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The Case for Anthocyanin Consumption to Promote Human Health: A Review

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Abstract: Anthocyanins belong to the flavonoid group of polyphenolic compounds, which are responsible for the red and blue colors of plant organs such as fruits, flowers, and leaves. Due to their frequent presence in plants, particularly berry fruits, vegetables, and grapes, they are key components of the human diet. Interest in anthocyanins has increased widely during the past decade. Numerous studies have suggested that anthocyanins have a wide range of health-promoting properties. These compounds are therefore considered to be a functional food factor, which may have important implications in the prevention of chronic diseases. The aim of this body of work is to investigate and review the current literature on anthocyanins, and particularly their pharmacokinetics and any health-promoting properties, in order to summarize existing knowledge and highlight any aspects that require further study and analysis.

Introduction

Anthocyanins (Greek anthos = flower and kyáneos = blue) belong to the flavonoid group of polyphenols, which are responsible for the red and blue colour of plant organs such as fruits, flowers, and leaves (Strack and Wray 1993). The anthocyanin molecule naturally occurs in plants as a glycoside (gly) where the anthocyanidin is bound to a sugar group, with glucose, galactose, rhamnose, xylose, or arabinose bound to an aglycon (Mazza and Miniati 1993). Chemically, anthocyanidins are polyhydroxy or polymethoxy derivatives of 2-phenylbenzopyrylium: 2 benzoyl rings (A and B) separated by a heterocyclic (C) ring (Figure 1; Mazza and Miniati 1993).

There are approximately 17 anthocyanidins found in nature, only 6 of which are widely distributed: cyanidin (cy), delphinidin (dp), petunidin (pt), peonidin (peo), pelargonidin (pl), and malvidin (mv; Figure 2) (Prior and Wu 2006). Differences between individual anthocyanidins come from: (i) the number and the position of hydroxyl (OH) groups, (ii) the degree of methylation of these OH groups, (iii) the nature, number, and location of sugars attached to the molecule, and (iv) the nature and number of aliphatic or aromatic acids attached to the sugar (Mazza and Miniati 1993). Glycosylation confers increased stability and water solubility to the parent anthocyanidins, and acylation of the sugar residues with cinnamic acid or an aliphatic acid further improves anthocyanin stability (Mazza and others 2004). Anthocyanins are unique in terms of their capacity to change structure following changes in the pH value. For example, at pH 1 to 3 they occur under flavylium cation form, which is the most stable form, and are red. At pH 5 they are converted into the less stable, colorless carbinol pseudobase which via a water-catalyzed tautomeric equilibrium could turn to a chalcone, whereas at pH 7 to 8 the blue–purple quinoidal base is formed (Brouillard and Delaporte 1977; Lapidot and others 1999). These natural compounds are well represented in the human diet, as they occur in berries (Table 1), other red, blue, or purple fruits, and in red wine (Mazza and Miniati 1993). Their content in red wine depends on different factors, including geographical origin, cultivar or variety (Makris and others 2006), and the age of the wine. For example, in 1-y-old red wine the total anthocyanins content ranges from 40 to 1269 mg/L and decreases about 60% in 4-y-old bottled wine (Mattivi and Nicolini 1997). Red wine generally contains glycosides of the 5 major anthocyanidin types: delphinidin, cyanidin, petunidin, peonidin, and malvidin, with pelargonidin occurring at trace levels (Waterhouse 2002).

Health-promoting properties are attributed to anthocyanidins (Clifford 2000). Many studies have linked these compounds with antioxidant, anti-inflammatory and anticarcinogenic properties, protection against both heart disease and cancer (of certain types), as well as a reduction in the risk of diabetes and cognitive function disorders. All have been substantially reviewed (Zafra-Stone and others 2007; He and Giusti 2010; Tsuda 2012).

Depending on the diet, the daily intake of anthocyanins in humans has been estimated to range from several milligrams to hundreds of milligrams (Morazzoni and Bombardelli 1996). Kühnau (1976) estimated an anthocyanin consumption of 200 mg/d in the U.S. population, but further studies demonstrated that this value was an overestimation due to inaccurate food intake data. Indeed, Wu and others (2006) estimated the average anthocyanin intake in U.S. adults as 12.5 mg/d. Zamora-Ros and others (2011) calculated the anthocyanin intake of people

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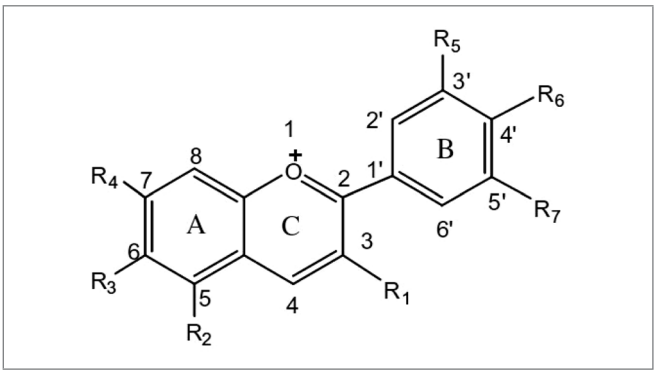
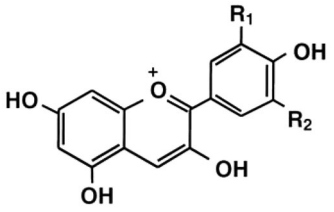


Figure 1–Chemical structure of anthocyanins.



R_1	R_2	Anthocyanidin
H	H	Pelargonidin
OH	H	Cyanidin
OCH_3	H	Peonidin
OH	OH	Delphinidin
OCH_3	OH	Petunidin
OCH_3	OCH_3	Malvidin

Figure 2–Structural classification of 6 most common anthocyanidins.

in European countries. The total mean intake of anthocyanidins for men ranged from 19.8 mg/d (Bilthoven, the Netherlands) to 64.9 mg/d (Turin, Italy), whereas for women the range was 18.4 mg/d (Granada, Spain) to 44.1 mg/d (Turin, Italy). Higher consumption by Italians may result from their Mediterranean diet, which includes berries, and other red and blue-colored fruits, and red wine. For example, Wu and others (2006) reported that a glass of wine provided approximately 20 to 35 mg of anthocyanins. Now-a-days, the dietary intake of anthocyanins may be considerably enhanced with the consumption of commercially available anthocyanin extract (Morazzoni and Bombardelli 1996).

There appears to be an inverse correlation between the consumption of dietary-derived phenolic compounds and the incidence of cardiovascular disease, certain cancers and other degenerative diseases (Wang and Stoner 2008), although these relationships are yet to be conclusively determined in animal and human clinical studies, which are limited to date. Anthocyanins have been purported to have cardioprotective, antidiabetic, anti-inflammatory, and anticarcinogenic properties, as well as enhancing weight loss (He and Giusti 2010).

The aim of this body of work was to investigate and review the current literature on anthocyanins, and particularly their pharmacokinetics and any health-promoting properties, in order to summarize existing knowledge and highlight any aspects that require further study and analysis.

Review Strategy

In order to assess the current data on anthocyanin pharmacokinetic and biological activity, a comprehensive search of the scientific literature was conducted. The search was applied to the PubMed electronic database. The search strategy used was an advanced search in "all fields" and with publication type "reviews" using these key words: (anthocyanin*); (anthocyanin* OR anthocyanidin*) AND bioavailability (anthocyanin* OR anthocyanidin*) AND biological activity. To search single journal articles, these expressions were used: for pharmacokinetics, ([anthocyanin* OR anthocyanidin*] AND bioavailability) AND animal*, ([anthocyanin* OR anthocyanidin*] AND bioavailability) AND human*, while for biological activity ([anthocyanin* OR anthocyanidin*] AND biological activity) AND *in vitro*, ([anthocyanin* OR anthocyanidin*] AND biological activity) AND in animal*, ([anthocyanin* OR anthocyanidin*] AND biological activity) AND Human*. Furthermore, to identify additional studies the reference lists of the selected publications were also analyzed. No language or publication date restrictions were imposed. The search of the PubMed database provided 250 review articles. After adjusting for duplicates, 210 remained. 28 review articles were selected by title and 17 were selected by abstract. Additionally, over 200 relevant publications were identified among the references of the selected review articles. The publications were then classified in 3 categories: studies *in vitro* in cell models, *in vivo* in animals, and *in vivo* in humans.

Pharmacokinetics

To validate the purported therapeutic properties of any compound it is necessary to understand its pharmacokinetics, as this is closely correlated with biological activity. Anthocyanins exhibit complex chemical behavior *in vitro* and *in vivo* (McGhie and Walton 2007), in terms of absorption, metabolism, distribution, and excretion (He and Giusti 2010; Cisowska and others 2011), each of which influences the extent and impact of any health-promoting properties.

Absorption

Animal model studies indicate that anthocyanins are rapidly absorbed, appearing in the bloodstream in few minutes (6 to 20 min) after consumption, and reaching maximum blood levels after 15 to 60 min (Table 2; Matsumoto and others 2001, 2005, 2006; Passamonti and others 2003; Wu and others 2004; Ichiyanagi and others 2005, 2006; McGhie and Walton 2007). The concentration of anthocyanins observed in plasma is in the nM to low μM range (Table 2).

Both in humans and animals, many ingested anthocyanins are absorbed intact and circulate in the plasma and pass into urine without undergoing metabolic changes (Morazzoni and others 1991; Cao and others 2001; Matsumoto and others 2001; Mazza and others 2002; Wu and others 2002; Felgines and others 2003; Felgines and others 2007). Indeed, Bub and others (2001) detected neither aglycons, nor glucuronidated or sulfated conjugates in human plasma and urine after the consumption of red wine or red grape juice, which indicates that mv-3-glc is absorbed in its glucosylated form.

These characteristics are consistent with observations that anthocyanins are actually absorbed from the stomach (Passamonti and others 2003; Talavéra and others 2003). Although absorption through the stomach mucosa is unusual for nutrients, it has been demonstrated via *in situ* gastric administration that anthocyanins come from the stomach as well as from the small intestine

Table 1–Dietary sources of anthocyanins.

Source	Antho.[a]	Concentration mg/100 g FW	Ref.[b]
Black grapes (*Vitis vinifera*)	mv-3-glc	39.23	Neveu and others 2010[c]
Red wine	mv-3-glc	9.97[d]	Neveu and others 2010[c]
Bilberries (*Vaccinium myrtillus*)	cy-3-glc	405.00	Ogawa and others 2008
	cy-3-gal	370.00	Ogawa and others 2008
Blueberries (*Vaccinium corymbosum*)	peo-3-glc	365.00	Ogawa and others 2008
Blackberries (*Rubus sp.*)	cy-3-glc	138.72	Neveu and others 2010[c]
	cy-3-rut	8.86	Neveu and others 2010[c]
Blackcurrants (*Ribes nigrum*)	dp-3-rut	304.91	Neveu and others 2010[c]
	cy-3-rut	160.78	Neveu and others 2010[c]
	dp-3-glc	86.68	Neveu and others 2010[c]
	cy-3-glc	25.07	Neveu and others 2010[c]
Chokeberries (*Aronia melanocarpa*)	cy-3-gal	557.67	Neveu and others 2010[c]
	cy-3-ara	252.76	Neveu and others 2010[c]
Strawberries (*Fragaria x ananassa*)	pl-3-glc	47.14	Neveu and others 2010[c]
Elderberries (*Sambucus nigra*)	cy-3-sam	462.96	Neveu and others 2010[c]
	cy 3-O-glc	794.13	Neveu and others 2010[c]

[a] Anthocyanins: cy, cyanidin; mv, malvidin; pl, pelargonidin; peo, peonidin; dp, delphinidin; pt, petunidin; 3-glc, 3-glucoside; 3-sam, 3-sambubioside; 3-ara, 3-arabinoside; 3-rut, 3-rutinoside; 3-gal, 3-galactoside.
[b] Ref., references.
[c] DATABASE phenol-explorer.
[d] In red wine mg/100 mL.

Table 2–Pharmacokinetic parameters of anthocyanins in animal studies.

Species	Source	Antho.[a]	Dose total antho.	C_{max}[b]	T_{max}[c] (h)	Urinary excretion (% of intake)	$T_{1/2}$ (h)[d]	Ref.[e]
Rat	Bilberries		400.00 mg	2 to 3 μg/mL	0.25			Morazzoni 1991
Rat	Elderberries	cy-3-glc	320.00 mg	1563 μg/L	0.25			Miyazawa and others 1999
Rat	cy-3-glc	cy-3-glc	900.00 μmol	0.31 μM	0.50			Tsuda and others 1999b
Rat	Blackcurrants	cy-3-glc	800 μmol/kg	0.84 μM	0.50		2.08	Matsumoto and others 2001
		cy-3-rut	800 μmol/kg	0.85 μM	0.50		1.36	
		dp-3-rut	800 μmol/kg	0.58 μM	2.00		0.79	
Rat	Purple black rice	cy-3-glc	100.00 mg	0.18 μM	0.25			Ichiyanagi and others 2005
Rat	Bilberries (8 d)*	cy 3-glc	1430.00 μmol		0.50	0.22 (24 h)		Talavéra and others 2006
Rat	dp-3-rut	dp-3-rut	152.00 μmol	0.29 μM	0.40		1.20	Matsumoto 2006
Rat	Bilberries		153.20 mg	1.20 μM	0.25			Ichiyanagi and others 2006
Rat	Blueberries (4 or 8 wk)*		24.00 mg		0.50			Del Bo and others 2010
Mice	cy-3-glc	cy-3-glc	500.00 mg	25.00 μM	0.50		1.60	Marczylo and others 2009
Mice	Bilberry extracts		100.00 mg	1.20 μM	0.25	1.88 (6 h)		Sakakibara and others 2009
			617.60 mg (2 wk)*	153.00 nM				
Pig	Dry marion blackberries	cy-3-glc	1452.00 μmol	36.30 nM	1.00	0.09 (24 h)		Wu and others 2004
Pig	Chokeberries		229.00 μmol			0.10 (24 h)		Wu and others 2005
	Blackcurrants		140.00 μmol			0.07 (24 h)		
	Elderberries		228.00 μmol			0.13 (24 h)		
Pig	Blackcurrants		100.00 mg	0.09 μg/mL	2.00-4.00			Walton and others 2006[a]
Rabbit	Blackcurrants		117.00 mg	780.00 ng/mL	0.50	0.04 (4 h)		Nielsen and others 2003

[a] Antho., anthocyanins, the main anthocyanin in the sources; Cy, cyanidin; mv, malvidin; pl, pelargonidin; peo, peonidin; dp, delphinidin; pt, petunidin; 3-glc, 3-glucoside; 3-sam, 3-sambubioside; 3-ara, 3-arabinoside; 3-rut, 3-rutinoside; 3-gal, 3-galactoside.
[b] C_{max}, maximal concentration in plasma.
[c] T_{max}, time to reach C_{max}.
[d] $T_{1/2}$, half-time.
[e] Ref, references.
*Duration of treatment.

(Passamonti and others 2003; Talavéra and others 2003). For example, in rats, anthocyanins such as mv-3-glc appear in the plasma within 6 min post *in situ* gastric administration and anthocyanin monoglycosides such as the glucoside (glc) and galactoside (gal) were measurable in portal and systemic plasma (Passamonti and others 2003; Talavèra and others 2003).

These and other studies suggest a plausible mechanism for the absorption of anthocyanins from the stomach, through involvement of the organic anion membrane carrier bilitranslocase located in the stomach (Battiston and others 1999; Passamonti and others 2002; Nicolin and others 2005). Although the referred *in vitro* assays were performed at pH 8.0, which is far from the gastric conditions, indicated that the transported anthocyanin species are the quinoidal ones, which support the hypothesis that bilitranslocase transport might also occur at the physiological intragastric conditions. In facts, at acidic pH values, flavylium species, though prevailing, are not unique (Brouillard and Delaporte 1977). Rather, they are in equilibrium with quinoidal species that can indeed be transported.

Anthocyanins are also absorbed from the small intestine of rats and are then excreted into bile and urine. Again, this was evaluated in rat small intestine using *in situ* perfusion (Talavèra and others 2004). In the gastrointestinal tract, the absorption of anthocyanins was highest from the jejunum tissue (55.3 ± 7.6%) and limited from duodenal tissue (10.4 ± 7.6%), while there was no absorption from the ileum or colon (Matuschek and others 2006). This is consistent with other observations where the uptake into the small intestine reached up to 7.5% of ingested anthocyanins, which is higher than previously observed bioavailability (He and others 2009).

Anthocyanins are large, highly water-soluble molecules (Hollman 2004), and this feature limits their absorption by passive

diffusion. In contrast, anthocyanidins, aglycones from anthocyanins, are more hydrophobic and passively diffuse across the mucosal epithelium (Hollman 2004; Arts and others 2004). Thus, the absorption of anthocyanins requires either a specific active transport mechanism, to transport glycosides across the intestine wall, or they need to be hydrolyzed to the aglycone in the small intestine through the action of β-glucosidase, β-glucuronidase, and α-rhamnosidase (Manach and others 2005; Kay 2006). Sodium–glucose cotransport (SGLT1) enables the transport of intact glycosides across enterocyte membranes (Mulleder and others 2002). This mechanism is also applicable to other flavonoids. For example, it has been observed that the presence of quercetin-3-glucoside reduced the adsorption of cy-3-glc (Walton and others 2006b), probably due to competition for the transport protein. According to another interpretation, the uptake of cy-3-glc was inhibited by the occurrence of quercetin aglycone, resulting from hydrolysis of quercetin-3-glucoside. This aglycone represents a substrate of bilitranslocase that is also located at the intestinal epithelium level (Passamonti and others 2009).

Kurilich and others (2005) suggested that anthocyanin absorption in humans was maximal at a dose of 350 μmol because of saturation of the absorption mechanism. Recently, a kinetic study of anthocyanin transport through gastric cell line model MKN-28 was reported and also suggested that anthocyanins are absorbed at the gastric barrier by a saturable mechanism (Fernandes and others 2012). This apparent saturation supports carrier involvement in anthocyanin absorption.

Distribution

In rats, anthocyanins appear to be rapidly taken up from blood into tissues ($T_{1/2} = 0.36$ min) where they accumulate up to their bioactivity threshold (Vanzo and others 2011), although their availability to target tissues appears limited (He and Giusti 2010). For example, rats on a blackberry anthocyanin-rich diet (14.8 mmol per kg) for 15 d only provided 68.6 nmol/g of total anthocynins in stomach tissue, 605 nmol/g in the jejunum, 0.38 nmol/g in the liver, 3.27 nmol/g in the kidney, and 0.25 nmol/g in the brain (Talavèra and others 2005).

When, however, Marczylo and others (2009) orally administered 500 mg/kg cy-3-glc to mice they observed a diverse tissue distribution of anthocyanins. Anthocyanin levels observed in the gastrointestinal mucosa, prostate gland, and kidneys were approximately 700 nmol/g, 50 nmol/g, and 400 nmol/g, respectively. These concentrations are of an order of magnitude consistent with pharmacological activity. As anthocyanins readily cross the blood–brain barrier, Kalt and others (2008) observed in pigs fed a 4% blueberry diet for 4 wk, 0.878 pmol/g total anthocyanins in the cortex and 0.664 pmol/g in the cerebellum, as well as 1.30 pmol/g in the liver. They also observed 1.58 pmol/g in the eyes which suggests that anthocyanins also readily cross the blood–retinal barrier, potentially contributing to eye as well as brain function.

Anthocyanins could be also taken up into human vascular endothelial cells. A recent study observed that the uptake of cy-3-glc occurs through bilitranslocase-mediated transport. Cy-3-glc enters the cells only 1 min after its application into the cells. Additionally, in the human endothelial cell line, cy-3-glc undergoes intracellular methylation, forming peo-3-glc, and this newly formed compound moves across the membrane in the opposite direction, from cell to medium. The detection of peo-3-glc in medium offers indirect evidence that its precursor was taken up into the cells (Ziberna and others 2012).

Similar observations have also been found *in vivo* in anesthetized rats 1 min after IV administration of cy-3-glc; peo-3-glc was found in the plasma, whereas both cy-3-glc and peo-3-glc were detected in the liver and the kidneys (Vanzo and others 2011). Recovery of cy-3-glc from tissues is never complete, because of strong molecular interactions between flavonoids and denatured proteins (Passamonti and others 2002). This fast conversion of cy-3-glc is catalyzed by catechol-O-methyl transferase (COMT), which in mammalian is known to naturally 1st methylate, the position 3′ of catechols, as in the degradation pathways of catecholamines. In the case of anthocyanins, according to *in vitro* experiments (Zimman and Waterhouse 2002; Fernandes and others 2009), COMT promotes the B-ring methylation of anthocyanidins both at their 3′ and 4′ positions. This means that 2 positional isomers (peo-3-glc or isopeo-3-glc) can be expected from the methylation of cy-3-glc. This observation raises the question of which of these 2 compounds is really formed *in vivo*. In the conditions reported by Vanzo and others (2011), with a ultra high performance liquid chromatography separation on the reversed phase column Acquity UPLC (ultra performance liquid chromatography) BEH C18, such positional isomers result in 2 nearby peaks, the 3′ isomer eluting at the retention time of standard peo-3-glc, and the 4′ isomer eluting later. Both can be unambiguously quantified via tandem MS in MRM mode at m/z 463 to >301 and confirmed at m/z 463 to >286. Extracting these information from the chromatograms of the experiment of Vanzo and others (2011), isopeo-3-glc was absent or below the detection limit in rat urine and plasma, while it was observed as a minor peak, around 3% and 17% of peo-3-glc, respectively in kidney and liver.

Some studies have investigated the uptake of anthocyanins into the liver. Sakakibara and others (2009) examined the tissue distribution of anthocyanins in male mice following a diet containing 0.5% bilberry anthocyanins for 2 wk. It was estimated that 51.5% of the anthocyanins within the body were located in the liver, suggesting that this organ may be the main target for the accumulation of absorbed anthocyanins. The liver bilitranslocase transport mechanism may be involved and this carrier is located at the level of the vascular pole of the hepatocyte (Passamonti and others 2005a).

Another important site of anthocyanin distribution is the brain; anthocyanins have the ability to cross the blood–brain barrier and thus play a role in brain function. Passamonti and others (2005b) found that grape anthocyanins (8 mg/kg body weight dose) were observed in the brains of rats, at a concentration of 192 ng/g, only 10 min after introduction into the stomach. The typical pattern observed in a rat's brain was quite different from that measured in plasma. This demonstrates the rapid and selective movement of grape anthocyanins from the stomach into the mammalian brain. Andres-Lacueva and others (2005) identified several anthocyanins in various regions of the brain, including the cerebellum, cortex, hippocampus, and striatum of elderly rats fed a daily 2% blueberry diet for 10 wk.

The kidneys are also a target of anthocyanins. Vanzo and others (2008) observed that grape anthocyanins administered into the stomach of anesthetized rats were detected in tissue only 10 min after gastric absorption, and in the kidneys, the total concentration was almost twice higher than in the systemic circulation (147 ng/g compared with 274 ng/mL, respectively). The concentration in rat kidneys was 2 to 4 times higher than in rat liver, showing that, in the short term, the kidney is more efficient than the liver at taking up anthocyanins. Indeed, bilitranslocase is also involved in the uptake of mv-3-glc in the kidney's tubular cells,

behaving as a pure competitive inhibitor, as observed in the liver (Passamonti and others 2002). Its calculated inhibition constant ($K_i = 4.8 \pm 0.2\ \mu$M) was, however, again higher than in the liver ($K_i = 1.42 \pm 0.13\ \mu$M) (Vanzo and others 2008).

Anthocyanins undergo metabolism in the kidney's tubular cells, with the involvement of COMT. Methylated anthocyanins are accumulated in rat kidneys and may also be transported back from the cells into the blood by bilitranslocase, which is a bidirectional carrier (Vanzo and others 2008).

Metabolism

Most anthocyanins do not appear to undergo extensive metabolism (Crozier and others 2009), because they have been detected in plasma and urine as intact glycosides, both in animals and in humans (Tsuda and others 1999b; Prior 2004). Anthocyanins are absorbed mainly in the intact glycosidic form, and in this form they rapidly reach the circulatory system (6 to 20 min) and are excreted in the urine (Table 2 and 3; Matsumoto and others 2001; Passamonti and others 2003; Prior and Wu 2006; McGhie and Walton 2007). Despite these observations, new evidence suggests that anthocyanins are generally absorbed and transported in human serum and urine primarily as metabolites (Kay 2006; Mullen and others 2008; Garcia-Alonso and others 2009). Researchers also administered a 721 mg oral dose of cy-3-gly to humans and detected only 32.7% of intact cy-3-gly and an average of 67.3% identified as conjugated metabolites in the serum. Similar results were observed in urine (Table 3; Kay and others 2005). It has been suggested that shortly after anthocyanin gastric absorption, the prevailing metabolic reaction involving grape anthocyanins appears to be O-methylation (Vanzo and others 2008). No anthocyanin metabolites (aglycones, sulfo/glucurono-conjugates) were detected in rat urine following ingestion of dp-3-rut, only the intact compound and its 4'-O-methyl derivative were detected (Matsumoto and others 2006).

Due to the unique ability of anthocyanins to change form in response to changing pH values, they undergo modification following the different pH regions of the gastrointestinal tract (Galvano and others 2009). In the stomach (at low pH) anthocyanins occur mainly as the flavylium cation, the most stable form (McDougall and others 2005). In the small and large intestine, at neutral pH, multiple molecular forms of anthocyanins will be present (hemiketal, chalcone, quinones) and these forms are much less stable. *In vivo*, at the pH value of the organism (7.3), the main anthocyanin form is the quinoidal base anion (Rossetto and others 2007).

Anthocyanins are little modified by gastric conditions, but are extensively modified by the gut microflora (McGhie and Walton 2007). Many *in vitro* studies have incubated anthocyanins with animal or human intestinal flora. Microflora isolated from the pig cecum can modify the structure of anthocyanins with rapid demethylation and deglycosylation to aglycones. Anthocyanidins are unstable in alkaline pH and degraded to phenolic acids and aldehydes (Keppler and Humpf 2005). These metabolites could be absorbed in small quantities by the colon. Anthocyanins and anthocyanidins incubated with human fecal microbiota are transformed into phenolic acids. Indeed, cy-3-rut and cy-3-glc were converted by gut microflora, where cy-3-glc hydrolysis was completed and only one-third of cy-3-rut remained after 2 h.

Protocatechuic acid was the major metabolite (Aura and others 2005) as detected in rat plasma by Tsuda and others (1999b). Many *in vitro* studies have reported diverse phenolic acids, such as the chemically derived metabolites of anthocyanins such as protocatechuic acid, syringic acid, vanillic acid, phloroglucinol aldehyde, phloroglucinol acid, and gallic acid, (Keppler and Humpf 2005; Aura and others 2005; He and others 2005; Forester and others 2008, 2010; Kay and others 2009), that may result from either enteric bacterial or chemical conversions. They are observed in humans as well as in animals (Vitaglione and others 2007; Azzini and others 2010).

These metabolites may contribute to the bioavailability of anthocyanins and may be responsible for protective and antioxidant effects (Aura and others 2005; Manach and others 2005; Vitaglione and others 2007; Crozier and others 2009).

Once in the bloodstream, the metabolites can be subjected to phase II metabolism with further conversions in the liver by phase II drug detoxification enzymes. These enzymes are also located in the intestine epithelium and kidneys (Felgines and others 2003). Through these pathways, anthocyanins undergo biotransformation including methylation, glucuronidation, and/or sulfation following ingestion, (Galvano and others 2009) thus decreasing their structural stability (Manach and others 2005). The enzymes involved are:

(i) COMT, by transference to the flavonoid aglycone of a methyl group (Ichiyanagi and others 2005). Indeed, 3-O-methylation can convert cyanidin to peonidin and delphinidin to petunidin, while 5'-O-methylation converts petunidin to malvidin (Zimman and Waterhouse 2002; Crozier and others 2009). Evidence of these pathways *in vivo* is scarce. Traces of pt-3-glc and mv-3-glc, likely derived from the methylation of dp-3-glc, a precursor transiently appearing as minor metabolite after injection of cy-3-glc, were quantified by UPLC-MS/MS in rat kidneys, plasma, liver, and urine by Vanzo and others (2011). The catechol structure is necessary for COMT activity so pelargonidin, peonidin, and malvidin anthocyanins have no methylated metabolites;

(ii) Uridine diphosphoglucose glucuronosyl transferase and uridine diphosphoglucose dehydrogenase (liver and intestine) catalyzed the glucuronidation of flavonoid aglycones (Yang and others 1998). Glucuronidation is affected by aglycone structure: cyanidin, peonidin, isopeonidin, and pelargonidin were detected *in vivo* as monoglucuronide derivatives (Felgines and others 2003, 2005; Bitsch and others 2004; Wu and others 2005; Kay and others 2005). Delphinidin was also converted *in vitro* to monoglucuronide derivatives (Fernandes and others 2009); and

(iii) Phenol sulfotransferase (SULT1) that sulfates flavonoids (Scalbert and others 2000). Sulfoconjugate of cyanidin or sulfoconjugate of pelargonidin were found in human urine after consumption of blackberries or strawberries, respectively (Felgines and others 2003, 2005).

Methylation appears faster than other pathways. Vanzo and others 2011 showed that anthocyanins disappear from blood rapidly ($T_{1/2} = 0.36$ min) and only 15 s after IV administration in rats. Both cy-3-glc and its methylated form, peo-3-glc, can be detected in plasma, kidneys, and liver. Traces of other minor metabolites of cy-3-glc (dp-3-glc and its methylated forms pt-3-glc and mv-3-glc) were also detected. In this case their positional isomers methylated at 4′ (isopt-3glc and isomv-3-glc), which are separated from their 3′ isomer (pt-3-glc and mv-3-glc) by reversed phase high-performance liquid chromatography (HPLC; Zimman and Waterhouse 2002), were not observed.

Recycling of the conjugated metabolites in the colon may occur through the enterohepatic circulation pathway (Donovan and others 2006) after excretion into the jejunum via bile.

Table 3–Pharmacokinetic parameters of anthocyanins in human studies.

Subj. (n)[a]	Source (dose)	Antho.[b]	Dose	C_{max}[c]	T_{max}[d] (h)	Urinary excretion (% of intake)	$T_{1/2}$ (h)[e]	Ref.[f]
6	Red wine (300 mL)		218.00 mg			5.10 (12 h)		Lapidot and others 1998
4	Elderberry extract (12 g)	Tot antho	720.00 mg	97.40 nM	1.10		2.20	Cao and others 2001
		cy-3-glc	863.00 μmol	42.50 nM	1.10		1.50	
		cy-3-sam	492.00 μmol	38.90 nM	1.20		2.20	
8	Blackcurrant concentrate (33 mg/kg)	Tot antho	6.24 μmol	5.00 to 73.40 nmol/L	1.25 to 1.75	0.11 (8 h)		Matsumoto and others 2001
		dp-3-rut	2.75 μmol	73.40 nM	1.80		3.18	
		cy-3-rut	2.08 μmol	46.30 nM	1.50		3.45	
		dp-3-glc	1.04 μmol	22.70 nM	1.50		4.19	
		cy-3-glc	0.37 μmol	5.00 nM	1.30		1.34	
6	Red wine (500 mL)	mv-3-glc	139.00 μmol	0.0014 μM	0.80	0.02 (6 h)		Bub and others 2001
	Dealcoholized red wine (500 mL)	mv-3-glc	118.00 μmol	0.0017 μM	1.50	0.02 (6 h)		
	Red grape juice (500 mL)	mv-3-glc	238.00 μmol	0.0028 μM	2.00	0.02 (6 h)		
4	Elderberry extract (12 g)	cy-3-glc, cy-3-sam	720.00 mg	n.d.		0.077 (4 h)		Wu and others 2002
6	Blueberries (189 g)		690.00 mg	n.d.		0.004 (6 h)		
5	Blueberry powder (100 g)		1200.00 mg	13.09 μgl/L	4.00			Mazza and others 2002
16	Elderberry extract (11 g)		1900.00 mg			0.012 (6 h)		Mulleder and others 2002
6	Strawberries (200 g)	peo-3-glc	179.00 μmol			1.80 (24 h)		Felgines and others 2003
5	Blackcurrants (300 mL)	Tot antho.	189.00 mg			0.06 (7 h)		McGhie and others 2003
		dp-3-glc	17.20 mg			0.042		
		dp-3-rut	72.20 mg			0.067		
		cy-3-glc	13.00 mg			0.04		
		cy-3-rut	85.80 mg			0.063		
	Blueberry extract (300 mL)	Tot antho	439.00 mg			0.02 (7 h)		
		dp-3-gal	39.50 mg			0.018		
		dp-3-glc	17.30 mg			0.03		
		dp-3-ara	53.80 mg			0.013		
		cy-3-glc	6.30 mg			0.019		
		cy-3-ara	11.00 mg			0.01		
		pt-3-gal	32.80 mg			0.027		
		pt-3-glc	19.30 mg			0.013		
		pt-3-ara	25.60 mg			0.012		
		mv-3-gal	99.60 mg			0.026		
		mv-3-glc	49.50 mg			0.02		
		mv-3-ara	81.60 mg			0.01		

(*Continued*)

Table 3–Continued.

Subj. (n)[a]	Source (dose)	Antho.[b]	Dose	C_{max}[c]	T_{max}[d] (h)	Urinary excretion (% of intake)	$T_{1/2}$ (h)[e]	Ref.[f]
9	Red wine (400 mL)	Tot antho.	279.00 mg	42.9 ng/mL	1.50	0.18 (7 h)	1.99	Bitsch and others 2004
		mv-3-glc	386.00 μmol	18.5 ng/mL	1.50	0.11	1.80	
		peo-3-glc	39.00 μmol	12.6 ng/mL	1.50	0.84	1.83	
		pt-3-glc	76.00 μmol	12.3 ng/mL	1.50	0.18	2.15	
	Red grape juice (400 mL)	Tot antho	283.50 mg	100.1 ng/mL	0.50	0.23 (7 h)	1.83	
		cy-3-glc	7. μmol	0.42 ng/mL	0.50	0.09	1.61	
		dp-3-glc	107.00 μmol	6.12 ng/mL	0.50	0.20	1.72	
		mv-3-glc	266.00 μmol	48.80 ng/mL	0.50	0.18	1.50	
		peo-3-glc	180.00 μmol	27.30 ng/mL	0.50	0.29	1.63	
		pt-3-glc	35.00 μmol	16.10 ng/mL	0.50	0.32	1.68	
5	Blackberries (200 g)	Tot antho	960.00 μmol			0.16 (24 h)		Felgines and others 2005
3	Chokeberry extract (7.1 g)	Tot antho	721.00 mg	96.08 nM	2.80	0.15 (24 h)		Kay and others 2005
		cy-3-gal	1094.00 μmol	23.40 nM	2.50		<1.35	
		cy-3-ara	418.00 μmol	8.90 nM	3.50		<1.67	
10	Blackcurrant juice (300 g)		33.60 mg			0.079 (48 h)		Hollands and others 2008b
	Frozen blackcurrants (100 g)		642.00 mg			0.075 (48 h)		
10	Strawberries (100 to 400 g)		57.10 μmol/100 g			2.35 (24 h)		Hollands and others 2008a
8	Strawberries (200 g)	pl-3-glc	222.00 μmol	274.00 nmol/L	1.10	1.00 (24 h)		Mullen and others 2008
7	Red grape extract (12 g)	Tot antho	183.80 mg		1.60	0.05 (24 h)		Garcia-Alonso and others 2009
		mv-3-glc	80.20 mg	4.20 nM	1.40	0.06	2.00	
		peo-3-glc	15.20 mg	0.80 nM	1.80	0.03	3.70	
13	Strawberries (300 g)	pl-3-glc	8.93 mg			0.90		Azzini and others 2010

[a] Subj., number of subjects.
[b] Antho., anthocyanins, the main anthocyanin in the sources; Cy, cyanidin; mv, malvidin; pl, pelargonidin; peo, peonidin; dp, delphinidin; pt, petunidin; 3-glc, 3-glucoside; 3-sam, 3-sambubioside; 3-ara, 3-arabinoside; 3-rut, 3-rutinoside; 3-gal, 3-galactoside.
[c] C_{max}, maximal concentration in plasma.
[d] T_{max}, time to reach C_{max}.
[e] $T_{1/2}$, half-time.
[f] Ref, references.

Excretion

In humans, anthocyanin clearance is rapid and after 6 h very little is detected in the plasma (Table 3; Cao and others 2001; Bub and others 2001). Anthocyanin glycosides appear to be quickly and efficiently absorbed in rats from the stomach and rapidly excreted into bile and urine as intact and metabolized forms (Passamonti and others 2003; Talavèra and others 2003). It has been demonstrated that in rat bile anthocyanidins and their metabolites are detected after 20 min, which confirms their rapid absorption and metabolism (Talavéra and others 2003). Intact glycosylated forms (Matsumoto and others 2001) and some methylated forms, but no aglycones or glucuronide forms, were detected in animal urine (Felgines and others 2002).

Measurement of urinary excretion has often been used to assess bioavailability and most studies on the pharmacokinetics of anthocyanins have reported low urinary excretion, in the range of 0.004% to 0.1% of intake (Table 2 and 3), although Lapidot and others (1998) measured higher levels of anthocyanins, up to 5%, after the consumption of red wine (Table 3). Furthermore, in a human feeding study with strawberries, Felgines and others (2003) reported urinary excretion equivalent to 1.8% of ingested pl-3-glc (Table 3). This suggests that pl-3 glc is absorbed more rapidly than other anthocyanins.

Unabsorbed flavonoids are excreted through feces (He and others 2005), the low permeability of anthocyanins across the epithelium of gastrointestinal tract causing them to be moved to the colon and eliminated from the organism in the feces. Feces are a major excretion route for ingested anthocyanins (Del Bò and others 2010). Conjugate flavonoid metabolites are likely to be excreted in the urine, but some of them may be recycled through the enterohepatic circulation pathway, excreted in feces or may re-enter the jejunum (Ichiyanagi and others 2006).

Bioavailability

The pharmacokinetics of anthocyanins suggests that these compounds are poorly bioavailable (Table 2 and 3). The proportion of total anthocyanins (native forms plus metabolites) absorbed and excreted in the urine is very low as compared to the ingested doses (McGhie and Walton 2007). The bioavailability of anthocyanins appears to be lower than that of other flavonoids (Manach and others 2005; McGhie and Walton 2007), considering the total urinary excretion of anthocyanins (less than 0.1%) and the maximum plasma concentrations calculated at nanomole or nanogram level both in animals and humans (Table 2 and 3; Cao and others 2001; McGhie and others 2003; Vitaglione and others 2007).

There may be several factors contributing to the low bioavailability observed, including:

(i) the concentration of metabolites may have been below the detection limits; and

(ii) the carbinol and chalcone forms of anthocyanins, present in blood and urine at neutral pH values, may escape detection or be chemically bound to other components and not be included in the analyzed fraction due to their inability to return into the flavylium cation on acidification during sample preparation (Mazza 2007).

Studies on the pharmacokinetics of anthocyanins are summarized in Table 2 and 3. Briefly, Kay (2006) observed in his review that the maximum plasma concentrations are reported in human studies as between 1.4 and 592 nmol/L and occurred at 0.5 to 4 h after consumption (doses 68 to 1300 mg). Average human urinary excretion is reported as between 0.03% and 4% of the ingested dose, with elimination half-lives of 1.5 to 3 h (Table 3).

The bioavailability of anthocyanins depends significantly on their chemical structure, being influenced by the nature of the sugar moiety and also by the structure of the anthocyanin aglycone.

In rat and human urine, the concentration of delphinidin-based anthocyanins is lower than that of malvidin-based anthocyanins. This results from a hydrophobicity difference; malvidin has fewer free hydroxyl groups and is thus more hydrophobic and can easily be transported to cells and tissues. Given that urinary excretion is an indicator of absorption, more delphinidin glycoside than malvidin glycoside is absorbed in rats (Table 3; McGhie and others 2003). This is supported by the fact that the plasma concentration was higher for delphinidin and cyanidin than for malvidin anthocyanins. In a rat model of *in situ* perfusion, for example, the rate of anthocyanin absorption ranged from 10.7% for mv-3-glc to 22.4% for cy-3-glc (Talavèra and others 2004).

Furthermore, the type of sugar moiety attached to the phenolic core influences anthocyanin pharmacokinetics. For example, galactoside was more extensively absorbed than arabinoside, so the plasma concentration was higher for galactoside and lower for arabinoside (Table 3; McGhie and others 2003). Anthocyanin glycosides have been shown to be hydrolyzed by the intestinal microflora within 20 min to 2 h after incubation, depending on the sugar moiety (Keppler and Humpf 2005). This was observed *in vivo* as well: losses in intestinal content were high for anthocyanin glucosides, moderate for galactosides, and negligible for arabinosides or xylosides. Talavèra and others (2004) noted that the disappearance of cy-3-glc in a rat's intestine was higher than for other glycosides of cyanidin. Although available data are limited, it suggests that the anthocyanins xyloside and arabinoside are preferentially retained in the cecal content and feces as compared to galactoside and glucoside.

Interestingly, acylation or diglucosylation enhanced anthocyanin stability and this resulted in a slower metabolization and enhanced excretion of the native compounds. Wu and others (2005) observed that cyanidin di-triglycoside exhibited a higher recovery. Anthocyanins containing more than 1 sugar in their structure are much more resistant to any transformation by intestinal microflora (Prior and others 2006).

Total urinary excretion depends on the sugar moieties of anthocyanidins. The urinary excretion of rutinosides (rut) was higher than that of glucosides, whereas the total urinary excretion of cy-3-samb (samb means sambubioside) and cy-3-samb-5-glc was higher than that of cy monoglycosides. Talavéra and others (2003) demonstrated a higher urinary excretion of cy-3-rut in rats as compared to cy-3-glc, because cy-3-rut increased stability. The absorption of cy-3-rut in rats was found to be lower than that of cy-3-glc (Talavéra and others 2005). Wu and others (2004) observed that the urinary recovery of acylated anthocyanin in pigs was lower than that of nonacylated anthocyanin. Acylation of cyanidin resulted in an 11- to 14-fold decrease in anthocyanin recovery in human urine and an 8- to 10-fold decrease in anthocyanin recovery in plasma (Kurilich and others 2005).

Additionally, the bioavailability of anthocyanins varies markedly depending on the food matrix, including other antioxidants, micronutrients, and macronutrients present in the foods consumed, which consequently affect the absorption and antioxidant capacity of anthocyanins (Yang and others 2011). Various types of food samples were used to determine the effects of the food matrix on anthocyanin bioavailability; Mullen and others (2008) reported that anthocyanins in strawberries were highly bioavailable, with urinary levels reaching more than 1% (Table 3). One study

suggested that anthocyanins in red wine were also highly bioavailable in humans, up to 5.1% of the ingested dose (Lapidot and others 1998), although these results are not consistent with several other observations in humans (low urinary excretion ranging from 0.03% to 0.2% of the ingested dose) (Table 3; Bub and others 2001; Bitsch and others 2004; Garcia-Alonso and others 2009).

Several structurally diverse anthocyanins are present in different food matrices. Kay and others (2009) noted an interaction between the food matrix and specific anthocyanins, for example, both blackberries and blood oranges have a high concentration of cy-3-glc, but urinary recovery in humans was very different (0.16% compared with 1.2%, respectively). In pigs, the total urinary recovery of pl-3-glc plus its related metabolites was 8-fold higher than that of cy-3-glc (Table 2; Wu and others 2004), which explains the greater bioavailability of anthocyanins from strawberries than blackberries (Prior and others 2006). In humans, pl-3-glc from strawberries was also more rapidly absorbed as compared to other anthocyanin forms (Table 3; Felgines and others 2003; Mullen and others 2008). Red grape juice anthocyanins were more rapidly absorbed than those from red wine in humans (urinary excretion 0.23% compared with 0.18%; Table 3). The presence of alcohol in red wine may contribute to the decrease in anthocyanin absorption (Bitsch and others 2004), or alcohol may have no effect on anthocyanin bioavailability but slow down the rate of absorption (Prior and others 2006). It is likely that compounds other than ethanol could account for the different absorption from wines and grape juices, since Bub and others (2001) observed that both wine and dealcoholized red wine at similar doses showed no differences in the absorption of mv-3-glc (Table 3). Another point of view was given by Faria and others (2009) who suggested that ethanol may enhance the cellular absorption of phenolic compounds and the transport of anthocyanins involving glucose transporter 2 (GLUT2) through intestinal epithelial cells.

The sugar content of the food matrix may also have an effect on the bioavailability of anthocyanin: it may facilitate the crossing of this compound through the intestinal brush border via SGLT1 and GLUT2 transporters (Mullder and others 2002; Faria and others 2009). For example, the sugar content in red grape juice may facilitate anthocyanin uptake via SGLT1 as compared to red wine (Bitsch and others 2004). Rice cakes, consumed by humans at the same time as an anthocyanin source, delayed the peak plasma concentration of anthocyanin but had no effect on bioavailability, suggesting that carbohydrates have no effect on bioavailability (Nielsen and others 2003).

Biological Activity

Numerous studies suggest that anthocyanins may be positively implicated in human health. They exert antidiabetic and antiobesity effects and they act as neuroprotective agents (Prior and Wu 2006; Tsuda 2012). These compounds may be useful in reducing inflammation and exerting cardiovascular protection (Mazza 2007; He and Giusti 2010). Additionally they seem active in preventing and inhibiting cancer growth (Cooke and others 2005; Wang and Stoner 2008 Thomaset and others 2009). All of anthocyanin's beneficial effects are summarized in Table 4 to 6.

It was shown that intravenous administration of cy-3-glc alters certain important cellular metabolites, such as bile acids, glutathione, oxidized glutathione and some lipids in the blood, kidneys, and liver of rats (Vanzo and others 2013).

Recently, a study by Vanzo and others (2013) supported the biological activity of anthocyanins in rats, where the capacity of anthocyanins to affect mammalian metabolism was demonstrated in an investigation of metabolomic changes in the brain and the plasma of adult rats after intravenously administration of cy-3-glc. It was shown that cy-3-glc alters certain important cellular metabolites, such as bile acids, glutathione, oxidized glutathione, and some lipids in the blood, kidneys, and liver of rats (Vanzo and others 2013). In view of high content of anthocyanins in blueberries, this fruit may be a health-enhancing or health-promoting food source. This was supported by Routray and Orsat (2011), in a study which summarized various aspects linked to the potential health benefits of anthocyanins, focusing on understanding the practical importance of this fruit.

Antioxidant activity

The antioxidant potential of anthocyanins depends on the chemical structure of the molecule, the phenolic structure giving antioxidant properties. This property is also influenced by: (i) the number of hydroxyl groups; (ii) the catechol moiety in the B ring; (iii) the oxonium ion in the C ring; (iv) the hydroxylation and methylation pattern; (v) acylation; and (vi) glycosylation (Yang and others 2011). Glycosylation of anthocyanin decreases radical scavenger activity as compared with aglycone, as it reduces the ability of anthocyanin radicals to delocalize electrons (Wang and Stoner 2008). The contribution of the B ring substitutes to the efficiency of antioxidant activity is $-OH > -OCH^3 >> -H$ (Rossetto and others 2007), and thus the potency of antioxidant activities is in the order of dp > pt > mv = cy > peo > pl (Rahman and others 2006). Furthermore, the positively charged oxygen atom in the anthocyanin molecule makes it a more potent and hydrogen−donating antioxidant, as compared to oligomeric proanthocyanidins and other flavonoids (Kong and others 2003).

The antioxidant activity of anthocyanins may be enhanced by other phytochemicals or vitamins that are also abundant in fruits. They may interact with each other synergistically or antagonistically (Niki and others 1988). For example, common dietary flavanols undergo synergistic antioxidant effects with common anthocyanins. Indeed, Rossetto and others (2002) demonstrated that mv-3-glc, investigated in the peroxidation of linoleic acid in micelles, when present together with catechin has a higher antioxidant potential, because it is regenerated by catechin. This mechanism was shown to be specific for mv-3-glc and peo-3-glc. Conversely, quercetin inhibits cy-3-glc uptake (Walton and others 2006b).

Anthocyanin metabolites such as protocatechuic acid have high bioaccessibility and a marked antioxidant effect and, accordingly, may contribute to the proposed health-promoting properties of anthocyanins (Vitaglione and others 2007).

In vitro. The possible mechanisms through which anthocyanins could exert their antioxidant effects include direct and indirect pathways. Anthocyanins have a direct free radical-scavenging capacity due to the hydrogen (electron) donation ability of the flavonoid molecule (Fukumoto and Mazza 2000; Borkowski and others 2005), which can bind with reactive oxygen species (ROs) such as super-oxide (O_2^-), singlet oxygen ($.O_2$), peroxide (ROO^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH). The overproduction of ROs can damages cells, which may lead to the development and progression of cardiovascular disease, cancers and other degenerative diseases (Allen and Tresini 2000).

Anthocyanins, through an indirect pathway, enhance endogenous antioxidant defences through numerous mechanisms such as: (i) restoring or increasing the activities of the antioxidant enzymes superoxide dismutase (SOD) and glutathione

Table 4–Biological activity of anthocyanins: *in vitro* studies.

Cell line	Source or antho.[a]	Conc.[b]	Other treatment	Effect[d]	Ref.[c]
Antioxidant					
Human erythrocytes	Red wine anthocyanin	10.00 μL	H2O2 and deprived of catalase activity by treatment with 4 mM sodium azide	↓ROS	Tedesco and others 2001
Cardiovascular protection					
Isolated rat hearts under ischemia-reperfusion conditions	Bilberry anthocyanins	0.01 to 1.00 mg/L		↓Rate of LDH ↑postischemic coronary flow, ↓the incidence and duration of reperfusion arrhythmias	Ziberna and others 2010
Antiobesity and antidiabetic					
Isolated rat adipocytes	cy-3- glc or cy [d]	100.00 μM (24 h)		Regulation of adipocytokine secretion, total 633 genes (cy-3-glc) or 427 genes (cy) were upregulated	Tsuda and others 2005
Human HepG2 cells.	cy-3-glc	5.00 to 100.00 μmol/L (24h)		↓Plasma cholesteryl ester transfer protein CETP activity	Qin and others 2009
Rodent pancreatic beta cell	pl-3- gal and Pl	50.00 μg/mL		↑1.4-fold in insulin secretion	Jayaprakasam and others 2005
Anti-inflammatory					
Human endothelium	Red wine anthocyanins	10.00 nmol/L		↑oxidant status and ↓MCP-1 ↓TNF-α-induced inflammation	Garcia-Alonso and others 2009
Anticancer activity					
T-lymphoblastoid, and HL-60 promyelocytic cells (leukemic cells)	cy-3-glc	12.50 to 200.00 μg/mL		↑Induction differentiation ↓cell proliferations ↑apoptosis IC50 175 ug/mL	Fimognari and others 2004
HT-29 and Caco-2 (colon cancer cells)	Muscadine grapes anthocyanin	1.00 to 7.00 mg/mL (IC_{50})		↓Cell growth ↑apoptosis	Yi and others 2005
Human oral (CAL-27, KB), colon (HT29, HCT-116), and prostate (LNCaP, DU145) cancer cells	Strawberry anthocyanins	100.00 μg/mL		↓Cell viability ↓cell growth	Zhang and others 2008
HT29 colon cancer cells	Antho. extracts of grapes, bilberries, or chokeberries	25.00 to 75.00 μg/mL		↓The growth cells	Zhao and others 2004
JB6 mice cells	dp, pt, cy, pl, peo, mv	0 to 20.00 μM		dp, pt, cy,↓ cell transormation but pl, peo, mv/	Hou and others 2003
Human promyelocytic leukemia cell (HL-60)	dp, pt, cy, pl, peo, mv	100.00 μM		dp, pt, cy,↑ apoptosis but pl, peo, mv /	Hou and others 2003b
Endothelioma	(Pretreatment) Optiberry (berry mix) powder	50.00 μg/mL		↓inhibit inducible MCP-1 expression and TNF-α transcription ↓ability to form hemangioma	Bagchi and others 2004
Human fibroblastoma HT1080 cell	Eggplants (dp)			↓Cell invasion	Nagase and others 1998
Human colon cancer cell lines HT 29 and HCT 116	Anthocyanidins from cerry and cy	Antho. 0 to 1000.00 mM cy. 0 to 250.00 mM		↓Cell growth	Kang and others2003
PC12 cells	(Pretreatment) mulberry fruit extract (cy-3-glc)	10.00, 20.00, and 30.00 g/ml	Hydrogen peroxide	↓Cell damage	Kang and others 2006
Caco2- cells	Anthocyanins microflora metabolites	140.00 μM		↓Cell proliferation ↑ apoptosis	Forester and others 2011
Antimicrobial activity					
Gram-positive and Gram-negative	Bilberry (cy) and blueberry (mv) extracts	50.00 μL		↓Growth	Burdulis and others 2009
E. coli	Cranberries	14.80 mg/L		↓Growth	Lacombe and others 2010
Staphylococcus aureus, Escherichia coli, Enterococcus faecium	Elderberry and blackcurrant concentrate	10.00 mL		↓Growth	Werlein and others 2005

[a] Antho., anthocyanins: cy, cyanidin; mv, malvidin; pl, pelargonidin; peo, peonidin; dp, delphinidin; pt, petunidin; 3-glc, 3-glucoside; 3-sam, 3-sambubioside; 3-ara, 3-arabinoside; 3-rut, 3-rutinoside; 3-gal, 3-galactoside.
[b] Conc, concentration.
[c] Ref., references.
[d] ↓, decrease; ↑, increase; /, no effects.

Table 5–Biological activity of anthocyanins in animal studies.

Animal model	Source of antho.[a]	Dose	Other treatment	Duration[b]	Results[c]	Ref.[d]
Antioxidant						
Rats	Glucoside dp, cy, pt, peo and mv	1 g/kg diet	Vitamin E-deficient diets	12 wk	↑ Plasma antioxidant capacity ↓ hepatic 8-hydroxy-2-deoxyguanoside	Ramirez-Tortosda and others 2001
Rats, hepatic ischemia-reperfusion model	cy-3-glc	2 g/kg diet		14 d	↓ Injury by ROS	Tsuda and others 1999a
Cardiovascular protection						
Hamsters	Optiberry		Hypercholesterolemic diet		↓ Atherosclerotic index ↓weight	Bagchi and others 2004
Rats	Antho. rich diet		Regional ischemia was induced in heart, coronary occlusion	8 wk	More resistant to regional ischemia reperfusion insult ↓ infarct size ↑ myocardial glutahione levels	Toufektsian and others 2008
Ischemia-reperfusion hamster model	Bilberry antho.				↓ Microvascular impairments ↑preservation endothelium and ↑capillary perfusion	Bertuglia and others 1995
Neuroprotection						
Brain injury mouse model	Mulberry fruit extract (cy-3-glc)	10 mg/kg	Middle cerebral artery occlusion (MCAO) was induced		Neuroprotective ↓ brain injury	Kang and others 2006
Rats	Lyophilised blueberries and bilberries	3.2 mg/kg d		30 d	↑Short-term memory and↑ working memory	Ramirez and others 2005
Aged F344 rats	Plum juice antho. (cy-3-glc)	0.043 mg		8 wk	↓ Decline of neural functions and ↑ cognitive functions	Shukitt-Hale and others 2009
Old F344 rats	Blueberries	2% diet		10 wk	↑ HSP70-mediated protection and ↓ deleterious effects of aging	Galli and others 2006
F344 rats	Blueberries	2% diet	Kainic acid induced cognitive and motor impairments	8 wk	↓Inflammation ↓ expression of IL-1b, TNF-α, and nuclear factor-kβ in the hippocampus	Shukitt-Hale and others 2008
Vision improvement						
Aging OXYS rats	Blueberry extract and Mirtilene forte	230 mg/kg			↓ Cataract ↓lipid peroxidation products in blood serum	Kolosova and others 2004
Chick with negative lenses	Black currant antho. (Currantex 20 S)	50, 100 mg/kg		3 d	↓ Transient myopia, ↓ eye fatigue, ↑ dark adaptation, and ↑retinal blood flow	Iida and others 2010
Antidiabetic and antiobesity activity						
Mice high-fat diet	Purple corn (cy-3-glc)	2 g/kg diet	High-fat diet	12 wk	↓ Insulin resistance ↓ body weight ↓ triacylglycerol synthesis	Tsuda and others 2003
Rats	Grape skin antho.	21 mg/kg	Fed a 60%-enriched fructose	6 wk	↓ Triglycerides and ↑ HDL	Al-Awwadi and others 2005
Diabetic rats	Boysenberry (cy-3-glcrut, cy 3-diglc and cy3-glc)	0.1% of diet	Streptozotocin (STZ)	28 d	↓ Biomarker oxidation	Sugimoto and others 2003
Mice	Strawberries Blueberries	2.9 mg/g 27.8 mg/g	High-fat diet	8 wk	↓ Body weight gain	Prior and others 2008
Mice	Cherries	1g/kg diet	High-fat diet	12 wk	↓ 24% in weight gain and ↓ lipid accumulation ↓in liver triacyglycerol concentration	Jayaprakasam and others 2005
Mice	Blueberry extract	0.49 mg	High-fat diet	72 d	↓ Weight gain ↓ body fat accumulation	Prior and others 2010
Mice	Blueberry powder		High-fat meals		⁄ Body weight gain but, ↓obesity-induced inflammation in adipose tissue ↓ hyperglycemia ↓adipocyte death	DeFuria and others 2009

(*Continued*)

Table 5–Continued.

Animal model	Source of antho.[a]	Dose	Other treatment	Duration[b]	Results[c]	Ref.[d]
Type-2 diabetes mouse model	Bilberry extract	27 g of BBE/kg diet			↓ Hyperglycemia ↓ elevation of blood glucose levels and↑ insulin sensitivity, activated AMPK in white adipose tissue, in skeletal muscle and liver.	Takikawa and others 2010
Type-2 diabetes mouse model	cy-3-glc	2 g/kg diet			↓ Elevation of blood glucose levels ↑ insulin sensitivity upregulated the glucose transporter 4 (GLUT4), downregulated RBP4 and downregulation of the inflammatory adipocytokines in the white adipose tissue	Sasaki and others 2007
Anti-inflammatory						
Rats with lung inflammation	Blackberry antho. (cy-3-glu 80%)*	10 or 30 mg/kg	Carrageenan		↓ Inflammation	Rossi and others 2003
Anticancer activity						
ApcMin mice, intestinal cancer model	Cherry extract (cy)	375 to 3000 mg/kg diet		10 wk	↓ Cyclooxygenase enzymes ↓ cecal tumors 74%	Kang and others 2003
ApcMin mice, intestinal cancer model	cy-3-glc	9 mg		12 wk	↓ Adenoma 45%	Cooke and others 2006
ApcMin mice, intestinal cancer model	Bilberry antho. (Mirtoselect)	9 mg		12 wk	↓ Adenoma 30%	Cooke and others 2006
Fischer 344 male rats	Bilberry, chokeberry or grape antho.	3.85 g /kg diet	Colon carcinogen azoxymethane		↓ Aberrant crypt foci by 26% to 29%	Lala and others 2006
Rats	Antho. extract*	4 g/kg diet	Carcinogen (azoxymethane-induced colonic aberrant crypt foci)	1 wk	↓ Number of aberrant crypt foci by inhibition of COX2 gene	Magnuson and others 2003
Rats (female)	Antho. concord grape juice	10, 15 20 mg	7,12-Dimethylbenz (a)anthracene (DMBA) induces mammary tumor	20 wk	↓ Mammary tumors	Singletary and others 2003
Rats (female)	Grape juice	830 mg/L drinking water	7,12-Dimethylbenz (a)anthracene	3 wk	↓ Final tumor mass, ↓DMBA_DNA adducts	Jung and others 2006
Rats	Chokeberries*	5, 10, 20 mg/kg	Indomethacin-induced gastric mucosal damage		↓ Gastric lesions ↓plasma and gastric malondialdehyde	Valcheva-Kuzmanova and others 2005
Rats	Purple sweet potato and red cabbage antho.	0.5% diet	1,2 Dimethylhydrazine (DMH)	32 wk	↓ Lesion development	Hagiwara and others 2002
Rat	Freeze-dried black raspberries	0.3% diet	N-nitrosomethylbenzylamine (NMBA)-induced esophagus tumor	25 wk	↓ Tumor incidence ↓tumor mulitplicity ↓COX-2	Chen and others 2006

[a] Antho., anthocyanidins: cy, cyanidin; mv, malvidin; pl, pelargonidin; peo, peonidin dp, delphinidin; pt, petunidin; 3-glc, 3-glucoside; 3-sam, 3-sambubioside; 3-ara, 3-arabinoside; 3-rut, 3-rutinoside; 3-gal, 3-galactoside.
[b] d, days; wk, weeks; mo, months.
[c] ↓, decrease; ↑, increase; /, no effects.
[d] Ref., references.
*Pretreatment.

Table 6–Biological activity of anthocyanins in human studies.

Subj.[a]	Study design	Source or antho.[b]	Treatment (dose)	Duration	Results[c]	Ref.[d]
Antioxidant						
9		Red wine	300 mL	30 min	↑ Antioxidant capacity in serum 18% and 11% after 1 h and 2 h	Whitehead and others 1995
22 Hemodialysis patients	Multiple dose: twice daily	Red grape juice	50 mL	14 d	↓ Oxidized LDL and ↓ activity of NADPH oxidase ↑ HDL	Castilla and others 2008
8 Elderly women		Strawberries	240 g		↑ Antioxidant capacity in serum and urine ↑ plasma vitamin C level	Cao and others 1998
		Red wine	300 mL		↑ Antioxidant capacity in serum	
5	Single dose	Freeze-dried blueberry powder	1.2 g		↑ Serum antioxidant capacity	Mazza and others 2002
Cardiovascular protection						
20		Grape juice	7 mL/kg	14 d	↓ Platelet aggregation ↑ NO ↓ O2-	Freedman and others 2001
Middle-aged unmedicated subjects ($n = 72$) with cardiovascular risk factors	Single-blind, randomized, placebo-controlled intervention trial	Berries	100 g	8 wk	↓ Platelet function and ↑ HDL-cholesterol ↓ systolic BP	Erlund and others 2008
120 Dyslipidemic subjects	Double-blind, randomized, placebo-controlled trial, multiple dose: twice daily	Medox	160 mg	12 wk	↑ HDL-cholesterol concentrations ↓ LDL-cholesterol concentrations ↑ cellular cholesterol efflux to serum.	Qin and others 2009
52 Postmenopausal women	Parallel designed, randomized, placebo-controlled study	Elderberry extract (cy glycosides)	500 mg/d	12 wk	No significant change in biomarkers of CVD risk, but ↑ postprandial metabolism	Curtis and others 2009
8 Males	Single oral dose	Nagano purple grapes (dried fruit)	50 g		↓ LDL oxidation	Kamiyama and others 2009
Neuroprotective						
Older adults with memory decline	Double-blind trial	Concord grape juice	6 and 9 mL/kg	12 wk	↑Verbal learning ↑cognitive function	Krikorian and others 2010 b
Vision improvement						
16 Young normal volunteers	Single oral dose	Blueberry extract and beta-carotene	12, 24, 36 mg	24 h	/ In 1st 24 h	Levy and Glovinsky 1998
18 Young normal volunteers	Multiple oral dose (twice a day)	Blueberry extract and beta-carotene	12, 24 mg	4 d	/	Zodok and others 1999
8 Young healthy males	Multiple oral dose (3 times a day)	Bilberry extract	160 mg	21 d	/	Muth and others 2000
6 Subjects	Double-blind placebo-controlled study	Bilberries		3 h	Adapted to the light within 6.5 min	Camire 2000
50 Patients with senile cataracts	Multiple oral dose (twice a day)	Bilberry extract	180 mg	4 mo	Progression of cataracts was stopped in 96% of the subjects treated	Bravetti 1989
21 Slightly myopic subjects, 2 h of continuous work on a personal computer	Double-blind, placebo controlled crossover design study	Blackcurrant anthocyanins	50 mg		↓ Diopter values	Nakaishi and others 2000
30 Glaucoma patients		Blackcurrant anthocyanins	50 mg/d	6 mo	↑ Retinal blood flow	Ohguro and others 2007
60 Patients with asthenopia	Randomized, double-blind, placebo-controlled trial (twice daily)	Grape pulp and skin	85 mg	4 wk	73% of patients ↑ symptoms	Lee and others 2005
Anti-inflammatory						
120	Parallel-designed, placebo-controlled clinical trial	Bilberries and blackcurrants (Medox)	300 mg/d	3 wk	↓ NF-kβ, ↓ plasma concentrations of proinflammatory chemokines, cytokines, and inflammatory mediators	Karlsen and others 2007
Anticancer activity						
25 Colon cancer patients	Multiple dose (20 g, 3 times a day)	Black raspberry powder	60 g/d	2 to 4 wk	↓ Proliferation ↑ apoptosis	Wang and others 2007
20 Healthy females		Cranberry juice	750 mL/d	2 wk	/ On DNA damage of leukocytes	Duthie and others 2006

[a] Subj, subjects.
[b] Antho., anthocyanidins.
[c] ↓, decrease; ↑, increase; / no effects.
[d] Ref., references.

peroxidase, consequently increasing glutathione content (Toufeksian and others 2008); (ii) activation of genes that code for these enzymes (Shih and others 2005); and (iii) a reduction in the formation of oxidative abducts in DNA, reducing the formation of endogenous ROs by inhibiting NADPH oxidase and xanthine oxidase, or by modifying mitochondrial respiration and arachidonic metabolism (Ferràndiz and Alcaraz 1991; Steffen and others 2008).

The antioxidant activity of anthocyanidins has been measured using different methods. Using ferric reducing ability of plasma method , the 3-glucosides of delphinidin, petunidin, and malvidin, were observed to be 2 to 2.5 times more antioxidant than ascorbic acid (Garcia-Alonso and others 2004). Using Trolox equivalent antioxidant capacity or oxygen radical-absorbing capacity (ORAC), the antioxidant capacity of anthocyanins was however observed to be 3 to 6 times that of the standard Trolox (water-soluble vitamin E analog). Given these data, anthocyanins appear to be better antioxidants than the classic antioxidants such as butylated hydroxytoluene, butylated hydroxyanisole, and α-tocopherol (Wang and others 1997; Fukumoto and Mazza 2000). This powerful antioxidant effect is associated with the presence of hydroxyl groups in ring B. Noda and others (2002) investigated the antioxidant effects of 3 major anthocyanidins (delphinidin, cyanidin, pelargonidin) by using an electron spin resonance technique. The ID_{50} (50% inhibitory dose) values of delphinidin, cyanidin, and pelargonidin were 2.4, 22, and 456 μM, respectively. Using the method of linoleic acid peroxidation in micelles, Rossetto and others (2002) reported that also in this system the radical-scavenging efficiency of pelargonidin 3-glucoside was much lower than those characterizing the other anthocyanins.

In vitro *cellular models*. Recently, Bornsek and others (2012) demonstrated that bilberry and blueberry anthocyanins are powerful intracellular antioxidants in different mammalian cells at very low concentrations (EC_{50} < 1 μg/L; nM range). These observations were confirmed by another study of the same group, in which cy-3-glc, transported via bilitranslocase into endothelial cell (EA.hy926), acted as an intracellular antioxidant displaying a half-maximal effect (EC_{50}) at 0.9 nM (Ziberna and others 2012). These studies showed that anthocyanins are active antioxidant agents *in vivo*, even though the plasma concentration reached after oral consumption is low.

***In vivo* in animal models.** The antioxidant properties of anthocyanins have also been demonstrated *in vivo* in animal models (Table 5). Rats receiving a diet supplemented with anthocyanin-rich extract had a superior plasma antioxidant capacity and lower hepatic 8-hydroxy-2-deoxyguanoside, as compared to the control group (Ramirez-Tortosda and Andersen 2001). Tsuda and others (1999a) observed the activity of cy-3-glc under oxidative stress in rats with hepatic ischemia-reperfusion. This efficiently attenuated biomarker changes in liver injury. Indomethacin-induced gastric mucosa damage in rats, developed by oxidative stress, was reduced by pretreatment with chokeberry fruit (Valcheva-Kuzmanova and others 2005). The antioxidant activity of chokeberry polyphenols was extensively reviewed by Denev and others (2012), focusing on anthocyanin action mechanisms, summarizing both *in vitro* and *in vivo* literature.

***In vivo* in humans.** Epidemiological studies suggest that anthocyanin consumption may reduce certain parameters of oxidative damage (Weisel and others 2006). Individuals who consumed anthocyanin/phenolic-rich fruit juice were observed to have reduced oxidative DNA damage and a significant increase in reduced glutathione content when compared to control subjects (Weisel and others 2006). Cao and others (1998) observed an increased serum antioxidant capacity after the consumption of strawberries or red wine, while Mazza and others (2002), using the ORAC test, observed a positive correlation between postprandial serum anthocyanin content and antioxidant status (Table 6).

Cardiovascular protection

Cardiovascular disease development is due to platelet aggregation, hypertension, high-plasma LDL cholesterol, and vascular endothelium dysfunction. Epidemiological studies suggest that the consumption of red wine may be cardioprotective (Renaud and others 1992; Strandberg and others 2007; Streppel and others 2009; Hansen-Krone and others 2011; Levantesi and others 2013). The association between grape and wine phenolic compounds and coronary heart disease has been attributed partly to the presence of anthocyanins in red wine (Frankel and others 1993; Kanner and others 1994). The cardioprotective effect of anthocyanins may involve increasing serum antioxidant capacity, protecting against LDL oxidation and anti-inflammatory and antiplatelet activities (Erlund and others 2008).

***In vitro*.** Anthocyanins can improve the endothelial function (Table 4). According to the results obtained by Ziberna and others (2012), anthocyanins, in addition to decreasing oxidative stress in the vascular endothelium, exerted cardioprotection under ischemia-reperfusion conditions. A previous study also showed that vascular endothelial cells can incorporate anthocyanins into the membrane and cytosol, providing significant protective effects against oxidative insult, preserving endothelial function and preventing vascular diseases (Youdim and others 2000).

***In vivo* in animal models.** Ziberna and others (2010) investigated the acute direct effect of bilberry anthocyanins on whole rat hearts under ischemia-reperfusion conditions. The results showed that perfusion with a low concentration of bilberry anthocyanins (0.01 to 1 mg/L) significantly attenuated ischemia-reperfusion injury, decreasing the rate of lactase dehydrogenase, increasing postischemic coronary flow, and decreasing the incidence and duration of reperfusion arrhythmias. Conversely, a high concentration of anthocyanins (5 to 50 mg/L) was cardiotoxic, acting pro-oxidatively to increase the formation of damaging ROs (Halliwell 2008). Under reperfusion conditions, anaerobic metabolism may lead to a decrease in intracellular pH values, which can further influence the radical-scavenging properties of anthocyanins (Cvorovic and others 2010). This study also showed that bilberry anthocyanins were strongly antioxidant in the low-concentration range (<0.5 mg/L). All these observations may suggest that, despite low bioavailability, anthocyanins may have significant cardioprotective properties (Ziberna and others 2010). An earlier study of bilberry anthocyanins (Table 5) investigated an *in vivo* model of ischemia-reperfusion on hamsters, reducing microvascular impairments with preservation of the endothelium and improved capillary perfusion (Bertuglia and others 1995). Toufektsian and others (2008) also investigated long-term (8 wk) dietary consumption of plant-derived anthocyanins in rats. Consumption was observed to make the myocardium less susceptible to ischemia-reperfusion injury *ex vivo* as well as *in vivo*. The protection mechanisms are thus possibly related to improved endogenous antioxidant defences of the heart, increasing myocardial glutathione concentration.

***In vivo* in humans.** In a human clinical study (Table 6) of the consumption of anthocyanin-containing foods (blackcurrants, bilberries, and blueberries), it was shown that anthocyanins have the capacity to reduce the concentration of LDL-cholesterol and increase plasma antioxidant capacity (Erlund and others 2008).

Freedman and others (2001) fed healthy subjects 300 to 500 mL/d of anthocyanin-containing grape juice for a period of 7 to 28 d and observed inhibition of platelet aggregation, while another study of red grape juice supplements in dialysis patients showed that the concentration of oxidized LDL was reduced as was NADPH activity (Castilla and others 2008).

The consumption of 300 mL of anthocyanin-containing red wine has also been shown to increase serum antioxidant capacity by 18% after 1 h and by 11% after 2 h (Whitehead and others 1995), while the consumption of 300 mL of an anthocyanin-rich red wine (304 μmol anthocyanins) with a meal has been shown to prevent the postprandial increase in plasma lipid hydroperoxides and oxycholesterols, thus protecting the organism from their potential proatherogenic effect (Natella and others 2011).

Cardioprotection has also been attributed to the anthocyanin metabolites (Tsuda 2012). For example, anthocyanin colonic metabolites (such as protocatechuic acid) improved atherosclerosis progression, inhibiting the inflammation process, and also demonstrated antiplatelet activity (Rechner and others 2005; Wang and others 2011).

Neuroprotection

The review of Rendeiro and others (2012) attempted to explain the impact of a flavonoid-rich, diets such as those containing berries and anthocyanin compounds, on modulating neuronal functions. Anthocyanin-rich fruit diets may also have beneficial effects in combating cognitive decline and neurodegeneration associated with ageing (Tsuda 2012).

***In vivo* in animal models.** In animal models (Table 5), blackberry and plum anthocyanin extracts were observed to delay the onset of neural function decline and to improve both cognitive and motor function (Shukitt-Hale and others 2009), by inhibiting neuroinflammation and modulating neural signaling. Another plausible mechanism may be an improvement in cerebral blood flow (Spencer and others 2010).

In rats, blocking of nuclear factor-kβ (NF-kβ) upregulation provided the mechanism for inhibiting neuroinflammation (Goyarzu and others 2004). The kainic acid challenge-induced cognitive and motor function impairments were inhibited by the intake of blueberries by rats, which also demonstrated that IL-1b, tumor necrosis factor-alpha (TNF-α), and NF-kβ expression in the hippocampus was suppressed (Table 5; Shukitt-Hale and others 2008). Berries can reverse age-related and oxidative stress-induced decline in neural function, enhancing dopamine release in the brain, which improves the ability of neurons to enhance intracellular communication (Table 5; Youdim and others 2000; Galli and others 2006). For example, a mulberry fruit extract containing cy-3-glc in a brain injury mouse model with middle cerebral artery occlusion had a neuroprotective effect (Kang and others 2006). Bilberry-fed animals also showed better memory, better vision, and better control of sensory input than control animals (Galli and others 2006) while rats-fed lyophilized berries showed enhanced short-term memory and improved working memory (Table 5; Ramirez and others 2005). Rendeiro and others (2012) cited several studies that collectively suggest that blueberries can improve memory and learning in elderly animals, and that these improvements are linked to the modulation of important structural and synaptic plasticity markers. A blueberry-rich diet was shown to have a positive impact on neuronal function and in protecting from the development of Alzheimer's disease (amyloid precursor protein/PSI transgenic mice), preventing spatial memory deficits along with enhancement of memory-associated neuronal signaling. In this study, it was also observed that blueberry supplementation can regulate synaptic plasticity and consolidation of learning and memory (Joseph and others 2003). Blueberry flavonoids may improve memory by acting on the hippocampus and stimulating neurogenesis (Rendeiro and others 2012).

Interestingly, in a human clinical study (Table 6), a male subject aged 76.2 y consumed blueberry juice for 12 wk and improved his memory performance (Krikorian and others 2010).

Vision improvement

It has been suggested that vision may be improved by anthocyanin consumption (Kramer 2004). Indeed, anthocyanins from berries are currently used in ophthalmology due to their capacity to improve vision and prevent diabetic retinopathy (Ghosh and Konishi 2007). Berry anthocyanins appear to benefit vision in several ways by: (i) improving night vision by enhanced generation of retinal pigments; (ii) increasing circulation within the capillaries of the retina; (iii) decreasing molecular degeneration and diabetic retinopathy; and (iv) improving or preventing glaucoma, retinitis pigmentosa, and cataracts (Camire 2000).

***In vivo* in animal models.** In ophthalmology, the antioxidant effects of anthocyanins have been demonstrated on retinal pigment epithelium (Milbury and others 2007). Indeed, bilberry anthocyanins improve night vision through interaction with rhodopsin (Bastide and others 1968) or phosphodiesterase (PDE) on phototransduction (Ferretti and others 1988; Virmaux and others 1990). Inhibition of PDE (human PDE5A1) by grape anthocyanins has been observed *in vitro* as one of the mechanisms inducing smooth muscle cells vasorelaxation (Dell'Agli and others 2005).

Transient myopia has been observed to be inhibited by blackcurrant anthocyanins while glaucoma-associated retina blood flow was increased, dark adaption improved and eye fatigue reduced (Table 5; Iida and others 2010). The stimulation of the endothelin-1B receptor associated with the production of nitric oxide, ciliary smooth muscle relaxation, and thinning of the eye lens all act to inhibit myopia. For example, Matsumoto and others (2005) observed that anthocyanins such as dp-3-rut, at a concentration of 10^{-8} to 10^{-7} M, relaxed ciliary smooth muscle, which regulates, through constriction and relaxation, accommodation and module refraction of the lens. Matsumoto and others (2006) investigated the ocular distribution of blackcurrant anthocyanins (dp-3-glc, dp-3-rut, cy-3-glucuronide, cy-3-rut) in rabbits and rats after oral, intravenous, and intraperitoneal administration. They were absorbed and distributed in ocular tissue as intact forms crossing the ocular barriers. The concentration in the eye tissue was approximately 100-times higher than in the blood (Matsumoto and others 2006). The ocular distribution of blueberry anthocyanins was also investigated in pigs after oral administration where they were similarly observed to accumulate in eye tissue although in low concentration (pmol/g) (Kalt and others 2008). Given these results, anthocyanins become a potential drug for treating ophthalmological diseases such myopia and glaucoma.

***In vivo* in humans.** The effect on vision was one of the 1st reported properties of anthocyanins. British Royal Air Force aviators in World War II ate bilberry jam at breakfast as, it was argued, bilberry jam improves night vision. In their review, Ghosh and Konishi (2007) reported some studies on the effects of anthocyanins on human vision. Early studies seemed to offer positive results, whereas more recently studies have provided controversial

results. Some of these studies on human subjects are reported in Table 6. Another study investigated the effect of anthocyanins from grape skins on nocturnal visual function using 60 patients with asthenopia, with 73.3% of patients having improved symptoms (Lee and others 2005). The effect of blackcurrant anthocyanins (50 mg) on ocular fatigue was investigated by Nakaishi and others (2000) in 21 partially myopic subjects using a double-blind, placebo-controlled, crossover study design. Significantly decreased dioptre values were observed for the blackcurrant anthocyanin group compared to the control group after 2 h of continuous or prolonged computer work suggesting that the anthocyanins were able to prevent a myopic refractory shift.

Another double-blind placebo-controlled study showed that oral doses of anthocyanins were important for the generation of visual purple, which helps to convert light into electrical signals for the brain (Table 6). Adapto-electroretinograms of 2 sets of 6 subjects were done before treatment and 1 and 3 h postadministration; the subjects given the bilberries adapted to the light within 6.5 min, compared to 9 min for the control group (Camire 2000). In another trial, 50 patients with senile cataracts were given a combination of bilberry extract, standardized to contain 25% anthocyanins (180 mg twice daily) and vitamin E (100 mg twice daily) administered for 4 mo. The progression of cataracts was halted in 96% of the subjects treated, as compared to 76% in the control group (Head 2001). Similar to that observed in animal studies, in 30 glaucoma subjects retinal blood flow was observed to be significantly increased following administration of 50 mg anthocyanins per day for 6 mo (Table 6; Ohguro and others 2007).

The conflicting conclusions and results are, however, due to differences in the type of subject, the methods for evaluating night vision and the concentration, as well as the dose and source of the anthocyanin samples (Ghosh and Konishi 2007).

Antidiabetic and antiobesity properties

Type-2 diabetes is is associated with insulin resistance and relative insulin deficiency and is characterized by a high-blood glucose level. Obesity is associated with an imbalance of energy intake and expenditure and is characterized by the excessive accumulation of adipose tissue. Both are considered to be metabolic disorders and metabolic syndrome.

Fruit and vegetables may reduce the risk of obesity and decrease the incidence of type-2 diabetes associated with insulin resistance (Anderson and others 2004). Dietary constituents can regulate blood glucose levels or induce insulin production through pancreatic β-cells in type-2 diabetes (Ghosh and Konishi 2007). One of these constituents is anthocyanins which, by attenuating adipocyte dysfunction, may act to prevent obesity and metabolic syndrome (Tsuda 2008). Anthocyanins interact with adiponectin, which is one of the most important adipocytokines. In the case of obesity and insulin resistance, this molecule is decreased (Arita and others1999), but anthocyanins may act on this target in order to prevent obesity and diabetes (Table 4).

In vitro. Jayaprakasam and others (2005) (Table 4) demonstrated that several anthocyanins and anthocyanidins stimulate insulin secretion from rodent pancreatic β-cells in the presence of 4 and 10 mM of glucose. For example, pl-3-gal and its aglycone, pelargonidin, caused a 1.4-fold increase in insulin secretion at 4-mM glucose concentration, while other anthocyanins had only a marginal effect. Dietary antioxidants, including anthocyanins, protected pancreatic β-cells from glucose-induced oxidative stress (Al-Awwadi and others 2005). Cyanidin and cy-3-glc induced some lipid metabolism-related genes and modulated gene expression of adipocytokines in human adipocytes (Table 4; Tsuda and others 2006). The same research group (Tsuda and others 2005) evaluated the gene expression profile in isolated rat adipocytes treated with anthocyanins (100 nM cy-3-glc or cyanidin) for 24 h *in vitro*. A total of 633 genes or 427 genes were upregulated through treatment of adipocytes with cy-3-glc or cyanidin, respectively. The upregulated genes included lipid metabolism and signal transduction related genes.

***In vivo* in animal models.** In animal studies (Table 5), anthocyanins seem to have a significant α-glucosidase inhibitory effect, suppressing the increase in postprandial glucose (Matsui and others 2004). For example, the intake of anthocyanins improved insulin sensitivity and hence inhibited an increase in blood glucose level in a type-2 diabetic model (Table 5; Sasaki and others 2007; Takikawa and others 2010). The insulin sensitivity improvement mechanism is correlated to downregulation of retinol-binding protein 4 (RBP4), an adipocytokine involved in insulin resistance. The expression of GLUT4 was upregulated by Cy-3-gly with a consequent improvement in insulin resistance, which reduced the release of glucose following excessive gluconeogenesis (Table 5; Sasaki and others 2007).

Takikawa and others 2010 observed that bilberry extract in mice-activated AMP-activated protein kinase (AMPK) in white adipose tissue, skeletal muscle, and the liver. This is a crucial factor in cellular energy homeostasis and a potential therapeutic target in the prevention and treatment of type-2 diabetes. In white adipose tissue the upregulation of GLUT4 is induced by the activation of AMPK, which leads to enhanced glucose uptake as well as utilization in these tissues. This is also observed in skeletal muscle. In the liver, however, activated AMPK induces phosphorylation of acetylCoA carboxylase, and upregulation of acylCoA oxidase that contribute to decreased lipogenesis and to upregulate catabolic pathways that generate ATP. In addition, activation of AMPK resulted in downregulation of expression of gluconeogenic enzymes that are involved in hepatic glucose production. Thus, an improvement in hyperglycemia and a reduction in the lipid concentration in liver and serum occurred, while there was an increase in insulin sensitivity by reducing lipotoxicity (Table 5; Takikawa and others 2010).

Anthocyanin intake may also help prevent obesity and improve insulin resistance in mice. In 24 male mice, purple corn extract (cy-3-glu) for 12 wk suppressed a high-fat diet induced body weight gain compared to controls by downregulating mRNA level enzymes involved in fatty acid and triacylglycerol synthesis and regulated insulin sensibility associated with adipocytokine secretion (Tsuda and others 2003). Similarly in male mice, fed a high-fat diet, purified blueberry anthocyanin supplementation for 8 wk resulted in lower body weight gain and body fat compared to controls. In contrast, whole blueberry supplementation increased obesity, perhaps as a result if additional calorie intake from sugar (Table 5; Prior and others 2008). Similarly, black raspberries (cy-3-rut) did not prevent body weight gain or the accumulation of body fat in mice fed a high-fat diet (Prior and others 2010). Thus only purified anthocyanins can contribute to combating obesity, significantly reducing serum triglyceride and cholesterol concentration, and markedly increasing HDL concentration (Tsuda and others 2003; Al-Awwadi and others 2005). In addition, the lack of any effects may be also due to a different anthocyanin composition among berries. Thus, the sugar conjugated with anthocyanidins may modulate the functional expression, but it is unclear which anthocyanin molecular structures are responsible for "antiobesity" effects (Prior and others 2010).

Discrepancies in the results of different studies may be due to different experimental conditions such as the percentage of lipid-to-energy contribution in a high-fat diet, and the dosage of test samples used as a supplement. There are, unfortunately, no extensive *in vivo* studies and clinical evaluations to validate antidiabetic and "antiobesity" observations (Ghosh and Konishi 2007).

Anti-inflammatory effects

Inflammation is a complex biological response of vascular tissues to injuries, irritants, or stimulants, and is associated with the initiation, development, and progression of cancers or tumors. Consequently, drug therapies that reduce inflammation also have the potential to prevent the initiation, development, and progression of tumors (Coussens and Werb 2002). The stimulation of inflammation is due to cyclooxygenase (COX) enzymes that convert arachidonic acid to prostaglandins.

In vitro. *In vitro,* anthocyanins had the ability to inhibit mRNA and/or protein expression levels of COX-2, NF-kβ, and various interleukins, in multiple cell types (Afgaq and others 2005; Boivin and others 2007). Anthocyanins and their aglycons could also inhibit the activities of the human prostaglandin synthase. Compared to aspirin, cyanidin appears to have more significant anti-inflammatory effects such as COX activities (Wang 1999), and was able to decrease COX-1 and COX-2 activities by 52% and 74%, respectively (Seeram and others 2001). Delphinidin and cyanidin have been shown to inhibit COX-2 expression, while pelargonidin, peonidin, and malvidin did not. Delphinidin suppressed the activation of the mitogen-activated protein kinase (MAPK) involved in directing cellular responses to a wide range of stimuli such as mitogens and proinflammatory cytokines (Hou and others 2005). Only anthocyanins with the *ortho*-dihydroxyphenyl structure may have anti-inflammatory properties, through the inhibition of MAPK-mediated COX-2 expression (Hou and others 2005).

***In vivo* in animal and human models.** In rats with lung inflammation, induced by carrageenan, blueberry anthocyanins (cy-3-glc 80%) have been observed to reduce all inflammation parameters in a dose-dependent manner (Table 5; Rossi 2003), while in humans, red wine-derived anthocyanins have been observed to inhibit TNF-α-induced inflammation through modulation of endothelial monocyte chemoattractant protein-1 (Table 4; Garcia Alonso and others 2009).

Chemoprevention and cancer protection

These properties have been extensively reviewed by different authors, however, there remains a lack of human evidence in current studies (Cooke and others 2005; Wang and Stoner 2008; Thomaset and others 2009).

In vitro. The anticancer properties of anthocyanins have been established largely based on *in vitro* evidence (He and Giusti 2010) and may be attributed to additive multiple mechanisms. These mechanisms include the following: (i) anthocyanins arrest the cell cycle through arresting or blocking the G1/G0 and G2/M phases; (ii) they induce apoptosis and antiangiogenesis; (iii) anthocyanins inhibit oxidative DNA damage; (iv) they induce phase II enzymes for detoxification; (v) they have an antimutagenic effect and inhibit carcinogens; and (vi) they inhibit COX-2 enzymes.

The antiproliferative activity of anthocyanins occurs in multiple cell types *in vitro* (Table 4) (Zhao and others 2004; Zhang and others 2005; Yi and others 2005). They block various stages of the cell cycle via effectors on cell cycle regulatory proteins as well as selectively inhibiting the growth of cancer cells, with relatively little or no effect on the growth of normal cells. For example, anthocyanin extracts of grapes, bilberries, or chokeberries at 25 to 75 μg/mL inhibited the growth of human malignant HT29 colon cancer cells, but not that of nonmalignant colon-derived NCM460 cells (Zhao and others 2004). The mechanism involved in this selective effect is not known. Red wine anthocyanins appear to be more effective in inhibiting cell growth than nonanthocyanin flavonoids in HCT-15 cell lines (intestinal carcinoma-derived) and AGS cell lines (human gastric adenocarcinoma cell line) (Kamei and others 1998).

Anthocyanins isolated from strawberries reduce the cell vitality of human oral, colon, and prostate cancer cells at the 100 μg/mL dose level, but each individual compound had a different sensitivity (Table 4; Zhang and others 2008). The chemical structure of the individual anthocyanin compounds such as the type of aglycone, glycosylation pattern, and acylation determine its antiproliferative effects (Jing and others 2008). Indeed, anthocyanidins are better inhibitors of cell proliferation than anthocyanins (Zhang and others 2005), by blocking activation of the MAPK pathway. Delphinidin had the best growth inhibition, due to the presence of hydroxyl groups on ring B of the anthocyanidin molecule (Hou 2003). Cyanidin and delphinidin, though neither pelargonidin nor malvidin, exert cytotoxicity in the metastatic human colorectal cancer cell lines (LoVo and LoVo/ADR) through inactivation of the glutathione antioxidant system and promotion of oxidative stress (Cvorovic and others 2010). Jing and others (2008) also observed the additive effects of anthocyanins and other phenolic compounds, where the most active against all cancer cell lines was the anthocyanin fraction plus proanthocyanidin.

The metabolites of anthocyanins also seem to have anticancer properties. Indeed, a study by Forester and others (2011) aimed to measure the effects on cell proliferation, cytotoxicity, and the apoptosis of anthocyanin metabolites from Cabernet Sauvignon grapes (syringic acid, 2,4,6- trihydroxybenzaldehyde, 3-O-methylgallate, and gallic acid) in Caco2 cells (human colon cancer cells; Table 4). These metabolites showed limited toxicity for cells, but they clearly suppressed cell proliferation and induced apoptosis at 140 μM. The activity may be due to their pro-oxidant activity, releasing hydrogen peroxide, which would oxidize any enzymes present (Forester and others 2011).

Apoptosis plays an important role in the elimination of damaged tumor cells and is induced by chemopreventive agents (Thompson and others 1995; Galati and others 2000). Anthocyanins cause apoptosis in premalignant and malignant cells, while they have a proapoptotic effect in multiple cells *in vitro*, through both intrinsic pathways (increasing mitochondrial membrane potential cytochrome c release and modulating proapoptotic proteins) (Chang and others 2005) and extrinsic pathways (modulating expression of FAS and FAS ligand in cancer cells resulting in apoptosis; Table 4). They may act as pro-oxidants and lead to an accumulation of ROS in cancer cells (Feng and others 2007). Hou and others (2003) used human leukemia cells (HL-60) to investigate the ability of anthocyanidin to induce apoptosis at 100 μM for 6 h. Delphinidin, petunidin, and cyanidin induced apoptosis and DNA fragmentation, while pelargonidin, peonidin, and malvidin showed no induction of apoptosis. The best anticancer properties were observed with delphinidin and involved gene expression and activation of caspase-3, which is responsible for apoptosis. Delphinidin may also trigger an apoptotic program in HL-60 cells through oxidative stress-induced signaling pathways. Thus, anthocyanidins can act as pro-oxidants and induce the production of ROS (Hou and others 2003).

Furthermore, anthocyanins have anti-invasive properties, thus they can inhibit the metastasis process. This occurs when tumor cells invade the surrounding tissue and distant tissue following penetration of the blood vessels. This mechanism includes chemotactic motility. For example, tumor cells secrete proteolytic enzymes to facilitate degradation of the extracellular matrix barrier for successful tumor invasion. Anthocyanins inhibit the invasion of multiple cancer cell types, reducing the expression of matrix metalloproteinase (MMP, which regulates the degradation of basement membrane) and stimulate inhibitors of MMP (Brandstetter and others 2001). Nagase and others (1998) observed that anthocyanins also inhibit human fibroblastoma HT1080 cell invasion (Table 4).

Angiogenesis, forming new blood vessels from the existing vascular network, is an important factor in tumor growth and metastasis (Huang and others 2006). Anthocyanins appear to suppress angiogenesis through several mechanisms such as by: (i) inhibiting H_2O_2 and the TNF-α-induced vascular endothelial growth factors (VEGF), a biomarker of angiogenesis, and playing a crucial role in the vascularization of tumors (Bagchi and others 2004); (ii) inhibiting neovascularization (Favot and others 2003); and (iii) downregulation of VEGF (Huang and others 2006). OptiBerry® (InterHealth Nutraceuticals, Benicia, CA, USA), a synergistic combination of 6 berry extracts, has been shown to significantly inhibit both H_2O_2 and TNF-α-induced VEGF expression by human keratinocytes (Table 4; Bagchi and others 2004). This product was also studied in a model of proliferating hemangioma in which macrophages are commonly involved. The chemokine monocyte chemotactic protein-1 (MCP-1), a major accessory facilitating angiogenesis, has been shown to be responsible for recruiting macrophages to the infection or inflammation, and its transcription is mediated by NF-kβ. The hemangioma cells and elevated MCP-1 levels were significantly inhibited by pretreatment with OptiBerry (Nguyen 1997).

Hou (2003) investigated the molecular mechanisms of anticarcinogenesis in JB6 mouse epidermal cells. In the cells, the tumor promoters were 12-O-tetradecanoylphorbol-13- acetate (TPA), an epidermal growth factor (EGF), and TNF-α, which induce AP-1 activity and neoplastic transformation, activating the MAP kinase (Huang and others1998). Hou (2003) treated TPA-induced JB6 mouse cells with 6 anthocyanidins in the concentration range of 0 to 20 μM. The results showed that TPA-induced cell transformation and AP-1 transactivation were inhibited by delphinidin, petunidin, and cyanidin but not by pelargonidin, peonidin, and malvidin, suggesting that the *ortho*-dihydroxyphenyl structure on the B-ring of anthocyanidins may be essential. Delphinidin has the strongest inhibitory effect on AP-1 activation, through suppressed TPA-induced phosphorilation of the MAP kinase-signaling pathway. Furthermore, this anthocyanidin showed a synergistic effect with SOD in inhibiting AP-1 activity. Indeed, the superoxide anion promotes AP-1 activation and neoplastic transformation (Hou 2003).

***In vivo* in animal models.** Dietary anthocyanins exercise their main anticancer effects in the gastrointestinal tract, because they can reach relatively high concentrations in direct contact with the mucosa (He and others 2005), for example, black raspberries prevented the development of esophageal tumors in rats treated with carcinogen (N-nitrosomethylbenzylamine; see other example at Table 5). Anthocyanins may act by inhibiting the mRNA and protein expression levels of COX-2, inducible nitric oxide synthetase (iNOS), VEGF, and other genes associated with cell proliferation, inflammation, and angiogenesis (Stoner and others 2006). They also appear to be active against colon cancer, which develops through local irritation, producing a local inflammatory response and through an imbalance of electrolytes. This results from a defect in the epithelial barrier resulting in elevated ROS and COX-2 levels (Bruce and others 2000). As anthocyanins are widely available in the gastrointestinal tract, they may have a protective effect through direct contact with epithelial cells (He and others 2005), and may act to prevent or decrease damage to the epithelial barrier as well as inhibit protein expression levels of COX-2 and thereby inflammation, as well as quenching ROS in local cells.

Apc^{Min} intestinal cancer mouse models (the mice carry a Apc gene mutation that leads to development of adenomas in the small intestinal tract and reflects the human familial adenomatous polyposis) were fed with cherry extract anthocyanins (375 to 3000 mg/kg diet). It was observed that the treated mice had 74% fewer cecal tumors than the control group (Table 5; Kang and others 2003). The same model, fed with cy-3-glc or anthocyanin from bilberries, had adenomas which decreased by 45% and 30%, respectively (Cooke and others 2006). Rat colon cancer models fed with bilberry, chokeberry, or grape anthocyanins (3.85 g/kg diet) showed aberrant crypt foci reduced by 26% to 29%, by decreasing cell proliferation and COX-2 gene expression (Lala and others 2006). Commercially available anthocyanin-rich extract, 4 g/kg diet, administered to rats 1 wk before the carcinogen (azoxymethane-induced colonic aberrant crypt foci) reduced the number of aberrant crypt foci by inhibiting the COX-2 gene, compared to control rats (Table 5; Magnuson and others 2003).

Anthocyanins' anticancer properties may be related to the concentration of the compound in the blood. Indeed, anthocyanin availability in target organs depends on blood delivery (He and others 2005). Different research groups have tried to identify the concentration that may exert this beneficial effect in animal plasma. For example, female rats with induced mammary tumours received 10, 15, or 20 mg grape juice containing 15 different anthocyanins. The data, obtained from animal samples, showed that mammary tumors were reduced (Table 5; Singletary and others 2003).

***In vivo* in humans.** Epidemiological studies in humans have not provided convincing evidence of the anticancer effects of anthocyanins (Wang and Stoner 2008). A case-control study of 805 subjects with oral and pharyngeal cancer and 2081 hospital controls without neoplasia was conducted to examine the relationship between anthocyanidin intake and cancer risk. The results indicated no significant association between anthocyanidin intake and the risk of oral or pharyngeal cancer (Rossi and others 2007). Incident cases (1294) of prostate cancer and hospital controls (1451) without neoplasia were taken into account in a case-controlled study to evaluate the role of anthocyanin on prostate cancer risk. The results did not support the protective effect of anthocyanins on prostate cancer in this population (Bosetti and others 2006). Furthermore, interventional trials did not provide sufficiently strong results to suggest a positive correlation (Table 6). Indeed, dietary anthocyanins from cranberry juice had no effect on basal or induced oxidative DNA damage or cellular antioxidant status in leukocytes taken from treated individuals (Duthie and others 2006). A presurgical model of 25 colon cancer patients who had not received prior therapy consumed 60 g/d (20 g, 3 times a day) of black raspberry powder daily for 2 to 4 wk. Comparison of the results of biopsies taken before and after berry treatment showed that berries reduced proliferation rates and increased apoptosis in colon tumors, but not in normal-appearing crypts (Wang and others 2007).

However, anthocyanins have been shown to inhibit malignant cell growth, stimulate apoptosis and modulate oncogenic signaling events in a concentration range of 10^{-6} to 10^{-4} M in *in vitro* studies. Studies of the uptake of anthocyanins in humans after

consumption as mixtures suggest that they reach levels of 10^{-8} to 10^{-7} M in human blood, far below the levels required to exhibit anticarcinogenic effects *in vitro*. Thus, it is unclear whether the concentrations *in vivo* are sufficient to elicit anticarcinogenic effects in humans, and whether they exert chemopreventive efficacy by themselves or need to undergo hydrolysis to their aglyconic counterparts to be effective (Cooke and others 2005).

Antimicrobial activity

The antimicrobial activity of anthocyanins was extensively reviewed in a recent study by Cisowska and others (2011). There are different mechanisms through which anthocyanins can lead to microorganism toxicity. They can, for example, cause morphological damage to bacterial cells or destroy the structural integrity of the wall, membrane, and intracellular matrix. Anthocyanins may also cause cell deformation, breakage of the cell wall, and membrane condensation of cellular material with the presence of significant amounts of cytoplasmic material and membrane debris outside the cells (Lacombe and others 2010). Anthocyanins appear to inhibit enzymes through oxidized compounds, possibly through reaction with sulfhydryl groups or more nonspecific interactions with proteins, leading to their inactivation and loss of functioning (Naz and others 2007). In addition, anthocyanins may be responsible for destabilization of the cytoplasmic membrane, permeabilization of the plasma membrane, and inhibition of extracellular microbial enzymes. They may further have a direct effect on the microbial metabolism and on deprivation of the substrates required for microbial growth (Burdulis and others 2009).

In vitro. Bilberry and blueberry extracts showed inhibitory effects on the growth of Gram-positive bacteria (*Listeria monocytogenes, Staphylococcus aureus, Bacillus subtilis*, *and Enterococcus faecalis*) and Gram-negative bacteria (*Citrobacter freundii, Escherichia coli, Pseudomonas aeuruginosa*, *and Salmonella enterica ser. Typhimurium*). Of the bacteria tested, *C. freundii* and *E. faecalis* strains were the most sensitive, while *E. coli* showed the greatest resistance (Burdulis and others 2009). The European cranberry and the American cranberry had the same antimicrobial effect: they inhibited the growth of a wide range of human pathogenic bacteria, both Gram-negative and Gram-positive (Wu and others 2008; Cesoniene and others 2009). Cranberries led to an *E. coli* growth reduction below detectable limits after treatment with 14.8 mg/L anthocyanins at native pH. At neutral pH, anthocyanins showed reduced antimicrobial activity, possibly due to their instability (Table 4; Lacombe and others 2010). Cranberry extract did not have any effect on the growth of yeast species. Blackcurrant concentrates inhibited the growth of *S. aureus* and *Enterococcus faecium* strains, only mild effects were observed on *E. coli* and *S. cerevisiae* was slightly stimulated (Werlein and others 2005). Microbial strains had different susceptibilities to berry extracts; cloudberries had the most significant antimicrobial effect, followed by raspberries and strawberries (Nohynek and others 2006). *In vitro* berry extracts have bactericidal activities, inhibiting the growth of *Helicobacter pylori*, a Gram-negative bacterium that causes various gastrointestinal diseases including duodenal ulcer and gastric cancer. At 1% concentration, all extracts showed >70% inhibition, with cranberry, elderberry, bilberry, and blueberry extracts showing >90% inhibition (Chatterjee and others 2004).

Despite these observations on the antimicrobial activity of anthocyanins, Hidalgo and others (2012) observed that the incubation of mv-3-glc with fecal bacteria mainly resulted in the formation of gallic, syringic, and p-coumaric acids. All the anthocyanins and their metabolites tested significantly enhanced the growth of *Bifidobacterium spp.* and *Lactobacillus-Enterococcus spp.* These results suggest that anthocyanins and their metabolites may exert a positive modulation of the intestinal bacterial population.

Tolerability and Safety

The consumption of anthocyanins has not been associated with adverse health effects (Brouillard 1982).

The risk of anthocyanin toxicity from the food supply, in spite of a high dietary consumption of phenolic compounds in certain countries, is relatively low, largely due to their overall low absorption (Martin 2010). Nevertheless, the increasing use of dietary supplements rich in phenolic compounds could make consumption potentially problematic (Bueno and others 2012).

Bilberry extracts, containing 36% anthocyanidins, were used to test the tolerability and safety of anthocyanins in animals (Morazzoni and Bombardelli 1996). The LD_{50} values were over 2000 mg/kg, without toxic symptoms. In dogs, a single dose of 3000 mg/kg did not lead to mortality or any adverse effects, except for a marked darkening of urine (demonstrating the absorption of the product) and feces. Also, rats given doses of 125 to 500 mg/kg and dogs given doses of 80 to 320 mg/kg daily for 6 mo did not suffer any mortalities or toxic effects, and it did not influence fertility in rats. The extract did not show any teratogenic effects or mutagenic activity in different mutagenesis tests. Clinical safety was confirmed (Morazzoni and Bombardelli 1996).

Most people taking 160 mg twice daily for 1 to 2 mo tolerated the extract well or very well, with only 4% of people complaining of side effects, mainly gastrointestinal, related to the skin and cutaneous annexes and the nervous system (He and Giusti 2010).

A two-generation reproduction study (WHO 1982; Clifford 2000) with 18 weeks of observation demonstrated that anthocyanin extracts were associated with a low level of toxicity such that the no-observed-effect-level (NOEL) was calculated as 225 mg/kg body weight for young rats.

Concluding Remarks and Future Perspectives

The prevention of disease has become increasingly important in modern society. Health is one of the most essential aspects for a good life and the main priority for human beings, and public policy bodies are strongly encouraging preventative health measures as a means of reducing reliance on expensive treatments. As part of this trend, both governments and individuals are making dietary recommendations and choices based on scientific research. It is generally understood that certain food sources and compounds found naturally in foods can play a role in helping an organism to remain healthy. In the case of anthocyanins, they are a class of natural bioactives whose presence in food and beverages can be visually appreciated, which is clearly facilitating the transfer of information from the nutritional and pharmacological research into practical advice toward health-concerned consumers, as it has been suggested, for example, in the case of the color diet (Joseph and others 2002). Anthocyanins, phytopigments associated with health-promoting properties, can be found in several "functional foods" such as red, blue, and purple berries and red wine. Their biosynthesis is now well understood and considerable research work has been done in order to improve their presence in our plant food, such as in the case of red fruit-flesh apples (Chagnè and others 2013). Anthocyanins-rich extracts from grape skin, blackcurrant, purple corn, or red cabbage (Codex Alimentarius INS 163) are natural colorants approved in Europe, Australia, and New Zealand and are widely used in the food industry. The studies summarized in this review suggest a low toxicity for this class

of compounds, and given that natural anthocyanins have been ingredients of approved drugs for over 30 years, their high level of safety is supported by long-term extensive pharmaco-vigilance (Morazzoni and Bombardelli 1996).

This paper describes the health-promoting effects of anthocyanins and highlights current knowledge about the pharmacokinetics of anthocyanins.

For future work, a standardized set of analytical methodologies is clearly desirable. The availability of rigorous methods providing more homogeneous results would promote more rapid and productive comparisons between different studies. Current analytical methods have some limitations, such as underestimating anthocyanin levels in plasma and urine. Analysis of biological fluids is carried out through indirect quantification, using the HPLC-detected anthocyanin concentration as red flavilyum cations at 530 nm. This structure is not likely to exist in neutral physiological environments where, according to Rossetto and others (2007), the main form of anthocyanin at physiological pH is the quinoidal base anion. Anthocyanins transformed during metabolism are unable to return to the red flavylium cation following reacidification during sample preparation, and thus will not be detected by current methods of analysis using HPLC. For these reasons, flavylium cation cannot be considered as the starting point for metabolism. Different pictures of the anthocyanin metabolism could emerge when methods to directly observe and measure the quinoidal base form are available (McGhie and Walton 2007).

Moreover, recent results demonstrating the ultra-fast distribution and metabolism of IV- administered anthocyanins in rats (Vanzo and others 2011, 2013) suggest that in mammalian plasma the sink could largely exceed the capacity of the source; and this raised the question if the fast-changing concentrations of anthocyanins measured in plasma can be at all a wise indicator to estimate their putative bioefficacy.

Multiple and complex mechanisms other than a direct antioxidant activity have been proposed to explain most of the health-promoting mechanisms so far elucidated for this class of natural bioactive flavonoids. Moreover, animal and human bioavailability studies have reported concentrations in tissues and biofluids well below those required for direct antioxidant action. The case for a direct antioxidant role of anthocyanins is far from fully established.

Although research on anthocyanins has evolved rapidly, more research studies are required to fully catalogue and understand the effects of the anthocyanins on health. Indeed, much of the detail is missing regarding their pharmacokinetics and the mechanisms involved in their biological activities. More studies are needed in order to establish the real implications of anthocyanins and their metabolites and the specific mechanisms through which anthocyanins exercise their health-promoting properties.

In the experimental procedures, it should also be considered that dietary sources of anthocyanins, such as berries, are rich in a wide range of phenolic compounds. On the one hand, anthocyanins can exercise health-promoting effects with synergistic actions in combination with other compounds, due to interaction between substances. On the other hand, anthocyanins are present in berries in different structures and this heterogeneity leads to different pharmacological outcomes. In some cases, such as for the antiobesity properties of anthocyanins, conflicting results have been obtained in experiments with pure compounds in respect to those involving the administration of the whole food matrix. There is a need to confirm those putative health-promoting effects observed using anthocyanin-rich food and beverages, using the pure compounds, or comparing spiked against nonspiked food matrices.

Despite the low bioavailability of anthocyanins, the literature suggests that the metabolites may actually be responsible for much of their health-promoting properties. This hypothesis should be easily included in new nutritional studies, given that the pool of anthocyanin metabolites is largely simplified in comparison to the sheer complexity of natural anthocyanins. Mass spectrometry-based metabolomics in particular is quickly becoming a useful and promising tool for measuring in the same experiment both the native compounds, their main metabolites produced in the organism, and the perturbations induced by the transitory presence of anthocyanins on endogenous metabolic pathways.

Abbreviations

Cy cyanidin
mv malvidin
dp delphinidin
pl pelargonidin
pt petunidin
peo peonidin
gly glycosides
glc glucoside
rut rutinosides
gal galactoside
samb sambubioside

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