Lipid Digestion and Bioaccessibility of Lipid-Soluble Compounds

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Summary

Understanding the digestion of lipids and bioaccessibility of their derivate (lipid-soluble compounds/molecules) after digestion is very important to know as these compounds constitute a main part of the human's diet. In this chapter, two main aspects of digestion and bioaccessibility of the major lipid-soluble materials are discussed; a) the structure, metabolism, absorption, bioavailability, and bioaccessibility of lipid-soluble compounds/molecules (e.g. vitamins, polyphenols, sterols, etc.), b) the role of digestion in regard to the relationship between lipid digestion and bioaccessibility of fat-soluble compounds/molecules. It appears that the main aspects of the role of gastrointestinal digestion on bioavailability/bioaccessibility of lipophilic compounds are related to their molecular properties. While the role of the molecular form and linkage of the compounds during digestion is clear, the knowledge about gastrointestinal metabolites of most of the lipophilic compounds, their generation, and absorption is limited. Furthermore, it appears that the interactions between other nutrients and lipophilic compounds during gastrointestinal digestion, beside the organization of the mixed micelles in the real situation of multiple nutrients are yet to be fully described.

Keywords: Lipid digestion; bioaccessibility; lipid-soluble compounds; fat-soluble vitamins; lipophilic polyphenols; sterols.

1. Introduction

Food digestion within the gastrointestinal tract involves many processes, which are mainly mechanical and chemical, although some are physical and biological. The complexity of the interactions between food components and the gastrointestinal environment makes it difficult to understand the exact role each component plays to favour health or disease. For decades, nutrients and micronutrients were only studied through their absorption and biological fate, often in isolation from the food. However, it is now recognized that the nature and structure

of the food play important roles in the production and liberation of nutrients, and in some cases even dictate the subsequent kinetics of absorption. In particular, efficient lipid digestion and liberation from the food within the gastrointestinal tract are known to be essential for the optimal assimilation of lipid-soluble molecules (such as fat-soluble vitamins, carotenoids, polyphenols, etc.). But this relation is not fully understood, as some of the factors involved were not studied sufficiently. In this chapter, the goal is thus to review the current knowledge on these factors, making the link between *in vitro* gastrointestinal digestion and *in vivo* absorption studies with respect to the structure of the fat-soluble materials. To this end, a few definitions will first be given, then the main lipid-soluble molecules found in food will be described, together with what is currently known about their fate within the gastrointestinal tract and after absorption. Then, in the last part of the chapter, the role of lipid digestion will be highlighted, by presenting research findings obtained from both *in vitro* and *in vivo* experiments, as well as numerical simulations.

2. Definitions for lipid-soluble molecules in the framework of lipid digestion

The term 'lipid solubility' or 'lipophilicity' is generally referred to as the ability of a compound/substance/chemical to dissolve in lipids (fats and oils). Such compounds, which are known as 'lipophilic compounds', also dissolve in non-polar solvents (e.g. hexane, toluene, etc.), but do not dissolve in polar/hydrophilic (water-loving) solvents. However, the solubility of lipophilic compounds in an aqueous-based medium depends on several different factors, such as temperature, pH, ionic strength, and presence of solubilizing compounds (e.g. bile salts). These factors are crucial when digestion of the lipid-soluble molecules/compounds is considered.

Bioaccessibility of a lipophilic compound is defined as the amount incorporated in mixed micelles relative to the amount in the food. Mixed micelles form in the small intestine (duodenum), composed of a) bile secreted by the gallbladder and the liver (bringing bile salts, phospholipids, and cholesterol), and of b) free fatty acids, monoglycerides, and lysophospholipids, produced by hydrolysis of the lipids (triglycerides and phospholipids, respectively). Several steps are needed before this incorporation is achieved: i) mechanical and chemical breakdown of food structures, releasing nutrients and lipophilic compounds, ii) hydrolysis of some esterified lipophilic compounds by specific or non-specific enzymes, iii) transfer of the lipophilic compounds from the lipid phase to the mixed micelles. These steps will be discussed in Section 4.

In regard to lipid solubility of the fat-soluble compounds and their behaviour after digestion in the gastrointestinal tract, bioavailability is an important step in ensuring that bioefficacy of the fat-soluble compounds. There are several different stages that are associated with the bioavailability of hydrophobic/fat-soluble compounds. These include the liberation of the specific compound from the lipid structure in the digestive tract, its absorption from the intestinal lumen, as well as its distribution and metabolism in the body and the elimination of its excess amount from the body. Of course, a good understanding of the metabolic and absorptive fate of the fat-soluble compounds can help with their impact on the promotion of their effect on human health. Various factors such as food matrix (especially fat content and fat type), protein transporters, molecular structure, bioaccessibility, and metabolizing enzymes can affect the bioavailability of the fat-soluble compounds in the body (Rein et al., 2013). In the following section, we discuss different types of lipid-soluble compounds, their structure, solubility properties in the human digestion system, in addition to their metabolism, absorption, bioavailability and bioaccessibility.

3. Lipid-soluble molecules; structure, metabolism, bioaccessibility, absorption, and bioavailability

3.1.1. Lipid-soluble vitamins

There are four lipid-soluble vitamins known in humans that are needed to maintain good health. These include Vitamins A, D, E, and K. The main characteristics of these vitamins and their vitamers (provitamins) are presented in Table 1 (Patrick Borel, 2009). Unlike water-soluble vitamins that are substantially excreted from the body, and so their concentrations in urine is a robust predictor of their consumption or deficiencies, fat-soluble vitamins are absorbed through the digestion system with the help of different kinds of lipids/fats (Fukuwatari & Shibata, 2008; Maqbool & Stallings, 2008). Accordingly, the deficiency and toxicity of such vitamins (i.e. fat-soluble vitamins) are more complex than water-soluble vitamins. While the deficiency of fat-soluble vitamins due to their malabsorption can be significant in several cases, the accumulation of some of these vitamins in the body (e.g. Vitamins A and D) can result in hypervitaminosis (Maqbool & Stallings, 2008). Fortunately, there is not a significant loss of fat-soluble vitamins due to cooking, but human body needs to have access to a source of these vitamins every day and it can store excess amounts in the liver and adipose tissues.

Table 1: Main characteristics of fat-soluble vitamins and their vitamers. Modified from Patrick Borel (2009).

Common Name	Main molecular species found in the Western diet	RDA[1]	Mean/median daily intake[2]
Pre-formed Vitamin A	Retinyl palmitate	900 REA[3]	598–682 μg
Provitamin A carotenoids	β-carotene	–	2.15–2.62 mg
	α-carotene	–	0.39 mg
	β-cryptoxanthin	–	0.12–0.14
Vitamin E	d-alpha-tocopherol	15 mg	9.8–10.3 mg
	d-γ-tocopherol	–	–
Vitamin D	Cholecalciferol	5 μg	2.9 μg
Vitamin K	Phylloquinone	120 μg	70–80 μg
	Menaquinone	–	21 μg
Non-provitamin	Lycopene	–	6.6–12.7 mg
A carotenoids	Lutein/Zeaxanthin	–	2.0–2.3[4]
Phytosterols	Sitosterol	–	167–437 mg
	Stigmasterol	–	
	Campesterol	–	

[1]US RDA (recommended dietary allowances), or adequate intake (Vitamin D), for the adult male population.

[2]Mean/median daily intake for the adult male population.

[3]REA: Retinol Activity Equivalents.

[4]Lutein + zeaxanthin.

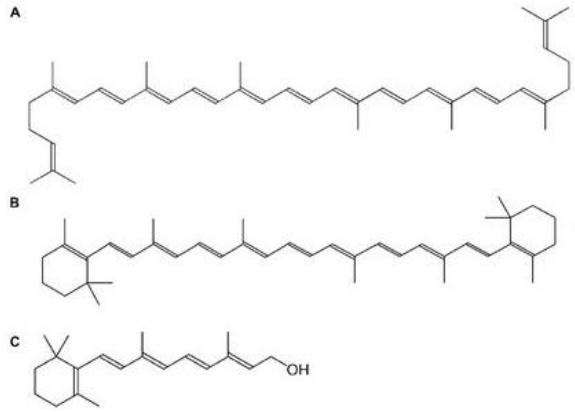
In the small intestine, fats and fat-associated compounds can move across the mucosal membranes and enter the bloodstream, and finally, they enter the liver, their storage site. Therefore, if the amount of fat in one's diet is inadequate, the absorption of the fat-soluble vitamins in the body may be impaired (Ravisankar et al., 2015).

3.1.2. Vitamin A and carotenoids

Vitamin A, which exists in foods in two principal forms (i.e. retinol or the carotenes), can be found in foods such as liver (and some other meat products) of different animals, ghee and butter (and other dairy foods), vegetables with orange and dark colours (e.g. sweet potato, carrot, pumpkin, broccoli, spinach, etc.), eggs, and different fruits (e.g. apricots, papayas, mangos, etc.) (USDA, 2017). Structurally speaking, this vitamin (Vitamin A) contains the class composed of retinol, retinal, retinoic acid, carotenoids, and lycopene (carotenes are also known as provitamin A, the main one being beta-carotene). In the human body, there are several important functions known for Vitamin A, such as, its role in growth and development (in form of retinoic acid, a hormone-like growth factor for different cells), vision (in the form of retinal), and maintenance of the immune system (Tanumihardjo, 2011;

Wolf, 2001). Figure 1a shows the different chemical structure of Vitamin A and its provitamins (Strobbe, De Lepeleire, & Van Der Straeten, 2018).

a



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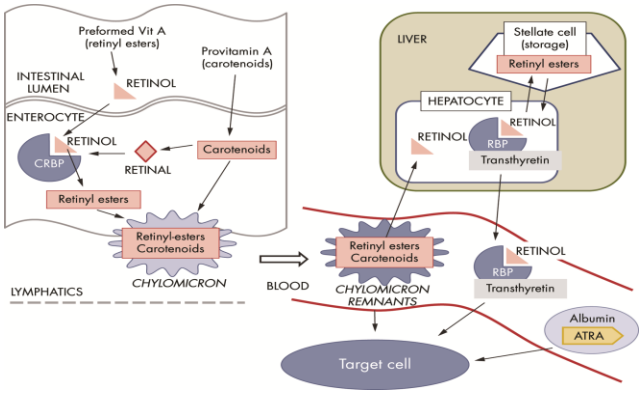


Figure 1: a) Chemical structure of Vitamin A and its precursors. (A) Lycopene, (B) β-carotene, (C) all-trans-retinol (Strobbe et al., 2018), b) dietary uptake and transport of Vitamin A redrawn from Conaway, Henning, and Lerner (2013) with permission.

Since the pure alcohol form of Vitamin A has low stability, this vitamin is found in a form of retinyl ester in different animal tissues, which is also the commercially produced form of Vitamin A (DeMan, 1999). Retinyl palmitate, the major form of Vitamin A in animal-based food products, is an ester that can be converted to retinol in the small intestine. Retinol, which is also the storage form of Vitamin A, when required, can be converted to active aldehyde form, retinal (DeMan, 1999). The carotenoids family such as alpha-, beta-, and gamma-carotene, as well as the xanthophyll beta-cryptoxanthin, are the provitamins of Vitamin A in both herbivores and omnivores that have the enzyme beta-carotene 15,15'-

dioxygenase to cleave the carotene molecule (i.e. beta-carotene) and convert it to retinol in the intestinal mucosa (WHO, 2009).

The dietary uptake and transport of Vitamin A are shown in Figure 1b (Conaway et al., 2013). Some specific pancreatic and intestinal enzymes can hydrolyse the dietary retinyl esters to retinol, which is then taken up by the cells in the intestinal mucosa (i.e. enterocytes) (Blaner et al., 1994). Fat plays several important and substantial functions in this regard; it is a stimulator for the enzymes responsible for retinyl esters hydrolysis, it is also an important factor for the formation of the micelle required for solubilisation of carotenoids and retinol in the intestinal lumen, and it promotes the formation of chylomicron (Harrison, 2012). As a hydrophobic molecule, in the enterocyte, retinol is bound by a specific compound called 'cellular retinol binding protein, CRBP II', which is able to bind most of the existing retinol in intestinal cells (D'Ambrosio, Clugston, & Blaner, 2011). Almost half of the provitamin A carotenoids is oxidized and converted to retinal and finally, reduced to retinol; whereas, the rest is absorbed in mucosa cells in their intact form (Blomstrand & Werner, 1967). For more efficient absorption, due to its fat-loving nature, retinol is first esterified with long-chain fatty acids. Then, the esters of retinyl, along with the original/intact carotenoids, are incorporated into chylomicrons with other fat-soluble compounds (e.g. triglycerides, cholesterol, cholesterol esters, etc.) (Harrison & Hussain, 2001).

After hydrolysis of chylomicron in the bloodstream, the chylomicron remnants containing retinyl esters, which are made by lipoprotein lipase (D'Ambrosio et al., 2011), are taken up by hepatocytes through the receptor-mediated endocytosis (Cooper, 1997). Retinol becomes re-esterified and remains in the liver stellate cells (as the main storage place for Vitamin A), if there is an excess amount of it in the body. However, some smaller quantities of retinyl esters and/or carotenoids may also be transferred by chylomicrons to extrahepatic tissues for storage and further utilization (D'Ambrosio et al., 2011). Once the retinyl esters are hydrolyzed in liver stellate cells, retinol can be transported back to hepatocytes in order to be bound by retinol binding protein (RBP), a specific transport protein for this lipid-soluble molecule (Sporn, 1994). Afterwards, the complex of retinol-RBP can enter the bloodstream and combine with transthyretin, which is another protein synthesized in the liver (Episkopou et al., 1993). Such binding of the retinol-RBP complex to transthyretin results in the prevention of retinol-RBP clearance induced by the kidneys (van Bennekum et al., 2001).

3.1.3. Vitamin D

Vitamin D, as shown in Figure 2a, in humans exists in two forms including D3 (cholecalciferol) D2 (ergocalciferol) and involves a class of fat-soluble secosteroids. These secosteroids play some important roles in increasing the intestinal absorption, homeostasis, and metabolism of the important minerals such as calcium, phosphate, and magnesium, as well as being responsible for several other biological effects in the body, such as immunity and cell growth (Holick, 2004). Only a few foods (e.g. milk and Vitamin D-fortified dairy products, oily fish, and cod liver) contain Vitamin D. The principal natural source of this vitamin is a chemical reaction that happens under the skin and results in the synthesis of cholecalciferol from cholesterol. This synthesis process is highly dependent on one's exposure to the sunlight, particularly, UVB radiation. Nevertheless, in different populations over the world and during different seasons and climates, sun exposure can be very variable, making recommendations about the safe amount of exposure very unreliable; the dietary recommendations for Vitamin D characteristically assume that all of the Vitamin D requirement is taken orally (Supplements, 2016).

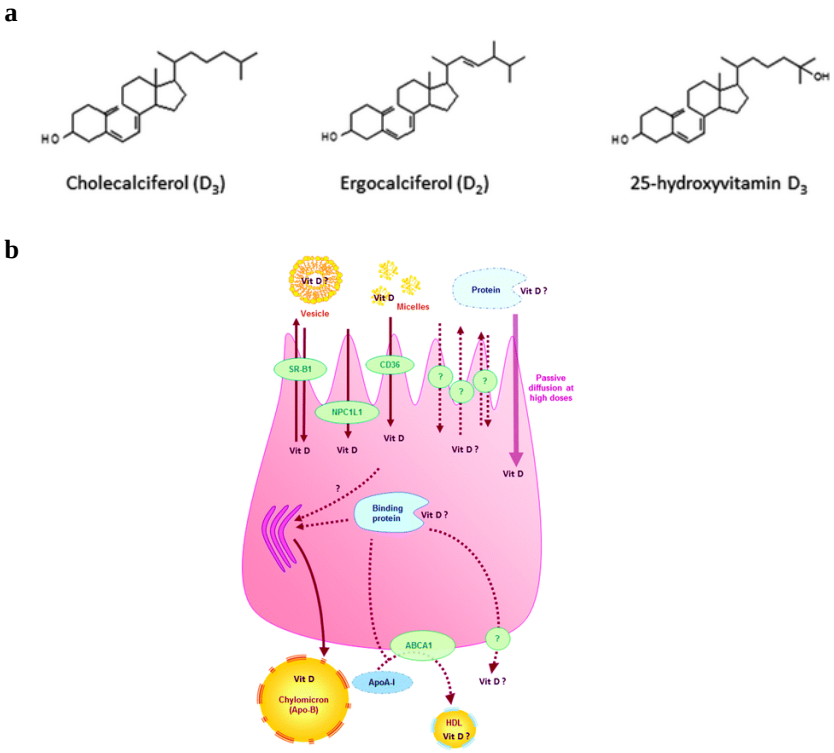


Figure 2: a) the chemical structure of the main molecular forms of Vitamin D., b) proteins which are involved in the transport of Vitamin D through the enterocyte. Redrawn from Reboul (2015), with permission.

Regardless of the source that Vitamin D comes from (i.e. from skin synthesis or the diet), it is biologically inactive, and it requires a specific enzyme (binding protein) to hydroxylate and convert it to its active form. Such conversion occurs in the liver as well as in the kidneys (Norman, 2008). Previously, it was believed that there existed a passive process for the intestinal absorption of Vitamin D, but new data shows this is a complex process (Reboul, 2015). The fate of Vitamin D in the upper part of the human's gastrointestinal tract was reviewed by Reboul (2015). It was concluded that the micelle binding to the membrane receptors of Vitamin D could be an important initiator in regard to the entry of this fat-soluble vitamin into the enterocyte (Reboul, 2015).

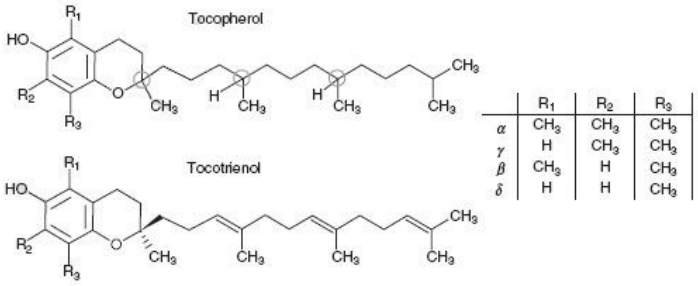
As it is shown in Figure 2b, there are several different proteins involved in the transport of Vitamin D across the enterocyte, some of which and their mechanism of action (and pathways) is yet unknown (Reboul, 2015). According to this process, firstly, mixed micelles (or maybe vesicles and/or proteins) help Vitamin D to be taken up. This happens with some apical membrane transporters, including SR-BI, NPC1L1, and CD36. Generally, a part of the dietary Vitamin D may enter the cell through passive diffusion; this is more relevant when pharmacological doses of Vitamin D are administered. Then, apical membrane transporters (such as SR-BI) can help a fraction of the Vitamin D to be effluxed back to the intestinal lumen, while another part is transported to the chylomicron incorporation site. Finally, the free form of Vitamin D is secreted in the lymph and into chylomicrons; in other words, it enters the apolipoprotein B-dependent route. However, this is only one of the possible way of secretion pathways for this vitamin and some non-apolipoprotein B-dependent routes (e.g. via ABCA1 and HDL) may also exist (Reboul, 2015). As mentioned before, although the intracellular transport of Vitamin D involves its binding to some specific proteins, the whole mechanism is yet to be clearly identified, especially in relation to the transporters, the associated pathways, and the molecular mechanisms underlying this such phenomenon.

3.1.4. Vitamin E

Vitamin E is found in different foods such as oil from different sources (e.g. wheat germ, hazelnut, canola, etc.), meat products (e.g. fish, oysters, chicken), and dairy products (e.g. milk, cheese, butter, ghee), and it includes a group of various fat-soluble compounds. This group includes four tocopherols as well as four tocotrienols; their chemical structures are presented in Figure 3 (Galli et al., 2017). This vitamin expresses some antioxidant functions in cell membranes in the human body and there are theories to support that Vitamin E may be involved in controlling gene expression, regulating enzyme activity (e.g. protein kinase C)

and transduction of cell signals. Vitamin E deficiency problems reported to date, are rarely related to a diet low in Vitamin E rather being associated to an underlying cause related to impaired dietary fat digestion (Azzi, 2018; Brigelius-Flohe & Traber, 1999).

a



	R_1	R_2	R_3
α	CH_3	CH_3	CH_3
γ	H	CH_3	CH_3
β	CH_3	H	CH_3
δ	H	H	CH_3

b

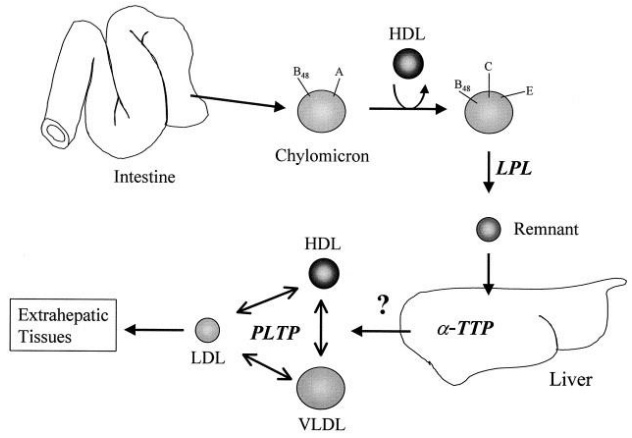


Figure 3: a) The chemical structures of tocopherols and tocotrienols. The circles mark the three chiral centres in tocopherols, which include 2, 4', and 8', b) absorption, transport, and distribution of tocopherols in the human body. LPL: lipoprotein lipase, PLTP: plasma phospholipid transfer protein, LDL: low-density lipoprotein, VLDL: very low-density lipoprotein.Redrawn from Manolescu et al. (2008) and Hosomi et al. (1997), respectively, (free access)

Both known sources of Vitamin E (i.e. tocopherols and tocotrienols) are present in α (alpha), β (beta), γ (gamma), and δ (delta) forms, depending on the number of methyl groups and the position they are located on the chromanol ring (Brigelius-Flohe & Traber, 1999). All of the vitamers of Vitamin E possess a chromane double ring, along with a hydroxyl group. This feature allows them to donate a hydrogen atom, by which free radicals are reduced. They also contain a hydrophobic side chain that enables such vitamers to penetrate into biological membranes.

The metabolism of tocopherols (including the stereoisomers of synthetic alpha-tocopherol) and tocotrienols, starts with their absorption from the intestinal lumen. Together with the fat-soluble components present in the food, Vitamin E is emulsified. Later, the lipolysis and emulsification of the newly-formed lipid droplets can lead to the production of mixed micelles. Through passive diffusion, these micelles are then absorbed in the mucosa's brush border membrane of Golgi of the mucosa cell, which can help re-assembly of tocopherols together with triglycerides, phospholipids, apolipoproteins, and cholesterol to chylomicrons (Hosomi et al., 1997). After the absorption, they are incorporated into chylomicrons, then are secreted into the portal vein and transferred to the liver, where they can be stored. It is estimated that 51% to 86% of Vitamin E-related compounds (i.e. tocopherols and tocotrienols) are absorbed from the intestinal lumen. The portion of Vitamin E (and its vitamers) that is not absorbed, is excreted via faeces. Moreover, excretion of this vitamin by the liver via bile and into the intestinal lumen is also possible. After this, the vitamin is either reabsorbed or excreted (via faeces), while all of the vitamers of Vitamin E are excreted via urine (Azzi, 2018; Monsen, 2000). Figure 3b shows a schematic view of the absorption, transport, and distribution of tocopherols in the human body (Hosomi et al., 1997).

After alpha-tocopherol reaches the liver, it is taken up by a specific transferring protein called 'α-TTP'. α-TTP is known as the key intracellular transport protein for vitamin E. With the help of this protein, alpha-tocopherol secretion is mediated into the bloodstream via a non-Golgi-dependent pathway (Kaempf-Rotzoll, Traber, & Arai, 2003). However, the other forms of vitamin E can be degraded to a compound called '2'-carboxethyl-6-hydroxychromane (CEHC)'. During such a process, the phytic tail of the molecule is truncated, then it might be sulfated or glycuronidated, which results in rendering the molecules water-soluble and leading them to the excretion through urine. In the case of alpha-tocopherol, the same process is also applied for its degradation, by which '2,5,7,8-tetramethyl-2-(2 ′-carboxyethyl)-6-hydroxychromane (α-CEHC)' is produced. Nevertheless, this process is slower than what happens in the case of other forms of vitamin E, due to the protection mechanism of α-TTP. Accordingly, if a large amount of alpha-tocopherol is taken, it will result in an increased amount of α-CEHC in the urine, which is an efficient mechanism for disposing of the excess Vitamin E from the human body (Azzi, 2018; Monsen, 2000).

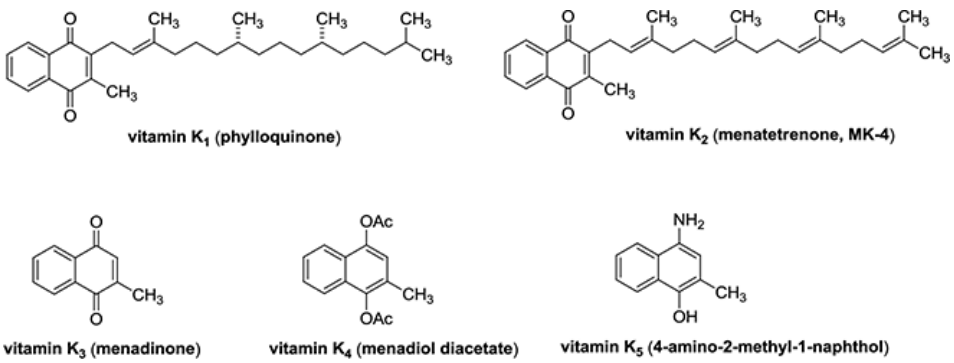
3.1.5. Vitamin K

Vitamin K is mostly found in leafy green vegetables (e.g. lettuce, Swiss chard, spinach, etc.), Brassica vegetables (e.g. kale, broccoli, cabbage, cauliflower, etc.), and Brussels sprouts),

some fruits (e.g. kiwifruit, avocados, grapes, etc.), and vegetable oils (particularly, soybean oil). It includes a group of structurally-similar hydrophobic compounds that are required for the human body for the complete synthesis and function of specific proteins, which act as the prerequisites for blood coagulation. In addition, these proteins are also required for controlling the binding of the elements, such as calcium in bones and other tissues (Data, 2013; Sankar et al., 2016b). The lack of Vitamin K is often associated with an impaired blood coagulation system and the subsequent uncontrolled bleeding. Various clinical studies have shown that Vitamin K deficiency can result in weakened bones in the skeletal system, and accordingly, can lead to osteoporosis, as well as promotion of the calcification in arteries and soft tissues in the human body (Eby, 2016; Kuroda, Uenishi, Ohta, & Shiraki, 2019; Sankar et al., 2016a).

Chemically speaking, Vitamin K belongs to a family containing '2-methyl-1,4-naphthoquinone' derivatives (Figure 4a), including Vitamin K1 and Vitamin K2 as the natural vitamers and the synthetic vitamers. As can be seen in Figure 4b, the dietary sources of these different vitamers (e.g. Vitamin K1 vs Vitamin K2) are different. For example, Vitamin K1 is found in leafy greens while Vitamin K2 is mostly sourced from the foods such as natto (the most important source) and some cheeses. Vitamin K1 can be converted into Vitamin K2 by the bacteria in the human gut. In addition, some homologues of Vitamin K2 (most notably, the MK-7 to MK-11) may also be produced by the gut bacteria typically through lengthening the isoprenoid side chain of Vitamin K2.

a



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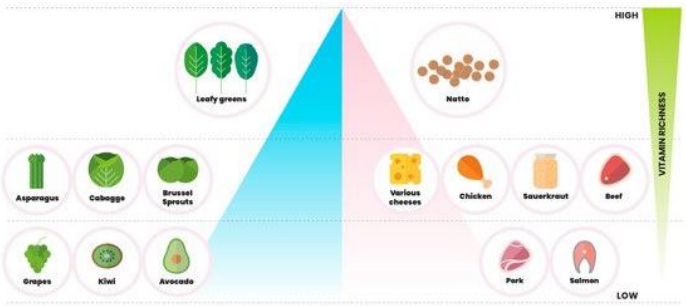


Figure 4: a) the chemical structures of different vitamers of Vitamin K, including natural vitamers (Vitamin K1 and Vitamin K2) and synthetic vitamers (Vitamin K3, Vitamin K4, and Vitamin K5), , b) dietary sources of Vitamin K1 (left) vs Vitamin K2 (right). Modified from Halder et al. (2019) and Halder et al. (2019), respectively.

Although Vitamin K is well-absorbed from a typical diet, total vitamin K levels are dependent of the status of the digestive health (Halder et al., 2019; Kurosu, 2017). Therefore, for healthy humans (i.e. with healthy intestines), Vitamin K deficiency is considered to be quite rare, regardless of the fact that the K1 form is abundant in a normal diet containing leafy and salad vegetables, herbs, etc. This is different in the case of newborns, who have not been fed with Vitamin K-rich foods and their gut has not been well-developed yet; in most countries, newborns usually receive a Vitamin K injection or oral Vitamin K drops upon birth in order to prevent bleeding or the development of haemorrhagic disease (Halder et al., 2019; Kurosu, 2017).

Like the other fat-soluble vitamins, Vitamin K and its vitamers also are absorbed by a pathway similar to that for fat/triacylglycerols (Giammanco, Cefalù, Noto, & Averna, 2015). In the food containing Vitamin K, therefore, this vitamin is initially dissolved within the small lipid droplets which contain triacylglycerols. When the food is ingested, the lipid droplets and lipophilic compounds undergo alterations in their composition, size, and dimensions as a result of the changes in the conditions of the digestive tract (e.g. pH, ionic strength, mechanical forces, the activity of digestive enzymes, and molecular interactions) (Mao & Miao, 2015; McClements & Xiao, 2012). The triacylglycerols are digested by intestinal lipase, which results in the liberation of the free fatty acids (FFAs) and monoacylglycerols (MAGs), which in turn can associate with bile salts and phospholipids and form mixed micelles (Porter, Trevaskis, & Charman, 2007). Here, Vitamin K becomes incorporated into the hydrophobic interior of the formed mixed micelles. Such Vitamin K-loaded mixed micelles can be absorbed by an active or a passive transport mechanism, but first, they need to be transported through the mucus layer and reach the epithelial cells. The

absorbed fatty acids and glycerols along with Vitamin E are reassembled into triacylglycerols after the absorption and then packed into chylomicrons (lipoproteins) (Kayden & Traber, 1993). The chylomicrons eventually reach the bloodstream, after they are excreted from the epithelial cells (the basolateral side) and go through the lymphatic system (McClements, Li, & Xiao, 2015).

3.2. Coenzyme Q10

Coenzyme Q10 is found in various foods, including meat products (e.g. beef, chicken, fish, etc.), oils (e.g. olive, grapeseed, sunflower, etc.), nuts (e.g. peanut, walnut, almond, pistachio, etc.), vegetables (e.g. broccoli, cauliflower, spinach, etc.), and fruits (e.g. avocado, grapes, blackcurrant, etc.). It is also known under some other names including coenzyme Q, ubiquinone, and ubidecarenone. Some abbreviations such as CoQ10, Q10, and CoQ have also been widely used. Chemically speaking, coenzyme Q10 comprises of a 1,4-benzoquinone, where the 'Q' states the chemical group of quinone and the number of isoprenyl subunits in the tail of the molecule is indicated by '10' (Erlanson & Borgström, 1968; Hernández-Camacho, Bernier, López-Lluch, & Navas, 2018; Quinzii, DiMauro, & Hirano, 2007).

Coenzyme Q10plays a number of different roles, in all respiratory eukaryotic cells. This coenzyme contributes to the aerobic cellular respiration as it is a component of the electron transport chain, so it has a vital effect on energy generation in the form of ATP (Dutton et al., 2000; Ernster & Dallner, 1995). CoQ10 is also considered as a potent and important antioxidant in the human body (Bhagavan & Chopra, 2006).

In regard to the absorption of coenzyme Q10 in the human digestive system, it is known that like other hydrophobic compounds/vitamins, its absorption occurs under the same process as that of lipids/triglycerides. In particular, the uptake mechanism of coenzyme Q10 is similar to that of Vitamin E (discussed in Section 3.3). Firstly, emulsification and micelle formation in the small intestine are facilitated by the secretion of pancreatic enzymes and bile (Bhagavan & Chopra, 2006). Of course, as in the case of other fat-soluble compounds, the presence of various lipids can stimulate excretion of bile acids, and accordingly enhance the coenzyme Q10 absorption greatly. In this regard, it has found that coenzyme Q10 from the exogenous sources is best absorbed from the small intestine, if it is taken with a meal containing fat (Ochiai et al., 2007; Weber, Bysted, & Hølmer, 1997).

Due to its hydrophobic nature as well as the large molecular weight, dietary coenzyme Q10 is absorbed slowly and to a limited extent. This coenzyme shows a Tmax and half-life

elimination of around 6 hours and 33 hours, respectively (Bhagavan & Chopra, 2006). There appears to be a rational correlation between the ingested doses of coenzyme Q10 (up to a certain point) and its plasma levels in humans (Chopra, Goldman, Sinatra, & Bhagavan, 1998). There is also evidence from the animal experiments confirming that this fat-soluble coenzyme can be taken up in high doses by all tissues (e.g. heart and brain mitochondria), (Alessandrì, Scalori, Giovannini, Mian, & Bertelli, 1988). The excess amount of coenzyme Q10 is eliminated through bile excretion and faeces as the major route for this purpose (Ozawa et al., 1986).

Recently, López-Lluch, del Pozo-Cruz, Sánchez-Cuesta, Cortés-Rodríguez, and Navas (2019) demonstrated that the bioavailability of CoQ10 in different supplemental formulations was significantly dependent on the individuals as well as the carrier lipids and solubilisation of the coenzyme Q10 products.. Even if the composition of a particular formulation was highly effective, the individuals showed different responses to the administered doses. Such responses depended on some unknown factors, such as lifestyle, body mass index, sex, weight, and age (López-Lluch et al., 2019).

3.3. Lipophilic polyphenols

Polyphenols (also known as polyhydroxyphenols, polyphenolic compounds, or phenolic compounds), the most abundant antioxidants in the human diet (especially in fruits, herbs, and vegetables), are a structural class of mainly natural and organic chemical compounds, which are categorised by the presence of phenol structural units. Every polyphenolic compound presents different and unique chemical, physical, and biological (i.e. metabolic and therapeutic health benefits, toxicity, etc.) properties, due to the difference in the number and properties of these phenol structures. Some examples of hydrophobic polyphenolic compounds include curcumin, quercetin, rutin, hesperidin, catechin, resveratrol, naringin, phenolic acids (caffeic acid, formic acid, gallic acid, etc.), and some tannins (Gomez & Martinez, 2018; Quideau, Deffieux, Douat-Casassus, & Pouységu, 2011).

There have been several epidemiological studies confirming the health-promoting effects of polyphenols/antioxidants as a result of the consumption of the foods rich in these natural compounds. In particular, they reduce the risk of chronic diseases, such as cardiovascular diseases, diabetes, and cancer through preventing the impairment caused by oxidative stress in certain biomolecules (e.g. nucleic acids and proteins) (Cilla et al., 2009). While some of the polyphenols with the large molecular weight (e.g. proanthocyanidins) are poorly absorbed

in humans, phenolic acids (e.g. caffeic acid) can be absorbed easily through the gut barrier. After the intestinal absorption, the conjugation of polyphenols to sulphate, glucuronide, and methyl groups in the inner tissues and gut mucosa occurs. Therefore, the non-conjugated polyphenols are not expected to be present in the plasma, which is a good functionality for facilitating the excretion of these phenolic compounds and limiting their possible toxicity (Scalbert, Morand, Manach, & Rémésy, 2002).

Although polyphenols show antioxidant activity *in vitro*, it is subsequent metabolism and absorption in the digestive tract which governs biological characteristics, including antioxidant activity (Tarko, Duda-Chodak, & Zajac, 2013). Only the fraction of the polyphenols released from the food matrix during digestion (in the small and/or large intestine) is considered to be part of the digesta. Food polyphenols in their native form mainly exist as polymers, esters, and glycosides which cannot be absorbed as such, and therefore, need to be hydrolysed by endogenous enzymes and/or microflora enzymes in the digestive tract (Williamson, Day, Plumb, & Couteau, 2000).

Regardless of their hydrophobicity, it has been shown that about 48% and 42% of polyphenols can be digested in the small and the large intestines, respectively. The nature of the food matrix itself (e.g. the presence of fat in the case of hydrophobic polyphenols) may also affect the bioavailability of polyphenols as they can react with some constituents in the food matrix (Claudine Manach, Scalbert, Morand, Rémésy, & Jiménez, 2004). Some other factors, such as pH, the gastrointestinal environment, and the presence of bile salts significantly affect the metabolism and bioavailability of polyphenols (Claudine Manach et al., 2004).

An overview of the general bioavailability of polyphenols, including the corresponding critical steps that can occur from ingestion to excretion is shown in Figure 5 (Bohn, 2014). There are several different proteins and transporters known to be involved in polyphenol bioavailability. Polyphenols, such as flavonoids, are absorbed in the human gastrointestinal tract, and metabolites and unchanged compounds are excreted in the urine and faeces (Cook & Samman, 1996). The gut bacteria can provide enzymes to split the heterocyclic ring of polyphenols and degrade them to other compounds such as phenyl acids, which then may be conjugated, absorbed, metabolized further by the bacteria, or excreted (Aherne & O'Brien, 2002). Soon after the ingestion, the polyphenols enter the bloodstream/plasma.

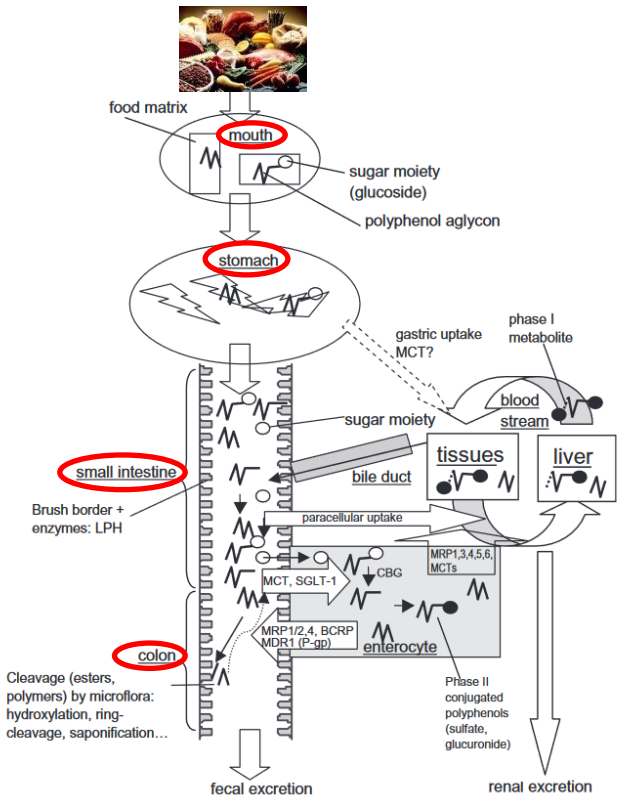


Figure 5: An overview of the bioavailability of polyphenols, showing critical steps that occur during release, digestion, uptake, transport, and excretion. Abbreviations: BRCP, breast cancer resistance protein; CBG, cytosolic β-glucosidase; LPH, lactase-phlorizin hydrolase; MCT, monocarboxylic acid transporter; MRP, multidrug resistance proteins; Pgp, P-glycoprotein; SGLT1, sodium-glucose linked transporter 1, MDR1, multidrug resistance protein. Modified from Bohn (2014).

As mentioned earlier, if the polyphenols could not be absorbed in the small intestine, they move into the colon, where the microflora can extensively metabolise them and convert them into a wide array of phenolic acids with lower molecular weight (Deprez, Mila, Huneau, Tome, & Scalbert, 2001; Fraga et al., 2002). The fate of undigested polyphenols has been reported in many studies (Déprez et al., 2000; Lee, Jenner, Low, & Lee, 2006; Rechner et al., 2002).The intestinal bacteria can hydrolyse glycosides to aglycones, which can subsequently be transformed into different acids by the action of esterases, β-glucosidase, and β-rhamnosidase. The enzymes within the gut microflora can catalyse the catabolism products of flavonoids into simple units such as gallic acid and catechin. Moreover, these enzymes are able to carry out actions such as hydrolysis, demethylation, decarboxylation, and dehydroxylation. From this action, a range of compounds can be formed according to the structure of the specific phenolic compound being digested (Lee et al., 2006). For example, flavonols will result in the production of hydroxyphenylacetic acids, whereas flavanones and flavones can be catabolised to hydroxyphenylpropionic acids. However, flavanols can be

degraded into both hydroxyphenylpropionic and phenylvalerolactone acids (Lee et al., 2006; Tarko et al., 2013). All of these metabolites formed from degradation of phenolic compounds will ultimately result in the synthesis of benzoic acid. These metabolites are able to be absorbed and enter blood circulation, bind to serum albumin, and finally be transported to the liver where they might undergo processes such as hydroxylation, o-methylation, and demethylation, or be conjugated to glucuronide and sulphated derivatives. A part of these may be secreted, together with bile, back into the small intestine and again undergo hydrolysis and subsequently go through either absorption and enter the circulation, or excreted into the faeces (Lee et al., 2006; Tarko et al., 2013).

3.4. Sterols

Sterols or steroid alcohols, which are found in both plant (including fungi) and animal foods, include a key class of organic molecules and a subgroup of steroids (with a hydroxyl group at the 3-position of the A-ring). The sterols from the plants and animal sources are called 'phytosterols' and 'zoosterols', respectively. These organic compounds can also be produced by some specific type of bacteria although the steroids produced this way may possess different functions (Fahy et al., 2005; Wei, Yin, & Welander, 2016). The basic chemical structure of sterol as well as its different derivatives is shown in Figure 11 (Segura, Javierre, Lizarraga, & Ros, 2006).

From animal sources, cholesterol is the main sterolthat makes a vital contribution to the structure of cell membrane, as well as its important function as a precursor to steroid hormones and fat-soluble vitamins. Campesterol, stigmasterol, and sitosterol are the most familiar phytosterols, while ergosterol is a well-known sterol found in different types of fungi (Kreis & Müller-Uri, 2018). Sterols are in fact amphipathic lipids with a quite flat molecular structure, where the hydroxyl group on the A ring is polar and the rest of the aliphatic chain being non-polar (Fahy et al., 2005).

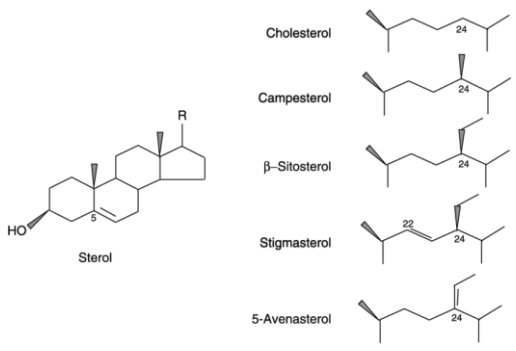


Figure 11: Chemical structure of sterols. Redrawn from Segura et al. (2006) with permission.

The process of sterol absorption in the small intestine is a selective process, by which phytosterols and ergosterol are absorbed poorly or may not be absorbed at all, while cholesterol can be absorbed efficiently (Turley & Dietschy, 2003). The intestinal cholesterol absorption is the main means for cholesterol entry into the blood which accordingly can impact the LDL plasma levels (Kramer et al., 2003; Kramer et al., 2000). Nevertheless, it appears that the molecular mechanisms of cholesterol absorption are yet to be fully understood. A transported called 'ATP-binding cassette (ABC) transporter' or 'ABCG5/ABCG8', as the apical cholesterol export pumps has been identified post-absorption. This mechanism promotes the almost complete efflux of phytosterols and partial efflux of cholesterol from enterocytes into the intestinal lumen. This may explain the reason for the selective process of cholesterol absorption in the human body in regards with phytosterols and other non-cholesterol sterol discussed above (Kramer et al., 2000). Additionally, there is a putative protein for import of cholesterol, but it is yet to be fully characterized (Kramer et al., 2000).

4. Role of lipid digestion in lipophilic compound bioaccessibility and bioavailability

Lipid-soluble molecules are obviously not alone in the gastrointestinal tract, and they interact with all other nutrients and food compounds during digestion. In this section, we will review the general factors controlling their bioavailability, highlighting the role of lipids, especially important to enhance their bioaccessibility.

4.1. The SLAMENGHI factors

Many factors contribute to lipophilic compounds bioaccessibility during digestion. A general classification was developed to evaluate micronutrient bioavailability, first applied to iron (West, 1996), then to carotenoids (Castenmiller & West, 1998). In this scheme, bioavailability is explained by the so-called SLAMENGHI factors which are: Species of the compound, molecular Linkage (e.g. esterified), Amount consumed in a meal, Matrix in which the compound is incorporated, Effectors of absorption and bioconversion (e.g. other nutrients, digestive juice compounds), Nutrient status of the host, Genetic factors (e.g. for different ethnic groups), Host-related factors (e.g. health status), Interactions between all factors. The first five factors (the physicochemical ones) can be applied to explain the bioaccessibility of most lipophilic compounds. These were reviewed in the specific case of emulsions (Sebastien Marze, Gaillard, & Roblin, 2015). Here, the physicochemical factors controlling lipophilic

compound bioaccessibility will be discussed for foods, with some illustrative examples regarding their effect on human bioavailability.

4.1.1. Amount and type of lipophilic compound

Most of the *in vitro* digestion protocols being static, the amounts of lipophilic compound and of bile salt (and their ratio) are fixed, and this could limit bioaccessibility when the first is in excess, as lipophilic compounds usually incorporate in bile salt mixed micelles. In a bioaccessibility study, this ratio should thus be based on physiological values, which are normal intake of the lipophilic compound, and amount of secreted bile salt, neglecting bile salt recirculation. Several studies showed that the amount of lipophilic compound (or reciprocally the amount of bile salt) modifies bioaccessibility. In three studies, increasing beta-carotene amount in emulsion decreased its percent bioaccessibility, whereas increasing bile salt amount increased beta-carotene percent bioaccessibility (Tyssandier, Lyan, & Borel, 2001; Wright, Pietrangelo, & MacNaughton, 2008). All the other studies showed that an increasing amount of bile salt increased carotenoid bioaccessibility (Corte-Real et al., 2018; Hou, Liu, Lei, & Gao, 2014). These trends are usually linear in a specific range then reach a plateau. This confirms that bioaccessibility is limited by the lipophilic compound/bile salt ratio. *In vivo*, percent bioavailability was found to decrease with increasing doses of phenolic compounds fed to humans, because the amount of bioavailability only increases sublinearly with the dose (Feliciano, Mills, Istas, Heiss, & Rodriguez-Mateos, 2017; Manach, Morand, Gil-Izquierdo, Bouteloup-Demange, & Remesy, 2003; Stalmach, Williamson, & Crozier, 2014). Although many chemical and biological processes are involved *in vivo*, bioaccessibility studies could be useful to optimize the amount fed as mixed micelle incorporation is a prerequisite to absorption.

It is well known that bioavailability and bioaccessibility vary widely among lipophilic compounds, due to their specific molecular properties (Borel, 2003; Marze, 2015). Less is known about the effects of the molecular linkage of specific compounds. One human study reported variable lutein bioavailability for free lutein compared to lutein di-ester, free lutein being more bioavailable only when completely dissolved in oil (Bowen, Herbst-Espinosa, Hussain, & Stacewicz-Sapuntzakis, 2002). This trend was also seen in the case of an *in vitro* study for lutein and lutein esters bioaccessibility from milk or yogurt, where free lutein was more bioaccessible than lutein esters (Xavier, Mercadante, Garrido-Fernandez, & Perez-Galvez, 2014). From cajá pulp beverages, free carotenoids were also found to be more bioaccessible (in both percent and amount) compared to carotenoid esters (da Costa &

Mercadante, 2018). Similarly, mainly non-esterified zeaxanthin was found in mixed micelles after *in vitro* digestion of various vegetables containing zeaxanthin esters, in the presence of carboxyl ester lipase hydrolysing the esters. Moreover, mainly non-esterified zeaxanthin was uptaken by Caco-2 cells (Goltz, Campbell, Chitchumroonchokchai, Failla, & Ferruzzi, 2012). With this knowledge, the main limiting factor for absorption appears to be ester hydrolysis. However, more work is needed to understand the interplay between ester hydrolysis and micellarization of both esterified and free lipophilic compounds.

4.1.2. Amount and type of lipid

There is a considerable amount of literature on the effect of lipid type on lipophilic compound bioaccessibility. Most *in vitro* studies on beta-carotene showed that a higher final bioaccessibility is reached at a given intestinal digestion time (usually 2 hours) for long chain triglycerides compared to medium chain triglycerides (Meroni & Raikos, 2018; Shah, Zhang, Li, & Li, 2016; Zhou et al., 2018). However, Nguyen et al. (2019) reported the complete kinetics of beta-carotene bioaccessibility from droplets of various oils and found an inverse trend after 40 min intestinal digestion. At the end of droplet digestion (spanning 40 min to 4 hours), the final bioaccessibility value was similar for all oils (Nguyen, Marquis, Anton, & Marze, 2019). For various vegetable salad carotenoids (carotenes, lycopene), it has been reported that bioaccessibility increased with the chain length of the added triglyceride, but not with its unsaturation degree. No significant trend was observed for lutein + zeaxanthin (Huo, Ferruzzi, Schwartz, & Failla, 2007). This was confirmed in other studies where no significant trend or an inverse trend were observed for these xanthophylls (Gleize et al., 2013). For lutein, one significant inverse trend of higher bioaccessibility with medium chain triglyceride compared to long chain triglyceride (Yuan, Liu, McClements, Cao, & Xiao, 2018) and one significant trend of higher bioaccessibility with long chain triglyceride compared to medium chain triglyceride (Nidhi & Baskaran, 2011) were reported. These inverse trends might originate from the duration of the intestinal digestion phase *in vitro*, that is usually too short (2 hours) for the slowly digested long chain triglycerides (such as those in fish oils). This aspect will be discussed further in the next section. For lycopene, bioaccessibility was also found to be higher in long chain triglycerides (Colle, Van Buggenhout, Lemmens, Van Loey, & Hendrickx, 2012). All the other lipophilic compounds studied (curcumin, cholecalciferol, alpha-tocopherol, eugenol) follow the same trend of higher bioaccessibility from long chain triglycerides compared to medium chain triglycerides (Ahmed, Li, McClements, & Xiao, 2012; Majeed et al., 2016; Zou et al., 2016).

This latter trend was the only one reported in bioavailability studies in the human. The fasting blood serum concentration of cholecalciferol was indeed found to be higher when humans were fed peanut oil compared to medium chain triglyceride (Holmberg et al., 1990). Retinyl esters and beta-carotene concentrations were found to be higher in blood chylomicron when humans were fed long chain triglycerides (olive oil or sunflower oil) compared to medium chain triglyceride (butter) (Borel et al., 1998; Sauvant et al., 2003). Carotenoids concentration was found to be higher in the triacylglycerol-rich fraction of blood when humans were fed canola oil compared to butter (Goltz et al., 2012).

The amount of lipid also plays an important role in enhancing lipophilic compounds bioaccessibility. However, all *in vitro* studies showed that this is only true up to an optimal amount, beyond which bioaccessibility decreases (Ahmed et al., 2012; Colle et al., 2012; Huo et al., 2007). This is again likely due to the static nature of *in vitro* digestion protocols, where triglyceride digestion products compete with lipophilic compounds for mixed micelle incorporation, especially with long chain triglycerides.

In humans, the carotenoids concentration in blood chylomicron was found to be higher from a vegetable salad with 28 g added fat compared to 6 g added fat or to fat-free salad (Brown et al., 2004). The same trend was reported for all carotenoids in the triacylglycerol-rich fraction of blood after a vegetable salad with 20 g added fat or 8 g added fat (Goltz et al., 2012). For a vegetable salad with 0, 2, 4, 6, 8, or 32 g added fat, there was an increasing trend for the lipophilic compounds concentration in blood chylomicron. This trend was linear for alpha-carotene, lycopene, retinyl palmitate, and phylloquinone, and nonlinear for lutein, tocopherols, and beta-carotene (White et al., 2017). Thus, in the normal range investigated, there seems to be no significant competition effect between triglyceride digestion products and lipophilic compounds *in vivo*.

4.1.4. Droplet size

When food and/or digesta contain emulsified lipid droplets, most of the *in vitro* digestion studies showed that lipophilic compound bioaccessibility of lipophilic compounds significantly increases when the mean droplet size decreases (Hou et al., 2014; Salvia-Trujillo et al., 2019; F. Xu, Pandya, Chung, McClements, & Kinchla, 2018). This is because, at a given lipid concentration, more surface area is available for mass transfer from the lipid phase to the aqueous micellar phase. However, for curcumin this trend was found to be

significant in only one study (Shah et al., 2016), with two other studies reporting no significant effect of the droplet size (Ahmed et al., 2012; Zou et al., 2016).

In vivo, a human study found no significant effect of the droplet size on vitamin A and vitamin E bioavailability (P. Borel, 2003). In rats, no significant effect was found for ergocalciferol (Salvia-Trujillo et al., 2019) or ubiquinone (Cho et al., 2014), and only one significant trend was found for the maximum plasma concentration of astaxanthin (Affandi, Julianto, & Majeed, 2012). This absence of droplet size effect is likely due to a change in the droplet size distribution in the gastrointestinal tract. For example, in the work of P. Borel et al. (2001), the mean droplet size was not significantly different in the duodenum. It is thus essential to measure droplet size distribution throughout digestion in vitro, to ensure the initial size difference is retained during the intestinal phase, where the release takes place. With a stable droplet size distribution, an effect is nevertheless expected for lipophilic compound absorption *in vivo*, as a cellular study with stable emulsions of different mean droplet sizes showed that 5-demethyltangeretin uptake is much more efficient for mean droplet diameters of 134 nm and 250 nm compared to 406 nm (Zheng et al., 2014).

4.1.5. Molecular interactions

All the effects described above may occur simultaneously, influencing each other. These are the so-called Interactions in the SLAMENGHI acronym. Molecular interactions, involved in the Matrix and in the Effectors of absorption factors, should be discussed separately. These are the interactions between the different nutrients and compounds from the food, and also with the digestive juice compounds from the host. For example, it is known that divalent minerals strongly interact with lipids, bile salts, and many lipophilic compounds, reducing their bioaccessibility/bioavailability (Joana Corte-Real & Bohn, 2018). Nutrient-nutrient interactions at the lipid droplet interface were already discussed in Marze (2015). Briefly, these nutrients (lipids, proteins, polysaccharides mainly) associate or compete at the droplet interface. Depending on the exact interaction, this may result in a) droplet destabilization by flocculation, coalescence, creaming, and/or b) inhibition of the interfacial activity of lipases and/or bile salts. These effects influence lipid digestion and in turn lipophilic compound bioaccessibility.

Molecular interactions were also described in the bulk, summarized in Marze (2013). In this context, the most studied compound appears to be dietary fiber. Some fibers were initially found to bind bile salts *in vitro* (Kritchevsky & Story, 1974; Story & Kritchevsky, 1976),

while all lipids in the mixed micelle were in the bound form (Vahouny, Tombes, Cassidy, Kritchevsky, & Gallo, 1980). The mechanism was similar to that exerted by synthetic bile salt sequestrates. Bioaccessibility studies were conducted to evaluate the effect of various dietary fibers. First, sugar beet pectin was found to reduce beta-carotene bioaccessibility (Xu, et al., 2014). In other studies, the reduction was found to be larger with a lower degree of methyl-esterification for citrus pectin (Verrijssen et al., 2014; Verrijssen et al., 2016), but the inverse trend was observed in the presence of phosphatidylcholine (Verrijssen, Verkempinck, Christiaens, Van Loey, & Hendrickx, 2015). Beta-carotene bioaccessibility was also decreased when a nanoemulsion was encapsulated in alginate beads (Zhang et al., 2016), or when a nanoemulsion was entrapped in a kappa-carrageenan gel (Soukoulis et al., 2017), all the more with increasing dietary fiber concentration. A decrease was also found for curcumin bioaccessibility when a nanoemulsion was encapsulated in either alginate or kappa-carrageenan beads (Zhang et al., 2016).

In vivo, some soluble dietary fibers were found to reduce cholesterol and lipid absorption in the rat (Ebihara & Schneeman, 1989). In a meta-analysis, a significant effect was confirmed in humans for fasting plasma cholesterol but not for lipid concentrations (Brown, Rosner, Willett, & Sacks, 1999). In two studies of carotenoid absorption in humans, it was found that some soluble dietary fibers did not change plasma cholesterol and lipid concentrations significantly, but nevertheless reduced plasma carotenoid concentrations significantly, except for canthaxanthin (Riedl, Linseisen, Hoffmann, & Wolfram, 1999; Rock & Swendseid, 1992). Although the in vitro results clearly show that soluble dietary fibers sequester bile salts and/or lipids, their effect *in vivo* is still not fully understood, and the few studies involving lipophilic compounds are thus the subject of speculations (Palafox-Carlos et al., 2011).

4.2. Relation between lipid digestion and lipophilic compound bioaccessibility

4.2.1. Simulation of lipophilic compound bioaccessibility

In order to study the interplay between triglyceride digestion and lipophilic vitamin bioaccessibility, a numerical simulation was developed (Marze, 2014, 2015). This was based on multi-agent modeling, which represents each molecular species as an autonomous particle with specific properties and rules. One static oil droplet with triglyceride and lipophilic vitamin particles and a continuous aqueous phase with lipase or lipase-bile salt particles was modeled, with an interface where digestion products compete with lipase/bile salt. Final

digestion products (monoglyceride and fatty acid particles) were produced after a given number of contacts with lipase at the interface, inversely proportional to experimental lipolysis rates for various triglycerides. Final digestion products and lipophilic vitamin were solubilized in simple bile salt micelles after a given number of contacts with bile salt at the interface, inversely proportional to experimental solubilization ratios (mass of solubilizate per mass of bile salt) for various fat-soluble vitamins and lipophilic drugs. All particles were set to diffuse according to their molecular mass and the viscosity of the oil phase. Lipase-bile salt particles were moved randomly to reflect their faster movements due to the low viscosity of water and some convective flows in the aqueous phase. Bile salt micelles were set to i) saturate at the solubilization ratio (static in vitro case), or ii) recycle by discharging their solubilizates (*in vivo* case).

The results showed that the bioaccessibility kinetics of lipophilic vitamin always followed the bioaccessibility kinetics of digestion products, slower with slowly digested triglycerides. The final lipophilic vitamin bioaccessibility was always lower in the saturated case compared to the recycled case. It decreased with the triglyceride chain length in the saturated case, whereas it increased with the triglyceride chain length in the recycled case. This was explained by an incomplete triglyceride digestion in the saturated case, whereas triglyceride digestion was complete in the recycled case. Whenever triglyceride digestion was complete or when comparing at a given triglyceride digestion degree, lipophilic vitamin bioaccessibility was always higher for longer chain triglycerides.

To validate these results experimentally, a semi-dynamic microfluidic digestion system was developed (Nguyen et al., 2019). Isolated droplets of triglycerides containing a lipophilic vitamin could be generated, immobilized, and digested under continuous digestive fluid flow and renewal, reflecting the recycled case of the simulation. The release kinetics of the digestion products and of the lipophilic vitamin could be monitored by measuring the droplet size and the vitamin autofluorescence using confocal fluorescence microscopy. The microfluidic digestion results were in very good qualitative agreement with the simulation results in the recycled case, except for the final lipophilic vitamin bioaccessibility, which was always underestimated in the first version of the simulation (Marze, 2014). A second version improved this discrepancy by accounting for additional factors, the formation of mixed micelles (digestion products-bile salt) being the most significant one (Marze, 2015).

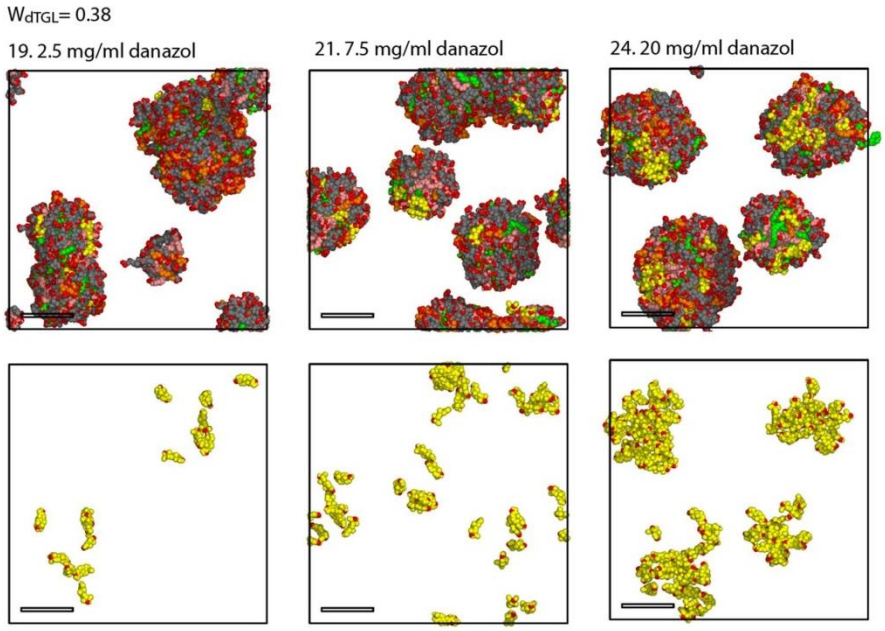
These findings suggest that triglyceride digestion should be complete when comparing lipophilic compound bioaccessibility for various triglycerides at a given intestinal digestion time (usually 2 hours). As the amount of bile salt is fixed in static in vitro protocols, this can be achieved by decreasing the amount of triglyceride. An alternative is to compare lipophilic compound bioaccessibility for various triglycerides at a given triglyceride digestion degree. This may require longer digestion duration for long chain triglycerides, which should be kept within the realistic limit of 4 hours (average small intestine transit time).

4.2.2. Simulation of mixed micelle incorporation

The last step making one lipophilic compound bioaccessible is its incorporation into mixed micelles. It is important to note that various other terms are used for this concept, such as solubilization, micellization, and of course bioaccessibility (which is relative to the initial amount). Mixed micelle is a generic term referring to nanoscale particles composed of bile constituents (bile salts, phospholipids, cholesterol) and digested lipids (fatty acids, monoglycerides, lysophospholipids). It generally refers to a spherical particle, although it was repeatedly shown that various structures are formed in vitro and *in vivo* (Hernell, Staggers, & Carey, 1990; Kossena, Boyd, Porter, & Charman, 2003). The major ones are the spherical micelle and vesicle, but ellipsoidal, disk-like, cylindrical structures (among others) were also reported (Euston, 2017; Sebastien Marze et al., 2015), depending on the relative amounts of the constituents. This is an important aspect, as these particles are likely to transport lipids and lipophilic compounds differently in the small intestine.

For lipophilic compound incorporation, the seminal works of Kossena et al. (2003) in the fasted state showed that various particles form as the fatty acid/monoglyceride amount varies in the bile mixture. Each particle was found to have a specific incorporation capacity for lipophilic drugs, increasing with the fatty acid/monoglyceride amount, and depending on their chain length and unsaturation degree (Kossena et al., 2003). Subsequent *in vivo* experiments demonstrated that lipophilic drug absorption in the rat also greatly varies depending on the type of particle infused into the duodenum, increasing proportionally with the solubilization capacity (Kossena, Charman, Boyd, & Porter, 2005). Such a relation is currently under systematic investigation for food lipophilic compounds in the fed state (Marze, project AssemBiles). There is indeed a substantial amount of data on solubilization capacity for lipophilic drugs in the fasted state (as compiled by Wiedmann and Kamel (2002)), but very few results for food lipophilic compounds in the fed state.

Mixed micelle incorporation of lipophilic compounds was stressed to be a key step toward absorption. However, mostly macroscopic data are available to quantify this incorporation, although it is controlled by interactions at the molecular scale. As studying these interactions precisely is challenging experimentally, computer simulations were also developed, mostly using molecular dynamics. Initially, bile salt, bile salt-phospholipid, or bile salt-phospholipid-cholesterol assemblies were modeled to characterize their structures (Euston, 2017). Recently, other lipids such as fatty acid were included in the simulations (Turner, Yin, Kindt, & Zhang, 2010), in order to study the incorporation of lipophilic compounds for more realistic assemblies. For this purpose, only two articles were published, about lipophilic drug incorporation in bile salt-phospholipid assemblies in the fasted state (Holmboe, Larsson, Anwar, & Bergstrom, 2016), or in bile salt-phospholipid-fatty acid-monoglyceride assemblies in both the fasted and fed states (Birru et al., 2017). The results in the fed state confirm that when the fatty acid-monoglyceride concentration increases, the assembly structure changes from micellar to lamellar, with a progressive particle size growth. These changes were accompanied by an increased incorporation of danazol (a lipophilic drug). Above the incorporation capacity, danazol became aggregated, hence not in its solubilized form anymore. Figure 6 illustrates these findings for several conditions. This type of simulation should be applied to the incorporation of food lipophilic compounds into mixed micelles, in order to study the universality of this behavior.



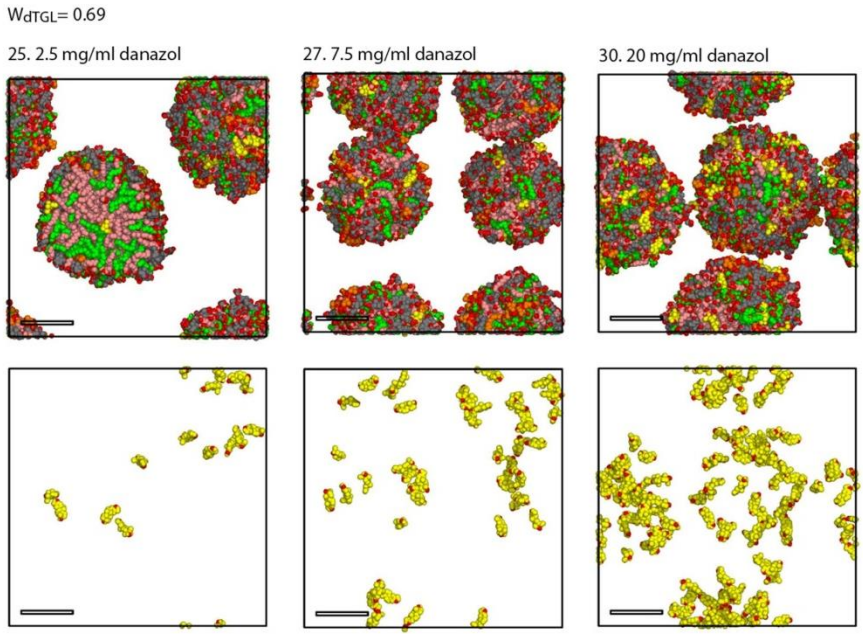


Figure 6: localization of danazol within the final frames of selected simulations, with changing danazol concentration. Danazol is colored yellow, glycodeoxycholate is grey, lysophosphocholine is orange, oleic acid is pink, glyceryl monooleate is green and oxygen atoms are red. The box indicates the periodic boundary and the scale bar length is 3.0 nm. Water atoms have been omitted. Reprinted with permission from Birru et al. (2017).

4.3. Lipid digestion in natural foods

Lipids in natural foods occur generally as in the form of complex structure in which triglycerides particles are coated with a solubilizing, stabilizing layer or multi-layer of membrane phospholipids and proteins. Breaking down the surrounding structures and releasing the lipid droplets from the cells, seed bodies or whatever locating matrix, has a profound influence on our ability to digest the lipid and use its components efficiently and effectively.

4.3.1. Milk fat globules

As it is well-known, milk is an oil-in-water emulsion containing milk fat globules (MFGs), which are constituted by a core rich in triglycerides and stabilized by the milk fat globule membrane (MFGM). MFGs have a mean diameter around 4 μm with a size range of 0.2 to 10 μm (Heid & Keenan, 2005; Lopez, 2011). The MFGM has a thickness of about 4–10 nm (trilayer) and is composed of polar lipids, specific membrane proteins, glycoproteins, cholesterol, and some other minor components (Lopez, 2011).

So far, the digestion behavior of different types of milks including raw milk has been studied *in vitro* and the effect of digestion on the microstructure, proteolysis, and lipolysis has been described (Berton et al., 2012; S. Gallier, Ye, & Singh, 2012; Ye, Cui, & Singh, 2011). S. Gallier et al. (2012) reported that due to a high zeta-potential, under *in vitro* gastric conditions, there was no coalescence or aggregation of MFGs observed. Since phospholipids present in the structure of MFGs are not digested in the stomach, the integrity of these globules is remained during gastric digestion (Gallier et al., 2012; Hamosh et al., 1999). Under *in vitro* intestinal conditions, however, the lipolytic products are solubilized when a lamellar phase of these products becomes accumulated at the surface of MFGs, and bile salt micelles formed (Gallier et al., 2012). Bile salts can displace some of the membrane on MFGs in order to allow the pancreatic lipase-colipase complex to anchor at the surface of the milk fat globule, as a result of the fact that the intestinal lipolysis of MFGs does not present a lag phase and is efficient (Gallier et al., 2012). The components of MFGs that are solubilized by bile salts, are then absorbed by the intestinal cells. Short and medium-chain fatty acids are quickly absorbed by the enterocytes and transported by the portal vein for orientation towards β-oxidation, while long-chain fatty acids are absorbed by the small intestinal enterocytes and transported though the lymphatics as chylomicrons (Gallier et al., 2012; Michalski, 2009).

4.3.1.2. Oil bodies (oleosomes)

Oil bodies, which may also be known as oleosomes, sphaerosomes, or spherosomes, are responsible for storage and synthesis of lipids in plant cells (about 98% of an oleosome is lipid), especially oil seeds. Oil bodies provide the required energy for the seed during germination and have a size in the range of 0.2 to 2.5 μm (Tzen & Huang, 1992). In fact, oleosomes are small cell organelles containing a single membrane. These organelles arise from endoplasmic reticulum and are bounded by a single (but half unit) membrane with a phospholipid monolayer (Tzen & Huang, 1992; Zielbauer et al., 2018). The phospholipids are composed of polar heads and hydrophobic tails towards the cytosol and the inner side, respectively. The membrane of oleosomes is stabilized by oleosins (proteins) and that is the reason for giving them the name ‘oleosomes’. However, only 2% of oleosomes is composed of the membrane proteins; some of these proteins have enzymatic functions by which they contribute to the synthesis of lipids (De Chirico, di Bari, Foster, & Gray, 2018; Zielbauer et al., 2018). There are three basic structural domains in every oleosin, including; a) an amphipathic domain present at both N-terminal and C-terminal, and b) a highly-hydrophobic domain which penetrates into the triacylglycerols matrix. The amphipathic domain resides on

the surface of oil bodies resulting in their stability via steric hindrance and electronegative repulsion (Frandsen, Mundy, & Tzen, 2001; Qu & Huang, 1990).

In regard with the digestion of oleosomes and oil bodies, there have been some research carried out *in vitro*. One interesting example is the work by Gallier and Singh (2012), who studied the physicochemical and structural changes of the almond oil body in an emulsion formulation under simulated gastrointestinal digestion environment. It was found that the emulsion containing almond oil body showed a similar behavior to that of a protein-stabilized emulsion, where the flocculation of the oil bodies occurred during the simulated gastric conditions. Throughout the gastric digestion, the surface of the oil bodies was covered by proteins, peptides, and phospholipids. Under simulated intestinal digestion, the interfacial peptides and phospholipids were displaced by bile salts so that the flocs were disrupted. A negative impact on the efficiency of gastric digestion was observed by the oil body membrane, which in turn led to the accusation of long chain fatty acids (the main lipolytic products) at the surface of the oil bodies, and correspondingly, limiting the activity of pancreatic lipase (Gallier & Singh, 2012).

5. Conclusions

The structure, metabolism, absorption, bioavailability, and bioaccessibility of lipid-soluble compounds, as well as the role of digestion regarding the relationship between lipid digestion and bioaccessibility of lipophilic compounds were discussed. To the best of our knowledge, the main aspects that still need to be studied in order to understand the role of gastrointestinal digestion on the bioavailability of lipophilic compounds are related to the molecular properties. First, the role of the molecular form and linkage of the compounds is not always clear. For example, the hydrolysis kinetics of esters is known only for a few compounds, and the effect of dietary fiber linkage to some polyphenols is mostly unknown. Second, the knowledge of the gastrointestinal metabolites of lipophilic compounds, how they are produced and how they are absorbed, lacks most of the time, except for a few model carotenoids and polyphenols. This hinders the understanding of the whole fate of the parent molecules from ingestion to excretion. Third, the interactions between nutrients other than lipids and lipophilic compounds, as well as the organization of the mixed micelles in the real situation of multiple nutrients are not well described. Competition between molecules for incorporation indeed seems to be more frequent than currently thought. Finally, the effect of natural or designed food structures (at all scales) on the liberation of some lipophilic

compounds should be accounted for, as it constitutes a major barrier to digestion and absorption in some cases.

References

Affandi, M., Julianto, T., & Majeed, A. B. A. (2012). Enhanced Oral Bioavailability of Astaxanthin with Droplet Size Reduction. *Food Science and Technology Research, 18*(4), 549-554.

Aherne, S. A., & O'Brien, N. M. (2002). Dietary flavonols: chemistry, food content, and metabolism. *Nutrition, 18*(1), 75-81.

Ahmed, K., Li, Y., McClements, D. J., & Xiao, H. (2012). Nanoemulsion- and emulsion-based delivery systems for curcumin: Encapsulation and release properties. *Food Chemistry, 132*(2), 799-807.

Alessandrì, M. G., Scalori, V., Giovannini, L., Mian, M., & Bertelli, A. A. (1988). Plasma and tissue concentrations of coenzyme Q10 in the rat after intravenous administration by a microsphere delivery system or in a new type of solution. *International journal of tissue reactions, 10*(2), 99-102.

Azzi, A. (2018). Many tocopherols, one vitamin E. *Molecular aspects of medicine, 61*, 92-103.

Berton, A., Rouvellac, S., Robert, B., Rousseau, F., Lopez, C., & Crenon, I. (2012). Effect of the size and interface composition of milk fat globules on their in vitro digestion by the human pancreatic lipase: Native versus homogenized milk fat globules. *Food Hydrocolloids, 29*(1), 123-134.

Bhagavan, H. N., & Chopra, R. K. (2006). Coenzyme Q10: Absorption, tissue uptake, metabolism and pharmacokinetics. *Free Radical Research, 40*(5), 445-453.

Birru, W. A., Warren, D. B., Han, S. F., Benameur, H., Porter, C. J. H., Pouton, C. W., & Chalmers, D. K. (2017). Computational Models of the Gastrointestinal Environment. 2. Phase Behavior and Drug Solubilization Capacity of a Type I Lipid-Based Drug Formulation after Digestion. *Molecular Pharmaceutics, 14*(3), 580-592.

Blaner, W. S., Obunike, J. C., Kurlandsky, S. B., Al-Haideri, M., Piantedosi, R., Deckelbaum, R. J., & Goldberg, I. J. (1994). Lipoprotein lipase hydrolysis of retinyl ester. Possible implications for retinoid uptake by cells. *Journal of Biological Chemistry, 269*(24), 16559-16565.

Blomstrand, R., & Werner, B. (1967). Studies on the intestinal absorption of radioactive β-carotene and vitamin A in man: conversion of β-carotene into vitamin A. *Scandinavian Journal of Clinical and Laboratory Investigation, 19*(4), 339-345.

Bohn, T. (2014). Dietary factors affecting polyphenol bioavailability. *Nutrition Reviews, 72*(7), 429-452.

Borel, P. (2003). Factors affecting intestinal absorption of highly lipophilic food microconstituents (fat-soluble vitamins, carotenoids and phytosterols). *Clinical Chemistry and Laboratory Medicine, 41*(8), 979-994.

Borel, P. (2009). CHAPTER 3 - Nutrigenetic Effect on Intestinal Absorption of Fat-Soluble Microconstituents (Vitamins A, E, D and K, Carotenoids and Phytosterols). In G. Barbosa-Cánovas, A. Mortimer, D. Lineback, W. Spiess, K. Buckle, & P. Colonna (Eds.), *Global Issues in Food Science and Technology* (pp. 31-42). San Diego: Academic Press.

Borel, P., Pasquier, B., Armand, M., Tyssandier, V., Grolier, P., Alexandre-Gouabau, M. C., Azais-Braesco, V. (2001). Processing of vitamin A and E in the human gastrointestinal tract. *American Journal of Physiology-Gastrointestinal and Liver Physiology, 280*(1), G95-G103.

Borel, P., Tyssandier, V., Mekki, N., Grolier, P., Rochette, Y., Alexandre-Gouabau, M. C., Azais-Braesco, V. (1998). Chylomicron beta-carotene and retinyl palmitate responses are dramatically diminished when men ingest beta-carotene with medium-chain rather than long-chain triglycerides. *Journal of Nutrition, 128*(8), 1361-1367.

Bowen, P. E., Herbst-Espinosa, S. M., Hussain, E. A., & Stacewicz-Sapuntzakis, M. (2002). Esterification Does Not Impair Lutein Bioavailability in Humans. *The Journal of Nutrition, 132*(12), 3668-3673.

Brigelius-Flohe, R., & Traber, M. G. (1999). Vitamin E: function and metabolism. *The FASEB Journal, 13*(10), 1145-1155.

Brown, L., Rosner, B., Willett, W. W., & Sacks, F. M. (1999). Cholesterol-lowering effects of dietary fiber: a meta-analysis. *American Journal of Clinical Nutrition, 69*(1), 30-42.

Brown, M. J., Ferruzzi, M. G., Nguyen, M. L., Cooper, D. A., Eldridge, A. L., Schwartz, S. J., & White, W. S. (2004). Carotenoid bioavailability is higher from salads ingested with full-fat than with fat-reduced salad dressings as measured with electrochemical detection. *American Journal of Clinical Nutrition, 80*(2), 396-403.

Castenmiller, J. J. M., & West, C. E. (1998). Bioavailability and bioconversion of carotenoids. *Annual Review of Nutrition, 18*, 19-38.

Cho, H. T., Salvia-Trujillo, L., Kim, J., Park, Y., Xiao, H., & McClements, D. (2014). Droplet size and composition of nutraceutical nanoemulsions influences bioavailability of long chain fatty acids and Coenzyme Q10. *Food Chemistry, 156*, 117-122.

Chopra, R. K., Goldman, R., Sinatra, S. T., & Bhagavan, H. N. (1998). Relative bioavailability of coenzyme Q10 formulations in human subjects. *International journal for vitamin and nutrition research. Internationale Zeitschrift fur Vitamin- und Ernahrungsforschung. Journal International de Vitaminologie et de Nutrition, 68*(2), 109-113.

Cilla, A., De Palma, G., Lagarda, M. J., Barberá, R., Farré, R., Clemente, G., & Romero, F. (2009). Impact of Fruit Beverage Consumption on the Antioxidant Status in Healthy Women. *Annals of Nutrition and Metabolism, 54*(1), 35-42.

Colle, I. J. P., Van Buggenhout, S., Lemmens, L., Van Loey, A. M., & Hendrickx, M. E. (2012). The type and quantity of lipids present during digestion influence the in vitro bioaccessibility of lycopene from raw tomato pulp. *Food Research International, 45*(1), 250-255.

Conaway, H. H., Henning, P., & Lerner, U. H. (2013). Vitamin A Metabolism, Action, and Role in Skeletal Homeostasis. *Endocrine Reviews, 34*(6), 766-797.

Cook, N., & Samman, S. (1996). Flavonoids—chemistry, metabolism, cardioprotective effects, and dietary sources. *Journal of Nutrition Biochemestry, 7*(2), 66-76.

Cooper, A. D. (1997). Hepatic uptake of chylomicron remnants. *Journal of Lipid Research, 38*(11), 2173-2192.

Corte-Real, J., & Bohn, T. (2018). Interaction of divalent minerals with liposoluble nutrients and phytochemicals during digestion and influences on their bioavailability–a review. *Food Chemistry, 252*, 285-293.

Corte-Real, J., Desmarchelier, C., Borel, P., Richling, E., Hoffmann, L., & Bohn, T. (2018). Magnesium affects spinach carotenoid bioaccessibility in vitro depending on intestinal bile and pancreatic enzyme concentrations. *Food Chemistry, 239*, 751-759.

D'Ambrosio, D. N., Clugston, R. D., & Blaner, W. S. (2011). Vitamin A metabolism: an update. *Nutrients, 3*(1), 63-103.

da Costa, G. A., & Mercadante, A. Z. (2018). In vitro bioaccessibility of free and esterified carotenoids in cajá frozen pulp-based beverages. *Journal of Food Composition and Analysis, 68*, 53-59.

Data, S. N. (2013). Nutrition facts, calories in food, labels, nutritional information and analysis.

De Chirico, S., di Bari, V., Foster, T., & Gray, D. (2018). Enhancing the recovery of oilseed rape seed oil bodies (oleosomes) using bicarbonate-based soaking and grinding media. *Food Chemistry, 241*, 419-426.

DeMan, J. M. (1999). Principles of food chemistry—3rd ed. A Chapman & Hall Food Science BookAspen Publishers. *Inc. Gaithersburg, Maryland*.

Déprez, S., Brezillon, C., Rabot, S., Philippe, C., Mila, I., Lapierre, C., & Scalbert, A. (2000). Polymeric proanthocyanidins are catabolized by human colonic microflora into low-molecular-weight phenolic acids. *Journal of Nutrition, 130*(11), 2733-2738.

Deprez, S., Mila, I., Huneau, J.-F., Tome, D., & Scalbert, A. (2001). Transport of Proanthocyanidin Dimer, Trimer, and Polymer Across Monolayers of Human Intestinal Epithelial Caco-2 Cells. *Antioxidants & Redox Signaling, 3*(6), 957-967.

Dutton, P. L., Ohnishi, T., Darrouzet, E., Leonard, M. A., Sharp, R. E., Cibney, B., Moser, C. C. (2000). Coenzyme Q oxidation reduction reactions in mitochondrial electron transport. *Coenzyme Q: Molecular Mechanisms in Health and Disease*, 65-82.

Ebihara, K., & Schneeman, B. O. (1989). Interaction of bile-acids, phospholipids, cholesterol and triglyceride with dietary-fibers in the small-intestine of rats. *Journal of Nutrition, 119*(8), 1100-1106.

Eby, C. S. (2016). Bleeding and Vitamin K Deficiency. In J. Teruya (Ed.), *Management of Bleeding Patients* (pp. 145-150). Cham: Springer International Publishing.

Episkopou, V., Maeda, S., Nishiguchi, S., Shimada, K., Gaitanaris, G. A., Gottesman, M. E., & Robertson, E. J. (1993). Disruption of the transthyretin gene results in mice with depressed levels of plasma retinol and thyroid hormone. *Proceedings of the National Academy of Sciences, 90*(6), 2375-2379.

Erlanson, C., & Borgström, B. (1968). The identity of vitamin A esterase activity of rat pancreatic juice. *Biochimica et Biophysica Acta (BBA) - Enzymology, 167*(3), 629-631.

Ernster, L., & Dallner, G. (1995). Biochemical, physiological and medical aspects of ubiquinone function. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1271*(1), 195-204.

Euston, S. R. (2017). Molecular simulation of biosurfactants with relevance to food systems. *Current Opinion in Colloid & Interface Science, 28*, 110-119.

Fahy, E., Subramaniam, S., Brown, H. A., Glass, C. K., Merrill Jr., A. H., Murphy, R. C., Dennis, E. A. (2005). A comprehensive classification system for lipids. *European Journal of Lipid Science and Technology, 107*(5), 337-364.

Feliciano, R. P., Mills, C. E., Istas, G., Heiss, C., & Rodriguez-Mateos, A. (2017). Absorption, Metabolism and Excretion of Cranberry (Poly) phenols in Humans: A Dose Response Study and Assessment of Inter-Individual Variability. *Nutrients, 9*(3).

Fraga, C. G., Schramm, D. D., Schmitz, H. H., Hammerstone, J. F., Sullards, M. C., Zhu, Q. Y., Keen, C. L. (2002). Procyanidin dimer B2 [epicatechin-(4β-8)-epicatechin] in human plasma after the consumption of a flavanol-rich cocoa. *The American Journal of Clinical Nutrition, 76*(4), 798-804.

Frandsen, G. I., Mundy, J., & Tzen, J. T. (2001). Oil bodies and their associated proteins, oleosin and caleosin. *Physiologia Plantarum, 112*(3), 301-307.

Fukuwatari, T., & Shibata, K. (2008). Urinary water-soluble vitamins and their metabolite contents as nutritional markers for evaluating vitamin intakes in young Japanese women. *Journal of nutritional science and vitaminology, 54*(3), 223-229.

Galli, F., Azzi, A., Birringer, M., Cook-Mills, J. M., Eggersdorfer, M., Frank, J., Özer, N. K. (2017). Vitamin E: Emerging aspects and new directions. *Free Radical Biology and Medicine, 102*, 16-36.

Gallier, S., & Singh, H. (2012). Behavior of almond oil bodies during in vitro gastric and intestinal digestion. *Food & Function, 3*(5), 547-555.

Gallier, S., Ye, A., & Singh, H. (2012). Structural changes of bovine milk fat globules during in vitro digestion. *Journal of Dairy Science, 95*(7), 3579-3592.

Giammanco, A., Cefalù, A. B., Noto, D., & Averna, M. R. (2015). The pathophysiology of intestinal lipoprotein production. *Frontiers in Physiology, 6*(61).

Gleize, B., Tourniaire, F., Depezay, L., Bott, R., Nowicki, M., Albino, L., Borel, P. (2013). Effect of type of TAG fatty acids on lutein and zeaxanthin bioavailability. *British Journal of Nutrition, 110*(1), 1-10.

Goltz, S. R., Campbell, W. W., Chitchumroonchokchai, C., Failla, M. L., & Ferruzzi, M. G. (2012). Meal triacylglycerol profile modulates postprandial absorption of carotenoids in humans. *Molecular Nutrition & Food Research, 56*(6), 866-877.

Gomez, M., & Martinez, M. M. (2018). Fruit and vegetable by-products as novel ingredients to improve the nutritional quality of baked goods. *Critical Reviews in Food Science and Nutrition, 58*(13), 2119-2135.

Halder, M., Petsophonsakul, P., Akbulut, A. C., Pavlic, A., Bohan, F., Anderson, E., Schurgers, L. (2019). Vitamin K: Double Bonds beyond Coagulation Insights into Differences between Vitamin K1 and K2 in Health and Disease. *International Journal of Molecular Sciences, 20*(4), 896.

Hamosh, M., Peterson, J. A., Henderson, T. R., Scallan, C. D., Kiwan, R., Ceriani, R. L., Hamosh, P. (1999). Protective function of human milk: The milk fat globule. *Seminars in Perinatology, 23*(3), 242-249.

Harrison, E. H. (2012). Mechanisms involved in the intestinal absorption of dietary vitamin A and provitamin A carotenoids. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1821*(1), 70-77.

Harrison, E. H., & Hussain, M. M. (2001). Mechanisms involved in the intestinal digestion and absorption of dietary vitamin A. *The Journal of nutrition, 131*(5), 1405-1408.

Heid, H. W., & Keenan, T. W. (2005). Intracellular origin and secretion of milk fat globules. *European Journal of Cell Biology, 84*(2-3), 245-258.

Hernández-Camacho, J. D., Bernier, M., López-Lluch, G., & Navas, P. (2018). Coenzyme Q10 Supplementation in Aging and Disease. *Frontiers in Physiology, 9*(44).

Hernell, O., Staggers, J. E., & Carey, M. C. (1990). Physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. 2. Phase analysis and aggregation states of luminal lipids during duodenal fat digestion in healthy adult human beings. *Biochemistry, 29*(8), 2041-2056.

Holick, M. F. (2004). Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *The American Journal of Clinical Nutrition, 80*(6), 1678S-1688S.

Holmberg, I., Aksnes, L., Berlin, T., Lindback, B., Zemgals, J., & Lindeke, B. (1990). Absorption of a pharmacological dose of vitamin-d3 from 2 different lipid vehicles in man - comparison of peanut oil and a medium chain triglyceride. *Biopharmaceutics & Drug Disposition, 11*(9), 807-815.

Holmboe, M., Larsson, P., Anwar, J., & Bergstrom, C. A. S. (2016). Partitioning into Colloidal Structures of Fasted State Intestinal Fluid Studied by Molecular Dynamics Simulations. *Langmuir, 32*(48), 12732-12740.

Hosomi, A., Arita, M., Sato, Y., Kiyose, C., Ueda, T., Igarashi, O., Inoue, K. (1997). Affinity for α-tocopherol transfer protein as a determinant of the biological activities of vitamin E analogs. *FEBS letters, 409*(1), 105-108.

Hou, Z. Q., Liu, Y. W., Lei, F., & Gao, Y. X. (2014). Investigation into the in vitro release properties of beta-carotene in emulsions stabilized by different emulsifiers. *Lwt-Food Science and Technology, 59*(2), 867-873.

Huo, T., Ferruzzi, M. G., Schwartz, S. J., & Failla, M. L. (2007). Impact of fatty acyl composition and quantity of triglycerides on bioaccessibility of dietary carotenoids. *Journal of Agricultural and Food Chemistry, 55*(22), 8950-8957.

Kaempf-Rotzoll, D. E., Traber, M. G., & Arai, H. (2003). Vitamin E and transfer proteins. *Current Opinion in Lipidology, 14*(3), 249-254.

Kayden, H. J., & Traber, M. G. (1993). Absorption, lipoprotein transport, and regulation of plasma concentrations of vitamin E in humans. *Journal of Lipid Research, 34*(3), 343-358.

Kossena, G. A., Boyd, B. J., Porter, C. J. H., & Charman, W. N. (2003). Separation and characterization of the colloidal phases produced on digestion of common formulation lipids and assessment of their impact on the apparent solubility of selected poorly water-soluble drugs. *Journal of Pharmaceutical Sciences, 92*(3), 634-648.

Kossena, G. A., Charman, W. N., Boyd, B. J., & Porter, C. I. H. (2005). Influence of the intermediate digestion phases of common formulation lipids on the absorption of a poorly water-soluble drug. *Journal of Pharmaceutical Sciences, 94*(3), 481-492.

Kramer, W., Girbig, F., Corsiero, D., Burger, K., Fahrenholz, F., Jung, C., & Müller, G. (2003). Intestinal cholesterol absorption: identification of different binding proteins for cholesterol and cholesterol absorption inhibitors in the enterocyte brush border membrane. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, 1633*(1), 13-26.

Kramer, W., Glombik, H., Petry, S., Heuer, H., Schäfer, H.-L., Wendler, W., Weyland, C. (2000). Identification of binding proteins for cholesterol absorption inhibitors as components of the intestinal cholesterol transporter. *FEBS letters, 487*(2), 293-297.

Kreis, W., & Müller-Uri, F. (2018). Biochemistry of Sterols, Cardiac Glycosides, Brassinosteroids, Phytoecdysteroids and Steroid Saponins *Annual Plant Reviews Online* (pp. 304-363).

Kritchevsky, D., & Story, J. A. (1974). Binding of bile-salts in-vitro by nonnutritive fiber. *Journal of Nutrition, 104*(4), 458-462.

Kuroda, T., Uenishi, K., Ohta, H., & Shiraki, M. (2019). Multiple vitamin deficiencies additively increase the risk of incident fractures in Japanese postmenopausal women. *Osteoporosis International, 30*(3), 593-599.

Kurosu, M. (2017). Vitamin K2 Biosynthesis: Drug Targets for New Antibacterials *Vitamin K2-Vital for Health and Wellbeing*: IntechOpen.

Lee, H. C., Jenner, A. M., Low, C. S., & Lee, Y. K. (2006). Effect of tea phenolics and their aromatic fecal bacterial metabolites on intestinal microbiota. *Research in Microbiology, 157*(9), 876-884.

López-Lluch, G., del Pozo-Cruz, J., Sánchez-Cuesta, A., Cortés-Rodríguez, A. B., & Navas, P. (2019). Bioavailability of coenzyme Q10 supplements depends on carrier lipids and solubilization. *Nutrition, 57*, 133-140.

Lopez, C. (2011). Milk fat globules enveloped by their biological membrane: unique colloidal assemblies with a specific composition and structure. *Current Opinion in Colloid & Interface Science, 16*(5), 391-404.

Majeed, H., Antoniou, J., Hategekimana, J., Sharif, H. R., Haider, J., Liu, F., Zhong, F. (2016). Influence of carrier oil type, particle size on in vitro lipid digestion and eugenol release in emulsion and nanoemulsions. *Food Hydrocolloids, 52*, 415-422.

Manach, C., Morand, C., Gil-Izquierdo, A., Bouteloup-Demange, C., & Remesy, C. (2003). Bioavailability in humans of the flavanones hesperidin and narirutin after the ingestion of two doses of orange juice. *European Journal of Clinical Nutrition, 57*(2), 235-242.

Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: food sources and bioavailability. *The American Journal of Clinical Nutrition, 79*(5), 727-747.

Manolescu, B., Atanasiu, V., Cercasov, C., Stoian, I., Oprea, E., & Buşu, C. (2008). So many options but one choice: the human body prefers α-tocopherol. A matter of stereochemistry. *Journal of Medicine and Life, 1*(4), 376.

Mao, L., & Miao, S. (2015). Structuring Food Emulsions to Improve Nutrient Delivery During Digestion. *Food Engineering Reviews, 7*(4), 439-451.

Maqbool, A., & Stallings, V. A. (2008). Update on fat-soluble vitamins in cystic fibrosis. *Current Opinion in Pulmonary Medicine, 14*(6), 574-581.

Marze, S. (2013). Bioaccessibility of Nutrients and Micronutrients from Dispersed Food Systems: Impact of the Multiscale Bulk and Interfacial Structures. *Critical Reviews in Food Science and Nutrition, 53*(1), 76-108.

Marze, S. (2014). A coarse-grained simulation to study the digestion and bioaccessibility of lipophilic nutrients and micronutrients in emulsion. *Food & Function, 5*(1), 129-139.

Marze, S. (2015). Bioaccessibility of lipophilic micro-constituents from a lipid emulsion. *Food & Function, 6*(10), 3218-3227.

Marze, S., Gaillard, C., & Roblin, P. (2015). In vitro digestion of emulsions: high spatiotemporal resolution using synchrotron SAXS. *Soft Matter, 11*(26), 5365-5373.

McClements, D. J., Li, F., & Xiao, H. (2015). The Nutraceutical Bioavailability Classification Scheme: Classifying Nutraceuticals According to Factors Limiting their Oral Bioavailability. *Annual Review of Food Science and Technology, 6*(1), 299-327.

McClements, D. J., & Xiao, H. (2012). Potential biological fate of ingested nanoemulsions: influence of particle characteristics. *Food & Function, 3*(3), 202-220.

Meroni, E., & Raikos, V. (2018). Physicochemical stability, antioxidant properties and bioaccessibility of beta-carotene in orange oil-in-water beverage emulsions: influence of carrier oil types. *Food & Function, 9*(1), 320-330.

Michalski, M. C. (2009). Specific molecular and colloidal structures of milk fat affecting lipolysis, absorption and postprandial lipemia. *European Journal of Lipid Science and Technology, 111*(5), 413-431.

Monsen, E. R. (2000). Dietary reference intakes for the antioxidant nutrients: vitamin C, vitamin E, selenium, and carotenoids. *Journal of the Academy of Nutrition and Dietetics, 100*(6), 637.

Nguyen, H. T., Marquis, M., Anton, M., & Marze, S. (2019). Studying the real-time interplay between triglyceride digestion and lipophilic micronutrient bioaccessibility using droplet microfluidics. 2 application to various oils and (pro)vitamins. *Food Chemistry, 275*, 661-667.

Nidhi, B., & Baskaran, V. (2011). Influence of Vegetable Oils on Micellization of Lutein in a Simulated Digestion Model. *Journal of the American Oil Chemists Society, 88*(3), 367-372.

Norman, A. W. (2008). From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. *The American Journal of Clinical Nutrition, 88*(2), 491S-499S.

Ochiai, A., Itagaki, S., Kurokawa, T., Kobayashi, M., Hirano, T., & Iseki, K. (2007). Improvement in intestinal coenzyme Q10 absorption by food intake. *Yakugaku Zasshi, 127*(8), 1251-1254.

Ozawa, Y., Mizushima, Y., Koyama, I., Akimoto, M., Yamagata, Y., Hayashi, H., & Murayama, H. (1986). Intestinal absorption enhancement of coenzyme Q10 with a lipid microsphere. *Arzneimittel-Forschung, 36*(4), 689-690.

Porter, C. J., Trevaskis, N. L., & Charman, W. N. (2007). Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. *Nature reviews Drug discovery, 6*(3), 231.

Qu, R., & Huang, A. (1990). Oleosin KD 18 on the surface of oil bodies in maize. Genomic and cDNA sequences and the deduced protein structure. *Journal of Biological Chemistry, 265*(4), 2238-2243.

Quideau, S., Deffieux, D., Douat-Casassus, C., & Pouységu, L. (2011). Plant Polyphenols: Chemical Properties, Biological Activities, and Synthesis. *Angewandte Chemie International Edition, 50*(3), 586-621.

Quinzii, C. M., DiMauro, S., & Hirano, M. (2007). Human Coenzyme Q10 Deficiency. *Neurochemical Research, 32*(4), 723-727.

Ravisankar, P., Reddy, A. A., Nagalakshmi, B., Koushik, O., Kumar, B., & Anvith, P. (2015). The comprehensive review on fat soluble vitamins. *IOSR Journal of Pharmacy, 5*(11), 12-28.

Reboul, E. (2015). Intestinal absorption of vitamin D: from the meal to the enterocyte. *Food & Function, 6*(2), 356-362.

Rechner, A. R., Kuhnle, G., Bremner, P., Hubbard, G. P., Moore, K. P., & Rice-Evans, C. A. (2002). The metabolic fate of dietary polyphenols in humans. *Free Radical Biology and Medicine, 33*(2), 220-235.

Rein, M. J., Renouf, M., Cruz-Hernandez, C., Actis-Goretta, L., Thakkar, S. K., & da Silva Pinto, M. (2013). Bioavailability of bioactive food compounds: a challenging journey to bioefficacy. *British Journal of Clinical Pharmacology, 75*(3), 588-602.

Riedl, J., Linseisen, J., Hoffmann, J., & Wolfram, G. (1999). Some dietary fibers reduce the absorption of carotenoids in women. *Journal of Nutrition, 129*(12), 2170-2176.

Rock, C. L., & Swendseid, M. E. (1992). Plasma beta-carotene response in humans after meals supplemented with dietary pectin. *American Journal of Clinical Nutrition, 55*(1), 96-99.

Salvia-Trujillo, L., Verkempinck, S., Rijal, S. K., Van Loey, A., Grauwet, T., & Hendrickx, M. (2019). Lipid nanoparticles with fats or oils containing beta-carotene: Storage stability and in vitro digestibility kinetics. *Food Chemistry, 278*, 396-405.

Sankar, M. J., Chandrasekaran, A., Kumar, P., Thukral, A., Agarwal, R., & Paul, V. K. (2016a). Vitamin K prophylaxis for prevention of vitamin K deficiency bleeding: a systematic review. *Journal of Perinatology, 36*, S29.

Sankar, M. J., Chandrasekaran, A., Kumar, P., Thukral, A., Agarwal, R., & Paul, V. K. (2016b). Vitamin K prophylaxis for prevention of vitamin K deficiency bleeding: a systematic review. *Journal of Perinatology, 36*(S1), S29.

Sauvant, P., Mekki, N., Charbonnier, M., Portugal, H., Lairon, D., & Borel, P. (2003). Amounts and types of fatty acids in meals affect the pattern of retinoids secreted in human chylomicrons after a high-dose preformed vitamin A intake. *Metabolism-Clinical and Experimental, 52*(4), 514-519.

Scalbert, A., Morand, C., Manach, C., & Rémésy, C. (2002). Absorption and metabolism of polyphenols in the gut and impact on health. *Biomedicine & Pharmacotherapy, 56*(6), 276-282.

Segura, R., Javierre, C., Lizarraga, M. A., & Ros, E. (2006). *Other relevant components of nuts: Phytosterols, folate and minerals* (Vol. 96 Suppl 2).

Shah, B. R., Zhang, C. L., Li, Y., & Li, B. (2016). Bioaccessibility and antioxidant activity of curcumin after encapsulated by nano and Pickering emulsion based on chitosan-tripolyphosphate nanoparticles. *Food Research International, 89*, 399-407.

Soukoulis, C., Tsevdou, M., Andre, C. M., Cambier, S., Yonekura, L., Taoukis, P. S., & Hoffmann, L. (2017). Modulation of chemical stability and in vitro bioaccessibility of beta-carotene loaded in kappa-carrageenan oil-in-gel emulsions. *Food Chemistry, 220*, 208-218.

Sporn, M. (1994). The Retinoids, Sporn MB, Roberts AB, Goodman DS eds: New York Raven Press.

Stalmach, A., Williamson, G., & Crozier, A. (2014). Impact of dose on the bioavailability of coffee chlorogenic acids in humans. *Food & Function, 5*(8), 1727-1737.

Story, J. A., & Kritchevsky, D. (1976). Comparison of binding of various bile-acids and bile-salts invitro by several types of fiber. *Journal of Nutrition, 106*(9), 1292-1294.

Strobbe, S., De Lepeleire, J., & Van Der Straeten, D. (2018). From in planta function to vitamin-rich food crops: the ACE of biofortification. *Frontiers in plant science, 9*.

Tanumihardjo, S. A. (2011). Vitamin A: biomarkers of nutrition for development. *The American Journal of Clinical Nutrition, 94*(2), 658S-665S.

Tarko, T., Duda-Chodak, A., & Zajac, N. (2013). Digestion and absorption of phenolic compounds assessed by in vitro simulation methods. A review. *Roczniki Państwowego Zakładu Higieny, 64*(2), 79-84.

Turley, S. D., & Dietschy, J. M. (2003). Sterol absorption by the small intestine. *Current Opinion in Lipidology, 14*(3), 233-240.

Turner, D. C., Yin, F. C., Kindt, J. T., & Zhang, H. L. (2010). Molecular Dynamics Simulations of Glycocholate-Oleic Acid Mixed Micelle Assembly. *Langmuir, 26*(7), 4687-4692.

Tyssandier, V., Lyan, B., & Borel, P. (2001). Main factors governing the transfer of carotenoids from emulsion lipid droplets to micelles. *Biochimica Et Biophysica Acta-Molecular and Cell Biology of Lipids, 1533*(3), 285-292.

Tzen, J., & Huang, A. (1992). Surface structure and properties of plant seed oil bodies. *The Journal of Cell Biology, 117*(2), 327-335.

USDA. (2017). Rank order of vitamin A content in foods per 100 g. *USDA National Nutrient Database, March 2017*.

Vahouny, G. V., Tombes, R., Cassidy, M. M., Kritchevsky, D., & Gallo, L. L. (1980). Dietary-fibers .5. Bindaing of bile-salts, phospholipids and cholesterol from mixed micelles by bile-acid sequestrants and dietary-fibers. *Lipids, 15*(12), 1012-1018.

van Bennekum, A. M., Wei, S., Gamble, M. V., Vogel, S., Piantedosi, R., Gottesman, M., Blaner, W. S. (2001). Biochemical basis for depressed serum retinol levels in transthyretin-deficient mice. *Journal of Biological Chemistry, 276*(2), 1107-1113.

Verrijssen, T. A. J., Balduyck, L. G., Christiaens, S., Van Loey, A. M., Van Buggenhout, S., & Hendrickx, M. E. (2014). The effect of pectin concentration and degree of methyl-esterification on the in vitro bioaccessibility of beta-carotene-enriched emulsions. *Food Research International, 57*, 71-78.

Verrijssen, T. A. J., Christiaens, S., Verkempinck, S. H. E., Boeve, J., Grauwet, T., Van Loey, A. M., . . . Hendrickx, M. E. (2016). In vitro -Carotene Bioaccessibility and Lipid Digestion in Emulsions: Influence of Pectin Type and Degree of Methyl-Esterification. *Journal of Food Science, 81*(10), C2327-C2336.

Verrijssen, T. A. J., Verkempinck, S. H. E., Christiaens, S., Van Loey, A. M., & Hendrickx, M. E. (2015). The effect of pectin on in vitro beta-carotene bioaccessibility and lipid digestion in low fat emulsions. *Food Hydrocolloids, 49*, 73-81.

Weber, C., Bysted, A., & Hølmer, G. (1997). Intestinal absorption of coenzyme Q10 administered in a meal or as capsules to healthy subjects. *Nutrition Research, 17*(6), 941-945.

Wei, J. H., Yin, X., & Welander, P. V. (2016). Sterol Synthesis in Diverse Bacteria. *Frontiers in Microbiology, 7*(990).

West, C. E. (1996). Strategies to control nutritional anemia. *American Journal of Clinical Nutrition, 64*(5), 789-790.

White, W. S., Zhou, Y., Crane, A., Dixon, P., Quadt, F., & Flendrig, L. M. (2017). Modeling the dose effects of soybean oil in salad dressing on carotenoid and fat-soluble vitamin bioavailability in salad vegetables. *American Journal of Clinical Nutrition, 106*(4), 1041-1051.

WHO. (2009). Global prevalence of vitamin A deficiency in populations at risk 1995–2005. WHO Global Database on Vitamin A Deficiency. *Newsletter, 4*(2), 5–6, 8.

Wiedmann, T. S., & Kamel, L. (2002). Examination of the solubilization of drugs by bile salt micelles. *Journal of Pharmaceutical Sciences, 91*(8), 1743-1764.

Williamson, G., Day, A., Plumb, G., & Couteau, D. (2000). Human metabolic pathways of dietary flavonoids and cinnamates. *Biochemical Society Transactions, 28*(2), 16-21.

Wolf, G. (2001). The discovery of the visual function of vitamin A. *The Journal of nutrition, 131*(6), 1647-1650.

Wright, A. J., Pietrangelo, C., & MacNaughton, A. (2008). Influence of simulated upper intestinal parameters on the efficiency of beta carotene micellarisation using an in vitro model of digestion. *Food Chemistry, 107*(3), 1253-1260.

Xavier, A. A. O., Mercadante, A. Z., Garrido-Fernandez, J., & Perez-Galvez, A. (2014). Fat content affects bioaccessibility and efficiency of enzymatic hydrolysis of lutein esters added to milk and yogurt. *Food Research International, 65*, 171-176.

Xu, D., X.,, Yuan, F., Gao, Y. X., Panya, A., McClements, D. J., & Decker, E. A. (2014). Influence of whey protein-beet pectin conjugate on the properties and digestibility of beta-carotene emulsion during in vitro digestion. *Food Chemistry, 156*, 374-379.

Xu, F., Pandya, J. K., Chung, C., McClements, D. J., & Kinchla, A. J. (2018). Emulsions as delivery systems for gamma and delta tocotrienols: Formation, properties and simulated gastrointestinal fate. *Food Research International, 105*, 570-579.

Ye, A., Cui, J., & Singh, H. (2011). Proteolysis of milk fat globule membrane proteins during in vitro gastric digestion of milk. *Journal of Dairy Science, 94*(6), 2762-2770.

Yuan, X., Liu, X. J., McClements, D. J., Cao, Y., & Xiao, H. (2018). Enhancement of phytochemical bioaccessibility from plant-based foods using excipient emulsions: impact of lipid type on carotenoid solubilization from spinach. *Food & Function, 9*(8), 4352-4365.

Zhang, Z. P., Zhang, R. J., Zou, L. Q., Chen, L., Ahmed, Y., Al Bishri, W., McClements, D. J. (2016). Encapsulation of curcumin in polysaccharide-based hydrogel beads: Impact of bead type on lipid digestion and curcumin bioaccessibility. *Food Hydrocolloids, 58*, 160-170.

Zheng, J. K., Li, Y., Song, M. Y., Fang, X., Cao, Y., McClements, D. J., & Xiao, H. (2014). Improving intracellular uptake of 5-demethyltangeretin by food grade nanoemulsions. *Food Research International, 62*, 98-103.

Zhou, X. H., Wang, H., Wang, C. N., Zhao, C., Peng, Q., Zhang, T. H., & Zhao, C. H. (2018). Stability and in vitro digestibility of beta-carotene in nanoemulsions fabricated with different carrier oils. *Food Science & Nutrition, 6*(8), 2537-2544.

Zielbauer, B. I., Jackson, A. J., Maurer, S., Waschatko, G., Ghebremedhin, M., Rogers, S. E., Vilgis, T. A. (2018). Soybean oleosomes studied by small angle neutron scattering (SANS). *Journal of Colloid and Interface Science, 529*, 197-204.

Zou, L. Q., Zheng, B. J., Zhang, R. J., Zhang, Z. P., Liu, W., Liu, C. M., McClements, D. J. (2016). Influence of Lipid Phase Composition of Excipient Emulsions on Curcumin Solubility, Stability, and Bioaccessibility. *Food Biophysics, 11*(3), 213-225.