Aspects of anthocyanin absorption, metabolism and pharmacokinetics in humans

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Interest in the health-promoting properties of berry anthocyanins is intensifying; however, findings are primarily based on in vitro characteristics, leaving mechanisms associated with absorption, metabolism and clearance largely unexplored. The present review integrates the available anthocyanin literature with that of similar flavonoids or polyphenols in order to form hypotheses regarding absorption, metabolism and clearance in humans. Of the limited available literature regarding the absorption and clearance kinetics of anthocyanins, maximum plasma concentrations are reported anywhere between 1.4 and 592 nmol/l and occur at 0.5–4 h post-consumption (doses; 68–1300 mg). Average urinary excretion is reported between 0.03 and 4 % of the ingested dose, having elimination half-lives of 1.5–3 h. In addition, much is unknown regarding the metabolism of anthocyanins. The most commonly cited conjugation reactions involved in the metabolism of other flavonoids include glucuronidation, methylation and sulfation. It is reasonable to suspect that anthocyanins are metabolised in much the same manner; however, until recently, there was little evidence to suggest that anthocyanins were metabolised to any significant extent. New evidence now suggests that anthocyanins are absorbed and transported in human serum and urine primarily as metabolites, with recent studies documenting as much as 68–80 % of anthocyanins as metabolised derivatives in human urine. Further research is required to resolve mechanisms associated with the absorption, metabolism and clearance of anthocyanins in order to establish their true biological activities and health effects. The presented evidence will hopefully focus future research, refining study design and propagating a more complete understanding of anthocyanins' biological significance in humans.

Anthocyanins: Flavonoids: Pharmacokinetics: Metabolites

Introduction

Numerous studies have suggested that anthocyanins (Fig. 1) are protective against many chronic degenerative diseases (Kamei et al. 1995; Laplaud et al. 1997; Andriambeloson et al. 1998; Trevithick & Mitton, 1999; Mazza, 2000; Parthasarathy et al. 2001). However, the health-promoting properties of anthocyanins are based primarily on in vitro evidence, with information regarding bioavailability, metabolism, and pharmacokinetics in humans remaining largely unknown. The limited available evidence seems to indicate that anthocyanins are poorly absorbed and not metabolised to the same extent as other flavonoids. It has therefore been suggested that anthocyanins' bioavailability is relatively low. Conversely, some researchers have suggested that anthocyanin metabolites may be overlooked using conventional methods of identification (Day & Williamson, 2001; Felgines et al. 2003; Mazza et al. 2004; Kay et al. 2005; Manach et al. 2005). In fact, two recent studies identifying anthocyanin metabolites confirm that the absorption of anthocyanins has been underestimated (Felgines et al. 2003; Kay et al. 2005). Moreover, our work suggests that anthocyanins share common metabolic properties with other flavonoids, undergoing extensive glucuronidation and methylation in humans (Mazza et al. 2002; Kay et al. 2005). Even though anthocyanins are reported to be rapidly absorbed and eliminated, their absorption efficiency cannot be sufficiently estimated until the movement of all metabolites (intestinal, hepatic, and microbial) can be traced within the human body. Additionally, it must be considered that anthocyanins are absorbed and metabolised, and their metabolites either contribute to or retrace from their overall bioactivities.

Anthocyanin metabolism in humans has yet to be adequately characterised partly due to the large variation in findings in the literature. This is probably the result of variations in dosages used in clinical studies. Since metabolic pathways that rely on cofactors are saturatable, large doses of

Abbreviations: EHC, enterohepatic circulation; \( t_{\text{max}} \), time to reach maximum concentration; \( t_{1/2} \), half-life of elimination.

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anthocyanins (pharmacological doses) will probably result in the saturation of metabolic pathways and consequently large amounts of unconjugated parent compounds will enter the circulation. Alternatively, anthocyanins fed in lower doses, more similar to the levels found in the diet, would not be expected to saturate metabolic pathways and the circulating species would most probably be largely conjugated. For this reason, studies feeding a single dose of anthocyanins or flavonoids will not establish a complete representation of their absorption, metabolism and pharmacokinetics. More advanced multiple-dosing studies are required to establish a true understanding of the metabolism of these compounds.

The use of insufficient extraction and identification procedures has further limited the progress of anthocyanin metabolism research. Insufficient extraction and identification procedures limit the quantification of metabolites, thereby underestimating the total absorption and bioactivity of anthocyanins. Evidence presented by Murkovic et al. (2000) further suggests that the analytical recovery of anthocyanins may be hindered as a result of binding with plasma proteins. As well, it has recently been observed that anthocyanin metabolites may degrade under various storage conditions (Felgines et al. 2003). All of the above factors probably contribute to the underreporting of anthocyanin absorption and metabolism in the literature. Once the complete metabolism of anthocyanins is established their true health-promoting properties can be sufficiently explored.

The present paper will review the absorption, metabolism and bioavailability of anthocyanins. Since there are limited data on the absorption, metabolism and bioavailability of anthocyanins to date, the review will consider mechanisms for the absorption and metabolism of the flavonoids quercetin and catechin, as they are structurally similar to anthocyanins (Fig. 2).

Absorption

Flavonoids are mostly present in foods as glycosides (Hollman & Katan, 1998a). Glycosylation influences their biological properties, having the greatest effect on partitioning coefficients. This property is important in determining mechanisms of absorption, i.e. whether a compound will passively diffuse across a biological membrane and how it might partition internally within various cell phases. Aglycones are primarily hydrophobic and can passively diffuse through biological membranes. The linkage with sugars increases their water solubility and limits passive diffusion. Diffusion of a hydrophilic flavonoid glycoside across biological membranes is not likely to occur. Therefore, the absorption of an anthocyanin glycoside probably requires either a specific active transport mechanism or hydrolysis of the β-glycoside before absorption (Brown et al. 1998; Gee et al. 2000; Walgren et al. 2000; Day & Williamson, 2001; Manach et al. 2005) (Fig. 3).

Fig. 1. Common anthocyanidin structures.

<table>
<thead>
<tr>
<th>Aglycone</th>
<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delphinidin</td>
<td>OH</td>
<td>OH</td>
<td>OH</td>
</tr>
<tr>
<td>Cyanidin</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
</tr>
<tr>
<td>Petunidin</td>
<td>OCH₃</td>
<td>OH</td>
<td>OH</td>
</tr>
<tr>
<td>Peonidin</td>
<td>OCH₃</td>
<td>H</td>
<td>OH</td>
</tr>
<tr>
<td>Malvidin</td>
<td>OCH₃</td>
<td>OCH₃</td>
<td>OH</td>
</tr>
<tr>
<td>Pelargonidin</td>
<td>H</td>
<td>H</td>
<td>OH</td>
</tr>
</tbody>
</table>

Fig. 2. Structures of cyanidin (a), quercetin (b) and catechin (c).
Human studies within the last decade have established that the absorption of most flavonoids occurs in the small intestine (Hollman et al. 1995, 1996; Scalbert & Williamson, 2000; Williamson et al. 2000); however, the exact mechanisms involved in flavonoid absorption are a matter of much debate. It is speculated that the absorption of non-glycosylated flavonoids occurs in the small intestine via passive diffusion (Hollman et al. 1997; Donovan et al. 2001), while it has yet to be definitively established how flavonoid glycosides enter the enterocyte. The question is whether flavonoid glycosides enter the enterocyte as intact structures and are cleaved before passage across the basolateral membrane or if they are cleaved at the cell interface and move passively across the luminal membrane (Fig. 3). The two proposed mechanisms involved in the transport of flavonoid glycosides are: either the transport of the intact glucoside by a sodium–glucose co-transporter, or the extracellular hydrolysis of the glycoside via lactate phlorizin hydrolase at the brush border, followed by passive diffusion of the aglycone (Hollman et al. 1999; Gee et al. 2000; Williamson et al. 2000; Manach et al. 2005). In addition, a fraction of flavonoids will escape absorption in the upper small intestine and undergo bacterial metabolism in the lower intestine where the compounds will be deglycosylated and the aglycones will be subjected to transport or further metabolism (Scalbert & Williamson, 2000; Williamson et al. 2000; Manach et al. 2005).

The mechanisms involved in the absorption and metabolism of anthocyanins are probably similar to those of other hydrophilic flavonoid glycosides, and may occur by one or both of the above suggested routes (Fig. 3). First, anthocyanin glycosides may be hydrolysed at the mucosal brush-border membrane by lactate phlorizin hydrolase (Gee et al. 2000; Williamson et al. 2000). Once at the brush border, the aglycone may diffuse into the enterocyte where it could either enter the portal circulation as an aglycone, or be conjugated before transference across the basolateral membrane into the serosal fluid (Laitinen & Watkins, 1986; Hollman, 2001). Currently there is little direct evidence of anthocyanin aglycones existing in the circulation or urine of humans (Felgines et al. 2003). Also, if anthocyanin glycosides are hydrolysed at the mucosal brush-border membrane by lactate phlorizin hydrolase before absorption and conjugation with glucuronic acid, one would not expect to find parent anthocyanin glycosides in biological fluids (Table 1). Therefore a second pathway must exist, as many investigators have identified anthocyanin glycosides in human blood and urine (Table 1).

The second possible route of absorption is likely to occur via transport of the intact anthocyanin glycoside into the enterocyte, possibly by sodium–glucose con-transporter, as suggested for other flavonoids (Hollman et al. 1995; Wolffram et al. 1995; Hollman & Katan, 1998a; Williamson et al. 2000; Mülleder et al. 2002). Supplementation of anthocyanins with various sugars has been observed to reduce the excretion of anthocyanins (Mülleder et al. 2002), suggesting the potential role of sodium–glucose co-transporter in the transport of anthocyanin glycosides. Once inside the cell, the intact glycoside could either directly cross the basolateral membrane into the portal circulation, or be hydrolysed by cytosolic β-glucosidase (Day et al. 1998; Walle et al. 2000; Williamson et al. 2000; Mülleder et al. 2002) before intestinal metabolism and transport (Hollman, 2001). Saturation of the above detailed pathways could account for the large variation in findings presented in the literature. The theory of a saturatable transport mechanism involving an Na-dependent transporter has been previously proposed for the absorption of quercetin glycosides (Mizuma et al. 1994; Hollman et al. 1995; Wolffram et al. 1995; Hollman & Katan, 1998a). Even...
Table 1. Pharmacokinetics of anthocyanins following oral consumption in human subjects

<table>
<thead>
<tr>
<th>Anthocyanin Source</th>
<th>Period (h)</th>
<th>Dose (mg)</th>
<th>Urinary recovery (%)</th>
<th>C_{max} (nmol/l)</th>
<th>t_{max} (h)</th>
<th>t_{1/2} (h)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mixed (C-3-gly)</td>
<td>24</td>
<td>721</td>
<td>0.15</td>
<td>96.1</td>
<td>2.8</td>
<td>1.5</td>
<td>Kay et al. (2005)</td>
</tr>
<tr>
<td>Mixed Hibiscus extract</td>
<td>7</td>
<td>147</td>
<td>0.018</td>
<td>7.6†</td>
<td>1.5</td>
<td>2.6</td>
<td>Frank et al. (2005)</td>
</tr>
<tr>
<td>Mixed Red grape juice</td>
<td>7</td>
<td>283</td>
<td>0.23</td>
<td>222.7†</td>
<td>0.5</td>
<td>1.8</td>
<td>Bitsch et al. (2004b)</td>
</tr>
<tr>
<td>Mixed Red wine</td>
<td>5</td>
<td>3570</td>
<td>0.053</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Bitsch et al. (2004b)</td>
</tr>
<tr>
<td>Mixed Blackcurrant</td>
<td>7</td>
<td>145</td>
<td>0.04</td>
<td>–</td>
<td>–</td>
<td>1.7</td>
<td>Bitsch et al. (2004a)</td>
</tr>
<tr>
<td>Mixed Elderberry</td>
<td>7</td>
<td>147</td>
<td>0.37</td>
<td>–</td>
<td>–</td>
<td>1.7</td>
<td>Bitsch et al. (2004a)</td>
</tr>
<tr>
<td>Mixed Red wine</td>
<td>24</td>
<td>77</td>
<td>1.9</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Kay et al. (2004)</td>
</tr>
<tr>
<td>Mixed Strawberries</td>
<td>24</td>
<td>720</td>
<td>0.23</td>
<td>222.7†</td>
<td>0.5</td>
<td>1.83</td>
<td>Frank et al. (2003)</td>
</tr>
<tr>
<td>Mixed Red grape juice</td>
<td>7</td>
<td>283</td>
<td>0.18</td>
<td>95.5†</td>
<td>1.5</td>
<td>2.0</td>
<td>Frank et al. (2003)</td>
</tr>
<tr>
<td>Mixed Blueberry</td>
<td>4</td>
<td>1200</td>
<td>0.003</td>
<td>29.2†</td>
<td>1.0</td>
<td>1.83</td>
<td>Mazzara et al. (2002)</td>
</tr>
<tr>
<td>Mixed Elderberry</td>
<td>6</td>
<td>1900</td>
<td>0.03</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Mülleder et al. (2002)</td>
</tr>
<tr>
<td>Mixed Blackcurrant</td>
<td>7</td>
<td>1000</td>
<td>0.039</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Rechner et al. (2002)</td>
</tr>
<tr>
<td>Mixed Elderberry</td>
<td>24</td>
<td>720</td>
<td>0.08</td>
<td>97.4</td>
<td>–</td>
<td>–</td>
<td>Wu et al. (2002)</td>
</tr>
<tr>
<td>Mixed Blueberry</td>
<td>6</td>
<td>690</td>
<td>0.004</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Wu et al. (2002)</td>
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<td>6</td>
<td>68</td>
<td>0.03</td>
<td>1.4</td>
<td>0.3</td>
<td>–</td>
<td>Bub et al. (2001)</td>
</tr>
<tr>
<td>Mixed De-alcoholised red wine</td>
<td>6</td>
<td>56</td>
<td>0.03</td>
<td>1.7†</td>
<td>1.5</td>
<td>–</td>
<td>Bub et al. (2001)</td>
</tr>
<tr>
<td>Mixed Red grape juice</td>
<td>6</td>
<td>117</td>
<td>0.03</td>
<td>2.8‡</td>
<td>3.0</td>
<td>–</td>
<td>Bub et al. (2001)</td>
</tr>
<tr>
<td>Mixed (C-3-gly) Elderberry</td>
<td>24</td>
<td>720</td>
<td>0.05</td>
<td>97.4</td>
<td>1.2</td>
<td>2.2</td>
<td>Cao et al. (2001)</td>
</tr>
<tr>
<td>Mixed Blackcurrant</td>
<td>6</td>
<td>236§</td>
<td>0.11</td>
<td>60.0</td>
<td>1.5</td>
<td>3.0</td>
<td>Matsumoto et al. (2001)</td>
</tr>
<tr>
<td>Mixed Elderberry</td>
<td>–</td>
<td>500</td>
<td>0.05</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Murkovic et al. (2001)</td>
</tr>
<tr>
<td>Mixed Blackcurrant</td>
<td>5</td>
<td>153</td>
<td>0.03</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Netzel et al. (2001)</td>
</tr>
<tr>
<td>Mixed Mixed berries</td>
<td>–</td>
<td>162§</td>
<td>–</td>
<td>29.0</td>
<td>1.0</td>
<td>–</td>
<td>Miyazawa et al. (1999)</td>
</tr>
<tr>
<td>Mixed Red wine</td>
<td>12</td>
<td>218</td>
<td>3.3</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Lapidot et al. (1998)</td>
</tr>
</tbody>
</table>

C_{max}, maximum concentration derived from serum or plasma data; t_{max}, time to reach maximum concentration derived from serum or plasma data; t_{1/2}, half-life of elimination; C-3-gly, mixed cyanidin 3-glycosides; –, unavailable data in original manuscript.

* Values represent total anthocyanins and include anthocyanin metabolites when identified.
† Molar equivalents derived from molecular weight of given anthocyanin. If multiple species listed in paper, cyanidin glucoside (wt 449) was used to derive molar equivalents.
‡ Data interpreted visually from a concentration v. time graph (exact values were not identified in manuscript).
§ Dose derived from conversion of mg/kg to mg based on the reported average subject weight.
though evidence presented by Németh et al. (2003) suggests that cyanidin and delphinidin glucosides are not substrates for lactate phlorizin hydrase and cytosolic β-glucosidase, the fact remains that cyanidin glycosides are absorbed and appear as parent glycosides as well as glucuronide derivatives in the circulation (Mazza et al. 2002; Wu et al. 2002; Felgines et al. 2003; Galvano et al. 2004; Kay et al. 2004, 2005), suggesting both transport of intact compounds and hydrolysis before transport are possible. As both anthocyanin glycosides and glucuronide derivatives have been identified in human plasma and serum the current evidence suggests that both pathways are involved in the absorption of anthocyanins. It is clear that much further research is needed to resolve the mechanisms of anthocyanin absorption. The stomach has also been proposed as a site of absorption of anthocyanins (Passamonti et al. 2003; Talavéra et al. 2003); however, direct evidence of this occurring in humans has not been presented and requires further investigation.

**Metabolism**

Many flavonoids are extensively metabolised in humans with less than 5–10 % of the ingested parent (intact) compounds (following a low to moderate oral dose) excreted in the urine (Bravo 1994). It has been reported that as much as 52 % of an oral dose of flavonoids is excreted in the urine (Bravo 1994). It has been recently documented, with investigations reporting both transport of intact compounds and hydrolysis before transport are possible. As both anthocyanin glycosides and glucuronide derivatives have been identified in human plasma and serum the current evidence suggests that both pathways are involved in the absorption of anthocyanins. It is clear that much further research is needed to resolve the mechanisms of anthocyanin absorption. The stomach has also been proposed as a site of absorption of anthocyanins (Passamonti et al. 2003; Talavéra et al. 2003); however, direct evidence of this occurring in humans has not been presented and requires further investigation.

Many flavonoids are extensively metabolised in humans with less than 5–10 % of the ingested parent (intact) compounds (following a low to moderate oral dose) excreted in the urine (Bravo et al. 1994). It has been reported that as much as 52 % of an oral dose of radiolabelled quercetin was exhaled as 14CO2 (Walle 1980), making the highly hydroxylated flavonoids prime targets for glucuronidation. The glucuronidation reaction is catalysed by UDP-glucuronosyltransferases which is found in high concentrations in the liver, intestine and kidneys. Of all the tissues, the liver has the greatest capacity for glucuronidation (Mojarrabi & Mackenzie, 1998; Strassburg et al. 1998), although more and more evidence points toward the intestine as being the initial and principal site for flavonoid glucuronidation in humans following typical dietary consumption (Cheng et al. 1999; Crespy et al. 1999; Donovan et al. 2001).

Methylation appears to be the second most significant conjugation reaction involving flavonoids (Kuhnle et al. 2000; Williamson et al. 2000). Methylation is driven by a group of enzymes referred to as methyltransferases. These non-specific enzymes are found in many tissues including the liver and intestine (Laitinen & Watkins, 1986). The most common methylation reaction associated with flavonoid metabolism is O-methylation. O-methylation is catalysed by catechol-O-methyltransferase utilising S-adenosyl methionine as a cofactor. The liver has the highest catechol-O-methyltransferase activity and is the main organ responsible for methylation (Piskula & Teroa, 1998). The hydroxylation pattern of a flavonoid’s ring structure will determine the primary site of methylation. Studies reveal that quercetin is extensively methylated following low oral doses in humans and in animals (Manach et al. 1998; Williamson et al. 2000).

Sulfation or glucuronidation are also common conjugation reactions which predominately control the metabolism of phenolic drugs. Sulfation reactions are catalysed by sulfotransferases, which are a small group of cytosolic enzymes widely distributed throughout the body. They utilise phosphoadenosine-5′-phosphosulfate as a cofactor and their known substrates include phenols and polyphenols (i.e. flavonoids), iodothyronines, 4-nitrophenol, and hydroxylamines (Dutton, 1980; Coughtrie et al. 1998). Additionally, sulfation, as a conjugation reaction, is relatively costly in ATP and sulfate and is more likely to be rapidly limited by aglycone loading than glucuronidation (Dutton, 1980). Therefore, sulfate conjugation is regarded as a highly saturable pathway, making it difficult to identify flavonoid sulfates as a result of their relatively low concentrations in the blood and urine.

The body of knowledge regarding the subject of anthocyanin metabolism is much less extensive than that of many other flavonoids, with the metabolic consequence(s) of anthocyanins still a matter of much debate (Fig. 4; potential pathways in the body). The presence of unmetabolised (parent) anthocyanin glycosides in human blood and urine has been extensively documented (Table 1). However, even though many investigators suggest that anthocyanins are not metabolised before release into the systemic circulation, recent evidence indicates otherwise. The detection of glucuronide (Wu et al. 2002; Felgines et al. 2003; Kay et al. 2004, 2005), methyl (Wu et al. 2002; Kay et al. 2004, 2005) and sulfon conjugates (Felgines et al. 2003) has recently been documented, with investigations reporting...
between 68% (Kay et al. 2005) and 80% (Felgines et al. 2003) of anthocyanins in the urine as metabolised derivatives. Studies identifying anthocyanins exclusively as unmetabolised parent compounds probably result from either saturation of metabolic pathways following mega-dose interventions, insufficient extraction procedures, and misidentification as a result of insufficient detection methods (i.e. using UV-visible HPLC exclusively for identification).

Elimination and reabsorption

The route of elimination of anthocyanins and flavonoids (Fig. 4) will ultimately depend on the type of conjugates produced and their site of production. Glucuronides formed in the intestine tend to enter the systemic circulation directly and are not readily available for biliary excretion unlike those newly formed in the liver, which are predominantly excreted into the bile. This implies that glucuronidation in the intestinal cells facilitates the loss of flavonoids from enterohepatic circulation (EHC) towards the systemic circulation. Compounds undergoing EHC may also undergo further sulfation in the intestine or hepatocyte, which would divert them from biliary excretion toward eventual urinary elimination, as enteral reabsorption of strongly polar compounds is negligible (Gregus & Klassen, 1986).

EHC prolongs the residence of a chemical or xenobiotic in the body by reducing its faecal excretion. If a compound undergoing EHC is also available to the systemic circulation, this will result in a longer plasma elimination half-life ($t_{1/2}$). EHC is likely to be a major factor in the metabolism of many flavonoids and is responsible for their low but consistent levels in the circulation long after their time to reach maximum concentration ($t_{\text{max}}$) is achieved (for example, quercetin $t_{\text{max}}$ 1–2 h; $t_{1/2}$ 11–28 h) (Manach et al. 2005). Although it appears that various forms of quercetin may undergo EHC, current evidence suggests that this is probably not a major route for the elimination for highly polar anthocyanin glycosides as their $t_{1/2}$ is reported to be relatively short ($t_{\text{max}}$ 1–4 h; $t_{1/2}$ 1–4 h) (Table 1). This, however, is only speculative as the biliary excretion of anthocyanins in humans is currently unexplored.

The urinary excretion of most flavonoids is relatively low (for example, quercetin about 2–5%) with the exception of the isoflavonoids (for example, genistein and daidzein about 5–50%) (Manach et al. 2005). Similarly the urinary excretion of anthocyanins is generally reported between 0.1% and 3% of the ingested dose (Table 1). In addition, the urinary elimination of colonic metabolites of anthocyanins is largely unknown and may be significant.

Colonic bacteria are likely to play a major role in the metabolism of anthocyanins. Many flavonoids are extensively metabolised by colonic bacteria, leaving a range of phenolic acids which may be reabsorbed (Das & Sothy, 1971; Shaw & Griffiths, 1980; Hackett, 1986; Hollman et al. 2001; Manach et al. 2005). Colonic bacteria cleave glycosides from their parent compounds for catabolism, leaving the resulting aglycones for reabsorption through the intestinal wall or further degradation (Boulton et al. 1999; Schneider et al. 1999; Skibola & Smith, 2000). In a study conducted by Das & Sothy (1971) utilising radiolabelled catechin, [14C]phenolic acid metabolites were found to be reabsorbed and excreted in the urine of rats post-bacterial degradation. Conversely, in a study by Schneider et al. (1999) utilising colonic bacteria isolated from human faeces, quercetin-3-glucoside was observed to be degraded to quercetin; however, the majority of quercetin aglycone was left intact. The extent to which flavonoids are eliminated in the faeces of humans is undetermined for many flavonoids including anthocyanins.

Tsuda et al. (1999) reported a significant increase in plasma concentrations of protocatechuic acid following the administration of cyanidin glycosides to rats. The
researchers suggested that the protocatechuic acid may be derived from the breakdown of anthocyanins. The concentration of protocatechuic acid in the plasma was reported to be eight-fold higher than the parent anthocyanins. Additionally a recent in vitro fermentation study has confirmed that protocatechuic acid is a major metabolic by-product of anthocyanins by human faecal bacteria (Aura et al. 2004). Radiolabelling studies are required to establish the extent to which this occurs in humans in vivo. If anthocyanins are readily absorbed and less than 2 % are generally identified in the urine, it is possible that a significant proportion of anthocyanins exist as colonic metabolites in the circulation. Additionally, if a large conversion of anthocyanins to phenolic acids and phenolic acid residues does occur, these compounds would not be easily identified in biological fluids using traditional HPLC techniques and would probably go undetected.

Varying proportions of flavonoids present in food will also pass through the gastrointestinal tract unabsorbed and be eliminated in the faeces (Rozman, 1986). Studies feeding oral doses of radiolabelled quercetin (Ueno et al. 1983) and catechin (Matsumoto et al. 2001) to rats reported 30–32 % of the radiolabelled compounds to be excreted in the faeces unmetabolised. Empirical evidence for the excretion of anthocyanins in human faeces is currently not available.

The lung is responsible for the elimination of volatile substances from the body and is a major site of excretion for many xenobiotics. In a study by Petrikis et al. (1959), 44 % of the radioactivity of 14C-labelled quercetin was detected in the gastrointestinal tract of rats and 11 % was detected in the lungs. More recently, it has been reported that as much as 52 % of an oral dose of radiolabelled quercetin in humans was exhaled as 13CO2 (Walle et al. 2001). Unfortunately, the relative respiratory excretion of many flavonoids, including anthocyanins, is still unknown.

Pharmacokinetics
The pharmacokinetics of flavonols, flavanones, flavanols and isoflavones has been extensively studied and reviewed elsewhere (Hollman & Katan, 1999; Ross & Kasum, 2002; Scalbert et al. 2002; Karakaya, 2004; Manach & Donovan, 2004; Walle, 2004; Manach et al. 2005; Williamson & Manach, 2005). Complete pharmacokinetic data regarding anthocyanins are limited. The available data suggest that maximum plasma concentrations of anthocyanins in humans are generally between 1 4 and 592 nmol/L and are reported to occur between 0.5–4 h post-consumption of doses ranging between 68 and 1300 mg. Additionally, the average urinary excretion of parent glycosides is generally reported between 0.03 and 4 % of the ingested dose, with a maximum rate of urinary excretion occurring between 1 and 4 h and having a t1/2 of 1.5–3 h (Table 1). Even though the reported elimination rates for quercetin are slow (t1/2 11–28 h), suggesting the possibility for bioaccumulation (Manach et al. 2005), the elimination of anthocyanins is quite rapid (t1/2 1.5–3 h) and accumulation is not likely to occur to any significant extent following normal dietary consumption. However, the significance of the accumulation of colonic metabolites and aglycone breakdown products has yet to be explored.

Summary and conclusion
The literature suggests that anthocyanins may have important health effects; however, from the present review, it is quite apparent that basic aspects of their absorption and metabolism are not fully established. Complete metabolic studies involving compound labelling and the collection of blood, urine, CO2 and faeces are required. Furthermore, multi-dose interventions are necessary to determine the complete pharmacokinetic profiles of all major anthocyanins and anthocyanin metabolites. The limited available data as presented suggest that anthocyanins are absorbed and transported in human serum and urine primarily as metabolites. The contribution of each individual metabolite to the reported biological activity of anthocyanins should be a focus of future research. Once the complete metabolism of anthocyanins is established their true biological activities and heath effects can be explored.

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