate is CD36, which has been suggested to play a role in apical uptake of β-carotene and vitamin D. Indeed, this scavenger receptor has been detected in both the apical membrane and the Golgi apparatus [145]. Finally, SR-BI, which is involved in apical uptake of several FSV&C, has been found in apical and basolateral membranes of enterocytes, in cytoplasmic lipid droplets and in tubulovesicular membranes. Furthermore, SR-BI is mainly localized in the microvillus membrane in the fasting state and seems to be endocytosed during absorption of dietary fat [146]. We hypothesize that some FSV&C might stick to these transporters, or to membrane microdomains containing these transporters, at the apical side of the enterocyte and follow them within the cell to be transferred to either other intracellular transporters or to intracellular membranes.

The fact that apical membrane transporters can participate in the intracellular transport of FSV&C obviously does not exclude the possibility that proteins dedicated to the intracellular transport of these compounds are also involved. This is supported by the fact that proteins involved in intracellular transport of some FSV&C have been identified in several cell types, as well as in enterocytes. When such proteins from other tissues are also expressed in the enterocyte, it is tempting to hypothesize that they play the same role in the enterocyte, but this must be confirmed in dedicated studies.

4.1 SPECIFIC INTRACELLULAR TRANSPORTERS

4.1.1 Vitamin A

Intracellular transporters of vitamin A (retinol and its metabolites: retinal and retinoic acid) were discovered many years ago. These proteins are called retinoid-binding proteins [147, 148]. They are expressed in numerous tissues and are involved in both intracellular transport and targeting of the essential vitamin A metabolite to the appropriate site in the cell, e.g. retinoic acid to the nucleus or retinal to opsin. A retinoid-binding protein of special interest for vitamin A transport within the enterocyte is CRBPII (Cellular Retinol-Binding Protein II). CRBPII is primarily expressed in the intestinal mucosa, and it is one of the most abundant soluble proteins in the jejunum, representing 0.4–1.0% of the total enterocyte cytosolic proteins [149]. The hypothesis that this protein is important for the intracellular transport of retinol in the enterocyte was first prompted by the finding that its mRNA expression increased in the small intestine of rats under a retinoid-deficient diet [150]. Further investigations, using CRBPII-deficient mice, have definitively demonstrated that CRBPII plays an important role in vitamin A metabolism in the intestine [151, 152]. The involvement of another CRBP, CRBPI [153], cannot be ruled out as this protein is also found in the enterocyte, but there is no evidence that it plays an important role in vitamin A metabolism in this cell type. Concerning carotenoids, a carotenoid-binding protein (CBP) assumed to transport carotenoids in the cytosol has been described in the midgut of the silkworm *Bombyx mori* [109, 154]. Furthermore, two lutein-binding proteins have been described in human retina: GSTP1 (Glutathione S-Transferase Pi 1) [155] and HR-LBP (Human Retinal Lutein-Binding Protein) [156] that shows good cross-reactivity with antibodies raised against *Bombyx mori* silkworm CBP. If expressed in human enterocytes, LBP would be a good candidate as intracellular transporter of xanthophylls.

4.1.2 Vitamin E

In humans, a specific intracellular protein that binds tocopherol, the α-tocopherol-transfer protein (α-TTP), is mainly expressed in the liver. Mutations in the gene encoding this protein can disrupt tocopherol transfer from the liver into plasma lipoproteins, resulting in a

clinical syndrome called Ataxia with isolated Vitamin E deficiency. Although a specific binding protein is needed for α-tocopherol transfer onto nascent hepatic VLDL [157], it is assumed that other vitamin E-binding proteins are involved in the intracellular transport of vitamin E in various tissues [158]. A vitamin E-binding protein called TAP (tocopherol-associated protein) has been identified in both bovine [159] and human tissues [160]. This protein belongs to a family of hydrophobic ligand-binding proteins that share the same *cis*-retinal binding motif sequence. Northern analyses showed that TAP mRNA is ubiquitously expressed. Thus, TAP could be a good candidate for intracellular transport of vitamin E within the enterocyte, although its expression in intestinal cells has apparently not been measured [159]. Other candidates could be the sec14p-like proteins (hTAP1, 2 and 3), which are ubiquitously detectable in several tissues and which enhance the *in vitro* transport of α-tocopherol to mitochondria at the same rate as α-TTP [161]. Since there is no discrimination between the various forms of vitamin E, e.g. α, β, γ, δ tocopherols and tocotrienols, in terms of their absorption, we suggest that if a vitamin E-binding protein is implicated in intracellular transport of vitamin E within the enterocyte, it is a protein that has no specificity for RRR-α-tocopherol, conversely to α-TTP.

Finally, the fact that the basolateral membrane transporter ABCA1 is implicated in basolateral efflux of a fraction of vitamin E in apo-AI lipoparticles (see section 5) prompts the question "Is there one specific intracellular vitamin E-binding protein that channels vitamin E to the site where it is incorporated into chylomicrons, and another that channels vitamin E to the site where it is secreted in intestinal HDL via the ABCA1 transporter"?

4.1.3 Vitamin D

Apart from VDR, the nuclear receptor involved in the well-known effect of vitamin D on gene expression, the first intracellular vitamin D-binding protein (CDBP – cytosol vitamin D-binding protein) immunologically related to DBP that is responsible for vitamin D transport in the blood was found in rat kidney [162]. Fifteen years later, an intracellular

vitamin D binding protein (called IDBP) was identified in new-world primate cell extracts [163, 164]. It is not known whether CDBP and IDBP are the same protein. IDBP bind s25OHD and is putatively responsible for translocation of 25OHD to the vitamin D 1- and 24-hydroxylase enzymes located in the inner mitochondrial membrane. In humans, two IDBP (IDBP-1 and 2), which were related to the IDBP identified in new-world primates, have been cloned and expressed. [165]. Finally, in rat enterocytes, an intracellular binding protein with distinct characteristics from primate IDBP has been characterized [166]. This protein is named cytosolic vitamin D metabolite binding protein (cDBP), and because it preferentially binds 25OHD, it has been suggested that it would transport 25OHD across the enterocyte via the chylomicron-independent route [167]. However, vitamin D is mainly ingested as cholecalciferol which is incorporated into chylomicrons, and there is no data on the expression of cDBP or other IDBP in human enterocytes or on their putative role in cholecalciferol transport across the enterocyte.

4.1.4 Vitamin K

Only one study has suggested the existence of an intracellular-specific vitamin K-binding protein, which was described in the nucleus of osteoblasts. It binds MK-4 (menatetrenone), which is produced via conversion of phylloquinone in the body [168], and phylloquinone and its molecular structure is close to human GAPDH [169]. There is too little relevant data to allow any identification of specific candidate protein(s) for the intracellular transport of vitamin K across the human enterocyte.

In summary, although several candidate binding proteins exist for most FSV&C, only retinol presents an identified specific intracellular protein (CRBPII) which has been involved in its transport across the human enterocyte. Dedicated studies are therefore required to investigate this important aspect of FSV&C metabolism.

4.2 NON-SPECIFIC INTRACELLULAR TRANSPORTERS: THE FATTY ACID-

BINDING PROTEINS

The hypothesis that fatty acid-binding proteins (FABPs) can participate in the intracellular transport of FSV&C comes from studies by Hollander's group. This team was the first to suggest, from results showing interactions between phylloquinone and linoleic acid, that a FABP might also be a carrier for phylloquinone [14]. The fact that FABPs are also able to bind different hydrophobic molecules [170, 171] supports this hypothesis, and suggests that enterocyte FABPs might be involved in intracellular transport of some FSV&C. Two FABPs are co-expressed in the human enterocyte: intestinal FABP (IFABP) and liver FABP (LFABP) [172]. Both exhibit high-affinity binding of long-chain fatty acids [173]. Utilizing mice genetically lacking either IFABP or LFABP, it has been demonstrated that each of the enterocyte FABPs participates in specific pathways of intestinal lipid metabolism [174]. In particular, LFABP appears to target fatty acids toward oxidative pathways and dietary monoacylglycerols toward anabolic pathways, while IFABP targets dietary fatty acids toward triacylglycerol synthesis. It is thus hypothesized that the enterocyte FABPs allow a specific trafficking of their ligands to their respective metabolic fates. Although dedicated studies should be performed to verify whether IFABP and/or LFABP are involved in FSV&C metabolism, the fact that a genetic association study found that a genetic variant in IFABP was associated to fasting plasma lycopene concentrations [175] can be considered as a first argument that the FABPs could well be involved in intracellular transport of some FSV&C.

5. BASOLATERAL SECRETION OF FAT-SOLUBLE VITAMINS & CAROTENOIDS

It is assumed that most of the newly-absorbed FSV&C are incorporated into chylomicrons that are secreted into the lymph via an apoB-dependent route [176]. Vitamin A is assumed to be recovered as retinyl esters [140, 177] and as non-cleaved provitamin A carotenoids.

The other fat-soluble vitamins and the non-provitamin-A carotenoids are assumed to be recovered as non-esterified molecules. However, a growing body of evidence now suggests that the intestine secretes important amounts of HDL during the postprandial period. Indeed, it has been shown in differentiated Caco-2 cells and in rat enterocytes that free cholesterol was secreted at the basoteral side by two distinct routes: an apolipoprotein B-dependent pathway (chylomicrons) and a non-apo-B-dependent pathway. Conversely, cholesterol esters were exclusively secreted into chylomicrons. As this non-apo-B pathway was i) inhibited by glyburide and ii) increased by agonists of the nuclear receptors LXR/RXR (liver X receptor/retinoid X receptor), it was assumed to be an ABCA1/HDL-dependent pathway [176]. Results from animal studies led to the conclusion that intestinal ABCA1 would contribute to approximately 30% of steady-state plasma cholesterol levels [178]. ABCA1 is a protein of 240 kDa, and its expression during embryonic development has been correlated to apoptosis areas. Defects in the ABCA1 gene were subsequently shown to be the cause of Tangier disease, which is characterized by a lack, or abnormally low levels of HDL and a markedly increased risk of coronary artery diseases. Studies using knock-out mice have confirmed that ABCA1 plays a pivotal role in reverse cholesterol transport. Increased expression of ABCA1 in a number of cell types results in elevated net efflux of cellular cholesterol and phospholipids. This efflux is dependent on the presence of an acceptor such as lipid-poor HDL or apolipoprotein AI (Apo AI) [179]. The molecular functioning of ABCA1 remains unclear, but it has been suggested that the ligation/hydrolysis of ATP on the protein induces a change in the conformation of the transporter, which then binds to a lipid-poor Apo AI. Phospholipids then transfer from the cell membrane to Apo AI, which in turn induces cholesterol transfer [180]. Proteins other than Apo AI may also act as acceptors, although their physiological relevance is presently unclear. In addition to cholesterol and phospholipids, ABCA1 has also been reported to promote secretion of α-tocopherol, apoE and interleukin-1β at tissue level [181]. ABCA1 is

highly expressed in the liver and the intestine [182], especially at the basolateral side of the cell [134], where it can play a role in intestinal HDL production and thus in basolateral cholesterol and phytosterol secretion [134-136]. As ABCA1 controls HDL formation by the cellular efflux of a broad range of lipids [136, 183-185] and is associated with α-tocopherol secretion in macrophages [186], it was not surprising that *in vitro* models showed that a fraction of α-tocopherol was also secreted with intestinal HDL by an ABC transporter [187, 188]. The use of ABCA1-deficient mice definitively confirmed that intestinal ABCA1 is significantly involved in the intestinal absorption of vitamin E (α- and γ-tocopherol) but not vitamin A (retinyl palmitate) [189]. It has also been suggested that free retinol efflux from intestinal cells was probably facilitated by ABCA1 [99], but this result needs to be confirmed.

Additionally to ABCA1, other transporters may play a role in FSV&C basolateral efflux. The first candidate would be ABCG1, which is also expressed in the intestinal cell [190]. Indeed, its activity is sometimes difficult to differentiate from ABCA1 activity, although it is thought to efflux lipid to mature HDL and not to lipid-free apo AI [191]. Another example would be SR-BI, as it is also partly expressed at the basolateral membrane of the intestinal cell [58, 92] and is clearly involved in lipid and FSV&C efflux [56, 88, 98, 124, 192]. However, further studies are required to confirm the contributions of these transporters to intestinal FSV&C basoteral secretion.

6. HYPOTHESIS ON THE MODULATION OF FAT-SOLUBLE VITAMIN & CAROTENOIDS ABSORPTION VIA MODULATION OF THE EXPRESSION OR ACTIVITY OF PROTEINS INVOLVED IN FAT-SOLUBLE VITAMIN & CAROTENOID METABOLISM WITHIN THE ENTEROCYTE

The fact that intestinal proteins are involved in FSV&C absorption raises the question of the consequences of modulations in their expression or activity on FSV&C bioavailability. Two

main factors modulate the expression and activity of intestinal proteins involved in nutrient absorption: 1) the nutrients absorbed via these proteins themselves (negative feedback regulation) and 2) genetic variations in the genes encoding these proteins.

The first nutrients that can likely modulate the expression of proteins involved in absorption of FSV&C are FSV&C themselves. To support this hypothesis, the expression of SR-BI, the transporter involved in apical uptake of several FSV&C, is modulated by both vitamin A [61] and vitamin E [193, 194]. Moreover, vitamin E is also able to downregulate the expression of SR-BI post-transcriptionally [96]. Vitamin E also downregulates CD36 [195-197], a transporter involved in intestinal uptake of β-carotene [93], and ABCA1 [190, 193, 194], a transporter involved in its basolateral secretion. These observations suggest that some FSV&C can modulate their own absorption by a negative feedback regulation, which further supports a key role of the intestinal proteins regulated by FSV&C in the absorption of these compounds. Because we have recently found that SR-BI, CD36 and NPC1-L1 are involved in intestinal uptake of vitamin D [56], and because this vitamin has a well-known effect on gene expression, it would be interesting to assess whether this vitamin modulates the expression of proteins involved in its own absorption.

The fact that several intestinal proteins involved in FSV&C absorption are also involved in fatty acid and cholesterol absorption makes it is reasonable to hypothesize that dietary fatty acids, or cholesterol, modulate the expression of these proteins. This is supported by the fact that NPC1L1, as well as the ATP-binding cassette proteins ABCA1, ABCG5 and ABCG8 are downregulated by a cholesterol-free high-fat diet [198]. For other transporters, there is little data at the intestinal level, but it has been shown that heart CD36 and hepatic SR-BI expressions are regulated by dietary fat [199, 200]. We can thus suggest that the fat and cholesterol content in the diet may affect the absorption efficiency of FSV&C by modulating the expression of lipid transporters involved in their absorption. Finally, the expression of an intestinal protein assumed to be specific for the transport of a FSV&C, i.e.

CRBPII that transport retinol, is modulated by diets containing long-chain fatty acids [150]. The hypothesis that genetic variants in genes encoding proteins involved in FSV&C absorption may affect FSV&C absorption comes from the observation that there is a huge inter-individual variability in FSV&C assimilation [62, 63], that the "responder characteristic" is apparently an intrinsic characteristic of the subjects, and that proteins are involved in FSV&C absorption. Indeed, the finding that intestinal proteins are involved in absorption of FSV&C leads to the hypothesis that genetic variations in their related genes may affect their expression (promoter region of the gene) or their activity (modification of amino acid sequence) and, in turn, their ability to absorb/transport these compounds. This exciting hypothesis needs to be verified in specific studies, but data on the associations between genetic variants SR-BI and CD36 and blood concentrations of carotenoids [201, 202] and between CD36 and vitamin E [203] support the proposal. Nevertheless, it should be stressed that these associations may be due to the effect of genetic variants on the expression or activity of the proteins in tissues other than the intestine. Indeed, these scavenger receptors are expressed in several tissues, and it is possible that the association with fasting blood concentration of FSV&C is also due to an effect of the genetic variants on uptake or efflux of carotenoids and vitamin E in other tissues.

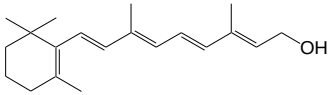
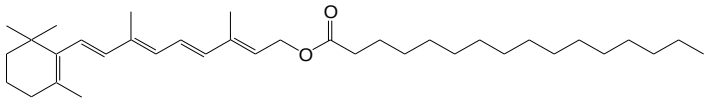
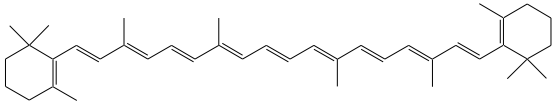
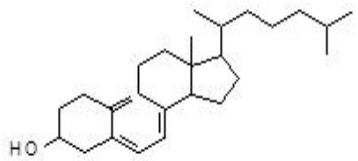
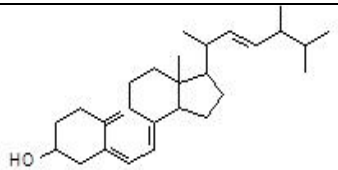
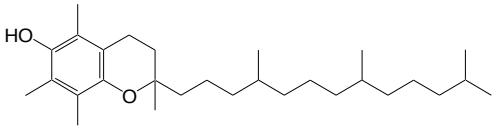
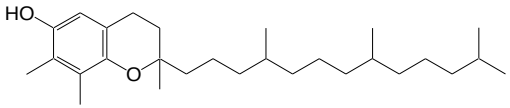
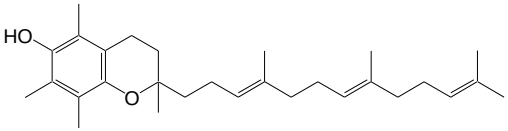
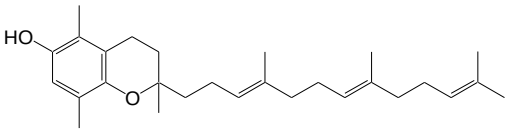
If the hypothesis that genetic variants affect FSV&C absorption efficiency is verified, it would prompt the following questions: "do groups of subjects with low responder genotypes need higher dietary intakes of FSV&C?", and conversely "do groups of subjects with high responder genotypes need to avoid high intakes of some FSV&C due to the risk of toxic effects [204]?". Finally, because some intestinal proteins are involved in absorption of different FSV&C (e.g. SR-BI is involved in absorption of vitamin E), carotenoids and vitamin D, we can ask whether genetic variants can simultaneously affect the absorption efficiency of these different FSV&C — or, in other words, whether certain subjects could be at risk of micronutrient multideficiency.

PERSPECTIVES

Numerous gaps need to be filled before we can gain a full understanding of the uptake, intracellular transport and basolateral secretion of FSV&C by the enterocyte. Although some specific proteins such as CRBPII, and several non-specific proteins such as SR-BI, NPC1-L1, and ABCA1 have been identified, other transporters, (e.g. those involved in apical uptake of retinol and phylloquinone) remain to be identified. Another important point to unravel is how some proteins can be involved in the metabolism of FSV&C but display strikingly different chemical structures (SR-BI and CD36 are implicated in the metabolism of carotenoids, vitamin E and vitamin D). Finally, the discovery that proteins are involved in FSV&C absorption has not only opened a new field of research on the topic, but has also allowed the proposal of an explanation for the huge inter-individual variability observed in FSV&C absorption. Moreover, this discovery has led to the hypothesis that genetic variants in genes encoding these proteins may affect the absorption efficiency of these micronutrients, leading to suggestions that future Recommended Dietary Allowances of FSV&C may need to take these genetic variants into account. This would constitute a first step toward personalized nutrition based on the genetic characteristics of individuals.

Tables

Table 1: Molecules belonging to the different fat-soluble vitamin groups

Vitamin group	Structures of the main molecules provided by the diet	Main food sources (µg/ 100 g) [205, 206]
Vitamin A	Retinol  Retinyl palmitate  β-carotene 	Liver: 10800 - 23500 Fatty fish: 800 -1000 Butter: 700
Vitamin D	 Cholecalciferol  Ergocalciferol	Fatty fish: 10 - 20
Vitamin E	 RRR-α-tocopherol  RRR-γ-tocopherol  RRR-α-tocotrienol  RRR-β-tocotrienol	Sunflower oil: 55000-80000 Wheat germ oil: 205000

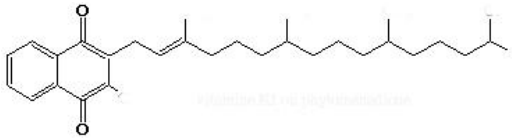
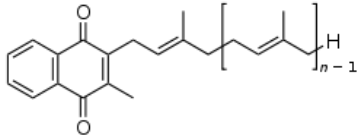
Vitamin K	 Phylloquinone  Menaquinone	Sauerkraut: 1540 Broccoli: 100 – 1000 Canola oil: 100 - 1000

Table 2: Main dietary carotenoids

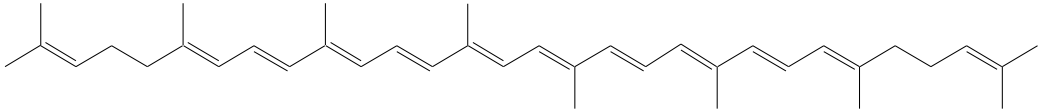
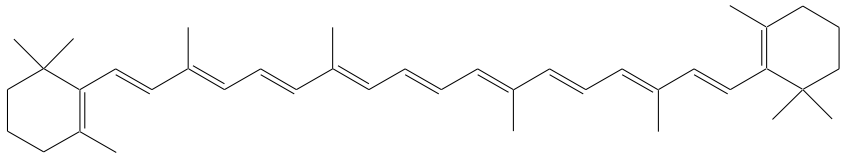
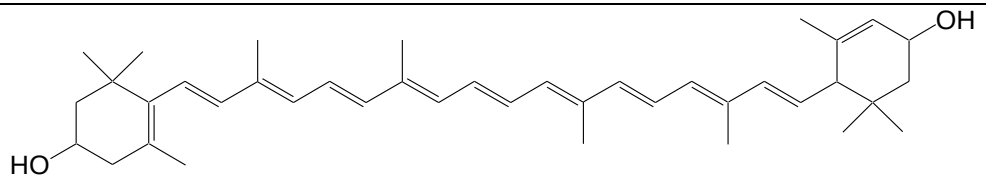
Main carotenoids	Structure of the molecules	Main food sources (µg/100 g) [205, 206]
Lycopene		Tomato sauce: 15 920 Tomatoes: 3030 Watermelon: 4870
β-carotene		Raw carrot: 8840 Canned carrot: 5780 Cooked spinach: 5240
Lutein		Cooked spinach: 7040 Lettuce: 2640 Canned green beans: 660

Table 3: Proteins identified as involved in fat-soluble vitamin and carotenoid transport in the enterocyte

	Apical uptake	Apical efflux	Cytosolic transport	Basolateral efflux
Vitamin A (retinol)			CRBPII	ABCA1 (?)
Vitamin D (cholecalciferol)	SR-BI CD36 NPC1L1	SR-BI		
Vitamin E (tocopherol)	SR-BI NPC1L1	SR-BI		ABCA1
Vitamin K (phylloquinone)				
β-carotene	SR-BI CD36			
Lutein	SR-BI	ABCG5 (?)		
Lycopene	SR-BI			

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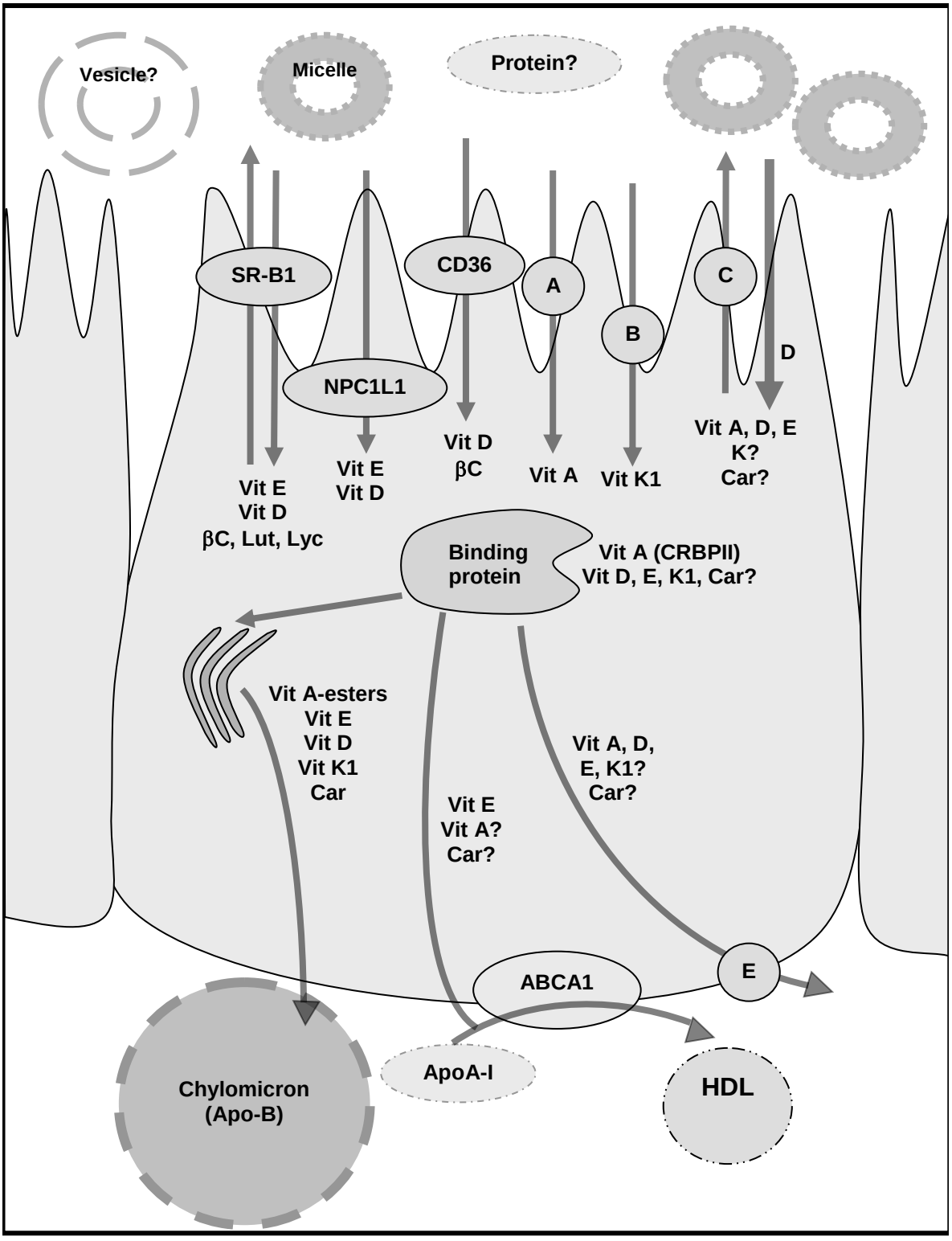

<u>Figure 1</u>: Proteins involved in uptake, transport and secretion pathways of vitamin A, D, E, and K and of carotenoids across the enterocyte.

Vit = vitamin ; βC = β-carotene ; Lut = lutein ; Lyc = Lycopene ; Car = carotenoids ; A = Retinol putative specific transporter; B = Phylloquinone putative specific transporter; C = Unidentified apical efflux transporter ; D =

Passive diffusion ; E = Unidentified basolateral efflux transporter ; ? = Putative pathway. Vitamins D and E, as well as carotenoids, are captured from mixed micelles by apical membrane transporters: SR-BI (Scavenger Receptor class B type I), CD36 (Cluster Determinant 36) and NPC1L1 (Niemann-Pick C1-Like 1). Apical membrane proteins involved in apical uptake of retinol and phylloquinone have not been identified yet. A fraction of the vitamins and carotenoids might then be effluxed back to the intestinal lumen *via* apical membrane transporters (SR-BI and possibly other transporters). Another fraction is transported to the site where they are incorporated into chylomicrons. It is hypothesized that proteins are involved in intracellular transport of fat-soluble vitamins and carotenoids, although only CRBPII (cellular retinol binding protein II) has clearly been identified. Non-metabolized vitamins and carotenoids are secreted in the lymph into chylomicrons, while a part of the more polar vitamins and carotenoid metabolites may be secreted via the portal route. It has been shown that vitamin E can also be secreted at the basolateral side via ABCA1 (apoAI pathway).