REVIEW

Dietary anthocyanin-rich plants: Biochemical basis and recent progress in health benefits studies

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Anthocyanins are one type of flavonoid phytopigment. Although the role of anthocyanins as a functional food factor remains relatively less established than that of other flavonoids, progress in this area has been made at the molecular level in recent years. This review discusses the potential health benefits of plant-derived anthocyanin-rich foods, with a focus on the role of anthocyanins in obesity control, diabetes control, cardiovascular disease prevention, and improvement of visual and brain functions, areas that have attracted much attention. Such health benefits are not necessarily derived from the antioxidant effect of anthocyanins, but in fact are produced by currently unestablished chemical properties beyond the antioxidant capacity of the molecules. However, a better understanding of the physiological functionality of anthocyanins remains to be elucidated. It is desirable, therefore, to clarify the molecular type and composition of the anthocyanins that confer specific health benefits and to conduct further investigation into the underlying molecular mechanisms. The pharmacological actions of anthocyanins could not be fully established without knowledge on the effects of treatment of anthocyanins alone, the effects of non-anthocyanin components, and the possible interactions between anthocyanin and non-anthocyanin species.

Keywords:
Anthocyanin / Brain function / Diabetes / Eye vision / Obesity

1 Introduction

Anthocyanins are one of the flavonoid phytopigments [1]. Previous research has focused on chemical analysis of the structure and hue of flower pigments, and pigment expression and stabilization. Efforts have been made to identify genes for anthocyanin synthesis in plants and elucidate the regulatory pathways for gene expression. In horticulture, color conversion of flower pigments has been made possible by genetic engineering, and while the role of anthocyanins as a functional food factor remains relatively less established than other flavonoids, exciting progress has been made at the molecular level in this area in recent years. This review aims to discuss the potential health benefits of plant-derived anthocyanin-rich foods, with a focus on the role of anthocyanins in obesity control, diabetes control, cardiovascular disease (CVD) prevention, and improvement of visual and brain functions, areas that have attracted much attention.

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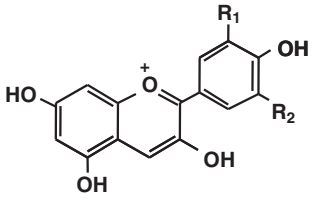
Abbreviations: AMPK, AMP-activated protein kinase; **BA,** blackcurrant anthocyanins; **BBE,** bilberry extract; **CVD,** cardiovascular disease; **C3G,** cyanidin 3-glucoside; **C3R,** cyanidin 3-rutinoside; **Glut4,** glucose transporter 4; **PPAR,** peroxisome proliferator-activated receptor; **RBP4,** retinol-binding protein 4; **TNF-α,** tumor necrosis factor-α; **WAT,** white adipose tissue; **WBP,** whole blueberry powder

2 Chemistry and source of anthocyanins

Anthocyanins occur naturally in plants in the form of glycosides in which the anthocyanidin molecule is coupled with a sugar. The part of the pigment that exists free of sugar (generically known as aglycone) is called an anthocyanidin. Anthocyanins can be classified into many types based on modifications, such as substituent groups on the B ring, type and number conjugated sugar, and the presence or absence of an acyl group. There are at least six principal types of anthocyanins: pelargonidins, cyanidins, delphinidins,

peonidins, petunidins, and malvidins (Fig. 1). The hue of anthocyanins may vary according to different substituent groups present on the B ring, and color saturation increases with increasing number of hydroxyl groups and decreases with the addition of methoxyl groups. Under highly acidic conditions, anthocyanins assume the form of a flavylium ion, exhibit a red color, and are relatively stable. Under weakly acidic or neutral pH conditions, however, anthocyanins are converted into colorless pseudo-bases upon reaction with water, thereby becoming unstable (Fig. 2) [2]. The relative abundance of anthocyanins varies greatly according to plant species and timing of harvest, and anthocyanins can be found in many plant foods such as cereals, tubers and roots, vegetables, pulses, and fruits. Cyanidins (which contain two hydroxyl groups on the B ring) are the most widely distributed species, followed by delphinidins. Extensive research has been done on the distribution of anthocyanins in major plant foods [3–5]. Table 1 shows the anthocyanin composition of some common berries [4]. While it is known that fruits (e.g. berries and grapes), cereals, and vegetables constitute good sources of anthocyanins, considerable individual variability exists in daily consumption levels of total anthocyanins [6, 7].



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 1. Chemical structures of the anthocyanidins in plant-based foods.

3 Absorption and metabolism and intake dosage for functional expression of anthocyanins

A number of excellent reviews have been written on the subject of anthocyanin absorption and metabolism [8–10]. Here, we summarize findings from recent studies with a focus on interactions with other compounds.

It has been reported that protocatechuic acid derived from the B ring of cyanidin 3-glucoside (C3G) was detected as an anthocyanin metabolite [11]. More recently, phenolic acids including protocatechuic acid, syringic acid, vanillic acid, phloroglucinol aldehyde, phloroglucinol acid, and gallic acid have been confirmed as chemically derived metabolites of anthocyanins [12–19]. These phenolic acids are thought to be formed during anthocyanin metabolic processing by enteric bacteria or in chemical reactions [20].

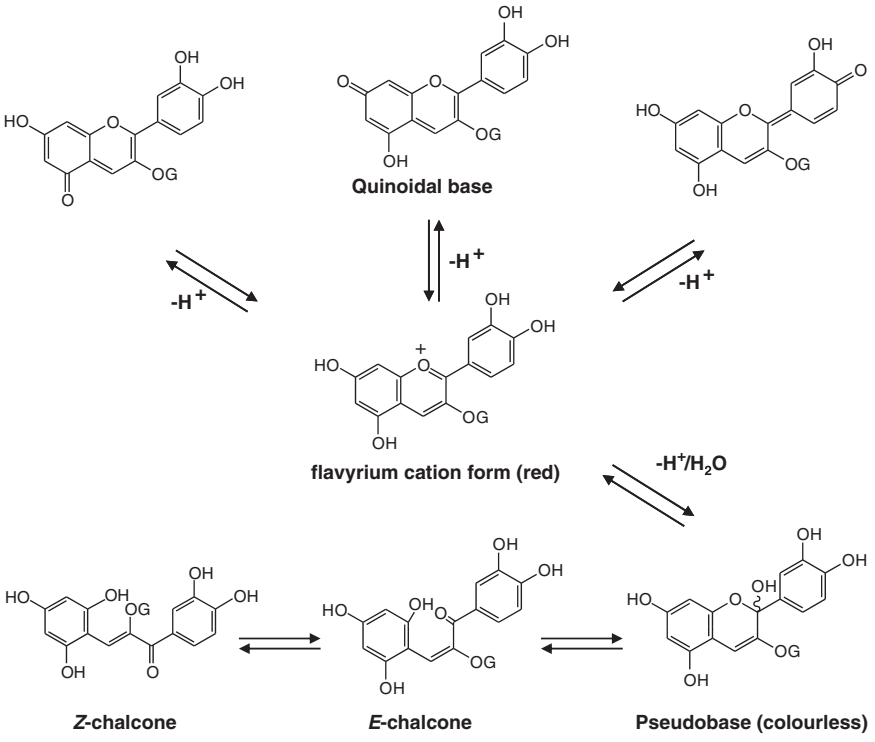


Figure 2. Structural changes of anthocyanins (C3G) in aqueous solution (G; glucose).

Table 1. Anthocyanin content in fresh berries [4]

Berries	Anthocyanins	mg/g extract[a)]
Bilberry	Cyanidin 3-galactoside	3.70
	C3G	4.05
	Cyanidin 3-arabinoside	2.54
	Delphinidin 3-galactoside	4.58
	Delphinidin 3-glucoside	4.73
	Delphinidin 3-arabinoside	3.53
	Peonidin 3-galactoside	0.46
	Petunidin 3-halactoside	1.52
	Petunidin 3-glucoside	2.94
	Petunidin 3-arabinoside	0.84
	Malvidin 3-arabinoside	0.81
	Peonidin 3-glucoside/malvidin 3-Galactoside	3.48
	Peonidin 3-arabinoside/malvidin 3-glucoside	3.62
Blackberry	C3G	7.17
	C3R	0.06
	Cyanidin 3-arabinoside	0.05
	Cyanidin 3-xyloside	0.47
	Cyanidin 3-(6-malonoyl)glucoside	0.30
	Cyanidin 3-dioxaloylglucoside	2.05
Blackcurrant	C3G	1.10
	C3R	7.08
	Delphinidin 3-glucoside	2.94
	Delphinidin 3-rutinoside	9.79
	Peonidin 3-rutinoside	0.11
	Petunidin 3-rutinoside	0.18
Blueberry	Cyanidin 3-galactoside	0.28
	C3G	0.04
	Cyanidin 3-arabinoside	0.12
	Delphinidin 3-galactoside	1.37
	Delphinisin 3-glucoside	0.13
	Delphinidin 3-arabinoside	0.74
	Peonidin 3-galactoside	0.15
	Petunidin 3-galactoside	1.07
	Petunidin 3-glucoside	0.11
	Petunidin 3-arabinoside	0.46
	Marlidin 3-arabinoside	1.75
	Peonidin 3-glucoside/malvidin 3-galactoside	3.65
	Peonidin 3-arabinoside/malvidin 3-glucoside	0.43
Strawberry	C3G	0.09
	Pelargonidin 3-glucoside	50.7

a) Values are expressed as mean of triplicate analyses for each sample. This table was reprinted from [4], with permission from American Chemical Society.

These anthocyanin metabolites have also been found in humans [21–23]. It is therefore reasonable to take into account the potential effects of such metabolites when assessing the health benefits of anthocyanins. In terms of interactions with other compounds, quercetin was found to inhibit C3G uptake [24]. On the other hand, when blackcurrant anthocyanins (BA) and phytic acid were simultaneously administered, the blood concentration of anthocyanin was significantly increased in both rats and humans compared with the administration of BA alone [25]. In humans, intake of cream did not affect the C_{max} of pelargonidin-*O*-glucuronide (an anthocyanin metabolite), but raised its T_{max} [26]. In studies using male pigs, it was found that intake of cereal and milk did not affect the C_{max} of BA during absorption, but increased the T_{max} and absorption rate constant by 0–8 h [27]. In human studies, intake of rice cake was reported to have no effect on BA [28]. As discussed later, in clinical research on visual functions, anthocyanin intake at a daily dose of 50 mg reportedly confers some health benefits [29]. For other physiological functions, there have been few reports on the optimal intake dose for granting beneficial effects in humans.

4 Recent progress in studies of the health benefits of anthocyanins

So far, the potential health benefits of anthocyanins have been articulated in the contexts of their antioxidant properties. Recent efforts have been directed toward elucidating the molecular mechanisms underlying some of anthocyanins' novel health benefits. Such novel functions are not necessarily dependent on the antioxidant effects of anthocyanins, but in fact are produced by currently unestablished chemical properties beyond the antioxidant capacity of the molecules. In the following sections, we discuss recent findings of the effects of anthocyanins on obesity and diabetes prevention, CVD prevention, and improvement of visual and brain functions.

4.1 Obesity

The inhibitory effects of anthocyanins on body fat accumulation were first reported by Tsuda et al. in 2003 [30]. In C57BL/6J mice, a C3G-containing diet (2 g/kg) was found to significantly reduce body fat accumulation induced by high-fat meals (60% of energy), when compared with controls [30, 31]. This effect was probably due to suppression of lipid synthesis in the liver and in white adipose tissue (WAT). In addition, a C3G-containing diet also significantly reduced plasma glucose concentration, which was elevated by high-fat meals. Anthocyanins may act on adipocytes and modulate the expression levels of adipocytokines. C3G (or its agylcone cyanidin) was reported to upregulate the expression of adiponectin, which can increase insulin sensitivity in human adipocytes [32, 33]. However, this effect was not observed in in vivo (mouse) models [34].

Berries are a rich source of anthocyanins. Interestingly, when anthocyanin extract from blueberries or whole blueberry powder (WBP) was added as a supplement to high-fat meals (45% of energy) in C57BL/6 mice, intake of anthocyanin extract significantly inhibited weight gain and body

fat accumulation, whereas intake of WBP actually promoted body fat accumulation [35]. In another study by the same group, it was found that intake of a blueberry juice did not produce any statistical difference in body weight or percentage of WAT (epididymal and retroperitoneal fat) in mice fed high-fat meals (45% of energy) [36]. In this case, the sugar content in blueberry juice was found to be not linked to the progression of obesity. In a similar study with C57BL/6 mice by DeFuria et al., intake of WBP supplemented to high-fat meals (60% of energy) failed to inhibit body weight gain [37]. On the other hand, intake of WBP did show inhibitory effects on obesity-induced inflammation in WAT in this study. Specifically, mRNA levels of tumor necrosis factor-α (TNF-α) and monocyte chemoattractant protein-1 were upregulated in the high-fat meal control group, although expression was significantly reduced in the blueberry group. Additionally, intake of blueberries restored glutathione peroxidase 3 levels, which were otherwise significantly reduced by high-fat meals as in the control group.

As another example of anthocyanin source, black raspberries (black raspberry juice or black raspberry powder) were unable to inhibit body fat accumulation or body weight gain in mice fed with high-fat meals (60% of energy) [38, 39]. However, other studies have reported that intake of mulberry extract in an aqueous phase significantly inhibited body weight gain [40], while intake of tart-cherry powder extract inhibited body weight gain, reduced retroperitoneal fat amount, suppressed proinflammatory cytokine (IL-6, TNF-α) expression, and upregulated mRNA expression of peroxisome proliferator-activated receptor (PPAR) α and γ in Zucker fatty rats [41]. For non-berry species, intake of blood orange (Moro orange), which contains anthocyanins, reportedly inhibited body weight gain and body fat accumulation, but intake of anthocyanin extract fractions was less effective [42].

Based on evidence from the above studies, intake of anthocyanins could inhibit high-fat meal-induced body weight gain and body fat accumulation to some extent in animal (mouse) models, though the effects of whole powder intake remain controversial. Discrepancies in findings may have arisen from different experimental conditions used, including the percentage of lipid to energy contribution in a high-fat diet, dosage of test sample used as supplement, and anthocyanin content and composition. In particular, the lack of effects by black raspberries [39] may be due to anthocyanin compositions different from those of blueberries; in black raspberries, cyanidin 3-rutinoside (C3R) is the principal anthocyanin. This finding also suggests that the conjugating sugar may modulate the functional expression of the anthocyanin molecule. To date, the majority of studies evaluating the antiobesity role of anthocyanins have relied on anthocyanin-rich crude extracts from plant sources, and it is unclear which molecular structures of anthocyanins are responsible for antiobesity. To obtain a better understanding of the drug effects of anthocyanins, it is necessary to clarify the most potent anthocyanins by characterizing their properties at the molecular level, and it is necessary to take into account the effects of co-existing compound species in extracts, as well as the effects that occur in parallel during anthocyanin treatment.

4.2 Diabetes

Various studies have reported that intake of anthocyanins could inhibit elevation of blood glucose levels induced by a high-fat diet in mouse models of obesity [31, 35, 37, 43]. In a type 2 diabetes model, intake of anthocyanins was found to inhibit elevation of blood glucose levels and improve insulin sensitivity. This was achieved by intake of high-purity anthocyanin (C3G) [31, 34] or bilberry extract (BBE) anthocyanin [44], which contain a diverse variety of anthocyanins. In a type 2 diabetes mouse (KK-A^y) model, anthocyanin (high-purity C3G) was found to inhibit elevation of blood glucose levels and improve insulin sensitivity via downregulation of retinol-binding protein 4 (RBP4) [34]. In a 2005 study by Yang et al., RBP4 was identified as an adipocytokine linked to type 2 diabetes pathogenesis and as a factor implicated in insulin resistance [45]. While this finding may be insufficient to conclude the role of RBP4 in diabetes in humans, it provides some insight into how C3G exhibits inhibitory effects against diabetes in mouse models. More specifically, C3G intake upregulated glucose transporter 4 (Glut4) expression, which in turn led to downregulated RBP4 expression. Consequently, this process constitutes an inhibitory effect on the reduction of insulin sensitivity in peripheral tissue and glucose release following excessive gluconeogenesis [34]. However, because only a low level of C3G is contained in BBE, the antidiabetic effects of BBE (which contains multiple anthocyanins) cannot be established on the basis of downregulated RBP4 expression. Recent studies have shown that AMP-activated protein kinase (AMPK) is one of the crucial factors for cellular energy homeostasis. It is therefore recognized as a potential therapeutic target in the prevention and treatment of type 2 diabetes [46]. In type 2 diabetes therapies, AMPK could be activated by some small-molecule drugs (for example, metformin and thiazolidinediones) [47, 48]. Indeed, activation of AMPK by exercise is one of the most effective interventions in type 2 diabetes patients [49]. Likewise, dietary BBE activates AMPK in WAT, skeletal muscle, and liver. In WAT and skeletal muscle, activation of AMPK induces upregulation of Glut4, which results in enhanced glucose uptake and utilization in these tissues. In the liver, dietary BBE clearly reduces glucose production via AMPK activation. This reduction efficiently ameliorates hyperglycemia in type 2 diabetic mice. Furthermore, BBE-induced AMPK activation in the liver results in significantly decreased liver and serum lipid content via upregulation of PPARα and acylCoA oxidase. Upregulation of carnitine

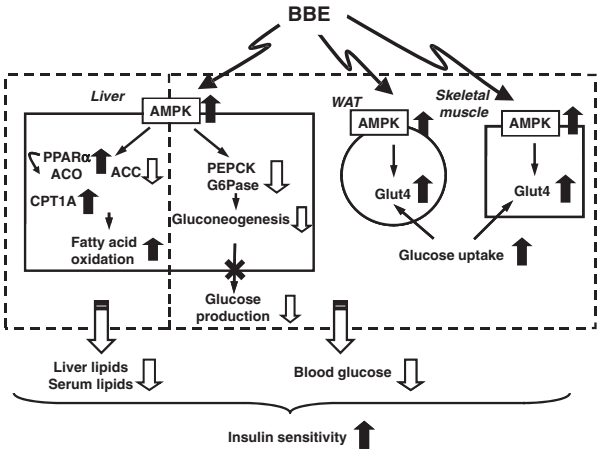


Figure 3. Proposed mechanism for amelioration of hyperglycemia and insulin sensitivity by dietary BBE. BBE activates AMPK in the WAT and skeletal muscle. This activation induces upregulation of Glut4 and enhancement of glucose uptake and utilization in these tissues. In the liver, dietary BBE reduces glucose production via AMPK activation, which ameliorates hyperglycemia in type 2 diabetic mice. The AMPK activation induces phosphorylation of acetylCoA carboxylase (ACC) and upregulation of PPARα, acylCoA oxidase (ACO), and carnitine palmitoyltransferase-1A (CPT1A) gene expression in the liver. These changes lead to reductions in lipid content and increases in insulin sensitivity via reduction of lipotoxicity [44]. PEPCK, phosphoenol pyruvate carboxykinase; G6Pase, glucose-6-phosphatase.

palmitoyltransferase-1A by dietary BBE enhances the decrease in lipid content via enhancement of fatty acid oxidation. Such changes may also contribute to the antidiabetic effect of BBE (Fig. 3) [44]. It is important to further clarify how complex formulations (such as BBE) containing multiple anthocyanins can exhibit antidiabetic effects through the activation of AMPK pathways.

Regarding the mechanisms underlying anthocyanins' actions in antidiabetic effects, our knowledge remains limited owing to the paucity of empirical studies. In addition to the above-mentioned RBP4 and AMPK pathways, it has been suggested that anthocyanins may also work as antidiabetes food factors via inhibition of α-glucosidase activity in the small intestinal endothelium. The inhibitory effects of anthocyanins on α-glucosidase activity vary with the molecular structure of drugs. Among the anthocyanins, glycosides such as that of C3G were found to be very weak inhibitors of α-glucosidase [50]. Matsui and colleagues established a unique assessment method for testing the inhibition of α-glucosidase activity, with which they examined the inhibition of α-glucosidase by acylated anthocyanins. In their results, purple potato-derived acylated anthocyanins were shown to inhibit α-glucosidase activity [51, 52]. This same group also studied the inhibitory effects acylated anthocyanins on the elevation of blood glucose levels in rats [53], and found that the molecular structure responsible for α-glucosidase inhibition was not anthocyanidin itself, but caffeoylsophorose, which is a component of the acylated anthocyanin molecule [54–56]. Caffeoylsophorose can be absorbed in vivo in an intact form and subsequently produces caffeic acid and its conjugate [57]. It is possible that absorbed caffeoylsophorose (or its metabolites) can be explored as new antidiabetic agents.

4.3 CVD

The Kuopio ischemic heart disease risk factor study demonstrated that a group of people who had consumed a large amount of berries rich in anthocyanins (>408 g/day) had a significantly lower risk of CVD-related death than those in the low-consumption group (<133 g/day) [58]. A significant association between strawberry consumption and mortality due to CVD was also revealed in the Iowa women's health study involving postmenopausal women [59]. On the other hand, Curtis et al. reported that the intake of cyanidin glycosides (500 mg/day) did not affect the levels of inflammatory biomarkers, platelet reactivity, or lipid levels involved in CVD in postmenopausal women [60]. With regard to human intervention trials [59, 61, 62], consumption of anthocyanin-containing plant foods, such as blackcurrants, bilberries, and blueberries, reduced LDL-cholesterol levels and increased plasma antioxidant capacity. Furthermore, the intake of red grape juice rich in anthocyanin reduced the concentrations of oxidized LDL and the activity of NADPH oxidase in dialysis patients [63], and the inhibitory effects of fruits containing anthocyanins on atherosclerosis were reported in elderly men [64]. Recent studies, however, showed no effects of blackcurrant juice [65] and blood orange juice [66] on cardiovascular risk markers.

Anthocyanins are thought to exert their cardiovascular protective effects through anti-inflammation and antiplatelet activity, the latter which is reported to mediate anthocyanins or their putative colonic metabolites [61]. In addition, there are reports that anthocyanins significantly inhibit TNF-α-induced inflammation through monocyte chemoattractant protein-1 in human endothelium [67], suppress myocardial ischemia-reperfusion injury by delphinidin through the inhibition of signal transducers and activators of transcription 1 activity in cardiac muscle [68], and potentially inhibit the effects of C3G or its metabolite, protocatechuic acid, on atherosclerosis progression via inhibition of inflammation [69, 70].

The above studies clearly demonstrate the beneficial cardiovascular protective effects of polyphenols, including anthocyanins, in humans. However, because of a number of negative reports, further studies are needed to elucidate whether anthocyanins alone can exhibit significant cardiovascular protection. Moreover, we believe that certain cardiovascular protective effects of anthocyanins are attributed to anthocyanin metabolites such as protocatechuic acid.

4.4 Visual functions

Antioxidant effects are one of the well-documented physiological functions of anthocyanins. In ophthalmological research, these antioxidant effects have been demonstrated in retinal pigment epithelium [71]. In animal models, blueberry extract reportedly reduced cataract and significantly reduced lipid oxidation in blood [72]. Recent studies investigating the effect of anthocyanins on the improvement of visual functions have revealed an interesting insight into BA. For example, intake of BA was found to inhibit transient myopia, reduce eye fatigue, improve dark adaptation, and enhance retinal blood flow with glaucoma.

4.4.1 Inhibitory effects on myopia and mitigative effects on eye fatigue

In a study involving 21 weakly myopic subjects, a BA dose of 50 mg was administered in juice (enabling uptake for 2 h), followed by 2 h of continuous work on a personal computer. The BA-juice group was found to have significantly decreased diopter values, thus suggesting that BA was able to prevent myopic refractory shift after prolonged visual tasks [29]. These trials employed a double-blind, placebo-controlled crossover design.

In a study designed to clarify the underlying molecular mechanisms, anthocyanin (at concentrations of 10^{-8} to 10^{-7} M) was shown to have relaxing effects on ciliary smooth muscle, which countered the effects of endothelin-1 in ciliary muscle constriction [73]. Ciliary smooth muscle is responsible for regulating accommodation and modulating refraction of the lens through constriction and relaxation. Such relaxing effects are unique to anthocyanins and are not observed with other flavonoids [73]. Among anthocyanins, delphinidin 3-rutinoside is particularly potent in relaxing ciliary smooth muscle, as compared with other anthocyanins. Myopia inhibition is possibly achieved by stimulation of endothelin-1B receptor, induction of nitric oxide production, relaxation of ciliary smooth muscle, and subsequent thinning out of the eye's lens. In a recent study using a chick model of myopia, BA was reported to show inhibitory effects on myopia [74]. Intriguingly, in terms of distribution in rats, BA concentrations in the eye tissue of sclera and choroid membrane were about 100-fold higher than blood concentration. Similarly, BA concentration in the ciliary body, retina, iris, and cornea were also higher than that in the blood [75].

Regarding BA's mitigative effects on eye fatigue, a visual analogue scale method in the previously mentioned study was used to rate subjectively the reported degree of fatigue. Results revealed that eye and back fatigue were significantly reduced in the BA-juice group [29]. In this regard, studies have reported that intake of BA induces vasorelaxation of the aorta [76] and an increase in peripheral blood flow [77].

4.4.2 Improvement of dark adaptation

In a clinical study on the effect of anthocyanins on dark adaptation in humans, a double-blind crossover design was adopted [29]. In 12 healthy subjects, intake of BA at a dose of 50 mg significantly lowered the threshold values of dark adaptation, indicating that BA intake improved eye function under such conditions. To account for the effects of anthocyanins on improving low-light adaptation performance, it has been suggested that anthocyanins work by promoting regeneration of rhodopsin [78]. In a study by Matsumoto and colleagues, the synthesis rate of rhodopsin prepared from frog retinal rod outer segment and of rhodopsin derived from 11-*cis*-retinal was measured. In the presence of 20 μM C3R and C3G, rhodopsin regeneration was enhanced; a process possibly mediating improvements in dark adaptation. Interestingly, using NMR spectrometry, Yanamala and colleagues showed that C3G binds to light-activated as well as non-light-activated states of rhodopsin and opsin, thereby modulating their structures. Furthermore, molecular docking studies have suggested that the C3G binding pocket is located in the cytoplasmic domain of the protein and that this binding is likely to be pH-dependent [79–81].

4.4.3 Enhancement of retinal blood flow in eyes with glaucoma

Glaucoma is a condition of visual impairment precipitated by elevated intraocular pressure and atrophy of the optic nerve under compression. In recent years, there has been an increase in the incidence of glaucoma in patients with a normal range of intraocular pressure. One possible cause for this may involve circulatory impairments at the optic disc and dysregulated endothelin-1 signaling. In one clinical study, 30 glaucoma patients were treated with BA at a dosage of 50 mg/day for 6 months. Retinal blood flow was found to be significantly increased in the BA intake group, while blood endothelin-1 concentration was also significantly increased [82]. However, during the treatment period, there was no statistical difference in blood pressure or ocular pressure between the groups. This result suggests that BA may be used in glaucoma patients with a normal range of ocular pressure as a supplement with neuroprotective effects.

4.4.4 Comparison of the efficacy of BBE and BA

There has been scant evidence for the effectiveness of blueberries or bilberries in improving visual function [83–86]. Nevertheless, in recent studies using cellular and animal models by Hara and colleagues, blueberry anthocyanins were found to exhibit neuroprotective effects by reducing oxidative stress in eye tissue and preventing

symptoms of diabetic retinopathy [87–89]. In addition, they also reported that purple rice-derived anthocyanins exhibited similar effects [90, 91]. For comparative analysis on in vivo absorption of blueberry anthocyanins and BA, experiments in rats have demonstrated that BA was more efficiently absorbed into blood circulation compared with blueberry anthocyanins. The amount of BA circulating in the blood stream was also higher than that of blueberry anthocyanins. Additionally, BA uptake preferentially occurred in eye tissue whereas uptake of blueberry anthocyanins could not be detected [92]. Likewise, Sakakibara and colleagues also reported that anthocyanins could not be detected in mouse eye tissue following blueberry anthocyanin administration [93]. On the other hand, Kalt et al. reported that in pigs treated with blueberries, anthocyanins could be detected in eye tissue [94]. Since blood BA concentration is known to decay slowly following intake [95], it may explain the seemingly differential absorption rates of various anthocyanins. Perhaps not surprisingly, for improvement of visual functions, only BA was reported to exhibit a significant relaxing effect, as demonstrated in studies comparing the effects of blueberry anthocyanins and BA on endothelin-1-induced constriction of ciliary smooth muscle [92].

Despite controversies arising from divergent results in individual studies, it is worthwhile to establish standardized assessment methodologies for large-scale clinical trials, and to conduct more conclusive comparisons on the efficacy of the various anthocyanin-containing foods. So far, in studies focusing on the role of anthocyanins in improving visual functions, current evidence supports the notion that a series of anthocyanins with delphinidins as the parent aglycone are most effective for relaxing ciliary smooth muscle, while cyanidins are most effective for promoting rhodopsin regeneration. The underlying mechanisms for these differences remain obscure. To provide a better understanding of the mediating mechanisms, it is desirable to clarify the molecular types or compositions of anthocyanins that are most effective for visual functions, and to conduct further investigation on the underlying molecular mechanisms.

4.5 Brain function

Age-related decline in brain function is a concerning problem. Various studies have suggested that intake of anthocyanin-rich fruits confers some beneficial effects against age-related neurodegeneration and cognitive decline. In one recent study in humans, aged 76.2 ± 5.2 years, Krikorian et al. reported that intake of blueberry juice for 12 wk improved memory performance [96]. This same group also reported that concord grape juice produced similar effects on brain function [97, 98]. In animal models, studies have suggested that intake of freeze-dried fruits or anthocyanin fruit extracts (plum and blackberry) delays the onset of decline of neural functions and improves cognitive and motor performance [99, 100].

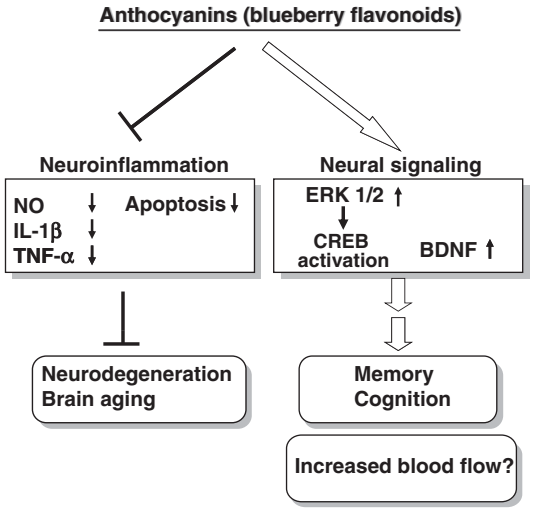


Figure 4. Mechanism for prevention of neurodegeneration and brain aging, and enhancement of memory and cognition, by dietary anthocyanins-rich plants. BDNF, brain-derived neurotrophic factor; ERK1/2, extracellular signal-related kinase1/2.

In terms of mechanism of action, the effects of anthocyanins might be mediated through inhibition of neuroinflammation. For example, anthocyanins reportedly blocked age-related upregulation of nuclear factor-κB (NF-κB) expression in Fischer rats [101]. Intake of blueberries inhibited cognitive and motor impairments induced by kainic acid challenge, as evidenced by the suppression of expression of IL-1β, TNF-α, and nuclear factor-κB in the hippocampus [102]. Moreover, production of nitric oxide, IL-1β, and TNF-α in microglia was reported to be inhibited following blueberry intake [103]. In a study using a rat model, Williams et al. showed that intake of blueberries led to activation of cyclic AMP-response element-binding protein (CREB) and upregulation of brain-derived neurotrophic factor [104]. Within the hippocampus, activation of cyclic AMP-response element-binding protein may be mediated through extracellular signal-related kinase1/2 signaling rather than calcium calmodulin kinase II and IV or protein kinaseA pathways. Based on evidence from these studies, berry-derived effects of anthocyanins on improvement of brain function might involve the inhibition of neuroinflammation and modulation of neural signaling, while a collateral effect on the improvement of cerebral blood flow may well be another plausible factor [105]. Although there have been no brain imaging studies conducted on anthocyanin or berry intake, there are good examples of cocoa intake reportedly improving cerebral blood flow in functional MRI [106, 107]. Figure 4 shows the mechanism for prevention of neurodegeneration and brain aging, and enhancement of memory and cognition, by dietary anthocyanins-rich plants.

A point that deserves some attention is that while berries are rich natural sources of anthocyanins, it may be more practical to use crude extracts or freeze-dried fruit

powder as experimental reagents for testing brain functions. In addition, it is important to consider questions such as whether the drug affects the result from anthocyanins alone, whether non-anthocyanin components are also essential, and whether co-treatment with multiple anthocyanins and non-anthocyanins is required for improvement in brain function.

5 Concluding remarks and perspective

Research on anthocyanins has rapidly evolved from a secluded niche focusing on the chemical analysis of enigmatic flower pigments, chemical states of occurrence in plants, and biosynthesis to a diverse investigation devoted to physiological functions at the molecular level, molecular structure, metabolism and absorption, and dietary applications. Nevertheless, much remains to be elucidated before a comprehensive understanding of the effects of anthocyanins and related functions emerge. It is currently unclear which molecular structures of anthocyanins are responsible for pleiotropic health benefits. In some cases, intake of multiple anthocyanin species can prove more effective. In order to achieve specific health benefits, however, it is still necessary to clarify which anthocyanins are most potent by characterizing their properties and functions at the molecular level. To date, while some studies have used discrete purified anthocyanins for evaluating its health benefits, the majority of studies have relied on anthocyanin-rich crude extracts from plant sources. As stated previously, the pharmacological actions of anthocyanins could not be fully established without knowledge on the effects of treatment with anthocyanins alone, the effects of non-anthocyanin components, and the possible interactions between anthocyanin and non-anthocyanin species. In addition, robust clinical evidence supporting the health benefits of anthocyanins in human has remained insufficient. Further efforts in this area are therefore necessary. Thanks to advancement in analytical technologies, the metabolic pathways of anthocyanins can now be properly mapped to provide vital information on the roles and significance of metabolites. Conventionally, the bioavailability of anthocyanins was thought to be very low. The amount of anthocyanin metabolites formed in the gastrointestinal tract may therefore be explored as a measure to evaluate anthocyanin efficacy. However, at this stage, the link between functional doses of anthocyanin intake and metabolite concentrations remains poorly understood. In functional characterization, a standardized set of analytical methodologies is much desired, though its development is still in progress. Once available, such facile and robust methods would promote more rapid and productive comparisons of research findings across different studies.

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