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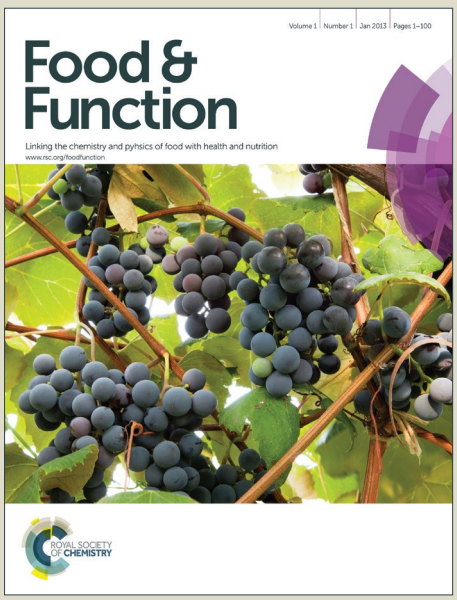

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The absorption, distribution, metabolism and excretion of procyanidins

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Liang Zhang[a]*, Yijun Wang[a], Daxiang Li[a], Chi-Tang Ho[b], Junsong Li[c], Xiaochun Wan[a]*

Procyanidins (PAs) are the polyphenols in plant food with many health benefits, including cancer prevention, cardiovascular protection and diabetes preventions. PAs have been known to have low oral-bioavilability. In this review, we summarized the published results about the ADME (absorption, distribution, metabolism and excretion) of PAs *in vivo* and *in vitro*. After oral administration, in the stomach, the decomposition of PAs is highly dependent on the pH value of gastric juice, which is also affected by food intake. In the small intestine, PAs polymers and oligomers with DP>4 are not directly absorbed *in vivo*, but minor PAs monomers and dimers could be detected in plasma. The methylated and glucuronidated PAs dimers and monomers are the main metabolites of PAs in plasma. In the colon, PAs are catabolized by colonic microflora into a series of low molecular weight phenolic acids, such as phenyl valerolactone, phenylacetic acids and phenylpropionic acids. We reviewed the degradation of PAs in gastric digestion, the absorption of PAs in small intestine and the metabolic pathway of PAs by colonic microflora. To clearly explain the pharmacokinetics of PAs *in vivo*, a systematic comparative analysis on the previously published data on PAs were conducted.

Introduction

Proanthocyanidins, also known as condensed tannins are one type of the most abundant phytochemicals in plants[1, 2]. They have the capacity to precipitate proteins, such as the salivary proteins in the oral cavity, and then produce astringent taste so that it could be easily recognized in tannin-rich food[3]. Usually, proanthocyanidins containing exclusively the (epi)-catechin are named procyanidins (PAs), whereas those containing afzelechin or gallocatechin are called propelargonidins or prodelphinidins, respectively. PAs have various isomers in term of stereo-chemical structures, such as the configuration of flavan-3-ol units and the linkage positions[4]. There are mainly two types of bonds linking two individual catechin or epicatechin, they are C_4-C_8 and/or C_4-C_6 bonds which are named B-type PAs, or C_4-C_8 and C_2-O_7 bonds which are named A-type PAs. In addition to these simple forms, there are more complicated structures containing gallic acid esters or doubly linked forms. According to the degree of polymerization (DP), PAs are classified into monomers, dimers, trimers or tetramers corresponding to the 1, 2, 3, and 4 units of flaval-3-ols, respectively[5]. PAs with DP>4 are usually named oligomers, and DP>10 polymers.

B-type PAs are the predominant PAs in many foods, such as berries, cereals, nuts, legumes, chocolates and wines[6-9]. They represent a major part of food flavonoids ingested from the plant foods[10]. A-type PAs mainly exist in limited varieties of foods, such as peanut, plums, cranberries, avocados and cinnamon[11-13]. As the elementary units of PAs, flavan-3-ols are more diversified in the tea plants (*Camellia sinensis* and *Camellia assamica*), they are epicatechin and its gallate, so the PAs 3/3′ gallates have been widely reported in tea plants[14]. For the PAs we consumed from dietary sources, most of those in cocoa, berries, brown sorghum, grape and pinto beans are polymers and oligomers.

There has been a phenol-explorer database (www.phenol-explorer.eu) for the polyphenols' contents in 452 kinds of foods and beverages. Grape seeds contain the highest contents of total PAs (3532.3±105.8 mg/100g), also the highest contents of PAs dimers (417.3±4.8 mg/100g)[15]. Furthermore, unsweetened baking chocolate contains the high contents of total PAs (1635.9±334.6 mg/100g). Hammerstone *et al.* also reported that chocolate contained the high contents of PAs in the dietary foods[16]. In the cereals and beans, sorghum and sumac bran contain high content of total PAs (3965.4±402.5 mg/100g), but the content of PAs dimers is much less than those of grape seed and baking chocolate.

It has been reported that dietary polyphenols could contribute to preventing various diseases[17, 18]. PAs have active phenolic groups, which possess antioxidant capacity. The *in vitro* and *in vivo* studies showed that PAs had various biological properties, such as radical scavenging, anti-ulcer, anti-allergy, anti-dental caries and anti-tumour activities[19, 20]. PAs and their

[a] *State Key Laboratory of Tea Plant Biology and Utilization, Anhui Agricultural University,* Hefei 230036, China Tel: +86-551-65786765; fax: +86-551-65786765, Email: zhli2091@sina.com (L., Zhang); xcwan@ahau.edu.cn (XC., Wan);
[b] *Department of Food Science, Rutgers University, 65 Dudley Rd., New Brunswick, NJ 08901, USA*
[c] *College of Pharmacy, Nanjing University of Chinese Medicine, Nanjing 210023, China*
* *Corresponding author.*

polymers can prevent the cancers of digestive tract[21]. Due to the high concentrations of catechins and PAs, cocoa and chocolate products may have the preventative effects against oxidative stress and chronic inflammation, which are the risk factors inducing cancer and other chronic diseases[22-24].

Recently, it was reported that grape seed polyphenols extract (GSPE) administration corrected all of the disruptions in the clock genes induced by obesity in the liver and GUT, the two organs that generate triglyceride-rich lipoproteins[25, 26]. A. Ribas-Latre *et al.*, reported that GSPE administration leaded to the overexpression of core clock genes, and acetylated BMAL1 protein in healthy animals and nicotinamide phosphoribosyl transferase (Nampt) was the most sensitive gene to GSPE[27].

Many epidemiologic studies have explored the beneficial effects of flavanol-rich cocoa[28, 29]. However, the extensive, preventive and dose-dependent health benefits of PAs do not match their low oral-availability. It has been suggested that many observed activities may not depend on the systemic bioavailability of native PAs. PAs' metabolites may play a very important role in the whole effects of native compounds of PAs. Furthermore, any activities locally in the gastro-intestinal tract including inhibition of digestive enzymes, modulation of gut microbiota, modulation of the gut immune response, gut epithelial architecture, modulation of gut hormone secretion may also lead to biological activities[30, 31].

Pharmacokinetics of PAs

The hydrolysis of PAs *in vitro*

It has been suggested that PAs would be hydrolysed to (-)-epicatechin (EC), and then absorbed in the small intestine[17]. After oral administration of PAs, the first pass of absorption is in gastric digestion *in vivo*. If the PAs could be largely degraded into monomers under the acidic condition, then it would be scarcely absorbed as intact molecules in small intestine, which is the main organ responsible for drug transportation. Conversely, if the PAs is stable in gastric juice, it may be absorbed in small intestine and detected in plasma.

Conflicting results on the stability of PAs are known. On one hand, it has been reported that PAs oligomers could be decomposed into dimers and monomers (epicatechin or catechin) in gastric juice at pH 2.0[32, 33]. PAs dimer B2 is almost completely degraded into EC in gastric juice at pH 1.8, but EC is quiet stable at pH 2.0 condition of gastric juice[34]. Under alkaline artificial duodenal juice, both PAs dimer B2 and its monomer EC are unstable[33].

On the other hand, when PAs oligomers were incubated in gastric juice at pH 7.4, the decomposition of PAs was scarcely observed[33]. PAs dimer B2 was not degraded in the ileostomy fluid either, which highlighted its stability in a near neutral milieu (pH 6.3)[34].

The hydrolysis of PAs in human *in vivo*

Laurent *et al.* reported the PAs' digestion *in vivo*[35]. After drinking 500 mL of cocoa beverage, the gastric sample was taken out from stomach every 10 min up to 50 min. No degradation of PAs was observed, neither the quantitative changes of monomeric PAs during ingestion. During the *in vivo* digestion, the pH value of gastric content was increased from 1.9±0.2 to 5.4±0.2 after cocoa beverage consumption. The real pH value is increased after consumption of cocoa beverage, which might dilute the gastric juice and increase the pH value. Therefore, it was confirmed that the pH value should be a critical factor for the degradation of PAs during gastric digestion.

The hydrolysis of PAs in animal *in vivo*

GSPE contains more complicated compositions of PAs. During the digestion of GSPE in gastric juice, the PAs trimers were found to be the most abundant compounds and the PAs dimers and tetramers were also highly increased after gastric digestion[36]. Co-administration with carbohydrate-rich food increased the degradation of PAs trimers and dimers in gastric juice, because food intake will stimulate the gastric acid secretion. Increased molecules of catechin and epicatechin were not quantitatively equal to the decreased molecules of PAs dimers and trimers in gastric juice. The results showed that EC was the main metabolites of PAs dimers, but the cleavage of C_4-C_8 or C_4-C_6 carbon bond may not produce double monomers.

The solubility PAs in consumed food affected the hydrolysis of PAs *in vivo*. If food was consumed as raw material, the PAs could be existed in food as non-dialysable or dialyzable fraction. During the duodenal digestion, most of PAs were stable and retained in non-dialysable fraction, which could be further conveyed to colon and then metabolized by colonic microflora[37-39]. Therefore, PAs' hydrolysis rests with multiple factors, such as pH condition, food intake and the solubility of PAs in gastric juice.

Pharmacokinetics study of PAs in animal *in vivo*

It has been doubted that the PAs dimers, trimers or other polymers could be directly absorbed *in vivo*[36]. There were few studies about the pharmacokinetics of PAs in animals and human following oral administration of pure PAs dimers or PAs-rich foods (**Table 1**).

Mostly, monomeric units such as catechin and epicatechin, and their methylated and conjugated forms are identified as the metabolites of PAs dimers in circulation. PAs dimer B2 and B5 could be absorbed as unmodified and methylated dimers by perfusion of jejunum, but with a very low content. EC and methyalted EC were the main metabolites of PAs dimers detected in serosal surface[40]. As the previous reports, the PAs dimers could competitively inhibit the activities of COMT, so that the methylation of EC would be decreased after high dose of PAs intake[41].

Most of PAs was circulated in the way of conjugated forms *in vivo*. Baba *et al.* studied the absorption and urinary excretion of PAs dimer B2 in rats[40]. After sulfatase treatment, free form of PAs dimer B2, EC and 3'-O-methyl-EC were detected and determined in plasma. Urinary excretion of total

PAs dimer B2 (conjugated and unconjugated forms) accounted for 0.340% of oral dose. The total urinary EC and 3'-O-methyl-EC were only 0.085% and 0.057% of the oral dose, respectively. Recently, Bittner *et al.* studied the absorption and metabolism of PAs B4 in pigs[42]. After oral administration of PAs B4, a very low level of intact PAs B4 was detected in urine and plasma. In these two studies, single PAs dimer B2 and B4 was given to rat and pig at the dose of 50 mg/kg and 10 mg/kg, respectively. The plasma maximum concentrations (C_{max}) of PAs dimer B2 (rat) and B4 (pig) were 0.5 μM and 3.68 nM, respectively. The higher C_{max} of intact PAs dimer B2 than B4 should be attributed to the PAs' chemical characteristics and animal differences.

Appeldoorn *et al.* compared the absorption and metabolism of A-type PAs dimer A1, A2 and B-type PAs dimer B2[43]. Perfusion with PAs dimer B2 didn't result in its absorption, but dimer A1 and A2 could be detected in plasma of rats after perfusion. In this study, the given dose of PAs dimer B2 was 100 μmol/L with the speed of 1 mL/min. Obviously, it is a very low dose comparing with the oral doses [40,42], so the absorption result of PAs may be influenced by low given dose and the sensitivity of analytical methods.

The above-mentioned reports were relied on the pure compunds of PAs orally given. According to the *in vitro* researches' results, PAs would be firstly degraded into monomeric flavan-3-ol in acidic gastric juice, and subsequently also degraded in colon. The oral-bioavailbility of PAs dimers was very low in rats and pigs. PAs dimer was degraded into epicatechin, and then metabolized into conjugated EC and methyl-EC conjugated (sulfated and glucuronidated). The methylation and glucuronidation of PAs and EC could be happened in both liver and intestinal mucosa[44-46]. By comparing the critical pharmacokinetics parameter, the time to maximum plasma concentration (t_{max}) of EC was delayed than PAs[42]. The ^{14}C-labelled PAs study gave a similar t_{max} of total ^{14}C radioactivity, which also confirmed PAs were less absorbed directly, but transferred into EC and other aromatic compounds for delayed absorption.

The analytical method also affected the determination of plasma levels of PAs in bio-samples. Using the electrochemical detection, trace amounts of PAs dimers may not be detected, but LC-MS highly improves the sensitivity on the detection of PAs in plasma. Furthermore, isotope labelling provides more accurate data for the total ^{14}C-labelled PAs in bio-samples, but the exact chemical structure of intact PAs or their metabolites were ambiguous. Integrating the isotope labelled PAs and LC-MS should provide more sensitive and accurate detection.

Table 1 The absorption and excretion of procyanidins *in vivo*.

Sample	Subject	Administration way	Dose (mg/kg)	Plasma analytes	Pharmacokinetics parameters	References
PB2	SD rats	i.g.	50	PB2, EC, 3'-O-methyl-EC	Plasma C_{max}, urinary excrection: PB2>EC>3'-O-methyl-EC	40
[^{14}C]PB2	Wister rats	i.v.	21	-	8-11% oral-bioavailability	47
		i.g.	21,10.5	-		
PB4	Pigs	i.g.	10	EC, 3'OMEC, 4'OMEC, PB4	AUC: EC>3'OMEC>4'OMEC>PB4	42
PB3, grape seed extract	Wister rats	Diet supplement	20(PB3),200 and 400 GSE	EC, C, methyl EC and C in GSE group	-	48
Grape seed extract	Wister rats	i.g.	1000	C, EC, (Methyl) glucuronidated C and EC, dimer, trimer	EC>C>dimer>EGCG>trimer	49
Grape seed extract	Wister rats	i.g.	1000	C, EC, dimer, trimer	Trimer>dimer>EC>C	36
Grape seed extract	SD rats	i.g.	300×2	EC, C, methyl EC and C, dimer, trimer	-	49
Grape seed extract	SD rats	i.g.	1000	(Methyl) Glucuronidated EC, C, methyl-sulfated EC and C	-	51

Procyanidins extract and cocoa cream	Wister rats	i.g.	1000+50	(Methyl) Glucuronidated EC, C, methyl-sulfated EC and C, dimer, trimer	EC>C>dimer>trimer	[52]
Cocoa	Human	i.g.	375	EC, C, PB2	EC>C>PB2	[53]
Apple procyanidins	Wister rats	Intragastric injections	1000	C, EC, PB1, PB2, PC1	-	[54]
B2, A1, A2, A-type DP3, A-type DP4,	Wister rats	In site perfusion of intestine	100 μmol/L	Dimer A1, A2, B2	5-10% absorption	[43]

i.v., intravenous; i.g., intragastric; PB2, procyanidins dimer B2; [^{14}C]PB2, ^{14}C-labelled procyanidins dimer B2; PB4, procyanidins dimer B4; PB3, procyanidins dimer B;

Pharmacokinetics study of PAs mixture in animal *in vivo*

Grape seed contains high contents of PAs polymers, oligomers and monomers. After oral administration of GSPE, PAs dimers B1, B2, B3 and B4 and trimers could be detected in plasma and urine and colon [50, 51, 49, 56]. It was reported that plasma levels of PAs dimer and trimer were higher than epicatechin and catechin after dosing GSPE[56]. Glucuronidated catechin and epicatechin could be detected after GSPE intake, and a tetramethylated metabolite of dimeric PAs in urine was also tentatively identified[49]. Most of PAs extract used in the ADME study was not well structurally characterized, and the analytical method highly affected the limit of determination of minor PAs and their metabolites in biological samples.

For example, the plasma concentrations of PAs dimer were detected as 2.40 μM at 2h post-administration[56], in the other study the concentrations was 0.57 nM[36]. During the absorption of PAs, these free forms appearing in the plasma are prior to the glucuronidated forms of the monomers. When human consumed 2.0 g of PAs-rich grape seed extract, PAs B1 were detected at the C_{max} of 10.6±2.5 nM[57]. In the above-mentioned researches, GSPE was given at oral high dose. For example, rats was given by gavage at the dose of 300 mg/kg[49], 1 g/kg[36, 51, 56], or was taken orally in hard capsule containing 2 g GSE for human[57].

In other researches, GSPE was also given to rats by mixing them in meal, but not directly gavaged to rats. When rats were given 200 or 400 mg of GSE in meal[48], PAs dimers could not be detected in plasma. It is apparent high dose and types of dosing would affect the absorption of PAs in plasma. To obtain the detectable levels of PAs, a single oral administration of high dose of PAs mixture is indispensable.

Apple peel extract was also applied to the pharmacokinetics study of PAs. After oral administration of apple condensed tannins, catechin, epicatechin, PAs B1 and B2, and PAs C1 were detected in rat's plasma by using a HPLC-MS/MS[54]. The t_{max} values for PAs dimers and trimers were about 2 h, and the methylated PAs trimers and dimers were also detected in plasma. These reports indicated that PAs dimer B2, the main PAs dimer in grape seed and apple, could be absorbed.

Sample preparation and analytical methods significantly affected the detection results of PAs and their metabolites in bio-samples. Lack of chemical standards may lead to the inaccurate detection levels of analytes, while bio-samples without enzymatic hydrolysis would miss the conjugated PAs. For example, Catherine Tsang *et al.*, found that the PAs could not be detected when samples were not hydrolysed[51], while the PAs dimers and trimers could be detected in plasma after sulfatase and glucosidase treatment[49]. Many researches also detected PAs monomers, dimers, and trimers in plasma samples after intake of procyanidin-rich extracts, such as grape seed extract, cocoa, apple and tea [40, 49, 53-55, 57, 58].

Serra *et al.* reported that epicatechin-glucuronide and methyl catechin-glucuronide were the main metabolites in the plasma after cocoa cream and hazelnut skin extract intake[52]. Furthermore, the PAs dimer and trimer in free forms were detected from 1 h to 2 h but cleared rapidly. The distribution results showed that *p*-hydroxybenzoic acid, trimethyluric acid, hydroxyphenyl acetic acid were the main metabolites in liver, other phase Ⅱ metabolites including glucuronidated catechin and epicatechin were the main metabolites in kidney and lung. Some phenolic compounds including phenyl acetic acid, hydroxyphenyl acetic acid, 5-dihydroxyphenyl valerolactone, vanillic acid and *p*-hyhydroxybenzoic acid were the main metabolites in heart and testicle.

B-type PAs have been widely reported, but the A-type PAs was less concerned. The A-type PAs dimers, trimers and tetramers have been separated from peanut skin. Appeldoorn *et al.* reported that A-type PAs dimers showed better absorption than B-type PAs dimer after *in situ* perfusion of the small intestine[43].

Pharmacokinetics study of PAs mixture in human *in vivo*

Cocoa is one of the most consumed PAs-rich foods [59]. Holt *et al.* studied the pharmacokinetics of PAs dimer B2 in the plasma of human after oral administration of 26.4 g cocoa[53]. Although there were only four blood samples after cocoa intake, the PAs dimer B2 could be detected at 0.5, 2 and 6 h, the plasma concentration of monomer epicatechin was significantly higher than dimer B2.

PAs dimers could enter into the circulation, but PAs trimers can rarely be absorbed. According to the "rule of 5"[60],

it was supposed that the higher degree of polymerization of PAs was poorly absorbed or permeable because their molecular weight and the sum of OHs were more than 500 and 5, respectively. These results suggested that PAs dimers could be absorbed *in vivo* and that polymer oral-bioavailability is limited to the gut lumen[61, 62].

The PAs were occasionally consumed with epicatechin in fruit juices. Comparing with single epicatechin consumption, PAs co-administration didn't increase the plasma levels of methyl-(-)-epicatechin metabolites[63]. Recent researches also indicated co-administration of high-fat food or physiological status, such as obesity and hyperlipidaemia also affected the pharmacokinetics of (epi)-catechin and their gallate *in vivo*[64, 65].

***In vitro* Caco-2 cells study**

Colonic carcinoma (caco-2) cell has been the most extensively characterized and useful *in vitro* model in the research of drug permeability and absorption[66]. PAs showed a very low P_{app} from apical to basolateral permeation. It was passively transported by paracellular transport and also refluxed by p-glycoprotein. Zumdick *et al.* also reported that the PAs dimer B2 and the PAs tetramer, pentamer, and hexamer were transported from apical to basolateral side by passive paracellular route[67]. 10% to 30% total PAs were up-taken into cell lysates. Permeability of various radiolabeled PAs differing in their molecular weight was compared with that of the radiolabeled catechin. The permeability of a PAs polymer with an average DP at 6 was 10 times less than catechin. Although the monomeric catechins and PAs were usually less permeable through caco-2, their intestinal microbiota metabolites could pass the caco-2 cell monolayer[68].

The metabolism of PAs

Shrestha *et al.* compared the hepatic metabolism of 3,3'-di-O-galloyl ester of PAs dimer B2 (B2G2), PAs dimer B2, epicatechin and 3-O-galloyl-epicatechin[69]. During the incubation of PAs dimer B2 with liver microsomes, most of the PAs dimer B2 remained unmetabolized in hepatic microsome, only minor glucuronidated products of PAs dimer B2 was detected. PAs dimer B2 methyl ether was presented in PAs dimer B2 of hepatic cytosol incubation. But in *in vivo* study, the pharmacokinetics study reported that PAs dimers were not methylated or conjugated in intestine by intestinal perfusion. PAs dimers A1 and A2 can be unmodified absorbed in small intestine, but PAs dimer B2 was not detected in rat plasma after perfusion, even using the HPLC-MS/MS analytical method[43].

Catechol-O-methyltransferase (COMT) catalyzed the O-methylation of endogenous and xenobiotic compounds including flavonoids[70]. The 3'-O-methylated catechin have been widely reported as the main metabolites of catechin *in vivo*[40]. COMT can methylate dimeric PAs *in vitro* by incubation with rat liver cytosol[71]. Monomethylated and dimethylated PAs metabolites have been identified. The PAs trimer C1 contains more hydroxyls, so liver cytosolic COMT yielded five monomethylated, nine dimethylated and four trimethylated metabolites of PAs trimer C1, but only two abundant metabolites were identified as monomethylated metabolites. PAs compete with epicatechin to limit the formation of *O*-methylated epicatechin. Furthermore, the high concentration of PAs can inhibit the COMT activity by dimer-protein interactions[72, 73].

Microbial metabolism of PAs

Because of the low oral-bioavailability of PAs, many approaches have been explored regarding the microflora metabolism of PAs in colon. To explain the metabolic efficiency of PAs, it was reported that the colonic fermentation of one molecule of PAs dimer B2 didn't produce two molecules of epicatechin[74]. Gonthier *et al.* compared the metabolism of PAs dimer B3, trimer C2 polymer and catechin *in vivo*[75]. The main metabolites of PAs dimer B2 were identified as epicatechin, phenylacetic acid, 4-hydroxyphenylacetic acid, protocatechuic acid and 4-hydroxybenzoic acid. Although the PAs could be degraded into epicatechin by colonic microflora, the epicatechin was subsequently rapidly metabolized into low molecular phenolic acids. Therefore, the epicatechin could be detected during fermentation, but 3,4-dihydroxyphenylacetic acid and 3,4-dihydroxyphenyl-γ-valerolactone were the main metabolites of PAs in urine. These phenolic metabolites could be quantitatively accounted for 12 mol % of dimers[43]. When rats were fed the diets supplemented with the procyanidin trimer C2 or polymer, the urinary excretion of 3-hydroxyphenylvaleric, 3-hydroxyphenylpropionic acid, and m-coumaric acids were increased.

Fulgencio *et al.* separated the insoluble polymerized PAs from *Ceratonia siliqua* L. PAs (CSPA), and separated soluble PAs from grape seeds[76]. Indigestible fraction of grape antioxidant dietary fiber (containing soluble PAs) presented greater ferment-ability (39%) than the CSPA (23%). The main metabolites of grape seed PAs and *Ceratonia siliqua* L. extract samples by colonic fermentation were hydroxyphenyl acetic acid with concentrations of 8.7 and 8.3 ng/mg of dry sample, respectively. Hydroxyphenyl valeric acid was the second main metabolite detected. These results showed that the main PAs metabolite detected in human plasma was 3,4-dihydroxyphenylacetic acid (also detected in its methylated and sulphated forms), followed by hydroxyphenylvaleric acids and hydroxyphenylpropinic acids.

The A-type PAs possess more complex structure in comparison to B-type PAs. Engemann *et al.* studied the metabolism of two A-type PAs dimer A2 and cinnamtannin B1 by pig cecum microbiota[77]. PAs dimer A2 was degraded by about 80% and cinnamtannin B1 was about 40% after 8h of incubation. The main metabolites of PAs A2 were 3,4-dihydroxyphenylacetic acid and 3-hydroxyphenylpropionic acid, the levels of which were increased along with the fermentation time. The main metabolites of cinnamtannin B1 were identified as 4-hydroxyphenylacetic acid, 3-hydroxyphenylpropionic acid and 3,4-dihydroxyphenylacetic acid.

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Table 2 The phenolic acids formed during the metabolism of procyanidins.

Phenolic acid type	Name	Metabolism way	Substrate	Reference
C_6-C_1 acid	3,4-dihydroxybenzoic acid	Human fecal microbiota	Procyanidin B2	[78]
	3-hydroxybenzoic acid			
	4-hydroxybenzoic acid			
C_6-C_2 acid	3,4-dihydroxyphenylacetic acid			
	3-hydroxyphenylacetic acid			
	4-hydroxyphenylacetic acid			
	Phenylacetic acid			
C_6-C_3 acid	3,4-dihydroxyphenylpropionic acid			
	3-hydroxyphenylpropionic acid			
	4-hydroxyphenylpropionic acid			
C_6-C_5 acid	Phenylvaleric acid			
	5-hydroxyphenylvaleric acid			
	4-hydroxyphenylvaleric acid			
C_6-C_2 acid	Hydroxyphenylacetic acid	Rat fecal microbiota	Grape procyanidins, *Ceratonia siliqua* L. procyanidins	[76]
C_6-C_5 acid	Hydroxyphenylvaleric acid			
C_6-C_3 acid	3- or 4-Hydroxyphenylpropionic acid			
C_6-C_3 acid	4-hydroxyphenylpropionic acid	Pig intestinal bacteria	Procyanidin A2/Cinnamtannin B1	[77]
	3-hydroxyphenylpropionic acid			
C_6-C_3 acid	3,4-dihydroxyphenylacetic acid		Cinnamtannin B1	
	4-hydroxyphenylacetic acid			
Flavnal-3-ol	Epicatechin	Rat colonic microflora	Procyannidin B2	[74]
C_6-C_2 acid	Phenylacetic acid			
	4-hydroxyphenylacetic acid			
	2-hydroxyphenylacetic acid			
C_6-C_1 acid	Protocatechuic acid			
	4-hydroxybenzoic acid			
C_6-C_5 acid	3-hydroxyphenylvaleric acid	Urine of rats orally administered procyanidins	Procyanidin B3, trimer C2 and polymer	[75]
C_6-C_3 acid	3,4-dihydroxyphenylpropionic acid			
	3-hydroxyphenylpropionic acid			
	Trans-3-Hydroxycinnamic acid			
C_6-C_1 acid	3-Hydroxybenzoic acid			
C_6-C_2 acid	3-hydroxyphenylacetic acid	Human colonic microflora	Procyanidins polymers	[79]
	4-hydroxyphenylacetic acid			

C_6-C_3 acid	3-hydroxyphenylpropionic acid 4-hydroxyphenylpropionic acid Phenylpropionic acid.			
C_6-C_5 acid	3-hydroxyphenylvaleric acid			
C_6-C_2 acid	3,4-dihydroxyphenylacetic acid 3-hydroxyphenylacetic acid, 4-hydroxyphenylacetic acid	Human fecal microflora	Procyanidin dimers	80
C_6-C_3 acid	3-hydroxyphenylpropionic acid			
C_6-C_5 acid	phenylvaleric acid			
C_6-C_5 (valerolactone)	Mono-hydroxylated phenyl valerolactone 5-(3,4-dihydroxyphenyl)-ɣ-valerolactone			

(-)-Epicatehin is the main compound of green tea, which has been widely reported as the substrate of gut microflora. Tea polyphenols were degraded into phenolic acids by colonic fermentation. Comparing the metabolic differences of epicatechin with PAs would contribute to explaining the exclusive features of PAs' metabolism. Stoupi *et al.* reported the comparative biotransformation of epicatechin and PAs dimer B2 by human faecal microbiota *in vitro*[78]. In this experiment, it was as-certained that PAs dimer B2 could be degraded into epicatechin by human fecal microflora, which suggested that the epicatechin was formed *via* the cleavage of the C_4-C_8 interflavan bond of procyanidin B2. The main metabolites of PAs dimer B2 were 3-hydroxyphenylvaleric acid, 3-hydroxyphenylpropionic acid and phenylacetic acid. After 11 hours' incubation, 3-hydroxyphenylpropionic acid, 3-hydroxyphenylacetic acid, phenylacetic acid and 3, 4-dihydroxyphenylpropionic acids were identified as the metabolites of PAs dimer B2 with authentic standards. These compounds are all phenyl acids with basic unit of C_6-C_3, C_6-C_2, and C_6-C_1 (**Table 2**). Furthermore, other metabolites of epicatechin and PAs dimer B2 were speculated by their fragment ions under negative mass mode. Two exclusive metabolites of PAs dimer B2 were tentatively identified as 2,4-dihydroxyphenyl-2-ene-valeric acid and 3,4-dihydroxyphenylvaleric acid. These metabolites were suggested by progressive shortening of aliphatic chain by α- and ß-oxidations to to generate C_6-C_5, C_6-C_3, C_6-C_2 and C_6-C_6 skeletons (**Table 2** and **Fig. 1**).

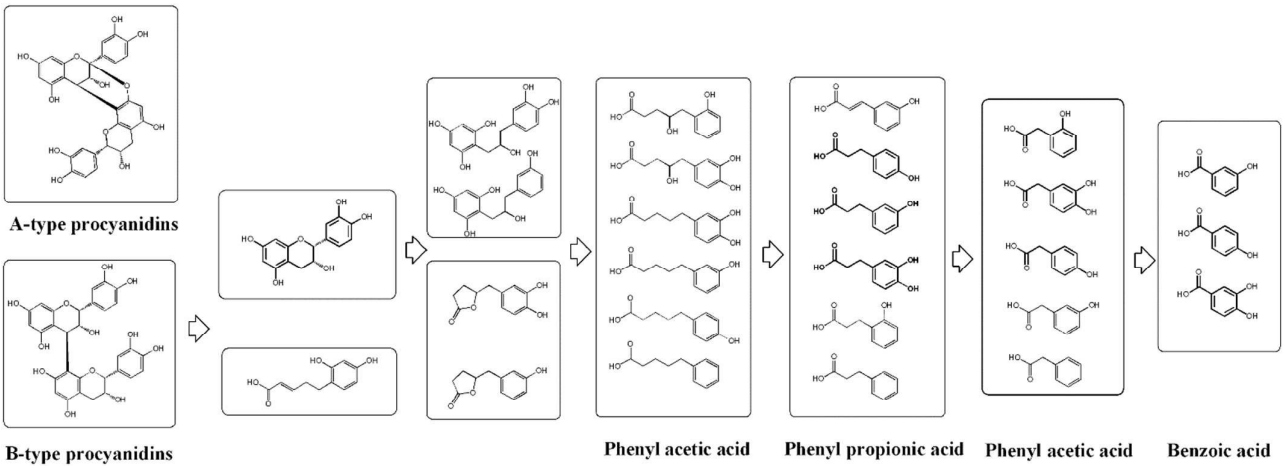

Fig. 1 The proposed metabolic pathway of A-type and B-type procyanidins. The bold chemical structures were the metabolites of A-type procyanidins; both bold and regular chemical structures are metabolites of B-type procyanidins.

The daily intake of PAs was usually from cocoa beverage or fruit juice, such as apple, grape and cranberry juice. The metabolites of PAs in these juices were more complicated than single PAs. Urpi-Sarda *et al.* studied the metabolism of epicatechin and PAs by intestinal microbiota[81]. A series of phenolic compounds were determined in the urine of rats or human after cocoa diet intake. The contents of ferulic acid (C_6-C_3), 3-hydroxyphenylacetic acids (C_6-C_2), 3-hydroxybenzoic acid (C_6-C_1) in urine were significantly increased after oral administration of cocoa.

Déprez *et al.* used gas chromatography coupled with mass spectrometry (GC-MS) to analyse the low molecular weight phenolic acids formed in the metabolism of polymeric PAs by human colonic microflora[63]. Three types of hydroxylation pattern were observed. They are 3-hydroxyphenyl group, non-hydroxylated aromatic acid and 4-hydroxyphenyl group acids. With the degradation of PAs polymers, the 3-hydroxyphenylpropionic acid and phenylic acid were the main aromatic acids, which were gradually increased, but the 3-hydroxyphenylacetic acid and 4-hydroxyphenylpropionic acid reached the highest abundance at 5 min, subsequently decreased after long time incubation.

Although the low molecular weight phenyl acids have been widely reported as the main metabolites, the content of phenolic acids wasn't equal to the reduction of PAs by fecal microflora fermentation. Stoupi *et al.* reported a detailed metabolic pathway of PAs dimer B2 under human fecal microflora fermentation[78]. A series of metabolites containing one whole molecule of epicatechin were identified. The PAs dimer B2 underwent an oxidative A-ring cleavage of flaval-3-ols. The results also showed that the conversion of PAs dimer B2 to epicatechin by cleavage of the interflavan bond did not account for more than 10% of the substrate, and the other 90% was degraded by the way of dimeric catabolites containing the part of a main body of flavnal-3-ol (**Fig. 2**).

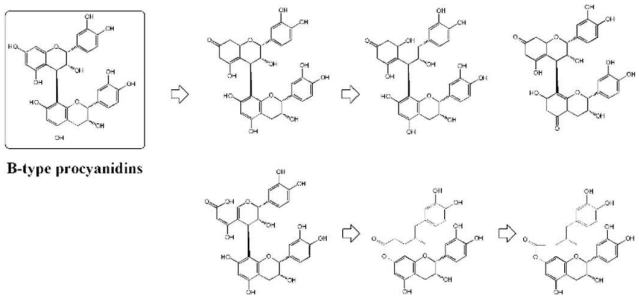

Fig. 2 The "dimeric" metabolites of procyanidin B2.

Excretion of PAs

Carbon-14 labelled is a potent tool to study the metabolism of PAs *in vivo*. Stoupi *et al.* used the single [^{14}C]-PAs dimer B2 to study the pharmacokinetics of PAs dimer B2 and evaluate the excretion of PAs dimer B2 in urine and feces[47]. In this paper, the total radioactivity of ^{14}C was calculated to represent the pure ^{14}C PAs dimer B2 *in vivo*. After intravenous administration, 76% of the dose was excreted via urine. After oral administration of ^{14}C-labelled PAs dimer B2, 58% of ^{14}C contained metabolites were excreted in urine.

Conclusion Remarks

Firstly, the PAs could be degraded into monomers, such as epicatechin and catechin under low pH (2.0) of gastric digestion. When the pH value of gastric juice was changed to 5.0, the degradation of PAs was scarcely detected. So, the pH value is critical for the cleavage of carbon-carbon bond of PAs.

In the small intestine, PAs dimers could be directly absorbed by passive paracellular transport, but the absorption was affected by the degree of polymerization. PAs with DP>4 are not absorbed. The efflux of PAs by p-glycoprotein lowers the permeation of PAs from the small intestine. The non-dialysable fraction containing PAs oligomers and polymers highly decreased the absorption of PAs in small intestine. COMT methylated a large part of PAs absorbed mainly in liver, but PAs reversely inhibited the activity of COMT and competed with catechin to decrease the methylation of catechins.

A large part of PAs were subjected to the metabolism of colonic microflora. These phenolic acids were mainly phenylvaleric acids, phenylpropionic acids, phenylacetic acids, benzoic acids derivatives and the phenyl valerolactone (one of the typical metabolites of catechins and gallocatechins). A small part of PAs dimers retained one intact molecular structure of catechin after colonic fermentation. So, the urinary metabolites of low molecular weight phenolic acids only accounted for a part of PAs intake. Using the ^{14}C labelled PAs, it was found that 58-78% PAs were excreted from urine after intravenous injection and oral administration, which indicated carbon dioxide after the oxidation of phenolic acids, was excreted by feces and aspiration. So far, the bioavailability of native PAs has been confirmed by various models *in vivo* and *in vitro*, but the mechanisms of health benefits of PAs have not been explored. The gut microflora may act as a mediate tool to evoke the forming of active metabolites of PAs, but still need more proofs. Furthermore, the typical bioavailability was mainly represented using prototype drug (compound), the integrated bioavailability of active phenolic metabolites of PAs should be more suitable.

The antioxidant ability of PAs was mainly relied on the B-ring dihydroxyl of flaval-3-ols, which could inhibit the gastrointestinal carcinogenesis *in vivo*. These compounds were hardly absorbed, so the main active compounds should be attributed to the low molecular phenolic acids after colonic metabolism. The human microbiota represents a whole systematic balance and health, the main metabolites of anaerobic bacteria produced a series of low molecular phenolic acids and short chain fatty acids. Until now, it is not clear how the polyphenols affect the properties and composition of colonic circumstance by phenolic acids, and then alter the human microbiota. From the reviewing on published results of absorption, metabolism and excretion of PAs, it was suggested that combination of the pharmacokinetics and pharmacology of metabolic products would be effective to exploring the mechanism of PAs' healthy benefits.

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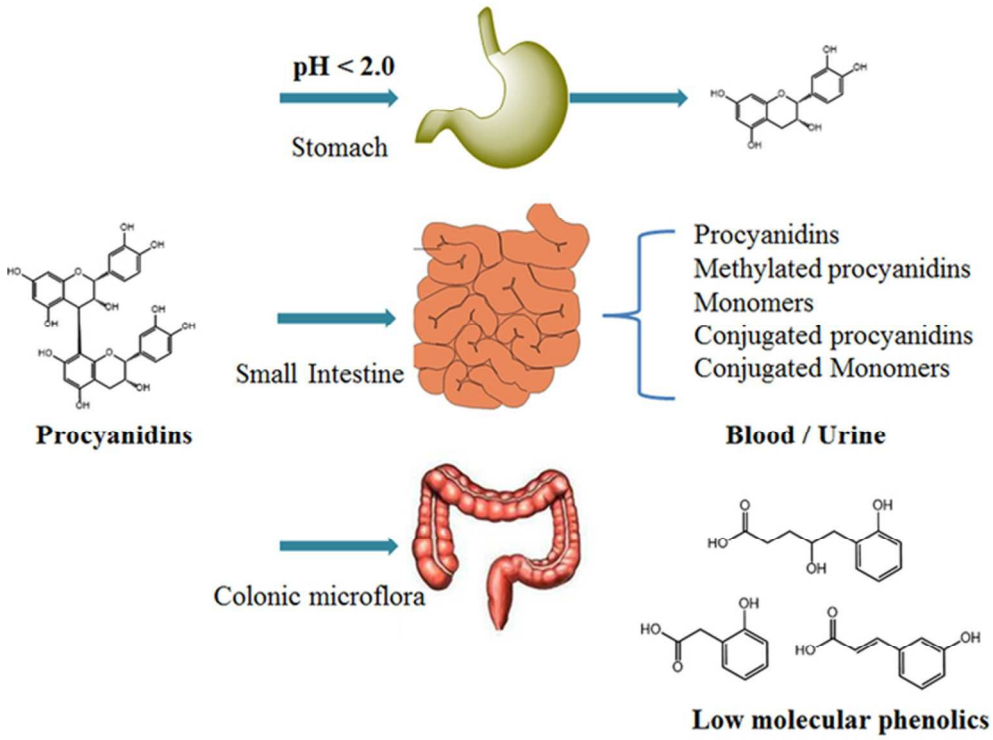

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