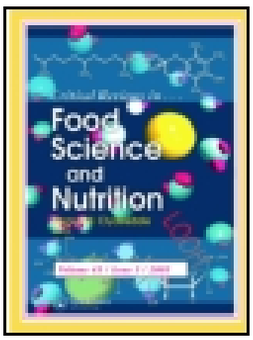

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REVIEW

Bioavailability and metabolism of selected cocoa bioactive compounds: A comprehensive review

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ABSTRACT

Cocoa beans and their co-products are a rich source of beneficial compounds for health promotion, including polyphenols and methylxanthines. Knowledge of bioavailability and *in vivo* bioactivity of these phytochemicals is crucial to understand their role and function in human health. Therefore, many studies concerning bioavailability and bioactivity of cocoa bioactive compound have been done in both *in vivo* animal models and in humans. This critical review comprehensively summarizes the existing knowledge about the bioavailability and the major metabolic pathways of selected cocoa bioactive compounds (i.e. monomeric flavan-3-ols, procyanidins, anthocyanins, flavonols, phenolic acids, *N*-phenylpropenoyl-L-amino acids, stilbenes, and methylxanthines). The compiled results indicated that many of these compounds undergo extensive metabolism prior to absorption. Different factors have been suggested to influence the bioavailability of polyphenols and methylxanthines among them the role of gut microbiota, structure of these compounds, food matrix and occurrence of other substances were the most often considered. Aforementioned factors decided about the site where these bioactive compounds are digested and absorbed from the alimentary tract, as well as the pathway by which they are metabolized. These factors also determine of the type of transport through the intestine barrier (passive, involving specific enzymes or mediated by specific transporters) and their metabolic path and profile.

KEYWORDS

Theobroma cacao L.; polyphenols; methylxanthines; cocoa metabolites; absorption; colonic catabolism

Introduction

Seeds of cocoa tree (*Theobroma cacao* L.) are a principal raw material for manufacturing of chocolate, cocoa powder and other cocoa-derived products (Badrie et al. 2015; Kongor et al. 2016). Cocoa products are widely consumed worldwide by different population groups, and the global cocoa consumption is still growing (Beg et al. 2017). For many years, chocolate was consumed purely for pleasure, but in the last years researchers have shown that cocoa-rich products have beneficial effect on human health (Bernaert et al. 2012; Latif 2013; Rusconi and Conti 2010; Smith 2013; Torres-Moreno et al. 2012). Increasing evidence from clinical and epidemiological studies, and associated meta-analyses suggests that the regular consumption of cocoa-derived products can contribute to preventing of chronic illnesses such as cardiometabolic diseases, cancers, and neurodegenerative diseases (Beg et al. 2017; Castell, Pérez-Cano, and Bisson 2013; De Araujo et al. 2016; Yuan et al., 2017). These health benefits have been attributed to the occurrence in cocoa beans and cocoa-derived products of polyphenols, mainly flavonoids (Gardea et al. 2017; Kris-Etherton and Keen 2002; Latif 2013; Smith 2013; Tomas-Barberan et al. 2007). Polyphenols constitute approximately 6–8% solid substance of cocoa beans (Table 1). Flavonoids comprise the most abundant class of phenolic compounds in cocoa beans, and include mainly flavan-3-ols, anthocyanins and flavonols (Aprotosoaie, Luca, and Miron 2016; Jalil and Ismail 2008; Oracz, Zyzelewicz, and Nebesny 2015; Tomas-Barberan et al. 2007). Recent reports indicate that cocoa beans and cocoa-derived products contain also substantial quantities of another group of polyphenols such as phenolic acids, stilbenes, and *N*-phenylpropenoyl-L-amino acids (NPAs) that belong to the family of polyphenol/amino acid conjugates (Table 2). These bioactive compounds are secondary cocoa metabolites that play fundamental role in plant protection against UV light, pathogens, parasites and plant predators (Belščak et al. 2009; Lechtenberg et al. 2012; Salvador et al. 2018). Additionally, they contribute substantially to the organoleptic properties of cocoa beans and cocoa-derived products (Aprotosoaie, Luca, and Miron 2016; Mojzer et al. 2016). Moreover, both cocoa beans and cocoa derived products contain considerable amounts of methylxanthines, which are another group of bioactive secondary cocoa metabolites derived from the purine base xanthine generated via repeated methylation (Belščak et al. 2009; Jalil and Ismail 2008; Monteiro et al. 2016). In addition to polyphenols and methylxanthines, cocoa beans are also a good source of

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Table 1. Chemical composition of cocoa products (g/100 g dry weigh).

Component	Raw cocoa nibs	Roasted cocoa nibs	Cocoa powder
Water	6.0–8.0	3.0–3.7	3.0–7.0
Fat	46.0–54.0	54.0	11.0–14.0
Protein	10.0–15.0	12.5	20.0–22.0
Polysaccharides	12.0	—*	16.0
Mono-and oligosaccharides	2.0–4.0	2.0	1.7
Fiber (insoluble/soluble)	3.1	2.5	33.2–34 (25.5/8.5)
Polyphenols	6.0–8.0	6.0	4.0
Theobromine	3.0	3.0	2.1
Caffeine	0.2	0.2	0.2
Ash	5.6	3.0	5.80
References	(Kongor et al. 2016; Jalil and Ismail 2008)	(Lima et al. 2011)	(Massot-Cladera et al. 2015; Wilson 2012)

*No data.

Table 2. Content of major polyphenols in cocoa beans (mg/g dry weigh).

Compounds	Raw cocoa beans	Roasted cocoa beans	Reference
Flavan-3-ol monomers and Procyanidins			
(−)-Epicatechin	0.97–4.82	0.91–3.94	Kothe et al. 2013
(+)-Catechin	0.07–0.26	0.21–0.62	
Procyanidin B1	0.018–0.027	0.015–0.022	
Procyanidin B2	0.43–2.03	0.43–1.70	
Procyanidin B5	0.12–0.57	0.10–0.45	
Anthocyanins			
Cyanidin-3-O-arabinoside	0–1.19	0–1.02	Oracz et al. 2015
Cyanidin-3-O-galactoside	0–0.81	0–0.75	
N-phenylpropenoyl-L-amino acids			
N-caffeoyl-L-aspartic acid	0.34–2.42		Lechtenberg et al. 2012
N-coumaroyl-L-aspartic acid	0.11–0.63		
N-caffeoyl-L-dopa	0.12–0.37		
N-coumaroyl-L-tyrosine	0.04–0.18		
N-caffeoyl-L-tyrosine	0.01–0.11		

dietary fibers (Table 1), such as cellulose, hemicellulose and pectic substances (Lecumberri et al. 2007; Lima et al. 2011; Massot-Cladera et al. 2015). Recently, it has been suggested that cocoa fiber also may be responsible for the health benefits of cocoa consumption (Lecumberri et al. 2007; Massot-Cladera et al. 2015). Despite mentioned earlier, other compounds with biological activity like biogenic amines, *N*-acylethanolamines, tetrahydro-β-carbolines and anandamide, an endogenous ligand for the cannabinoid receptor can be found in cocoa beans and cocoa-derived products (Nehlig 2013). Chemical structures of the most common bioactive compounds present in cocoa bean and its derived products are shown in Figure 1.

The presence of the aforementioned bioactive compounds renders cocoa beans an attractive source material for manufacturing of functional foods and nutraceuticals. Many recent studies have focused on cocoa polyphenols as a potential health-promoting compounds due to its abundance in the cocoa beans and cocoa derived-products (Berry et al. 2010; Cooper et al. 2008; Grassi, Desideri, and Ferri 2010; Ferri et al. 2015; Ostertag et al. 2013). It is well known, that biological function of cocoa polyphenols results from its high antioxidant activity and inhibition of certain enzymes, including those participating in the development of inflammatory processes (Kris-Etherton and Keen 2002; Latif 2013; Tomas-Barberan et al. 2007). Cocoa and dark chocolate polyphenols may reduce the risk of cardiovascular events by lowering the blood pressure, exerting metabolic and anti-atherosclerotic effects, as well as improving endothelial function (Aprotosoaie et al. 2016; Badrie et al. 2015; Castell, Pérez-Cano, and Bisson 2013; De Araujo et al. 2016). While methylxanthines have been reported to exert general stimulatory effects on the central nervous, cardiovascular systems and even other vegetative centers (Franco, Oñatibia-Astibia, and Martínez-Pinilla 2013; Briz, Ruiz, and Bravo-Clemente 2017; Monteiro et al. 2016). Recent evidence suggests that the bioavailability of phenolic compounds and methylxanthines is crucial to their potential beneficial effects on human health (Mojzer et al. 2016; Ozdal et al. 2016; Rusconi and Conti 2010; Visioli et al. 2009; Visioli et al. 2011). However, the accurate mechanisms of action, effects and bioavailability of these compounds *in vivo* are still not fully recognized. In order to understand this issue, it is worth to remind the terms of bioavailability, bioaccessibilty and bioactivity. Bioavailability is usually defined as the amount of analyzed compound or its metabolite that reaches the systemic circulation after administration of an acute or chronic dose of an isolated compound or a compound-containing food (Holst and Williamson 2008). Bioaccessibility describes the fraction of a component that is available for absorption in the gastrointestinal tract (GI tract). As a consequence, the bioactive component should be efficiently digested, assimilated, absorbed and finally exerts a positive or negative effect at the target organ (Stahl et al. 2002; Holst and Williamson 2008). Therefore, bioactivity can be described as the specific effect upon exposure to a substance including tissue uptake and the consequent physiological response, and may be evaluated *in vivo*, *ex vivo*, and *in vitro* methods (Carbonell-Capella et al. 2014; Fernández-García et al. 2009). The relation between bioaccessibility, bioavailability and bioactivity,

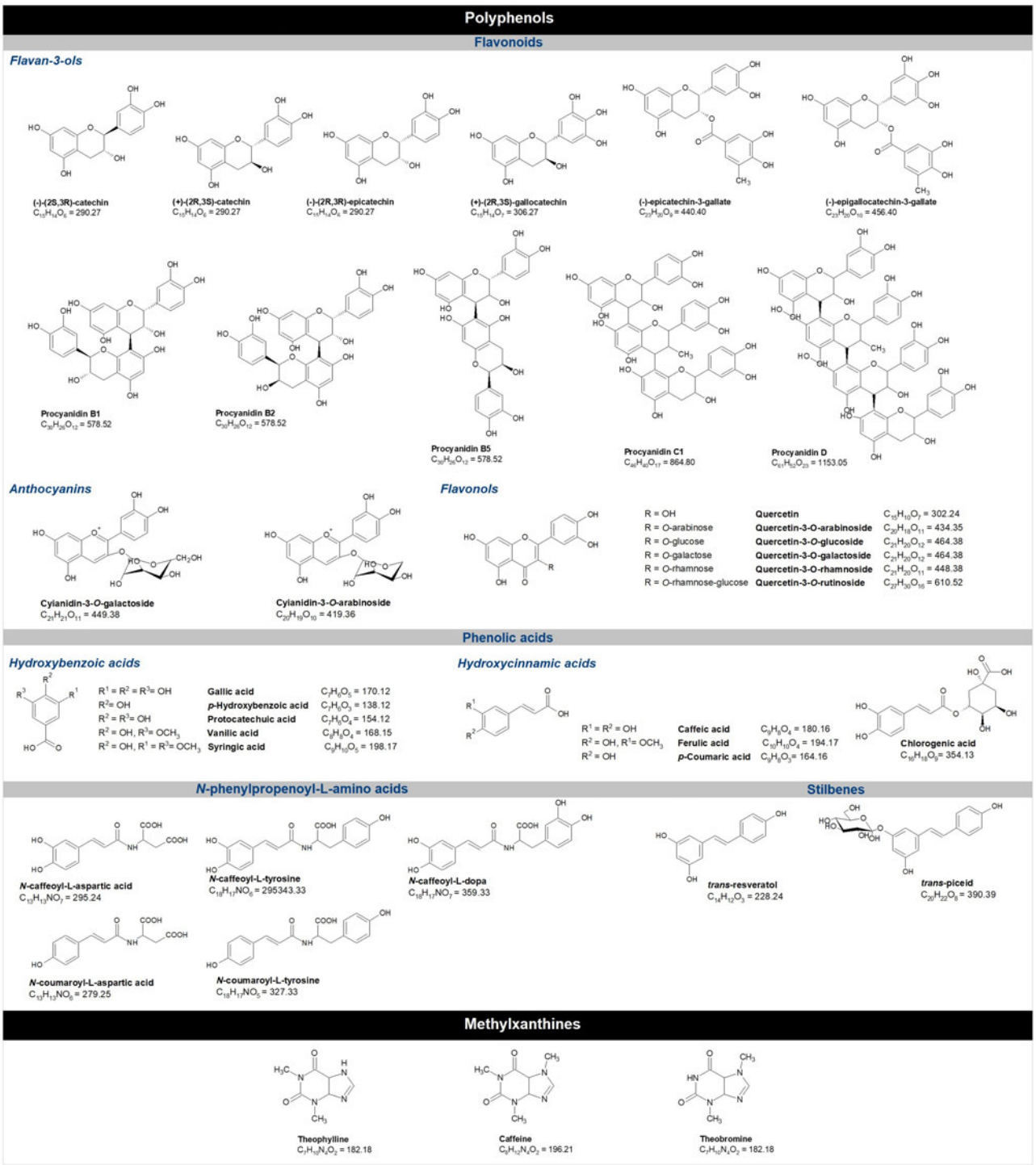

Figure 1. Chemical structures of polyphenols and methylxanthines present in cocoa and cocoa-derived products.

as well as their potential assessment methodologies are showed in Figure 2 (Carbonell-Capella et al. 2014; Fernández-García, Carvajal-Lérida, and Pérez-Gálvez 2009). Numerous studies on the pharmacokinetics and metabolism of cocoa polyphenols and methylxanthines including the different technical approaches and models (*in vitro, in vivo* or *ex vivo*) were tested by many authors out leading to variability in determined metabolites structures and profiles. Therefore, direct comparison of the metabolic pathways and metabolites profiles was problematic (Carbonell-Capella et al. 2014; Fernández-García, Carvajal-Lérida, and Pérez-Gálvez 2009; Smith 2013; Urpi-Sarda et al. 2010). The results of mentioned investigations revealed that bioactive cocoa compounds, due to great structural diversity greatly differ in the metabolites profile, its stability in the GI tract, degree of absorption and time of their metabolism. However, it should be emphasized that the number of biological and clinical studies of bioactive cocoa compounds and its influence on human health were focused on elucidation metabolism of the major cocoa flavan-3-ols and the factors that impact

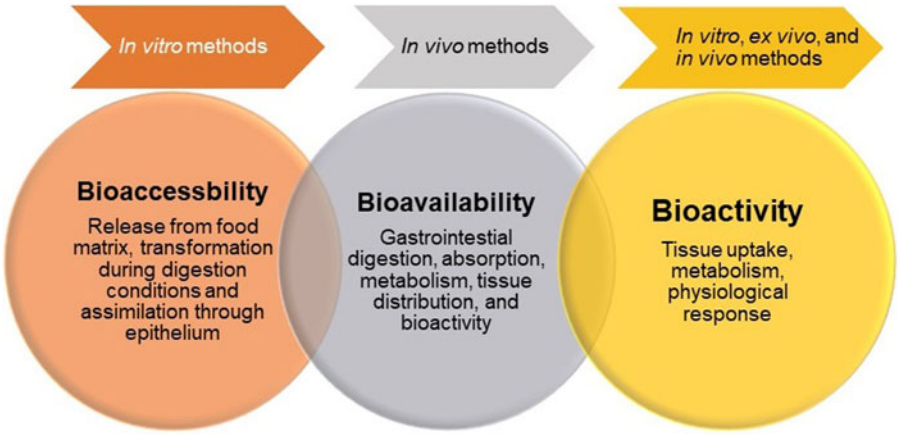

Figure 2. Schematic presentation interrelation of bioaccessibility, bioavailability and bioactivity and their potential assessment methodologies.

administration, distribution and metabolism of these substances (Cifuentes-Gomez et al. 2015; Dorenkott et al. 2014; Urpi-Sarda et al. 2010).

Polyphenols are recognized by the human body as xenobiotics, and their bioavailability is therefore relatively low in comparison to micro and macronutrients. Depending on their degree of structural complexity and polymerization the absorption pathway in the GI tract is different. The fraction of low-molecular-weight polyphenols such as monomeric and dimeric structures (5–10% total polyphenol intake) may be absorbed in the small intestine. The remaining polyphenols i.e. oligomeric and polymeric polyphenols such as condensed or hydrolyzable tannins, reaching molecular weight values close to 40,000 Da (90–95% of total polyphenol intake) reach the colon almost unchanged (Cardona et al. 2013; Borges et al. 2018). These high-molecular-weight polyphenols may accumulate in the colon up to the millimolar range, and be the subjects to enzymatic activities of the gut microbial community. The colonic microbiota are therefore responsible for the extensive breakdown of the original polyphenolic structures into a series of low-molecular-weight phenolics transforming into absorbable metabolites (Cardona et al. 2013).

Studies on bioavailability of cocoa methylxanthines revealed that the bioconversion of those compounds require multistep reaction including their *N*-demethylation to dimethylxanthines and monomethylxanthines, C8-oxidation to corresponding methyluric acids and ring opening reactions yielding substituted uracil metabolites (Arnaud 2011; Martínez-López et al. 2014; Ptolemy et al. 2010).

This review summarizes the bioavailability and metabolism of the selected bioactive compounds, like monomeric flavan-3-ols, procyanidins, anthocyanins, flavonols, phenolic acids, NPAs, stilbenes, and methylxanthines from cocoa-derived products, such as chocolate and cocoa powder, and provides clearly and systematically summarized insight into the mechanisms underlying the disposition, as well as interactions of these bioactive cocoa compounds with the human gut microbiota. Furthermore, the results of recent studies on the major factors affecting the bioaccessibility and bioavailability of these bioactive cocoa compounds have also been discussed.

Bioavailability and metabolism of polyphenols

Flavonoids

Numerous scientific reports on biological activities of flavonoids contained in cocoa beans, chocolate and cocoa powder paved the way to studies on their absorption, metabolism and secretion, which were conducted either *in vitro*, *ex vivo* or *in vivo* models (Rein et al. 2000; Roura et al. 2005; Tomas-Barberan et al. 2007; Ottaviani et al. 2011; Ottaviani et al. 2012a; Urpi-Sarda et al. 2010). The possible human metabolic and elimination pathways of selected flavonoids present in cocoa beans and cocoa derived products is shown in Figure 3. The bioavailability of the most common cocoa polyphenols and their main metabolites in humans are summarized in Table 3.

Flavan-3-ols

Flavan-3-ols have been the major group of flavonoids found in cocoa beans (Table 2). It is generally known that cocoa bean is a rich source of flavan-3-ols monomers, namely (−)-epicatechin [(−)-EC] and (±)-catechin [(±)-C] (approximately 37% of total polyphenols), as well as oligomers and polymers of these two monomers (approximately 58% of the total polyphenols) (Wollgast and Anklam 2000). Apart from these two main flavan-3-ol monomers [(−)-EC and (±)-C], cocoa beans contain also (+)-gallocatechin and (−)-epigallocatechin and their corresponding gallate esters, like (−)-epicatechin-3-*O*-gallate and (−)-epigallocatechin-3-*O*-gallate (Andres-Lacueva et al. 2008; Oracz, Zyzelewicz, and Nebesny 2015; Wollgast and Anklam 2000). Oligomeric and polymeric flavan-3-ols, also known as procyanidins (PCs) contained in cocoa seeds are represented by dimers, trimers, oligomers, and polymers composed of flavan-3-ol and flavan-3,4-diol units linked mainly through C4→C8 or C4→C6 bonds. Most abundant of them in cocoa beans, chocolate and cocoa powder are dimers [procyanidin B1 (PC B1), procyanidin B2 (PC B2), procyanidin B5 (PC B5)], trimers [procyanidin C1 (PC C1)], tetramers (cinnamtannin A2), and higher polymers (Aron and Kennedy 2008; Smith 2013; Oracz, Zyzelewicz, and Nebesny 2015). Some recent studies have indicated that PCs may constitute even over

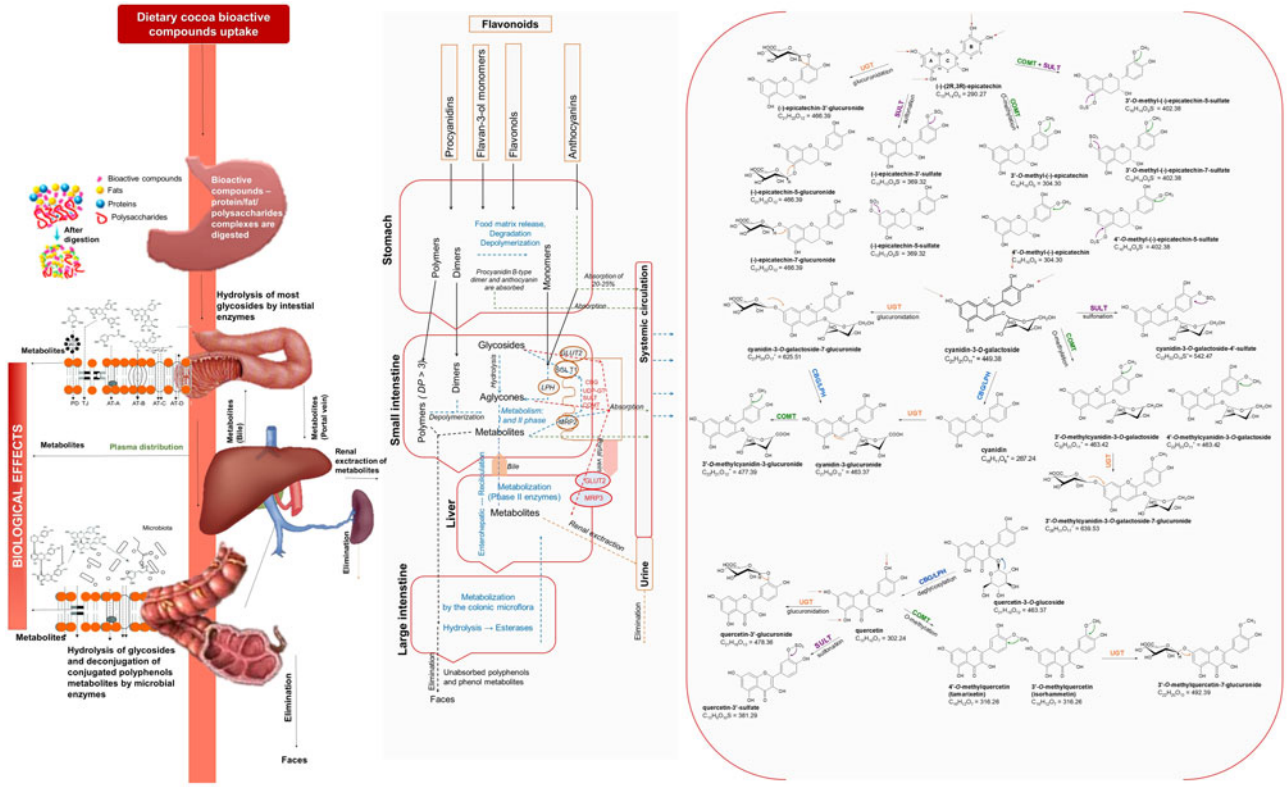

Figure 3. Proposed human metabolism of selected flavonoids found in cocoa beans and cocoa products; CBG, cytosolic β-glucosidase; COMT, catechol-*O*-methyl transferase; GLUT1/2, glucose transporters type 1 or 2; LPH, lactase-phlorizin hydrolase; MCT, monocarboxylate transporter; MDR, multiple drug resistance; MRP2/3, multidrug resistance-associated protein 2 or 3; SGLT1/2, sodium-dependent glucose transporter 1 or 2; SR-B1: Scavenger receptor class B type 1; ST, sulfotransferase; SULTs, sulfotransferases; UGT, uridine-5′-diphosphate glucuronosyltransferase (Crozier, Del Rio, and Clifford 2010; Marques et al. 2016; Ottaviani et al. 2012a; Serra et al. 2011).

90% of the total cocoa polyphenols, while flavan-3-ol monomers reach only 5–10% (Pedan et al., 2017).

Monomeric flavan-3-ols

Several studies have provided evidence that naturally occurring in cocoa bean and cocoa-derived products monomeric flavan-3-ols are absorbed and present in the systemic circulation of humans after consumption, and exert their biological effects, in a dose-dependent manner (Actis-Goretta et al. 2013; Borges et al. 2018; Cifuentes-Gomez et al. 2015; Mena et al. 2019; Ottaviani et al. 2018; Rodriguez-Mateos et al. 2014; Urpi-Sarda et al. 2010; Crozier 2013). A dose-response effect from chocolate or cocoa intake on plasma EC was indicated in 30 to 60 minutes after consumption. The maximum plasma concentration (C_{max}) of these compounds was reached 2–3 h after ingestion of flavan-3-ol-rich cocoa products (Baba et al. 2000; Del Rio et al. 2013; Richelle et al. 1999; Rodriguez-Mateos et al., 2015). According to Richelle et al. (1999), consumption of 80 g of dark chocolate containing 164 mg of EC by humans resulted in rapid absorption achieving EC plasma concentration of 0.7 µmol/L. Baba et al. (2000) found the highest concentrations of EC metabolites in blood plasma (3.46 µmol/L) 2 h after the oral administration of either chocolate or cocoa drink containing 220 mg EC. According to Holt et al. (2002) EC, C and PC B2 appeared in blood plasma (at concentrations of 16 ± 5 nmol/L, 2.61 ± 0.46 µmol/L and 0.13 ± 0.03 µmol/L, respectively) 30 minutes after the administration of 0.375 g cocoa/kg body mass (in the form of cocoa drink), while C_{max} of these compounds (41.0 ± 4.0 nmol/L, 5.92 ± 0.60 µmol/L and 0.16 ± 0.03 µmol/L, respectively) were observed 2 h after the intake. These results are consistent with data presented in other reports related to metabolism of flavan-3-ols after the intake of products derived from cocoa beans (Rein et al. 2000; Roura et al. 2005; Tomas-Barberan et al. 2007; Ottaviani et al. 2011; Ottaviani et al. 2012a).

However, the subsequent investigations have demonstrated that many factors may affect the bioavailability of flavan-3-ols in humans (Table 4). The key factor deciding of the absorption and systemic distribution of cocoa-derived monomeric flavan-3-ols is their stereo-isomeric form (Borges et al. 2018; Cifuentes-Gomez et al. 2015; Lau-Cam 2013; Ottaviani et al. 2011). Early *in vivo* studies indicate that (+)-C was relatively well absorbed in the small intestine in contrast to (−)-C (Donovan et al. 2006). Other studies revealed that the bioavailability of (−)-EC is higher than that of (+)-C (Monagas et al. 2010). Further studies on the relationship between the structure of monomeric flavan-3-ols and their bioavailability demonstrated that their different stereoisomers were released from food products after distinct periods of time, as well as their transport through intestinal mucosa to blood plasma, metabolism and excretion varied notably (Ottaviani et al. 2011; Lau-Cam 2013). Ottaviani et al. (2011) showed that 2 h after oral

Table 3. Summary of bioavailability and main metabolites and colonic catabolites of the most common cocoa polyphenols and methylxanthines.

Compounds	Bioavailability	Metabolites in humans	Colonic catabolites in humans	Reference
Monomeric flavan-3-ols and Procyanidins	Monomeric flavan-3-ols and procyanidins are stable at the acid environment of the stomach and reached the small intestine unchanged. Approximately 20% of monomeric flavan-3-ols are quickly absorbed in the small intestine and are circulate in the bloodstream after being rapidly and extensively metabolized into numerous structurally related metabolites such as *O*-glucuronidated, sulfated and *O*-methylated conjugates bearing an intact flavanol ring. The majority of unabsorbed flavan-3-ols reaches the colon almost intact, where they are extensively biotransformed by the gut microflora to several low molecular weight metabolites.	epicatechin-3′-β-D-glucuronide (−)-epicatechin-3′-sulfate (−)-epicatechin-5-sulfate (−)-epicatechin-7-sulfate 3′-*O*-methyl-(−)-epicatechin-5-sulfate 3′-*O*-methyl-(−)-epicatechin-7-sulfates 4′-*O*-methyl-(−)-epicatechin-5-sulfate 4′-*O*-methyl-(−)-epicatechin-7-sulfates	phenyl-γ-valerolactones hydroxyphenylvaleric acids phenylpropionic acids phenylacetic acids hydroxybenzoic acids hydroxyhippuric acid hippuric acid methyl, glucunoride and sulfate conjugates	Borges et al. 2018 Cifuentes-Gomez et al. 2015 Garcia-Aloy et al. 2015 Mena et al. 2019 Ottaviani et al. 2016 Rodriguez-Mateos et al., 2014 Urpi-Sarda et al. 2010
Anthocyanins	The bioavailability of anthocyanins is quite poor. After consumption, a large fraction of anthocyanins reach the colon and are subjected to pH-mediated degradation and/or hydrolysis by the gut microbiota with the β-glucosidase activity. The resulting aglycones are highly unstable at neutral pH and undergo spontaneous fission of the C-ring through various intermediates resulting in the formation of smaller phenolic acids and aldehydes.	cyanidin-glucuronide methylcyanidin-glucuronide methylcyanidin-3-*O*-glucoside-glucuronide	protocatechuic acid propionic acid hydroxyhippuric acid catechol pyrogallol phloroglucinaldehyde glucunoride conjugates sulfate conjugates	Czank et al. 2013 Del Rio et al. 2013 Faria et al. 2014 Morais et al. 2016
Flavonols	Quercetin aglycone exhibited lower bioavailability compared with its glycosides. Quercetin glycosides were absorbed after their deglycosylation into quercetin aglycones, and then undergoes phase II metabolism. Quercetin aglycone can then be further degraded by the colonic microbiota by carbon cleavage and ring fissions that lead to the production of several low molecular weight metabolites.	quercetin-3′-sulfate quercetin-3′-*O*-glucuronide 3′-*O*-methylquercetin 4′-*O*-methylquercetin	hydroxyphenylacetic acids hydroxyphenylpropionic acids protocatechuric acid hydroxybenzoic acid phloroglucinol	Braune and Blaut 2016 Pasinetti et al. 2018 Petersen et al. 2016 Wang and Sang 2018
N-phenylpropenoyl-L-amino acids	*N*-phenylpropenoyl-L-amino acids (NPAs) are supposed to be poorly absorbed also along the small intestine. After ingestion, NPAs are metabolically conjugated to give the corresponding glucuronide, sulfate, and/or *O*-methyl conjugates. NPAs during microbial degradation are finally transformed into phenolic acids.	*N*-coumaroyl-L-aspartic acid *N*-coumaroyl-L-glutamic acid *N*-coumaroyl-L-tyrosine *N*-feruloyl-L-aspartic acid	caffeic acid glucunoride conjugates	Gonthier et al. 2003 Stark et al. 2008
Stilbenes	Resveratrol is absorbed through the gastrointestinal tract and can further undergo rapid metabolism by both	resveratrol-4′-*O*-glucuronide resveratrol-3-*O*-glucuronide resveratrol-3-O-sulfate	dihydroresveratrol 3,4′-dihydroxy-trans-stilbene 3,4′-dihydroxybibenzyl	Bode et al. 2013 Rodriguez-Mateos et al. 2014 Walle et al. 2004 Wang and Sang 2018

(*continued*)

Table 3. Continued.

Compounds	Bioavailability	Metabolites in humans	Colonic catabolites in humans	Reference
	enterocytes and hepatocytes leading to formation of its corresponding glucuronides and sulfates. Resveratrol conjugates could be biotransformed by gut bacteria enzymes, such as β-glucuronidase and sulfatase. Additionally, the colon microbiota may metabolize resveratrol aglycone into dihydroresveratrol and other catabolites.	resveratrol-4′-O-sulfate resveratrol-3,4′-disulfate		
Methylxanthines	Theobromine and caffeine are rapidly absorbed from the gastrointestinal tract and then metabolized mainly by liver cytochrome P450 enzymes and xanthine oxidase. The major circulating component in plasma is unchanged TB, while the monomethylxanthine derivatives are the most common metabolites excreted in urine. Unlike to phenolics, the biotransformation of methylxanthines by the gut microflora is negligible.	theobromine caffeine paraxanthine 7-methylxanthine 3-methylxanthine 1-methyluric acid 6-amino-5(N-methylformylamino)-1-methyluracil	nd	Briz, Ruiz, and Bravo-Clemente 2017 Garcia-Aloy et al. 2015 Llorach-Asunción et al. 2010 Martínez-López et al. 2014 Rodriguez et al. 2015

administration of different stereoisomers of flavan-3-ols in a dose of 1.5 mg/kg body mass, the concentration of (−)-EC metabolites in blood plasma (889 ± 114 nmol/L) was 6-fold higher than the concentration of (−)-C metabolites (149 ± 18 nmol/L). These authors also noticed that 2 and 4 hours after the intake of the same amounts of four different stereoisomers of flavan-3-ols, relative concentrations of these compounds in blood plasma decreased in the following order: (−)-EC > (+)-C = (+)-EC > (−)-C. It was ascertained that metabolic transformations of different EC enantiomers were different, which in turn caused differences in concentrations of their metabolites in blood plasma and urine (Baba et al. 2001; Ottaviani et al. 2011). These results suggest that (−)-enantiomer undergoes rapid degradation and does not enter blood plasma while (+)-enantiomer is well absorbed. Additionally, the biological activity is also affected by stereo-isomeric form of flavan-3-ols, and the *in vivo* effects caused by different enantiomers could be either similar or different. According to several studies, differences in stereochemical configuration between enantiomers of flavan-3-ols contained in cocoa drinks may strongly affect their influence on cardiovascular system including relaxation of blood vessels (Baba et al. 2001; Donovan et al. 2006; Ferri et al. 2015; Grassi, Desideri, and Ferri 2010; Ottaviani et al. 2011).

Biotransformation of monomeric flavan-3-ols occurs mainly in the enterocytes lining the small intestine and in the liver and are catalyzed by phase II enzymes (Del Rio et al. 2013; Rodriguez-Mateos et al. 2015). Although in the liver, flavonoids may also undergo phase I metabolism (oxidation or *O*-demethylation) by cytochrome P450 monooxygenases, for the majority of these compounds, the *in vivo* contribution of phase I metabolism pathway is likely to be negligible. This might be at least partially due to the fact that phase II conjugation of hydrophilic functional groups in flavonoid molecules occurs before their phase I modification in the liver (Cassidy and Minihane 2017). Phase II enzymes, including uridine-5′-diphosphate glucuronosyltransferases (UGTs) and sulfotransferases (SULTs) catalyze condensation of flavan-3-ols with glucuronic acid and sulfate ions, respectively. Catechol-*O*-methyltransferases (COMTs) catalyze the transfer of a methyl group from S-adenosyl-L-methionine to 3′,4′-catechol ortho-dihydroxy moiety in the B-ring of (epi)catechin (Baba et al. 2000; Cifuentes-Gomez et al. 2015; Ottaviani et al. 2016). Despite the fact that theoretically *O*-glucuronidation of the (epi)catechin molecule may occur at five different sites (Borges et al. 2018; Cifuentes-Gomez et al. 2015; Ottaviani et al. 2012a), in humans, the glucuronidation of this compound occurs predominantly at the 3′ position of the B-ring (Figure 3). The transfer of a sulfate moiety from the 3′-phosphoadenosine-5′-phosphosulfate generally occurs at the 3′ position of the B-ring and at the 5,7-position of the A-ring of the (epi)catechin molecule. Methylation in humans occurs mainly at the 3′ position of the (epi)catechin molecule, and to a lesser extent (epi)catechin may also undergo *O*-methylation in the 4′ position of the B-ring (Ottaviani et al. 2012a). It was found that main products of all these processes such as glucuronic, sulfate, and 3′-*O*-methyl metabolites of flavan-3-ols and their combinations were absorbed by the intestinal mucosa (Cooper

Table 4. Summary of human and animal studies on bioavailability of cocoa polyphenols and methylxanthines.

Food matrix	Description of trial	Main metabolites in plasma	Main metabolites in urine	Detection	Findings	Reference
Flavonoids						
Chocolate	Trial on 8 healthy volunteers consuming chocolate with bread and water. The subjects were not randomized, so that all subjects ate first 40 g of chocolate and one week later 80 g of chocolate.	*After 2–3 h* EC (111 ng/ml (0.383 mmol/l) and 203 ng/ml (0.7 mmol/l) after consumption 40 and 80 g chocolate, respectively.	NA	HPLC	EC concentration increase after chocolates consumption, reaching a max. level (dose dependent) in 2–3 h and rapidly disappeared from the plasma ($t_{1/2}$ for elimination of 1.9 h, and 2.3 h for 40 g and 80 g chocolate, respectively).	Richelle et al. 1999
Cocoa and chocolate	Cross-over trial on five healthy volunteers consuming chocolate or cocoa (both contained 35 g of cocoa powder).	*After 2 h of cocoa intake* EC (0.22 ± 0.02 μmol/L); EC-SULF (1.14 ± 0.21 μmol/L); EC-GLU (0.91 ± 0.21 μmol/L); EC-SULF-GLU (1.19 ± 0.57 μmol/L); Me-EC-SULF (1.00 ± 0.34 μmol/L) *After 2 h of chocolate intake* EC (0.15 ± 0.04 μmol/L); EC-SULF (1.11 ± 0.43 μmol/L); EC-GLU (0.78 ± 0.28 μmol/L); EC- SULF-GLU (1.07 ± 0.24 μmol/L); Me-EC-SULF (0.95 ± 0.27 μmol/L)	*After 0–8 h of cocoa intake* Total EC (159 ± 53 μmoL) *After 8–24 h of cocoa intake* Total EC (33.4 ± 14.6 μmoL) *After 0–8 h of chocolate intake* Total EC (188 ± 33 μmoL) *After 8–24 h of chocolate intake* Total EC (39.5 ± 19.1 μmoL)	LC-MS	$C_{max.}$ of total EC metabolites was 4.77 + 0.94 ∼μmol/1 at 2 h after chocolate intake, and 4.92 + 0.94 ∼μmol/1 at 2 h after cocoa intake and eliminated in 24 h. Almost 80 % of the EC metabolites were excreted within 8 h. Main metabolites of EC in urine after chocolate or cocoa intake were SULF-GLU- and SULF- and Me-EC. Me-EC level was higher in the "chocolate group". Urinary excretion of all EC metabolites within 24 hours after chocolate and cocoa intake was 29.8 ± 5.3% and 25.3 ± 8.1% of total EC intake.	Baba et al. 2000
Flavanol-rich chocolate	Human trials consumption of a 80 g of rich in (−)-EC and PC oligomers chocolate.	*After 2 h* (−)-EC (257 ± 66 nmol/L) *After 6 h* (−)-EC (153 ± 69 nmol/L)	NA	HPLC	Within 2 h after the ingestion of the PC-rich chocolate, mean plasma antioxidant capacity values were 36% > than at baseline and returned to baseline in 6 h. In methodology, the GLU and SULF conjugates are degraded to free EC.	Rein et al. 2000
Cocoa	Human trail on 5 volunteers consuming 26.4 g cocoa per kg providing 323 mg flavan-3-ol monomers and 256 dimers.	*After 0.5 h* PC B2 (16 ± 5 nmol/L) (−)-EC (2.61 ± 0.46 μmol/L) (+)-C (0.13 ± 0.03 μmol/L) *After 2 h* PC B2 (41 ± 4 nmol/L) (−)-EC (5.92 ± 0.60 μmol/L) (+)-C (0.16 ± 0.03 μmol/L)	NA	LC-MS/MS	After PC B2 intake, (−)-EC, and (+)-C were detected reaching max. in 2 h after consumption. Monomers, dimers, and trimers can be transported across an *in vitro* cell layer while oligomers adhere to the cell surface. PC (DP 6) might be degraded to low-molecular-weight aromatic acids by colonic microflora. EC was the predominant plasma flavanol, with plasma C concentrations being only 3%.	Holt et al. 2002
Flavanol-rich chocolate	Human trial on 11 healthy volunteers consuming 80 g flavanol-rich chocolate with bread and water	NA	EC, Me-EC, diHPPA, HPPA, FA, CA, diHPAA, HPAA, 3,4-diHBA VA, HBA, HHA, HA	HPLC-DAD HPLC-ESI-MS/MS GC–MS	Despite VA, which showed a peak excretion shortly after consumption (0–3 h), all phenolic acids were maintained on higher levels even during the second day after consumption. The delayed excretion of other phenolic acids	Rios et al. 2003

(*continued*)

Table 4. Continued.

Food matrix	Description of trial	Main metabolites in plasma	Main metabolites in urine	Detection	Findings	Reference
					(9–48 h) of microbial origin. C and PC were extensively degraded by colon microflora to HPPA, HPAA, and HBA, diHPAA is most likely an intermediate between flavanols and the more dehydroxylated HPAA. FA probably originated from the metabolism of CA or from the metabolism of other polyphenols. VA probably originated from the VAN. (+)-C ingested by humans was shown to be metabolized to HPPA.	
Dark chocolate *milk chocolate*	Human trails on 12 volunteers consuming 100 g of dark chocolate, 100 g dark chocolate with 200 mL full-fat milk, or 200 g of milk chocolate (40 ml milk)	(−)-EC	NA	(−)-EC as (AUC, in ng mL^{-1} h^{-1})	Milk inhibits the *in vivo* antioxidant activity of chocolate. and the absorption into the bloodstream of (−)-EC	Serafini et al. 2003
Cocoa	Randomized, crossover trial on 5 volunteers consuming cocoa beverage containing 40 g of cocoa powder (54.4 mg of (−)-EC) in 250 mL of whole milk or 250 mL of whole milk in a random order	*After 2 h* (−)-EC and (−)-EC-GLU	(−)-EC-SULF, (−)-EC-GLU, O-Me-EC-GLU, EC-SULF-GLU and O-Me-EC-SULF-GLU	SPE-LC-ESI-MS/MS	At 2 h after cocoa drink consumption max. level of (−)-EC in plasma was detected. Main (−)-EC metabolites in urine were glucuronide and SULF conjugates of nonmethylated forms, while only the GLU form was found in plasma.	Roura et al. 2005
Flavanol-rich cocoa powder and conventional cocoa powder	Randomized double-blind crossover trial on 6 healthy volunteers consuming both flavonoid-enriched cocoa powder and conventional cocoa powder dissolved in 250 mL of semiskimmed milk	EC-GLU, EC-SULF, Me-EC-GLU and Me-EC-SULF	Me-EC-SULF, EC-GLU, EC-SULF, Me-EC-GLU and Me-EC-SULF-GLU	LC-ESI-MS/MS	$C_{max.}$ was reached between 1 and 2 h after the intake. Main GLUs were present in larger amounts than SULF. Other complex metabolites, such as EC- SULF-GLU and Me-EC-SULF-GLU, detected in volunteers that consumed flavonoid-rich drink, the accumulation of EPI-GLU and Me-EC-GLU in that group were ∼5-fold and 3-fold greater, respectively. The SULF metabolites were not detected in volunteers drinking regular cocoa. The total clearing of the metabolites was not always observed after 3 h. Main urine metabolites were EC-SULF, Me-EC-SULF, and EC-GLU with the highest excretion after 24 h of the intake. Late metabolism in the liver produced mainly SULF, while the early metabolism (1 and 2 h) produced mainly the GLU and Me-	Tomas-Barberan et al. 2007

(*continued*)

Table 4. Continued.

Food matrix	Description of trial	Main metabolites in plasma	Main metabolites in urine	Detection	Findings	Reference
					conjugates in the cells of the gastrointestinal tract.	
Milk chocolate drink and milk-free beverage and cocoa	A double blind cross-over study of 2 treatment conditions, trials on 24 volunteers consuming milk chocolate drink or milk-free chocolate drink contains 2.7 g polyphenols.	C 0.2 μmol/L EC 18 μmol/L	NA	HPLC-MS	Max. EC absorption after 1 h, max. C absorption after 2–3 h. Milk proteins have no impact on average plasma polyphenol level but their presence significantly decrease T_{max} .	Keogh, McInerney, and Clifton 2007
Cocoa beverage (in 100 *g–47 g of carbohydrates, 16 g of fiber, 5.4 g of fat)*	Open, prospective, randomized and crossover trials on 21 volunteers consuming 100 g of cocoa beverage (contains 70.5 mg (−)-EC, 63.75 mg of PC B2, 21 mg of C, 5 mg of flavonols) with 250 mL water or milk.	NA	(−)-EC-GLU and three (−)-EC-SULF	LC-MS/MS and TEAC assay	An antioxidant activity of urine samples increased 12 h after the intake of a cocoa beverage. Urinary excretion of (−)-EC metabolites corresponded to 1.6% of ingestion.	Roura et al. 2007a
Cocoa powder	Open, prospective, randomized and crossover trials on 21 volunteers consuming cocoa beverage. One group 40 g of cocoa powder in 250 ml of milk and second 40 g of cocoa powder in 250 ml of water. 40 g of cocoa powder contained: (−)-EC, 28·2 mg; PC B2, 25·5 mg; (+)-C, 8·4 mg; flavonols.	NA	Milk: (−)-EC-GLU, 112.8 μg (−)-EC/g creatinine; and three Σ(−)-EC-SULF$^{1-3}$, 154.1 μg (−)-EC/g creatinine. Water: (−)-EC-GLU, 195.0 μg (−)-EC/g creatinine; and three Σ(−)-EC-SULF$^{1-3}$, 215.2 μg (−)-EC/g creatinine.	HPLC–MS/MS	Four (−)-EC metabolites were detected in the urine samples. One (−)-EC-GLU reaching maximum 6 h after consumption, and three (−)-EC-SULF with a max. level in 6–12 h. Milk does not modify the total excretion of (−)-EC metabolites. After 12–24 h period, the metabolite concentrations returned to their base levels.	Roura et al. 2008
Cocoa drink	Cross over trials on 9 volunteers consuming 250 mL cocoa drink with hot water or hot milk. Cocoa powder contained 8.3 mg total flavan-3-ols/g.	(EC)C-SULF, *O*-Me-(EC)C-SULF	(EC)C-SULF, (−)-EC-GLU, (EC)C-SULF, *O*-Me-(EC)C-SULF	HPLC-PDA-MS	Main metabolite in plasma was (EC)C-SULF and in urine *O*-Me-(EC)C-SULF. Milk proteins extend the $t^1/_2$ of (EC)C-SULF. Milk also decreased the AUC for (EC)C-SULF. Level of flavan-3-ols urinary metabolites ingested in 0–24-h were 18.3 ± 1.9% of intake after ingestion of the cocoa-water drink compared with 10.5 ± 1.1% of intake after ingestion of the cocoa milk drink. The combined urinary excretion of the metabolites for the 0–2-h and 2–5-h samples was higher for the cocoa-water drink than for the cocoa-milk beverage. Almost 80% of the excretion occurred within 5 h of intake.	Mullen et al. 2009
Chocolate matrices varying in	Randomized cross-over study on six subjects consumed	C EC	nA		Physical form and sucrose content had significant effect on the	Neilson et al. 2009

(*continued*)

Table 4. Continued.

Food matrix	Description of trial	Main metabolites in plasma	Main metabolites in urine	Detection	Findings	Reference
macronutrient composition and physical form	of three solid confection (reference dark chocolate, high sucrose, high milk protein and two beverage (sucrose milk protein, non-nutritive sweetener milk protein) products providing commercially relevant levels of C and EC (approximately 36 mg C + EC/serving).	glucuronide and sulfate metabolites			pharmacokinetics of EC. The bioavailability of EC and C is enhanced by simultaneous ingestion of cocoa drink with sucrose. The area under the pharmacokinetic curves s were significantly increased for beverages.	
Flavanol-rich cocoa	**Human** trial on twenty-one nonsmoking healthy volunteers consumed 4.8 g natural cocoa powder/kg/day. Cocoa powder used in the study contained: 0.71 ± 0.09 mg/g of (−)-EC and 0.21 ± 0.01 mg/g of (+)-C, 0.64 ± 0.06 mg/g of PC B2, 3.87 μg/g isoquercitrin, 5.74 μg/g quercetin, 4.33 μg/g quercetin-3-glucuronide, and 36.32 μg/g quercetin-3-arabinoside, 57% of carbohydrates (sucrose, 46%; starch, 1%; complex carbohydrates, 10%), 16% of fiber, 5.4% of fat, 14.1% of protein, 3.97% of moisture, 1.3% of TB, 0.13% of CF, and 2% of ash. **Rats** trial with 15 day-old Wistar rats feed 4.8 g of natural cocoa powder/kg/day during 2 weeks.	NA	**Humans**: CA, FA, 3-HPAA, VA, 3-HBA, 4-HHA, HA, (−)-EC, and PC B2 **Rats:** diHPP, m-CuA, 3-HPA, PCA, VA, and (−)-EC CAFA, 3-Me-4-HPA, 3-HBA, PC B2	SPE-LC-MS/MS	19 microbial phenolic metabolites, among them monomeric and dimeric flavanols subjects urine samples collected after cocoa consumption were identified. **In humans**, the main metabolites observed in urine after consumption of cocoa products were CA, FA, 3-HPAA, VA, 3-HBA, 4-HHA, HA, (−)-EC, and PC B2. In trials on **rats**, the increase of concentration of 3,4-diHPPA, m-CuA, 3-HPAA, 3,4-diHBA, VA, and (−)-EC were found.	Urpi-Sarda et al. 2009
Cocoa-based drink	Human trial a randomized, double-masked, five times crossover study design, 7 volunteers, were given one of the test compounds (incorporated into the cocoa dairy drink matrix).	(−)EC, (+)EC, (−)C, 3′-*O*Me-(−)-EC, 3′-*O*Me-(+)-EC, 4′-*O*Me-(−)-EC, 4′-*O*Me-(+)-EC On	(−)-EC, (+)-EC, (+)-C	HPLC-FLD/UV	The renal excretion of flavanol metabolites – rapidly after ingestion, with 90 ± 5, 95 ± 2, 94 ± 7, and 97 ± 3% of the (−)-EC, (+)-EC, (−)-C, and (+)-C metabolites. The oral intake of (+) and (−)-EC resulted in appearance of 3′- and 4′*O*Me-EC derivatives in plasma, whereas the intake of (+) and (−)-C led to the presence of only the 3′-O-Me derivatives. Flavanol stereochemistry affects metabolic pathways other than *O*-Me, i.e. SUL and GLU. Urinary	Ottaviani et al. 2011

(*continued*)

Table 4. Continued.

Food matrix	Description of trial	Main metabolites in plasma	Main metabolites in urine	Detection	Findings	Reference
					excretion of (−)-EC metabolites detected in this study was 3% of intake. (−)-EC had a direct and immediate vasodilatory effect, acute consumption of (−)-EC causes an improvement in NO-dependent arterial dilation in human.	
70% chocolate and cocoa beverage	Human trials on 5 volunteers consumed in either 100 g of 70% chocolate or cocoa beverage containing 40 g of cocoa powder in milk.	(−)-EC-3′-GLU, (−)-EC-3′-SULF, 3′-*O*-Me-(−)-EC-4′-SULF and 3′-*O*-Me-(−)-EC-7-SULF	(−)-EC-3′-GLU, (−)-EC-3′-SULF, 3′-*O*-Me-(−)-EC-4′-SULF and 3′-*O*-Me-(−)-EC-7-SULF	UPLC–MS/MS	(−)-EC-3′-GLU, (−)-EC-3′-SULF, 3′-*O*-Me-(−)-EC-4′-SULF and 3′-*O*-Me-(−)-EC-7-SULF account for 85% in plasma and 72% in urine of all metabolites. Main plasma metabolites were GLUs, SULF, and *O*-Me-SULFs reached max. concentration 2 and 4 h after consumption, respectively, while in urine reached max. concentration (58%) 5–10 h after consumption.	Actis-Goretta et al. 2012
Cocoa drink	Human trail on 10 volunteers consuming cocoa drink containing (−)-EC in amount of 1.8 mg of (−)-EC/kg of BW.	(−)-EC, (−)-EC-GLU, (−)-EC-3′-GLU, 3′-*O*-Me-(−)-EC-7-GLU, 4′-*O*-Me-(−)-EC-5-GLU, 4′-*O*-Me-(−)-EC-7-GLU, 4′-*O*-Me-(−)-EC-3′-GLU, (−)-EC-5-SULF, (−)-EC-7-SULF, (−)-EC-3′-SULF, 3′-*O*-Me-(−)-EC and 4′-*O*-Me-(−)-EC	NA	SPE, HPLC-MS/MS	Predominant (−)-EC plasma metabolite was (−)-EC-GLU (46 ± 6%) and smaller amounts of 4′-*O*-Me-(−)-EC-7-GLU reached max. concentration 2 h after the consumption of the test drink. 3 distinct SULFs metabolites observed in plasma reaching max. amount in 2 h, 3′-*O*-Me-(−)-EC-5/7-SULF and 4′-*O*-Me-(−)-EC-5/7-SULF. Unconjugated (−)-EC present at low concentrations. (+)-EC is metabolized to the same metabolites but in 4 times lower amounts than detected for (−)-EC	Ottaviani et al. 2012a
Flavan-3-ol-enriched dark chocolate, standard dark chocolate and white chocolate bars	Observer-blinded randomized-controlled acute intervention trial on 42 healthy volunteers consuming 60 g of flavan-3-ol-enriched dark chocolate (EC 257.0 ± 1.06 mg; C, 53.6 ± 0.27 mg; PC B2 198.0 ± 1.22 mg; trimers, 168.0 ± 1.42 mg; tetramers, 105.5 ± 12.75 mg; pentamers, 125.4 ± 6.04 mg), standard dark chocolate (EC 84.1 ± 0.67 mg; C, 25.8 ± 1.02 mg; PC B2	C (0.435 ± 0.016 μmol/L)	C (0.713 ± 0.011 mmol/mol creatinine) PCB2 (0.112 ± 0.003 μmol/mol creatinine)	LC-MS/MS	Plasma concentrations of total EC aglycones increased in 2 h after consumption of enriched dark or standard dark chocolate compared with white chocolate. Levels of total plasma C decreased in 6 h. Urine concentrations of total C increased 2 and 6 h after consumption of dark chocolates compared with white chocolate. 6 h after consumption of dark chocolate urinary C amount was 13.4 mmol/mol creatinine. Similar effects were observed for urinary PC B2 reaching 57 μmol/mol creatinine after 6 h. Both dark and white chocolate, improved several measures of postprandial platelet	Ostertag et al. 2013

(*continued*)

Table 4. Continued.

Food matrix	Description of trial	Main metabolites in plasma	Main metabolites in urine	Detection	Findings	Reference
	74.4 ± 0.76 mg; trimers, 47.0 ± 2.23 mg; tetramers, 32.1 ± 4.40 mg; pentamers, 118.8 ± 39.50 mg) or white chocolate bars with 400 or 200 mL of water.				function in a gender-specific way, the beneficial anti-platelet effects was explained by individual differences in absorption or metabolism of flavan-3-ols by women and men.	
Aronia extract	Trials on 6 volounteers former smoker consumed ∼500 mg capsules of aronia extract with water. Aronia extract contained 45.1 mg anthocyanins, 41.9 mg, PC as C equivalents, 9.9 mg flavonols, and 36.9 mg HCAs.	CYN-3-*O*-GC, PEO-3-*O*-GAL, 3-4-diHPPA and HA	CYN-3-*O*-GC, CYN-3-*O*-GAL, CYN-3-*O*-AR, PEO-3-*O*-GAL, 3-4-diHPPA, 3,4-diHBA, FA and HA	UHPLC-MS	1–2 h after absorption CYN is absorbed from small intestine and its metabolites are present in plasma. Urine metabolites of CYN as GAL, GL and AR derivatives and acids were indicated after 4–6 h; CYN-3-*O*-GAL from aronia extract is *O*-Me faster than juice. The metabolites of anthocyanins generated from intestine and liver include phase I and phase conjugates. Colonic metabolites include HBA, HPPA, FA and HAs.	Xie et al. 2016
chocolate, cocoa powder or chocolate chip cookies	Human trials on 32 consumers of cocoa or derived products (CC) and 32 matched control subjects with no consumption of cocoa products (NC).	NA			Methoxyhydroxyphenylvalerolacton, 5-(3′,4′-diHP)-γ-VL glucuronides and -(3′,4′-diHP)-γ-VL sulfates	HPLC-q-ToF-MS
The discriminant biomarkers identified were mainly related to the metabolic pathways of theobromine and polyphenols, as well as to cocoa processing. The CC group there were higher urinary excretions of both host (epicatechin and vanillin metabolites) and microbial		(hydroxyphenylvalerolactones and hydroxyphenylvaleric acids).	Garcia-Aloy et al. 2015			
Radiolabeled and stereochemically pure [2-^{14}C](−)-*epicatechin* (^{14}C-EC)	Trials on eight male volounteers consumed 50 mL of a ^{14}C-EC-containing test drink, which delivered 60 mg	(−)-EC-3′-O-GLU; (−)-EC-7-O-GLU; (−)-EC-3′-SULF; (−)-EC-5-SULF; 3′-O-Me(−)-EC-4′-SULF; 3′-O-Me(−)-EC-5-SULF; 3′-O-Me(−)-EC-7-SULF; 4′-O-Me(−)-EC-5-SULF; 4′-O-Me(−)-EC-7-SULF; 3′-O-Me(−)-EC-	(−)-EC-3′-O-GLU; (−)-EC-3′-SULF; (−)-EC-5-SULF; 3′-O-Me(−)-EC-4′-SULF; 3′-O-Me(−)-EC-5-SULF; 3′-O-Me(−)-EC-7-SULF; 4′-O-Me(−)-EC-5-SULF; 4′-O-		The authors showed that 20 ± 2% of structurally related radiolabeled EC metabolites were absorbed into the circulatory system from the small intestine after the consumption of 50 mL of test	Ottaviani et al. 2016

(*continued*)

Table 4. Continued.

Food matrix	Description of trial	Main metabolites in plasma	Main metabolites in urine	Detection	Findings	Reference
	(207 μ mol) of EC, and 300 μ Ci of radioactivity.	5-O-GLU; 3′-O-Me(−)-EC-7-O-GLU; 5-(4′-HP)-γ-VL-3′-SULF; 5-(3′-HP)-γ-VL-4′-O-GLU; 5-(4′-HP)-γ-VL-3′-O-GLU; 5-(HP)-γ-HVA-SULF; 5-(3′-HP)-γ-HVA-4′-O-GLU	Me(−)-EC-7-SULF; 5-(Phenyl)-γ -VL-SULF-O-GLU; 5-(4′-HP)-γ-VL-3′-SULF; 5-(Phenyl)-γ -VL-3′-SULF; 5-(3′ -HP)-γ-HVA acid-4′-SULF; 5-(Phenyl)-γ -VL-SULF-O-GLU-II; 5-(3′-HP)-γ –HVA; 4′ -OGLU; 5-(Phenyl)-4-HVA acid-3′ -SULF; HPAA-SULF; 3-(3′-HP)-hydracrylic acid; HA; 3′-HHA		drink They also found that PVL and HPVA metabolites were excreted in urine in amounts corresponding to 42 ± 5% of the dose administered, while phenolic acids and HA metabolites accounted for 28 ± 3% of urinary radioactivity.	
Phenolic acids						
40 g of cocoa powder dissolve either in 250 mL of whole milk or in 250 mL of water.	21 humans, open, prospective, randomized, crossover trial of cocoa beverage consumption	NA	diHPPA, HPAA, 3-Me-4-HPA, 3-HPAA, PAA, 3,4-diHBA, 4-HBA, 3-HBA, 4-HHA, HPA, VA, CA, FA and *p*-CuA	SPE-LC-MS/MS	15 PAs in urine constructed the microbial degradation pathway. The highest ↑ was observed for diHPAc, PA, 4-HBA,4-HHA, HA, FA and CA reaching max. in 0–6 h. Concentrations of HCAs in urine, reaching 10.72–12.35 nmol/mg creatinine for FA and 0.52–0.54 nmol/mg creatinine for CA. Amounts of diHPAc, PA 4-HHA, HA, CA, and FA ↓ after the intake of cocoa with milk, while VA and PAc ↑. Milk partially affects the formation of microbial PAs derived from the colonic degradation of PCs and other compounds present in cocoa. PCA is formed through β-oxidation of diHPP or t α-oxidation of HPA. Microbial dehydroxylation of PCA could give ↑ to 4-HBA derivative that may undergo liver GLYC being converted to 4-HHA	Urpi-Sarda et al. 2010
Cocoa liquor, cocoa powder	Trials on 12 healthy volunteers consuming 3 control products in a single-blind study, and had a randomized, cross-over design with three arms.	FA, GA, CA, FA, 3,4-diHBA, HBA, VA, *p*-CuA	NA	HPLC-MS/MS	Clonic mociroflora is arranged in ring fission of monomeric C and conversion to phenolic acids and phenyl lactone derivatives. Compounds detected at high amounts in plasma were free and SULF conjugates of diHFA and diHPPA with C_{max} values ranging from 41 to 385 nmol/L.	Vitaglione et al. 2013
N-phenylpropenoyl-L-amino acids						
Cocoa drink	Clinical trials on 8 healthy volunteers consumed 100 g of cocoa drink containing 50.75 mg of cocoa powder in water.	NA	*N*-coumaroyl-L-aspartic acid (149.9 μg), *N*-coumaroyl-L-glutamic acid (44.6 μg), *N*-coumaroyl-L-tyrosine (44.0 μg) and *N*-feruloyl-L-aspartic acid (21.6 μg).	UV/Vis and RP-HPLC-MS/MS	It is interesting to notice that observed in urine NPAs metabolites that bearing either a *p*-coumaroyl or a feruloyl moiety in the molecule. The highest recovery rates of 57.3, 22.8, and 8.3% were observed for *N*-	Stark et al. 2008

(*continued*)

Table 4. Continued.

Food matrix	Description of trial	Main metabolites in plasma	Main metabolites in urine	Detection	Findings	Reference
					coumaroyl-L-glutamic acid, *N*-feruloyl-L-tyrosine, and *N*-feruloyl-L-aspartic acid, respectively. *N*-caffeoyl-L-amino acids were found in much lower amounts in urine when compared to the amides bearing *N*-feruloyl and *N*-*p*-coumaroyl moiety. Max. absorption of all NPAs observed after 2 h. NPAs are not enzymatically hydrolyzed and metabolically conjugated as *O*-GLUs or *O*-SULFs. Additionally, CF-GLUs are not the predominant metabolites of NPAs. Because of *N*-feruloyl – and N-*p*-coumaroyl-L-amino acids were found in urine with rather high recovery, possible these metabolites are formed by O-Me or by reduction of the corresponding caffeoyl-L-amino acids.	
Stilbenes						
trans-resveratol	Clinical 44 volunteers received RES daily for 29 days at daily doses of 0.5, 1.0, 2.5, or 5.0 g.	RES, RES-3-SULF, RES-4′-SULF, RES-diSULF, RES-3-*O*-GLU, RES-4′-*O*-GLU	ND	HPLC-MS/MS	The most abundant circulating RES metabolite was RES-3-SULF max. absorption of all RES observed after 1–2 h. Absorbed in the small intestine (mainly in duodenum and in lower amounts in jejunum), then metabolized via intestinal and hepatic conjugation (GLU and SULF). Secondary RES and its metabolites peaks was explained by enterohepatic recirculation of conjugated RES metabolites in liver and its conjugates are excreted in the bile, then reabsorbed in small intestine and extracted by faces. Inaccessible RES and its metabolites are mainly eliminated by urine.	Walle et al. 2004
Methylxanthines						
Chocolate	Trial on 8 healthy volunteers consuming **chocolate** with bread and water. The subjects were not randomized, so that all subjects ate first 40 g of chocolate and one week later 80 g of chocolate.	TB 6.2 mg/ml (34 mmol/l) and 11.3 mg/ml (63 mmol/l) for 40 g and 80 g chocolate, respectively	NA	HPLC	TB reached $C_{max.}$ at 2 h after chocolate consumption. After the maximum, plasma theobromine declined slowly, leading to a still elevated plasma concentration at 8 h ($t_{1/2}$ for elimination of 6.7 h and 8.2 h for 40 g and 80 g).	Richelle et al. 1999
Chocolate bars	Trials on 5 volunteers consuming two commercially available	TB, CF	TB, CF	LC–MS/MS	TB is intensively absorbed and detected after 1.5 h in urine, while CF was observed at low levels due	Ptolemy et al. 2010

(*continued*)

Table 4. Continued.

Food matrix	Description of trial	Main metabolites in plasma	Main metabolites in urine	Detection	Findings	Reference
	chocolate bars contained 188 and 26 mg of TB and CF respectively.				to its rapid enzymatic transformations to various MX and MU compounds.	
Cocoa powder: TB, 1.3% and CF, 0.13%	Trials on 10 volunteers consuming 40 g of cocoa powder with 250 ml of milk.	NA	N-Me-guanine, VG, DVL-GLU, FG, 7-MX, 3-MX, TB, XA.	LC-MS	The maximal concentration of cocoa metabolites in urine were observed at 6 h after consumption. The microbial metabolome is an important part of the urinary metabolome.	Llorach-Asunción et al. 2010
Cocoa products	Trials on 13 volunteers consumed the cocoa products dissolved in 200 mL of semi-skimmed milk.	CF (2.1 ± 1.3 μM/mL), PX (9.5 ± 1.3 μM/mL), TB (15.8 ± 3.3 μM/mL), TP (11.5 ± 2.6 μM/mL), 3-MX (0.6 ± 1.4 μM/mL), 7-MX (2.1 ± 1.4 μM/mL)	CF (2.1 ± 0.7 μM/mL), PX (3.5 ± 1.8 μM/mL), TB (50.4 ± 18.4 μM/mL), TP (1.0 ± 0.4 μM/mL), 1-MX (5.5 ± 2.7 μM/mL), 3-MX (34.8 ± 8.9 μM/mL), 7-MX (110.1 ± 40.1 μM/mL),1-MU (9.2 ± 4.1 μM/mL), 1,3-DMU (1.3 ± 1.1) μM/mL), 1,7-DMU (3.9 ± 2.7 μM/mL), 3,7-DMU (2.7 ± 1.1 μM/mL), 1,3,7-TMU (0.7 ± 0.3 μM/mL)	HPLC-DAD and LC-QTOF	CF and TB were observed in plasma ∼ 2 h after consumption while TP, 3-MX and 7-MX in 3 h and PX in 4 h after cocoa products administration. The main urinary metabolites were 7-MX, TB and 3-MX. A high excretion of 1-MU was observed, followed by 1-MX, 1,7-DMU, PX, 3,7-DMU, CF and small amounts of 1,3-DMU, TP and 1,3,7-TMU, ranged between 14.2 and 21.5 h. The higher MX intake resulted in shorter T_{max} times, ranging between 7.6 and 18 h; however, no significant differences were observed between products, except for 1-MX, 1-MU and 1,3,7-MU.	Martínez-López et al. 2014
Cocoa powder	Trials on 80 children, in 4 groups, 1) 26 not consuming cocoa; 2) 19 consuming one cocoa; 3) 12 consumed cocoa powder at breakfast, but no other cocoa products that day; and 4) 23 consumed chocolate, including cocoa powder, more than once a day.	NA	TB	HPLC with UV or MS	Ingested TB was extracted from urine in 3 days. TB excretion was directly related to cocoa consumption. 50% of TB intake is excreted during the next 12 h.	Rodriguez et al. 2015
Chocolate, cocoa powder or chocolate chip cookies	Human trials on 32 consumers of cocoa or derived products (CC) and 32 matched control subjects with no consumption of cocoa products (NC).	NA	AMMU, 3-MU, 7-MX, 3-MX, and TB	HPLC-q-ToF-MS	The CC group there were higher urinary excretions of xanthine, AMMU, 3-MU, 7- and 3-MX, 3,7-DMU, and TB.	Garcia-Aloy et al. 2015

NA, not applied; NO, nitric oxide; GLUT, glucuronosyl transferase; ST, sulfotransferase; EC, epicatechin; C, catechin; PC, procyanidin; Me-, methyl; GLYC, glycination; GAL, galactoside; GLU, glucuronide, GLY, glycoside; GL, glucoside, SULF, sulfate; AR, arabinoside; RU, rutinoside; QA, quinic acid; HNA, hydroxynicotinic acid; CuA, coumaric aicd; NPA, N-phenylpropenoyl-L-amino acid; HCA, hydroxycinnamic acids; GA, gallic acid; SA, Syringic acid; 3,4-diHBA, 3,4-dihydroxybenzoic acid; HBA, hydroxybenzoic aicd; FA, ferulic acid; iFA, isoferulic acid; FQA, feruloylquinic acid; HA, hippuric acid; pHHA, p-hydroxyhippuric acid; mHPAc, m-hydroxyphenylacetic acid; EV, ethylvanillin; ValA, valeric acid; VL, valerolactone; PVL, phenylvalerolactone; HPVL, hydroxyphenylvalerolactone; diHPVL, dihydroxyphenylvalerolactone;FG, Furolylglycine; DVL, Dihydrophenyl valerolactone; VG, Vanilloylglycine; VAN, vanillin; VA, vanillic acid; CF, caffeine; diHPPA, 3,4-dihydroxyphenylpropionic acid; diHPAA, 3,4-dihydroxyphenylacetic acid; HPPA, hydroxyphenylpropionic acid; HPAA; hydroxyphenylacetic acid; diHFA, dihydroferulic acid; TB, theobromine, TP, theophylline, PX, paraxanthine; MU, methyluric acid; DMU, dimethyluric acid; MXs, methylxanthines; EXs, ethylxanthines; diE-MPs, diethyl-methylpyrazines; XA, xanthurenic acid; HAccP, hydroxyacetophenone; AMMU, 6-amino-5-[N-methylformylamino]-1-methyluracil; RES, Resveratrol; CYN, Cyanidin; PEO, peonidin.

et al. 2008; Holt et al. 2002). It should be noted that the attachment of methyl groups to flavan-3-ol molecules may increase their lipophylic character and bioavailability. This finding is ascribed to the medium lipophylic character of methylated flavan-3-ol metabolites, which enables passing through biological membranes, including the blood-brain barrier, and draining into cells of the nervous tissues (Faria et al. 2011; Sokolov et al. 2013). Products of the first stages of flavan-3-ols metabolism are transported from the intestine to liver where they undergo further conversions, mainly to sulfate conjugates and methyl derivatives which are excreted either via the kidneys in urine or via bile or transported by ATP-binding cassette transporter-mediated back into the intestinal lumen (Monagas et al. 2010; Cassidy and Minihane 2017). The subsequent formation of anionic derivatives by conjugation of glucuronides and sulfates improves their urinary and biliary excretion ability and explains their rapid elimination (Ottaviani et al. 2012a). The most abundant metabolites present in human plasma and urine after cocoa or cocoa derived products consumption are presented in Table 4.

Ottaviani et al. (2012a) noticed that the most abundant metabolite in humans is (−)-epicatechin-3′-*β*-D-glucuronide, regardless of the enantiomer consumed. They showed that this compound accounted for about 46 ± 6% of all EC metabolites detected in plasma at 2 h after consumption of a cocoa-based test drink contained 476 mmol of (−)-EC and 66 mmol of (±)-C (Borges et al. 2018). The abundances of other metabolites identified in human plasma i.e. (−)-epicatechin-3′-sulfate, (−)-epicatechin-5-sulfate, (−)-epicatechin-7-sulfate and the group of 3′- and 4′-*O*-methyl-(−)-epicatechin-5/7-sulfates were 28 ± 6, 3.1 ± 0.8, 1.1 ± 0.3%, 17 ± 2 and 4.3 ± 0.7% of all EC, respectively. Quite different results were obtained by Actis-Goretta et al. (2012). The authors revealed that the other phase II metabolites detected in plasma after ingestion of 100 g of dark chocolate containing 241 mmol of (−)-EC and 90 mmol of (±)-C by healthy humans were 3′-*O*-methyl-(−)-epicatechin sulfates substituted in the 4′ and 7 positions, as well as (−)-epicatechin-4′-*β*-D-glucuronide and (−)-epicatechin-4′-sulfate (Actis-Goretta et al. 2012). The authors showed that (−)-epicatechin glucuronides, sulfates, and *O*-methyl sulfates accounted for 33 ± 4, 28 ± 5, and 33 ± 4% of the total metabolites in plasma.

A more recent study has provided interesting information about the post-absorptive metabolism of cocoa monomeric flavan-3-ols (Borges et al. 2016; Ottaviani et al. 2016). Ottaviani et al. (2016) who studied the absorption, metabolism, distribution and excretion of radiolabeled and stereochemically pure [2-^{14}C](−)-epicatechin (^{14}C-EC) in humans, revealed that the major metabolites of ^{14}C-EC recovered in plasma were (−)-epicatechin-3′-*O*-*β*-D-glucuronide, (−)-epicatechin-3′-sulfate, 3′-*O*-methyl-(−)-epicatechin-5-sulfate and 3′-*O*-methyl-(−)-epicatechin-7-sulfate. The C_{max} of 1223 nmoL/L EC metabolites was found 1.0 h after intake of ^{14}C-EC. In contrast to earlier findings (Actis-Goretta et al. 2012), however, (−)-epicatechin-4′-*β*-D-glucuronide and (−)-epicatechin-4′-sulfate were not found, despite the fact that the application of stable isotope tracers in metabolic studies in humans enables the detection of these compounds. According to Ottaviani et al. (2016) one possible explanation for this apparent discrepancy is that compounds recognized as EC metabolites by Actis-Goretta et al. (2012) are in fact metabolites of the EC stereoisomer, (−)-catechin. Ottaviani et al. (2016) investigated also the presence of oxidation products of EC, and any ortho-quinones or quinone-related adducts or derivatives of EC were either not detected or noted. Considering that monomeric flavan-3-ols are suspected to be oxidized to their corresponding ortho-quinones and their electrochemical behavior suggests that they are effective antioxidants. The authors concluded that the systemic biological activities of EC does not appear to be predominantly related to direct hydrogen atom transfer nor single electron transfer mechanisms (Ottaviani et al. 2016).

Like blood plasma also urine contains EC sulfates, methylsulfates and glucuronides (Ottaviani et al. 2011). According to a recent report by Ottaviani et al. (2016) the main urinary structurally related metabolites of EC were the same as in blood. The majority of these compounds are disposed of 24 h after the intake of both products derived from cocoa beans (Baba et al., 2000; Tomas-Barberan et al. 2007) and ^{14}C-label pure EC (Ottaviani et al. 2016). According to Baba et al. (2000) as much as 29.8% and 25.3% of EC metabolites are excreted in urine after the intake of chocolate or cocoa drink. Actis-Goretta et al. (2012) reported that the amount of EC metabolites excreted in urine after ingestion of chocolate by healthy humans corresponded to 21 ± 2% of the administered dose of EC. Subsequently Actis-Goretta et al. (2013) also studied the intestinal absorption, metabolism, and excretion of EC in eight healthy volunteers by means of intestinal perfusion method. Purified EC 50 mg (172 µmol) was administered directly into isolated jejunum segments, over a 0,5 h period, which were then continuously perfused with the perfusion buffer for the next 2 h. Upon perfusion, the mean amount of unchanged EC or phase II conjugates recovered in the perfusion fluid were 22 ± 4 mg and 0.8 ± 0.2 mg, respectively. This study demonstrated that about 46% of the administered dose of EC that reaches the intestinal cells are absorbed in humans. However, a high inter-individual variability has been reported in its absorption among the eight volunteers, ranging from 31 to 90% (Actis-Goretta et al., 2013; Rodriguez-Mateos et al., 2014). More recently Ottaviani et al. (2016) showed that 20 ± 2% of structurally related radiolabeled EC metabolites were absorbed into the circulatory system from the small intestine after the consumption of 50 mL of test drink containing 300 µ Ci (207 µmol) of ^{14}C-EC.

The bioavailability of flavan-3-ols is also modulated by the food matrix, their concentration and occurrence of other substances in foods (Table 4). The concentration of phytochemicals in cocoa beans differs depending on the variety and ripeness of the cocoa bean, the growing region, harvesting practices, and processing steps. Cocoa bean processing including fermentation, drying, roasting, and alkalization result in significant change of polyphenols content and thus antioxidant activity of cocoa products (Belščak et al. 2009; Oracz, Zyzelewicz, and Nebesny 2015). It is well known,

that epimerization of (−)-EC caused by the high temperature during roasting of cocoa beans gives rise to formation of its epimer such as (−)-C, while epimerization of (+)-C leads to formation of (−)-EC (Kofink, Papagiannopoulos, and Galensa, 2007; Lau-Cam 2013). Epimerization reactions are also induced by alkalization, which is one of unit operations of cocoa powder manufacturing. The latter reactions give rise to increased levels of (−)-C. Aforementioned processes caused that both cocoa and chocolate contain mainly (−)-EC and considerable amounts of (−)-C while concentration of (+)-C is very low, in contrast to raw cocoa beans (Donovan et al. 2006; Kofink, Papagiannopoulos, and Galensa, 2007). During process of chocolate preparation, composition and content of polyphenols are furtherly altered. However, nowadays mentioned processes are conducted in such manner to preserve as much polyphenol as possible (Quiroz-Reyes and Fogliano 2018). Due to the large variation in the polyphenol (flavan-3-ol) content of chocolate and cocoa products, it is critical to determine the concentration of these compounds in the foods used to evaluate the health effects of cocoa flavonoids. Thus, the influence of the complex food matrix e.g. chocolate or cocoa beverage on the bioavailability of cocoa flavan-3-ols has been intensively evaluating in a currently conducted *in vivo* studies (Mullen et al. 2009; Neilson et al. 2009; Ostertag et al. 2013; Schramm et al. 2003; Serafini et al. 2003).

In the interventional studies a large heterogeneity in terms of cocoa products like: cocoa powder, dark chocolate, milk chocolate, cocoa beverages were used and the researchers were concerned on flavan-3-ol metabolites as the main active compounds (Mullen et al. 2009; Neilson et al. 2009; Ostertag et al. 2013; Schramm et al. 2003; Serafini et al. 2003). Interestingly, Donovan et al. (2006) demonstrated that the absorption of flavan-3-ol monomers from chocolate or other cocoa containing products was lower than those contained in red wine or green tea. This apparent differences may be ascribed not only to the occurrence of different enantiomeric forms of flavan-3-ol monomer and their concentration in the digested foods, but also to the macronutrient and micronutrient composition, the synergisms and antagonisms of the different components, physical form of the matrix and processing conditions (Neilson and Ferruzzi 2011; Rein et al. 2013). In a previous study Serafini et al. (2003) noticed that intake of dark chocolate and milk or consumption of milk chocolate considerably decreased absorption of EC into the bloodstream. The theory of binding effect of flavonoids from chocolate to milk proteins and its influence on the bioavailability of flavonoids and therefore the antioxidant capacity of chocolate *in vivo* studies was also tested out by many authors. While Schramm et al. (2003) found that absorption of flavan-3-ols from cocoa drinks containing milk was not decreased and furthermore their bioavailability was improved compared to that related to cocoa drinks containing water instead of milk. Other authors did not report on such differences in absorption levels (Keogh, McInerney, and Clifton 2007; Roura et al. 2007b; Roura et al. 2008; Neilson et al. 2009). Roura et al. (2008) showed that the bioavailability of flavan-3-ols contained in cocoa drinks was the same in the presence of both water and milk. Similar results were revealed by Tomas-Barberan et al. (2007) who studied the influence of food matrix on bioavailability of flavan-3-ols contained in cocoa drinks with milk, which were prepared from either traditional powdered cocoa or powdered cocoa enriched with flavonoids. According to Mullen et al. (2009) administration of milk-based cocoa drinks to healthy volunteers varied only slightly in the pharmacokinetics of (epi)catechin sulfate content (compared to administration of water-based cocoa drinks), and had no effect on concentration of (epi)catechin *O*-methylsulfate in blood plasma. Additionally, it was found that consumption of milk-based cocoa extended the time of elimination of these metabolites from blood and caused a decrease in concentration of (epi)catechin sulfates and *O*-methylsulfates as well as *O*-glucuronides (from 18.3% to 10.5%) in urine (Mullen et al. 2009; Ostertag et al. 2013). According to Schramm et al. (2003) consumption of water-based cocoa supplemented with sucrose or simultaneous intake of bread accelerated the absorption of EC and increased its concentration in blood compared to the control group administered with milk-based cocoa. This finding was consistent with results of Neilson et al. (2009) who point the improved pharmacokinetics of EC and C out when cocoa drink was supplemented with carbohydrates (Table 4). They also revealed that the rate of dissociation of flavan-3-ols from the matrix depends on the form of chocolate (a drink or a bar) and strongly affects the pharmacokinetics of EC in blood plasma. Interestingly, the antioxidant activity of polyphenols incubated with the purified milk protein fractions decreased after 24 h of incubation, thus showing a significant effect of casein on polyphenol activity (Neilson et al. 2009). This decrease was more pronounced in the case of casein incubated with either C or EC. The polyphenols react with the free cysteine residues on the peptide, through strong (covalent, ionic) or weak (hydrogen bridges, π bonds, hydrophobic) bonds (Gallo et al. 2013). Sansone et al. (2017) recently showed that theobromine significantly increased the plasma concentration of structurally related (−)-EC metabolites after the co-ingestion of cocoa flavanols and methylxanthines, which resulted in a greater enhancement of flow-mediated vasodilation.

Procyanidins

Several studies revealed that cocoa procyanidins (PCs), oligomers and high molecular weight polymers of flavan-3-ols, are poorly absorbed and passing through the GI tract unchanged (Borges et al. 2018; Ottaviani et al. 2018; Wiese et al. 2015). The bioavailability of PCs is dependent on the location and stereochemistry of the interflavan linkage between the monomeric units, as well as their molecular size (Aprotosoaie et al. 2016; Dorenkott et al. 2014; Zumdick, Deters, and Hensel 2012). The efficiency of absorption of PCs decreased with increasing degree of polymerization. A number of studies showed that polymeric PCs are not absorbed from the GI tract (Mena et al. 2019; Ottaviani et al. 2012a; Wiese et al. 2015), and only small amounts of

intact oligomers of PCs might be partially absorbed in the intestinal mucosa (Mena et al. 2019). Therefore, compounds with low molecular weight like flavan-3-ol monomers and dimers can achieve higher concentrations in blood and reach the target organs in the body (Aprotosoaie et al. 2016).

Despite the increasing evidence for the bioavailability of flavan-3-ols with different degrees of polymerization, an in-depth knowledge of potential breakdown of the oligomeric or polymeric PCs into monomeric flavan-3-ols *in vivo* is still inconsistent. A first study examining the stability of cocoa PCs throughout the gastric transit in humans was developed by Rios et al. (2002). The authors evaluated the concentrations of flavan-3-ols in six healthy volunteers who consumed cocoa drink (500 mL) containing 733 mg polymeric PCs and 351 mg of monomeric flavan-3-ols. In contrast to previous *in vitro* study suggesting that oligomers of PCs (from trimers to decamers) isolated from cocoa beans and incubated in the artificial stomach juice (at pH 2) were quickly degraded to monomers and dimers (Spencer et al. 2000), they showed that the structure of PCs remained unchanged in the human stomach (Rios et al. 2002). These discrepancies might be ascribed to an increase in pH of stomach juice of the volunteers even up to 5.4 after dosing the cocoa drink, while in the *in vitro* tests where the artificial stomach juice is used the pH value is adjusted and stable. These results strongly suggest that hydrolysis of oligomers and polymers of PCs did not occur in the human stomach when these compounds are consumed with a meal. A study by Ottaviani et al. (2012b) also do not support the view that depolymerization of dietary PCs take place during the passage through the human stomach. In another *in vivo* study, Wiese et al. (2015) have shown that flavan-3-ol monomers are either not released in the GI tract of humans after ingestion of PC B1 and polymeric PCs or they are further rapidly degraded. These studies lead to the assumption that dietary PCs do not contribute to the systemic pool of flavan-3-ol monomers in humans (Ottaviani et al. 2012b; Rios et al. 2002; Wiese et al. 2015). On the other hand, some authors reported that depolymerization of polymeric PCs and transport of released monomers to blood may occur at the terminal portion of intestinal tract (Cooper et al. 2008; Fernandez and Labra 2013; Smith 2013; Oleaga et al. 2013). Spencer et al. (2001a) suggested that after passing through the intestinal mucosa, the B-type PC dimers were cleaved, mainly to EC monomer (95.8%). Some studies have demonstrated that dimeric PCs cross intestinal barriers, and can further undergo 3′-*O*-methylation, which leads to formation of small amounts of methyl derivative of PC dimer (Spencer et al. 2001b; Ottaviani et al. 2012a; Wiese et al. 2015). However, at higher concentrations these dimers act as inhibitors of COMTs activity, which retards methylation of dimers and monomers (Spencer et al. 2001a). According to another study, during the course of absorption, the PC dimers were not conjugated or methylated as compared to their monomeric units (epi)catechin (Appeldoorn et al. 2009b). As a result, mostly unconjugated PC B1 and B2, and only small amounts of methylated PC B1 forms, can be found in plasma within 1–4 h after ingestion (Ottaviani et al. 2012a; Wiese et al. 2015).

Some recent studies showed that the bioavailability of PCs is modulated not only by the degree of polymerization, but also by the presence of A-type linkages (Appeldoorn et al. 2009b; Ou et al. 2012). According to Appeldoorn et al. (2009b), the A-type PC dimers are better absorbed than B-type PC dimers in the rat small intestine. The A-type PCs, which are linked by an additional C2β→*O*→C7 or C2β →*O*→ C5 ether bond were better absorbed than B-type PCs, which contain single interflavan linkages (C4→C8 and/or C4→C6). The authors also revealed that the presence of A-type PC tetramers enhanced the absorption of B-type PC dimers (Appeldoorn et al. 2009b).

However, other research indicate that very weak absorption of oligomeric and polymeric PCs can be also connected with their high affinity to bind membrane proteins of intestinal mucosa and strengthen intercellular tight junction (Deprez et al. 2001; Ou et al. 2012; Ross and Kasum 2002). Only the B-type PC dimers, trimers, and tetramers pass through the intestinal mucosa, which was proved in the study employing a monolayer of human colon carcinoma cell line (Caco-2) (Deprez et al. 2001). The study on perfusion of the isolated small intestine revealed that less than 1% of PC B2 and PC B5 isolated from cocoa beans could pass through the enterocyte membrane (Spencer et al. 2001a). While, some authors suggests that also polymeric PCs with high molecular masses were absorbed in the small intestine without prior depolymerization, which was observed to occur in the large intestine and was mediated by the colonic microflora (Deprez et al. 2001; Holt et al. 2002; Khoo and Falk 2014).

Maintenance of the structure of PCs oligomers in the stomach and very limited absorption in the small intestine caused by specific binding to intestinal mucosa, may affect food digestion and intestine physiology. PCs are not released and absorbed, form complexes with proteins, starch and digestive enzymes in the small intestine, thereby decreasing the digestibility of proteins and starch (Santos-Buelga and Scalbert 2000; Rios et al. 2002). Phenol groups of flavan-3-ols can bind to protein molecules through hydrophobic interactions and hydrogen bonds. Proteins contained in the polyphenol-protein complexes are less accessible to the attack of proteolytic enzymes (Santos-Buelga and Scalbert 2000; Rios et al. 2002).

Anthocyanins

Anthocyanins consists approximately 4% of the total polyphenols in raw cocoa beans. Those compounds naturally occur mainly as glycosides of the anthocyanidin aglycones, which are derivatives of flavylium (2-phenylbenzopyrylium) salts (Akkarachiyasit et al. 2010; Kay et al. 2004). The major anthocyanins present in raw cocoa beans were derivatives of cyanidin, such as cyanidin-3-*O*-galactoside and cyanidin-3-*O*-arabinoside (Table 2) (Elwers et al. 2009; Oracz, Nebesny, and Żyżelewicz 2015).

Investigation of absorption and metabolism of anthocyanins from some natural sources revealed their weak bioavailability compared to other flavonoids (Czank et al. 2013; Rodriguez-Mateos et al. 2014; Wu, Cao, and Prior 2002). *In vivo* and *in vitro* studies have confirmed that anthocyanins are rapidly absorbed in their *O*-glycosyl forms and distributed into the systemic circulation as metabolites and catabolites (Czank et al. 2013; Wu, Cao, and Prior 2002; Xie et al. 2016). Some authors suggest that absorption of anthocyanins through the intestinal mucosa may be mediated by epithelial transporters, such as sodium-dependent glucose transporter 1 (SGLT1), analogously to absorption of flavonol glucosides (Ader et al. 2001; Braga et al. 2018; Wu, Cao, and Prior 2002; Xie et al. 2016). Wiczkowski et al. (2010) demonstrated that anthocyanins appeared in the plasma within 30 min after ingestion, which indicates that these compounds can be absorbed from the stomach via a saturable transport system without prior biotransformation (Xie et al. 2016). Additionally, Passamonti et al. (2003) suggest that bilitranslocase (TC 2.A.65.1.1), which acts as a carrier of organic anions and occurs in the stomach mucosa and liver, is involved in subjected transport. Substrates of this enzyme are aglycones and to a higher extent mono- and diglucoside derivatives.

After absorption into intestinal cells, anthocyanins may be subjected to phase II metabolism in the gut or liver (Wu, Cao, and Prior 2002; Xie et al. 2016). Proposed metabolic conversion of a selected anthocyanin present in cocoa beans and cocoa derived products are present in Figure 3. The metabolism of anthocyanins begins with their 3′-*O*-methylation (Marques et al. 2016). Xie et al. (2016) indicated, that among the anthocyanins, cyanidin-3-*O*-galactoside is rapidly metabolized to peonidin-3-*O*-galactoside (3′-*O*-methylcyanidin-3-*O*-galactoside). Some authors also found that concentration of methylated anthocyanins in rat liver was higher compared to non-methylated ones (Wu, Cao, and Prior 2002). The study of Wiczkowski et al. (2010) revealed that the native anthocyanins, as well as their glucuronidated and methylated derivatives were drained into the bloodstream and disposed in urine during 24 h. They showed that the total plasma concentration of anthocyanins reached the maximum (32.7 ± 2.9 nmol/L) in 1.3 ± 0.1 h after chokeberry juice consumption. Recently, Czank et al. (2013) performed an *in vivo* study concerning metabolism of cyanidin-3-*O*-glucoside by isotope tracer method with human volunteers. Participants of a trial consumed capsules contained 500 mg (1,114 µmoL) of $^{13}C_5$-labelled cyanidin-3-*O*-glucoside, and post-consumption blood, breath, urine, and feces samples were collected after 48 hours (Rodriguez-Mateos et al. 2014). The main metabolites identified were phase II conjugates, degradants, and several colonic metabolites (Czank et al. 2013). The presence of anthocyanin catabolites in the bloodstream can be ascribed to the pH-dependent decomposition of cyanidin to a retro-chalcone structure and further metabolism in the small intestine enterocytes or liver (Rodriguez-Mateos et al. 2014; Williamson, Kay, and Crozier 2018). Several studies suggest that approximately 85% of dietary anthocyanins reach the colon (Faria et al. 2014; Morais et al. 2016) and undergo substantial structural modifications through their spontaneous degradation under physiological conditions (Kay et al. 2004) or following microbial catabolism (Czank et al. 2013; Faria et al. 2014). However, structural differences between anthocyanins (the nature of the anthocyanin aglycone, and type of sugar moiety linked to anthocyanin aglycones) can cause discrepancies in their metabolic path and final profile (Olivas-Aguirre et al. 2016; Xie et al. 2016).

According to the study by Felgines et al. (2003), the overall amount of excreted metabolites corresponded to 1.8 ± 0.29% of cyanidin-3-*O*-glucoside dose. However, other researchers found that only minor amounts (<0.1%) of consumed anthocyanins or their metabolites were excreted in urine, which supports the hypothesis that anthocyanins undergoes extensive biotransformation before being excreted in urine (Akkarachiyasit et al. 2010; Del Rio et al. 2013; Fang 2014; Wiczkowski et al. 2010; Xie et al. 2016

Flavonols

The remaining cocoa flavonoids are represented by flavonols, such as quercetin and kaempferol derivatives. Similar to anthocyanins, flavonols are generally found in glycosylated rather than free form in cocoa beans and derived products. Therefore, it is noteworthy that cocoa beans and cocoa-derived products contain mainly quercetin and their glycosides, like quercetin-3-*O*-arabinoside, quercetin-3-*O*-glucoside, quercetin-3-*O*-galactoside, quercetin-3-*O*-rhamnoside, and quercetin-3-*O*-rutinoside (Sánchez-Rabaneda et al. 2003).

According to several studies, quercetin aglycone exhibited lower bioavailability compared with quercetin glycosides, probably due to its poor water solubility (Karakaya 2004). Investigation of pharmacokinetics of quercetin derivatives showed that after the oral administration quercetin glycosides (glucoside, rutinoside) are not absorbed in the stomach. Quercetin glycosides (i.e. glucoside, galactoside, arabinoside) were absorbed after their deglycosylation into quercetin aglycones, mediated by lactase-phlorizin hydrolase (LPH), a luminal brush border enzyme (Day et al. 2003; Nemeth et al. 2003; Petersen et al. 2016). Additionally, the proposed mechanism of quercetin glycosides absorption involves hydrolysis within the enterocytes by cytosolic β-glucosidase (Figure 3). Cytosolic β-glucosidase is synthesized by mammal cells of liver, kidneys and small intestine. Further, released quercetin aglycones are translocated through the intestinal mucosa by passive diffusion (Day et al. 2003; Nemeth et al. 2003; Stahl et al. 2002). Comparative studies on absorption mechanisms of quercetin glycosides showed that intact glucosides of quercetin are passing through the membrane of enterocytes via the active transport (Ader et al. 2001; Karakaya 2004; Stahl et al. 2002).

Comparatively higher bioavailability of quercetin glucosides than the free aglycone, is caused by participation of SGLT-1 in the process of their absorption through the intestinal mucosa. Active transport of quercetin 4′-*O*-glucoside involving SGLT-1 was demonstrated using the human colon

carcinoma cell line (Caco-2) (Petersen et al. 2016; Walgren et al. 2000). Further research showed that bioavailability of both quercetin-3-*O*-glucoside and 4′-*O*-glucoside was very similar (Olthof et al. 2000). In humans, glucosides are absorbed faster and the site of glucose binding to the aglycone is virtually meaningless for absorption of these derivatives (Olthof et al. 2000). However, the type of sugar moiety linked to the quercetin molecule played an important role in its absorption. Hollman et al. (1997) determined the level of absorption of various forms of quercetin from different food sources in healthy volunteers with colostromy, which prevented degradation of flavonoid derivatives by intestinal microflora. The level of quercetin absorption in the form of glucosides from onion was 52 ± 5% while levels of absorption of free aglycon and quercetin-3-*O*-rutinoside were 24 ± 9% and 17 ± 15%, respectively (Hollman et al. 1997).

After ingestion and absorption, quercetin aglycone undergoes phase II metabolism in the small intestine, liver, colon and kidney (Petersen et al. 2016). In liver, hydroxyl groups attached to the ring B of quercetin and its glucosides are methylated, which leads to formation of derivatives like for instance isorhamnetin (3′-*O*-methylquercetin) (Figure 3). Neither blood plasma nor urine contained free quercetin (Stahl et al. 2002). Principal metabolites occurring both in urine and bile were glucuronides of quercetin, 3′-*O*-methylquercetin and 4′-*O*-methylquercetin (Stahl et al. 2002). For example, after the intake of onion, the main metabolites occurring in blood plasma were quercetin-3′-sulfate and 3-glucuronide (Day et al. 2001). According to Olthof et al. (2000), only 3% of ingested quercetin is excreted in urine. Complete extraction of quercetin from the organism is slow and its high-life is around 25 h mainly due to formation of conjugates with blood plasma proteins, and is eliminated through intestine-liver circulation metabolism.

Phenolic acids

Phenolic acids naturally occurring only in small amounts in cocoa beans are, in vast majority, derivatives of either hydroxybenzoic [gallic acid (GA), hydroxybenzoic acid (HBA), protocatechuic acid, vanillic acid (VA), syryngic acid (SA)] or hydroxycinnamic acids [caffeic acid (CA), ferulic acid (FA), coumaric acid (CuA) and chlorogenic acid (CHA)] (Figure 1) (Ortega et al. 2008). Specifically, the GA accounted for almost half of the total phenolic acids content of various cocoa products (Belščak et al. 2009).

Phenolic acids are generally bioavailable, and their free forms are mainly absorbed in the upper parts of the GI tract via passive paracellular diffusion (Adam et al. 2002; Konishi, Zhao, and Shimizu 2006). As acknowledged, simple phenolic acids, like CA are absorbed across the intestinal epithelium via monocarboxylic acid transporter (MCT) mediated transport (Lafay and Gil-Izquierdo 2008). Poquet, Clifford, and Williamson (2008) studied transport and metabolism of FA through the colonic epithelium, by measuring its transepithelial transport in Caco-2 and mucus-producing HT29-MTX cells. These authors showed that FA was primarily transported as the free form via intestinal epithelium by transcellular diffusion. Interestingly, an active absorption of numerous phenolic acids takes place in other tissues, such as the gastric mucosa. GA, CA, FA, CuA and CHA can be absorbed from the stomach (Konishi, Zhao, and Shimizu 2006; Lafay and Gil-Izquierdo 2008). Their rapid absorption is observed in 1–2 h after the intake.

After absorption in the small intestine, free phenolic acids are transported to the blood circulation or quickly conjugated by phase II enzymes for excretion, analogously to flavonoids (Figure 4). The most important circulating forms of phenolic acids in plasma or urine are their glucuronide, sulfate, and sulfoglucuronide conjugates. The linkage of glucuronic acid with phenolic acids might involve either the carboxyl group in the side chain (ester linkage) or the hydroxyl group on the aromatic ring (ether linkage) (Nardini et al. 2006). The presence of a methoxy group in addition to the hydroxyl group on the aromatic ring of both VA and FA decreases their hydrophilicity and these compounds were present in blood mainly as conjugated (sulfates and glucoronates) forms. CA, in spite of the presence of two hydroxyl groups in the ortho position on the aromatic ring (3,4-dihydroxyl moiety), was present in plasma mainly as conjugated (mainly sulfates) forms (87–100% of the total).

GA compared to other polyphenols is extremely well absorbed, rapidly metabolized and excreted after ingestion (Kaliora, Kanellos, and Kalogeropoulos 2013; Mennen et al. 2006). The most abundant metabolite of GA identified in humans in both intervention and observational studies is 4-*O*-methylgallic acid. This compound may be formed by methylation of GA in various human tissues, mainly in the liver (Mennen et al. 2006). GA was determined as a methylated form in plasma 4 h after the consumption of tea, red wine or dealcoholized red wine, (Kaliora, Kanellos, and Kalogeropoulos 2013). The major compounds detected in urine after consumption of 3 cups of black tea were the methyl ethers of GA, including 4-*O*-methylgallic acid, 3-*O*-methylgallic acid and 3,4-*O*-dimethylgallic acid, while unmethylated GA was not detected in any of the urine samples (Hodgson et al. 2000). Animal studies the first revealed that the main urinary metabolite of GA is 4-*O*-methylgallic acid, followed by pyrogallol (conjugated and unconjugated), and small amounts of conjugated 2-*O*-methylpyrogallol (Shahrzad et al. 2001). 4-*O*-Methylgallic acid was positively associated with polyphenol-rich foods intake, indicating that GA was absorbed in its methylated form (Hodgson et al. 2000; Mennen et al. 2006).

However, plants contain not only free phenolic acids but also their derivatives, which are either building blocks of the complex structures of lignin and hydrolyzable tannins or esters and glycosides. Large part of phenolic groups associated with the fiber fraction in consumed cocoa and its derived products are not released in stomach (Kern et al. 2003; Adam et al. 2002; Lafay and Gil-Izquierdo 2008). For example, Kern et al. (2003) showed that soluble FA is rapidly absorbed in the small intestine whereas insoluble FA (esterified with other compounds like arabinoxylans) passes through stomach and small intestine reaching the colon, where its metabolized by microflora occurred. Generally, the

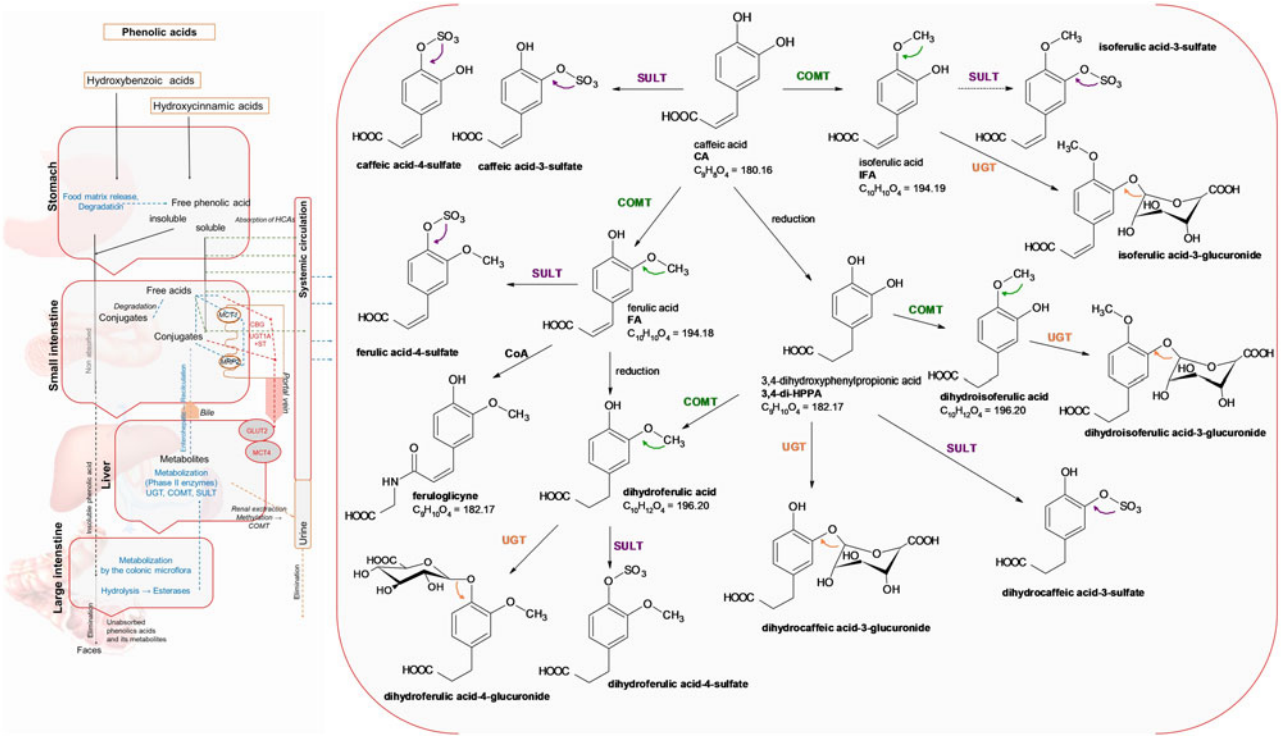

Figure 4. Proposed gastrointestinal distribution and metabolic pathway of phenolic acids based on the metabolites detected in humans. COMT, *O*-methyl transferases; SULT, sulfotransferase; UGT, uridine-5′-diphosphate glucuronosyltransferase (Crozier, Del Rio, and Clifford 2010).

bioavailability of esterified phenolic acids is only 0.3–0.4% of the original intake. It can be explained that the esterified phenolic acids are hydrolyzed in the enterocytes before reaching the blood circulation and the enzymes of intestinal cells are not so efficient to hydrolyze the ester bonds (Adam et al. 2002; Lafay and Gil-Izquierdo 2008). Finally, esterified phenolic acids reach the colon where are metabolized by colon microflora and manifest themselves in plasma between 7 and 8 hours after the intake.

N-phenylpropenoyl-L-amino acids

Besides flavonoids and phenolic acids, phenolic/amino acid conjugates were also detected in cocoa and cocoa-derived products (Stark et al. 2008; Lechtenberg et al. 2012). Most abundant NPAs from cocoa beans are *N*-caffeoyl-L-aspartic acid, *N*-coumaroyl-L-aspartic acid, *N*-caffeoyl-L-dopa, *N*-coumaroyl-L-tyrosine, and *N*-caffeoyl-L-tyrosine (Table 2).

There are only a few studies about the bioavailability of cocoa NPAs (Stark et al. 2008; Urpi-Sarda et al. 2010). The amide linkage present in NPAs are stable enough under physiological conditions (more stable than ester linkage). However, increasing number of evidences suggest that NPAs might exhibit low accessibility. Proposed the GI distribution and metabolic pathway of NPAs present in cocoa beans and cocoa derived products are present in Figure 5. NPAs can be absorbed through the small intestinal epithelium reaching the bloodstream. Rios et al. (2003) proposed that NPAs during microbial degradation are finally transformed into phenolic acids, for example, the urinary excretion of FA after consumption of chocolate was associated with *N*-caffeoyl-L-dopa content (Urpi-Sarda et al. 2010). Generally, it would be difficult to distinguish metabolites of NPAs from each other and cocoa matrix components. Stark et al. (2008) studied the absorption of NPAs by healthy volunteers, and revealed that the maximum concentration of NPAs excreted in urine was observed 2 h after oral administration of cocoa drink. Among investigated NPAs, the highest level in the total urine volume was found for *N*-coumaroyl-L-aspartic acid (149.9 µg), followed by *N*-coumaroyl-L-glutamic acid (44.6 µg), *N*-coumaroyl-L-tyrosine (44.0 µg) and *N*-feruloyl-L-aspartic acid (21.6 µg). The highest recovery rates (57.3 and 22.8%) were observed for *N*-coumaroyl-L-glutamic acid and *N*-feruloyl-L-tyrosine. In comparison, only 8.3, 7.5, and 5.3% of the amount of *N*-feruloyl-L-aspartic acid, *N*-coumaroyl-L-aspartic acid, and *N*-coumaroyl-L-tyrosine present in the ingested cocoa drink were excreted in urine (Stark et al. 2008). The total level of the other conjugates in urine was below 1.6% of the administered dose. In addition, after cocoa intake, urinary *N*-caffeoyl-L-amino acids concentrations were much lower than that found for NPAs with either a *p*-coumaroyl or a feruloyl groups attached to amino acid molecules. *N*-caffeoyl-L-dopa, *N*-caffeoyl-L-tyrosine, *N*-coumaroyl-L-dopa, *N*-cinnamoyl-L-aspartic acid, *N*-feruloyl-L-tyrosine, and *N*-coumaroyl-L-tryptophan were present only in trace amounts (<0.1–1.9 µg), while *N*-caffeoyl-L-glutamic acid and *N*-caffeoyl-L-tryptophan were not detected in any of the collected urine samples. Thus, the recovery rates found for amino acid derivatives conjugated with CA were found to be below 0.4%. This observation was explained by either the enzymatic hydrolysis of *N*-caffeoyl-L-amino acids into CA and corresponding amino acid, or their poor absorption. The higher recovery rates of amino acid derivatives conjugated with FA and *p*-CuA could be also explained

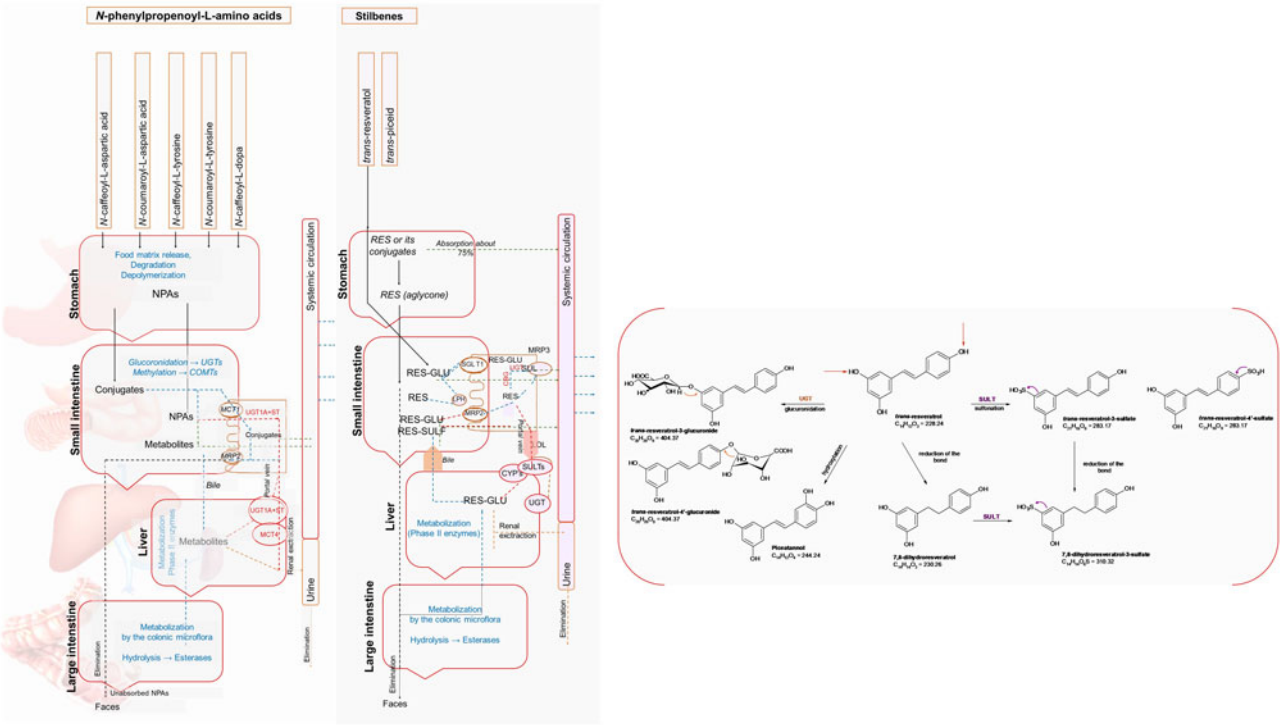

Figure 5. Proposed gastrointestinal distribution and metabolic pathway of *N*-phenylpropenoyl-L-amino acids and stilbenes based on the metabolites detected in humans. CBG, cytosolic *β*-glucosidase; MCT, monocarboxylate transporter; MDR, multiple drug resistance; MRP2/3, multidrug resistance-associated protein 2 or 3; SULT, sulfotransferase; UGT, uridine-5′-diphosphate glucuronosyltransferase (Rocha-González, Ambriz-Tututi, and Granados-Soto 2008).

by the formation of feruloyl- or *p*-coumaroyl-L-amino acid derivatives as the result of *O*-methylation or by reduction of the corresponding *N*-caffeoyl-L-amino acids (Stark et al. 2008). Moreover, there is lack of evidences that the NPAs are enzymatically hydrolyzed, conjugated or degraded by intestinal microbes. Concerning metabolic pathway the quantitative data obtained from enzyme treatment studies revealed the same amounts of the NPAs in the corresponding urine samples, thus indicating that neither glucuronides nor sulfates of these phenolic amides were formed upon metabolic conjugation (Gonthier et al. 2003; Stark et al. 2008). The authors reported that CA released from *N*-caffeoyl-L-amino acids by enzyme-catalyzed hydrolysis, can undergo further conjugation with glucuronic acid leading to formation of its corresponding glucuronides. However, irrespective of the urine sample not even trace amounts of caffeoyl-glucuronides were detected. Based on this finding, it seems that CA glucuronides are not the predominant metabolites of NPAs in humans. After ingestion of NPAs, *N*-caffeoyl-amides are metabolically conjugated to give the corresponding glucuronides, sulfates, and/or *O*-methyl ethers as reported for polyphenols including CA and CA esters such as CHA (Gonthier et al. 2003; Stark et al. 2008).

Stilbenes

Another bioactive compounds encountered in products derived from cocoa beans are resveratrol (3,5,4′-trihydroxystilbene), that can be found either in *cis*- or *trans*-configurations, however the *trans*-configuration is the dominant form, and its glycosides, like *trans*-piceid (*trans*-resveratrol-3-glucoside) (Counet, Callemien, and Collin 2006; Hurst et al. 2008; Jerkovic et al. 2010). Resveratrol is one of the secondary metabolites with a structure of stilbene and belongs to the group of phytoalexins found in a few edible materials, such as grape skins, peanuts, and red wine (Bhat, Kosmeder, and Pezzuto 2001). Chemical structure of this compound is similar to synthetic estrogen diethylstilbestrol (4,4′-dihydroxy-trans-α,β-diethylstilbene), and therefore it is also regarded as one of phytoestrogens – plant hormones with estrogen-like activity (Fulda 2010; Rauf et al. 2018). The biological activity of resveratrol is a consequence of the planar structure of stilbene skeleton, and the presence of 4-hydroxyl groups in the trans conformation on the 4- and 4′-positions of the stilbenic backbone (Fulda 2010). Resveratrol and its derivatives have been reported to exert a wide variety of biological activities including chemopreventive, antioxidant, antiproliferative, anti-inflammatory, and cardioprotective activities (Bhat, Kosmeder, and Pezzuto 2001; Fulda 2010; Rauf et al. 2018). Several studies indicate that this compound may act as an antioxidant, promote nitric oxide production, decreases the pressure of blood vessels walls, inhibits platelet aggregation, and enhances the levels of high-density lipoprotein (Bhat, Kosmeder, and Pezzuto 2001; Delmas, Jannin, and Latruffe 2005; Ostertag et al. 2013).

A recent study indicates that bioavailability of *trans*-resveratrol depends on its binding affinity and accessibility, however, in general is relatively low (<1%) resulting in low biological efficacy (Sergides et al. 2016; Tsai, Ho, and Chen 2017). Therefore, despite the fact that absorption of resveratrol administered with the diet reaches 75%, the blood contains only trace amounts of free resveratrol (Sergides et al. 2016; Walle et al. 2004). This phenomenon could be

explained by the dominant contribution of transepithelial diffusion in resveratrol absorbtion in the human small intestine (Sergides et al. 2016) and its subsequent rapid metabolism in enterocytes and human liver by phase II conjugation enzymes (Figure 5) (Chachay et al. 2011; Rocha-González, Ambriz-Tututi, and Granados-Soto 2008; Wang and Sang 2018). The UGT1A1 and A9 enzymes catalyze the transfer of a glucuronic acid to the 3 and 4′ hydroxyl group of resveratrol, respectively. This biotransformation alters the biological activity of resveratrol, as well as enhances its elimination from the body (Wang and Sang 2018). Resveratrol-4′-*O*-glucuronide and resveratrol-3-*O*-glucuronide have been identified as the major glucuronides of resveratrol, however their formation was significantly inhibited at higher resveratrol concentrations (Maier-Salamon et al. 2011). It was shown that the human SULTs 1A1, 1A2, 1A3, and 1E1 catalyzes the sulfation of resveratrol mainly to resveratrol-4′-*O*-sulfate, and resveratrol-3,4′-disulfate (Brown et al. 2010; Wang and Sang 2018). Walle et al. (2004) investigated the absorption, bioavailability, and metabolism of radiolabeled resveratrol after oral doses in six human volunteers. After oral administration of 25 mg ^{14}C-resveratrol, concentration of resveratrol-3-*O*-sulfate in plasma was approximately 3-fold higher than the concentration of resveratrol-3-*O*-glucuronide, while unchanged resveratrol was encountered in trace amounts (<1%) (Walle et al. 2004). These compounds are circulated in the bloodstream for even 9 h, and disposed of either in urine or in faces (Delmas, Jannin, and Latruffe 2005). The maximum plasma concentration for resveratrol after its oral consumption was in the range of 0.8–1.5 h, indicating fast absorption of this compound (Wang and Sang 2018). Secondary resveratrol and its metabolites were observed due to enterohepatic recirculation of conjugated resveratrol metabolites in liver that were excreted in the bile, and then reabsorbed in small intestine and extracted by faces. Inaccessible resveratrol and its metabolites are mainly eliminated by urine (Wenzel et al. 2005).

Recently, studies on the bioavailability and pharmacokinetic of resveratrol confirmed that, similarly to other polyphenols, absorption of the resveratrol in humans is strongly dependent on the food matrices. According to Rotches-Ribalta et al. (2012) the peak plasma concentrations of resveratrol-*O*-glucuronides was observed 2–2.5 h after ingestion of red wine. In contrast, the absorption rate of resveratrol from grape tablets was significantly slower, and the peak plasma concentrations of the same resveratrol metabolites occur between 4 and 7 h after consumption (Rodriguez-Mateos et al. 2014). The same finding was reported previously, as in studies by la Porte et al. (2010) showed that the oral administration of resveratrol mixed with breakfast cereal flakes, which are rich in fat, may retard and decrease its absorption.

Bioavailability and metabolism of methylxanthines

The most abundant methylxanthines in cocoa beans is theobromine (TB) and the second one is caffeine (CF), while theophylline (TP) is present in much lesser amount (Afoakwa et al. 2008; Jalil and Ismail 2008; Martínez-López et al. 2014).

Methylxanthines are not cumulated in human organism but quickly absorbed in the GI tract and metabolized mainly in the liver to its metabolites, which are finally excreted in urine (Arnaud 2011; Martínez-López et al. 2014). The metabolism of methylxanthines begins with their *N*-methylation, C8-oxidation and ring opening reactions, as illustrated in Figure 6 (Arnaud 2011; Briz, Ruiz, and Bravo-Clemente 2017; Martínez-López et al. 2014). The bioavailability of the most common cocoa methylxanthines and their main metabolites in humans are summarized in Table 3. Arnaud (2011) described the metabolic pathways of methylxanthines and indicated that cytochrome P450 superfamily 1A2 isoenzymes and xanthine oxidase are involved in their liver metabolism. The main metabolite of CA (1,3,7-trimethylxanthine) is *N*3-demethylation product – paraxanthine (PX, 1,7-dimethyloxanthine). TB (3,7-dimethylxanthine) and TP (1,3-dimethylxanthine) are also believed to be metabolites resulting from the *N*1- and *N*7-demethylation of CF, respectively. In contrast, TB can be converted neither to other dimethylxanthines (TP and PX) nor to CF. The conversion of CF into PX is mainly mediated via CYP1A2 enzyme, whereas other enzymes are involved in the biotransformation to TB and TP (Arnaud 2011; Briz, Ruiz, and Bravo-Clemente 2017; Martínez-López et al. 2014; Smith 2011). TB is metabolized mainly to 3-methylxanthine (3-MX) and 7-methylxanthine (7-MX). PX undergoes *N*1- and *N*7-demethylation and yielding 7-MX and 1-methylxanthine (1-MX), respectively. 3-MX and 1-MX are also metabolites resulting from TP *N*-demethylation, catalyzed by CYP1A2 isoenzyme (Arnaud 2011; Briz, Ruiz, and Bravo-Clemente 2017; Martínez-López et al. 2014; Smith 2011). The bioconversion of dimethylxanthines via C8-oxidation to their corresponding dimethyluric acids [1,3-methyluric (1,3-DMU), 1,7-dimethyluric (1,7-DMU), and 3,7-dimethyluric acids (3,7-DMU)] is catalyzed by the various cytochrome P450 isoenzymes. Although to a lesser extent, CF is also metabolized to trimethyluric acid [1,3,7-trimethyluric (1,3,7-TMU)] by hydroxylation via CYP3A4 and CYP1A2 isoenzymes. The intermediate metabolites of cocoa methyloxanthines are further degraded by xanthine oxidase and converted to their corresponding monomethyluric acids [1-methyluric (1-MU), 3-methyluric (3-MU), and 7-methyluric acids (7-MU)] (Arnaud 2011; Briz, Ruiz, and Bravo-Clemente 2017; Martínez-López et al. 2014). Another important pathway of TB metabolism is its hydrolytic conversion to 6-amino-5(*N*-methylformylamino)-1-methyluracil (AMMU), catalyzed by non-microsomal hepatic *N*-acetyl-transferases (Briz, Ruiz, and Bravo-Clemente 2017; Martínez-López et al. 2014). All these metabolites are extracted through urinary system (Llorach et al. 2009; Ptolemy et al., 2010; Llorach-Asunción et al. 2010; Vuong 2014; Rodriguez et al. 2015).

The predominant metabolites of methylxanthines present in human plasma and urine after cocoa or cocoa derived products consumption are presented in Table 4. Ptolemy et al. (2010) investigated the urine, plasma and saliva concentrations of TB and CF in five healthy volunteers who

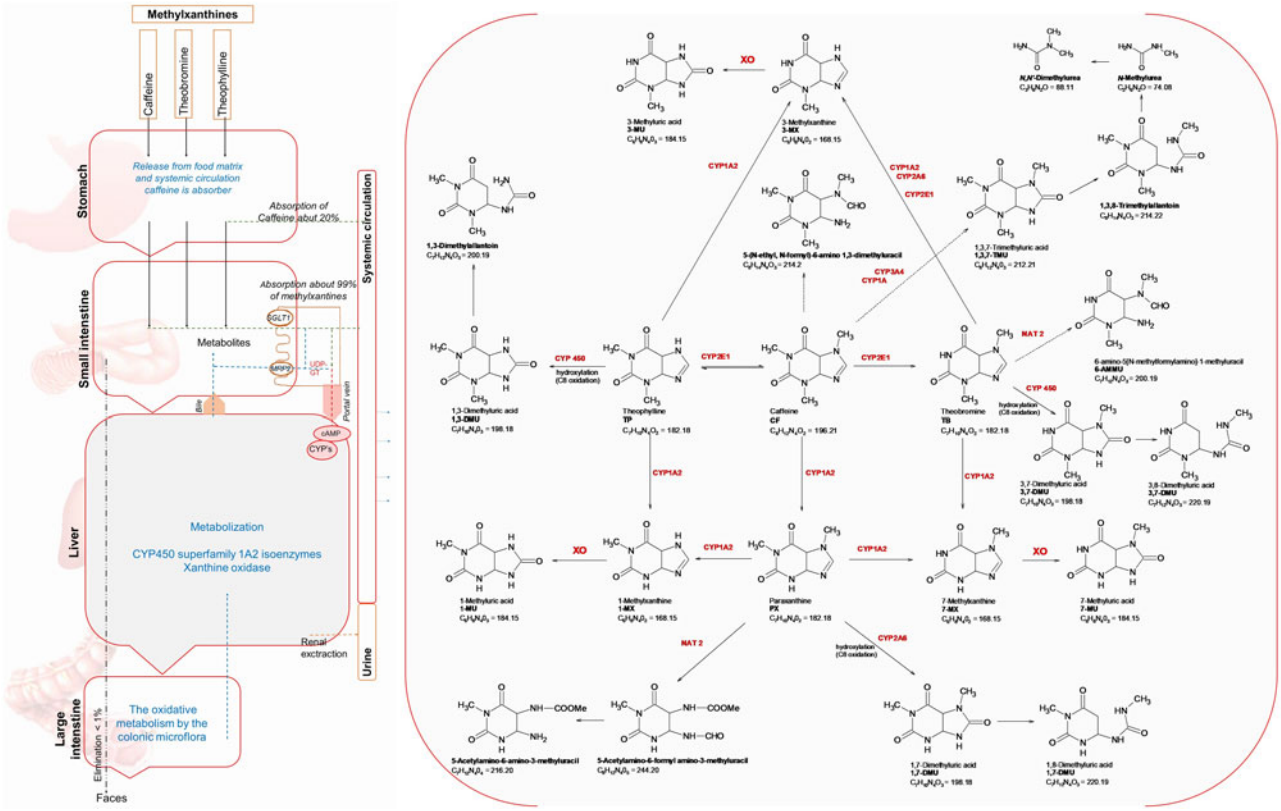

Figure 6. Proposed gastrointestinal distribution and metabolic pathway of methylxanthines based on the metabolites detected in humans. cAMP, cyclic adenosine monophosphate; CYP's, cytochromes P450; NAT 2, *N*-acetyl-transferase 2; XO, xanthine oxidase (Arnaud 2011; Briz, Ruiz, and Bravo-Clemente 2017; Martínez-López et al. 2014).

consumed two commercially available dark chocolate bars contained 188 and 26 mg of TB and CF, respectively. The oral administration of 376 mg of cocoa-derived TB increases the level of this compound in saliva, plasma and urine samples. Richelle et al. (1999) showed that TB appeared in blood serum at maximum concentrations in 2 h after the administration of chocolate with bread and water. Although the level of this compound declined slowly, leading to a still elevated plasma concentration at 8 h after the intake. Ptolemy et al. (2010) detected the highest levels of TB in urine after 1.5 h of ingestion. After consumption of 50 mg of cocoa-derived CF, the parent compound was observed at low levels due to its rapid enzymatic transformations to various methylxanthines and methyluric acids (Ptolemy et al. 2010). Around 90% of CF is rapidly absorbed and distributed throughout as an undissociated forms throughout the whole organism (including the fetus). The concentration of CF in blood plasma reaches maximum after 30–70 minutes of ingestion (Mandel 2002). The medium lipophylic nature of this compound causes that CF easily pass through all biological membranes. The residual fraction is absorbed in further fragments of the GI tract and in 10–30% bound to blood plasma proteins and only around 2% of ingested dose is excreted in urine in the original form. The methylxanthines affinity to human tissues depends on their hydration and plasma concentration. Their deposition in humans varies between 4 and 10 h and is by 20–30% shorter in females than in males (Smith 2011; Mandel 2002; Ptolemy et al. 2010; Martínez-López et al. 2014). As compared to CF, the bioavailability and blood-brain barrier penetration of TB is much lower (Baggott et al. 2013). Absorption of TB took place mainly in small intestine (Ellam and Williamson 2013). Martínez-López et al. (2014) also evaluated the levels of methylxanthines and their metabolites in the plasma and urine of thirteen healthy subjects after the ingestion of cocoa and cocoa enriched in methylxanthines. The HPLC–DAD analysis revealed that TB was the major compounds detected in plasma samples after cocoa ingestion, followed by TP, PX, CF, and two monomethylxanthines (3-MX and 7-MX). All metabolites appeared in plasma from 0.5 to 1.5 h after cocoa intake, indicating its rapid absorption and metabolization. Based on the plasma analysis, the maximum levels of most of these compounds were observed between 2 and 4 h after the ingestion of cocoa enriched in methylxanthines. Thus, the maximal concentration of methylxanthines occurred approximately 1.8–4.6 h after dosing. While excretion of all metabolites ranged from 0.5–8 h to 12–24 h after cocoa consumption. The major metabolites excreted in urine after digestion of cocoa and cocoa enriched in methylxanthines were 7-MX (32–50%) and 3-MX (15–17%). Excretion of unmetabolized TB represented 23–30% of this compound ingested after cocoa products administration (Martínez-López et al. 2014). Dimethylxanthines have lower renal clearance than monomethylxanthines and methyluric acids (Arnaud 2011). Martínez-López et al. (2014) suggested that TB biotransformation pathways might be saturated in case of TB overdose. The low urinary excretions of CF and PX compared to other metabolites confirm their almost complete biotransformation.

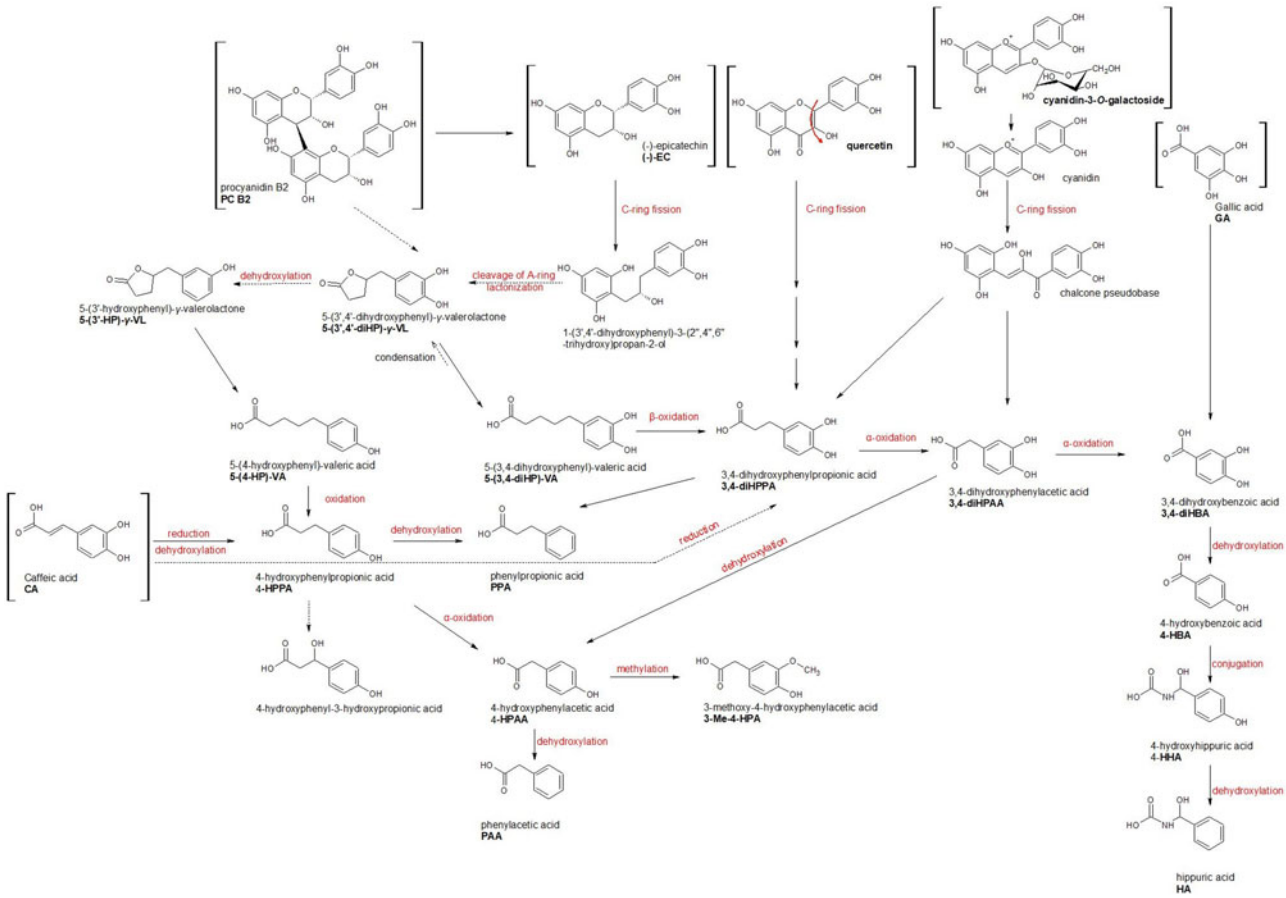

Figure 7. Proposed pathway involved in the colonic catabolism of selected cocoa flavonoids and phenolic acids (Ou et al. 2014; Serra et al. 2011).

A few studies have indicated that bioavailability of methylxanthines is dependent not only on structure of these compounds but also food matrix may affect their absorption and metabolism (Mumford et al. 1996; Mitchell et al. 2011). Mumford et al. (1996) found that as compared to capsules, absorption of TB after oral administration of chocolate was more rapid, while absorption of CF from chocolate was delayed. Indeed, some studies have argued that carbohydrates contained in the chocolate can reduce absorption of CF (Mitchell et al. 2011). A more recent studies also evaluated urine TB concentrations by HPLC-UV-MS after consumption of cocoa powder by 80 healthy children (Rodriguez et al. 2015). The authors concluded that TB excretion was directly related to cocoa consumption, and perceived that a single intake of TB during the morning is not sufficient to maintain a constant urinary excretion of this compound throughout the day. Although, beneficial effects of methylxanthines on human organism were evidenced by many studies (Franco, Oñatibia-Astibia, and Martínez-Pinilla 2013; Monteiro et al. 2016), the low daily methylxanthines doses may be necessary to maintain protective effect of those compounds.

The role of the colonic microflora in the cocoa bioactive compounds metabolism

A large part of ingested cocoa phenolics remains unabsorbed in the small intestine and reaches the colon. It is now more than clear that the gut microbiota plays an important role in the inter-individual variability existing in the biochemical transformations of several phenolic compounds (Mena et al. 2018). Some recent studies suggested that the observed biological effect of consumed with food polyphenols is more likely due to metabolites derived from the colon microbiota rather than to the parent compounds (Heleno et al. 2015). The latest research indicate the influence of high inter-individual variability on the absorption and excretion of their derivatives observed in plasma and urine samples (Williamson and Clifford 2017; Castello et al. 2018; Mena et al. 2018; Murota et al. 2018). The differences in gut metabotypes or polyphenols metabolism patterns result in varying bioactivity and health benefits associated with cocoa polyphenols consumption. Therefore, the identification of specific human microbiome involved in flavonoids and other cocoa polyphenols transformation is also very important.

Biotransformation of cocoa flavonoids by human colonic microflora in lower parts of the large intestine causes the cleavage of heterocyclic C-ring and formation of phloroglucinol and phenolic acids with different hydroxylation profiles and length of side chain, as illustrated in Figure 7 (Appeldoorn et al. 2009a; Cifuentes-Gomez et al. 2015; Fogliano et al. 2011; Mena et al. 2019; Ou et al. 2014; Serra et al. 2011). Bacterial enzymes catalyze such reactions as hydrolysis of glucuronides, sulfates and glycosides, oxidation, dehydroxylation, demethylation, reduction of the double bond, and further degradation by fission of the C-ring to

yielding small phenolic acids and aromatic catabolites (Appeldoorn et al., 2009a; Ottaviani et al. 2011; Roura et al. 2007a). Recent reports indicate that both cocoa monomeric flavan-3-ols or B-type PCs (degree of polymerization ranging from 2 to 10) are extensively biotransformed by the gut microflora in the colon into phenyl-γ-valerolactones (PVLs) and other low molecular weight phenolic metabolites, such as 4-hydroxy-5-(3′,4′-dihydroxyphenyl)-valeric acid [4-H-5-(3,4-diHP)-VA], 5-(3′,4′-dihydroxyphenyl)-valeric acid [5-(3,4-diHP)-VA], 5-(4-hydroxyphenyl)-valeric acid [5-(4-HP)-VA], 3,4-dihydroxyphenylpropionic acid (3,4-diHPPA), and 3,4-dihydroxyphenylacetic acid (3,4-diHPAA), 3-hydroxyphenylpropionic acid (3-HPPA), 3-hydroxyphenylacetic acid (3-HPAA), phenylacetic acid (PAA) and phenylpropionic acid (PPA), 3-metoxy-4-hydroxyphenylacetic acid (homovanillic acid, 3-Me-4-HPA), 3,4-dihydroxybenzoic acid (protocatechuic acid, 3,4-diHBA), HBA, 4-hydroxy-3-methoxybenzoic acid (vanillic acid, VA), hippuric acid (HA), and 4-hydroxyhippuric acid (4-HHA) (Fogliano et al. 2011; Rios et al. 2003; Urpi-Sarda et al. 2009; Urpi-Sarda et al. 2010). The principal colonic metabolites of flavan-3-ol monomers and procyanidins are given in Table 3.

The recent studies indicate the species of gut bacteria which are involved in microbial catabolic processes of monomeric cocoa flavan-3-ols, such as (−)-EC and (+)-C. These compounds can be biotransformed via the C-ring fission mechanisms by *Eggerthella lenta* rK3 into 1-(3,4-dihydroxyphenyl)-3-(2,4,6-trihydroxyphenyl)-propan-2-ol (3,4-diHPP-2-ol). The cleavage of the C-ring of flavan-3-ols was found to be also catalyzed by a *Lactobacillus plantarum* strain (Braune and Blaut 2016). 3,4-DiHPP-2-ol is further converted by *Flavonifractor plautii* aK2 to 5-(3′,4′-dihydroxyphenyl)-γ-valerolactone [5-(3′,4′-diHP)-γ-VL] and/or 4-H-5-(3,4-diHP)-VA (Mena et al. 2019). Wiese et al. (2015) reported that 5-(3′,4′-diHP)-γ-VL and 4-H-5-(3,4-diHP)-VA participating in the same metabolic pathway and exhibited a very similar blood plasma and urine kinetic courses. These metabolites are then subject to the γ-valerolactone ring opening and/or dehydroxylation resulting in the formation of 4-H-5-(3,4-diHP)-VA and 5-(3,4-diHP)-VA (Appeldoorn et al. 2009a; Mena et al. 2018; Mena et al. 2019). The direct degradation of the lower unit of PC B2 also involves the formation of 5-(3′,4′-diHP)-γ-VL (Figure 7), while the cleavage of the upper unit yielding 3,4-diHPAA (Mena et al. 2019). However, the bacterial species responsible for the fragmentation of PCs in the human gut have not yet been fully identified (Braune and Blaut 2016).

Hydroxyphenylvaleric acids (HPVAs) derived from PVLs may undergo β-oxidative removal of successive carbon atoms from the side chain leading to the formation of 3,4-diHPPA and HPPA (Mena et al. 2019). According to several studies, 3,4-diHPPA is most likely an intermediate between the most of flavonoids and their colonic metabolites (Fogliano et al. 2011; Rios et al. 2003; Stoupi et al. 2010). The authors suggested that putative source of 3,4-diHPAA is α-oxidation of 3,4-diHPPA, while HPAA is a product of α-oxidation of HPPA. In addition, 3,4-diHPPA may undergo β-oxidation yielding 3,4-diHBA (Fogliano et al. 2011). Further dehydroxylation of 3,4-diHBA conducted by intestinal microflora yields the HBA. Literature data indicate that 4-HBA can be produced by the degradation of PC B3 dimer and (±)-C by intestinal microflora (Gonthier et al. 2003; Urpi-Sarda et al. 2010). HHA and HA are mainly generated by hepatic glycination of benzoic acid derivatives but noteworthy, these compounds disposed in urine may be also the byproducts of breakdown of various polyphenols such as anthocyanins, flavonols and hydroxycinnamic acid (HCAs).

The study of Ottaviani et al. (2016) demonstrated that 70% of the ingested ^{14}C-EC reaches the colon where the colonic microbiota metabolized EC mainly into low molecular phenolic catabolites, which are absorbed into the circulatory system (Mena et al. 2019; Ottaviani et al. 2016). PVLs, HPVAs, and other low molecular weight phenolic acid derivatives may be absorbed to blood plasma and act as biologically active substances or undergo subsequent phase II modification by enzymes present in the wall of the colon and/or the liver (Ottaviani et al. 2016), which yield metabolites that are removed in urine (Table 3) (Holt et al. 2002; Mena et al. 2018; Mena et al. 2019; Oleaga et al. 2013).

Anthocyanins are metabolized by the human gut bacteria with β-glucosidase activity, including *Bifidobacterium lactis* and *Lactobacillus casei*. The deglycosylation of anthocyanins into aglycones, which are highly unstable at neutral pH and undergo spontaneous fission of the C-ring through various intermediates resulting in the formation of smaller phenolic acids and aldehydes (Braune and Blaut, 2016; Czank et al. 2013; Faria et al. 2014; Morais et al. 2016), as indicated in Figure 7. The *in vitro* studies showed that the main degradation product of cyanidin-3-*O*-glucoside after fecal fermentation is 3,4-diHBA (Han et al. 2009; Rodriguez-Mateos et al. 2014). Further *in vivo* studies provide evidence that the most abundant catabolites of cyanidin-3-*O*-glucoside identified in humans after anthocyanin intake include 3,4-diHBA, phloroglucinaldehyde, 3,4-diHPAA, 4-HPAA, 3-Me-4-HPA, VA, CA, FA, 4-HHA and HA, as well as and phase II conjugates of 3,4-diHBA. Authors revealed that, FA, HA, PPA and PAA were detected in serum, urine, and feces as end products (Czank et al. 2013; Rodriguez-Mateos et al. 2014; Xie et al. 2016). Recently, Xie et al. (2016) indicated that among the urinary metabolites of anthocyanins, HA accounted for 98.5% of the total polyphenols after anthocyanins supplementation. However, full and accurate data on the fragmentation of anthocyanins to phenolics derivatives in humans are still limited.

A large part of consumed quercetin (neither absorbed in the small intestine nor secreted with the bile) is metabolized by the microflora of large intestine (Figure 7). Biotransformation of quercetin by human colonic microbiota occurs through the reduction of the C2-C3 double bond yielding dihydroquercetin (taxifolin). Quercetin may undergo conversion by *Eubacterium ramulus* and *F. plautii* leading to the formation of the intermediates taxifolin and alphitonin, which may be cleaved further by *Eggerthella* (former *Eubacterium*) sp. SDG-2 into hydroxydihydrochalcone (Braune and Blaut 2016). Quercetin can be metabolized following ring scission by *Enterobacteria* spp. in the colon and

then enterocyte phase II transformation such as dehydration or reduction into 3-HPPA and 3-HBA (Pasinetti et al. 2018). The B-ring metabolites of the quercetin are 3,4-diHPAA HPAA, and 3,4-diHBA, while phloroglucinol, 3,4-diHPPA and 3-HPPA are metabolites arising from the A-ring. The *in vivo* conversion of quercetin by for *E. ramulus* was demonstrated in a gnotobiotic rats associated with human intestinal bacteria (Braune and Blaut 2016; Schneider et al. 2000).

Similar to anthocyanins, the quercetin glycosides may undergo *O*-deglycosylation by human gut microbiota prior to their absorption and/or further conversion, which improves their bioavailability. The *β*-glucosidase activity has been reported for certain types of bacteria, including *Bacteroides uniformis, Bacteroides ovatus, Bifidobacterium adolescentis, Bifidobacterium longum, Bifidobacterium dentium*, *Bifidobacterium pseudocatenulatum, Eubacterium ramulus Parabacteroides distasonis, Enterococcus casseliflavus,* and *Enterococcus avium* (Braune and Blaut 2016). For example, quercetin-3-*O*-rutinoside cannot be hydrolyzed in the small intestine due to a lack of human intestinal α-L-rhamnosidase. Therefore, quercetin-3-*O*-rutinoside can only be converted to its 3-*O*-glucoside by detachment of rhamnose unit by bacteria strains of *L. acidophilus, L. plantarum* and *B. dentium* with α-L-rhamnosidase and β-glucosidase activities (Mueller et al. 2017). This observation confirmed the participation of intestinal bacteria in absorption of quercetin glycosides. In the consequence of these processes, quercetin is released from its glycosides and may be degraded further to HPAAs as described above, which can be further absorbed (Crozier, Del Rio, and Clifford 2010; Olthof et al. 2000; Stahl et al. 2002).

Human colonic microflora were found to conduct biotransformation of HCAs. Bacterial conversion of HCAs and their corresponding esters in the human intestine, includes the hydrolysis of ester bonds by esterase enzymes, and further conversion of the resulting phenolic acids. For example, free CA was detected 5 h after ingestion of its ester. CA is further metabolized by reduction of the double bond in the aliphatic chain and then by dehydroxylation of phenyl ring mainly into 3-HPPA and to minor extent to BA (Figure 7). Additionally, CA may undergo decarboxylation and transformation to 4-ethylcatechol (Ozdal et al. 2016). FA may be derived either from the diet or through metabolism of CA via *O*-methylation. Poquet, Clifford, and Williamson (2008) showed that mainly free form of FA and only a small percentage of conjugated and reduced FA are available to the blood after passage across the colonic barrier. FA can be biotransformed further to 3,4-diHPPA, 3,4-diHPAA, 3-PPA, and BA (Ozdal et al. 2016).

Recent studies also showed that the resveratrol conjugates, mainly the glucuronides, are secreted by the biliary pathway into the duodenum, in the distal segments of the intestine where they may be subjected to microbial transformation by the colonic bacteria enzymes, such as *β*-glucuronidase and sulfatase (Walle et al. 2004). The resveratrol aglycone is biotransformed through human colon microbiota into dihydroresveratrol, 3,4′-dihydroxy-trans-stilbene, and 3,4′-dihydroxybibenzyl (lunularin) (Bode et al. 2013). Furthermore, it was reported that the resulting metabolite, dihydroresveratrol, may be absorbed from the colon, and further metabolized to form phase II conjugates, before to urinary excretion (Rodriguez-Mateos et al. 2014). However, it has to be highlight, that the huge inter-individual variability in the metabolism rate and extent, as well as metabolic routes of resveratrol between human donors were observed (Bode et al. 2013).

According to the literature, cocoa methylxanthines can potentially be metabolized by the colonic microflora yielding phenolic acids, including PAA, HPAA, 5-HP-γ-VL, and 5-(3′,4′-diHP)-γ-VL (Madyastha and Sridhar 1998; Serra et al. 2011). Madyastha and Sridhar (1998) suggested that the oxidation is the major pathway for the degradation of CF by colonic microflora. However, Serra et al. (2011) claimed that the metabolism of these particular methylxanthines by the colonic microflora is very low. Some recent studies have argued that the genetic polymorphisms may also contribute to the inter-individual variation in bioavailability of CF (Milenkovic et al. 2017).

After consumption of soluble cocoa powder, cocoa drinks or chocolate by humans an increase in the concentration of 5-(3′,4′-diHP)-γ-VL, HPVAs, HCAs (mainly CA and FA), 3,4-diHPPA, 3-HPPA, 3-HPAA, 3,4-diHPAA, PAA, VA, 3-HBA, 4-HBA, 4-HHA, and HA, as well as their methyl glucunoride and sulfate conjugates in urine was observed showing a significant rise after 24 h of consumption (Garcia-Aloy et al. 2015; Goodrich et al. 2014; Heleno et al. 2015; Rios et al. 2003; Urpi-Sarda et al. 2010 Vitaglione et al. 2013,;).

Numerous studies on the cocoa polyphenol metabolites represent the differences in technical approaches (Table 4), models (*in vitro* or *in vivo*), as well as various type of metabolites profiles (Mena et al. 2018; Rios et al. 2003; Urpi-Sarda et al. 2010; Wiese et al. 2015). The most reliable data can be obtained from *in vivo* bioavailability trials.

Generally, studies using human subjects typically analyze concentrations of parent compounds, conjugates, and microbial metabolites in blood and urine samples. Rios et al. (2003) evaluated the levels of phenolic acids formed by the microflora and excreted in the urine of human subjects after consumption of polyphenol-rich chocolate containing 439 mg PCs and 147 mg flavan-3-ol monomers. The HPLC-ESI-MS/MS analysis of urine samples revealed the presence of 3-HPPA, 3-HPAA, FA, 3,4-diHPAA, VA and 4-HBA. Urpi-Sarda et al. (2010) found that HA and PAA were the dominant *in vivo* metabolites in blood and urine. They also showed that phenolic acids reached the highest amount in urine 6 h after consumption, nevertheless concentrations higher than initial were observed in the 0–6 h for 3,4-diHPAA, HBAs, and HCAs (Urpi-Sarda et al. 2010; Vitaglione et al. 2013). Wiese et al. (2015) evaluated the biokinetics and the metabolic fate of pure monomeric, dimeric, and polymeric flavan-3-ols in the seven healthy male subjects after the ingestion of a hard gelatin capsule containing EC, PC B1 and PPCs. Both blood and urine samples showed the highest concentrations of *O*-glucuronides, sulfate esters and *O*-methyl ethers of (−)-EC and 5-(3′,4′-diHP)-γ-VL.

Garcia-Aloy et al. (2015) used an untargeted HPLC-q-ToF-MS based metabolomics screening approach to discriminate the urinary metabolome of regular cocoa product consumption in a free-living population. Using this approach, the authors identified a total of 31 discriminating metabolites, and ten of them were chosen as valid biomarkers of cocoa consumption. In agreement with previous data, the authors suggested that the discriminant cocoa biomarkers were mainly related to the metabolic pathways of theobromine (AMMU, 3-MU, 7-MX, 3-MX, 3,7-diMA and TB) and polyphenols microbiota metabolism (methoxyhydroxyphenylvalerolactone, and glucuronide and sulfate conjugates of 5-(3′,4′-diHP)-γ-VL), as well as to cocoa processing (Garcia-Aloy et al. 2015; Mena et al. 2019). Subsequently Ottaviani et al. (2016) also studied the role of the human gut microbiome in EC catabolism on eight male volunteers who were administered to ^{14}C-EC. The metabolic profile in urine indicated that EC was excreted primarily as sulfates and glucuronides of 5-(3′,4′-diHP)-γ-VL and 4-H-5-(3,4-diHP)-VA, with a small amounts of 3-(3′-hydroxyphenyl)hydracrylicacid, 3-HHA, and HA. They found that PVL and HPVA metabolites were excreted in urine in amounts corresponding to 42 ± 5% of the dose administered, while phenolic acids and HA metabolites accounted for 28 ± 3% of urinary radioactivity.

The large majority of valerolactone metabolites, colon-derived polyphenol catabolites, were also found in plasma or urine after consumption of tea and coffee products (Mena et al. 2018) or apple products (Trost et al. 2018). Mena et al. (2018) found three human metabotypes which released in high amounts could be treated as the specific biomarkers after tea consumption: 5-(3,4-diHP)-γ-VL-*O*-glucuronide, 5-HP-γ-VL-*O*-glucuronide, and 3-PPA-sulfate. The similar conclusions could be drawn from human *in vivo* studies on the production of polyphenol metabolites derived from apple (Trost et al. 2018). Among these HP-γ-VL, diHP-γ-VL and methoxy(hydroxyphenyl)-γ-valerolactones were conjugated to (methyl)glucuronide, (methyl)sulfate moieties, and diHP-γ-VL glucuronide isomers were the most abundant compounds within the group of proanthocyanidin metabolites (Trost et al. 2018). Additionally, the results from this human *in vivo* study indicate on the association of intestinal bacterial genera with specific microbial catabolites derived from dietary polyphenols. They found the main metabolites of phloretin, epicatechin, as well as phenolic acids (VA sulfate, FA sulfate, feruloylquinic acid isomers) in plasma and urine in the first hours post-dose. These data suggest that this fraction of native polyphenols is quickly absorbed and metabolized in the upper gut with little or no contribution from the colonic microbiota and rapidly excreted in urine. On the contrary, the derivatives of valerolactones, catechol, hippuric, propionic and acetic acids are metabolized slowly by gut microbiota and their concentration increased in urine over the 24 h period (Trost et al. 2018).

In vivo studies using human and animals indicate the key role of gut microbiota for the transformation of dietary polyphenols into bioactive and bioavailable compounds (Borges et al. 2016; Ottaviani et al. 2016; Urpi-Sarda et al. 2009). Urpi-Sarda et al. (2009) investigated EC, PCs, and other phenolic microbial metabolites after cocoa intake in humans and rats, and showed that the types of the determined metabolites were similar but the concentrations in urine were different especially for PC B2 (Table 4). The study of Ottaviani et al. (2016) revealed marked species-dependent differences in the metabolism of EC. Alternatively, the pig is considered as a useful *in vivo* model of human food consumption and metabolism because of similarities between the physiology and microbial composition of the GI tract. The pig cecum has been used in studies targeting delivery of metabolites to the colon and has been shown to be suitable for studies of the metabolism of several classes of flavonoids (Labib et al. 2004). Similarly, pig urinary metabolomics studies have detected several metabolites commonly found in humans (Engemann et al. 2012). Jang et al. (2016) reported that *O*-methyl-epicatechin-glucuronide conjugates dose-dependently increased in the urine, serum, and adipose tissue of pigs fed cocoa powder. Additionally, this study demonstrated that consumption of cocoa powder by pigs could contribute to gut health by enhancing the abundance of *Lactobacillus* and *Bifidobacteriu*m species and modulating markers of localized intestinal immunity (Jang et al. 2016).

Similarly, there are a few scientific reports discuss the effect of food matrix on the digestibility and stability of the phenolic acids fraction during the gastric and duodenal (Table 4). FA may be either a component of diet or an intermediate metabolite of CA. Occurrence of FA in urine after the intake of cocoa drinks or chocolate may also result from conversions of phenolics conducted by intestinal microflora like for instance dehydrogenation of 3,4-diHPPA and degradation of CHA, N-caffeoyl-L-dopa (colvamide) and other amides of hydroxycinnamic acids (NPAs) (Rios et al. 2003; Tomas-Barberan et al. 2007; Urpi-Sarda et al. 2009; Urpi-Sarda et al. 2010).

Urpi-Sarda et al. (2010) proved that the type of food matrix (milk or water) strongly influenced the urinary concentrations of certain phenolic acids, generated in the large intestine in processes mediated by its microflora. The intake of milk-based cocoa drink decreased concentrations of 3,4-diHPAA, 3,4-diHBA, 4-HBA, 4-HHA, CA and FA. Additionally, authors revealed that VA and PAA was more abundant in a presence of milk proteins, majorly excreted over the first 6 h after consumption (Urpi-Sarda et al. 2010; Vitaglione et al. 2013). The increased level of VA in urine could be a consequence of addition of vanillin used as a flavoring. The lipophylic vanillin is easier and faster absorbed from milk-based cocoa beverage than from water-based cocoa drink. VA may be formed in the liver via vanillin oxidation, which is catalyzed by aldehyde oxidase or by methylation of 3,4-diHBA. An increase in concentration of PAA in urine may be caused by the presence of phenylethylamine in cocoa. This amine is quickly absorbed with milk and oxidized in the liver by aldehyde dehydrogenase and oxidase to phenylacetaldehyde, which is further metabolized to PAA (Panoutsopoulos, Kouretas, and Beedham 2004). The discrepancies between results could be explained by high inter-individual variability in the bioavailability of polyphenols in

humans, as well as to the small number of subjects selected in the studies (D'Archivio et al. 2010).

In vitro methods are good alternative and generally consist of a simulation of the GI digestion prior to analytes determination. Fogliano et al. (2011) combined *in vitro* simulated digestion with the GI enzymes with bacterial fermentation in a human colonic model system to investigate the bioaccessibility and gut biotransformation of phenolic compounds present in the water-insoluble cocoa fraction. In contrast to several *in vivo* studies, the authors in this study showed that biotransformation of flavonoids from cocoa by human gut microflora in a three-stage continuous culture colonic model system of lower parts of the large intestine generates only three phenolic acids as the major *in vitro* metabolites of flavan-3-ol monomers and dimers. The LC-MS/MS analysis of all samples revealed the presence of 3-HPPA, 3-HPAA and 3,4-diHBA, but PVLs and di HPAAs were not detected. The authors claim that this results could be associated with the conversion of γ-valerolactones and of 3,4-diHPAA and the increase in their metabolites in the fermentation vessel (Fogliano et al. 2011; Stoupi et al. 2010). Moreover, Pastoriza et al. (2011) noticed that significant amounts of parent compounds and metabolites may remain in the residues after *in vitro* digestion and usually are ignored. In recent decades, *in vitro* methods have been improved by incorporating enterocyte-like cell cultures, such as the Caco-2 cell line (Jailani and Williamson 2014). Caco-2 cell line exhibits many properties of the normal intestinal epithelium, and it has been used as a suitable model to study the absorption of phenolic compounds and antioxidant cellular response (Jailani and Williamson 2014; Kaulmann and Bohn, 2016). Kern et al. (2003) noticed that Caco-2 cells are able to metabolize polyphenols to several metabolites including ferulic acid–sulfate, synapic acids–sulfate, *p*-coumaric acid–sulfate, and methylferulate–sulfate, while Yi et al. (2006) revealed that anthocyanins added to Caco-2 cells can be degraded and demethylated during absorption and transport by the cells. *In vitro* experimental protocols are fast and reproducible approach for the assessment of bioaccessibility of specific compounds under controlled environmental conditions. Although *in vitro* models are less expensive and less time-consuming than *in vivo* counterparts, they have limitations as methods for evaluating bioactive compounds absorption and metabolism, due to the lack of host cells. Furthermore they do not fully replicate the *in vivo* models. Thus, the most reliable data can be obtained from *in vivo* bioavailability trials. Some recent studies showed that the TNO *in vitro* the GI model (TIM) represent a valuable computer-controlled model simulates the conditions in the both stomach and small intestine (TIM-1 system) and the large intestine (TIM-2 system). TIM model has been used to assess the availability of a specific compound for absorption through the intestinal wall (bioaccessibility). This type of model show a good predictability compared to *in vivo* experiments (Etcheverry, Grusak, and Fleige 2012; Carbonell-Capella et al. 2014; Minekus, 2015). Based on the literature review, many authors suggest that it is non-trivial choice to point the most appropriate method out. In consequence, the differences between laboratories and procedures of sample preparation and analytical methods may represent a key contributing factor in the substantial differences with regard to the type of metabolites, as well as their reported and relative levels (Ottaviani et al. 2012a; Aprotosoaie et al. 2016).

The interactions between the gut microbiota and cocoa bioactive compounds

As it was shown, the relationship between polyphenols and microbiota is complex. The phenolic substrates may modulate and cause fluctuations in the composition of the microflora populations through selective prebiotic effects and antimicrobial activities against gut pathogenic bacteria. Moreover, the interpersonal differences in the gut microbiota cause formation of the variable polyphenol metabolites which stimulate the growth of selected bacteria species simultaneously inhibiting other microbiota species like it was observed in obesity (Jamar et al. 2017). It has been shown that anthocyanins from fruits, metabolized by certain types of bacteria such as *Bifidobacterium spp*. and *Lactobacillus spp*. in the colon, were associated with beneficial changes in the gut microbiota as they might promote intestinal colonization by these specific groups of bacteria (Boto-Ordonez et al. 2014, Faria, et al. 2014, Jamar et al. 2017). Other data also indicated that the same effects were observed for proanthocyanidin-rich extracts, flavan-3-ol rich sources, resveratrol or quercetin (Cardona et al. 2013, Etxeberria et al. 2013). Williamson and Clifford rewieved the studies on the polyphenol-microbiota interactions and underlined that modulation of the human gut microbiota composition by supplementation with some (poly)phenol-rich commodities depends on the treatment, length of time and on the individual metabotype (Williamson and Clifford 2017).

The interactions between cocoa bioactive compounds and the gut microbiome may increases the bioavailability of phenolics and therefore affects their health-promoting effects in the humans. For example, the main products of microbial degradation of polyphenols, such as 3,4-diHPAA and 3,4-diHPPA display the stronger anti-aggregation and anti-inflammatory activities than the parent compounds (Edwards et al. 2017; Larrosa et al. 2009; Crozier et al. 2010). Other cocoa phenolic metabolites, such as 3,4-diHBA, HBA, FA and CA has been reported to have antioxidant, antimicrobial, cytotoxic, chemopreventive and antimutagenic properties (Heleno et al. 2015).

Aforementioned results suggest that the nature and form of food matrix and presence of other nutrients (milk proteins, sucrose, starch and dietary fiber) influences mastication, gastric emptying rates, digestibility and liberation of flavan-3-ols and other bioactive compounds from the ingested food (Neilson et al. 2009; Ottaviani et al. 2016). Therefore, the type of food matrix carrying cocoa bioactive compounds into the organism decides of the rate and extent of their absorption, distribution, metabolism and excretion. Thus also the beneficial impact on human health caused by ingestion of small amounts of bioactive compounds

contained in chocolate and cocoa drinks may be weaker or stronger.

Cocoa powder is a good source of dietary fibers (DF), including mainly insoluble DF and soluble DF (Lecumberri et al. 2007; Massot-Cladera et al. 2015). Cocoa-based dietary fiber that resist hydrolysis and digestion in the upper GI tract and reaching the colon intact (Massot-Cladera et al. 2015) could potentially manipulate the mechanism of microbial catabolism of polyphenols (Edwards et al. 2017). The interactions between dietary fibers and phenolic compounds play an important role in the release of phenolics from food matrices prior to absorption. It was found that some fibers could bind phenolic compounds in the food matrix, which decrease their absorption in the small intestine and could in turn increase bioavailability of polyphenols through bacterial metabolism (Edwards et al. 2017; Perez-Jimenez et al. 2013). The colonic bacterial populations and their metabolic activities can be influenced by fermentable fibers via their microbial metabolites, such as short chain fatty acids (SCFAs). In addition, polyphenols may modulate the composition of colonic microbiota through both prebiotic effects and selective antimicrobial activities against gut pathogenic bacteria, and could in turn influence the fermentation of the dietary fibers (Edwards et al. 2017).

Concluding remarks

In the present review, while going through the literature, it was observed that there is a great diversity of the bioavailability and metabolism of high value bioactive compounds in cocoa and their co-products. The bioavailability and pro-healthy potency of cocoa bioactive compounds is dependent on their molecular mass, chemical structures and concentration in food, as well as food matrix and their digestion pathways. Studies on the relationship between the structure of monomeric flavan-3-ols and their bioavailability showed that their stereoisomers were released from food products at different rates, as well as the transport through intestinal mucosa to blood plasma, metabolism and excretion also varied. Strong influence on the bioavailability has the character of conjugated form, for example some polyphenols, like anthocyanins and flavonols, are strongly affected by the type of attached sugar. After absorption into the small intestine, flavonoids, phenolic acids and their derivatives, and stilbenes may undergo extensive Phase I and particularly Phase II biotransformation in the small intestine and then the liver and kidneys to a series of conjugate metabolites (methyl, glucuronide, sulfate and methyl sulfate/glucuronide derivatives) rapidly liberated to the systemic circulation for further tissue distribution and excretion in urine. The key role of human enzymes and gut, typically colonic, microbiota in metabolism of polyphenols was confirmed. They are involved in many polyphenol transformations like hydrolysis of glycosides, glucuronides, sulfates, amides, and esters associated with further ring-fission, as well as opposite reactions like reduction, lactonization, decarboxylation, demethylation, dehydroxylation, deglycosylation, glucuronidation, sulfation, and possibly deesterification. This microbial biotransformation of cocoa polyphenols produces the large variety of lactones and aromatic and phenolic acids. The evaluated studies that investigated the metabolism and excretion of methylxanthines suggest that the major liver metabolites in plasma and urine after consumption of cocoa co-products were TB, followed its principal metabolites, such as 7-MX and 3-MX.

The results of research into bioavailability of different bioactive compounds are often difficult to evaluate because the *in vitro* methods fail to regard the role of the individual microbiota present in the human body. However, *in vivo* and *ex vivo* methods also have its limitations, since each subject has own microbiota that clearly interferes with the bioavailability of polyphenols and other bioactive compounds due to inter-individual differences. The number of studies concerning on metabolites present in humans after cocoa consumption show significant discrepancies with regard to their structure and concentration. Variability in the particular metabolites abundance despite mentioned inter-individual differences in metabolism may lie in the methodological differences, such as sample preparation techniques and detection methods. Additionally, variations in cocoa components, bioactive compounds concentrations, and matrix effects should be considered by standardizing the results to the maximum value of the factors involved to ensure that the results are comparable, reproducible and reliably related to the actual in vivo conditions. Although the flavonoids are widely distributed in cocoa and its derived products their concentration in blood plasma after consumption is much lower compared to the administered dose, which is ascribed to its weak absorption. Still, there has been much interest in the recognition of their physiological mechanism of action and bioactivities *in vivo*. Thus, the accurate estimation of cocoa polyphenols and methylxanthines intake is of high importance in order to determine the bioavailability of these compounds and to be able to calculate these compounds doses that could be related to certain health effects. Therefore the health benefits of cocoa bioactive compounds are attributed to the additive and synergistic interactions of the phytochemicals present in cocoa and its co-products. Thus, the intake of TB and CF from cocoa and chocolate, which are rich source of a range of nutrients and other bioactive compounds including flavonoids, phenolic acids and their derivatives, as well as stilbenes, might also carry health benefits well beyond of those offered by methylxanthines alone.

Abbreviations

3,4-diHBA	protocatechuic acid
3-Me-4-HPA	3-metoxy-4-hydroxyphenylacetic acid
AMMU	6-amino-5(*N*-methylformylamino)-1-methyluracil
C	catechin
CA	caffeic acid
CF	caffeine
CHA	chlorogenic acid
COMT	catechol-*O*-methyltransferase
CuA	coumaric acid

EC	epicatechin
FA	ferulic acid
GA	gallic acid
HA	hippuric acid
HBA	hydroxybenzoic acid
HCA	hydroxycinnamic acid
HHA	hydroxyhippuric acid
HPAA	hydroxyphenylacetic acid
HPPA	hydroxyphenylpropionic acid
HPVA	hydroxyphenylvaleric acid
HP-γ-VL	hydroxylphenyl-γ-valerolactone
IFA	isoferulic acid
LPH	lactase-phlorizin hydrolase
MCT	monocarboxylic acid transporter
MU	methyluric acid
MX	methylxanthine
NPA	*N*-phenylpropenoyl-L-amino acid
PAA	phenylacetic acid
PC	procyanidin
PPA	phenylpropionic acid
PX	paraxanthine
ROS	reactive oxygen species
SGLT1	sodium-dependent glucose transporter 1
SULT	sulfotransferase
TB	theobromine
TP	theophylline
UGT	uridine-5′-diphosphate glucuronosyltransferase
VA	vanillic acid

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References

Actis-Goretta, L., A. Léveques, F. Giuffrida, F. Romanov-Michailidis, F. Viton, D. Barron, M. Duenas-Paton, S. Gonzalez-Manzano, C. Santos-Buelga, G. Williamson, and F. Dionisi. 2012. Elucidation of (−)-epicatechin metabolites after ingestion of chocolate by healthy humans. *Free Radical Biology and Medicine* 53 (4):787–95. doi: 10.1016/j.freeradbiomed.2012.05.023.

Actis-Goretta, L., A. Lèvéques, M. Rein, A. Teml, C. Schäfer, U. Hofmann, H. Li, M. Schwab, M. Eichelbaum, and G. Williamson. 2013. Intestinal absorption, metabolism, and excretion of (−)-epicatechin in healthy humans assessed by using an intestinal perfusion technique. *The American Journal of Clinical Nutrition* 98 (4):924–33. doi: 10.3945/ajcn.113.065789.

Adam, A., V. Crespy, M.-A. Levrat-Verny, F. Leenhardt, M. Leuillet, C. Demigné, and C. Rémésy. 2002. The bioavailability of ferulic acid is governed primarily by the food matri rather than its metabolism in intestine and liver in rats. *The Journal of Nutrition* 132 (7):1962–8. doi: 10.1093/jn/132.7.1962.

Ader, P., M. Blöck, S. Pietzsch, and S. Wolffram. 2001. Interaction of quercetin glucosides with the intestinal sodium/glucose co-transporter (SGLT-1). *Cancer Letters* 162 (2):175–80. doi: 10.1016/S0304-3835(00)00645-5.

Afoakwa, E. O., A. Paterson, M. Fowler, and A. Ryan. 2008. Flavor formation and character in cocoa and chocolate: A critical review. *Critical Reviews in Food Science and Nutrition* 48 (9):840–57. doi: 10.1080/10408390701719272.

Akkarachiyasit, S., P. Charoenlertkul, S. Yibchok-Anun, and S. Adisakwattana. 2010. Inhibitory activities of cyanidin and its glycosides and synergistic effect with acarbose against intestinal α-glucosidase and pancreatic α-amylase. *International Journal of Molecular Sciences* 11 (9):3387–96. doi: 10.3390/ijms11093387.

Andres-Lacueva, C., M. Monagas, N. Khan, M. Izquierdo-Pulido, M. Urpi-Sarda, J. Permanyer, and R. M. Lamuela-Raventtos. 2008. Flavanol and flavonol contents of cocoa powder products: Influence of manufacturing process. *Journal of Agricultural and Food Chemistry* 56 (9):3111–7. doi: 10.1021/jf0728754.

Appeldoorn, M. M., J. P. Vincken, A. M. Aura, P. C. Hollman, and H. Gruppen. 2009a. Procyanidin dimers are metabolized by human microbiota with 2-(3,4-dihydroxyphenyl)acetic acid and 5-(3,4-dihydroxyphenyl)-γ-valerolactone as the major metabolites. *Journal of Agricultural and Food Chemistry* 57 (3):1084–92. doi: 10.1021/jf803059z.

Appeldoorn, M. M., J. P. Vincken, H. Gruppen, and P. C. H. Hollman. 2009b. Procyanidin dimers A1, A2, and B2 are absorbed without conjugation or methylation from the small intestine of rats. *The Journal of Nutrition* 139 (8):1469–73. doi: 10.3945/jn.109.106765.

Aprotosoaie, A. C., S. V. Luca, and A. Miron. 2016. Flavor chemistry of cocoa and cocoa products – An overview. *Comprehensive Reviews in Food Science and Food Safety* 15 (1):73–91. doi: 10.1111/1541-4337.12180.

Aprotosoaie, A. C., A. Miron, A. Trifan, V. S. Luca, and I. I. Costache. 2016. The cardiovascular effects of cocoa polyphenols – An overview. *Diseases* 4 (4):39. doi: 10.3390/diseases4040039.

Arnaud, M. J. 2011. Pharmacokinetics and metabolism of natural methylxanthines in animal and man. In *Handbook of experimental pharmacology: Methylxanthines*, ed. B. B. Fredholm 1st ed., 33–91. New York, NY: Springer.

Aron, P. M., and J. A. Kennedy. 2008. Flavan-3-ols: Nature, occurrence and biological activity. *Molecular Nutrition & Food Research* 52 (1):79–104. doi: 10.1002/mnfr.200700137.

Baba, S., N. Osakabe, A. Yasuda, M. Natsume, T. Takizawa, T. Nakamura, and J. Terao. 2000. Bioavailability of (−)-epicatechin upon intake of chocolate and cocoa in human volunteers. *Free Radical Research* 33 (5):635–41. doi: 10.1080/10715760000301151.

Baba, S., N. Osakabe, M. Natsume, Y. Muto, T. Takizawa, and J. Terao. 2001. In vivo comparison of the bioavailability of (+)-catechin, (−)-epicatechin and their mixture in orally administered rats. *The Journal of Nutrition* 131 (11):2885–91. doi: 10.1093/jn/131.11.2885.

Badrie, N., F. Bekele, E. Sikora, and M. Sikora. 2015. Cocoa agronomy, quality, nutritional, and health aspects. *Critical Reviews in Food Science and Nutrition* 55 (5):620–59. doi: 10.1080/10408398.2012.669428.

Baggott, M. J., E. Childs, A. B. Hart, E. de Bruin, A. A. Palmer, J. E. Wilkinson, and H. de Wit. 2013. Psychopharmacology of theobromine in healthy volunteers. *Psychopharmacology* 228 (1):109–18. doi: 10.1007/s00213-013-3021-0.

Beg, M. S., S. Ahmad, K. Jan, and K. Bashir. 2017. Status, supply chain and processing of cocoa – A review. *Trends in Food Science and Technology* 66:108–16. doi: 10.1016/j.tifs.2017.06.007.

Belščak, A., D. Komes, D. Horzic, K. Kovačević Ganić, and D. Karlović. 2009. Comparative study of commercially available cocoa products in terms of their bioactive composition. *Food Research International* 42 (5–6):707–16. doi: 10.1016/j.foodres.2009.02.018.

Bernaert, H., I. Blondeel, L. Allegaert, and T. Lohmueller. 2012. Industrial treatment of cocoa in chocolate production: Health implications. In *Chocolate and health*, eds. R. Paoletti, A. Poli, A. Conti, and F. Visioli, 17–30. Berlin, Germany: Springer-Verlag.

Berry, N. M., K. Davison, A. M. Coates, J. D. Buckley, and P. R. Howe. 2010. Impact of cocoa flavanol consumption on blood pressure responsiveness to exercise. *British Journal of Nutrition* 103:1480–4. doi: 10.1017/S0007114509993382.

Bhat, K. P. L., J. W. Kosmeder, 2nd, and J. M. Pezzuto. 2001. Biological effects of resveratrol. *Antioxidants & Redox Signaling* 3 (6):1041–64. doi: 10.1089/152308601317203567.

Bode, L. M., D. Bunzel, M. Huch, G. S. Cho, D. Ruhland, M. Bunzel, A. Bub, C. M. Franz, and S. E. Kulling. 2013. In vivo and in vitro metabolism of trans-resveratrol by human gut microbiota. *The American Journal of Clinical Nutrition* 97 (2):295–309. doi: 10.3945/ajcn.112.049379.

Borges, G., J. J. van der Hooft, and A. Crozier. 2016. A comprehensive evaluation of the [2-^{14}C](−)-epicatechin metabolome in rats. *Free Radical Biology & Medicine* 99:128–38. doi: 10.1016/j.freeradbiomed.2016.08.001.

Borges, G., J. I. Ottaviani, J. J. van der Hooft, H. Schroeter, and A. Crozier. 2018. Absorption, metabolism, distribution and excretion of (−)-epicatechin: A review of recent findings. *Molecular Aspects of Medicine* 61:18–30. doi: 10.1016/j.mam.2017.11.002.

Boto-Ordonez, M., M. Urpi-Sarda, M. I. Queipo-Ortuno, S. Tulipani, F. J. Tinahones, and C. Andres-Lacueva. 2014. High levels of *Bifidobacteria* are associated with increased levels of anthocyanin microbial metabolites: A randomized clinical trial. *Food & Function* 5 (8):1932–8. doi: 10.1039/C4FO00029C.

Braga, A. R. C., D. C. Murador, L. M. de Souza Mesquita, and V. V. de Rosso. 2018. Bioavailability of anthocyanins: Gaps in knowledge, challenges and future research. *Journal of Food Composition and Analysis* 68:31–40. doi: 10.1016/j.jfca.2017.07.031.

Braune, A., and M. Blaut. 2016. Bacterial species involved in the conversion of dietary flavonoids in the human gut. *Gut Microbes* 7 (3):216–34. doi: 10.1080/19490976.2016.1158395.

Briz, M. R. M., B. S. Ruiz, and L. Bravo-Clemente. 2017. Methylxanthines: Dietary sources, bioavailability, and health benefits. In *Fruit and vegetable phytochemicals: Chemistry and human health*, ed. E. M. Yahia, 2nd ed., 183–97. Hoboken, NJ: John Wiley & Sons Ltd.

Brown, V. A., K. R. Patel, M. Viskaduraki, J. A. Crowell, M. Perloff, T. D. Booth, G. Vasilinin, A. Sen, A. M. Schinas, G. Piccirilli, et al. 2010. Repeat dose study of the cancer chemopreventive agent resveratrol in healthy volunteers: Safety, pharmacokinetics, and effect on the insulin-like growth factor axis. *Cancer Research* 70 (22):9003–11. doi: 10.1158/0008-5472.

Carbonell-Capella, J. M., M. Buniowska, F. J. Barba, M. J. Esteve, and A. Frígola. 2014. Analytical methods for determining bioavailability and bioaccessibility of bioactive compounds from fruits and vegetables: A review. *Comprehensive Reviews in Food Science and Food Safety* 13 (2):155–71. doi: 10.1111/1541–4337.12049.

Cardona, F., C. Andres-Lacueva, S. Tulipani, F. J. Tinahones, and M. I. Queipo-Ortuno. 2013. Benefits of polyphenols on gut microbiota and implications in human health. *Journal of Nutritional Biochemistry* 24 (8):1415–22. doi: 10.1016/j.jnutbio.2013.05.001.

Cassidy, A., and A. M. Minihane. 2017. The role of metabolism (and the microbiome) in defining the clinical efficacy of dietary flavonoids. *The American Journal of Clinical Nutrition* 105 (1):10–22. doi: 10.3945/ajcn.116.136051.

Castell, M., F. J. Pérez-Cano, and J. F. Bisson. 2013. Clinical benefits of cocoa: An overview. In *Chocolate in health and nutrition*, eds. R. Watson, V. R. Preedy, and S. Zibadi, 265–76. New York, Heidelberg, Dordrecht London: Humana Press.

Castello, F., G. Costabile, L. Bresciani, M. Tassotti, D. Naviglio, D. Luongo, P. Ciciola, M. Vitale, C. Vetrani, G. Galaverna., et al. 2018. Bioavailability and pharmacokinetic profile of grape pomace phenolic compounds in humans. *Archives of Biochemistry and Biophysics* 646:1–9. doi: 10.1016/j.abb.2018.03.021.

Chachay, V. S., C. M. J. Kirkpatrick, I. J. Hickman, M. Ferguson, J. B. Prins, and J. H. Martin. 2011. Resveratrol – Pills to replace a healthy diet? *British Journal of Clinical Pharmacology* 72 (1):27–38. doi: 10.1111/j.1365-2125.2011.03966.x.

Cifuentes-Gomez, T., A. Rodriguez-Mateos, I. Gonzalez-Salvador, M. E. Alañon, and J. P. Spencer. 2015. Factors affecting the absorption, metabolism, and excretion of cocoa flavanols in humans. *Journal of Agricultural and Food Chemistry* 63 (35):7615–23. doi: 10.1021/acs.jafc.5b00443.

Cooper, K. A., J. L. Donovan, A. L. Waterhouse, and G. Williamson. 2008. Cocoa and health: A decade of research. *British Journal of Nutrition* 99 (1):1–11. doi: 10.1017/S0007114507795296.

Counet, C., D. Callemien, and S. Collin. 2006. Chocolate and cocoa: New sources of trans-resveratrol and trans-piceid. *Food Chemistry* 98 (4):649–57. doi: 10.1016/j.foodchem.2005.06.030.

Crozier, A., D. Del Rio, and M. N. Clifford. 2010. Bioavailability of dietary flavonoids and phenolic compounds. *Molecular Aspects of Medicine* 31 (6):446–67. doi: 10.1016/j.mam.2010.09.007.

Crozier, A. 2013. Absorption, metabolism, and excretion of (–)-epicatechin in humans: An evaluation of recent findings. *The American Journal of Clinical Nutrition* 98 (4):861–2. doi: 10.3945/ajcn.113.072009.

Czank, C., A. Cassidy, Q. Zhang, D. J. Morrison, T. Preston, P. A. Kroon, N. P. Botting, and C. D. Kay. 2013. Human metabolism and elimination of the anthocyanin, cyanidin-3-glucoside: A ^{13}C-tracer study. *The American Journal of Clinical Nutrition* 97 (5):995–1003. doi: 10.3945/ajcn.112.049247.

Day, A. J., F. Mellon, D. Barron, G. Sarrazin, M. R. Morgan, and G. Williamson. 2001. Human metabolism of dietary flavonoids: Identification of plasma metabolites of quercetin. *Free Radical Research* 35 (6):941–52. doi: 10.1080/10715760100301441.

Day, A. J., J. M. Gee, M. S. DuPont, I. T. Johnson, and G. Williamson. 2003. Absorption of quercetin-3-glucoside and quercetin-4′-glucoside in the rat small intestine: The role of lactase phlorizin hydrolase and the sodium-dependent glucose transporter. *Biochemical Pharmacology* 65 (7):1199–206. doi: 10.1016/S0006-2952(03)00039-X.

D'Archivio, M., C. Filesi, R. Varì, B. Scazzocchio, and R. Masella. 2010. Bioavailability of the polyphenols: Status and controversies. *International Journal of Molecular Sciences* 11 (4):1321–42. doi: 10.3390/ijms11041321.

De Araujo, Q. R., J. N. Gattward, S. Almoosawi, Md Silva, P. A. 2. Dantas, and Q. R. De Araujo Júnior. 2016. Cocoa and human health: From head to foot – A review. *Critical Reviews in Food Science and Nutrition* 56 (1):1–12. doi: 10.1080/10408398.2012.657921.

Del Rio, D., A. Rodriguez-Mateos, J. P. Spencer, M. Tognolini, G. Borges, and A. Crozier. 2013. Dietary (poly)phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxidants & Redox Signaling* 18 (14):1818–92. doi: 10.1089/ars.2012.4581.

Delmas, D., B. Jannin, and N. Latruffe. 2005. Resveratrol: Preventing properties against vascular alterations and ageing. *Molecular Nutrition & Food Research* 49 (5):377–95. doi: 10.1002/mnfr.200400098.

Deprez, S., I. Mila, J. F. Huneau, D. Tome, and A. Scalbert. 2001. Transport of proanthocyanidin dimer, trimer, and polymer across monolayers of human intestinal epithelial caco-2 cells. *Antioxidants & Redox Signaling* 2001 (3):957–67. doi: 10.1089/152308601317203503.

Donovan, J. L., V. Crespy, M. Oliveira, K. A. Cooper, B. B. Gibson, and G. Williamson. 2006. (+)-catechin is more bioavailable than (−)-catechin: Relevance to the bioavailability of catechin from cocoa. *Free Radical Research* 40 (10):1029–34. doi: 10.1080/10715760600868545.

Dorenkott, M. R., L. E. Griffin, K. M. Goodrich, K. A. Thompson-Witrick, G. Fundaro, L. Ye, J. R. Stevens, M. Ali, S. F. O'Keefe, M. W. Hulver, and A. P. Neilson. 2014. Oligomeric cocoa procyanidins possess enhanced bioactivity compared to monomeric and polymeric cocoa procyanidins for preventing the development of obesity, insulin resistance, and impaired glucose tolerance during high-fat feeding. *Journal of Agricultural and Food Chemistry* 62 (10):2216–27. doi: 10.1021/jf500333y.

Edwards, C. A., J. Havlik, W. Cong, W. Mullen, T. Preston, D. J. Morrison, and E. Combet. 2017. Polyphenols and health: Interactions between fibre, plant polyphenols and the gut microbiota. *Nutrition Bulletin* 2 (4):356–60. doi: 10.1111/nbu.12296.

Ellam, S., and G. Williamson. 2013. Cocoa and human health. *Annual Review of Nutrition* 33 (1):105–28. doi: 10.1146/annurev-nutr-071811-150642.

Elwers, S., A. Zambrano, C. Rohsius, and R. Lieberei. 2009. Differences between the content of phenolic compounds in criollo, forastero and trinitario cocoa seed (*Theobroma cacao* L.). *European Food Research and Technology* 229 (6):937–48. doi: 10.1007/s00217-009-1132-y.

Engemann, A., F. Hubner, S. Rzeppa, and H. U. Humpf. 2012. Intestinal metabolism of two A-type procyanidins using the pig

cecum model: Detailed structure elucidation of unknown catabolites with Fourier transform mass spectrometry (FTMS). *Journal of Agricultural and Food Chemistry* 60 (3):749–57. doi: 10.1021/jf203927g.

Etcheverry, P., M. A. Grusak, and L. E. Fleige. 2012. Application of in vitro bioaccessibility and bioavailability methods for calcium, carotenoids, folate, iron, magnesium, polyphenols, zinc, and vitamins B6, B12, D, and E. *Frontiers in Physiology* 3:317. doi: 10.3389/fphys.2012.00317.

Etxeberria, U., A. Fernandez-Quintela, F. I. Milagro, L. Aguirre, J. A. Martinez, and M. P. Portillo. 2013. Impact of polyphenols and polyphenol-rich dietary sources on gut microbiota composition. *Journal of Agricultural and Food Chemistry* 61 (40):9517–33. doi: 10.1021/jf402506c.

Fang, J. 2014. Bioavailability of anthocyanins. *Drug Metabolism Reviews* 46 (4):508–20. doi: 10.3109/03602532.2014.978080.

Faria, A., D. Pestana, D. Teixeira, P. O. Couraud, I. Romero, B. Weksler, V. de Freitas, N. Mateus, and C. Calhau. 2011. Insights into the putative catechin and epicatechin transport across blood-brain barrier. *Food & Function* 2 (1):39–44. doi: 10.1039/C0FO00100G.

Faria, A., I. Fernandes, S. Norberto, N. Mateus, and C. Calhau. 2014. Interplay between anthocyanins and gut microbiota. *Journal of Agricultural and Food Chemistry* 62 (29):6898–902. doi: 10.1021/jf501808a.

Felgines, C., S. Talavéra, M.-P. Gonthier, O. Texier, A. Scalbert, J.-L. Lamaison, and C. Rémésy. 2003. Strawberry anthocyanins are recovered in urine as glucuro- and sulfoconjugates in humans. *The Journal of Nutrition* 133 (5):1296–301. doi: 10.1093/jn/133.5.1296.

Fernandez, K., and J. Labra. 2013. Simulated digestion of proantho-cyanidins in grape skin and seed extracts and the effects of diges-tion on the angiotensin I-converting enzyme (ACE) inhibitoryactivity. *Food Chemistry* 139:196–202. doi: 10.1016/j.foodchem.2013.01.021.

Fernández-García, E., I. Carvajal-Lérida, and A. Pérez-Gálvez. 2009. In vitro bioaccessibility assessment as a prediction tool of nutritional efficiency. *Nutrition Research* 29 (11):751–60. doi: 10.1016/j.nutres.2009.09.016.

Ferri, C., G. Desideri, L. Ferri, I. Proietti, S. Di Agostino, L. Martella, F. Mai, P. Di Giosia, and D. Grassi. 2015. Cocoa, blood pressure, and cardiovascular health. *Journal of Agricultural and Food Chemistry* 63 (45):9901–9. doi: 10.1021/acs.jafc.5b01064.

Fulda, S. 2010. Resveratrol and derivatives for the prevention and treatment of cancer. *Drug Discovery Today* 15 (17–18):757–65. doi: 10.1016/j.drudis.2010.07.005.

Fogliano, V., M. L. Corollaro, P. Vitaglione, A. Napolitano, R. Ferracane, F. Travaglia, M. Arlorio, A. Costabile, A. Klinder, and G. Gibson. 2011. In vitro bioaccessibility and gut biotransformation of polyphenols present in the water-insoluble cocoa fraction. *Molecular Nutrition & Food Research* 55 (S1):S44–S55. doi: 10.1002/mnfr.201000360.

Franco, R., A. Oñatibia-Astibia, and E. Martínez-Pinilla. 2013. Health benefits of methylxanthines in cacao and chocolate. *Nutrients* 5 (10):4159–73. doi: 10.3390/nu5104159.

Gallo, M., G. Vinci, G. Graziani, C. De Simone, and P. Ferranti. 2013. The interaction of cocoa polyphenols with milk proteins studied by proteomic techniques. *Food Research International* 54 (1):406–15. doi: 10.1016/j.foodres.2013.07.011.

Gardea, A. A., M. L. García-Bañuelos, J. A. Orozco-Avitia, E. Sánchez-Chávez, B. Sastré-Flores, and G. Ávila-Quezada. 2017. Cacao (*Theobroma cacao* L.). In *Fruit and vegetable phytochemicals: Chemistry and human health*, ed. E. M. Yahia, 2nd ed., 921–40. Hoboken, NJ: John Wiley & Sons Ltd.

Gonthier, M.-P., M.-A. Verny, C. Besson, C. Rémésy, and A. Scalbert, 2003. Chlorogenic acid bioavailability largely depends on its metabolism by the gut microflora in rats. *The Journal of Nutrition* 133 (6):1853–9. doi: 10.1093/jn/133.6.1853.

Goodrich, K. M., M. R. Dorenkott, L. Ye, S. F. O'Keefe, M. W. Hulver, and A. P. Neilson. 2014. Dietary supplementation with cocoa flavanols does not alter Colon tissue profiles of native flavanols and their microbial metabolites established during habitual dietary exposure in C57BL/6J mice. *Journal of Agricultural and Food Chemistry* 62 (46):11190–9. doi: 10.1021/jf503838q.

Garcia-Aloy, M., R. Llorach, M. Urpi-Sarda, O. Jáuregui, D. Corella, M. Ruiz-Canela, J. Salas-Salvadó, M. Fitó, E. Ros, R. Estruch, and C. Andres-Lacueva. 2015. A metabolomics-driven approach to predict cocoa product consumption by designing a multimetabolite biomarker model in free-living subjects from the PREDIMED study. *Molecular Nutrition & Food Research* 59 (2):212–20. doi: 10.1002/mnfr.201400434.

Grassi, D., G. Desideri, and C. Ferri. 2010. Blood pressure and cardiovascular risk: What about cocoa and chocolate? *Archives of Biochemistry and Biophysics* 501 (1):112–5. doi: 10.1016/j.abb.2010.05.020.

Han, S. J., S. N. Ryu, H. T. Trinh, E. H. Joh, S. Y. Jang, M. J. Han, and D. H. Kim. 2009. Metabolism of cyanidin-3-*O*-beta-D-glucoside isolated from black colored rice and its antiscratching behavioral effect in mice. *Journal of Food Science* 74:253–8. doi: 10.1111/j.1750-3841.2009.01327.x.

Heleno, S. A., A. Martins, M. J. R. P. Queiroz, and I. C. F. R. Ferreira. 2015. Bioactivity of phenolic acids: Metabolites versus parent compounds: A review. *Food Chemistry* 173:501–13. doi: 10.1016/j.foodchem.2014.10.057.

Hollman, P. C. H., J. M. P. van Trijp, M. N. C. P. Buysman, M. S. V.D. Gaag, M. J. B. Mengelers, J. H. M. de Vries, and M. B. Katan. 1997. Relative bioavailability of the antioxidant flavonoid quercetin from various foods in man. *FEBS Letters* 418 (1–2):152–6. doi: 10.1016/S0014-5793(97)01367-7.

Holst, B., and G. Williamson. 2008. Nutrients and phytochemicals: From bioavailability to bioefficacy beyond antioxidants. *Current Opinion in Biotechnology* 19 (2):73–82. doi: 10.1016/j.copbio.2008.03.003.

Holt, R. R., S. A. Lazarus, M. C. Sullards, Q. Y. Zhu, D. D. Schramm, J. F. Hammerstone, C. G. Fraga, H. H. Schmitz, and C. L. Keen. 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. doi: 10.1093/ajcn/76.4.798.

Hodgson, J. M., L. W. Morton, I. B. Puddey, L. J. Beilin, and K. D. Croft. 2000. Gallic acid metabolites are markers of black tea intake in humans. *Journal of Agricultural and Food Chemistry* 48 (6):2276–80. doi: 10.1021/jf000089s.

Hurst, W. J., J. A. Glinski, K. B. Miller, J. Apgar, M. H. Davey, and D. A. Stuart. 2008. Survey of the trans-resveratrol and trans-piceid content of cocoa-containing and chocolate products. *Journal of Agricultural and Food Chemistry* 56 (18):8374–8. doi: 10.1021/jf801297w.

Jang, S., J. Sun, P. Chen, S. Lakshman, A. Molokin, J. M. Harnly, B. T. Vinyard, J. F. Urban, Jr., and C. D. Davis, G. Solano-Aguilar. 2016. Flavanol-enriched cocoa powder alters the intestinal microbiota, tissue and fluid metabolite profiles, and intestinal gene expression in pigs. *The Journal of Nutrition* 146 (4):673–80. doi: 10.3945/jn.115.222968.

Jailani, F., and G. Williamson. 2014. Effect of edible oils on quercetin, kaempferol and galangin transport and conjugation in the intestinal Caco-2/HT29-MTX co-culture model. *Food & Function* 5 (4):653–62. doi: 10.1039/c3fo60691k.

Jalil, A. M., and A. Ismail. 2008. Polyphenols in cocoa and cocoa products: Is there a link between antioxidant properties and health? *Molecules* 13 (9):2190–219. molecules. doi: 10.3390/molecules13092190.

Jamar, G., D. Estadella, and L. P. Pisani. 2017. Contribution of anthocyanin-rich foods in obesity control through gut microbiota interactions. *Biofactors* 43 (4):507–16. doi: 10.1002/biof.1365.

Jerkovic, V., M. Bröhan, E. Monnart, F. Nguyen, S. Nizet, and S. Collin. 2010. Stilbenic profile of cocoa liquors from different origins determined by RP-HPLC-APCI(+)-MS/MS. Detection of a new resveratrol hexoside. *Journal of Agricultural and Food Chemistry* 58 (11):7067–74. doi: 10.1021/jf101114c.

Kaliora, A. C., P. T. Kanellos, and N. Kalogeropoulos. 2013. Gallic acid bioavailability in humans. In *Handbook on gallic acid*, eds. M. A.

Thompson and P. B. Collins, 301–12. Hauppauge, NY: Nova Science Publishers, Inc.

Kaulmann, A., and T. Bohn. 2016. Bioactivity of polyphenols: Preventive and adjuvant strategies toward reducing inflammatory bowel diseases-promises, perspectives, and pitfalls. *Oxidative Medicine and Cellular Longevity* 2016:9346470. doi: 10.1155/2016/9346470.

Karakaya, S. 2004. Bioavailability of phenolic compounds. *Critical Reviews in Food Science and Nutrition* 44 (6):453–64. doi: 10.1080/10408690490886683.

Kay, C. D., G. Mazza, B. J. Holub, and J. Wang. 2004. Anthocyanin metabolites in human urine and serum. *British Journal of Nutrition* 91 (6):933–42. doi: 10.1079/BJN20041126.

Keogh, J. B., J. McInerney, and P. M. Clifton. 2007. The effect of milk protein on the bioavailability of cocoa polyphenols. *Journal of Food Science* 72 (3):S230–S233. doi: 10.1111/j.1750-3841.2007.00314.x.

Kern, S. M., N. R. Bennett, P. W. Needs, F. A. Mellon, P. A. Kroon, and M. T. Garcia-Conesa. 2003. Characterization of metabolites of hydroxycinnamates in the *in vitro* model of human small intestinal epithelium caco-2 cells. *Journal of Agricultural and Food Chemistry* 51 (27):7884–91. doi: 10.1021/jf030470n.

Khoo, C., and M. Falk. 2014. Cranberry polyphenols: Effects on cardiovascular risk factors. In *Polyphenols in human health and disease*, eds. R. R. Watson, V. R. Preedy and S. Zibadi, 1049–65. Cambridge, MA: Academic Press.

Kofink, M., M. Papagiannopoulos, and R. Galensa. 2007. (−)-Catechin in cocoa and chocolate: Occurence and analysis of an atypical flavan-3-ol enantiomer. *Molecules* 12 (7):1274–88. doi: 10.3390/12071274.

Kongor, J. E., M. Hinneh, D. V. de Walle, E. O. Afoakwa, P. Boeckx, and K. Dewettinck. 2016. Factors influencing quality variation in cocoa (*Theobroma cacao*) bean flavor profile – A review. *Food Research International* 82:44–52. doi: 10.1016/j.foodres.2016.01.012.

Konishi, Y., Z. Zhao, and M. Shimizu. 2006. Phenolic acids are absorbed from the rat stomach with different absorption rates. Journal *of Agricultural and Food Chemistry* 54 (20):7539–43. doi: 10.1021/jf061554+.

Kothe, L., B. F. Zimmermann, and R. Galensa. 2013. Temperature influences epimerization and composition of flavanol monomers, dimers and trimers during cocoa bean roasting. *Food Chemistry* 141 (4):3656–63. doi: 10.1016/j.foodchem.2013.06.049.

Kris-Etherton, P. M., and C. L. Keen. 2002. Evidence that the antioxidant flavonoids in tea and cocoa are beneficial for cardiovascular health. *Current Opinion in Lipidology* 13 (1):41–9. doi: 10.1097/00041433-200202000-00007.

la Porte, C., N. Voduc, G. Zhang, I. Seguin, D. Tardiff, N. Singhal, and D. W. Cameron. 2010. Steady-State pharmacokinetics and tolerability of trans-resveratrol 2000 mg twice daily with food, quercetin and alcohol (ethanol) in healthy human subjects. *Clinical Pharmacokinetics* 49 (7):449–54. doi: 10.2165/11531820-000000000-00000.

Labib, S., A. Erb, M. Kraus, T. Wickert, and E. Richling. 2004. The pig caecum model: A suitable tool to study the intestinal metabolism of flavonoids. Molecular *Nutrition* & *Food Research* 48 (4):326–32. doi: 10.1002/mnfr.200400022.

Lafay, S., and A. Gil-Izquierdo. 2008. Bioavailability of phenolic acids. Phytochemistry *Reviews* 7 (2):301–11. doi: 10.1007/s11101-007-9077-x.

Larrosa, M., C. Luceri, E. Vivoli, C. Pagliuca, M. Lodovici, G. Moneti, and P. Dolara. 2009. Polyphenol metabolites from colonic microbiota exert antiinflammatory activity on different inflammation models. Molecular *Nutrition* & *Food Research* 53 (8):1044–54. doi: 10.1002/mnfr.200800446.

Latif, R. 2013. Health benefits of cocoa. *Current Opinion in Clinical Nutrition & Metabolic Care* 16 (6):669–74. doi: 10.1097/MCO.0b013e328365a235.

Lechtenberg, M., K. Henschel, U. Liefländer-Wulf, B. Quandt, and A. Hensel. 2012. Fast determination of *N*-phenylpropenoyl-L-amino acids (NPA) in cocoa samples from different origins by ultra-performance liquid chromatography and capillary electrophoresis. *Food Chemistry* 135 (3):1676–84. doi: 10.1016/j.foodchem.2012.06.006.

Lecumberri, E., R. Mateos, M. Izquierdo-Pulido, P. Rupérez, L. Goya, and L. Bravo. 2007. Dietary fibre composition, antioxidant capacity and physico-chemical properties of a fibre-rich product from cocoa (*Theobroma cacao* L.). *Food Chemistry* 104 (3):948–54. doi: 10.1016/j.foodchem.2006.12.054.

Lima, L. J. R., M. H. Almeida, M. J. R. Nout, and M. H. Zwietering. 2011. *Theobroma cacao* L., "the food of the gods": Quality determinants of commercial cocoa beans, with particular reference to the impact of fermentation. *Critical Reviews in Food Science and Nutrition* 51 (8):731–61. doi: 10.1080/10408391003799913.

Llorach, R., M. Urpi-Sarda, O. Jauregui, M. Monagas, and C. Andres-Lacueva. 2009. An LC-MS-based metabolomics approach for exploring urinary metabolome modifications after cocoa consumption. Journal *of Proteome Research* 8 (11):5060–8. doi: 10.1021/pr900470a.

Llorach-Asunción, R., O. Jauregui, M. Urpi-Sardaa, and C. Andres-Lacueva. 2010. Methodological aspects for metabolome visualization and characterization. A metabolomic evaluation of the 24 h evolution of human urine after cocoa powder consumption. *Journal of Pharmaceutical and Biomedical Analysis* 51 (2):373–81. doi: 10.1016/j.jpba.2009.06.033.

Lau-Cam, C. A. 2013. The absorption, metabolism, and pharmacokinetics of chocolate polyphenols, in chocolate. In *Health and nutrition*, eds. R. R. Watson, V. R. Preedy, and S. Zibadi, 201–46. Totowa, NJ: Humana Press.

Maier-Salamon, A., M. Bohmdorfer, T. Thalhammer, T. Szekeres, and W. Jaeger. 2011. Hepatic glucuronidation of resveratrol: Interspecies comparison of enzyme kinetic profiles in human, mouse, rat, and dog. *Drug Metabolism and Pharmacokinetics* 26 (4):364–73. doi: 10.2133/dmpk.DMPK-11-RG-006.

Madyastha, K. M., and G. R. Sridhar. 1998. A novel pathway for the metabolism of caffeine by a mixed culture consortium. *Biochemical and Biophysical Research Communications* 249 (1):178–81. doi: 10.1006/bbrc.1998.9102.

Mandel, H. G. 2002. Update on caffeine consumption, disposition and action. *Food and Chemical Toxicology* 40 (9):1231–4. doi: 10.1016/S0278-6915(02)00093-5.

Marques, C., I. Fernandes, S. Norberto, C. Sá, D. Teixeira, V. de Freitas, N. Mateus, C. Calhau, and A. Faria. 2016. Pharmacokinetics of blackberry anthocyanins consumed with or without ethanol: A randomized and crossover trial. Molecular *Nutrition* & *Food Research* 60 (11):2319–30. doi: 10.1002/mnfr.201600143.

Martínez-López, S., B. Sarriá, M. Gómez-Juaristi, L. Goya, R. Mateos, and L. Bravo-Clemente. 2014. Theobromine, caffeine, and theophylline metabolites in human plasma and urine after consumption of soluble cocoa products with different methylxanthine contents. *Food Research International* 63:446–55. doi: 10.1016/j.foodres.2014.03.009.

Massot-Cladera, M., A. Costabile, C. E. Childs, P. Yaqoob, A. Franch, M. Castell, and F. J. Perez-Cano. 2015. Prebiotic effects of cocoa fibre on rats. *Journal of Functional Foods* 19:341–52. doi: 10.1016/j.jff.2015.09.021.

Mena, P., I. A. Ludwig, V. B. Tomatis, A. Acharjee, L. Calani, A. Rosi, F. Brighenti, S. Ray, J. L. Griffin, L. J. Bluck, and D. Del Rio. 2018. Inter-individual variability in the production of flavan-3-ol colonic metabolites: Preliminary elucidation of urinary metabotypes. *European Journal of Nutrition*. doi: 10.1007/s00394-018-1683-4.

Mena, P., L. Bresciani, N. Brindani, I. A. Ludwig, G. Pereira-Caro, D. Angelino, R. Llorach, L. Calani, F. Brighenti, M. N. Clifford, et al. 2019. Phenyl-γ-valerolactones and phenylvaleric acids, the main colonic metabolites of flavan-3-ols: Synthesis, analysis, bioavailability, and bioactivity. *Natural Product Reports*. doi: 10.1039/c8np00062j.

Mennen, L. I., D. Sapinho, H. Ito, S. Bertrais, P. Galan, S. Hercberg, and A. Scalbert. 2006. Urinary flavonoids and phenolic acids as biomarkers of intake for polyphenol-rich foods. *British Journal of Nutrition* 96 (1):191–8. doi: 10.1079/BJN20061808.

Milenkovic, D., C. Morand, A. Cassidy, A. Konic-Ristic, F. Tomás-Barberán, J. M. Ordovas, P. Kroon, R. De Caterina, and A. Rodriguez-Mateos. 2017. Interindividual variability in biomarkers of cardiometabolic health after consumption of major Plant-Food

bioactive compounds and the determinants involved. Advances *in Nutrition* (*Bethesda*, Md.) 8 (4):558–70. doi: 10.3945/an.116.013623.

Minekus, M. 2015. The TNO Gastro-Intestinal Model (TIM). In *The impact of food bioactives on health*, eds. K. Verhoeckx, P. Cotter, I. López-Expósito, C. Kleiveland, T. L. A. Mackie, T. Requena, D. Swiatecka, and H. Wichers, 37–46. Cham, Heidelberg, New York, Dordrecht, London: Springer.

Mitchell, L. E. S., M. Slettenaar, N. Vd Meer, C. Transler, L. Jans, F. Quadt, and M. Berry. 2011. Differential contributions of theobromine and caffeine on mood, psychomotor performance and blood pressure. *Physiology & Behavior* 104 (5):816–22. doi: 10.1016/j.physbeh.2011.07.027.

Mojzer, E. B., M. K. Hrnčič, M. Škerget, Ž. Knez, and U. Bren. 2016. Polyphenols: Extraction methods, antioxidative action, bioavailability and anticarcinogenic effects. *Molecules* 21 (7):901–39. doi: 10.3390/molecules21070901.

Monagas, M., M. Urpi-Sarda, F. Sánchez-Patán, R. Llorach, I. Garrido, C. Gómez-Cordovés, C. Andres-Lacueva, and B. Bartolomé. 2010. Insights into the metabolism and microbial biotransformation of dietary flavan-3-ols and the bioactivity of their metabolites. *Food & Function* 1 (3):233–53. doi: 10.1039/c0fo00132e.

Monteiro, J., M. Alves, P. Oliveira, and B. Silva. 2016. Structure-bioactivity relationships of methylxanthines: Trying to make sense of all the promises and the drawbacks. *Molecules* 21 (8):974. doi: 10.3390/molecules21080974.

Morais, C. A., V. V. de Rosso, D. Estadella, and L. P. Pisani. 2016. Anthocyanins as inflammatory modulators and the role of the gut microbiota. *Journal of Nutritional Biochemistry* 33:1–7. doi: 10.1016/j.jnutbio.2015.11.008.

Mullen, W., G. Borges, J. L. Donovan, C. A. Edwards, M. Serafini, M. E. Lean, and A. Crozier. 2009. Milk decreases urinary excretion but not plasma pharmacokinetics of cocoa flavan-3-ol metabolites in humans. *The American Journal of Clinical Nutrition* 89 (6):1784–91. doi: 10.1007/s002280050205.

Mumford, G. K., N. L. Benowitz, S. M. Evans, B. J. Kaminski, K. L. Preston, C. A. Sannerud, K. Silverman, and R. R. Griffiths. 1996. Absorption rate of methylxanthines following capsules, cola and chocolate. *European Journal of Clinical Pharmacology* 51 (3–4):319–25. doi: 10.1007/s002280050205.

Murota, K., Y. Nakamura, and M. Uehara. 2018. Flavonoid metabolism: The interaction of metabolites and gut microbiota. *Bioscience, Biotechnology, and Biochemistry* 82 (4):600–10. doi: 10.1080/09168451.2018.1444467.

Nardini, M., F. Natella, C. Scaccini, and A. Ghiselli. 2006. Phenolic acids from beer are absorbed and extensively metabolized in humans. *Journal of Nutritional Biochemistry* 17 (1):14–22. doi: 10.1016/j.jnutbio.2005.03.026.

Nehlig, A. 2013. The neuroprotective effects of cocoa flavanol and its influence on cognitive performance. British *Journal of Clinical Pharmacology* 75 (3):716–27. doi: 10.1111/j.1365-2125.2012.04378.x.

Neilson, A. P., J. C. George, E. M. Janle, R. D. Mattes, R. Rudolph, N. V. Matusheski, and M. G. Ferruzzi. 2009. Influence of chocolate matrix composition on cocoa flavan-3-ol bioaccessibility in vitro and bioavailability in humans. Journal *of Agricultural and Food Chemistry* 57 (20):9418–26. doi: 10.1021/jf902919k.

Neilson, A. P., and M. G. Ferruzzi. 2011. Influence of formulation and processing on absorption and metabolism of flavan-3-ols from tea and cocoa. Annual *Review of Food Science and Technology* 2 (1):125–51. doi: 10.1146/annurev-food-022510-133725.

Nemeth, K., G. W. Plumb, J. G. Berrin, N. Juge, R. Jacob, H. Y. Naim, G. Williamson, D. M. Swallow, and P. A. Kroon. 2003. Deglycosylation by small intestinal epithelial cell beta-glucosidases is a critical step in the absorption and metabolism of dietary flavonoid glycosides in humans. *European Journal of Nutrition* 42 (1):29–42. doi: 10.1007/s00394-003-0397-3.

Oleaga, C., C. J. Ciudad, M. Izquierdo-Pulid, and V. Noé. 2013. Cocoa flavanol metabolites activate HNF-3β, Sp1, and NFY-mediated transcription of apolipoprotein AI in human cells. Molecular *Nutrition & Food Research* 57 (6):986–95. doi: 10.1002/mnfr.201200507.

Olivas-Aguirre, F. J., J. Rodrigo-García, N. D. Martínez-Ruiz, A. I. Cárdenas-Robles, S. O. Mendoza-Díaz, E. Álvarez-Parrilla, G. A. González-Aguilar, L. A. de la Rosa, A. Ramos-Jiménez, and A. Wall-Medrano. 2016. Cyanidin-3-*O*-glucoside: Physical-chemistry, foodomics and health effects. *Molecules* 21 (9):1264. doi: 10.3390/molecules21091264.

Olthof, M. R., P. C. Hollman, T. B. Vree, and M. B. Katan. 2000. Bioavailabilities of quercetin-3-glucoside and quercetin-4′-glucoside do not differ in humans. *The Journal of Nutrition* 130 (5):1200–3. doi: 10.1093/jn/130.5.1200.

Ortega, N., M.-P. Romero, A. Macià, J. Reguant, N. Anglès, J.-R. Morelló, and M.-J. Motilva. 2008. Obtention and characterization of phenolic extracts from different cocoa sources. Journal *of Agricultural and Food Chemistry* 56 (20):9621–7. doi: 10.1021/jf8014415.90/molecules21091264.

Oracz, J., E. Nebesny, and D. Żyżelewicz. 2015. Changes in the flavan-3-ols, anthocyanins, and flavanols composition of cocoa beans of different *Theobroma cacao* L. groups affected by roasting conditions. European *Food Research and Technology* 241 (5):663–81. doi: 10.1007/s00217-015-2494-y.

Oracz, J., D. Zyzelewicz, and E. Nebesny. 2015. The content of polyphenolic compounds in cocoa beans (*Theobroma cacao* L.), depending on variety, growing region, and processing operations: A review. *Critical Reviews in Food Science and Nutrition* 55 (9):1176–92. doi: 10.1080/10408398.2012.686934.

Ostertag, L. M., P. A. Kroon, S. Wood, G. W. Horgan, E. Cienfuegos-Jovellanos, S. Saha, G. G. Duthie, and B. de Roos. 2013. Flavan-3-ol-enriched dark chocolate and white chocolate improve acute measures of platelet function in a gender-specific way – A randomized-controlled human intervention trial. Molecular *Nutrition & Food Research* 57 (2):191–202. doi: 10.1002/mnfr.201200283.

Ottaviani, J. I., T. Y. Momma, C. Heiss, C. Kwik-Uribe, H. Schroeter, and C. L. Keen. 2011. The stereochemical configuration of flavanols influences the level and metabolism of flavanols in humans and their biological activity *in vivo*. *Free Radical Biology and Medicine* 50 (2):237–44. doi: 10.1016/j.freeradbiomed.2010.11.005.

Ottaviani, J. I., T. Y. Momma, G. K. Kuhnle, C. L. Keen, and H. Schroeter. 2012a. Structurally related (−)-epicatechin metabolites in humans: Assessment using de novo chemically synthesized authentic standards. *Free Radical Biology & Medicine* 52 (8):1403–12. doi: 10.1016/j.freeradbiomed.2011.12.010.

Ottaviani, J. I., C. Kwik-Uribe, C. L. Keen, and H. Schroeter. 2012b. Intake of dietary procyanidins does not contribute to the Pool of circulation flavanols in humans. *The American Journal of Clinical Nutrition* 95 (4):851–8. doi: 10.3945/ajcn.111.028340.

Ottaviani, J. I., G. Borges, T. Y. Momma, J. P. Spencer, C. L. Keen, A. Crozier, and H. Schroeter. 2016. The metabolome of [2-^{14}C](−)-epicatechin in humans: Implications for the assessment of efficacy, safety, and mechanisms of action of polyphenolic bioactives. *Scientific Reports* 6:29034. doi: 10.1038/srep29034.

Ottaviani, J. I., C. Heiss, J. P. E. Spencer, M. Kelm, and H. Schroeter. 2018. Recommending flavanols and procyanidins for cardiovascular health: Revisited. *Molecular Aspects of Medicine* 61:63–75. doi: 10.1016/j.mam.2018.02.001.

Ou, K., S. S. Percival, T. Zou, C. Khoo, and L. Gu. 2012. Transport of cranberry A-type procyanidin dimers, trimers, and tetramers across monolayers of human intestinal epithelial caco-2 cells. Journal *of Agricultural and Food Chemistry* 60 (6):1390–6. doi: 10.1021/jf2040912.

Ou, K., P. Sarnoski, K. R. Schneider, K. Song, C. Khoo, and L. Gu. 2014. Microbial catabolism of procyanidins by human gut microbiota. Molecular *Nutrition & Food Research* 58 (11):2196–205. doi: 10.1002/mnfr.201400243.

Ozdal, T., D. Sela, J. Xiao, D. Boyacioglu, F. Chen, and E. Capanoglu. 2016. The reciprocal interactions between polyphenols and gut microbiota and effects on bioaccessibility. *Nutrients* 8 (2):78. doi: 10.3390/nu8020078.

Quiroz-Reyes, C. N., and V. Fogliano. 2018. Design cocoa processing towards healthy cocoa products: The role of phenolics and

melanoidins. *Journal of Functional Foods* 45:480–90. doi: 10.1016/j.jff.2018.04.031.

Panoutsopoulos, G. I., D. Kouretas, and C. Beedham. 2004. Contribution of aldehyde oxidase, xanthine oxidase, and aldehyde dehydrogenase on the oxidation of aromatic aldehydes. Chemical *Research in Toxicology* 17 (10):1368–76. doi: 10.1021/tx030059u.

Pasinetti, G. M., R. Singh, S. Westfall, F. Herman, J. Faith, and L. Ho. 2018. The role of the gut microbiota in the metabolism of polyphenols as characterized by gnotobiotic mice. *Journal of Alzheimer's Disease* 63 (2):409–21. doi: 10.3233/JAD-171151.

Passamonti, S., U. Vrhovsek, A. Vanzo, and F. Mattivi. 2003. The stomach as a site for anthocyanins absorption from food. *FEBS Letters* 544 (1–3):210–3. doi: 10.1016/S0014-5793(03)00504-0.

Pastoriza, S., C. Delgado-Andrade, A. Haro, and J. A. Rufián-Henares. 2011. A physiologic approach to test the global antioxidant response of foods. The GAR method. *Food Chemistry* 129 (4):1926–32. doi: 10.1016/j.foodchem.2011.06.009.

Pedan, V., N. Fischer, K. Bernath, T. Hühn, and S. Rohn. 2017. Determination of oligomeric proanthocyanidins and their antioxidant activity from different chocolate manufacturing stages using the NP-HPLC-online DPPH methodology. *Food Chemistry* 214: 523–32. doi: 10.1016/j.foodchem.2016.07.094.

Perez-Jimenez, J., M. E. Dıaz-Rubio, and F. Saura-Calixto. 2013. Nonextractable polyphenols, a major dietary antioxidant: Occurrence, metabolic fate and health effects. Nutrition *Research Reviews* 26:118–29. doi: 10.1017/S0954422413000097.

Petersen, B., S. Egert, A. Bosy-Westphal, M. J. Müller, S. Wolffram, E. M. Hubbermann, G. Rimbach, and K. Schwarz. 2016. Bioavailability of quercetin in humans and the influence of food matrix comparing quercetin capsules and different apple sources. *Food Research International* 88 (Pt A):159–65. doi: 10.1016/j.foodres.2016.02.013.

Poquet, L., M. N. Clifford, and G. Williamson. 2008. Transport and metabolism of ferulic acid through the colonic epithelium. *Drug Metabolism & Disposition* (1):190–197. doi: 10.1124/dmd.107.017558.

Ptolemy, A. S., E. Tzioumis, A. Thomke, S. Rifai, and M. Kellogg. 2010. Quantification of theobromine and caffeine in saliva, plasma and urine via liquid chromatography–tandem mass spectrometry: A single analytical protocol applicable to cocoa intervention studies. *Journal of Chromatography B* 878 (3–4):409–16. doi: 10.1016/j.jchromb.2009.12.019.

Rauf, A., M. Imran, M. S. Butt, M. Nadeem, D. G. Peters, and M. S. Mubarak. 2018. Resveratrol as an anti-cancer agent: A review. *Critical Reviews in Food Science and Nutrition* 58 (9):1428–47. doi: 10.1080/10408398.2016.1263597.

Rein, D., S. Lotito, R. R. Holt, C. L. Keen, H. H. Schmitz, and C. G. Fraga. 2000. Epicatechin in human plasma: In vivo determination and effect of chocolate consumption on plasma oxidation status. *The Journal of Nutrition* 130 (8):2109S–14S. doi: 10.1093/jn/130.8.2109S.

Rein, M. J., M. Renouf, C. Cruz-Hernandez, L. Actis-Goretta, S. K. Thakkar, and M. da Silva Pinto. 2013. Bioavailability of bioactive food compounds: A challenging journey to bioefficacy. British *Journal of Clinical Pharmacology* 75 (3):588–602. doi: 10.1111/j.1365-2125.2012.04425.x.

Richelle, M., I. Tavazzi, M. Enslen, and E. A. Offord. 1999. Plasma kinetics in man of epicatechin from black chocolate. European *Journal of Clinical Nutrition* 53 (1):22–6. doi: 10.1038/sj.ejcn.1600673.

Rios, L. Y., Bennett, R. N., Lazarus, S. A., Rémésy, C., Scalbert, A., and G. Williamson. 2002. Cocoa procyanidins are stable during gastric transit in humans. *The American Journal of Clinical Nutrition* 76 (5): 1106–1110. doi: 10.1093/ajcn/76.5.1106.

Rios, L. Y., M.-P. Gonthier, C. Rémésy, I. Mila, C. Lapierre, S. A. Lazarus, G. Williamson, and A. Scalbert. 2003. Chocolate intake increases urinary excretion of polyphenol-derived phenolic acids in healthy human subjects. *The American Journal of Clinical Nutrition* 77 (4):912–8. doi: 10.1093/ajcn/77.4.912.

Rocha-González, H. I., M. Ambriz-Tututi, and V. Granados-Soto. 2008. Resveratrol: A natural compound with pharmacological potential in neurodegenerative diseases. *CNS Neuroscience & Therapeutics* 14 (3): 234–47. doi: 10.1111/j.1755-5949.2008.00045.x.

Rodriguez-Mateos, A., D. Vauzour, C. G. Krueger, D. Shanmuganayagam, J. Reed, L. Calani, P. Mena, D. Del Rio, and A. Crozier. 2014. Bioavailability, bioactivity and impact on health of dietary flavonoids and related compounds: An update. Archives *of Toxicology* 88 (10):1803–53. doi: 10.1007/s00204-014-1330-7.

Rodriguez-Mateos, A., T. Cifuentes-Gomez, I. Gonzalez-Salvador, J. I. Ottaviani, H. Schroeter, M. Kelm, C. Heiss, and J. P. E. Spencer. 2015. Influence of age on the absorption, metabolism, and excretion of cocoa flavanols in healthy subjects. Molecular *Nutrition & Food Research* 59 (8):1504–12. doi: 10.1002/mnfr.201500091.

Rodriguez, A., A. Costa-Bauza, C. Saez-Torres, D. Rodrigo, and F. Grases. 2015. HPLC method for urinary theobromine determination: Effect of consumption of cocoa products on theobromine urinary excretion in children. *Clinical Biochemistry* 48 (16–17):1138–43. doi: 10.1016/j.clinbiochem.2015.06.022.

Rotches-Ribalta, M., C. Andres-Lacueva, R. Estruch, E. Escribano, and M. Urpi-Sarda. 2012. Pharmacokinetics of resveratrol metabolic profile in healthy humans after moderate consumption of red wine and grape extract tablets. *Pharmacological Research* 66 (5):375–82. doi: 10.1016/j.phrs.2012.08.001.

Roura, E., C. Andrés-Lacueva, O. Jauregui, E. Badía, R. Estruch, M. Izquierdo-Pulido, and R. M. Lamuela-Raventós. 2005. Rapid liquid chromatography tandem mass spectrometry assay to quantify plasma (−)-epicatechin metabolites after ingestion of a standard portion of coca beverage in humans. Journal *of Agricultural and Food Chemistry* 53 (16):6190–4. doi: 10.1021/jf050377u.

Roura, E., M. P. Almajano, M. L. Bilbao, C. Andrés-Lacueva, R. Estruch, and R. M. Lamuela-Raventós. 2007a. Human urine: Epicatechin metabolites and antioxidant activity after cocoa beverage intake. *Free Radical Research* 41 (8):943–9. doi: 10.1080/10715760701435236.

Roura, E., C. Andrés-Lacueva, R. Estruch, M. L. Mata-Bilbao, M. Izquierdo-Pulido, A. L. Waterhouse, and R. M. Lamuela-Raventós. 2007b. Milk does not affect the bioavailability of cocoa powder flavonoid in healthy human. Annals *of Nutrition and Metabolism* 51 (6):493–8. doi: 10.1159/000111473.

Roura, E., C. Andrés-Lacueva, R. Estruch, M. L. Mata-Bilbao, M. Izquierdo-Pulido, and M. Lamuela-Raventós. 2008. The effects of milk as a food matrix for polyphenols on the excretion profile of cocoa (−)-epicatechin metabolites in healthy human subjects. British *Journal of Nutrition* 100 (4):846–51. (doi: 10.1017/S0007114508922534.

Ross, J. A., and C. M. Kasum. 2002. Dietary flavonoids: Bioavailability, metabolic effects, and safety. Annual *Review of Nutrition* 22 (1): 19–34. doi: 10.1146/annurev.nutr.22.111401.144957.

Rusconi, M., and A. Conti. 2010. *Theobroma cacao* L., the food of the goods: Ascientific approach beyond myths and claims. *Pharmacological Research* 61 (1):5–13. doi: 10.1016/j.phrs.2009.08.008.

Salvador, I., A. P. Massarioli, A. P. Silva, H. Malaguetta, P. S. Melo, and S. M. Alencar. 2018. Can we conserve trans-resveratrol content and antioxidant activity during industrial production of chocolate? *Journal of the Science of Food and Agriculture* 99 (1):83–9. doi: 10.1002/jsfa.9146.

Sánchez-Rabaneda, F., O. Jáuregui, I. Casals, C. Andrés-Lacueva, M. Izquierdo-Pulido, and R. M. Lamuela-Raventós. 2003. Liquid chromatographic/electrospray ionization tandem mass spectrometric study of the phenolic composition of cocoa (*Theobroma cacao*). Journal *of Mass Spectrometry* 38 (1):35–42. doi: 10.1002/jms.395.

Sansone, R., J. I. Ottaviani, A. Rodriguez-Mateos, Y. Heinen, D. Noske, J. P. Spencer, A. Crozier, M. W. Merx, M. Kelm, H. Schroeter, and C. Heiss. 2017. Methylxanthines enhance the effects of cocoa flavanols on cardiovascular function: Randomized, double-masked controlled studies. The *American Journal of Clinical Nutrition* 105 (2): 352–60. doi: 10.3945/ajcn.116.140046.

Santos-Buelga, C., and A. Scalbert. 2000. Proanthocyanidins and tannin-like compounds-nature, occurrence, dietary intake and effects on nutrition and health. Journal *of the Science of Food and*

Agriculture 80 (7):1094–117. doi: 10.1002/(sici)1097-0010(20000515)80:7 < 1094::aid-jsfa569 > 3.0.co;2-1.

Serafini, M., R. Bugianesi, G. Maiani, S. Valtuena, S. De Santis, and A. Crozier. 2003. Plasma antioxidants from chocolate. *Nature* 424 (6952):1013. doi: 10.1038/4241013a.

Sergides, C., M. Chirilă, L. Silvestro, D. Pitta, and A. Pittas. 2016. Bioavailability and safety study of resveratrol 500 mg tablets in healthy male and female volunteers. *Experimental and Therapeutic Medicine* 11 (1):164–70. doi: 10.3892/etm.2015.2895.

Serra, A., A. Macià, M.-P. Romero, N. Anglés, J.-R. Morelló, and M.-J. Motilva. 2011. Metabolic pathways of the colonic metabolism of procyanidins (monomers and dimers) and alkaloids. *Food Chemistry* 126 (3):1127–37. doi: 10.1016/j.foodchem.2010.11.145.

Shahrzad, S., K. Aoyagi, A. Winter, A. Koyama, and I. Bitsch. 2001. Pharmacokinetics of gallic acid and its relative bioavailability from tea in healthy humans. *The Journal of Nutrition* 131 (4):1207–10. doi: 10.1093/jn/131.4.1207.

Schneider, H., R. Simmering, L. Hartmann, H. Pforte, and M. Blaut. 2000. Degradation of quercetin-3-glucoside in gnotobiotic rats associated with human intestinal bacteria. Journal *of Applied Microbiology* 89 (6):1027–37. doi: 10.1046/j.1365-2672.2000.01209.x.

Schramm, D. D., M. Karim, H. R. Schrader, R. R. Holt, N. J. Kirkpatrick, J. A. Polagruto, J. L. Ensunsa, H. H. Schmitz, and C. L. Keen. 2003. Food effects on the absorption and pharmacokinetics of cocoa flavanols. *Life Science* 73 (7):857–69. doi: 10.1016/S0024-3205(03)00373-4.

Smith, D. S. 2013. Benefits of flavanol-rich cocoa-derived products for mental well-being: A review. *Journal of Functional Foods* 5 (1):10–5. doi: 10.1016/j.jff.2012.09.002.

Smith, J. H. 2011. Theobromine and the pharmacology of cocoa. In *Handbook of experimental pharmacology: Methylxanthines*, ed. B. B. Fredholm, 1st ed., 201–34. New York, NY: Springer.

Sokolov, A. N., M. A. Pavlova, S. Klosterhalfen, and P. Enck. 2013. Chocolate and the brain: Neurobiological impact of cocoa flavanols on cognition and behavior. *Neuroscience & Biobehavioral Reviews* 37 (10):2445–53. doi: 10.1016/j.neubiorev.2013.06.013.

Stoupi, S., G. Williamson, F. Viton, D. Barron, L. J. King, J. E. Brown, and M. N. Clifford. 2010. In vivo bioavailability, absorption, excretion, and pharmacokinetics of [14C] procyanidin B2 in male rats. *Drug Metabolism & Disposition* 38 (2):287–91. doi: 10.1124/dmd.109.030304.

Spencer, J. P., F. Chaudry, A. S. Pannala, S. K. Srai, E. Debnam, and C. Rice-Evans. 2000. Decomposition of cocoa procyanidins in the gastric milieu. *Biochemical and Biophysical Research Communications* 272 (1):236–41. doi: 10.1006/bbrc.2000.2749.

Spencer, J. P., H. Schroeter, B. Shenoy, S. K. Srai, E. S. Debnam, and C. Rice-Evans. 2001a. Epicatechin is the primary bioavailable form of the procyanidin dimers B2 and B5 after transfer across the small intestine. *Biochemical and Biophysical Research Communications* 285 (3):588–93. doi: 10.1006/bbrc.2001.5211.

Spencer, J. P., H. Schroeter, A. J. Crossthwaithe, G. Kuhnle, R. J. Williams, and C. Rice-Evans. 2001b. Contrasting influences of glucuronidation and *O*-methylation of epicatechin on hydrogen peroxide-induced cell death in neurons and fibroblasts. *Free Radical Biology & Medicine* 31 (9):1139–46. doi: 10.1016/S0891-5849(01)00704-3.

Stahl, W., H. van den Berg, J. Arthur, A. Bast, J. Dainty, R. M. Faulks, C. Gärtner, G. Haenen, P. Hollman, B. Holst, et al. 2002. Bioavailability and metabolism. *Molecular Aspects of Medicine* 23 (1–3):39–100. doi: 10.1016/S0098-2997(02)00016-X.

Stark, T., R. Lang, D. Keller, A. Hensel, and T. Hofmann. 2008. Absorption of N-phenylpropenoyl-L-amino acids in healthy humans by oral administration of cocoa (*Theobroma cacao*). Molecular *Nutrition & Food Research* 52 (10):1201–14. doi: 10.1002/mnfr.200700447.

Tomas-Barberan, F. A., E. Cienfuegos-Jovellanos, A. Marín, B. Muguerza, A. Gil-Izquierdo, B. Cerda, P. Zafrilla, J. Morillas, J. Mulero, A. Ibarra, et al. 2007. A new process to develop a cocoa powder with higher flavonoid monomer content and enhanced bioavailability in healthy humans. Journal *of Agricultural and Food Chemistry* 55 (10):3926–35. doi: 10.1021/jf070121j.

Torres-Moreno, M., A. Tarrega, E. Costell, and C. Blanch. 2012. Dark chocolateacceptability: Influence of cocoa origin and processing conditions. Journal *of the Science of Food and Agriculture* 92 (2):404–11. doi: 10.1002/jsfa.4592.

Trost, K., M. M. Ulaszewska, J. Stanstrup, D. Albanese, C. De Filippo, K. M. Tuohy, F. Natella, C. Scaccini, and F. Mattivi. 2018. Host: Microbiome co-metabolic processing of dietary polyphenols – An acute, single blinded, cross-over study with different doses of apple polyphenols in healthy subjects. *Food Research International* 112:108–28. doi: 10.1016/j.foodres.2018.06.016.

Tsai, H. Y., C. T. Ho, and Y. K. Chen. 2017. Biological actions and molecular effects of resveratrol, pterostilbene, and 3′-hydroxypterostilbene. *Journal of Food and Drug Analysis* 25 (1):134–47. doi: 10.1016/j.jfda.2016.07.004.

Urpi-Sarda, M., M. Monagas, K. Nasiruddin, R. M. Lamuela-Raventos, C. Santos-Buelga, E. Sacanella, M. Castell, J. Permanyer, and C. Andres-Lacueva. 2009. Epicatechin, procyanidins, and phenolic microbial metabolites after cocoa intake in humans and rats. Analytical and Bioanalytical Chemistry 394 (6):1545–56. doi: 10.1007/s00216-009-2676-1.

Urpi-Sarda, M., R. Llorach, N. Khan, M. Monagas, M. Rotches-Ribalta, R. Lamuela-Raventos, R. Estruch, F. J. Tinahones, and C. Andres-Lacueva. 2010. Effect of milk on the urinary excretion of microbial phenolic acids after cocoa powder consumption in humans. Journal of Agricultural and Food Chemistry 58 (8):4706–11. doi: 10.1021/jf904440h.

Visioli, F., H. Bernaert, R. Corti, C. Ferri, S. Heptinstall, E. Molinari, A. Poli, M. Serafini, H. J. Smit, J. A. Vinson, et al. 2009. Chocolate, lifestyle, and health. *Critical Reviews in Food Science and Nutrition* 49 (4):299–312. doi: 10.1080/10408390802066805.

Visioli, F., C. A. De La Lastra, C. Andres-Lacueva, M. Avira, C. Calhau, A. Cassano, M. D'Archivio, A. Faria, G. Favé, V. Fogliano, et al. 2011. Polyphenols and human health: A prospectus. *Critical Reviews in Food Science and Nutrition* 51 (6):524–46. doi: 10.1080/10408391003698677.

Vitaglione, P., R. B. Lumaga, R. Ferracane, S. Sellitto, J. R. Morelló, J. R. Miranda, E. Shimoni, and V. Fogliano. 2013. Human bioavailability of flavanols and phenolic acids from cocoa-nut creams enriched with free or microencapsulated cocoa polyphenols. British Journal of Nutrition 109 (10):1832–43. doi: 10.1017/S0007114512003881.

Vuong, Q. 2014. Epidemiological evidence linking tea consumption to human health: A review. *Critical Reviews in Food Science and Nutrition* 54 (4):523–36. doi: 10.1080/10408398.2011.594184.

Walgren, R. A., J.-T. Lin, K.-H. Kinne, and T. Walle. 2000. Cellular uptake of dietary flavonoid quercetin-4′-*β*-glucoside by sodium-dependent glucose transporter SGLT1. *Journal of Pharmacology and Experimental Therapeutics* 294 (3):837–43.

Walle, T., F. Hsieh, M. H. DeLegge, J. E. Oatis, Jr., and U. K. Walle. 2004. High absorption but very low bioavailability of oral resveratrol in humans. *Drug Metabolism & Disposition* 32 (12):1377–82. doi: 10.1124/dmd.104.000885.

Wang, P., and S. Sang. 2018. Metabolism and pharmacokinetics of resveratrol and pterostilbene. *Biofactors* 44 (1):16–25. doi: 10.1002/biof.1410.

Williamson, G., and M. N. Clifford. 2017. Role of the small intestine, Colon and microbiota in determining the metabolic fate of polyphenols. *Biochemical Pharmacology* 139:24–39. doi: 10.1016/j.bcp.2017.03.012.

Williamson, G., C. D. Kay, and A. Crozier. 2018. The bioavailability, transport, and bioactivity of dietary flavonoids: A review from a historical perspective. *Comprehensive Reviews in Food Science and Food Safety* 17 (5):1054–112. doi: 10.1111/1541-4337.12351.

Wenzel, E., T. Soldo, H. Erbersdobler, and V. Somoza. 2005. Bioactivity and metabolism of trans-resveratrol orally administered to Wistar rats. Molecular Nutrition & Food Research 49 (5):482–94. doi: 10.1002/mnfr.200500003.

Wiczkowski, W., E. Romaszko, and M. K. Piskula. 2010. Bioavailability of cyanidin glycosides from natural chokeberry (*Aronia melanocarpa*) juice with dietary-relevant dose of anthocyanins in humans. Journal of Agricultural and Food Chemistry 58 (23):12130–6. doi: 10.1021/jf102979z.

Wiese, S., T. Esatbeyoglu, P. Winterhalter, H. P. Kruse, S. Winkler, A. Bub, and S. E. Kulling. 2015. Comparative biokinetics and metabolism of pure monomeric, dimeric, and polymeric flavan-3-ols: A randomized cross-over study in humans. Molecular Nutrition & Food Research 59 (4):610–21. doi: 10.1002/mnfr.201400422.

Wilson, P.K. 2012. Chocolate as medicine: A changing framework of evidence throughout history. In *Chocolate and health*, eds. R., Paoletti, A. Poli, A. Conti, and F. Visioli, 1–16. Milano, Italia: Springer Verlag Italia.

Wollgast, J., and E. Anklam. 2000. Review on polyphenols in *Theobroma cacao*: Changes in composition during the manufacture of chocolate and methodology for identification and quantification. *Food Research International* 33 (6):423–47. doi: 10.1016/S0963-9969(00)00068-5.

Wu, X., G. Cao, and R. L. Prior. 2002. Absorption and metabolism of anthocyanins in elderly women after consumption of elderberry or blueberry. *The Journal of Nutrition* 132 (7):1865–71. doi: 10.1093/jn/132.7.1865.

Xie, L., S. G. Lee, T. M. Vance, Y. Wang, B. Kim, J.-Y. Lee, O. K. Chun, and B. W. Bolling. 2016. Bioavailability of anthocyanins and colonic polyphenol metabolites following consumption of aronia berry extract. *Food Chemistry* 211 (15):860–8. doi: 10.1016/j.foodchem.2016.05.122.

Yuan, S., X. Li, Y. Jin, and J. Lu. 2017. Chocolate consumption and risk of coronary heart disease, stroke, and diabetes: A meta-analysis of prospective studies. *Nutrients* 9 (7):688. doi: 10.3390/nu9070688.

Yi, W., C. C. Akoh, J. Fischer, and G. Krewer. 2006. Absorption of anthocyanins from blueberry extracts by caco-2 human intestinal cell monolayers. Journal of Agricultural and Food Chemistry 54 (15):5651–8. doi: 10.1021/jf0531959.

Zumdick, S., A. Deters, and A. Hensel. 2012. In vitro intestinal transport of oligomeric procyanidins (DP 2 to 4) across monolayers of Caco-2 cells. *Fitoterapia* 83 (7):1210–7. doi: 10.1016/j.fitote.2012.06.013.