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Review

The bioavailability and absorption of anthocyanins: Towards a better understanding

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Evidence that anthocyanin compounds have beneficial effects for health are increasingly being reported in the scientific literature and these compounds are now widely recognised as potential therapeutic compounds. Berry fruit are rich sources of anthocyanins and berry fruit products or derived beverages can provide 10s to 100s of milligrams of anthocyanins in a single serve. Anthocyanins exhibit complex chemical behaviours *in vitro* and this will result in complex behaviour *in vivo*. This review attempts to summarize some aspects of anthocyanin biochemistry and discusses these in the context of what is currently known about bioavailability and absorption. Compared with other flavonoid groups, such as flavonols, relatively little is known about details and mechanisms of anthocyanin absorption and transport and much remains to be discovered.

Keywords: Absorption / Anthocyanins / Berryfruit / Bioavailability / Metabolism

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1 Introduction

Interest in the biochemistry and biological effects of anthocyanin compounds has increased substantially during the last decade because of increasing evidence demonstrating potential therapeutic effects. Anthocyanins have been reported to be strong antioxidants [1, 2], inhibit the growth of cancerous cells [3], inhibit inflammation [4], be vasoprotectors [5], and have anti-obesity effects [6]. The therapeutic properties of anthocyanins have been recently reviewed [7–9]. The occurrence of anthocyanins is widespread in plants and these compounds are consumed as part of a normal diet. Consumption was thought to be as much as 180–255 mg/day in the United States [10]; a value that far exceeds the consumption of most other flavonoids. Recent studies suggest that the average anthocyanin consumption is in the order of 82 mg/day in Finland and 12.5 mg/day in the United States [11]. Berry fruit are rich sources of dietary anthocyanins [12–15] and can contribute 10s to 100s of milligrams of anthocyanins in a single serving. Therefore, dietary choice can make a substantial impact on the amount of anthocyanin consumed. Many of the health benefits associated with berry fruit may be due to the high concentrations of anthocyanins that they contain.

The majority of evidence supporting a therapeutic effect of anthocyanins is *in vitro* or mechanistic in nature and there is still a lack of *in vivo* evidence from animal or human intervention studies. Any therapeutic effects that anthocyanins have are dependant on sufficient bioavailability both as exposure to cells and as exposure to a whole organism through the diet. Anthocyanins exhibit complex biochemistry and much still remains to be discovered about the biochemical activity of these compounds. This review will present and discuss what is currently known about the biochemistry of anthocyanins and discuss possible impacts on their therapeutic effects, with an emphasis on bioavailability and metabolism.

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Abbreviations: **BW**, body weight; **GIT**, gastrointestinal tract

2 Biochemistry of anthocyanins

Anthocyanins (Greek anthos = flower and kyanos = blue) are a group of over 500 compounds that provide the red, purple and blue colours of many vegetables and fruits [16]. Anthocyanins belong to a larger group of compounds collectively known as flavonoids, which are a subgroup of an even larger group of compounds known as polyphenolics.

Chemically, anthocyanins are glycosylated, polyhydroxy or polymethoxy derivatives of 2-phenylbenzopyrylium and

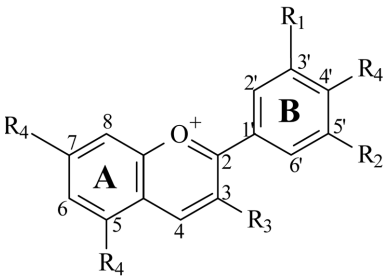

Figure 1. The flavylium cation. R_1, R_2 = H, OH or OCH_3; R_3 = OH or glycosyl; R_4 = OH or glycosyl.

contain two benzoyl rings (A and B) separated by a heterocyclic (C) ring (Fig. 1) [17]. The structural variations of anthocyanins are due to differences in the number of hydroxyl groups in the molecule, the degree of methylation of these hydroxyl groups, the nature and number of the sugar moiety attached to the phenolic (aglycone) molecule and the position of the attachment, as well as the nature and number of aliphatic or aromatic acids attached to the sugars [17]. Anthocyanins most frequently occur as 3-monosides, 3-biosides and 3-triosides as well as 3,5-diglycosides and more rarely 3,7-diglycosides associated with the sugars glucose, galactose, rhamnose, arabinose, and xylose [17].

Anthocyanins are reactive compounds and readily degrade, or react with other constituents in mixtures to form colourless or brown compounds. Loss of anthocyanin pigmentation also occurs in the presence of oxygen and various enzymes, and as a result of high temperature processing. Besides oxygen, temperature, light, and enzymes, pH has a marked effect on anthocyanin stability, and on the colour of media containing these pigments [18]. Although anthocyanins are flavonoids, their biochemistry is more complex than that of other flavonoid compounds. In aqueous solutions, anthocyanins exist as a number of different molecular forms that are in dynamic equilibrium. Originally it was thought that anthocyanins existed in aqueous solutions as three molecular interconvertable forms [19], but this increased to four [20], and recently eight distinct molecular structures were recorded [21]. The structures and interconversions between the different molecular structures of anthocyanins are summarized in Fig. 2. The red flavylium cation is the most abundant molecular form when the pH is <2. As the pH increases, there is a rapid loss of a proton to generate the blue quinonoidal structure. At the same time, a much slower hydration of the flavylium cation occurs to yield the colourless hemiketal form that further tautomerises through an opening of the C-ring to generate the chalcone (*cis* and *trans*) forms. The relative composition of the different molecular structures of anthocyanins coexisting in aqueous solution at any given time will be dependant on pH, temperature and time. This is a particularly important point as during the preparation of manufactured foods, product shelf-life and passage through the gastrointestinal tract (GIT), anthocyanins are exposed to different pH and temperature environments and the chemical forms and therefore bioactive properties of anthocyanins are likely to vary. Although anthocyanins are present usually as the red flavylium in foods, at neutral pH the flavylium cation concentration is very low and the other molecular forms will dominate. (*e.g.* quinonoidal bases, hemiketals, and chalcones) and will be responsible for the bioactivity observed. This feature of anthocyanin biochemistry is a substantial complication for efforts to understand the health benefits of anthocyanins. For example, standard antioxidant assays are performed at different pH and the outcomes are likely to depend on the pH of the assay, and the temperature and time between dilution of the substance to be tested and the actual assay.

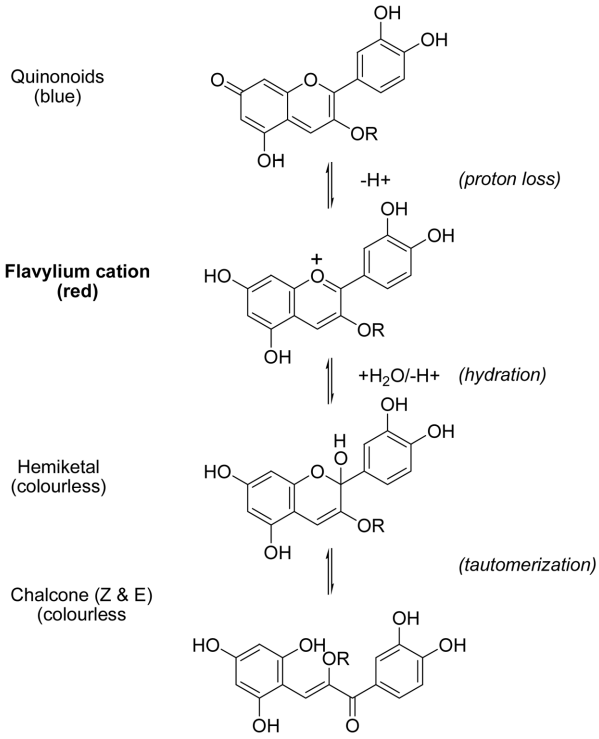

Figure 2. The various anthocyanin molecular structures that are known to be generated under different pH conditions.

3 Bioavailability of anthocyanins

Bioavailability is defined in various ways. With nutrients, for which metabolism is usual and appropriate and the route of administration is nearly always oral, the notion of bioavailability generally designates simply the quantity or fraction of the ingested dose that is absorbed [22]. The commonly accepted definition of bioavailability is the proportion of the nutrient that is digested, absorbed and metabolised through normal pathways. Consequently, it is not only

Table 1. Animal bioavailability studies

Species	Anthocyanin source	Dose[a] (per kg body weight)	C_{max}[b]	t_{max}[c] (h)	Urinary excretion [d] (%)	Reference
Rat	Bilberry	400 mg	2–3 μg/mL	0.25		[26]
Rat	Elderberry	360 mg	3.80 μmol/L	0.25		[27]
	Blackcurrant					
Rat	Purple corn	400 mg	0.31 μmol/L	0.5		[28]
Rat	Blackcurrant	359 mg Cy-3-glu[e]	0.84 μmol/L	0.5		[29]
		476 mg Cy-3-rut[f]	0.85 μmol/L	0.5		
		489 mg Dp-3-rut[g]	0.58 μmol/L	2.0		
Rabbit	Blackcurrant	117 mg	780 ng/mL	0.5	0.035 (4 h)	[48]
		164 mg	100 ng/mL	0.25	0.009 (4 h)	
		53 mg	450 ng/mL	0.5	0.023 (4 h)	
Rat	Blackcurrant	100 mg Dp-3-glu[h]	0.4 μmol/L	0.25		[32]
Pig	Marion berry	74 mg	0.103 μmol/L	1	0.088 (24 h)	[35]
Rat	Purple black rice	100 mg C3G[e]			0.005 (4 h)	[33]
Rat	Blackcurrant	100 mg Cy-3-glu[e]	0.18 μmol/L	0.25		[34]
Rat	Blackcurrant		0.36 μmol/L	3	0.190 (24 h)	[37]
Pig	Chokeberry	229 μmol			0.096 (24 h)	[36]
	Blackcurrant	140 μmol			0.067 (24 h)	
	Elderberry	228 μmol			0.131 (24 h)	
Pig	Blackcurrant	100 mg	0.09 μg/mL	2–4		[80]
Pig	Raspberry	50 mg			0.073 (4 h)	[81]

a) Total anthocyanins, if not stated otherwise.
b) Maximal plasma concentration.
c) Time to reach C_{max}.
d) % of intake.
e) Cy-3-glu = cyanidin-3-glucoside.
f) Cy-3-rut = cyanidin-3-rutinoside.
g) Dp-3-rut = delphinidin-3-rutinoside.
h) Dp-3-glu = delphinidin-3-glucoside.

important to know how much of a nutrient is present in a food or dietary supplement; but even more important to know how much of that present is bioavailable [23]. Bioavailability is sometimes defined simply as the proportion of a nutrient of bioactive component that was absorbed from the GIT, while others include metabolism, excretion, utilisation and a measure of efficacy in their definitions. However, bioavailability is often just characterised as plasma concentration [24]. The bioavailability of anthocyanins has recently been summarised [25] as part of a review of the bioavailability of flavonoids. Here, we will review the developing understanding of the bioavailability of anthocyanin despite the fact that the definition of bioavailability varies among authors.

3.1 Animal studies

Animal studies have been frequently used to investigate the bioavailability of anthocyanins and reported studies are summarized in Table 1. Most animal studies have found that anthocyanins are absorbed mainly in their intact glycosidic form, and rapidly reach the circulatory system within 0.25–2 h. For example, in rats, after a single oral administration [400 mg/kg body weight (BW)] of *Vaccinium myrtillus* anthocyanins the plasma concentrations reached peak level (2–3 μg/mL) after only 15 min and then rapidly declined within 2 h [26]. The fast appearance of intact anthocyanin in plasma [C_{max}: 3.8 μmol/L (1.8 μg/mL) at 15 min] after oral administration of red fruit anthocyanin (320 mg cyanidin-3-glucoside/kg BW) via stomach intubation into rats was confirmed [27]. However, neither aglycones nor conjugates of anthocyanins were present in rat plasma, suggesting that the flavylium cation structure is much more stable to bacterial hydrolysis than other flavonoids. Those results are supported by a study of Tsuda and colleagues [28]. After oral administration of cyanidin-3-glucoside (400 mg/kg BW) to rats, the intact form appeared rapidly in the plasma [C_{max}: 0.31 μmol/L (0.14 μg/mL) at 30 min], but again the aglycone cyanidin was not detected, although it was present in the jejunum. In addition, the authors found protocatechuic acid in the plasma, which they suggest to be produced by degradation of cyanidin, and its concentration was eight times higher than that of cyanidin-3-glucoside. Since a maximum cyanidin-3-glucoside concentration after only 15 min in stomach tissue was found, anthocyanin may already be absorbed from this organ.

Measurement of urinary excretion has also often been used to assess bioavailability. Blackcurrant anthocyanins were directly absorbed in the blood (t_{max} at 0.5–2 h), and

Table 2. Human bioavailability studies

Material	Anthocyanin dose[a] (total intake)	C_{max}[b]	t_{max}[c] (h)	Urinary excretion[d] (%)	Reference
Red wine (300 mL)	218 mg			5.10 (12 h)	[38]
Elderberry extract (25 g)	1.5 g	100 ng/mL	0.5		[39]
Blackcurrant	236 mg	0.120 μmol/L	1.25 – 1.75	0.11 (8 h)	[29]
Elderberry juice (spray dried capsules)	180 mg	35 ng/mL	1		[82]
Blackcurrant juice (200 mL)	153 mg			0.02-0.05 (5 h)	[41]
Red wine (500 mL)	68 mg Mv-3-glu[e]	0.0014 μmol/L	0.8	0.02 (6 h)	[42]
Dealcoholized red wine	56 mg Mv-3-glu[e]	0.0017 μmol/L	1.5	0.02 (6 h)	
Red grape juice (500 mL)	117 mg Mv-3-glu[e]	0.0028 μmol/L	2.0	0.02 (6 h)	
Elderberry (11 g)	1.9 g			0.003 – 0.012 (6 h)	[43]
Blueberry powder (100g)	1.2 g	0.029 μmol/L	4		[83]
Elderberry extract (12 g)	720 mg	0.097 μmol/L	1.2	0.06 (24 h)	[84]
Elderberry extract (12 g)	720 mg			0.08 (4 h)	[61]
Blueberry (189 g)	690 mg			0.004 (6 h)	[61]
Red wine (400 mL)	180 mg	43 ng/mL	1.5	0.23 (7 h)	[45]
Red grape juice (400 mL)	284 mg	100 ng/mL	0.5	0.18 (7 h)	[45]
Blackcurrant juice	1.24 g	53 ng/mL	0.75	0.07 (4 h)	[48]
	0.72 g	16 ng/mL	0.75	0.05 (4 h)	
	0.75 g	32 ng/mL	1.5	0.05 (4 h)	
Blackcurrant concentrate (300 mL)	189 mg			0.06 (7 h)	[44]
Boysenberry concentrate (300 ml)	345 mg			0.03 (7 h)	[44]
Blueberry extract (300 mL)	439 mg			0.02 (7 h)	[44]
Strawberries (200 g)	76 mg			1.80 (24 h)	[46]
Chokeberry extract (7.1 g)	721 mg	0.096 μmol/L	2.8	0.15 (24 h)	[58]
Blackberries (200 g)	431 mg			0.16 (24 h)	[47]

a) Total anthocyanins if not stated otherwise.
b) Maximal plasma concentration.
c) Time to reach C_{max}.
d) % of intake.
e) Mv-3-glu = malvidin-3-glucoside.

excreted into urine as the glycosylated forms in rats after oral administration of three purified anthocyanins (delphinidin-3-*O*-β-rutinoside, cyanidin-3-*O*-β-rutinoside, cyanidin-3-*O*-β-glucoside). No other peaks, or potential metabolites were detected [29].

Felgines and colleagues [30] have shown that blackberry anthocyanins are excreted in urine as intact as well as methylated forms, but no aglycones or conjugated forms were detected. Furthermore, they detected low amounts of anthocyanins as well as aglycones in caecal contents, suggesting an adaptation of microflora for anthocyanin degradation. In addition, anthocyanins and their metabolites have been reported in bile for the first time after 20 min, which suggests rapid absorption and metabolism [31].

More recent anthocyanin absorption studies in rats reported the occurrence of methylated anthocyanins in plasma [32]. After an oral administration of 100 mg delphinidin-3-glucoside/kg BW, a maximum concentration (0.4 μmol/L) appeared in plasma within 15 min. The methylated form of delphinidin-3-glucoside showed a maximum plasma concentration after 1 h. Further studies detected the glucuronides of anthocyanins in rat plasma, and it was suggested that these metabolites are mainly produced in the liver, rather than by intestinal flora [33, 34]. Both studies applied a 100 mg cyanidin-3-glucoside/kg BW dose to rats, and reported maximum concentrations for the original compound (0.18 μmol/L; t_{max}: 15 min), methylated metabolites (<0.021 μmol/L; t_{max}: 15 – 120 min), and glucuronidated compounds (<0.07 μmol/L; t_{max}: 15 – 60 min). The glucuronide and methylated conjugates of anthocyanins have also been shown to be the two major types of metabolites that appear in urine in pigs [35]. The original anthocyanins showed a maximum plasma concentration of 0.103 μmol/L after 1 h. The urinary recovery of the original anthocyanins and their related metabolites was 0.088%. Wu and colleagues [36] administered three kinds of berry types with different anthocyanin profiles to pigs and suggested that the aglycone and the sugar moieties alter the absorption and metabolism of anthocyanins. Talavera and colleagues [37] were the first to report the original as well as methylated and glucuronidated metabolites of anthocyanins in the jejunum, liver and kidneys of rats.

3.2 Human studies

The number of human studies investigating the bioavailability of anthocyanins has increased rapidly over the last decade and these are summarised in Table 2. In an early

study, Lapidot and colleagues [38] investigated the bioavailability of red wine anthocyanins (218 mg anthocyanin/300 mL) after ingestion of a normal amount of red wine (two glasses). They measured a urinary excretion rate of 5% of the dose over a 12-h period; a rate much greater than in more recent studies. In another early study Cao and Prior [39] reported direct evidence of the absorption of anthocyanins (intake total anthocyanins: 1.5 g) in their glycosidic form in humans. The plasma anthocyanin concentration was reported to be at least 100 μg/L 30 min after consumption of the elderberry extract.

Matsumoto and colleagues [29] found blackcurrant anthocyanins to be directly absorbed, distributed to the blood and excreted into urine as the glycosylated forms in rats and humans. In their human study, they detected blackcurrant anthocyanins in both the plasma (0.120 nM) and the urine (0.11% of the dose ingested) also as the intact form, after subjects ingested a single dose of blackcurrant concentrate (3.57 mg cyanidin-3-glucoside/kg BW). Their results indicate that anthocyanin glycosides can be absorbed rapidly within 2 h of ingestion and are excreted in urine as the intact form. The extremely low bioavailability of anthocyanins has also been shown in a human study by Murkovic and colleagues [40]. After ingestion of 180 mg anthocyanin (spray-dried elderberry juice as gelatinous capsules), the maximum plasma concentration was found to be 35 ng/mL. Besides low bioavailability, the authors suggest a quick degradation or excretion of the compounds. Another study, using 200 mL blackcurrant juice (153 mg of anthocyanin), found that only 0.02–0.05% of the oral dose was excreted in urine [41].

Malvidin-3-glucoside, an anthocyanin occurring in red wine and red grape juice was studied by Bub and colleagues [42]. After ingestion of red wine (68 mg malvidin-3-glucoside/500 mL) or red grape juice (117 mg malvidin-3-glucoside /500 mL), malvidin-3-glucoside was found in plasma (C_{max}: 1.4 nM at 20 min, red wine; C_{max}: 2.8 nM at 180 min, red grape juice) and urine (<0.03% of the ingested amount of red wine, and red grape juice, respectively) of human volunteers. Neither aglycones nor glucuronate or sulphate conjugates were found in plasma and urine samples, indicating that malvidin-3-glucoside is absorbed in its glucosylated form.

More recent studies still confirm a low bioavailability of anthocyanins. After ingestion of 11 g elderberry (containing 1.9 g anthocyanin), very low recoveries were found in urine (0.003–0.012% of the oral dose) [43]. In addition, our group has found low anthocyanin-excretion in urine (0.01–0.06%) over a 7-h period after ingestion of Boysenberry concentrate (345 mg anthocyanin), blackcurrant concentrate (189 mg anthocyanin), and blueberry extract (439 mg anthocyanin) [44]. Frank and colleagues [45] found urinary excretions of anthocyanins between 0.18 and 0.23%, after the ingestion of a single oral dose of either 400 mL red grape juice (283.5 mg total anthocyanins), or 400 mL red wine (279.6 mg total anthocyanins). Felgines and colleagues [46] on the other hand reported a urinal excretion of 1.80%, after consumption of 200 g strawberries providing 179 μM pelargonidin-3-glucoside, which is to date the highest reported recovery. In a more recent study, investigating blackberry anthocyanins (mainly cyanidin-3-glucoside), the same authors showed a urinary excretion of only 0.16% [47]. The considerable difference in urinal excretion indicates a possible difference in the bioavailability of individual anthocyanins, as had been shown in pigs by Wu and colleagues [36]. A recent study, which compared the absorption and excretion of blackcurrant anthocyanins in humans and rabbits, found no differences between the two species in the percentage of the ingested dose excreted in urine at 4 h after ingestion [48].

3.3 Anthocyanin bioavailability: Where to from here?

The bioavailability of anthocyanins differs from other flavonoids such as the flavonols (*e.g.* quercetin glycosides) in a number of key aspects. First, the apparent bioavailability is consistently very low across all studies with often less than 0.1% of the ingested dose appearing in the urine. Secondly, bioabsorption occurs quickly following consumption. The t_{max} in plasma is 15–60 min. and excretion is complete within 6–8 h. These observations have led to the suggestion that anthocyanins are absorbed from the stomach. Thirdly, intact anthocyanin glycosides appear to be absorbed, distributed into the circulatory systems and excreted in the urine.

The most pressing issue for the bioactivity of anthocyanins is to confirm the apparent low bioavailability. This will continue to be difficult to achieve, since anthocyanins exist in a number of different molecular structures and there are a number of potential metabolites that can be generated both *in* vitro and in the GIT. One approach to help to address this bioavailability issue is the use of isotopically labelled anthocyanins to estimate bioavailability and subsequent transportation, accumulation into various tissue, and excretion.

Most, if not all, studies on anthocyanin bioavailability and absorption have used detection methods that are based on the measurement of anthocyanins as red flavylium cations by HPLC. From the preceding discussion, it is clear that the flavylium cation form of anthocyanin is unlikely to exist *in vivo*. Consequently, all current assessments have *indirectly* measured anthocyanin bioavailability by relying on the measurement of the flavylium cation when it is probably the hemiketal and chalcone forms of anthocyanins that are likely to be present *in vivo* and participate in absorption and metabolism reactions. As presented in Fig. 3, anthocyanin structures that fail to regenerate the flavylium cation because of *in vivo* metabolism or modification will not be detected by analytical methods based on HPLC separation

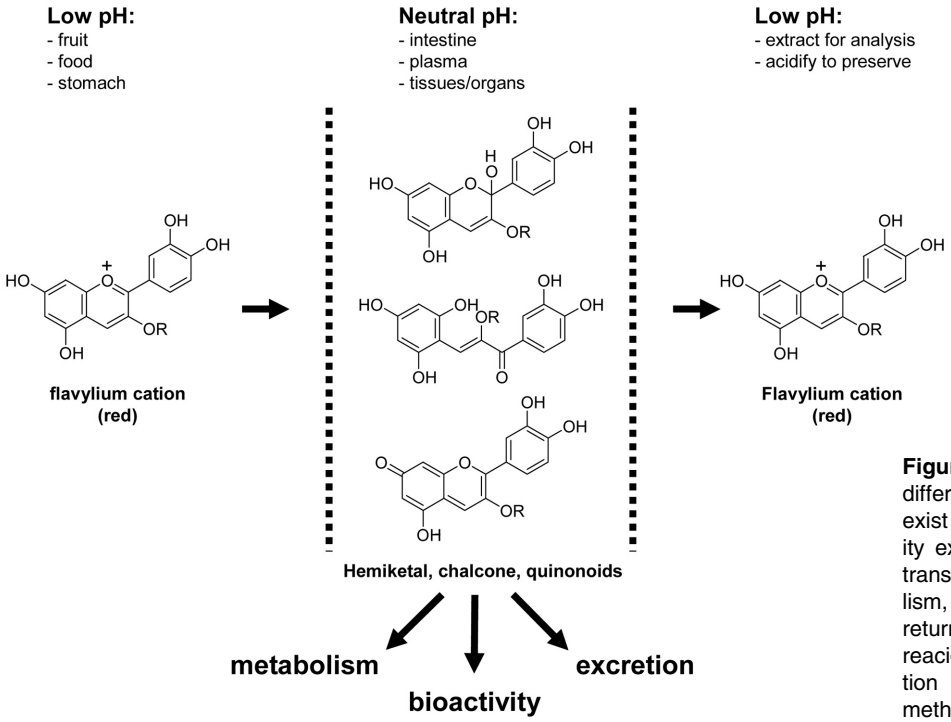

Figure 3. Schematic representing the different forms of anthocyanins that exist during an absorption/bioavailability experiment. Anthocyanins that are transformed during bioactivity, metabolism, or excretion and are unable to return to the red flavylium cation upon reacidification during sample preparation will *not* be detected by current methods of analysis by HPLC.

and detection at 530 nm. Much progress towards understanding the bioavailability and bioactive behaviour of anthocyanins might be made if selective and sensitive methods for determining the alternative molecular structures of anthocyanins (quinoniodal bases, hemiketals, and chalcones) were available. Currently, such methods do not exist.

4 Metabolism and molecular modification

The anthocyanins present in food have a range of different molecular structures as a result of biosynthesis and accumulation in plant tissues. Many fruit contain monoglycosides of the six main aglycones (Fig. 1), but some foods, particularly red vegetables also contain acylated anthocyanins. Recently, anthocyanin-flavan-3-ol conjugates have also been reported [49]. These naturally occurring anthocyanins can be modified before consumption, particularly during the processing and storage/shelf life of manufactured foods. For example, red wine anthocyanins undergo a number of molecular modifications during wine maturation [50]. Therefore, in processed foods, the anthocyanins consumed may not be the same compounds that were present in the plants used for manufacture.

On entry into the body, the components of food experience numerous different environments and physiochemical conditions that may lead to molecular modification. Within the GIT there are distinct environments (*e.g.* stomach, small intestine, colon). Following absorption from the GIT, physiological compartments and tissues have distinct physiochemical environments.

4.1 Metabolism in the GIT

There appears to be no information on the effect of saliva and the upper GIT on anthocyanins. However, some catechol-containing flavonoids have been shown to increase nitric oxide bioavailability when exposed to saliva, suggesting that anthocyanin of the cyanidin types might have a similar effect [51]. The GIT is characterised by regions with different pH and different microbial populations; both features which may modify anthocyanin compounds. The pH of the stomach is low, in the range of 1–2, which should ensure that anthocyanins are maintained as the flavylium cation, which is the most stable form of anthocyanins. The stability of anthocyanins in conditions under the gastric conditions of the stomach has been confirmed by *in vitro* digestion studies [52, 53]. In contrast to the stomach, the environment of the small and large intestines is largely at neutral pH, where multiple molecular forms of anthocyanin will be present and where anthocyanins are much less stable [4]. Furthermore, the microbial populations, in particular in the colon, are likely to modify the molecular structures of anthocyanins. Although only a limited number of studies have been carried out, Keppler and Humpf [54] have shown in an *in vitro* model using microflora isolated from pig caecum that anthocyanins might be substantially modified. They investigated five different anthocyanins and showed

that exposure to gut microflora resulted in rapid deglycosylation and demethylation to the corresponding aglycones. The aglycones were unstable at neutral pH and rapidly degraded to their corresponding phenolic acids and aldehydes through cleavage of the C-ring. Similar results were obtained in studies using two cyanidin anthocyanins and an acylated anthocyanin and incubation with human faecal microbiota [55, 56]. The studies to date show that anthocyanins are little modified by gastric conditions, but probably extensively modified by gut microflora, and that anthocyanins that are not absorbed in the stomach or small intestine are substantially modified to phenolic acids in the colon. Modification by conditions in the small intestine is more difficult to assess and remains to be described.

4.2 Metabolism during or after absorption

Although anthocyanins are reactive compounds, initial studies on bioabsorption found the same anthocyanins that were consumed were also present in the urine [28, 29, 44]. However, in most of these studies additional anthocyanin-like peaks were also detected by HPLC and were eventually identified as a variety of glucuronide and methylated derivatives, indicating that anthocyanins, like other flavonoids are subject to extensive conjugation *in vivo* [47, 57, 58]. The *in vivo* metabolism of anthocyanins has been discussed in two recent reviews by Prior and Wu [59] and Kay [60] and should be consulted further. Even though the flavylium cation form of anthocyanins is not likely to be present *in vivo* at neutral pH, many discussions on anthocyanin metabolism consider the flavylium cation as the starting point for metabolism [35, 36, 61]. This is probably because most of the metabolites identified so far have been flavylium cation-based metabolites that were present in pH neutral biological samples. Analysis usually involves acidification and measurement by HPLC or LCMS methods that are specific for the flavylium cations. It is possible that a different picture of anthocyanin metabolism will emerge when methods to directly observe an measure the quinoidal, hemiketal, and chalcone forms (and their metabolites) are available.

5 Absorption and transport mechanisms

Anthocyanins are large, highly water-soluble molecules that have been considered unlikely to be absorbed in cells or into the circulatory systems of animals and humans [62]. However, the bioavailability data discussed above demonstrate that anthocyanins are apparently absorbed from the GIT, transported into the circulatory system and excreted in urine. Although there have been numerous studies investigating the absorption/bioavailability of anthocyanins, compared with other classes of flavonoids, such as the flavonols, comparatively little is known about absorption and transport mechanisms of anthocyanins.

5.1 Anthocyanins are absorbed through cell membranes

There are many studies demonstrating the bioactivity of anthocyanins towards mammalian cells such as protection against oxidative stress [63]; induction of apoptosis and growth inhibition [64–66], and changes in inflammatory response [67]. Although such studies are not proof of the absorption of anthocyanins into cells, they do demonstrate that anthocyanins have the potential to interact with cells directly. Despite the high water solubility of anthocyanins, it has been demonstrated that when cells are exposed to anthocyanins they do indeed cross the cell membrane and can be detected in the interior of cells [68]. Recently, the absorption of blueberry anthocyanins into Caco-2 human intestinal monolayer was investigated [69]. Although transport efficiency was low, variations in absorption were observed between different phenolic aglycones and sugar moieties of the anthocyanins studied. For example, comparison between delphinidin-3-glucoside and malvidin-3-glucoside showed that the malvindin anthocyanin had the highest transport efficiency. Comparison between galactoside and glucosides with the same aglycone showed that glucosides had higher transport efficiencies. In general, although many studies have investigated the effects of anthocyanin on cells, few studies have attempted to measure the absorption of anthocyanins into cells or the metabolites that might be present following exposure.

5.2 Anthocyanin glycosides affect absorption and are absorbed intact without modification

Many of the bioavailability studies discussed above have used mixtures of anthocyanins and demonstrated that absorption and excretion vary between the different anthocyanin structures. For example, when an anthocyanin rich extract of boysenberry was administered to rats, less cyanidin-3-glucoside was recovered in the urine relative to the other anthocyanin components. This suggests that the bioabsorption of cyanidin-3-glucoside differs from the other anthocyanins (cyanidin bio- and triosides) in boysenberry [44]. Blueberry contains a variety of anthocyanins that are monoglucosides (galactoside, glucoside, and arabinosides) of five aglycones (delphinidin, cyanidin, petunidin, peonidin, malvidin) and these have been used in studies to investigate differences in bioabsorption. Considering urinary excretion as an indication of absorption, in rats relatively more delphinidin glycoside is absorbed than malvidin glycoside and relatively more galactoside is absorbed than arabinoside, indicating that both phenolic aglycone and sugar moiety affects absorption [44]. In support of these results a recent study using similar anthocyanins, but sourced from bilberry, determined the pharmacokinetic of 15 anthocyanins. Plasma concentrations were highest for galactosides and lowest for arabinosides, and among the aglycones,

plasma concentrations were higher for the delphinidins and cyanidins than for the malvidin anthocyanins [70].

All studies investigating anthocyanin absorption and bioavailability have reported the absorption of intact anthocyanin glycosides. This appears to be a key difference between the absorption of anthocyanins and other flavonoids. Unmodified absorption of unglycosylated flavan-3-ols (*e.g.* catechin) has also been observed when high doses were given and this is believed to be because of the saturation of the metabolic pathways. Some of the reported studies with anthocyanins also used large doses, but other studies have used realistic doses that can be achieved by normal dietary intake. Since some food sources contain substantial amounts of the anthocyanins, it needs to be determined if these 'normal' doses do indeed saturate metabolic pathways and lead to absorption of the intact anthocyanin glycosides. Recently, Passamonti and colleagues [71] detected intact malvidin-3-glucoside in portal plasma after the introduction of 2 mg malvidin-3-glucoside to the stomach of a rat. The absorption of intact anthocyanin glycoside was confirmed with a level of dosing that would be unlikely to saturate *in vivo* metabolism.

5.3 The stomach and jejunum are sites for anthocyanin absorption

Bioavailability studies have shown that anthocyanins appear rapidly in the circulatory system and in various tissues following dosing. This has been interpreted as being because of absorption of anthocyanins from the stomach. However, the gastric absorption of nutrients is relatively unusual and the rapid absorption of quercetin glycosides has been interpreted as being due to absorption from the small intestine. Additionally, residence time of dose in the stomach may be short, as many of the anthocyanin bioavailability studies have been performed with fasted animals or humans and with liquid formulations. Direct evidence of the absorption from the stomach has been demonstrated using *in situ* gastric exposure to anthocyanins [31, 71 – 73]. Additionally, anthocyanins have been shown to interact with bilitranslocase, providing a plausible mechanism for the absorption of anthocyanins from the stomach [74]. There is also direct evidence that anthocyanins are absorbed from the small intestine of rats. Using an *in situ* perfusion of rat intestines, it was shown that anthocyanins were efficiently absorbed and excreted into the bile and urine [75]. Our group has measured the absorption of cyanidin-3-glucosides from various tissues of the mouse GIT using an *in vitro* method [76]. Cyanidin-3-glucoside was significantly absorbed by tissue from the jejunum and slightly absorbed by duodenal tissue. No absorption was measured for tissue from the ileum and colon. These results corroborate the *in situ* studies and suggest that an active transport mechanism for anthocyanins is present in the jejunum but absent in the ileum and colon. We further studied the mechanism of absorption in the presence of glucose and phloridzin, compounds that inhibit transport by the sodium-dependent glucose transporter (SGLT1) and inhibit lactase phloridzin hydrolase (LPH) activity. Both of these are believed to be involved in the absorption of quercetin glycosides from the small intestine [77, 78]. Neither of these compounds substantially affects the absorption of cyanidin-3-glucoside by jejunum tissue, suggesting that the transport mechanisms of quercetin glycosides are unrelated to those for anthocyanins. However, in this study we also found that the presence of quercetin-3-glucoside significantly reduced the absorption of cyanidin-3-glucoside [79]. This result suggests that the absorption of anthocyanins and flavonols is related. Further research is needed to clarify these findings, but a follow up *in vivo* study using pigs found that the presence of rutin (quercetin rutinoside) had no effect on the absorption of blackcurrant anthocyanins [80].

5.4 Are anthocyanins mainly absorbed from the colon as metabolites?

Since only a small percentage of the anthocyanins consumed by either animals and or humans is excreted in the urine, there is a possibility that most of the anthocyanins that are consumed remain in the GIT. However, a number of studies have shown that anthocyanins are absorbed from the stomach [31, 71, 73], and the small intestine [31, 76, 79, 81]. While these studies demonstrate the potential for substantial absorption, they invariably assess absorption as disappearance of anthocyanin from the test system. There is a possibility that anthocyanin is transformed into molecular structures that are not detected by the current analytical methods, rather than being absorbed. Recent studies have shown that anthocyanins can be extensively transformed by microbial populations taken from the gut [55, 56], suggesting the possibility that large concentrations of anthocyanin-derived compounds might be present in GIT. Support for this view was provided by Tsuda and colleagues [28], who found a plasma concentration of protocatechuic acids eight times greater than that of cyanidin-3-glucoside. Protocatechuic acid is a potential degradation product of cyanidin-based anthocyanins. There are a large number of compounds that potentially could be formed from the degradation of anthocyanins in the GIT, which presents a significant analytical challenge. To our knowledge, a comprehensive study of anthocyanin transformation products present in the GIT has not been reported. Such a study would be a valuable contribution towards the understanding of anthocyanin bioactivity.

6 Summary of current knowledge

As the evidence of therapeutic effects of phytochemicals, and anthocyanins in particular, continues to accumulate, it

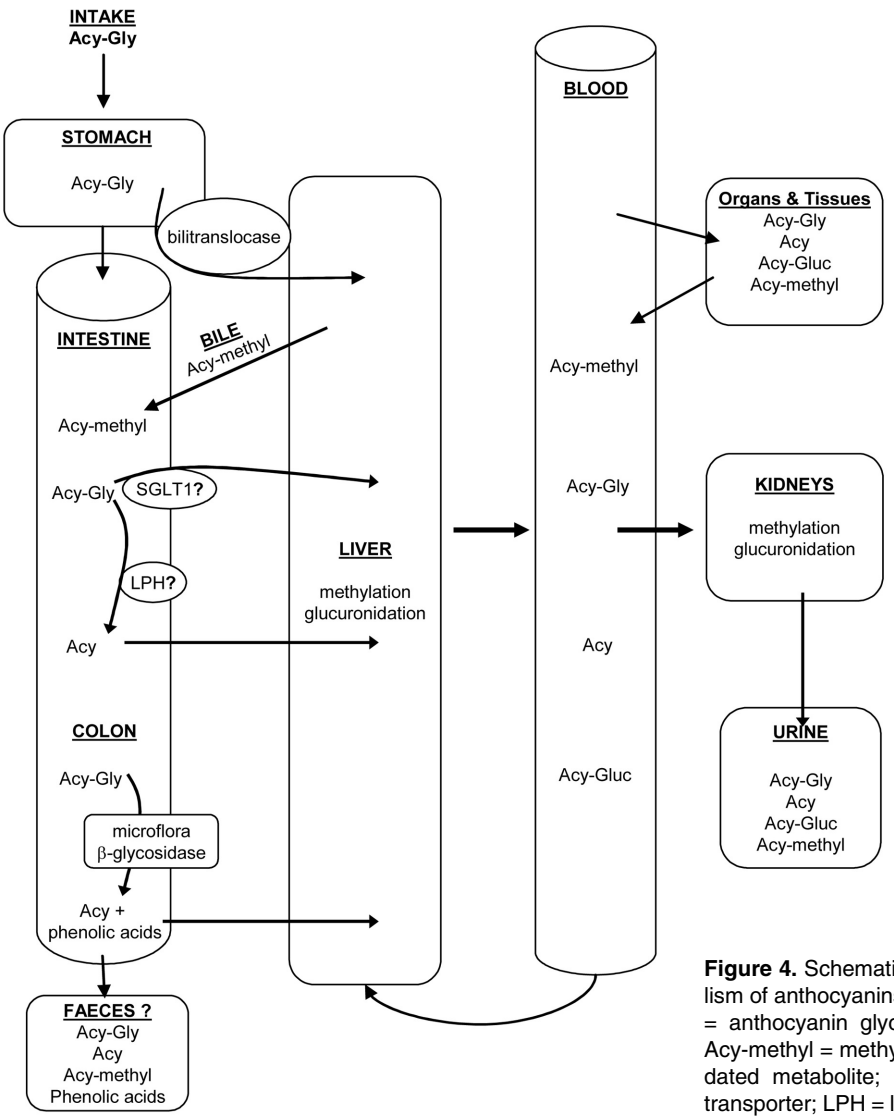

Figure 4. Schematic depicting the absorption and metabolism of anthocyanins based on current information. Acy-Gly = anthocyanin glycosides; Acy = anthocyanin aglycone; Acy-methyl = methylated metabolite; Acy-Gluc = glucuronidated metabolite; SGLT1 = sodium dependent glucose transporter; LPH = lactase phloridzin hydrolase.

is becoming more important to understand the nature of absorption and metabolism *in vivo*. Greater understanding of these processes will enable the development of new food products, both fresh and manufactured, with greater therapeutic efficacy. Currently, relatively little is known about how anthocyanins and compounds derived from them enter the body, distribute to tissues and exert beneficial health effects. Figure 4 is a representation of the absorption of anthocyanins based on current knowledge.

Anthocyanin glycosides can be rapidly absorbed from the stomach after ingestion by a process that may involve bilitranslocase, and they enter the systemic circulation after passing through the liver. A portion of the anthocyanin is metabolised by methylation and glucuronidation reactions and some of the metabolites are transported to the intestine as bile. Anthocyanin glycosides that are not absorbed from the stomach move into the small intestine where, because of the higher pH they convert to a combination of hemiketal, chalcone, and quinonoidal forms. Further absorption appears to take place in the jejunum. The transport mechanism involved has not been identified, but if similar to flavonols, may involve hydrolysis of the glycosides by various hydrolases and absorption of the phenolic aglycone. Absorbed anthocyanins enter the systemic circulation after passage though the liver and may be metabolised. Anthocyanins that reach the colon are exposed to a substantial microbial population and may be degraded to sugar and phenolic components, with the phenolic components further degraded by disruption of the C-ring to yield phenolic acids and aldehydes. These products, derived from the ingested anthocyanins, may contribute to the health effect of anthocyanins either directly in the GIT or after absorption from the colon.

Much of the detail is missing about how anthocyanins are absorbed, how the variation of molecular structures consumed in food, and the forms generated *in vivo* contribute to

the health benefits. Greater understanding will generate the potential for consumers to gain even more health benefits for high anthocyanin-containing foods such as berry fruit, than is currently the case.

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7 References

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