Bioavailability of bioactive food compounds: a challenging journey to bioefficacy

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Bioavailability is a key step in ensuring bioefficacy of bioactive food compounds or oral drugs. Bioavailability is a complex process involving several different stages: liberation, absorption, distribution, metabolism and elimination phases (LADME). Bioactive food compounds, whether derived from various plant or animal sources, need to be bioavailable in order to exert any beneficial effects. Through a better understanding of the digestive fate of bioactive food compounds we can impact the promotion of health and improvement of performance. Many varying factors affect bioavailability, such as bioaccessibility, food matrix effect, transporters, molecular structures and metabolizing enzymes. Bioefficacy may be improved through enhanced bioavailability. Therefore, several technologies have been developed to improve the bioavailability of xenobiotics, including structural modifications, nanotechnology and colloidal systems. Due to the complex nature of food bioactive compounds and also to the different mechanisms of absorption of hydrophilic and lipophilic bioactive compounds, unravelling the bioavailability of food constituents is challenging. Among the food sources discussed during this review, coffee, tea, citrus fruit and fish oil were included as sources of food bioactive compounds (e.g. (poly)phenols and polyunsaturated fatty acids (PUFAs)) since they are examples of important ingredients for the food industry. Although there are many studies reporting on bioavailability and bioefficacy of these bioactive food components, understanding their interactions, metabolism and mechanism of action still requires extensive work. This review focuses on some of the major factors affecting the bioavailability of the aforementioned bioactive food compounds.

Introduction

Food is for our sustenance, nourishment and enjoyment. More than this, people are increasingly looking to non-nutrient food components for added benefits from their food which may play a role in health promotion, disease prevention and performance improvement [1]. When considering these properties of food, we aim for long term physiological effects, as opposed to acute effects as in the case of most of the pharmaceutical drugs [2].

The majority of bioactive food compounds responsible for the positive effects on well-being are predominantly derived from the plant kingdom with some from animal sources. Several epidemiological studies throughout the years have suggested that diets rich in fruits and vegetables promote health and reduce the risk of certain chronic and neurodegenerative diseases [3]. Five portions of fruits or vegetables a day has become a rule of thumb for healthy lifestyle dietary habits [4]. Many of these fruits and vegeta-

bles contain polyphenols which are secondary metabolites in plants, and are considered as non-essential for sustenance of life but potentially contribute to the maintenance of human health [5]. Similar to hydrophilic bioactive molecules, lipophiles derived from various plant and animal sources may also exert beneficial effects on health. Long chain polyunsaturated fatty acids derived from marine animals or plant sources, and carotenoids of plant origin are examples of lipophilic bioactive nutrients. Among the food sources discussed in this review, coffee, tea, citrus fruit and fish oil are included as examples of important ingredients for the food industry. Cereals, dairy products, nuts, red wine and olive oil are also interesting sources of bioactive food compounds, but will not be considered in this review.

In order to exert a health benefit, the compound of interest, whether hydrophilic or lipophilic, needs to withstand food processing, be released from the food matrix post-ingestion and be bioaccessible in the gastrointestinal tract, undergo metabolism and reach the target tissue of

action [2]. In other words, the bioactive compound in question needs to be bioavailable before it can have an effect [6]. It may be considered redundant to study the health effects of dietary bioactive compounds if their bioavailability is not also fully elucidated. Bioavailability is a key step regarding functional foods and health claims related to food components, followed by knowledge of the circulating metabolites, which leads to understanding of the mechanisms of action in relation to the benefit.

Due to the complexity of food compounds, the many factors affecting their transition during digestion, and also to the different mechanisms of absorption of water soluble and lipid soluble molecules, unravelling the bioavailability of food constituents is challenging when compared with pharmaceutical drugs. In addition, understanding, for example, the polyphenol–gut microbiota interactions and gut microbial bioconversion capability will facilitate studies on bioavailability of bioactive food compounds in the host and provide more insight into the health effects of these molecules. Williamson & Clifford [7] reported that since microbial metabolites could be present in very high concentrations, colonic metabolites could be considered as the missing link between the consumption of certain polyphenols and their biological activity.

From a pharmacological perspective, bioavailability is the rate and extent to which the bioactive compound or a drug is absorbed and becomes available at the site of action [8]. From a nutritional perspective, bioavailability is the fraction of a given food that the body can utilize, and is therefore a matter of nutritional efficacy [9, 10]. Bioavailability addresses several processes such as liberation from a food matrix, absorption, distribution, metabolism and elimination phases (LADME).

The assessment of bioaccessibility and bioavailability of health associated compounds is important for the understanding of the relationship between food and nutrition. The rate and extent of absorption can vary widely between individuals. The inter-individual variability in bioavailability depends on several key factors including diet, genetic background, gut microbiota composition and activity. Some bioactive food compounds such as polyphenols, are relatively poorly absorbed, the absorption ranging from 0.3% to 43%, and the circulating plasma concentrations of their metabolites can be low [6]. Limited bioavailability hinders the use of bioactive food compounds as functional ingredients [11, 12]. Only by understanding the mechanisms of absorption of food derived compounds, can their bioavailability be enhanced and thus the potential for greater health benefits be realized.

In this review we focus on the nutrient bioavailability of different bioactive food compounds, polyphenols and PUFAs, two very different classes of compounds that have been reported to contribute to a healthy diet. As groups of bioactive food compounds, they differ greatly in their origin, physicochemical properties and absorption mechanisms, and yet they have similarities with regard to various

health related benefits, such as cardio vascular health [13–17]. We also focus on the challenges faced with food matrices, effects of processing, absorption mechanisms, and selected technologies to improve the bioavailability of bioactive food molecules, all in comparison with pharmaceutical drugs.

Bioaccessibility: the first step of bioavailability

Bioaccessibility has been defined as the fraction of a compound which is released from the food matrix in the gastrointestinal lumen and thereby made available for intestinal absorption [18]. Mastication in the mouth initiates the process and several digestive fluids containing different enzymes continue to break down the food matrix in the stomach and throughout the remainder of the gastrointestinal lumen [19]. Bioaccessibility is influenced by the composition of the digested food matrix, the synergisms and antagonisms of the different components [10], but also by physicochemical properties, such as pH, temperature and texture of the matrix [20]. The digested food is predominantly broken down in the small intestine by bile, pancreatic and other enzymes secreted from the intestinal mucosa [19]. These digestive aids are crucial for lipid soluble bioactive compounds, such as some vitamins (A, D, E and K), carotenoids and PUFAs. The sequential obligatory steps of lipid digestion prior to absorption are partial gastric hydrolysis, emulsification by bile and further lipolysis by pancreatic lipases. These enzymes release free fatty acids and monoacylglycerol which form micelles so that the lipids can be absorbed across the water barrier and into the intestinal enterocytes [21–24].

The caloric content and the volume of the food matrix can cause physiological changes in the gastrointestinal tract which affects the bioaccessibility of digested compounds [25–27]. Walsh *et al.* [28] observed in an *in vitro* model that the bioavailability of isoflavonoids from foods containing fat and protein exceeds that of isoflavonoid supplements consumed without food. Brown *et al.* [29] showed that both full fat and reduced fat salad dressing improved carotenoid absorption in human subjects when compared with consumption of salad with fat free salad dressing.

Processing of plant foods can influence the bioaccessibility of nutrients, mainly through changes in the plant cell wall structure and properties [30]. Since plant cell walls are largely resistant to degradation in the upper gut, they represent an important barrier for the release of bioactive compounds. One example is ferulic acid in whole grain wheat, where ferulic acid has a limited bioavailability due to its high binding affinity to polysaccharides. Anson *et al.* [31] studied the bioaccessibility of ferulic acid from wheat fractions and breads consumed by human subjects. The authors observed that wheat ferulic acid had a low bioac-

cessibility (< 1%). However, the bioaccessibility was higher when free ferulic acid was added to flour (~60%). Also fermentation of wheat prior to baking broke the ferulic acid ester links to fibre, thus releasing ferulic acid and subsequently improving its bioavailability [32].

By contrast, pharmaceutical drugs can be taken without a meal, keeping the drug dissolution less complicated than the bioaccessibility of a bioactive food compound, since limiting factors are less and orally taken drugs are mostly water soluble. The effects of food on drug bioavailability are usually studied by evaluating the rate and extent of absorption of a drug when administered after a meal vs. no co-digested meal. Changes observed are related to transit time, luminal dissolution, permeability and systemic availability [8]. Drug dissolution has been extensively studied in the pharmaceutical industry. However, with foods, bioaccessibility has not been investigated to the same extent, although there is a lot of potential for improving bioefficacy via greater liberation of bioactive compounds from food matrices beyond that of the matrix effect *per se* [5].

Bioavailability

After bypassing the challenge of being released from the food matrix and becoming bioaccessible, bioactive food compounds can be absorbed in the gastrointestinal tract. The absorption of these compounds can be influenced by solubility, interaction with other dietary ingredients, molecular transformations, different cellular transporters, metabolism and the interaction with the gut microbiota, resulting in changes to their bioavailability [20]. The different solubility of lipophilic and hydrophilic compounds results in different absorption mechanisms [33]. Before it was thought that dietary lipids pass unaltered through the intestinal wall since structure wise similar lipid particles were found both in the gut and in the systemic and lymphatic circulation. However, lipid bioavailability is not so simple and even today it is not completely elucidated [34]. Our food contains diverse classes of lipids, the main ones being triacylglycerides, phospholipids, glycolipids, free fatty acids, sterols, vitamins and their precursors [35]. Owing to the physiology of the small intestine, with the presence of an unstirred water layer across the intestinal barrier, lipid absorption can be compromised [36]. To overcome the intestinal water barrier, the size of dietary lipid particles is reduced, and after these digestion products form micelles where bile salts and other amphiphilic nutrients act as emulsifiers. Lipases of the gastric juice hydrolyze lipids at the emulsion–water interphase resulting in diacylglycerols and free fatty acids [34]. Uptake of lipids by the enterocyte is believed to take place through passive diffusion but also through facilitated diffusion via transporters [37]. Once in the enterocyte, fatty acids are re-esterified with monoacylglycerols to form triacylglycer-

ols prior to secretion into the lymphatic circulation *via* triacylglycerol rich lipoproteins also called chylomicrons [23]. Unlike hydrophilic compounds, lipid soluble compounds are not readily excreted from our body. Lipids are either stored within the liver or re-excreted into the circulation as lipoproteins and further stored in the adipose tissue.

Hydrophilic compounds, such as polyphenols and most drugs have a more simplistic mechanism of absorption than lipids. Most polyphenols found in foods exist as esters, glycosides or polymers which cannot be absorbed as such [11]. Enzymatic hydrolysis takes place with most polyphenols at the brush border of small intestine epithelial cells. This liberates the aglycone which can then enter the enterocyte. Aglycones can be liberated also in the enterocyte by cytosolic β -glucosidase-mediated hydrolysis [38, 39]. In the enterocytes flavonoid aglycones can be conjugated by the phase II enzymes, resulting in methylated and/or glucuronidated forms. Some of the metabolites can be effluxed back from the enterocyte into the intestinal lumen by ABC-transporters [11, 38–40]. The absorbed metabolites as well as the flavonoids which escaped conjugation in the enterocyte pass next into hepatocytes via the hepatic portal vein where further conjugation takes place. From the liver, bioactive metabolites can be excreted either into systemic circulation or into the bile. Polyphenol metabolites present in the systemic circulation are finally excreted into urine [38, 39, 41]. Since a large portion of polyphenols and some larger molecules are not absorbed in the small intestine, these compounds reach the large intestine where they are metabolized by the microbiota into smaller molecules.

The difference and similarities of food bioactives (lipophilic and hydrophilic) and pharmaceutical drugs with regards to bioavailability are highlighted in Figure 1. Taken together, the metabolism of bioactive compounds of foods, as with drugs, affects their physical and chemical properties, which make them more water soluble and more readily excreted at the end of the bioavailability pathway [42]. This process, also referred to as metabolic detoxication, leads to the near absence of aglycones in the systemic circulation. Previous thinking that the aglycones were the active forms of flavonoids should be re-considered since the conjugated forms are the ones most likely to express a biological activity [6, 11, 41, 43].

Factors affecting bioavailability

Structure of bioactive molecules

The molecular structure of a bioactive compound affects its absorption considerably [44]. For example, it is well known that high molecular weight compounds, such as the oligomeric proanthocyanidins and complex lipids, do not pass through the intestinal cells unless they are firstly broken down [45]. Also the sugar moiety of flavonoids has been suggested to be an important determinant in their

Figure 1

General scheme on the absorption, metabolism and excretion for xenobiotics (A) and examples of compounds discussed in this review (drugs, polyphenols and PUFAs) (B). ■, drug; ■, polyphenols; ▲, other food ingredients; ★, micelles; ★, PUFAs in form of TAG; ■, Metabolites (from drugs and polyphenols); ●, microbial metabolites; -OH -Conj, phase I and phase II metabolites; ■, PUFA hydrolyzed from TAG; ■, PUFA re-esterified as TAG and released as chylomicron

absorption in humans [46, 47]. Flavonoids attached to β -glucosides, one of the most predominant forms in nature [48], can be absorbed to a very small extent as such and/or metabolized by enzymes (e.g. β -glucosidases and lactase-phlorizin hydrolase) in the small intestine [42, 46, 47, 49]. However, when flavonoids are attached to an additional rhamnose moiety, as in the case of quercetin from tea, they need to reach the large intestine to have the sugar moieties cleaved off by the intestinal microbiota before absorption [50, 51].

Moreover, it is not only the chemical structure of bioactive food compounds, but also their isomeric configuration that can affect their absorption. As for some drugs, flavonoids with different stereochemistry exhibit different bioavailability and bioefficacy. This is the case for (–)-epicatechin and (+)-catechin bioavailability [52, 53], the *cis*-isomers and all-*trans* isomer of lycopene bioavailability [54], the biological activity of (R/S) equol [55], and for the metabolism of (R/S) hesperidin [56, 57]. In the last example, hesperitin-7-glucoside was found to have an R : S ratio of 39 : 69 in human plasma and urine samples, suggesting that the S configuration could be more bioavailable [57]. Another example is that of lycopene, a bioactive carotenoid from tomato, which is present as 95% all-*trans* isomer

in tomatoes, yet the *cis* isomers represent around 50% of lycopene in human plasma through a combination of isomerization in the gastrointestinal tract and greater bioavailability of *cis*-isomers in crossing the intestinal wall [58, 59].

Transport mechanisms

The different transport mechanisms which take place in the intestinal lumen are one of the most important factors affecting the bioavailability of ingested food compounds and drugs. These include passive diffusion, facilitated diffusion and active transport. The first two mechanisms involve diffusion towards a concentration gradient through the intestinal cells into the blood circulation. The later mechanism works against the concentration gradient and can result either in the increase of compounds in the blood circulation or in the efflux of the ingredients back to the intestinal lumen [60].

As many drugs and bioactive food compounds do not have the optimal physicochemical properties necessary for passive diffusion, trans-membrane transporters are needed for enhancing their permeability. Membrane transporters are involved in two mechanisms related to permeability of compounds, uptake and efflux. Vitamin

transporters, GLUT-family, SGLT-family and organic anion transporters (OAT1), amongst others, are involved in the uptake of compounds and enhance their transport across the intestine [61]. The ABC (ATP Binding Cassette) family of transporters, including P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), on the other hand, are examples of efflux mechanisms, and can hinder the bioavailability of drugs and bioactive food compounds [61]. The activity of different efflux transporters affects the disposition of drugs and bioactive compounds through several different mechanisms, such as limitation of absorption, facilitation of the elimination by secretion into bile and urine, and limitation of distribution to target tissues [62].

Intestinal transport systems have different selectivity towards different bioactive compounds. The transporters can be blocked by certain nutrients, significantly affecting the bioavailability of other xenobiotics [61]. It has been reported that competition for transport by organic anion transporters can cause retention of certain drugs in cell culture models, leading potentially to longer plasma half-lives [63]. Whitley *et al.* [64] studied the ability of organic anion transporters and organic anion-transporting polypeptides to transport ellagic acid. These authors reported that there is a very high affinity of ellagic acid to organic anion transporters, especially hOAT1, indicating a potential interaction of ellagic acid with drugs such as β -lactam antibiotics, angiotensin converting enzyme inhibitors, non-steroidal anti-inflammatory drugs, antiviral drugs, prostaglandins and diuretics. Itakagi *et al.* [65] described the food bioactive compound–drug interaction between ferulic acid and nateglinide (a hypoglycaemic agent) involving a transport system. The concomitant administration of nateglinide with ferulic acid-rich sources could lead to inhibition of absorption of the drug, reducing its oral bioavailability. Although these examples are from *in vitro* cell culture models, it has been reported that flavonoids are well known substrates for the ABC family of transporters involved in the efflux mechanism, and affinity with these transporters has been suggested as one of the main reasons for poor bioavailability of these bioactive compounds [66–68].

Although the uptake of lipids and lipid digestion products has been hypothesized to be passive for many decades, scientific evidence reported that transport proteins like FATP4, FABPpm and CD36 may be involved in lipid transport across the intestinal barrier [23]. This relatively new discovery has propagated a hypothesis that if one family of membrane transporters is common to multiple nutrients, there might be competitive inhibition of absorption of other nutrients using the same transporter. An example of this phenomenon is reported by Richelle *et al.* [69], where plant sterols reduced absorption of cholesterol, carotenoids and alpha-tocopherol in normocholesterolaemic subjects, most likely by competing with lipid membrane transporters.

Metabolism and food–drug interactions

Once the drug or bioactive food molecule has entered into an enterocyte, it may be subjected to metabolism by cytochrome 450 enzymes (CYP) (phase I) which modify the xenobiotic structure by reactions such as oxidation and reduction. In general, polyphenols are not considered as substrates for CYP enzymes, unlike drugs, but they are subjected to several phase II enzymes leading to conjugation with methyl (catechol-O-methyltransferases – COMT), sulfate (sulphotransferases – SULT) and glucuronyl groups (uridine-5'-diphosphate glucuronosyl-transferases – UDPGT). This results in molecular forms, which are different from the original constituents of the digested food [70]. The activity of CYP family enzymes can be inhibited or activated by co-administered bioactive compounds or drugs. For example, inhibition of CYP enzymes can increase circulating amounts of lipid soluble vitamins, as is the case for the sesame seed lignin, sesamin, which can considerably increase γ -tocopherol concentrations in humans [71, 72]. Grapefruit juice has also been reported in many scientific papers in respect to food–drug interactions [73–75]. It has been proposed that bergamottin (a furanocoumarin) and derivatives could be responsible for the interaction [76, 77]. Grapefruit juice can produce clinically important increases in oral drug bioavailability when co-administered with substrates of cytochrome P450 3A4 (CYP3A4), leading to an increase in the plasma concentration of the drug [76, 78]. This in turn should be considered as it would lead to toxic supra-pharmacologic plasma concentrations. It has been reported in an animal study that the presence of lipids could also promote drug absorption. An example of this is the use of eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids as a vehicle for ciclosporin A, an immunosuppressive agent, for increasing its bioavailability. The suggested mechanism involved here was the inhibition of CYP3A by DHA [79]. An opposite example, *i.e.* decrease in the plasma concentration of the drug, is the food–drug interaction reported in the case of St John's wort (*Hypericum perforatum*) and drugs such as ciclosporin and indinavir, where the interaction led to a low plasma concentration of the drug, thus compromising its therapeutic effect [80, 81].

Improvement of bioavailability

Improving the bioavailability of bioactive food compounds is fundamental to improve their bioefficacy. Several approaches have been evaluated for the improvement of bioaccessibility and bioavailability of bioactive ingredients and drugs. These include technological and chemical modifications of the molecules to improve their solubility or the site of absorption, design of colloidal systems (micelles and vesicles), and use of nanosystems (nanoparticles), among others [82]. Examples of materials commonly used to improve the bioavailability of drugs

include chitosan, polymers, cyclodextrins and dendrimers. These materials are also applicable to many bioactive food compounds.

Nanotechnology is currently one of the major fields studied in pharmaceutical research [83]. Although there are many cases where systemic bioavailability is improved by means of nanotechnology, the main reason for the design of such nanosystems is to improve local bioavailability, or the delivery of a certain drug to a target tissue [83]. In principle, the use of nanosystems could facilitate the entering of the drug or bioactive compound through biological barriers, besides avoiding the metabolic modifications that could lead to low absorption. However, it is very important to understand, from an efficacy perspective, that bioavailability parameters might be influenced by the composition material and the physico-chemical properties of the nanoparticles [84, 85]. Therefore, human studies to identify potential risks of the use of nanoparticles should be taken into consideration. Tzeng *et al.* [86] reported the use of a nanoparticle engineering process for the enhancement of antioxidant activity and solubility of kaempferol. These authors suggested that kaempferol nanoparticles could be considered as a low dose alternative in health foods and future clinical research. Curcumin, the major curcuminoid compound, is well known for many potential health benefits [87, 88]. However, due to its insolubility in water, this compound has a very low bioavailability [89]. A method to improve the bioavailability of curcumin in rats was achieved by introducing PLGA [poly (lactic-co-glycolic acid)] nanoparticles that had 5.6 fold higher bioavailability and longer half-life when compared with free curcumin [84]. Yu *et al.* [90] developed a food grade curcuminoid organogel with high bioaccessibility and high loading of curcumin. They demonstrated that in *in vitro* models this preparation could be regarded as an alternative to the nanoparticle approach for improving bioavailability.

Several technologies for encapsulation of food bioactive molecules are aimed to improve bioavailability. These technologies include spray drying, coacervation, liposome entrapment, inclusion complexation, cocrystallization, nanoencapsulation, freeze drying, yeast encapsulation and emulsion [91]. For example, Fernandez-Garcia *et al.* [92] evaluated in several *in vitro* digestion models an emulsifier system to improve the bioaccessibility of carotenoids. The use of microencapsulation, liposomes, gel emulsions and plant spore exines as delivery systems for PUFAs has demonstrated improved bioavailability when compared with fish oil, a typical representation of normal dietary intake [35, 93–96].

Other potential ways to improve the bioavailability of bioactive compounds and drugs could be achieved through the competition and inhibition of intestinal cell transporters [97]. The intestinal P-glycoprotein efflux pump has been reported as one of the major contributors to the low oral bioavailability of a number of compounds

reported in animal studies [84]. *In vitro* experiments suggest that the bioavailability of the flavonoid hesperidin may be enhanced by inhibiting the ABC transporters by competitive exposure to other flavonoids, such as quercetin, resulting in a decrease of the efflux of hesperidin [98]. The same approach has been suggested for (–)-epigallocatechin 3-gallate (EGCG), where the combination of the bioactive compound with naturally occurring inhibitors of efflux proteins resulted in increased cytosolic levels of the compound [99].

Other approaches for improving bioavailability are structural modifications of bioactive compounds and the processing of the food matrix. For example, it has been reported in animal and human studies that quercetin, the aglycone form of rutin, is more bioavailable than the parent flavonol [50, 100]. Carotenoids, such as lycopene and zeaxanthin, generally have a low bioavailability in humans [101]. By processing raw tomatoes into tomato paste, the bioavailability of lycopene is increased due to release from the food matrix, but also due to isomerization of *trans* lycopene into the more bioavailable *cis* form [102, 103]. Alternatively, entrapping lycopene with whey proteins improves its bioavailability to the same level as that of tomato paste [104], and zeaxanthin bioavailability is improved three-fold by homogenization with hot milk [105]. Another example for improved bioavailability through improved bioaccessibility is phytosterols, plant sterols with chemical structures similar to that of cholesterol. When phytosterols are added to food products, changes in product texture are observed due to re-crystallization. The insoluble crystalline phytosterols cannot be absorbed in the intestine thus resulting in a very low bioavailability. Crystallization retardation, emulsification technologies [106] and synthesis of colloidal phytosterols leads to an enhanced solubilization of phytosterols [107] thereby improving the bioaccessibility and consequently the bioavailability of phytosterols.

Examples on the complexity of bioavailability of food bioactive components: (poly)phenols

Coffee

Coffee contains high levels of phenolic compounds called hydroxycinnamates, consisting principally of chlorogenic acids. They are a family of esters formed between a phenolic acid, *e.g.* caffeic or ferulic acid, and quinic acid. The main chlorogenic acid in coffee is 5-caffeoylquinic acid [108], although other caffeoylquinic, feruloylquinic and di-caffeoylquinic acids are present in significant quantities. Phenolic metabolites of chlorogenic acids have been studied for potential bioefficacy, and controversy still remains as the results are not entirely clear [109, 110]. In addition, only a few studies have investigated the bioavailability of coffee phenolic and chlorogenic acids.

Due to the intricate and complex metabolic pathways in humans [111, 112], chlorogenic acids may be transformed into phenolic acids (caffeic, ferulic and isoferulic moieties), and subsequently into colonic metabolites (dihydrocaffeic and dihydroferulic acids). With extensive conjugation at the level of the intestine and the liver, many different metabolites (aglycone, sulfate, glucuronide and methyl) could then be identified from a single cup of coffee. Because of this complexity, many studies have used enzymatic cleavage to reduce the number of metabolites measured, to increase the accuracy of the quantification, thus dismissing the diversity of the coffee metabolites [113]. Recently, conjugated metabolites were measured in great numbers [112, 114] although validated quantification of these metabolites is yet to be performed using proper standards [114, 115].

Research on the effect of the food matrix on the bioavailability of coffee phenolic compounds has been limited. Some reports have investigated the potential effect of proteins, especially from milk, on the bioavailability of coffee phenolics. For example, using *in vitro/ex vivo* modelling of the digestive process showed some binding of caffeoylquinic acid to proteins (albumin, casein), but the relevance of those findings to an *in vivo* setting remains to be determined. In the framework of human bioavailability studies, the main effect investigated was also the addition of milk in coffee. Renouf *et al.* [116] investigated the effect of adding 10% whole milk or sugar/non-dairy creamer to coffee on the bioavailability of phenolic acids equivalents. The milk treatment showed no significant difference in the area under the curve (AUC), maximum concentration (C_{max}) or time when maximum concentration was reached (t_{max}) of caffeic acid, ferulic acid and isoferulic acid equivalents compared with coffee alone. With respect to the sugar/non-dairy creamer treatment, the AUC of caffeic acid, ferulic acid and isoferulic acid equivalents were similar to coffee alone. However, C_{max} of caffeic acid and isoferulic acid equivalents were significantly lower and t_{max} of ferulic acid and isoferulic acid equivalents were significantly longer for the sugar/non-dairy creamer group compared with coffee. Therefore, even if the delivery of chlorogenic acid metabolites was not significantly different between treatments, the addition of sugar/non-dairy creamer to coffee led to significant changes in plasma appearance of those metabolites ('flatter', but 'longer' curves). More recently, a report by Duarte & Farah [117] showed that urinary excretion of chlorogenic acids and metabolites was significantly lowered when soluble coffee was reconstituted in 100% whole milk ($40 \pm 27\%$ ingested dose) compared with coffee reconstituted in water ($68 \pm 20\%$ ingested dose).

Tea

Tea (*Camellia sinensis* L.) is the second most consumed beverage worldwide after water. The different types of tea

(green, oolong and black) differ depending on the extension of the oxidative processing, leading to the presence of different compounds such as flavan-3-ols in green tea (minimally processed), theaflavins and thearubigins in oolong (partial oxidation) and black tea (complete oxidation). Several epidemiological studies have suggested an inverse correlation between tea consumption and chronic and degenerative diseases, possibly related to their polyphenol content [118].

As most of the studies on tea report benefits related to the consumption of green tea, the proposed beneficial effects have been attributed to the presence of flavan-3-ols such as (–)-epigallocatechin 3-gallate (EGCG). EGCG is the main flavan-3-ol present in green tea and it has been reported to be present in human plasma mainly in its native form [115]. Flavan-3-ols are suggested to be absorbed in the small intestine and undergo extensive metabolism. Following consumption of green tea, flavan-3-ols appear to be rapidly absorbed having a maximum concentration (C_{max}) of between 0.5 to 2 h, followed by rapid metabolism and excretion.

One limiting factor for a better absorption of flavan-3-ols, is the affinity of these compounds by P-glycoprotein and multidrug resistance proteins (MRPs) leading to a significant percentage of these compounds being effluxed back into the lumen [119]. The importance of colonic metabolism for EGCG bioavailability should not be underestimated as there is a substantial recovery of colonic metabolites in human urine [120]. Del Rio *et al.* [121] have suggested that when all the metabolites are taken into account (conjugates and microbial metabolites) the green tea flavan-3-ols could be considerably more bioavailable than previously reported.

Tea is usually consumed during a meal, or accompanied by creamers (dairy or non-dairy) or sugars, and sweeteners are added in tea products such as ready-to-drink beverages. Many studies have reported the effect of milk addition on the bioavailability and bioaccessibility of flavan-3-ols from black and green teas. In general these studies have found that the addition of milk to tea had a negligible effect on bioavailability and bioaccessibility of tea flavan-3-ols [122, 123].

As previously described, due to low bioaccessibility and bioavailability of most polyphenols, there have been many attempts to improve the bioavailability of these compounds. Several approaches to improve the bioavailability of EGCG have been tested, such as chemical modifications, dosing formulation and combination with other food ingredients [82]. Another strategy to improve the absorption of flavan-3-ols is their administration during the fasting state; however, it is important to note that some human studies have shown that high doses of green tea preparations can be potentially hepatotoxic [124]. Although there are many studies describing green tea bioavailability and associated health benefits, there are still many aspects that need further investigation.

Cocoa

Historically, cocoa products have been extensively used for a vast array of medicinal purposes. The long list of its medical applications includes from treatment of mental fatigue to strengthening of gums [125]. Cocoa extract is a complex intrinsic matrix that contains many potentially bioactive compounds [126]. These cocoa compounds include not only polyphenols but also methylxanthines such as theobromine and caffeine [127]. Similar to tea, flavan-3-ols are the most important group of polyphenols in cocoa products [128]. Specifically, (–)-epicatechin and procyanidins (oligomers of (–)-epicatechin compounds) are the main flavan-3-ols found in these foods. Contrary to some other polyphenols like quercetin and hesperetin, (–)-epicatechin and procyanidins are not found attached to sugar moieties in plants or plant-derived foods.

Following consumption of cocoa products, (–)-epicatechin is absorbed within 1 to 4 h, with $t_{\max}$ between 1 to 2 h. Once absorbed, (–)-epicatechin is completely glucuronidated, sulfated and/or methylated [129, 130]. Several beneficial effects have been attributed to the consumption of (–)-epicatechin [128]; and the improvement of cardiovascular biomarkers is the most consistent beneficial effect demonstrated in human intervention studies [16, 131–133].

According to Baba *et al.* [134], (–)-epicatechin bioaccessibility is similar when a cocoa beverage or a chocolate bar is consumed. The authors reported that $29.8 \pm 5.3\%$ and $25.3 \pm 8.1\%$ of the initial intake was absorbed when five volunteers consumed equal amounts of (–)-epicatechin in a beverage or chocolate form, respectively.

As a beverage, cocoa is regularly consumed with milk. Many controversial scientific articles have been published about the effect of milk proteins on the bioavailability of (–)-epicatechin. Serafini *et al.* [135] reported a detrimental effect of milk over the bioavailability of (–)-epicatechin, showing a lower (–)-epicatechin AUC (approximately –40%) when 100 g chocolate was ingested with 200 ml of whole milk. In contrast, several other groups have demonstrated that milk has no effect on the bioavailability of cocoa polyphenols [136–139]. Recently, it has been proposed that milk decreases urinary excretion of cocoa flavan-3-ol metabolites in humans but not the plasma pharmacokinetics [140].

In addition to the possible effect of milk, it has been demonstrated that (–)-epicatechin bioavailability can be improved by the co-ingestion of cocoa products with other foods. Schramm *et al.* [141] showed that (–)-epicatechin AUC and $C_{\max}$ can be increased by ingestion of carbohydrates and Neilson *et al.* [142] concluded that the physical form of the food and also the sucrose content may influence the $t_{\max}$ and $C_{\max}$ of cocoa flavan-3-ols.

Procyanidins are also highly present in cocoa extracts. Because of their large structure and molecular weight, procyanidins are poorly absorbed [143, 144]. Currently, only procyanidin B2 has been reported to be detected in

human plasma after consumption of cocoa products [145], suggesting that the highest molecular weight procyanidins, which are not absorbed in the small intestine, can reach the large intestine and interact with the gut microbiota. Recently, it has been suggested that procyanidins could modulate the growth of selected gut microorganisms with potential prebiotic benefits in humans [146]. Consequently, further investigations of the interaction of procyanidins with the gut microbiota and especially the bioavailability of the breakdown products produced by these interactions are warranted.

Citrus fruits

Hesperidin belongs to the flavanone group of flavonoids, and it is mostly present in citrus fruits such as oranges, lemons, and grapefruits. The highest amount of hesperidin is found in the solid tissues, mainly in the pith of the fruit. Hesperidin is associated with benefits on bone health and cardiovascular health [147, 148]. Similarly to other more complex flavonoid glycosides, hesperidin is absorbed in the large intestine where it is hydrolyzed by the gut microbiota and the aglycone is then absorbed by passive transport [6, 149]. Several bioavailability studies on hesperidin have shown a late $t_{\max}$ value (4.4 h–7.0 h) suggesting that absorption takes place in the colon [150–154]. However, modification of hesperidin to hesperetin-7-glucoside, by enzymatically cleaving the rhamnose moiety with a α -L-rhamnose, has been known, not only to increase the bioavailability of this flavanone [155, 156], but also to alter the absorption kinetics by having a much earlier $t_{\max}$ (0.6 h vs. 7 h) [154] suggesting that the site of absorption has changed.

Dosage also affects the bioavailability of hesperidin. It has been shown that the relative absorption is increased with higher doses of hesperidin. This is the case also for the relative urinary excretion of the flavanone [152, 154].

The food matrix has not been shown to have an impact on hesperidin bioavailability. Consumption of orange juice with full-fat yogurt did not significantly affect the $C_{\max}$ or $t_{\max}$ values [153], nor did food processing have an effect on hesperidin bioavailability when comparing the consumption of orange fruits with the consumption of orange juice [150].

Lipids

PUFAs

Bioactive PUFA, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are lipids that may exert beneficial biological functions in the human body such as prevention of heart disorders and maintenance of cognitive function [157–160]. Dietary sources of PUFA are usually limited to single cell oils, marine derived fatty fishes, fish oil and related fractions, and are incorporated into a variety of food products [157]. The underlying mechanism of apical

uptake of lipid digestion products still remains to be fully elucidated. However both active (apical membrane proteins: fatty acid binding protein and fatty acid translocase) and passive transporters have been proposed [161]. Intestinal cell models have suggested saturable transport for long chain fatty acids but not for short chain fatty acids [162].

Hypothetically, the bioavailability of matrix embedded PUFA may differ from its supplemental counterpart. Many studies have focused on the bioavailability of EPA and DHA but the mechanism of their uptake into absorptive epithelial cells and intracellular events leading to basolateral secretion is still derived from knowledge obtained for long chain fatty acids. In some pathological states, such as malabsorptive conditions, absorption of PUFA is reduced. Similarly, Orlistat™, a lipase inhibitor, may also lead to a reduction in the absorption of total lipids by as much as 30% [163]. Therefore, in order to modulate bioavailability of these bioactive PUFA a clear understanding of physiologically orchestrated steps of pre-absorptive and absorptive events is essential.

Conclusions

Identifying the bioavailability of bioactive food compounds and pharmaceutical drugs is essential when evaluating their potential health benefits as well as their toxicity. The bioavailability of orally ingested drugs includes the same steps as in bioactive food compounds (LADME) and faces some of the same challenges including transporters, molecular structures, and enzymes. However, unravelling the bioavailability of bioactive food compounds is even more challenging than with drugs since many other factors such as bioaccessibility, food matrix effect and the gut microbiota can affect bioactive food compounds during digestion.

Bioactive food molecules are a diverse group of compounds. They include lipophilic and hydrophilic molecules with complex chemical structures resulting in a number of different absorption mechanisms. For polyphenols, even within the same subclass of compounds, there are significant differences in absorption. For PUFAs, their bioaccessibility is a challenge since these lipids can be represented in different dietary forms and structures, *i.e.* triacylglycerides or phospholipids. Liberation of PUFA from these constituents varies, affecting their extent of bioavailability. Although there are many studies reporting the bioavailability and the bioefficacy of these bioactive food components, understanding their interactions, metabolism and mechanism of action warrants further work. In addition, it should be considered that current analytical methods for estimating bioavailability in humans through identification of main metabolites have limitations. For example, if we only look at urinary excretion data we underestimate the actual bioavailability, as

for most of the bioactive food compounds their metabolism has not been fully characterized.

The next important step in understanding the bioavailability of bioactive food compounds is to identify properly the circulating metabolites in order to have a better understanding of the fate of the parent compounds. Only when the circulating forms of a bioactive food molecule or a drug are known, a more complete picture related to bioavailability and possible correlation to bioefficacy can be obtained. For drugs, this is a requirement when performing bioavailability studies and this type of approach can be translated to nutrition research too.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare MJR, MR, CC-H, L A-G, SKT and MSP had support from Nestle Research Center, Nestec Ltd. for the submitted work, MJR, MR, CC-H, L A-G, and SKT are and have been employees with Nestle Research Center, Nestec Ltd. in the previous 3 years, MSP is and has been an employee with Nestle Research Center, Nestec Ltd. in the previous 1 year and declares no financial relationships with any other organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work

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