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Interactions Affecting the Bioavailability of Dietary Polyphenols *in Vivo*

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Abstract: Polyphenols are widely abundant dietary constituents in plants that are associated with health-promoting effects. This review summarizes factors influencing the bioavailability of polyphenols, specifically flavanols, flavonols, flavanones, flavones, and hydroxycinnamic (phenolic) acids. Most factors tested so far indicate that bioaccessibility, defined as the amount of compound reaching the enterocyte in a form suitable for absorption, is the most important factor determining the absorption in the gut. Factors leading to an improved absorption of flavonols, notably quercetin and its metabolites, are primarily the nature of the attached sugar, and secondly, the solubility as modified by ethanol, fat, and emulsifiers. The absorption of flavanols, notably green tea catechins, is affected by epimerization reactions occurring during processing, the presence of lipid and carbohydrate, and is improved by the presence of piperine and tartaric acid. Flavanones, such as hesperidin, are strongly affected by the type of attached sugar. Phenolic acids are affected by the attached sugar, which can covalently link these compounds to the cereal bran matrix. In the few examples tested, absorption of polyphenols is dependent on release from the food matrix. There are only a few examples reported, but where information is available, the absorption increases with dose but is sometimes linear and sometimes saturated. The lack of systematic information on the effects of other components on the bioavailability of polyphenols needs to be addressed, and more human studies should be conducted in this field to establish general principles affecting absorption *in vivo*. Information derived from such experiments could be useful for the optimal design of future bioefficacy studies.

Key words: Quercetin, catechin, food matrix, tea, absorption, pharmacokinetics, antioxidant, lipid, emulsifier, ethanol

Introduction

Polyphenols are a group of substances occurring naturally in plants and, in the diet, have proposed health-promoting effects such as reducing the risk of degenerative diseases [1–4]. After intake of polyphenols, absorption from the gastrointestinal tract and subsequent bioavailability is a prerequisite for positive influence on human health. A number of factors have been reported to affect the oral bioavailability of polyphenols:

Modification or cleavage of one or more attached sugar
Solubility, delivery, and food matrix
Dose and adaptation to dose
Inhibition of any chemical changes that might occur during processing or in the GI tract
Competition and interaction with other compounds

The aim of the present paper is to summarize data derived from animal and human studies (Tables I–III) focusing on

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interactions affecting the bioavailability of different classes of polyphenols [flavanols, flavonols, flavanones, flavones, and hydroxycinnamic (phenolic) acids], (Figure 1) mainly with respect to plasma bioavailability parameters. Isoflavones and anthocyanins are not covered in this review. Furthermore, polyphenol and milk interactions are not considered, because information in this specific field is contradictory and still needs to be clarified. Whether milk proteins affect absorption of various polyphenol classes is not resolved [5–7].

Table Ia: Flavonols[1]

Type of interaction	Studied polyphenols	Results on bioavailability (BV)	Dose	Sample size and design	Remarks	Ref
Quercetin rutinoside Pure (rutin) vs. plant matrix (buckwheat tea)	Quercetin rutinoside	Plasma total quercetin c_{max} ↑ by 100% and AUC ↑ by 52%(tea) vs. rutin (only a trend)	Single doses of 200 mg quercetin equivalents	n = 12 healthy subjects; randomized cross-over	BV from tea higher due to enhancing factors in matrix?	[18]
Red wine (W), fried onions (O), or black tea (T)	Quercetin	Plasma concentration of total flavonols ↑ by 138% (W), by 288% (O) and by 169% (T) vs. baseline; plasma concentration significantly ↑ after O vs. W and T	Repeated doses of 14–16 mg	n = 12 healthy subjects; randomized cross-over	Difference in absorption reflects nature of attached sugars rather than food matrix effect	[8]
Matrix effects (white wine, grape juice, vegetable juice)	Quercetin	Plasma total quercetin $AUC_{(4h)}$ significantly ↑ after white wine; t_{max} = 30 min	Single doses of quercetin (10 mg/ 70 kg) given in 3 matrices	n = 12 healthy subjects (4 subjects per group)	Possible increase due to higher solubility of quercetin in ethanol	[11]
Ground beef	Quercetin-3-*O*-glucoside	Plasma total AUC (quercetin and metabolites) significantly ↑ by 140% and C_{max} ↑ by 118% after ground beef vs. standard diet; t_{max} = 30 min	Single dose of 29.6 µmol/kg quercetin-3-*O*-glucoside	n = 3 pigs	Absorption of quercetin from meat matrix higher	[15]
Fat in the diet (17%)	Quercetin and quercetin-3-*O*-glucoside	Plasma total AUC significantly ↑ by 57% (aglycone) and 32% (glucoside) vs. 3% fat diet (control)	Single dose of 30 µmol/kg quercetin and quercetin-glucoside in 3%, 17% and 32% fat diet	n = 7 pigs; sequential design	Fat increases quercetin absorption but no further BV ↑ with 32% fat; quercetin elimination delayed with higher fat diet	[16]
Combination of lipids and emulsifiers	Quercetin and metabolites	Plasma c_{max} significantly ↑ by 58% to 110% vs. control group (water + quercetin)	Single dose of 150 µmol/kg quercetin in 2 mL water	n = 5 rats per group (16 groups)	Lipids or emulsifier alone gave no significant effects on quercetin absorption: combination required	[12]
Soybean-, fish oil, beef tallow or lecithin; Co-ingestion of soybean oil and emulsifiers	Quercetin and metabolites	Plasma concentration of quercetin metabolites: significantly ↑ with soybean oil (> 0.75 g/d) and significantly ↑ through co-ingested emulsifiers	Repeated doses (2 weeks) with 1 g onion powder/d (~3.9 mg quercetin aglycone equivalent/g dry weight)	n = 5 rats per group (10 groups)	Lecithin most effective type of lipid (0.75 g/d) for enhancement	[17]

Table Ia: Flavonols[1] (Continue)

Type of interaction	Studied polyphenols	Results on bioavailability (BV)	Dose	Sample size and design	Remarks	Ref
Single compounds vs. mixture	Quercetin, rutin and αG-rutin vs. mixture of αG-rutin and rutin	Plasma quercetin AUC for αG-rutin ↑ by 390% and 103% vs. quercetin and rutin; αG-rutin comparable to the mixture	Single doses of 50 μmol/kg in sodium carboxy-methyl cellulose (CMC-Na)	n = 4 rats per group	Possibly a solubility enhancement effect	[19]
Propylene glycol	Quercetin	Plasma total quercetin c_{max} 70.3 μM after 0.5 h (propylene glycol) and 15.5 μM after 0.25 h (propylene glycol/water) vs. 5.5 μM (water)	Single doses of 50 mg/kg quercetin in either 2 mL propylene glycol, propylene glycol/ water or water	n = 3 rats per group	Probably additional impact of stomach fluids (may cause precipitation)	[13,14]

[1] ↑ Increase/increased; BV, bioavailability; AUC, area under the plasma concentration-time curve; c_{max}, maximal plasma concentration; t_{max}, time to reach c_{max}

Table Ib: Flavanols[1]

Type of interaction	Studied polyphenols	Results on bioavailability (BV)	Dose	Sample size and design	Remarks	Ref
Carbohydrate consumption	Cocoa flavanols (catechin and epicatechin)	Sugar: Plasma flavanol AUC sign. ↑ by 44% (c_{max} ↑ by 40%); Cocoa and bread: AUC sign. ↑ by 30–40% (c_{max} ↑ by 28–37%); Cocoa and grapefruit juice: AUC sign. ↑ by 21% vs. cocoa alone (at 17.5 kJ/kg)	Single dose of 0.125 g/kg cocoa (= 1.53 mg/kg flavanols)	n = 6 healthy subjects per group; 4 cross over trials with different foods	No changes in t_{max} and gastric emptying; 8.75 and 17.5 kJ/kg tested	[23]
Matrix effects (white wine, grape juice, vegetable juice)	Catechin	Plasma total catechin $AUC_{(4h)}$ significantly ↑ after grape juice t_{max} = 30 min	Single doses of catechin (25 mg/ 70 kg) given in 3 matrices	n = 12 healthy subjects (4 subjects per group); randomised	Ethanol does not affect flavanol uptake	[11]
Green tea extract (free vs. phospho-lipid complex form)	EGCG	Plasma c_{max} ↑ by 100% for complex vs. free form; t_{max} = 2 h	Single dose of 400 mg EGCG	n = 12 healthy subjects (6 per group); parallel design	Phospholipid enhancement mechanism unclear	[22]
Piperine	EGCG	Plasma total EGCG c_{max} significantly ↑ by 106% and AUC ↑ by 121% vs. EGCG alone	Single doses of 163.8 μmol/kg EGCG and 70.2 μmol/kg piperine vs. EGCG alone	n = 12 mice (6 per group); parallel design	Mechanism proposed to be competition for conjugation and transit time	[24]
Tartaric acid	Catechin	Plasma AUC of catechin-5-*O*-β-glucuronide ↑ by ~60% (catechin + 1% or 2% tartaric acid) vs. control (catechin + water)	Single oral dose of 100 mg/kg polyphenols (catechin, epicatechin) in water	Rats n not stated		[25]
Single/double dose vs. repeated dosing of green tea extract	EGCG	Significant ↑ of conjugated EGCG plasma concentration after repeated dosing (1 week) vs. before ingestion	Single dose (164 mg catechins) vs. double dose; Repeated doses	Healthy subjects: Single/double dose: n = 5; repeated dose: n = 16	No change in plasma antioxidant activity	[30]

Table Ib: Flavanols[1] (Continue)

Type of interaction	Studied polyphenols	Results on bioavailability (BV)	Dose	Sample size and design	Remarks	Ref
EGCG isolated or extract (once vs. twice daily)	EGCG	Plasma free EGCG AUC significantly ↑ by 52% (800 mg EGCG) and by 61% (800 mg EGCG as extract) once daily for 4 weeks vs. first intake	800 mg EGCG once or 400 mg twice daily	n = 40 healthy subjects; randomized, placebo-controlled	4 week adaptation study	[31]
Fasting/fed conditions	EGCG	Plasma free EGCG AUC significantly ↑ by 246% (400 mg), 180% (800 mg), and 129% (1200 mg) when fasting; Significant ↑ in c_{max}	Single doses of 400 mg, 800 mg, or 1200 mg EGCG	n = 30 healthy subjects; randomized, cross-over	Oral BV of free catechins ↑ (empty stomach)	[26]
Green tea (GT), black tea (BT), and green tea extract supplement (GTS)	Tea flavanols	GTS: EGCG c_{max} significantly ↑ by 60% (vs. BT) and by 100% (vs. GT); t_{max} significantly ↑ by about 1 h for all tea flavanols	Single doses of 697 mg total flavanols from GT (214 mg EGCG), 547 mg from BT (231 mg EGCG), and 462 mg from GTS (193 mg EGCG)	n = 30 healthy subjects; randomized, cross-over	Standardized EGCG content	[27]
Dose dependency	Tea flavanols (EGCG, EGC, and EC)	For 3.0 g vs. 1.5 g green tea powder: Plasma AUC significantly ↑ by 148% (EGCG), 300% (EGC) and 280% (EC); Plasma c_{max} significantly ↑ by 174% (EGCG), 143% (EGC), and 144% (EC)	1.5 g, 3.0 g, or 4.5 g of decaffeinated green tea solids (= 282 mg, 564 mg, and 846 mg total flavanols)	n = 18 healthy subjects	Saturation phenomenon observed	[29]

[1] AUC, area under the plasma concentration-time curve; ↑ Increase/increased; c_{max}, maximal plasma concentration; t_{max}, time to reach c_{max}; EGC, epigallocatechin; EC, epicatechin; BV, bioavailability

Table I c: Other flavonoids[1]

Type of interaction	Studied polyphenols	Results on bioavailability (BV)	Dose	Sample size and design	Remarks	Ref
Ferulic acid (FA) vs. wheat bran diet	Ferulic acid (FA)	Plasma c_{max} ↑ by 138% after free FA ingestion vs. wheat bran; t_{max} = 30 min	Single dose (5 mg pure FA/kg) or wheat bran (4 mg FA /kg) vs. standardized diet (no FA)	n = 66 rats	Plasma kinetics: bran more efficient than pure FA	[39]
Sodium Carboxy-methyl cellulose/ propylene glycol	Luteolin and luteolin-7-*O*-β-glucoside	Plasma c_{max} of total luteolin ↑ by ~300% for luteolin in propylene glycol vs. luteolin in sodium carboxy-methyl cellulose; t_{max} = 30 min	Single doses of 50 µmol/kg in 0.5% carboxy-methyl cellulose sodium or propylene-glycol	n = 3–5 rats per group; n = 2 healthy subjects	Free luteolin and mono-glucuronide in rat/human plasma	[42]

Table Ic: Other flavonoids[1] (Continue)

Type of interaction	Studied polyphenols	Results on bioavailability (BV)	Dose	Sample size and design	Remarks	Ref
Domestic cooking	Naringenin (N); Chlorogenic acid (CA)	N: Plasma c_{max} significantly ↑ after 2 h for cooked tomatoes CA: $_{cmax}$ significantly ↑ after 6 h for cooked tomatoes (vs. fresh tomatoes)	Single dose of 46 mg CA + 19 mg N (fresh tomatoes) vs. 31 mg CA + 17 mg N (cooked tomatoes)	n = 5 healthy subjects; cross-over	500 g fresh vs. cooked tomatoes	[43]
Enzymatic modification	Hesperidin/ Hesperetin-7-glucoside (H-7-G)	Plasma AUC of total hesperetin after H-7-G juice significantly ↑ by 2-fold vs. low-dose hesperidin juice	Single doses of 2 mg/kg, 6 mg/kg and 1.52 mg/kg hesperidin	n = 16 healthy subjects; randomized, cross-over	H-7-G: c_{max} higher and t_{max} reached faster	[38]

[1] ↑ Increase/increased; BV, bioavailability; AUC, area under the plasma concentration-time curve; c_{max}, maximal plasma concentration; t_{max}, time to reach c_{max}

Table II: Factors without effect on the bioavailability of polyphenols[1]

Type of interaction	Studied polyphenols	Dose	Sample size and design	Remarks	Ref
Addition of antioxidants (vitamins and carotenoids)	Gallic and caffeic acids, catechin, naringenin	Single dose of 50 μM gallic-/caffeic acid or 25 μM catechin, naringenin; 150 μM in total	n = 6 rats per group; 3 treatments	Intestinal perfusion model; 150 μM ≈ 1 g (estimated daily intake)	[37]
Ethanol	Catechin	Single dose of 35 mg catechin (120 mL dealcoholized and reconstituted red wine)	n = 9 healthy subjects; randomized, cross-over	AUC, c_{max}, and t_{max} not affected	[20,21]
Single compound vs. cocoa powder matrix	Epicatechin	Single dose of 1, 5, and 10 mg/kg epicatechin alone or in cocoa powder matrix	n = 30 rats (5 per group)	Cocoa powder composition has no effect on BV; dose dependency shown	[32]
Single meal vs. diet adaptation (10 days)	Ferulic acid (FA) and *p*-coumaric acid (PCA)	Single dose (4.5 mg FA + 0.44 mg PCA); diet adaptation (30 mg FA + 3 mg PCA per day)	n = 12 rats (single meal); n = 4 rats (diet adaptation)	No BV improvement through adaptation; refined corn bran diet given	[41]

[1] AUC, area under the plasma concentration-time curve; c_{max}, maximal plasma concentration; t_{max}, time to reach c_{max}; BV, bioavailability

Table III: Factors decreasing the bioavailability of polyphenols[1]

Type of interaction	Studied polyphenols	Results on bioavailability (BV)	Dose	Sample size and design	Remarks	Ref
Co-administration	Quercetin and catechin	Co-administration: quercetin metabolites significantly ↓ by 35% and catechin metabolites ↓ by 28%	Repeated dose for 3 weeks (45 mg quercetin or catechin equivalents daily)	n = 8 rats per group	Competitive interaction suggested	[36]
Single compounds vs. mixture	Catechin and epicatechin	Mixture: competitive absorption suggested; plasma metabolite AUC not significantly different (for separate administration vs. mixture)	Single dose of 172 µmol/kg catechin or epicatechin separately or a mixture (345 µmol/kg) vs. control (10 mL/kg water)	n = 20 rats (5 per group)	Separate administration: epicatechin absorption better than catechin absorption	[35]
Epimerization reactions	Green tea catechins	Plasma AUC significantly ↓ by 73.5% for (–)-catechin epimer and by 36% for (–)-catechin gallate epimer compared to the precursors	Single dose of 4000 mg/kg (orally) or 200 mg/kg (intravenously) green tea epicatechin-epimer mixture	n = 5 rats per group	BV of epimers compared with respective precursors	[33]
Ferulic acid (FA) (supplemented diets vs. cereal matrices)	Ferulic acid (FA)	Plasma concentration ↓ by ~70–80% after intake of different cereal meals (14–16 mg/d FA) vs. the comparable supplemented diet (12 mg/d FA)	Repeated dose for 3 weeks (11–16 mg/d FA in different cereal matrices or 3, 12, and 63 mg/d FA in enriched diets vs. control diet)	n = 8 rats per group	Blood collection at the end of the intervention	[40]

[1] ↓ decreased; AUC, area under the plasma concentration-time curve; BV, bioavailability

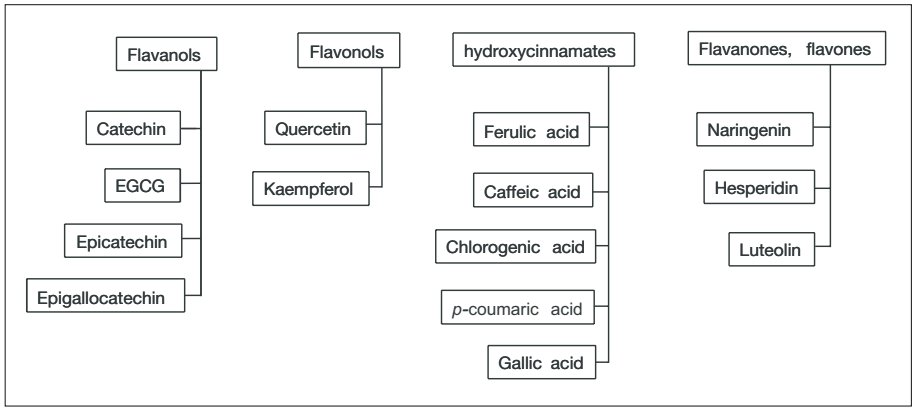

Figure 1: Classification of polyphenols covered in this review.

1. Flavonols

Modification or cleavage of one or more attached sugar

When polyphenols are present in food as glycosides, the sugar moiety probably has the greatest effect on absorption compared to any other factor. Quercetin is present in onions as a glucoside, but in tea as the rutinoside (glucose and rhamnose), and quercetin from these two sources is absorbed with very different pharmacokinetics. The much better absorption of quercetin when it is attached to glucose rather than rutinose has been reported by several authors [8–10] (Table IV). The reason is that quercetin glucoside hydrolysis takes place in the small intestine, catalyzed by brush-border glucosidases (especially lactase phloridzin hydrolase) followed by efficient absorption, whereas rhamnoside hydrolysis takes place in the colon catalyzed by microflora enzymes and is accompanied by some quercetin degradation. Although the sugar moiety has a big effect, quercetin is actually absorbed as the aglycone after removal of the sugar, and then conjugated in the enterocyte. Quercetin has a long half-life in the plasma, a short t_{max} (time to reach maximum plasma concentration, c_{max}) when given as a glucoside, and is found in plasma entirely in conjugated forms.

Solubility, delivery and food matrix

Depending on the exact conditions, the food matrix can also affect bioavailability, although to a lesser extent than the attached sugar. The key issue here is the solubility, which determines the bioaccessibility of the compound to the enterocyte. Water and fat solubility, as indicated by "Log P values" (where P is the partition coefficient between *n*-octanol and water) are important for determining bioaccessibility. A highly fat-soluble molecule, such as lycopene, a carotenoid, requires incorporation into mixed micelles for absorption. A very water-soluble molecule, such as vitamin C, simply requires solubilization in an aqueous matrix for efficient absorption. Polyphenols are in between these two examples, and range from the water-soluble flavanols to the much less water-soluble flavonols. However, the latter are usually glycosylated in foods, which increases their water solubility. Hence compounds that have lower solubility in the gut lumen could be affected by solubilizing agents, whereas very soluble compounds will be less affected by such agents.

To illustrate the effect of ethanol acting as a possible solubilizing agent, a single dose of quercetin was dissolved in three aqueous matrices (grape juice, white wine, and vegetable juice) and given to subjects to compare absorption [11]. This is an unusual design in that the compound was not already present in the food, but was added and dissolved in the different drinks. A significantly higher (~two-fold) plasma quercetin was obtained after the administration of quercetin in white wine compared to the other two matrices. One of the factors responsible for this could be the ethanol, so that quercetin is better solubilized and delivered to the enterocyte in a more bioaccessible form. The effect of ethanol (10%, 20%, 30% or 50%) on quercetin absorption in rats [12] supports this, where a concentration of 30% ethanol or more showed an absorption-enhancing effect. The absorption correlated well with the increased solubility of quercetin in higher ethanol concentrations.

Piskula *et al* [13, 14] investigated the influence of propylene glycol on the bioavailability of quercetin in rats.

Table IV:

Parameter	Flavonols	Flavanols	Flavanones and flavones	Phenolic acids
Modification or cleavage of one or more attached sugar	↑↑↑↑↑	–	↑↑↑↑↑	↑↑↑↑↑
Solubilization (e.g. ethanol, propylene glycol)	↑↑↑	↑	↑↑↑↑	?
Lipids and emulsifiers	↑↑↑	↑↑↑	?	?
Carbohydrate	?	↑↑↑	?	?
Other food matrix	↑	↑↑↑	↑↑↑	↑↑↑
Epimerization	–	↑↑↑	?	–
Other compounds	↑↑	↑↑	?	?
Dose response of absorption	Rutin linear, quercetin saturated in humans	EGCG linear when pure, EGCG saturated when in green tea in humans, epicatechin linear in rats	?	Ferulic acid linear in rat perfusion model

Relative effects from weak (↑) to strong (↑↑↑↑↑)
– not relevant
? not yet determined

Single doses were administered either in propylene glycol, a propylene glycol/water mixture, or water alone. Quercetin in pure propylene glycol showed the highest solubility and was reflected in the plasma concentration, but a direct correlation between absorption extent and solubility could not be assessed. Therefore, the authors suggest that other factors such as propylene glycol itself or the particle size might also be important regarding quercetin absorption.

The presence of a meat matrix or of fat content on quercetin absorption in pigs has also been examined. There was preferential quercetin-3-*O*-glucoside absorption from the meat matrix versus the standard diet. A lower glucose and higher fat content of the meat as well as a higher concentration of quercetin-3-*O*-glucoside in the meat portion could serve as possible explanations for the better absorption [15]. The effect of fat content on quercetin absorption in pigs was also examined [16]. The highest absorption of quercetin was for the 17% fat-containing diet compared to a 3% fat-containing control diet. A higher 32% fat diet, however, gave no further bioavailability increase. The presence of fat could have led to a better incorporation of quercetin in mixed micelles and thus a facilitated absorption through the brush border membrane. However, it has not been proven that quercetin requires mixed micelles for absorption, or that the presence of mixed micelles favors quercetin absorption. Again, as for ethanol, absorption may simply reflect improved solubility and bioaccessibility. In rats, only a combination of lipid and emulsifier enhanced the plasma concentration of quercetin and its metabolites, whereas lipids or emulsifiers separately showed no significant effects; again this could reflect differences in solubility [12]. Lecithin and soybean oil were also effective enhancers of quercetin absorption in rats, and co-ingestion of emulsifiers was also effective [12, 17].

The concept of the food matrix affecting absorption of rutin was tested in humans, given either as a pure compound or as naturally present in buckwheat tea. Only a small effect (a trend) was seen on absorption. This weak effect presumably was observed because rutin is quite water-soluble, and it is well solubilized in the gastrointestinal (GI) tract when given as a solid [18]. A different approach to improve quercetin solubility was tested in rats. α-G-rutin (a synthetic derivative of rutin consisting of α-D-glucopyranosyl-rutin (82%) and isoquercitrin (13%)) was significantly better absorbed than rutin based on area-under-the-curve (AUC) measurements [19], which could be explained by a greater water solubility of α-G-rutin compared to rutin.

These results all indicate that quercetin absorption is affected by solubility, although the effects are not as dramatic as the effect of the sugar moiety. The presence of fat in the diet, or the combined presence of lipids and emulsifiers, is effective at increasing absorption. Furthermore, the nature of the food or beverage matrix might be an important determinant for the absorption, but this may again be an issue of solubility.

Dose and adaptation to dose

Rutin and quercetin in different doses were given to volunteers, and the concentration in plasma measured. There was a linear increase in plasma quercetin concentration with dose of rutin, but with quercetin, the plasma concentration of quercetin was saturated [10]. This could be related to solubility, since rutin is quite water-soluble and so bioaccessible at all concentrations, whereas quercetin aglycone is potentially not.

2. Flavanols

Flavanols are catechin, epicatechin, and their oligomeric forms, such as procyanidins, and include epicatechin derivatives from green tea, such as epigallocatechin gallate (EGCG). Typically, the flavanols are not glycosylated and so the effect of attached sugars, as discussed above for flavonols, has not been tested. Flavanols are less well absorbed than quercetin, and appear in the blood with a T_{max} of around 2 hours. The procyanidins are poorly absorbed, and also the absorption of EGCG is quite low. Owing to chiral centers in the molecules, the flavanols exist in enantiomeric forms, usually referred to as (+) and (–).

Solubility, delivery and food matrix

Alcohol appears to have only a minimal effect on the absorption of flavanols. After consumption of catechin in wine or in dealcoholized wine, there was no difference in plasma concentration in healthy volunteers in two studies [20, 21]. Additionally, catechin was better absorbed when dissolved in grape juice compared to white wine or vegetable juice [11]. However, the addition of phospholipids to a green tea preparation increased the plasma concentration of EGCG [22], indicating that lipids could be important.

Schramm *et al* [23] investigated the influence of other foods on catechin and epicatechin absorption from cocoa, and showed that concurrent consumption of bread, sugar, or grapefruit juice affected the bioavailability in humans. Carbohydrate-rich food resulted in significant increases in plasma flavanols versus cocoa alone, whereas protein-flavanol or lipid-flavanol interactions had minimal effects on bioavailability.

The effect of some small molecules on absorption has been tested, although the original rationale for testing these particular compounds is not obvious. Piperine, a component of pepper, increased EGCG absorption in mice [24]. Both the inhibition of small intestinal glucuronidation of EGCG (although this is already low) and a delay in the gastrointestinal transit time by piperine may be responsible for this increase. Tartaric acid also increased the amount of plasma catechin-5-*O*-β-glucuronide when given to rats after a single dose of catechin in water [25]. Furthermore, an increase in plasma AUC is suggested by the authors, but was not explicitly determined.

Single doses of a green tea extract were consumed fasting or with a light breakfast. For all three dose levels applied (400–1200 mg), a significant increase in plasma free EGCG was observed in the absence of food compared to the presence of breakfast. Similarly, c_{max} of free forms of EGCG, epigallocatechin, and epicatechin gallate obtained without food were significantly higher compared to the breakfast-associated concentrations [26]. This demonstrates that consumption of green tea catechins with food under certain conditions may reduce the absorption of EGCG. It has been reported that EGCG is better absorbed from a green tea supplement in a capsule compared to black or green tea drink, although the mechanism of this is unclear [27].

Dose and adaptation to dose

Various doses of pure EGCG (up to 1.6 g) were given to humans, and resulted in plasma concentrations proportional to the dose, indicating a linear dose response even up to high levels of EGCG [28]. However, another study on volunteers given three doses of decaffeinated green tea containing up to 330 mg EGCG did not find a linear increase in plasma with dose. Instead, the study found that the highest dose (330 mg EGCG equivalents) was apparently saturated [29]. In another study, volunteers were given a dose of green tea catechins (164 mg) and compared to 2 doses of the same green tea catechins (each of 164 mg) separated by 2 hours. Plasma concentrations of EGCG after this double ingestion were slightly but not significantly higher than after the single dose (180 minutes after intake). In an additional experiment, the effect of repeated intakes of green tea catechins 3 times a day for 1 week was investigated. After repeated dosing, concentrations of conjugated EGCG were significantly increased after 1 week compared to the time point before the first ingestion. Therefore, continuous daily intake seems to be important for the maintenance of a high plasma concentration [30]. Chow *et al* [31] also investigated repeated dosing. Eight subjects received either 400 mg EGCG isolated or as green tea extract twice a day or 800 mg EGCG or green tea extract once. After intake of 800 mg EGCG or green tea extract once daily for 4 weeks, a significant increase in plasma EGCG compared to the measurement on the first study day was observed. By comparing the results on the last treatment days, a significant difference between the 400 mg dose given twice daily and the 800 mg dose once daily could also be observed. No significant changes in the AUC were seen after administration of the 400 mg dose twice daily for 4 weeks compared with the time point of the first intake.

Single doses of 1, 5, and 10 mg/kg epicatechin compared to the same dose administered in a cocoa powder were given to rats [32]. A linear dose response (correlation coefficient, $r^2 = 0.99$ for epicatechin from cocoa, $r^2 = 0.93$ for epicatechin as pure compound) for c_{max} in plasma was observed, and epicatechin present in the cocoa powder matrix was absorbed as efficiently as the flavanol administered alone.

Inhibition of any chemical changes that might occur during processing or in the GI tract

Flavanols, unlike flavonols, possess 2 chiral centers in each monomeric unit. The naturally occurring forms are (–)-epicatechin and (+)-catechin, and the oligomeric procyanidins are made from these compounds. However, during certain processes and under conditions such as high temperature, pH, etc., (–)-epicatechin can be converted to (–)-catechin, and (+)-catechin to (+)-epicatechin. In the same way, (–)-EGCG can be converted to (–)-gallocatechin gallate. The influence of these epimerization reactions induced by heat treatment was examined by comparing the bioavailability of generated epimers with their corresponding precursors in rats [33]. A single dose of a mixture containing catechins and their epimers was administered orally or intravenously. (–)-Catechin, (–)-catechin gallate, and (–)-gallocatechin gallate were less efficiently absorbed compared to their precursors (–)-epicatechin, (–)-epicatechin gallate, and EGCG respectively. This was also seen in a direct small intestinal perfusion experiment in rats, where the (–) form of catechin was absorbed to a much lower extent than the naturally-occurring (+) form, and in addition, the (–) form was much less methylated [34]. Additionally, (–)-epicatechin may be better absorbed than (+)-catechin in rats [35].

Competition and interaction with other compounds

Silberberg *et al* [36] compared the bioavailability and metabolism of quercetin and catechin when administered sep-

arately or in association in the diet of rats. Co-administration of these flavonoids revealed that the plasma concentration of metabolites decreased significantly by 35% for quercetin and 28% for catechin. According to the authors, a competitive interaction between the two compounds during digestion could have been responsible for the impaired bioavailability. The presence of mixed micelles and mixed micelles containing vitamin C, β-carotene, lutein, and α-tocopherol had no effect on catechin absorption in a rat intestinal perfusion model [37].

3. Other flavonoids and phenolic acids

There is less information in the literature on hydroxycinnamates (phenolic acids), flavones, and flavanones. The absorption of the flavanones, hesperidin and naringenin, follows the same pathway as the quercetin derivative, rutin: they are de-rhamnosylated by colonic microflora, the aglycone is absorbed in the colon, and appears as conjugates in the blood. It is less clear for hydroxycinnamates, such as ferulic and caffeic acids. Ferulic acid is often covalently bound to cell wall in cereals, and so is released in the colon by microflora and absorbed in the colon. The most consumed form of caffeic acid is as chlorogenic acid, and although caffeic acid conjugates are found in blood, the exact metabolism of chlorogenic acid is not clear.

Modification or cleavage of one or more attached sugar

The removal of rhamnose from hesperidin by enzymatic hydrolysis dramatically improved the absorption from orange juice in humans [38]. This is exactly comparable to the situation with quercetin-glucosides and rutin described above, since c_{max} was much higher and t_{max} was reached faster after ingestion of the hesperetin-7-glucoside-containing juice compared to hesperidin in orange juice.

Ferulic acid is also attached to a sugar (arabinose) in cereals, the best source of dietary ferulic acid. In this case, however, the arabinose is in turn covalently attached to the cereal matrix, which means that effectively the ferulic acid is insoluble and not available for absorption unless first released from the matrix. *In vivo*, the release from the cereal matrix occurs in the colon catalyzed by enzymes from the colonic microflora. Absorption from the cereal bran therefore follows very different pharmacokinetics compared to free ferulic acid in rats [39]. With free ferulic acid, the peak plasma concentration was reached in less than one hour, and the concentration declined to zero after 4 hours. When the ferulic acid was attached to the cereal matrix, there was a much smaller peak of absorption, but the ferulic acid was still present in plasma 24 hours after consumption of the cereal. This is due to slow but sustained release of the bound ferulic acid in the colon. Consistent with the plasma data, urinary recovery of ferulic acid after feeding free ferulic acid was about 50%, but only 3% after consumption of cereals in rats [40]. When ferulic acid was given to rats as corn bran, about 80% was found in the feces, and only about 0.5% in the urine. This did not change after 10 days adaptation to corn bran [41].

Solubility, delivery and food matrix

For the flavones, some information is available for luteolin [42]. Luteolin and luteolin-7-*O*-β-glucoside were given to rats by administering the flavonoids either in 0.5% carboxymethyl cellulose sodium or 0.5% propylene glycol. According to the pharmacokinetic profile, the maximal plasma concentration of total luteolin increased by around three-fold for luteolin in propylene glycol compared to luteolin administered in sodium carboxymethyl cellulose.

The effects of domestic cooking on the bioavailability of the flavanone naringenin and the phenolic acid chlorogenic acid in fresh or cooked cherry tomatoes was assessed in humans [43]. Both compounds were barely detected in plasma after consumption of fresh tomatoes. After eating cooked tomatoes, both naringenin and intact chlorogenic acid were significantly detected in plasma. This is clearly a significant effect of the food matrix and may be related to cell wall breakage.

Dose and interaction with other compounds

Over a five-fold concentration range, free ferulic acid was given in solution in a rat model of perfusion. The absorbed dose was proportional to the concentration given (r^2 = 0.997) [39]. The presence of mixed micelles and mixed micelles containing vitamin C, β-carotene, lutein, and α-tocopherol had no effect on caffeic acid and naringenin absorption in a rat intestinal perfusion model [37].

4. Conclusion

The bioavailability of dietary polyphenols is of great importance in determining any associated health-promoting effects. As presented in this review, several factors are known which could influence the bioavailability of different polyphenols in the diet. Several observations have been reported in this research area but there is not yet suf-

ficient information or evidence to prove general principles with any predictive capacity. Table IV summarizes the data qualitatively, although the conclusions may change as more information becomes available. Further studies are required to investigate more deeply and systematically the various types of interactions affecting the bioavailability of different classes of polyphenols. The consequences on the bioavailability need to be clarified and more human studies should be conducted in this field to establish more conclusive evidence *in vivo*. Information derived from such experiments could be useful for the design of future bioefficacy studies.

Abbreviations

AUC area under the plasma concentration-time curve
c_{max} maximal plasma concentration
t_{max} time to reach c_{max}
EGCG Epigallocatechin gallate

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