Anthocyanins are widely known as pigments responsible for the rich reds and purples in fruits, vegetables and legume seeds. Many flower colors are also attributed to anthocyanins. The physiological functions of anthocyanins as components in food have attracted attention, and research on their bioavailability and physiological functionality has progressed over the last 20 years. This review focuses on the health benefits of anthocyanins. It first describes the chemistry, metabolism, and absorption of anthocyanins and next summarizes the trends in research on the anti-diabetic and anti-obesity effects of anthocyanins. It then describes the other health benefits of anthocyanins, namely, the prevention of cancer and improvement of visual and brain functions, and finally discusses the challenges in and prospects of anthocyanin research.
Anthocyanin content varies markedly among plant species and cultivars and also within a single species, depending on harvest time. Anthocyanins are contained in many types of regularly consumed foods, such as cereals, tubers, roots, greens, pulses, and fruits. Cy and its derivatives, which possess two hydroxyl groups on the B-ring, are the most widely distributed, followed by Del and its derivatives. Although similar data for foods in Japan are unavailable, the typical concentrations of anthocyanins in foods commercially available in the United States have been reported (Wu et al., 2006). Berries are particularly rich in anthocyanins (Table 1), and daily anthocyanin intake in the United States is estimated at 12.5 mg/person. This figure is thought to be higher than the corresponding figure in Japan, albeit with significant differences among individuals.

Recently, results were reported in detail for the European Prospective Investigation into Cancer and Nutrition study, wherein the intake of anthocyanidins in 36,037 individuals (age range, 35 − 74 years) living across 10 European countries was investigated (Zamora-Ros et al., 2001). According to this report, the mean daily anthocyanidin intake in men ranged from 19.8 − 64.9 mg, while that in women ranged from 18.7 − 44.1 mg. There was a clear north-to-south gradi-

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

![Fig. 1. Chemical structures of the anthocyanidins.](image)

![Fig. 2. Structural changes of glucosyl anthocyanins in aqueous solution (G, glucose).](image)
Anthocyanins and Health Benefits

Phenolic acids have attracted much interest. Phenolic acids, such as protocatechuic acid, syringic acid, vanillic acid, phloroglucinol aldehyde, phloroglucinol acid, and gallic acid, have been reported in multiple studies as anthocyanin metabolites, depending on their structure (Gonthier et al., 2003; Aura et al., 2005; He et al., 2005; Keppler and Humpf, 2005; Borges et al., 2007; Forester and Waterhouse, 2008, 2010; Avila et al., 2009). These phenolic acids are likely to be produced during anthocyanin metabolism involving enteric bacteria, or by chemical conversion, and have also been found in humans (Kahle et al., 2006; Vitaglione et al., 2007; Azzini et al., 2010). The actions of these metabolites must be taken into consideration when discussing the health benefits of anthocyanins. However, whether these metabolites are solely responsible for the health benefits of anthocyanins remains a contentious topic. Recently, Lila et al. (2012) aimed to elucidate the metabolic fate of anthocyanins by employing two approaches. The first approach used a human gastrointestinal model for investigating the bioaccessibility of anthocyanins, which is defined as the potential for a substance to interact with or be absorbed by an organism. The second approach employed radio-labeled ($^{13}$C or $^{14}$C) anthocyanins synthesized by plant cells (Lila et al., 2012). Both approaches are crucial for revealing the metabolic pathway and absorption of anthocyanins in detail. To date, however, only a limited number of studies have reported anthocyanins as essential for human health benefits. A clinical ophthalmology study found possible visual improvement in response to a daily intake of 50 mg anthocyanins (Nakaishi et al., 2000).

### Health Benefits of Anthocyanins

Since their antioxidant effect was revealed, various health benefits of anthocyanins have been reported. In particular, marked advancements in research over the last 10 years have been made regarding the conferred health benefits of anthocyanins and the underlying molecular mechanisms. Recent findings in relation to the preventive and suppressive effects of anthocyanins against obesity and diabetes are outlined in the next section.

#### Obesity and diabetes

Suppression of body fat accumulation by anthocyanin intake was first reported by Tsuda et al. (2003). Supplementing high-fat meals (60% of energy) with 2 g/kg of C3G significantly reduced high-fat meal-induced body fat accumulation in C57BL/6J mice (Tsuda et al., 2003; Tsuda, 2008), and impaired lipid synthesis in white adipose tissue was considered a likely underlying mechanism. It was also shown that a diet supplemented with C3G significantly suppresses the elevation of the serum glucose levels induced by high-fat meals. Prior et al. (2008) reported that supplementing a high-fat diet (45% of energy) with anthocyanins extracted from blueberries inhibited weight gain and body fat...

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<table>
<thead>
<tr>
<th>Food</th>
<th>Total anthocyanins (mg/100 g of fresh weight or form consumed)</th>
<th>Total anthocyanins per serving (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Apple</td>
<td>1.3</td>
<td>1.8</td>
</tr>
<tr>
<td>(Fuji)</td>
<td>1.3</td>
<td>1.8</td>
</tr>
<tr>
<td>(Gala)</td>
<td>2.3</td>
<td>3.2</td>
</tr>
<tr>
<td>(Red Delicious)</td>
<td>12.3</td>
<td>17.0</td>
</tr>
<tr>
<td>2. Blackberry</td>
<td>245.0</td>
<td>353.0</td>
</tr>
<tr>
<td>3. Blueberry</td>
<td>(Cultivated) 386.6</td>
<td>529.0</td>
</tr>
<tr>
<td></td>
<td>(Wild) 486.5</td>
<td>705.0</td>
</tr>
<tr>
<td>4. Cherry, sweet</td>
<td>122.0</td>
<td>177.0</td>
</tr>
<tr>
<td>5. Chokeberry</td>
<td>1,480.0</td>
<td>2,147.0</td>
</tr>
<tr>
<td>6. Cranberry</td>
<td>140.0</td>
<td>133.0</td>
</tr>
<tr>
<td>7. Currant</td>
<td>(Black currant) 476.0</td>
<td>533.0</td>
</tr>
<tr>
<td></td>
<td>(Red currant) 12.8</td>
<td>14.3</td>
</tr>
<tr>
<td>8. Elderberry</td>
<td>1,375.0</td>
<td>1,993.0</td>
</tr>
<tr>
<td>9. Grape</td>
<td>(Red grape) 26.7</td>
<td>42.7</td>
</tr>
<tr>
<td></td>
<td>(Concord) 120.1</td>
<td>192.0</td>
</tr>
<tr>
<td>10. Nectarine</td>
<td>6.8</td>
<td>9.2</td>
</tr>
<tr>
<td>11. Peach</td>
<td>4.8</td>
<td>4.7</td>
</tr>
<tr>
<td>12. Plum</td>
<td>19.0</td>
<td>12.5</td>
</tr>
<tr>
<td>13. Strawberry</td>
<td>21.2</td>
<td>35.0</td>
</tr>
<tr>
<td>14. Black bean</td>
<td>44.5</td>
<td>23.1</td>
</tr>
<tr>
<td>15. Eggplant</td>
<td>85.7</td>
<td>35.1</td>
</tr>
<tr>
<td>16. Red cabbage</td>
<td>322.0</td>
<td>113.0</td>
</tr>
<tr>
<td>17. Red onion</td>
<td>48.5</td>
<td>38.8</td>
</tr>
</tbody>
</table>

This table is reproduced from Wu et al. (2006) with permission from the American Chemical Society.
accumulation, while supplementation with whole blueberry powder (WBP) promoted body fat accumulation in C57BL/6 mice. A separate study by the same group also demonstrated that consumption of blueberry juice caused a significant reduction in body weight gain and percentage of white adipose tissue (epididymal and retroperitoneal fat) in mice fed a high-fat diet (45% of energy) (Prior et al., 2010a). Similarly, DeFuria et al. (2009) reported that supplementing a high-fat diet (60% of energy) with WBP did not reduce body weight gain significantly in C57BL/6 mice. Contrary to these findings, interesting results were recently reported by Seymour et al. (2011), who demonstrated that supplementing a high-fat diet (45% of energy) with 2% WBP reduced abdominal fat mass and increased the activity of the adipose tissue and skeletal muscle peroxisome proliferator-activated receptor (PPAR) in Zucker fatty rats. However, the same study showed a WBP-induced body weight gain in Zucker lean rats (Seymour et al., 2011).

Anthocyanins from berries other than blueberries have also been tested for their anti-obesity and anti-diabetic effects. Intake of black raspberry anthocyanins (as a juice or in powder form) did not significantly reduce body fat accumulation or body weight gain induced by a high-fat diet (60% of energy) in mice (Prior et al., 2009, 2010b; Kaume et al., 2012). On the other hand, intake of mulberry water extracts containing a high concentration of anthocyanins reduced body weight gain (Peng et al., 2011). Intake of tart cherry powder also reduced body weight gain and the amount of retroperitoneal fat in Zucker fatty rats (Seymour et al., 2009). Intake of extracts of another anthocyanin-rich berry, chokeberry, also suppressed the increase in epididymal white adipose tissue and blood glucose level in mice fed a fructose-rich diet (Qin and Anderson, 2012).

Anthocyanins act on adipocytes, thereby modulating adipocytokine expression. C3G, or its aglycon Cy, was reported to upregulate the expression of adiponectin, which increases insulin sensitivity in rat white adipose tissue and in human adipocytes (Tsuda et al., 2004, 2006). However, these findings have not yet been supported by in vivo results. The study by DeFuria et al. (2009), introduced above, demonstrated the suppressive effect on obesity-associated inflammation in white adipose tissue in the group that received WBP. More specifically, the degree of upregulation of tumor necrosis factor-alpha (TNF-α) and monocyte chemotactic protein-1 mRNAs was significantly lower in the group fed a high-fat diet with blueberry supplementation than in the group without supplementation. It was also shown that a significant downregulation of glutathione peroxide 3 level, induced by the high-fat diet, was restored by blueberry intake. Furthermore, intake of tart cherry powder suppressed the upregulation of proinflammatory cytokines (IL-6 and TNF-α) associated with obesity, while upregulating PPARα and PPARγ mRNAs in Zucker fatty rats (Seymour et al., 2009). Similarly, chokeberry intake increased plasma adiponectin levels and decreased plasma TNF-α and IL-6 levels (Qin and Anderson, 2012).

Anthocyanin intake was also shown to reduce increases in blood glucose level and to improve insulin sensitivity in type 2 diabetes models. Similar effects were found in studies using high purity C3G (Sasaki et al., 2007; Tsuda, 2008) and also bilberry extract (BBE) containing different types of anthocyanins (Takikawa et al., 2010). Downregulation of retinol binding protein 4 (RBP4) was shown to constitute a part of the mechanism underlying such effects of isolated C3G in KK-A’ mice, a model for type 2 diabetes (Sasaki et al., 2007). Yang et al. (2005) identified RBP4 as a new adipocytokine linked to the pathogenesis of type 2 diabetes and a factor contributing to insulin resistance (Yang et al., 2005). Although the role of RBP4 in diabetes in humans remains debatable, the anti-diabetic effect of C3G in mice can be explained by this finding. More specifically, C3G intake upregulates the expression of glucose transporter 4 (Glut4), thereby decreasing RBP4 expression. This improves insulin sensitivity in peripheral tissues and suppresses glucose release induced by elevated gluconeogenesis (Sasaki et al., 2007). Further, C3G and its metabolite protocatechuic acid were recently shown to activate PPARγ and to induce upregulation of Glut4 and adiponectin in murine 3T3-L1 adipocytes and human adipocytes (Scaccia et al., 2011). However, C3G does not appear to serve as a ligand of PPARγ or to upregulate the expression of adiponectin in vivo (Sasaki et al., 2007). Thus, the anti-diabetic effect of C3G cannot be explained by the upregulated expression of PPARγ and adiponectin.

Considering the low C3G content in BBE, it is difficult to explain its anti-diabetic effect by the mechanism involving RBP4 downregulation. BBE intake was shown to activate AMP-activated protein kinase (AMPK) in skeletal muscle and white adipose tissue and the liver (Takikawa et al., 2010). BBE-induced AMPK activation leads to upregulation of Glut4 expression in skeletal muscle and white adipose tissue, as well as suppression of gluconeogenesis in the liver. At the same time, it also promotes lipid utilization, thereby suppressing increases in blood glucose level and improving insulin sensitivity (Fig. 3) (Takikawa et al., 2010). The fact that BBE contains many types of anthocyanins may be the key in the AMPK-mediated anti-diabetic effect, and this point needs to be addressed in future studies.

Another possible mechanism for anthocyanins’ preventive and suppressive effect on diabetes is the inhibition of
expression (Kwon et al., 2009). However, Del does not exist in the form of the flavylium cation and is unstable under neutral conditions; thus, whether it can exert an action on these target molecules in vivo remains unclear.

Recent attempts to create purple tomatoes by expressing high levels of anthocyanins (Butelli et al., 2008; Gonzali et al., 2009; Povero et al., 2011) are of particular interest. Martin and colleagues expressed snapdragon genes involved in anthocyanin biosynthesis in tomato and successfully obtained anthocyanin-rich tomatoes with intense purple coloration (Butelli et al., 2008). When cancer-susceptible mice (Trp53−/− mice) were fed a diet supplemented with purple tomatoes or normal tomatoes, the life span extension was significantly longer in the mice that consumed purple tomatoes than in those that consumed normal tomatoes (Butelli et al., 2008).

A similar approach may be useful for other health promoting purposes, as well as for cancer prevention. More precisely, establishing and cultivating vegetables that contain appropriate anthocyanin compositions and content, sufficient for exerting health benefits, will be useful for health improvement.

Other Health Benefits of Anthocyanins: Anthocyanins in Relation to Cancer, Visual Function, and Brain Function

Cancer The preventive and suppressive effects of anthocyanins on cancer have been demonstrated in many studies, and key findings in this area can be found in the literature (Thomasset et al., 2009; Prasad et al., 2010). In general, anthocyanins suppress inflammatory pathways associated with cancer pathogenesis. For example, Del, an aglycon, binds directly to mitogen-activated protein kinase 4 and phosphatidylinositol 3-kinase to inhibit both enzymes, thereby suppressing ultraviolet B-induced cyclooxygenase-2 expression (Kwon et al., 2009). However, Del does not exist in the form of the flavylium cation and is unstable under neutral conditions; thus, whether it can exert an action on these target molecules in vivo remains unclear.

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Visual function Improvement of visual function as well as antioxidant effects is a well-known health benefit of anthocyanins. A famous anecdote about the effect of anthocyanins on vision in Japan describes Royal Air Force pilots during World War II who experienced enhanced vision in the dark
after ingesting blueberry jam. However, this anecdote itself is not evidence-based, and several clinical studies failed to verify the above claim (Levy and Glovinsky, 1998; Zadok et al., 1999; Muth et al., 2000; Canter and Ernst, 2004). On the other hand, bilberry anthocyanins have been shown to exhibit neuroprotective effects in ocular tissue and suppressive effects on diabetic retinopathy via a reduction of angiogenesis (Matsunaga et al., 2010a, 2010b). Furthermore, several studies have demonstrated that blackcurrant anthocyanins (BA) improve visual function; for example, BA inhibit transient myopia, reduce eye fatigue, improve dark adaptation, and increase retinal blood flow in glaucoma (Nakaishi et al., 2000; Ohguro et al., 2007; Iida et al., 2010). The first three benefits have been shown in humans, and effective daily BA intake was reported as 50 mg (Nakaishi et al., 2000). Two studies have indicated the possible mechanism underlying inhibition of myopia. The BA concentration was higher in ocular tissues (sclera, choroid, ciliary body, retina, iris, and cornea) than in blood in a rat fed a diet containing BA, and in particular, concentrations in the sclera and choroid were 100-fold higher than the blood BA concentration (Matsumoto et al., 2006). In addition, it was shown that an anthocyanin in the concentration range of $10^{-7}$ to $10^{-8}$ M relaxed endothelin-1-induced contraction of bovine ciliary smooth muscle (Matsumoto et al., 2005), which plays an important role in modulating refraction of the lens through contraction and relaxation, which in turn control accommodation. Thus, it is suggested that anthocyanins stimulate the endothelin-1 receptor to induce production of NO, thereby relaxing ciliary smooth muscle, which in turn flattens the lens and consequently inhibits myopia.

**Brain function** The favorable effects of intake of anthocyanin-rich fruits on memory (Shukitt-Hale et al., 2006; Krikorian et al., 2010a, 2010b) and on cognitive and motor function by delaying deterioration of neural function in aged individuals (Shukitt-Hale et al., 2009a, 2009b) were studied mainly by the group lead by Joseph. Neural signaling and suppression of neuroinflammation are implicated in the mechanism for such effects. In Fisher rats, blueberry intake suppressed an aging-associated increase in nuclear factor-kappa B levels (NF-kB) (Goyarzu et al., 2004) and upregulation of TNF-α, IL-1β and NF-kB expression in the hippocampus (Shukitt-Hale et al., 2008).

Williams et al. (2008) demonstrated that blueberry intake induced activation of cyclic AMP-response element-bonding protein and an increase in the level of brain-derived neurotrophic factor, in which extracellular signal-related kinases 1 and 2 were involved. Improvement of cerebrovascular blood flow may also be implicated in the favorable effects of berries on brain function (Spencer, 2010).

**Challenges in and Prospect for Anthocyanin Research**

This review so far described the health benefits of anthocyanins, with an emphasis on their preventive and suppressive effects on obesity and diabetes, as well as their metabolism and absorption, and then outlined the trends in cancer prevention research and the improvement of visual and brain function by anthocyanins. Next, challenges in anthocyanin research are discussed to conclude this review.

Although evidence of the pleiotropic properties of anthocyanins, such as the suppressive effect on body fat accumulation and anti-diabetic action, continue to accumulate, only a limited number of studies have identified the specific molecular structures of anthocyanins responsible for their health benefits. Interestingly, in some cases, intake of a blend of multiple types of anthocyanins can be more effective than intake of a single molecular species, suggesting the necessity of clarifying the most effective molecular species and blend compositions of anthocyanins for exerting individual health benefits. For example, black raspberry anthocyanins do not appear effective for suppressing body fat accumulation (Prior et al., 2009, 2010b; Kaume et al., 2012), and this lack of effect may be attributed to the compositional difference between black raspberry and blueberry anthocyanins, as the main anthocyanin in black raspberry is cyanidin 3-rutinoside. If so, the sugar moieties of anthocyanins may influence the degree of function.

Investigations in the current literature used mainly anthocyanin-rich extracts, with a few exceptions of isolated and purified anthocyanins. In fact, anthocyanins, as a part of a diet, are more likely to be ingested with other dietary components such as polyphenols. Nevertheless, it is important to determine whether an anthocyanin (or anthocyanins) is solely responsible for exerting health benefits, whether other dietary components are the main mediators of the benefits, or whether co-intake of an anthocyanin (or anthocyanins) and other dietary components is necessary.

Current evidence to support the health benefits of anthocyanins in humans is insufficient. Although several studies showed the favorable effects of anthocyanins on visual function, they used a small number of subjects, and are thus not considered thorough. Further investigation is necessary to address the benefits of anthocyanins in humans.

Understanding anthocyanin metabolites on the basis of chemical structures of anthocyanins is of particular importance. The quantity of anthocyanin metabolites produced from processing by enteric bacteria or chemical reactions must be taken into account when considering the efficacy of anthocyanins. On the other hand, the effect of the anthocyanins directly absorbed in the form of glycosides, albeit with their low bioavailability, should not be neglected. Even
Anthocyanins and Health Benefits

though the quantities of absorbed anthocyanins, in the form of glycosides, or their metabolite are small, it is possible that the signals triggered by them are amplified in the digestive tract or on the cell surface, thereby exerting diverse functions. Advancements in research in this field may successfully depict mechanisms for health benefits of anthocyanins, despite their low absorption rates in the body. Nevertheless, the correlations between beneficial levels of anthocyanin intake and concentrations of their metabolites have not yet been clarified. If enteric bacteria-generated metabolites of anthocyanins are important for promoting health benefits, differences in the quality and quantity of gut flora will influence the levels of expected health benefits among individuals.

It was only a few decades ago that anthocyanins were regarded as highly degradable compounds, and the main research areas were their chemical structures, color stability, use as food constituents, and changes in foods during storage. Anthocyanins are now recognized as food constituents with potential health benefits, and research related to these properties has markedly progressed at the molecular level. Anthocyanins will continue to attract researchers across various disciplines, including those involved in the creation of new flower varieties with novel colors. Research on the health benefits of anthocyanins will provide information on underlying molecular mechanisms and absorption and metabolism. Moreover, once these benefits are proven in humans, development of foods and dietary supplements in capsule form can be accelerated to promote the proven functions.

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References


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