Bioavailability of Fat-Soluble Vitamins and Phytochemicals in Humans: Effects of Genetic Variation

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ABSTRACT

Recent data have shown that the interindividual variability in the bioavailability of vitamin A (beta-carotene), D, E, carotenoids (lutein and lycopene), as well as that of phytosterols is modulated by single nucleotide polymorphisms (SNPs). The identified SNPs are in or near genes involved in intestinal uptake/efflux of these compounds, as well as in genes involved in their metabolism and transport. The phenotypic effect of each SNP is usually low but combinations of SNPs can explain a significant part of the variability. Nevertheless, existing results should be considered as preliminary since they have not been validated in other cohorts yet. Guidelines for future studies are provided in order to obtain sound associations that could be used to build consolidated genetic scores that could allow the proposition of tailored dietary allowances, *i.e.* taking into account the multi-loci genotypic signature of groups of different ethnic origin or even of individuals.

INTRODUCTION

Vitamins are micronutrients: they are essential in minute amounts (< 1 g/day) to the normal development, growth and functioning of the body. These organic compounds cannot be endogenously synthesized, or at least not in adequate amounts, and must thus be obtained from the diet. Vitamins are divided into two classes: water-soluble and fat-soluble vitamins. The latter class contains four groups of compounds:

- vitamin A (preformed vitamin A, *i.e.* retinol and its esters, and provitamin A carotenoids, *i.e.* mainly β-carotene, α-carotene and β-cryptoxanthin)
- vitamin D (cholecalciferol and ergocalciferol)
- vitamin E (tocopherols and tocotrienols)
- vitamin K (phylloquinone and menaquinones).

Phytochemicals are naturally-occurring plant chemicals. Thousands are commonly found in the human diet, usually in minute amounts (< 1 g/day). However, they are not considered micronutrients since their essentiality has not been demonstrated. Nevertheless, some can exert biological actions and some are assumed to have beneficial effects on human health. Among phytochemicals, terpenoids, also known as isoprenoids, constitute a large and diverse class of fat-soluble organic compounds derived form 5-carbon isoprene units. They can be divided into 6 groups: terpenes, diterpenes, triterpenes, phytosterols (PS), saponines and carotenoids.

Among these groups, carotenoids and PS have received much attention and there is now a wealth of studies linking the intake, or the blood concentration, of these compounds with parameters of human health or specific diseases (*see* (111; 114) for review).

Fat-soluble vitamins and terpenoids are lipids and as such, their fate in the gastro-intestinal tract during digestion and their absorption by enterocytes share some common mechanisms (2; 72). The discovery of the involvement of proteins in the uptake and absorption of these compounds by the human body (115; 134) has led to bring forward new hypotheses to explain the relatively wide-ranging interindividual variability in their bioavailability and health effects. Indeed, the numerous proteins involved in their bioavailability has led to suggest that genetic variations in the genes encoding for these proteins could modulate the expression/activity of these proteins and could in turn affect the bioavailability of these compounds. The present review therefore aims to present what is currently known about the effects of genetic variations on the bioavailability of fat-soluble vitamins and phytochemicals (FSV&P) in humans, how future studies could be carried out, as well as the potential applications of this fast-moving field.

We will focus on the compounds for which there is sufficient data, *i.e.* vitamin A (β-carotene, βC), D and E, the carotenoids lycopene and lutein as well as PS.

1. FAT-SOLUBLE VITAMIN AND PHYTOCHEMICAL PRESENTATION

1.1. VITAMIN E

Vitamin E (VE) is the generic term that refers to compounds exhibiting qualitatively the biological activity of α-tocopherol. These include 8 naturally-occurring molecules: 4 tocopherols (α, β, γ and δ) and 4 tocotrienols (α, β, γ and δ) (**Figure 1**). Naturally-occurring and chemically-synthetized tocopherols are not identical: synthetic tocopherol is usually a racemic mixture of 8 stereoisomers and is named all-*rac*-tocopherol whereas natural tocopherols exists only in the *RRR* configuration. Additionally, synthetic tocopherol is often provided as an ester to protect the phenol group against oxidation and thus increase its shelf-life (129).

α- and γ-tocopherol are the most abundant VE forms in Western diets and are found at the highest concentrations in human blood and tissues (147). VE is found at relatively high concentrations in vegetable oils and nuts but it is also present in other food matrices. In the US, γ-tocopherol represents ≈70% of VE intake, due to the high consumption of food sources rich in γ-tocopherol in the typical diet (*e.g.* soybean oil, corn oil) (74). The current US recommended dietary allowance (RDA) for healthy adults is 15 mg/day but it is estimated that >90% of men and >96% women in the US do not consume the estimated average requirements (EAR) (128). Recent data point at similar inadequacies in several European countries (130). A recent systematic review of global α-tocopherol status has pointed at a relatively high prevalence of VE deficiency with 13% of the subjects exhibiting serum α-tocopherol concentration <12 µmol/l, which has been proposed as a criterion for VE deficiency (71), and only 21% of the subjects reaching serum α-tocopherol concentrations >30 µmol/l (110), which has been proposed as a criterion for VE adequacy (94).

VE is quantitatively the main lipid-soluble antioxidant in mammalian blood and tissues (30). It acts as a chain-breaking antioxidant, especially against peroxyl radicals, and is thus essential in maintaining the integrity of long-chain polyunsaturated fatty acids found in cell membranes (30). Recently, it has been shown to exert also non-antioxidant activities (149): modulation of gene expression (81; 148), inhibition of cell proliferation (75), regulation of bone mass (55)… Since oxidative stress has been implicated in the etiology of several diseases, *e.g.*

cardiovascular diseases and cancers, numerous epidemiological studies have investigated the association between VE dietary intake or status and the incidence of these diseases and reported negative associations (100; 109; 113). However, most randomized controlled trials have failed to show a benefit of VE supplementation on the incidence of these diseases (32; 127). Several explanations have been put forward, including an absence of effect of VE supplementation on these diseases (61), a negative effect of α-tocopherol supplementation on the bioavailability of other VE vitamers (39) or the absence of population stratification by VE status or oxidative stress (127). Recently, it has also been suggested that the high interindividual variability of α-tocopherol bioavailability may have interfered with the effects of VE supplementation (28) and that benefit from VE supplementation depends on a subject's genotype (16; 96; 150).

1.2. VITAMIN D

Vitamin D (VD) is the generic term that refers to compounds exhibiting antirachitic activity. The two main VD vitamers are cholecalciferol (vitamin D_3) and ergocalciferol (vitamin D_2) (**Figure 1**). Vitamin D_3 is synthesized by UVB light exposure of 7-dehydrocholesterol in the skin of animals, whereas vitamin D_2 is synthesized by UVB light exposure of ergosterol in fungi. In humans, VD photosynthesis varies widely depending on many factors, *e.g.* duration of sun exposure, time of day, latitude, season, atmosphere composition, clothing, sunscreen use and skin pigmentation, but it is acknowledged that a majority of individuals require some dietary VD, either from VD rich foods or from supplements, to reach an adequate VD status. Vitamin D_3 is the main dietary source of VD and is obtained in significant amounts from food items such as milk and dairy products, fatty fish, meat or eggs (66). The current US RDA for healthy adults is 15 µg/day (assuming minimal sun exposure) and recent data point at dietary intakes below recommended levels for more than 75% of the population of several Western countries (130). According to NHANES 2005–2006, 37% of the U.S. population uses a dietary supplement containing VD (10).

VD is a pro-hormone which needs two hydroxylations to become active. In the liver, it is converted by cytochrome P450, family 2, subfamily R, member 1 (CYP2R1) to 25-hydroxycholecalciferol ((25(OH)D), which is the main circulating VD form and whose concentration is measured to assess VD status. Following its transport via the bloodstream bound to vitamin D binding protein (DBP), it is converted in the kidney by cytochrome P450, family 27, subfamily B, member 1 (CYP27B1) to 1,25-dihydroxycholecalciferol ($1,25(OH)_2D$) (37). This metabolite exerts both non-genomic and genomic effects, via the vitamin D receptor (VDR), with far more than 1,000 target genes identified (33). VD is essential for bone health

and for regulating blood calcium and phosphate concentrations, but it is also involved in other biological functions such as immunity, cell proliferation, or apoptosis. In a recent Mendelian randomization analysis, VD status has been negatively associated with all-cause and cancer mortality (3).

1.3. CAROTENOIDS

Carotenoids are lipid phytochemicals belonging to tetraterpenes. They are split into 2 classes: carotenes that are non-oxygenated carotenoids (*e.g.* α- and β-carotene, lycopene), and xanthophylls that are oxygenated carotenoids (*e.g.* lutein, zeaxanthin, β-cryptoxanthin). Carotenoids are natural pigments, with colours ranging from red to yellow, produced in all photosynthetic organisms (bacteria, algae, and plants), as well as in some non-photosynthetic bacteria and fungi. They are involved in many biological functions such as photosynthesis, photoprotection, photomorphogenesis, development, hormone synthesis (105) and they also participate in the visual attraction of these organisms.

More than 700 different carotenoids have been identified but only about 40 are present in significant amounts in the human diet. Total and individual carotenoid intake is highly variable both within and between populations and largely reflects fruit and vegetable consumption. Data from 37,846 Europeans reported a mean total carotenoid intake of 10 ± 4 mg/day (122). The 6 most concentrated carotenoids found in the human blood are those found at the highest quantities in the human diet, *i.e.* ß-carotene, lycopene and lutein (**Figure 1**), as well as ß-cryptoxanthin, α-carotene and zeaxanthin (80). Although there are currently no RDA for carotenoids, a higher consumption thereof is usually recommended.

Following enzymatic cleavage by beta-carotene oxygenase 1 (BCO1), α- and ß-carotene, and ß-cryptoxanthin can be converted to retinol in the human body and are hence referred as provitamin A (proVA) carotenoids. The RDA for VA in France is 600 and 800 µg retinol activity equivalents (RAE) per day for women and men respectively (US: 700 and 900 µg/day). RAE have been established to take into account the variability in carotenoid bioavailability, depending on the food matrix in which they are incorporated, and the efficiency of their conversion to retinol following their enzymatic cleavage (61). Current RAE are as follows: 1 µg RAE = 1 µg retinol = 2 µg all-trans βC from supplements = 12 µg all-trans βC from food = 24 µg α-carotene or β-cryptoxanthin from food.

The contribution of proVA carotenoids as a source of VA depends on dietary habits but a recent meta-analysis has shown that they represent 35% of total VA intake (βC: 86%, α-carotene: 10%, β-cryptoxanthin: 4% thereof respectively) in developed countries (138).

Although frank VA deficiency is rare in developed countries, recent data point at dietary intakes below recommended levels in large parts of the population of several Western countries (130). Nevertheless, VA deficiency is still a public health problem in developing countries, mainly due to inadequate VA intake caused by insufficient access to VA-rich foods (*i.e.* animal products). It is estimated about one-third of children are concerned (93) but women of reproductive age are also at risk of deficiency. By compromising the immune system, it worsens the outcomes of common childhood infections (*e.g.* measles, malaria). The earliest symptom of VA deficiency is impaired night vision, leading in extreme cases to irreversible blindness. According to WHO, night blindness is estimated to affect 250,000–500,000 children each year, of which 50% die within the following year.

The xanthophylls lutein and zeaxanthin are present at high concentrations in the human macula (the yellow spot of the retina) and there is now a growing body of evidence suggesting that they exert a specific biological function in the eye. Several studies have demonstrated that they increase visual acuity and can quench 400-450 nm incident blue light, which is harmful for photoreceptors (90). Several other studies have also suggested that their consumption diminishes the incidence of cataract and age-related macular degeneration (7; 14).

Lycopene is the pigment responsible for the red colour of tomatoes and tomato products. Both its dietary intake and blood concentration have been negatively associated with prostate cancer (35; 136) and cardiovascular disease (36) risk. Since oxidative stress has been implicated in the etiology of these diseases, lycopene has been suggested to exert its protective effects through its antioxidant properties, which have been well characterized *in vitro* (78). However, lycopene also exhibits biological activities independent of its antioxidant effects: it modulates inflammation (52; 60; 89), reduces cholesterol absorption efficiency (151) and several studies suggest that its metabolic products also exert non-antioxidant biological effects (9; 59; 60; 95).

1.4. PHYTOSTEROLS

PS are plant-derived sterols with structural similarities with cholesterol, but with side chains modifications and ring saturations. PS are divided into 2 classes: sterols, also known as Δ^5-sterols, have an unsaturation at the 5 position in the sterol ring whereas stanols, also known as 5α-sterols, have an unsaturated sterol ring (**Figure 1**) (108). PS are found in fruits, vegetables, nuts and oils, with Δ^5-sterols the most abundant class.

PS intake is comprised between 200 and 400 mg/day in the typical Western diet, depending on dietary habits, which is close to the average cholesterol intake. Most ingested PS

are Δ^5-sterols with the most abundant being sitosterol. High PS concentrations are found in oil, such as corn and sunflower oil, but also in almonds, beans, corn and wheat.

Due to their structural similarity , PS and cholesterol compete for their incorporation into mixed micelles and subsequent uptake by the enterocyte. Overall, several meta-analyses have shown that PS added to food lead to a significant decrease in LDL-cholesterol (76; 111). No significant differences were observed between Δ^5-sterols and 5α-sterols.

2. FAT-SOLUBLE VITAMIN AND PHYTOCHEMICAL BIOAVAILABILITY

The bioavailability of an ingested molecule is usually defined as the relative amount of the molecule, or one of its metabolites, that reaches the systemic circulation, or its site of action. In practice, biologists can use different methods to assess it. For example, pharmacologists usually estimate the bioavailability of an orally administered drug by measuring the postprandial blood concentration of this drug, or one of its metabolite). While nutritionists also use this method, they also employ alternative methods. Indeed, they can estimate the bioavailability of a molecule by measuring the increase in its fasting blood concentration following its supplementation, either acute or chronic. They can also estimate its bioavailability by measuring the increase in its concentration in a tissue where it is assumed to exert its biological action, *e.g.* carotenoids in the skin. They can as well estimate its tissue bioavailability by quantifying a marker, whose level correlate with the tissue concentration of the studied molecule, *e.g.* macular pigment optical density, which is used as a marker of xanthophyll concentration in the macula. Techniques using stable isotopes of FSV&P allow nutritionists to measure more accurately the amount of dietary FSV&P absorbed (98; 131). It should be stressed that most approaches, with the exception of the last-mentioned, measure only the relative FSV&P bioavailability.

Different approaches can be used to assess the bioavailability of the same molecule. For example, lutein bioavailability can be estimated by measuring its postprandial chylomicron concentration (26) or plasma concentration after a lutein-rich test meal, by measuring the long term plasma lutein response to daily lutein supplementation (20), or by measuring variation in macular pigment optical density after long term lutein supplementation (63). The relative weights of the metabolic processes modulating FSV&P bioavailability differ according to the assessment method used. Thus, studies investigating the genetic variations associated with the variability in the bioavailability of a FSV&P are likely to yield different results if bioavailability assessment methods are different.

FSV&P bioavailability depends on several successive steps, from their transfer from the food/supplement in which they are ingested to the tissues where their concentration is measured. In order to put into perspective some of the genetic associations discussed in this review, we will present a short description of these steps, focussing on the proteins that can significantly impact FSV&P bioavailability. Readers can find more detailed information in the following reviews (17; 19; 21; 22). The first key step is the extraction of these compounds from food/supplements and their transfer to mixed micelles, *i.e.* their micellization (**Figure 2**). The efficiency of this step is only partial and the fraction of FSV&P thus made potentially available for absorption is referred as the bioaccessible fraction. The second step is the uptake of these compounds, or their metabolites produced during digestion, by the enterocyte (**Figure 3**). A third step is their resecretion in the intestinal lumen. This process can be quantitatively important for some FSV&P, *e.g.* PS which are excreted from the enterocyte by the ABCG5/G8 (ATP binding cassette subfamily G member 5 and 8) heterodimer. The compounds that remain in the intestinal cell are then transported across the enterocyte to be secreted at the basolateral side, mostly in chylomicrons, but also in intestinal HDL. Parent FSV&P and their metabolites are mostly secreted in the lymph, but some less apolar metabolites are also secreted in the portal vein. Following their uptake, metabolism and secretion in the bloodstream by the liver, the blood metabolism of the different FSV&P and their metabolites can be very different as some are transported in lipoproteins, *i.e.* VE, carotenoids and PS, while others are transported by specific proteins, *e.g.* the complex RBP4/TTR (retinol binding protein 4/ transthyretin) for retinol and DBP for 25(OH)D. Several very different factors can affect this complex processes and furthermore, the fact that numerous proteins are implicated in the bioavailability of FSV&P (**Figure 3**) likely explains the high interindividual variability that was observed for all studied FSV&P. Indeed, after giving standardized test meals rich in FSV&P to a group of healthy subjects, the CV of the blood response in FSV&P, which was used as a marker of their bioavailability, ranged from 47% for VD given as a supplement to 137% for lutein given in tomato puree (24-26; 28; 44).

De Pee and West were the first to propose a systematic review of the factors assumed to affect the bioavailability of a FSV&P, namely carotenoids (42). These factors have also been suggested to affect, although differently, the bioavailability of other FSV&P, *i.e.* fat-soluble vitamins and PS (18; 43), and are summarized in the acronym SLAMENGHI (139). Each letter stands for one factor, e.g. S for molecular species, L for molecular linkage… It is important to state that among all these factors, three are linked with the effect of the individual: N, which stands for the nutrient status of the host, which explains the effect of vitamin A status on βC

bioavailability and bioconversion for example (85), and G and H. H stands for host-related effects, *e.g.* gender, age, diseases, and G stands for effects of genetic factors. It is assumed that this last factor is particularly important with regard to the interindividual variability of FSV&P bioavailability (17). Its effect on this phenotype was first demonstrated for PS. Indeed, Berge *et al.* showed that mutations in *ABCG5* and *ABCG8* led to body accumulation of PS because these membrane transporters are responsible for sterol excretion in enterocytes and hepatocytes (12). Further studies have shown that single nucleotide polymorphisms (SNPs) can also have significant effects on blood concentration of PS (62), showing for the first time that genetic polymorphisms can significantly affect the bioavailability of a FSV&P. Nevertheless, the effect of genetic variations on the bioavailability of other FSV&P was addressed only recently. This is probably because the absorption of these compounds was thought to occur through simple passive diffusion. However, several studies have now clearly demonstrated that intestinal cell proteins are involved in the absorption of these compounds (115) (**Figure 3**). This discovery, together with affordable genotyping technologies, has led to the first studies investigating the association of genetic variations with the bioavailability of FSV&P other than PS (24-27; 44; 91; 135).

3. GENETIC VARIATIONS ASSOCIATED WITH FAT SOLUBLE VITAMIN AND PHYTOCHEMICAL BIOAVAILABILITY

3.1. VITAMIN E

Three clinical trials identified genetic variations associated with the variability in VE bioavailability but two fairly different methods were used to evaluate VE bioavailability, a source of potential result heterogeneity, as highlighted in the previous chapter. Major *et al.* (88) and Athinarayanan *et al.* (8) both measured VE blood concentration following VE supplementation. In the first study, 2112 male smokers received 50 mg/day all-*rac*-α-tocopheryl acetate (68 IU) for 3 years. Following a genome-wide association study (GWAS) approach (549,989 SNPs analyzed), 3 independent SNPs were significantly associated with the variability in VE response. The association with the SNP (rs964184), which is located between the *BUD13 homolog* (*BUD13*) and *zinc finger protein 259* (*ZNF259*), is likely due to a variation in *APOA5*, because this SNP is located close to the *APOA1/C3/A4/A5* cluster as suggested by Ferrucci *et al.* (53). Indeed, *ZNF259* and *BUD13* encode for proteins with no known role in VE metabolism. An association with a SNP (rs2108622) located in *CYP4F2* (*cytochrome P450, family 4, subfamily F, polypeptide* 2) was also significant. *CYP4F2* encodes for the rate-limiting

enzyme responsible for hepatic VE ω-oxidation. *NKAIN3* (*sodium/potassium transporting ATPase interacting 3*) encodes for a plasma membrane-bound Na/K-ATPase, whose activity is critical to the maintenance of cell viability and sensitive to radicals produced during lipid peroxidation. The inhibitory effect of VE on lipid peroxidation could thus explain the association with a SNP (rs7834588) in this gene. In the second study, 247 adults with nonalcoholic steatohepatitis and 173 children with nonalcoholic fatty liver disease received VE (*RRR*-α-tocopherol, 800 IU) for 96 weeks. Of the 2 nonsynonymous SNPs investigated, a SNP in *CYP4F2* (rs2108622) was significantly associated with a higher VE response following supplementation for 48 weeks (but not after 96 weeks). In a recent clinical trial from our group (28), the association of SNPs in genes involved in VE absorption and metabolism (3769 SNPs in 59 candidate genes) with the variability in VE bioavailability, measured as the postprandial chylomicron VE concentration, was assessed in a group of 38 healthy adult men who received a meal containing 100 IU *RRR*-α-tocopheryl acetate. A combination of 28 SNPs in 11 candidate genes was shown to be significantly associated with this variability. Of those 11 genes, 7 were previously associated with the interindividual variability in the postprandial chylomicron triglyceride response in the same group of subjects (46), while the remaining 4 encode for proteins which are more specifically involved in VE metabolism and transport. *ABCG1* (*ATP binding cassette subfamily G member 1*) could be involved in the basolateral efflux of VE from enterocytes to HDL (102; 107). Pancreatic lipase is a critical enzyme for the intestinal hydrolysis of dietary triglycerides and thus participates in the transfer of VE from oil droplets of dietary lipid emulsions to mixed micelles, a necessary step for VE absorption. The apical sodium bile acid transporter (ASBT), encoded by *SLC10A2* (*solute carrier family 10, member 2*), is responsible for the re-uptake of luminal bile acids in the ileum (41). Since bile acids are essential for normal VE absorption (121), SNPs in this gene could affect VE bioavailability. *SREBF2 (sterol regulatory element binding transcription factor 2)* encodes a transcription factor controlling the expression of many genes required for cholesterol synthesis (69) but also that of *SLC10A2* and *NPC1L1* (*NPC1 like intracellular cholesterol transporter 1*) (4), the main protein involved in the apical uptake of cholesterol (5) but also that of α-tocopherol (99) into enterocytes.

3.2. VITAMIN D

Several clinical trials have identified SNPs associated with the variability in VD bioavailability but all but one evaluated VD bioavailability as the circulating 25(OH)D concentration following VD supplementation (generally for several weeks). In summary, SNPs

in *CYP2R1* (11; 47; 106; 117; 137; 142), *cytochrome P450, family 24, subfamily A, member 1* (*CYP24A1*) (11), *VDR* (11; 117; 142), *vitamin D binding protein* (*GC*) (47; 106; 142), *calcium sensing receptor* (*CASR*) (117), and *retinoid X receptor alpha* (*RXRA*) (144) have been associated with the 25(OH)D response following VD supplementation. *CYP2R1* encodes for a hepatic hydroxylase responsible for the conversion of VD to 25(OH)D (146). *CYP24A1* encodes for an enzyme that catalyses the rate-limiting step in 25(OH)D and $1,25(OH)_2D$ catabolism in the kidney (37). *VDR* encodes for the nuclear receptor mediating the biological effects of $1,25(OH)_2D$ (37). *GC* encodes for VDR which is responsible for the blood transport of all VD metabolites (37). *CASR* encodes for a plasma membrane G protein-coupled receptor that senses small changes in circulating calcium concentration. Although VD involvement in calcium homeostasis is well-described and VD response elements have been identified in this gene (31), there is yet no explanation why SNPs in this gene could affect VD bioavailability. *RXRA* encodes for one of the nuclear receptors that mediate the biological effects of retinoic acid but no hypothesis has been put forward to explain the association of SNPs in this gene with VD bioavailability. Finally, Zhou *et al.* have shown that the methylation levels of *CYP2R1* and *CYP24A1* were associated with the response in serum 25(OH)D concentration following a 12-month long VD supplementation (145). Only one study has investigated the association between SNPs and the variability in the postprandial chylomicron VD concentration, which constitutes a more precise evaluation of VD bioavailability (44). In this study from our group, 39 healthy adult men received a meal containing 5 mg vitamin D_3 as a supplement and the association of SNPs in genes involved in VD absorption and metabolism (3791 SNPs in 61 candidate genes) with the variability in the VD response was assessed. A combination of 17 SNPs in 13 genes was shown to be significantly associated with this variability. Of those 13 genes, 5 (*ABCA1*, *APOB*, *Bet1 golgi vesicular membrane trafficking protein (BET1)*, *lipoprotein lipase (LPL)* and *N-acetyltransferase 2 (NAT2)*) were previously associated with the interindividual variability in the postprandial chylomicron triglyceride response in the same group of subjects (46). Nonetheless, 8 genes that encode for proteins that are apparently more specifically involved in VD metabolism and transport were also associated with this variability (*ATP-binding cassette, subfamily B, member 1 (ABCB1)*, *7-dehydrocholesterol reductase (DHCR7)*, *intestine specific homeobox (ISX)*, *microtubule-associated protein RP/EB family member 2 (MAPRE2)*, *pancreatic lipase (PNLIP)*, *SLC10A2*, *GC*, *scavenger receptor class B member 1 (SCARB1)*). Detailed explanations and hypothesis for these associations are discussed in the original paper (44) but it is interesting to note that SNPs were found in *ISX* and *SCARB1*: *ISX* has been shown to regulate *SCARB1* expression (140) and *SCARB1* encodes for

an apical membrane protein, SR-BI, which has been shown to participate in VD uptake by enterocytes (116). Although ASBT, encoded by *SLC10A2*, had been proposed to facilitate VD apical uptake by enterocytes, we were not able to confirm this hypothesis in a recent *in vitro* study and hence a more indirect effect, through its role in bile acid transport, is to be considered (45).

3.3. CAROTENOIDS

3.3.1. BETA-CAROTENE

Four clinical trials were dedicated to the identification of genetic variations associated with the interindividual variability in βC bioavailability, which was first described twenty years ago (29). In the first study, Leung *et al.* measured the postprandial triglyceride-rich lipoprotein βC and retinyl palmitate concentrations in 28 healthy adult women (non-smokers) who received a meal supplemented with 120 mg βC (83). They observed that 2 common nonsynonymous SNPs in *BCO1* (rs12934922 and rs7501331) were associated with a decrease in βC conversion capacity, as assessed by the retinyl palmitate:βC ratio in the triglyceride-rich lipoprotein fraction. These SNPs were also associated with an increase in the fasting blood βC concentration. This study hence provided some insights into the genetics of the poor βC converter phenotype. In another study on the same volunteers, Lietz *et al.* observed a significant association between 3 SNPs upstream from *BCO1* (rs6420424, rs11645428, and rs6564851, out of 6 SNPs tested) and βC conversion capacity (84). Interestingly, as highlighted by the authors, these SNPs exhibit highly different genotype frequencies in 11 HapMap populations, possibly pointing at selection pressure related to VA status. Wang *et al.* measured blood βC concentration in 23 healthy adult subjects (non-smokers) following consumption for 3 weeks of a watermelon juice, providing 2.5 mg βC per day (135). The 2 nonsynonymous SNPs previously investigated by Leung *et al.*, rs12934922 and rs7501331, were found to be associated with the blood βC response (measured as strong (n=17) *vs* weak (n=6), following a cluster analysis). It should be noted that the supplement used in this study provided a βC dose closer to normal nutritional intakes (as compared to the pharmacological dose used in the previous studies). The association of SNPs in other genes than *BCO1* with βC bioavailability was investigated in only one study (24). In this clinical trial from our group, 33 healthy adult men received a meal containing 100 g tomato puree, providing 0.4 mg βC. The association between the variability in the postprandial chylomicron βC concentration with genes involved in βC absorption and metabolism (2172 SNPs in 54 candidate genes) was assessed. A combination of 25 SNPs in 12 genes was shown to be associated with the variability in the βC response. Four

of these genes (*ABCA1*, *APOB*, *hepatic lipase* (*HL*), *transcription factor 7-like 2* (*TCF7L2*)) were previously associated with the interindividual variability in the postprandial chylomicron triglyceride response in the same group of subjects (46), while 8 were more specifically associated with the βC response. Detailed explanations and hypothesis for these associations are discussed in the original paper (24) but 2 genes deserve closer attention. Three SNPs were found in *ISX*, which encodes for a transcription factor involved in βC intestinal absorption and conversion, by regulating BCO1-mediated βC cleavage and SR-BI-mediated βC enterocyte apical uptake (140). Another study reported that a SNP in the ISX binding site in the *BCO1* promoter (rs6564851) was associated with decreased conversion rates of βC by 50% and increased fasting blood concentrations of βC (85). This data hence confirms the key role played by ISX in βC bioavailability (85). Additionally, a SNP in *BCO1* was found to be associated with the βC response in these 33 subjects. The second association of interest was found in *ELVOL2* (*ELOVL fatty acid elongase 2*). This gene encodes for an elongase responsible for the elongation of eicosapentaenoic acid to docosapentaenoic acid and subsequently that of docosapentaenoic acid to docosahexaenoic acid. Although βC is not considered to be a substrate for this enzyme, this association is possibly due to the inhibitory effect of eicosapentaenoic acid on carotenoid absorption, as shown for β-carotene (92).

3.3.2 LYCOPENE

Only 2 clinical trials were dedicated to the identification of genetic variations associated with the variability in lycopene bioavailability. Wang *et al.* measured blood lycopene concentration in 23 healthy adult subjects (non-smokers) following consumption for 3 weeks of a watermelon juice, providing 20 mg lycopene per day (135). The association of this response with 2 nonsynonymous SNPs in *BCO1* was tested but according to the authors, neither had a significant effect. It should however be noted that the sample size of this study was rather small and that lycopene responsiveness was only measured as strong (n=17) *vs* weak (n=6), following a cluster analysis. In the second clinical trial (25), carried out by our group, the association of SNPs in genes involved in lycopene absorption and metabolism (1885 SNPs in 49 candidate genes) with the variability in the postprandial chylomicron lycopene concentration was assessed in a group of 33 healthy adult men who received tomato puree, providing 9.7 mg all-trans lycopene. A combination of 28 SNPs in 16 genes was shown to be associated with the variability in the lycopene response. Seven of these genes (*ABCA1*, *LPL*, *insulin induced gene 2* (*INSIG2*), *solute carrier family 27 member 6* (*SLC27A6*), *CD36 molecule* (*CD36*), and *APOB*) were previously associated with the interindividual variability in the postprandial chylomicron

triglyceride response in the same group of subjects (39). Four genes were more specifically associated with lycopene bioavailability and exhibited significant *p*-values following multiple comparison correction. *ABCB1* encodes for the P-glycoprotein, an ATP-dependent drug efflux pump for xenobiotics with broad substrate specificity, highly expressed in enterocytes. This association suggests that it may modulate lycopene absorption efficiency by excreting a fraction of lycopene taken up back to the intestinal lumen. The potential interaction of SNPs in *ELOVL2* with carotenoid bioavailability has been addressed in the previous part about βC. *Microsomal triglyceride transfer protein* (*MTTP*) encodes for a protein that plays a critical role in the assembly of chylomicrons. SOD2 (encoded by *superoxide dismutase 2* (*SOD2*)) binds to the superoxide by-products of oxidative phosphorylation and converts them to hydrogen peroxide and diatomic oxygen. SNPs in this gene have been associated with prostate cancer risk (77) and lycopene has been suggested to modify the deleterious effect of these SNPs (126), possibly by quenching superoxide by-products, thereby leading to lycopene degradation.

3.3.3 LUTEIN

Since lutein status, together with that of another xanthophyll, zeaxanthin, has been associated with protection from age-related macular degeneration (56), studies investigating the association of genetic variations with the variability in lutein bioavailability have measured lutein bioavailability in both blood and *macula lutea*, *i.e.* lutein site of action. In a first study, Yonova-Doing *et al.* measured macular pigment optical density in 310 Caucasian female twins aged 20-50 before and after receiving 54 mg lutein and 7.2 mg zeaxanthin daily for 6 months (143). The association between changes in macular pigment optical density and candidate SNPs (12 SNPs in 8 genes), which were previously associated with circulating and macular levels of carotenoids, was investigated and 4 SNPs were found to be significantly associated with the macular pigment optical density response to supplementation. The SNP (rs1929841) found in *ABCA1* could be due to the role of this membrane protein in lipid influx in the retinal pigment epithelium (48) and/or to enterocyte basolateral secretion of HDL-lutein (104). A SNP (rs11057841) in *SCARB1* was associated with the variability in macular pigment optical density response, as well as with baseline serum lutein concentration. *FADS1* (*fatty acid desaturase 1*) encodes for a desaturase involved in the conversion of α-linolenic acid to eicosapentaenoic acid. The association of a SNP (rs174534) in this gene with macular pigment optical density variability could be due to the inhibitory effect of eicosapentaenoic acid on carotenoid absorption, as mentioned in the previous part. *SCARB1* encodes for SR-BI, a protein expressed ubiquitously participating in the apical uptake of lutein into enterocytes (49) but also in HDL-

lutein uptake into retinal pigment epithelium, although it has recently been shown that lutein is preferentially taken up in this tissue from LDL via a LDL receptor mediated mechanism (125). Finally, a SNP (rs4926339) in *RPE65* (*retinal pigment epithelium 65*) was also associated with the macular pigment optical density response following lutein supplementation. This gene encodes a protein highly expressed in the retinal pigment epithelium, working as a retinol isomerase of the visual cycle, which is involved in visual pigment regeneration. Interestingly, this protein was shown very recently to also act as an isomerase responsible for the production of *meso*-zeaxanthin, another xanthophyll found in the *macula lutea*, from lutein (120). In another clinical trial from our group (26), the association of SNPs in genes involved in lutein absorption and metabolism (1785 SNPs in 51 candidate genes) with the variability in the postprandial chylomicron lutein concentration was assessed in a group of 39 healthy adult men who received a meal supplemented with 15 mg free lutein. A combination of 29 SNPs in 15 genes was shown to be associated with the variability in the lutein response. Seven of these genes (*ABCA1*, *APOA1*, *APOB*, *cordon-bleu WH2 repeat protein like 1* (*COBLL1*), *HL*, *INSIG2*, *insulin receptor substrate 1* (*IRS1*), *LPL*, and *melanocortin 4 receptor* (*MC4R*)) were previously associated with the interindividual variability in the postprandial chylomicron triglyceride response in the same group of subjects (39). Four genes were more specifically associated with lutein bioavailability and exhibited significant *p*-values following multiple comparison correction: *ATP-binding cassette, subfamily G, member 2* (*ABCG2*), *ELOVL2*, *ISX* and *MTTP*. The potential implication of the last 3 genes with carotenoid metabolism has been discussed above. *ABCG2* encodes for a breast cancer resistance protein (BCRP), which is a multidrug transporter (101). This membrane protein has been suggested to be involved in lutein intestinal absorption but no clear mechanism has been demonstrated thus far.

3.4. PHYTOSTEROLS

As mentioned above, studies searching for mutations causing sitosterolemia, a rare autosomal recessive disorder characterized by elevated blood PS concentration and pathological accumulation of PS in several tissues, were the first studies dedicated to the identification of genetic variations associated with FSV&P bioavailability. They allowed researchers to link this disease to mutations in two genes, *ABCG5* and *ABCG8*, which encode for 2 ATP-binding cassettes, sterolin-1 and -2, working as hemitransporters (12; 82). These proteins are in charge of the excretion of PS, from enterocytes to the intestinal lumen and from hepatocytes to bile, a normally very efficient process. This discovery has led to hypothesize that genetic polymorphisms in these two genes could modulate PS bioavailability, albeit

probably to a lesser extent, *i.e.* asymptomatically. This was confirmed by several association studies, including one GWAS, which showed that SNPs, located mainly in *ABCG8* for an unknown reason, are associated with fasting blood PS concentration (13; 62; 68; 70; 73; 97; 118; 124). Nevertheless, knowing the dual role of these transporters in PS metabolism, *i.e.* in enterocytes and hepatocytes, these associations do not provide definite evidence that SNPs in these transporters affect PS intestinal absorption. Indeed, it is possible that the associations observed were mostly due to an effect on PS excretion by hepatocytes and not to an effect on PS excretion by enterocytes. Finally, Plat *et al.* showed that SNPs in *ABCG8* could modulate changes in PS blood concentration after consumption of PS, providing additional evidence that SNPs in *ABCG8* likely affect PS bioavailability (112).

PS bioavailability is not only under the control of ABCG5/G8: several other proteins/genes are involved directly or indirectly in the transport of PS across the enterocyte and in the blood and other tissues. For example, at the enterocyte level, PS have been shown to be taken up, at least partly, by a process mediated by NPC1L1 (40). It is thus likely that genetic variations in other genes than *ABCG5*/*G8* can modulate PS bioavailability. Yet, candidate gene association studies have focused only on *NPC1L1* and *APOE*. They have first confirmed what was expected, *i.e.* that SNPs in *NPC1L1* modulate PS bioavailability (38; 86; 87). Concerning *APOE*, it was observed in two studies that subjects with the *apoE* 3/4 or 4/4 alleles absorbed PS more effectively than subjects with the *apoE* 3/3 allele (86; 123). To the best of our knowledge, no biological mechanism has been suggested to explain how apoE can modulate PS absorption. Furthermore, although it was observed that intestinal cholesterol absorption, and thus likely PS absorption, was related to *apoE* genotype (79), another study did not confirm this finding (133). Thus, the elucidation of the role of genetic variations in *APOE* on PS bioavailability requires further investigations.

To be comprehensive, it should be mentioned that a SNP in a locus that was not expected to be involved in PS metabolism, the blood group *ABO* locus (*ABO, alpha 1-3-N-acetylgalactosaminyltransferase and alpha 1-3-galactosyltransferase*), was independently associated with fasting serum PS concentration in the only GWAS study dedicated to identify genetic variations associated with this phenotype (124). The authors have speculated that this association is perhaps related to the N-linked glycosylation of ABCG5 and/or ABCG8 proteins. Indeed, it has been hypothesized that these transporters can partly lose their activity when they are not glycosylated, as is the case with the blood *O* allele, leading to loss of the glycosyl transferase activity. In any case, it should be demonstrated that SNPs in the *ABO* locus can modulate not only fasting blood concentration of PS but also PS bioavailability.

4. FUTURE STUDIES TO PERFORM

As highlighted in the previous chapter, the identification of genetic variations that can affect FSV&P bioavailability has only been tackled recently and consequently only few studies are available. Several validations are necessary in genetic association studies before drawing definite conclusions so the results obtained to date should be considered preliminary. However, these studies serve as proof-of-concept studies, showing that methods and technologies are now available to identify these genetic variations. In this chapter, we will discuss the limits of the available association studies and suggest some guidelines for future studies (**Figure 4**), in order to obtain results that could translate into tailored dietary recommendations.

Association studies usually allow geneticists to conclude that an identified genetic variant modulates the phenotype of interest either directly or is in linkage disequilibrium with some nearby genetic variant that affects this phenotype. Thus, to definitely demonstrate the involvement of a genetic variant, it is necessary to perform functional studies where the effect of the different variant alleles on the studied phenotype can be evaluated, as what was done *in vitro* with *NPC1L1* variants on cholesterol absorption (50) or with *BCO1* variants on βC absorption and cleavage (83). Nevertheless, because most genetic variations identified to date have minor effects on bioavailability, *i.e.* they modulate only a few percent thereof, it is likely that it will be difficult to demonstrate significant biological effects in such studies. Thus, we believe that priority should rather be given to building genetic scores that could accurately predict this phenotype for an individual/group of the population.

The second observation is that all studies dedicated to identifying genetic variants associated with FSV&P bioavailability followed a candidate gene approach. The advantage of this approach is that it does not require a high number of subjects to limit the risk of false positive associations. However, many genes and SNPs with potential effect on FSV&P bioavailability are left out of the analysis. Thanks to the progress in high throughput genotyping, GWAS could be considered to identify genetic variations associated with variability in FSV&P bioavailability. GWAS display the advantage that they do not make any assumption on the genetic variations that can affect the studied disease/phenotype, allowing researchers to identify non-expected associations. Nevertheless, GWAS also have their drawback. Since a very large number of genetic variations is investigated, the sample size required to limit false positive associations is high (typically > 10,000 subjects). This can lead to false negative associations, *i.e.* to reject genetic variants that are marginally associated with the studied disease/phenotype.

Another drawback of the large sample size inherent to GWAS is the high cost. This is clearly a huge constraint if an accurate method to evaluate bioavailability is used, *i.e.* where the cost of phenotyping largely exceeds that of genotyping. We thus suggest either to diminish the cost of phenotyping, *e.g.* by decreasing the number of postprandial blood withdrawal. Another point that deserves attention is the fact that all studies on this topic looked at SNPs whose minor allele frequency was at least 1% in the studied populations. Yet, we hypothesize that SNPs that have a large effect on the phenotype are infrequent because they are under selective pressure due to the disadvantage they provide to the subjects who bear them. Thus, an interesting approach could be to perform an exome sequencing of key candidate genes involved in the phenotype, *e.g.* intestinal transporters of FSV&P, and to assess whether some less frequent SNPs are involved in the phenotype.

The third observation that arises from the studies published to date is that the methods used to evaluate FSV&P bioavailability were heterogeneous. As discussed in chapter 2, this factor can have huge consequences on the observed associations because different genes might be involved in the modulation of the FSV&P concentration actually measured. Thus, to compare/validate genetic associations observed in different studies, it is first necessary to check whether these studies used similar methods to evaluate FSV&P bioavailability. We therefore advise that future genetic studies use the same standardized method to evaluate bioavailability. Measuring the postprandial chylomicron response using 3-4 postprandial times could be a good compromise between accuracy and cost/practicality.

The fourth observation is that no validation cohort was used. Yet, genetic associations require to be observed in several independent groups of subjects. This lack of validation cohorts is likely due to the fact that measuring FSV&P bioavailability is complicated and costly compared to measuring *e.g.* fasting blood FSV&P concentration. Nevertheless future studies would need to meet the requirements of other studies, including replication cohorts (67).

The fifth observation is that most published studies were performed in male subjects from Caucasian origin. Because some genetic associations depend on gender, it is advised to perform studies both in females and males to conclude that the associations observed apply to a whole population. Furthermore, genotyping of different ethnic groups has shown that genetic variants are not linked similarly, *i.e.* haplotype blocks are not the same in different populations (1; 6; 119). Thus, it is possible that a polymorphism will be in linkage disequilibrium with a nearby allele that modulates FSV&P bioavailability in one population but not in another, leading to variable results of association studies. Moreover, SNPs involved in the variability of the bioavailability of a FSV&P might exhibit fairly different allele frequencies, depending on the

ethnic group investigated. Thus, studies are required in different ethnic groups to verify whether genetic variations are associated with FSV&P bioavailability in these groups or to identify genetic variations that are specific to some groups.

Although it is assumed that SNPs explain most of the genetic variability between individuals, it is not the only kind of genetic variations that happens in the human genome. Indeed, there are also microsatellites, variable number tandem repeats, copy number variants, insertion/deletion polymorphisms…(57). There is no reason to believe that some of these genetic variations do not also affect FSV&P bioavailability. On the contrary, we hypothesize that different number of copies of genes involved in the bioavailability of some FSV&P could have been selected in some populations and allowed them to adapt to the dietary availability of some FSV&P, as has been reported in the case of the alpha-amylase gene (51).

Another kind of genetic variations that could modulate FSV&P bioavailability is epigenetic modifications, which would add another level of complexity to the genetic regulation of FSV&P bioavailability. For example, SNPs that increase FSV&P bioavailability in one individual could have no effect in another individual due to epigenetic modifications, silencing these genetic variations.

5. POTENTIAL APPLICATIONS: TAILORED NUTRITIONAL RECOMMENDATIONS

Recent years have witnessed remarkable progress in genotyping and sequencing technology, leading to increased number of genetic variations investigated, increased coverage, together with a decrease in analysis time. The price of these technologies has seen a constant drop, making them affordable for individuals, allowing numerous companies now to offer direct-to-consumer genetic testing. With the increase in the number of studies aiming to identify genetic variations associated with the interindividual variability in FSV&P bioavailability, it is sensible to assume that the predictive quality of genetic scores combining these genetic variations will improve, *i.e.* these genetic scores will be able to predict more reliably the bioavailability of these FSV&P in specific segments of the population, or even at an individual's level. Altogether, these advances open the possibility to propose tailored nutritional recommendations so as to prevent deficiencies or to maximize the health benefits of these FSV&P in specific segments of the population or at the individual's level. We will now discuss the modalities and objectives of these applications.

5.1 AT THE POPULATION LEVEL

By harnessing high-throughput genetic data in several populations globally, projects such as the HapMap Projects (1; 6; 119) or the 1000 Genomes Project (57) have allowed geneticists to provide us with insights into the genetic structure of the human genome, revealing the extent of genetic diversity between populations, with differences in several hundred thousand SNPs with large allele frequency. Populations differing in their allele frequency at variations in genes involved in the bioavailability of a FSV&P could in turn exhibit different bioavailabilities of such compound. Current EAR and RDA have been established to cover the needs of at least 50, respectively 97.5%, of specific segments of the population (within one country) (**Figure 5**), based on age, gender and pregnancy/lactation status. Usually, this estimation is conducted in a population sample of limited size, which can obviously not account for population stratification. However, within a country, nutritional requirements might differ between segments of the population of different ethnic origin, *e.g.* between Hispanic Americans and African Americans, which could partly be due to differences in nutrient bioavailability. Moreover, in many countries, no RDA have been established and RDA established elsewhere, where population genetic structure might be fairly different, are used. Of course, RDA can then be empirically adjusted to meet a population requirement but *a priori* knowledge of a population ability to absorb a FSV&P could help save time and effort in order to establish tailored RDA. This could be absolutely crucial in countries with too few resources to empirically establish RDA, in countries with segments of the population of different ancestry, or for phenotypes with high age of onset, *e.g.* age related macular degeneration.

A good example of the application of population-tailored nutritional recommendations can be illustrated in the fight against VA deficiency. In spite of the numerous programs applied, VA deficiency is still a health issue in many developing countries: although more than 80% of 1-5 year-old children in these countries receive VA supplements, VA deficiency prevalence has diminished by only 3% in the last 10 years (93). WHO recommends a more diversified approach, including dietary modifications (by consuming local crops with higher levels of proVA carotenoids), breastfeeding, food fortification and supplementation. However, the bioavailability and conversion to VA of these carotenoids is very low and highly variable (64). We have recently shown that the variability in the bioavailability of βC, and probably of other proVA carotenoids, was associated with a combination of SNPs (23). A theoretical calculation also allowed us to show that, since the allele frequency of the genotypes involved in this bioavailability differs between ethnic groups, the bioavailability is likely to vary between different ethnic groups. Moreover, it has been shown that proVA carotenoid conversion to VA

is modulated by SNPs in the main conversion enzyme of these proVA carotenoids, BCO1 (54; 65; 141). The genotype frequency of these SNPs also varies in different ethnic groups. We thus hypothesize that different ethnic groups likely exhibit different absorption and conversion capacities for these proVA carotenoids. This knowledge could allow policy makers to define the best nutritional strategy to fight against VA deficiency in specific ethnic groups. For example, if the targeted ethnic group contains a significant proportion of individuals with genetic polymorphisms that impair the conversion of proVA carotenoids into VA, it would then be better to either recommend the consumption of foods rich in preformed VA or to provide this population with preformed VA supplements.

5.2 AT THE INDIVIDUAL LEVEL

The effect of the amount of vitamin consumption on health parameters follows a well-known curve: at low levels deficiency signs can occur and if consumption increases, FSV&P can then exert essential metabolic functions. If intakes increase more, FSV&P can exert other beneficial biological effects, *i.e.* they can participate in the prevention of some diseases. At even higher intakes, harmful or toxic effects can appear (usually at supplementation levels). RDA, which are defined to cover the requirements of 97.5% of the healthy population, are already enforced for specific subgroups (*e.g.* infants, children, elderly, pregnant women, breastfeeding women) (**Figure 5**) but these recommendations could be improved by taking into account more criteria in the segmentation of these subgroups. For example, this curve could be moved further to the left or to the right depending on the absorption phenotype of an individual for a specific FSV&P: an individual with a high capacity to absorb a FSV&P would have a lower requirement compared to the general RDA while an individual with a poor capacity to absorb a FSV&P would have a higher requirement compared to the general RDA (**Figure 5**). Most applications that come to mind involve personalized nutritional recommendation to prevent deficiencies or to maximize the health benefits of a FSV&P but it should be stressed that some FSV&P can also exert harmful effects, usually at chronic supplementation levels. For example, βC and VE supplement use have been reported to be associated with increased mortality (15). Thus, high absorbers of these FSV&P should be recommended not to consume chronic high doses of these FSV&P. The use of genetic scores combining genetic variations associated with the interindividual variability of a FSV&P bioavailability could predict the absorption phenotype of an individual and could thus provide insightful information to define a more personalized RDA. Moreover, the knowledge for an individual of his absorption phenotype, and thus his personal RDA, could help improve his dietary habits and maintain good adherence (103). This

approach is already proposed by several companies, albeit following single genetic variation predictions usually, with direct-to-consumer genetic testing and personalized dietary/supplement recommendations. Nonetheless, although there has been tremendous progress in the identification of genetic variations associated with the variability in the response to dietary compounds, several critics still consider that the current knowledge base is too limited in order to offer practical genotype-based nutritional recommendations (58). Additionally, other points require careful consideration and evaluation in order to define best practice, *e.g.* ethical considerations (including reporting of the results), analytical quality (34), complexity of information and practical guidance, communication of the results…

6. CONCLUSIONS

In summary, there is now sufficient evidences to state that FSV&P bioavailability is partly modulated by genetic variations in several genes. Nevertheless, the bioavailability of some FSV&P, such as VA (as retinol or its esters) and vitamin K or other terpenoids, has not been well characterized yet and, while it can be hypothesized that genetic variations are also involved in the variability of the bioavailability of these compounds, genetic association studies are still lacking. Although much work remains to be done to obtain a combination of genetic variations (SNPs but also other kinds of genetic variations) that will allow us to confidently predict the FSV&P bioavailability of different segments of the population or of an individual, the potential usefulness of this area of research is exciting regarding personalized RDA for FSV&P. Nevertheless, it should be reminded that genetic variations only represent one of the factors that affect FSV&P bioavailability, albeit stable over the lifespan, since other factors, such as epigenetic modifications or the factors described by the acronym SLAMENGHI, also affect this phenotype. Thus, a "DNA chip" that can determine crucial genotypes and accurately predict FSV&P bioavailability is unlikely to become a widespread and useful screening tool until numerous well-designed studies have provided validated genetic associations.

SUMMARY POINTS

Several factors are involved in the bioavailability of fat-soluble vitamins and phytochemicals (FSV&P), including an individual's genetic characteristics.

There is a high interindividual variability in the bioavailability of these compounds in healthy subjects and it is assumed that it is mainly due to genetic variations.

Available studies suggest that the bioavailability of FSV&P is modulated by combinations of genetic variations in genes involved in their metabolism and transport.

The method used to assess the bioavailability of these compounds can affect the results obtained, *i.e.* the genetic variations associated with their bioavailability.

To date, only single nucleotide polymorphisms (SNPs) have been associated with the bioavailability of these compounds but it is likely that other types of genetic variations, *e.g.* copy number variants, are also involved.

Replication cohorts are required to support the associations observed in order to establish in the future genetic scores that can help to reliably predict FSV&P bioavailability in a segment of the population or in an individual.

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FIGURE LEGENDS

Figure 1. Fat-soluble vitamin and phytochemical structure.

Only FSV&P discussed in the review are depicted. For the sake of space, only sitosterol is represented here to illustrate phytosterol general structure. Δ^5-sterols have an unsaturation at the 5 position in the sterol ring whereas 5α-sterols have an unsaturated sterol ring. For example, sitostanol is structurally identical to sitosterol except for the unsaturated B ring. Moreover, phytosterols also differ regarding the side chain at C24.

Figure 2. Fat-soluble vitamin and phytochemical fate in the lumen of the upper gastrointestinal tract during digestion.

Transfer of FSV&P between the different vehicles assumed to transport FSV&P in the human upper gastrointestinal lumen.

Figure 3. Proteins involved in uptake, transport and secretion pathways of fat-soluble vitamins and phytochemicals across the enterocyte.

Proteins involved in uptake, transport and secretion pathways of vitamin A, D, E, carotenoids and phytosterols across the enterocyte. Vit, vitamin; bC, b-carotene; Lut, lutein; Lyc, lycopene; PS, phytosterols; Car, carotenoids. (A) Retinol putative specific transporter; (B) unidentified apical efflux transporter; (C) passive diffusion; (D) unidentified basolateral efflux transporter; ? = putative pathway. Vitamin D and E, carotenoids and phytosterols are taken up from mixed micelles by apical membrane proteins: SR-BI (scavenger receptor class B type I), NPC1L1 (Niemann–Pick C1-Like 1), and CD36 (cluster determinant 36). Apical membrane protein(s) involved in apical uptake of preformed vitamin A (retinol) has(ve) not been identified yet. Most phytosterols are effluxed back to the intestinal lumen by the heterodimer ABCG5/G8 (ATP binding cassette subfamily G member 5 and 8). A fraction of vitamins and carotenoids might be effluxed back to the intestinal lumen via apical membrane transporters (SR-BI and possibly other transporters). The non-effluxed fraction of micronutrients is transported to the site where they are incorporated into chylomicrons. It is hypothesized that proteins are involved in intracellular transport of these non water-soluble compounds, although only CRBPII (cellular retinol binding protein II), which carries retinol, has clearly been identified. Non-metabolized vitamins, carotenoids and phytosterols are mostly secreted in the lymph into chylomicrons (apoB pathway), either as free or esterified molecules, while a part of the more polar vitamins and carotenoid metabolites may be secreted via the portal route. It has been shown that a minor

fraction of vitamin E can also be secreted at the basolateral side via ABCA1 (ATP binding cassette subfamily A member 1) and also possibly ABCG1 (ATP binding cassette subfamily G member 1), but it is not known whether this apoA-I pathway is also involved in the secretion of a fraction of the other micronutrients.

Figure 4. Procedure and some guidelines to perform studies dedicated to identifying genetic variations involved in the bioavailability of FSV (fat-soluble vitamins) and P (fat-soluble phytochemicals).

GWAS: genome wide association study. EWAS: exome wide association study. CNV: copy number variants. SNPs: single nucleotide polymorphisms. Indel: insertion/deletion.

Figure 5. Current population-wide requirement curve and theoretical population-tailored RDA curve.

A: Current population-wide requirement curve, which is assumed, by lack of sufficient knowledge, to be normally distributed. The estimated average requirement (EAR) theoretically meets the requirements of half the population. The recommended dietary allowance is usually set at two standard deviations above the EAR to theoretically meet the requirements of about 97.72 % of the targeted group of the population (*e.g.* healthy male adults, healthy pregnant female, children…). Nevertheless, note that this also means that about 2.28 % of the target group requires more than the RDA for the studied nutrient. B: Theoretical requirement if a single genetic variant significantly affects the bioavailability of a fat-soluble vitamin (FSV) in a specific population. The proportion of individuals in the three parts of the curve reflects the proportion of the three alleles in the studied group. In this hypothetical situation, new RDAs might be proposed to take into account the requirements of the subjects depending on their genetic signature: RDA 1 would cover the requirements of about 97.5% of individuals homozygous for the most frequent allele, RDA 2 would cover the requirements of about 97.5% of heterozygous individuals and RDA 3 would cover the requirements of about 97.5% of individuals homozygous for the least frequent allele.

TERMS AND DEFINITIONS LIST:

ABCA1, ATP binding cassette subfamily A member 1; **ABCB1**, ATP-binding cassette, subfamily B, member 1; **ABCG1**, ATP binding cassette subfamily G member 1; **ABCG2**, ATP-binding cassette, subfamily G, member 2; **ABCG5/G8**, ATP binding cassette subfamily G member 5 and 8; **ABO**, ABO, alpha 1-3-N-acetylgalactosaminyltransferase and alpha 1-3-galactosyltransferase; **ASBT**, apical sodium bile acid transporter; **βC**, beta-carotene; **BCO1**, beta-carotene oxygenase 1; **BET1**, Bet1 golgi vesicular membrane trafficking protein; **BUD13**, BUD13 homolog; **CASR**, calcium sensing receptor; **CD36**, CD36 molecule; **COBLL1,** cordon-bleu WH2 repeat protein like 1; **CY27B1**, cytochrome P450, family 27, subfamily B, member 1; **CYP24A1**, cytochrome P450, family 24, subfamily A, member 1; **CYP2R1**, cytochrome P450, family 2, subfamily R, member 1; **CYP4F2**, cytochrome P450, family 4, subfamily F, polypeptide 2; **DBP**, vitamin D binding protein; **DHCR7**, 7-dehydrocholesterol reductase; **EAR**, estimated average requirements; **ELOVL2**, ELOVL fatty acid elongase 2; **FADS1**, fatty acid desaturase 1; **FSV&P**, fat-soluble vitamins and phytochemicals; **GC**, vitamin D binding protein; **GWAS**, genome-wide association study; **HL**, hepatic lipase; **INSIG2**, insulin induced gene 2; **IRS1**, insulin receptor substrate 1; **ISX**, intestine specific homeobox; **LPL**, lipoprotein lipase; **MC4R**, melanocortin 4 receptor; **MTTP** (gene)/**MTP** (protein), microsomal triglyceride transfer protein; **NAT2**, N-acetyltransferase 2; **NKAIN3**, sodium/potassium transporting ATPase interacting 3; **NPC1L1**, NPC1 like intracellular cholesterol transporter 1; **PNLIP**, pancreatic lipase; **PS**, phytosterol; **RAE**, retinol activity equivalent; **RDA**, recommended dietary allowance; **RPE65**, retinal pigment epithelium 65; **RXRA**, retinoid X receptor alpha; **SCARB1** (gene)/**SR-BI** (protein), scavenger receptor class B member 1; **SL27A6**, solute carrier family 27 member 6; **SLC10A2**, solute carrier family 10, member 2; **SNP**, single nucleotide polymorphism; **SOD2**, superoxide dismutase 2; **SREBF2**, sterol regulatory element binding transcription factor 2; **TCF7L2**, transcription factor 7-like 2; **VD**, vitamin D; **VDR**, vitamin D receptor; **VE**, vitamin E; **ZNF259**, zinc finger protein 259.