3. Anthocyanins: Mechanism of action and therapeutic efficacy

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Abstract. The current status of research on anthocyanins, their biological effects in vitro and in vivo and their potential application in human therapy are reviewed. Anthocyanins are a class of compounds belonging to the larger flavonoids class which comprises a subset of the polyphenol class of compounds. Anthocyanins are almost exclusively found in higher plants. Major sources of anthocyanins are blueberries, cherries, raspberries, strawberries, black currants, purple grapes and red wine. Anthocyanins are included in the list of natural compounds known to work as powerful antioxidants. The properties of anthocyanins for human health are due to their peculiar chemical structure, as these substances are very reactive towards reactive oxygen species (ROS). Anthocyanins have been reported to exert positive effects in the treatment of various diseases and are prescribed as medicines in several countries for thousands of years. The available scientific evidence indicates that anthocyanins contained in a diet rich in fruits and vegetables are associated with a decreased risk of inflammation-related chronic diseases and that anthocyanins display a wide range of biological activities including antimicrobial, anti-carcinogenic and proapoptotic activities; improvement of vision

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and neuroprotective effects. In addition, anthocyanins display a variety of effects on blood vessels and platelets. The present review summarizes our knowledge on the bioavailability, antioxidant activity and health enhancing components of anthocyanin-rich foods and extracts, with a focus on the role of anthocyanins in obesity, diabetes, cardiovascular diseases, visual and brain functions and cancer protection. Since most health benefits are produced by chemical properties beyond the antioxidant capacity of the molecules, much remains to be elucidated before a comprehensive understanding of the effects of anthocyanins emerges.

**Introduction**

Anthocyanins are a class of compounds belonging to the larger flavonoids class which comprises a subset of the polyphenol class of compounds. The polyphenol class of compounds includes all molecules with more than one hydroxyl group on an aromatic ring. Flavonoids compounds share a common framework consisting of two aromatic rings (A and B), that are bound together by three carbon atoms forming an oxygenated heterocycle (ring C). Flavonoids are subsequently divided into several groups differing in the oxidation state of the heterocyclic pyran ring C. The subclasses consist of flavanols, flavanones, flavones, isoflavones, flavonols, and anthocyanins (listed in ascending order of oxidation) and within each of these subclasses, individual compounds are characterized by specific hydroxylation and conjugation patterns [1]. Most flavonoids are present in nature as the glycosidic form, with the exception of flavanols, and this contributes to their complexity and the large number of individual molecules that have been identified [2]. The group of anthocyanins represents the largest class of polyphenols. Anthocyanins are the most oxidized flavonoids with the C ring fully unsaturated and a hydroxyl at position 3. The basic structure is an aglycone, or anthocyanidin, with one or more sugars attached at most often C3, C5, or C7 and possibly esterification on the sugars. Anthocyanins are predominantly found in nature as water soluble glycosides of polyhydroxy and polymethoxy derivatives of 2-phenyl-benzopyrylium or flavylium salts. Individual members are differentiated by the number of hydroxyl and methoxyl groups of the B-ring, by the number of sugars attached to the aglycon and the position of attachment, and by the nature and number of aliphatic or aromatic acids attached to the sugar residues. Currently, there are 19 naturally occurring anthocyanidins. The six most common anthocyanidins found in edible plants include pelargonidin, peonidin, cyanidin, malvidin, petunidin, and delphinidin [3]. The prevalence of sugar occurrence in natural anthocyanins is glucose, rhamnose, xylose, galactose, arabinose, and fructose. Many anthocyanins have been found to be acylated by aliphatic or
anthocyanins, the most commonly seen acyl groups being coumaric, caffeic, ferulic, $p$-hydroxy benzoic, synapic, malonic, acetic, succinic, oxalic, and malic acids. Considering all these factors, the number of probable anthocyanin compounds is quite large, leading to over 600 having been identified from natural sources [4, 5]. Anthocyanins are responsible for much of the red, blue, and purple colors of fruits, vegetables, grains, flowers, and herbs, which explains their name, in Greek, *anthos* means flower and *kyanos* means blue. Anthocyanins are almost exclusively found in higher plants, although a few have been found in lower plants such as mosses and ferns [4, 6]. In general, the anthocyanins in most of the fruits and vegetables are observed in concentrations from 0.1% up to 1.0% dry weight [4, 7]. Anthocyanins are included in the list of natural compounds known to work as powerful antioxidants. In the last years, great attention was given to the possible protection exerted by natural antioxidants present in dietary plants, towards tissue injury mediated by reactive oxygen species (ROS). The anthocyanin-health properties are due to their peculiar chemical structure, as they are very reactive towards ROS because of their electron deficiency. They have been reported to have positive effects in the treatment of various diseases and are prescribed as medicines in any countries.

**Bioavailability and adsorption of anthocyanins**

The daily intake of anthocyanins in humans has been estimated at 180-215 mg/d in USA [8]. This value is considerably higher than the intake of other flavonoids such as flavones and flavonols as estimated in the Dutch diet (23 mg/d, measured as aglycones) [9]. Major sources of anthocyanins are blueberries, cherries, raspberries, strawberries, black currants, purple grapes and red wine. The early methods for testing anthocyanin absorption consisted of measuring the presence of red pigments in urine after oral administration. The evolution of testing methods has lead to observing anthocyanin absorption in plasma and urine to determine both location of absorption and rate of excretion. For many years, studies reported very low numbers for absorption of anthocyanins after oral administration, on the order of 0.004% to 0.1% of the intake, and indicated a rapid absorption and excretion with time to the maximum concentration to be 1.5h for plasma and 2.5 h for urine [10]. The metabolites persist in the urine for up to 24 h and may retain their basic anthocyanin structure [11]. However, most of the analyses were performed with UV-Vis detection after acidification, under the assumption all the anthocyanins would be converted into the colored flavylum form, and it is possible that some forms existing at neutral pH could not be colored due to chemical reactions within the plasma or urine. Also, the common technique
involved freezing and storing the urine and plasma samples before analysis but chromatograms of samples immediately after collection showed additional peaks that had degraded in the chromatograms of frozen samples [12]. Evidence from several laboratory indicates that anthocyanins are rapidly absorbed from both the stomach [13, 14] and small intestine [15], and appear in blood circulation and urine as intact, methylated, glucuronide derivatives and/or sulfoconjugated forms [12, 16-23]. In rats fed an anthocyanin- rich diet for 15 days, anthocyanins have been found in several organs, including stomach, small intestine (jejunum), liver, kidney and brain. In the brain, total anthocyanin content (blackberry anthocyanins and peonidin 3-O-glucoside) reached 0.25 ± 0.05 nmol/g of tissue [24]. Pharmacokinetic evidence suggests that the concentration of the parent glycosides and their glucuronide derivatives are prominent in early blood samples (0-5 h), with increasing methylation occurring over time (6-24 h). This evidence suggests that anthocyanins bioactivity is likely altered over time as a result of metabolic transformation post consumption.

As anthocyanins are rapidly degraded by intestinal microflora, this additional metabolism could account for the unrecovered anthocyanins, suggesting the possibility that large concentrations of anthocyanin derived compounds might be present in the gastrointestinal tract (GIT). Anthocyanidin glycosides are hydrolyzed by the microflora with cleavage of the protective 3-glycosidic linkage. The released aglycones are very unstable molecules under any condition, but in the neutral pH of the physiological conditions, they are spontaneously degraded into monomeric phenolic acids and aldehydes, specifically protocatechuic acid (3,4-dihydroxybenzoic acid), syringic acid, vanillic acid and phloroglucinol aldehyde, [25-29]. Support for this view was provided by Tsuda and colleagues [30], who found a plasma concentration of protocatechuic acids eight times greater than that of cyanidin-3-glucoside. The fate of anthocyanins was studied by González-Barrio and coworkers [31] following the consumption of 300 g of raspberries by healthy human volunteers and subjects with an ileostomy. Postingestion plasma and urine from the former and ileal fluid and urine from the latter group were collected and analyzed by HPLC-PDA-MS. Plasma from the healthy volunteers did not contain detectable quantities of either the native raspberry polyphenolics or their metabolites. The three main raspberry anthocyanins were excreted in urine in both healthy and ileostomy volunteers 0-7 h after ingestion, in quantities corresponding to <0.1% of intake. This indicates a low level of absorption in the small intestine. In subjects with an intact functioning colon, these compounds would pass to the large intestine.

In vitro anaerobic incubation of raspberry anthocyanins with fecal suspensions demonstrated conversion to phenolic acids [32]. In vivo
Anthocyanins

Urinary excretion of phenolic acids after ingestion of raspberries indicates that after formation in the colon some phenolic acids undergo phase II metabolism, resulting in the formation of products that do not accumulate when anthocyanins are degraded in fecal suspensions. Even with the observation of these anthocyanin metabolites, the majority of ingested anthocyanins are not recovered and therefore continued investigation is necessary to determine the fate of the compounds in the body.

The limited data that are available indicate that simultaneous intakes with foods can affect the absorption and excretion of flavonoids. It has been shown in a study with both rats and human subjects that phytic acid (myo-inositol hexaphosphate), a component of hulls of nuts, seeds and grain [33], increases the bioavailability of blackcurrant anthocyanins [34]. Urinary recovery of the anthocyanins from rats was enhanced 5.8-fold by co-ingestion with a 1% solution of phytic acid, which reduced gastrointestinal mobility and slowed the passage of the anthocyanins through the stomach, duodenum and jejunum, presumably thereby providing a longer time frame for the absorption of anthocyanins. Human plasma and urinary anthocyanin levels were also enhanced by phytic acid. The peak excretion was delayed until 4–8 h post-ingestion, and the recovery of anthocyanins increased 4.5-fold.

**Anthocyanin biological activities**

Numerous studies have shown that anthocyanins display a wide range of biological activities [35, 36] including antioxidant [16, 37-39], anti-inflammatory [40, 41], antimicrobial [42] anti-carcinogenic and proapoptotic activities [43-45], improvement of vision [46, 47] and neuroprotective effects [48, 49]. In addition, anthocyanins display a variety of effects on blood vessels [50, 51] and platelets [52, 53] that may reduce the risk of coronary heart disease [54].

**Antioxidant activity**

Anthocyanins are potent antioxidant superior to classical antioxidants such as butylated hydroxyanisole (BHA) [55, 56], butylated hydroxytoluene (BHT), α-tocopherol [37, 39] 6-hydroxy-2,5,7,8-tetramethyloxan-2-carboxylic acid (Trolox), catechin and quercetin [57]. Glycosylation of an anthocyanin decreases radical scavenger activity compared with the aglycone, as it reduces the ability of the anthocyanin radical to delocalize electrons. In accordance with this, Fukumoto and Mazza [39] reported increased antioxidant activity with increase in the hydroxyl groups and decreased
antioxidant activity with glycosylation of anthocyanidins. Since cyanidin and its glycosides represent one of the major groups of naturally occurring anthocyanins, their antioxidant and biological properties have been deeply investigated. Particularly, cyanidin 3-glucoside, alone or together with other cyanidin 3-glycosides and with the aglycone form, has been widely investigated with the aim to establish its antioxidant activity in different experimental conditions.

**In vitro studies**

Acquaviva [58] showed that cyanidin 3-glucoside and cyanidin had a protective effect on DNA cleavage, a dose-dependent free radical scavenging activity and significant inhibition of xanthine oxidase activity. Cyanidin 3-glucoside has been showed to be a scavenger of peroxynitrite and to be able to exert a protective effect against *in vitro* endothelial dysfunction and vascular failure induced by peroxynitrite tested on human umbilical vein endothelial cells (HUVEC) [59]. Duthie [60] found that cyanidin 3-glucoside protected against oxidative DNA damage in human colonocytes. Guerra [61] showed the ability of cyanidin 3-glucoside to reduce the production of ROS, and the inhibition of protein and DNA synthesis caused by aflatoxin B1 and ochratoxin A in a human hepatoma cell line (Hep G2) and a human colonic adenocarcinoma cell line (CaCo-2). Cyanidin 3-glucoside significantly reduced free radical species production and prevented genomic DNA damage due to ochratoxin A on human fibroblasts [62]. Cyanidin 3-glucoside has been showed to be able to modulate hepatic stellate cells proliferation and type I collagen synthesis induced by a ferric nitrilotriacetate complex as pro-oxidant agent, thus suggesting a potential role for this antioxidant compound in the prevention of fibrosis in chronic liver diseases [63]. In a cell culture study in which neuronal cells (PC 12) were exposed to a variety of sweet and tart cherry phenolic compounds, total phenolics, and predominantly anthocyanins, demonstrated a dose-dependent reduction in oxidant stress [64]. Cyanidin 3-glucoside from mulberry fruit extract [65] have been assumed as neuroprotective constituent on the PC12 cells exposed to cell-damaging oxidative stress.

Exposure to UV-A radiation is known to induce discrete lesions in DNA and the generation of free radicals that lead to a wide array of skin diseases. Two *in vitro* studies on human keratinocytes (HaCaT) concluded that cyanidin 3-glucoside could successfully be employed as a skin photoprotective agent against, respectively, ultraviolet-A and B [66, 67] (UVA and UVB) radiations. Tarozzi particularly, showed that UVA induced apoptosis and DNA fragmentation caused by the generation of ROS.
has been counteracted by cyanidin 3-glucoside by the inhibition of hydrogen peroxide (H2O2) release after UVA irradiation and by the enhancement of the resistance to the apoptotic effects of both H2O2 and the superoxide anion (O2-). The authors also suggested that cyanidin 3-glucoside protective effects could be attributed to the high membrane levels of incorporation. Giampieri and colleagues [68] very recently analyzed methanolic extracts from the strawberry Sveva cultivar for anthocyanin content and for their ability to protect human dermal fibroblasts against UV-A radiation. Five anthocyanin pigments were identified using high-performance liquid chromatography-diode array detection-electrospray ionization/mass spectrometry. Moreover, the strawberry extract showed a photoprotective activity in fibroblasts exposed to UV-A radiation, increasing cellular viability, and diminishing DNA damage.

The antioxidant activity exerted in a liposomal membrane system by different cyaniding glycosides (arabinoside, rutinoside, galactoside and glucoside) has been found to be higher than that of trolox in the case of Fe(II)-induced liposome oxidation and to be comparable with the action of trolox (a water-soluble tocopherol derivative) in the case of UV- and AA PH (2,2’-azobis[2-amidinopropane] dihydrochloride)-induced liposome membrane oxidation. The inhibitory effects of lipid peroxidation exerted by cyanidin 3-glucoside, cyanidin and cyanidin 3-galactoside have been showed and quantificated by Adhikari [69] who also found a good effect respect to commercial anti-oxidants butylated hydroxyanisole, butylated hydroxytoluene, and tert-butyldihydroxyquinone. As regards other cyaniding glycosides, cyaniding 3-galactoside showed a stronger activity respect to that of other flavonoids as well as vitamin E or Trolox from cranberries’ extracts in two antioxidant assays consisting in the evaluation of 1,1-diphenyl-2-picrylhydrazyl radical-scavenging activity and in the ability to inhibit low-density lipoprotein oxidation [70]. Cyaniding also showed its antioxidant activity. It was, in fact, the stronger superoxide radical scavenger among the polyphenols of different varieties of plums [71] and exerted a protective effect against the toxicity induced by linoleic acid hydroperoxide (LOOH) in cultured human fetal lung fibroblasts [72]. Similarly, cyaniding inhibited malonaldehyde formation in oxidized calf thymus DNA oxidized by Fenton’s reagent [73]. Lazze [74] showed that cyaniding was able to protect rat smooth muscle and hepatoma cell lines against cytotoxicity, DNA SSB formation and lipid peroxidation induced by tert-butyl-hydroperoxide (TBHP) whereas its glycoside and rutinoside derivatives did not work. The antioxidant activity exerted by cyaniding and by some glycosidic forms has been confirmed in three lipid containing models (human low-density lipoprotein – LDL – and bulk and emulsified methyl linoleate) by Kahkonen [57] who also linked the differences in the antioxidant power with the
different glycosylation patterns. The delphinidin and cyanidin anthocyanidins were found to be the most active inhibitors followed by malvidin, peonidin, pelargonidin and petunidin. Overall, the glucosylated forms or anthocyanins were less active than the free forms or anthocyanidins in the prevention of LDL oxidation, except for malvidin 3, 5-diglycoside. LDL does not form atherosclerotic plaques unless it is oxidised which may lead to a build up of plaque in the arteries. Therefore, the consumption of dietary antioxidants is beneficial in preventing cardiovascular diseases, particularly atherosclerosis [75]. In a recent study by Abdel-Aal and colleagues, the concentration of conjugated dienes (CD) formed due to LDL oxidation in vitro dropped significantly to varying extents depending upon the type of anthocyanin. The cyanidin-containing anthocyanins possessed a lower inhibition capacity against LDL oxidation compared to the delphinidin-based anthocyanins. The differences in the inhibition capacity between cyanidin and delphinidin could be due to the extra hydroxyl group at the C5-position in the delphinidin structure. The additional hydroxyl group would change the release of hydrogen ions and the hydration constant (pKH), thereby increasing inhibition effects against copper-induced human LDL cholesterol oxidation [55]. This findings further emphasize the degree to which antioxidant properties of anthocyanins can be influenced by their structure, that is, the site of glucosylation as well as type and degree of acylation with acid residues such as cinnamic and aliphatic acids. The mechanisms by which anthocyanins/anthocyanidins inhibit LDL oxidation in vitro remain unclear. It has been speculated that the protecting effects of phenolics and anthocyanins on LDL oxidation may be due to multiple factors such as scavenging of various radical species in the aqueous phase, interaction with peroxy radicals at the LDL surface and terminating chain-reactions of lipid peroxidation by scavenging lipid radicals and regenerating endogenous alpha-tocopherol back to its active antioxidative form [57].

Protocatechuic acid was able to suppresses (1-methyl-4-phenylpyridinium)þ-induced mitochondrial dysfunction and apoptotic cell death in PC12 cells, showing a potential clinical application to counteract neurodegeneration such as in Parkinson’s disease [76]. In cultured neural stem cells, protocatechuic acid decreased apoptosis, reducing the ROS and significantly suppressing the caspase cascade [77].

**In vivo studies**

Following consumption of anthocyanins, serum antioxidant status is significantly increased after 4 to 24 hr post-consumption [16, 48]. Antioxidant capacity in human serum has also been measured using various
methods following consumption of strawberries (240 g), spinach (294 g), red wine (300 mL) or vitamin C (1250 mg) in eight elderly women [78]. Total antioxidant capacity of serum increased significantly by 7-25% during 4 hr period after consumption of red wine, strawberries, vitamin C or spinach. The total antioxidant capacity of urine determined by ORAC also increased for all the treatments except red wine, with the largest increase for vitamin C (44.9%). Further studies report positive effects in older human subjects. Thirty-six grams of a lyophilised red grape powder was able to significantly reduce systemic oxidative stress as measured by urinary F2-isoprostanes in twenty postmenopausal women [79]. The supplement was also able to positively alter lipoprotein metabolism and inflammatory markers (TNFα), therefore supposedly reducing recognised cardiovascular risk factors. Consumption of tart cherry juice for 2 weeks was able to reduce the ischaemia/reperfusion-induced F2-isoprostane response in plasma and urinary excretion of oxidised nucleic acids, a measure of DNA oxidative damage, in twelve volunteers aged 69 years [80].

DNA was significantly protected against oxidative insult in twenty-one haemodialysis patients in a pilot intervention study by 200 ml/d intake for a 4-week period of an anthocyanin-rich red fruit juice [81]. Reduced DNA oxidation damage was accompanied by decreased protein and lipid peroxidation and an increase in glutathione. In twenty-six patients receiving haemodialysis and fifteen healthy subjects, daily consumption of 100 ml of red grape juice for a period of 14 d increased the antioxidant capacity of plasma, without affecting concentrations of uric acid or vitamin C, reduced oxidised LDL and increased the level of cholesterol-standardised a-tocopherol [82].

Finally, anthocyanins shows systemic or central antioxidant protection, as observed by Shih [83] who fed senescence-accelerated mice with a basal diet supplemented with a 0.18 and 0.9% mulberry extract for 12 weeks. Treated animals showed a higher antioxidant enzyme activity and reduced lipid oxidation in both the brain and liver.

**Eye health**

*In vitro* studies

The beneficial health effect that anthocyanins have on vision was one of the first reported health properties [84]. Jang [85] determined the ability of bilberry extract to modulate adverse effects of A2E on retinal pigment epithelial cells *in vitro*. A2E is an auto-fluorescence pigment that
accumulates in retinal pigment epithelial cells with age and can mediate a detergent-like perturbation of cell membranes and light-induced damage to the cell. This is significant since it is generally accepted that age-related macular degeneration begins with the death of retinal pigment epithelial cells, the degeneration of photoreceptor cells followed soon after by the loss of vision. The results showed that the bilberry extracts were able to suppress the photooxidation of pyridinium disretinoid A2E by quenching singlet oxygen. Additionally, cells that had taken up anthocyanins also exhibited a resistance to the membrane permeabilisation that occurs because of the detergent-like action of A2E.

In vivo studies

The role of anthocyanins on vision has also been demonstrated in a few animal studies. In a study by Kalt [86] using a pig model, the distribution of anthocyanins in tissues such as the liver, eye and brain tissue was investigated. The results suggest that anthocyanins can accumulate in tissues, even beyond the blood-brain barrier. The highest amount of anthocyanins was found in the eye tissue with maximum concentration of 700 pg of anthocyanins/g of fresh weight. Anthocyanins from blackcurrant, which only contains 4 anthocyanins (delphinidin-3-glucoside; delphinidin-3-rutinoside; cyanidin-3-glucoside and cyanidin-3-rutinoside) have extensively been examined for their effects on vision. Matsumoto investigated the ocular absorption, distribution and elimination of blackcurrant anthocyanins in rats after oral and intraperitoneal administration and in rabbits after intravenous administration. Blackcurrant anthocyanins are absorbed and distributed in ocular tissues as intact forms and pass through the blood-aqueous barriers and blood-retinal barriers in both rats and rabbits [20]. Nakaishi [87] report that an oral intake of 12.5, 20 or 50 mg of blackcurrant anthocyanins was significant to decrease the dark adaptation threshold in a dose-dependant manner. To examine the influence of the black currant anthocyanins on the disease progression of open-angle glaucoma (OAG), a randomized, placebo-controlled, double-masked trial was made in 38 patients with OAG treated by antiglaucoma drops [88]. Black currant anthocyanins (50 mg/day) or their placebos were orally administered once daily for a 24-month period. Systemic blood pressure, pulse rates, intraocular pressure, ocular blood circulation by laser-speckle flowgraphy, and Humphrey visual field mean deviation (MD) were measured during the 24-month period. A statistically significant difference was observed between the treatment groups in mean change from baseline in MD 24 months after therapy. Upon administration of black currant anthocyanins, the ocular blood flows
during the 24-month observational period increased in comparison with placebo-treated patients. In his study, Miyake and colleagues [89] generated a mouse model of endotoxin-induced uveitis (EIU) that shows retinal inflammation, as well as uveitis, by injecting lipopolysaccharide. He pretreated the mice with anthocyanin-rich bilberry extract and analyzed the effect on the retina. Anthocyanin-rich bilberry extract prevented the impairment of photoreceptor cell function, as measured by electroretinogram. At the cellular level, he found that the EIU-associated rhodopsin decreased and the shortening of outer segments in photoreceptor cells were suppressed in the bilberry-extract-treated animals. Moreover, the extract prevented both STAT3 activation, which induces inflammation-related rhodopsin decrease, and the increase in interleukin-6 expression, which activates STAT3. In addition to its anti-inflammatory effect, the anthocyanin-rich bilberry extract ameliorated the intracellular elevation of ROS and activated NF-κB, a redox-sensitive transcription factor, in the inflamed retina. In a randomized double-blind placebo-controlled study, Lee [90] investigated the effect of purified high-dose anthocyanin oligomer administration on nocturnal visual function and clinical systems in low to moderate myopia subjects. There was a significant improvement in the anthocyanin group (73.3% improved symptoms) compared to the placebo group. In addition, the anthocyanin group showed improved contrast sensitivity levels compared to the placebo group.

This findings strongly suggest that anthocyanins are absorbed and display several physiological activities and ocular health benefits. Oral administration of black currant anthocyanins may be a safe and promising supplement for patients with OAG in addition to antiglaucoma medication, while anthocyanin-rich bilberry extract has a protective effect on visual function during retinal inflammation.

**Obesity**

The inhibitory effects of anthocyanins on body fat accumulation were first reported by Tsuda in 2003 [91]. In C57BL/6J mice, a cyanidin 3-glucoside-containing diet (2 g/kg) was found to significantly reduce body fat accumulation induced by highfat meals (60% of energy), when compared with controls. This effect was probably due to suppression of lipid synthesis in the liver and in white adipose tissue. In addition, a cyanidin 3-glucoside-containing diet also significantly reduced plasma glucose concentration, which was elevated by highfat meals. Anthocyanins may act on adipocytes and modulate the expression levels of adipocytokines. cyanidin 3-glucoside (or its aglycone cyanidin) was reported to upregulate the expression of
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adiponectin, which can increase insulin sensitivity in human adipocytes [92-94]. When anthocyanin extract from blueberries or whole blueberry powder 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 [95]. In a similar study with C57BL/6 mice by DeFuria, intake of whole blueberry powder supplemented to high-fat meals (60% of energy) [96] showed inhibitory effects on obesity-induced inflammation in white adipose tissue in this study. Specifically, mRNA levels of tumor necrosis factor-a (TNFα) 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. Studies have reported that intake of mulberry extract in an aqueous phase significantly inhibited body weight gain [97], while intake of tart-cherry powder extract inhibited body weight gain, reduced retroperitoneal fat amount, suppressed proinflammatory cytokine (IL-6, TNFα) expression, and upregulated mRNA expression of peroxisome proliferator-activated receptor (PPAR) a and g in Zucker fatty rats [98]. For non-berry species, intake of blood orange (Moro orange), which contains anthocyanins, reportedly inhibited body weight gain and body fat accumulation [99].

Diabetes

Diabetes mellitus (DM) is a metabolic disorder in the endocrine system resulting from a defect in insulin secretion, insulin action or both of them. Wedick and coworkers [100] evaluated whether dietary intakes of major flavonoid subclasses (ie, flavonols, flavones, flavanones, flavan-3-ols, and anthocyanins) are associated with the risk of type 2 diabetes in US adults. They followed up a total of 70,359 women in the Nurses' Health Study (NHS; 1984-2008), 89,201 women in the NHS II (1991-2007), and 41,334 men in the Health Professionals Follow-Up Study (1986-2006) who were free of diabetes, cardiovascular disease, and cancer at baseline. During 3,645,585 person-years of follow-up, the authors documented 12,611 incident cases of type 2 diabetes. Higher intakes of anthocyanins were significantly associated with a lower risk of type 2 diabetes. Consumption of anthocyanin-rich foods, particularly blueberries and apples/pears, was also associated with a lower risk of type 2 diabetes. No significant associations were found for total flavonoid intake or other flavonoid subclasses.

The purpose of Lachin study [101] is to evaluate the effect of ethanolic extract of cherry fruit on alloxan induced diabetic rats. In this study 36 Male
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Wistar rats, body weight of 150-200gr were divided into 6 groups. Diabetes was induced by intra peritoneal injection of 120 mg/kg Alloxan. The duration of the cherries treatment was 30 days in which single dose of extracts (200mg/kg) were oral administered to diabetic rats. Blood glucose levels were estimated with glucometer before treatment, 2h and 1- 4 weeks after administration of extracts. Treatment with extracts of the cherries resulted in a significant reduction in blood glucose and urinary microalbumin and an increase in the creatinine secretion level in urea. Single anthocyanins have been tested for their potential antidiabetic activity. In a study with male streptozotocin-induced diabetic Wistar rats, intraperitoneal injection of pelargonidin normalised elevated glycaemia and improved serum insulin levels in diabetic rats. The typical biochemical symptoms of induced diabetes, such as lower serum levels of superoxide dismutase and catalase, increased concentrations of malondialdehyde and fructosamine, were effectively reverted to normal values after pelargonidin administration [102]. One possible mechanism is linked to the anthocyanin counteracting Hb glycation, consequent Fe release from the prosthetic group and Fe-mediated oxidative damage.

In a type 2 diabetes mutant mouse (KK-Ay) model, intake of anthocyanins was found to inhibit elevation of blood glucose levels and improve insulin sensitivity via downregulation of retinol-binding protein 4 (RBP4). This was achieved by intake of high-purity anthocyanin cyanidin 3-glucoside [91, 103] or bilberry extract anthocyanin [104], which contains a diverse variety of anthocyanins. Cyanidin 3-glucoside 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. However, because only a low level of cyanidin 3-glucoside is contained in bilberry extract, the antidiabetic effects of bilberry extract (which contains multiple anthocyanins) cannot be established on the basis of downregulated RBP4 expression. Likewise, dietary bilberry extract activates AMPK in white adipose tissue, skeletal muscle, and liver. In white adipose tissue 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 bilberry extract clearly reduces glucose production via AMPK activation. This reduction efficiently ameliorates hyperglycemia in type 2 diabetic mice. Furthermore, bilberry extract-induced AMPK activation in the liver results in significantly decreased liver and serum lipid content via upregulation of PPARa and acylCoA oxidase. Upregulation of carnitine palmitoyltransferase-1A by dietary bilberry extract enhances the decrease in lipid content via
enhancement of fatty acid oxidation. Such changes may also contribute to the antidiabetic effect of bilberry extract [104]. It is important to further clarify how complex formulations (such as bilberry extract) containing multiple anthocyanins can exhibit antidiabetic effects through the activation of AMPK pathways. 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. α-Glucosidase is the key enzyme catalyzing the final step in the digestive process of carbohydrates. Hence, α-glucosidase inhibitors can retard the liberation of d-glucose from dietary complex carbohydrates and delay glucose absorption, resulting in reduced postprandial plasma glucose levels and suppression of postprandial hyperglycemia. The inhibitory effects of anthocyanins on α-glucosidase activity vary with the molecular structure of drugs. Among the anthocyanins, glycosides such as that of cyanidin 3-glucoside were found to be very weak inhibitors of α-glucosidase [105, 106]. The IC₅₀ value of cyanidin-3-galactoside was 0.50 ± 0.05 mM against intestinal α-glucosidase sucrase [107]. A low dose of cyanidin-3-galactoside showed a synergistic inhibition on intestinal α-glucosidases (maltase and sucrase) when combined with α-glucosidase inhibitor acarbose [108]. 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 [109, 110]. This same group also studied the inhibitory effects acylated anthocyanins on the elevation of blood glucose levels in rats [111], and found that the molecular structure responsible for α-glucosidase inhibition was not anthocyanidin itself, but caffeoyl sophorose, which is a component of the acylated anthocyanin molecule [112-114].

**Cardiovascular disease (CVD)**

Cardiovascular disease or heart disease is a class of diseases that involve the heart or blood vessels (arteries and veins). While the term technically refers to any disease that affects the cardiovascular system, it is often used to refer to those related to atherosclerosis (AS) and/or hypertension. The causes, mechanisms, and treatments of these conditions often overlap. Cardiovascular diseases remain the biggest cause of deaths worldwide.

Cyaniding 3-glucose exerts a protective effect against peroxynitrite-induced endothelial dysfunction and vascular failure [59] acting as efficacious scavenger of peroxynitrite. However, its ability is not limited to the antioxidant activity but also results in the regulation of enzymes involved
in the NO synthesis. Indeed, the reduction of the levels of inducible nitric oxide synthase (iNOS) expression has been recorded in different experiments. The amelioration of endothelial dysfunction and the vasoprotective effects exerted by the regulation of the vascular tension regulator endothelial NO synthase (eNOS), a protective enzyme in the cardiovascular system, has been recorded in different conditions.

**In vitro studies**

Cyaniding 3-glucoside from blackberry extract showed an anti-inflammatory activity in J774 cells. At least some part of this activity is due to the suppression of NO production; iNOS expression was inhibited through the attenuation of NF-κB and/or MAPK activation [115]. In an *in vitro* test on bovine artery endothelial cells, cyanidin 3-glucoside enhanced eNOS expression and escalated NO production via an Src-ERK1/2-Sp1 signaling pathway [116] and enhanced eNOS activity by regulating its phosphorylation [117]. In a study on human endothelial cells Sorrenti [118], besides confirming that 3-glucoside upregulated e-NOS, also demonstrated that cyanidin 3-glucoside conferred an additional cytoprotective effect consisting in the induction of the stress protein heme oxygenase-1 (HO-1), that exerts an important cellular protective mechanism against oxidative injury. Cyanidin showed its vasoprotective effect in an *in vitro* experiment performed on cultured human umbilical vein endothelial cells (HUVECs) [119]. Cyanidin from red wine increased human eNOS in human endothelial cells [120]; moreover, inhibitory effect of cyanidin on TNFα-induced apoptosis has involved multiple pathways, such as eNOS and thioredoxin expression and Akt activation [121]. In a very recent study from Chen, [122] the effect of delphinidin on adhesion of monocytes to endothelial cells induced by ox-LDL was investigated. The results showed that the pre-treatment with delphinidin (50, 100, or 200 μM) dose-dependently decreased the ox-LDL-induced up-regulation of the expression of ICAM-1 and P-selectin, and the enhanced adhesion and transmigration of monocytes. Delphinidin attenuated ox-LDL-induced generation of ROS, p38MAPK protein expression, NF-κB transcription activity and protein expression, IκB-α degradation, NADPH oxidase subunit (Nox2 and p22phox) protein and mRNA expression in endothelial cells in a dose-dependent manner. These results suggest that delphinidin attenuates ox-LDL induced expression of adhesion molecules (P-selectin and ICAM-1) and the adhesion of monocytes to endothelial cells by inhibiting ROS/p38MAPK/NF-κB pathway.
*In vivo* studies

Anthocyanins are thought to exert their cardiovascular protective effects through anti-inflammation and antiplatelet coagulability [54, 123], the latter which is reported to mediate anthocyanins or their putative colonic metabolites [124]. Anthocyanins significantly inhibit TNFα-induced inflammation through monocyte chemoattractant protein-1 in human endothelium [125], suppress myocardial ischemia-reperfusion injury by delphinidin through the inhibition of signal transducers and activators of transcription 1 activity in cardiac muscle [126] and, via inhibition of inflammation, inhibit atherosclerosis progression by cyanidin-3-O-bgluicoside with the aid of its metabolite protocatechcuic acid [127]; cyanidin 3-glucoside orally administered in rats suppressed the zymosan-induced inflammatory response in the peritoneal exudate cells [128]. Other mechanisms such as inhibition of lipoprotein oxidation, free radical scavenging and modulation of eicosanoid metabolism [129-132] are also thought to play a role in the reduction of atherosclerosis.

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) [133]. A significant association between strawberry consumption and mortality due to CVD was also revealed in the Iowa women’s health study involving postmenopausal women [134]. With regard to human intervention trials [124, 134, 135], consumption of anthocyanin-containing plant foods, such as blackcurrants, bilberries, and blueberries, reduced LDL cholesterol levels and increased plasma antioxidant capacity.

Furthermore, the inhibitory effects of fruits containing anthocyanins on atherosclerosis were reported in elderly men [136]. Ahmet [137] reported that a blueberry enriched diet protected the myocardium of young male Fischer-344 rats against induced ischaemic damage and demonstrated the potential to attenuate the development of post-myocardial infarction chronic heart failure. The effects of raspberry, strawberry and bilberry juices on early atherosclerosis in hamsters have been investigated with the animals receiving a daily dose corresponding to the consumption of 275 ml of juice by a 70 kg human [138]. After 12 weeks on atherogenic diet, the berry juices inhibited aortic lipid deposition by approximately 90% and triggered reduced activity of hepatic antioxidant enzymes, which was not accompanied by lowered plasma cholesterol. The features and progression of the lesions observed in the hamster model of atherosclerosis are morphologically similar to atheromatous lesions observed in human subjects [139].
In a study using tart cherry seed extract, rat hearts were subjected to ischemic injury (which generally results in irregular and rapid heart beats and possibly heart attack) and exposed to cherry extract at variable doses. Extract at moderate doses was associated with reduced incidence of irregular and rapid heart rates as well significantly less cardiac damage as a result of heart attacks that did occur [140].

The intake of red grape juice rich in anthocyanin reduced the concentrations of oxidized LDL and the activity of NADPH oxidase in dialysis patients [141]. The association between grape phenolics and coronary heart disease has been ascribed in part to the presence of anthocyanins in red wine [142, 143]. In addition, several epidemiological studies have shown that coronary heart disease mortality can be decreased by moderate consumption of red wine [144, 145]. A study of the relationships between vasodilation capacity, antioxidant activity and phenolic content of 16 red wines reported that total phenol content correlated well with vasodilation capacity and antioxidant activity of the wines, but only anthocyanins were correlated with vasodilation capacity [146].

Hassellund and colleagues [147] assessed whether a purified anthocyanin supplement improves cardiovascular metabolic risk factors and markers of inflammation and oxidative stress in prehypertensive participants, and whether plasma polyphenols are increased 1-3h following intake. In all, 31 men between 35-51 years with screening blood pressure >140/90mmHg without anti-hypertensive or lipid-lowering medication, were randomized in a double-blinded crossover study to placebo versus 640mg anthocyanins daily. Treatment durations were 4 weeks with a 4-week washout. High-density lipoprotein (HDL)-cholesterol and blood glucose were significantly higher after anthocyanin versus placebo treatment. Several plasma polyphenols increased significantly 1-3h following anthocyanin intake. This study strengthens the evidence that anthocyanins may increase HDL-cholesterol levels [145, 148], and this is demonstrated for the first time in prehypertensive and non-dyslipidemic men.

Toufektsian [149] fed male Wistar rats an anthocyanin-rich diet for a period of 8 weeks. The hearts of these rats were more resistant to regional ischaemia and reperfusion insult ex vivo. With an in vivo model of coronary occlusion and reperfusion, infarct size was reduced compared to the anthocyanin-free diet. A parallel increase in myocardial glutathione levels indicates that anthocyanins or anthocyanin-derived products may modulate cardiac antioxidant defences. In male Sprague–Dawley rats, an anthocyanin-rich supplement significantly reduced brain infarct volume after focal cerebral ischaemic injury, and a putative mechanism was related to
interaction of phenolic compounds with phospho-c-Jun N-terminal kinase and the p53 signalling pathway [150].

This features provide a basis for the design of potent antiatherosclerotic and anti hypertensive agents that will have therapeutic potential in the prevention and treatment of AS and CVD.

**Brain health**

In their study in humans, aged 76.275.2 years, Krikorian and colleagues reported that intake of blueberry juice for 12 wk improved memory performance [151]. This same group also reported that concord grape juice produced similar effects on brain function [152, 153]. 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 [154 155]. The effects of anthocyanins might be mediated through inhibition of neuroinflammation. For example, anthocyanins reportedly blocked age-related upregulation of nuclear factor-kB (NF-kB) expression in Fischer rats [156]. Intake of blueberries inhibited cognitive and motor impairments induced by kainic acid challenge, as evidenced by the suppression of expression of IL-1b, TNFα, and nuclear factor-kB in the hippocampus [157]. Moreover, production of nitric oxide, IL-1b, and TNFα in microglia was reported to be inhibited following blueberry intake [158]. Williams showed that intake of blueberries led to activation of cyclic AMP-response element-binding protein (CREB) and upregulation of brain-derived neurotrophic factor [159]. 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 kinase A 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 [160]. Neuroprotective effects against cerebral ischemic damage in vivo has also been performed by Kang [161] using a mouse brain-injury model with a transient middle cerebral artery occlusion. Anthocyanins are able to improve learning and memory of ovariectomised rats [162]. Ovariectomy caused oestrogen deficit in the animals, which in turn is associated with mental health disorders, emotional difficulties, memory impairment and other cognitive failures; the supplementation with red grape anthocyanins resulted in memory-enhancing effects. Therefore, another possible mechanism involved in cognitive-related effects of anthocyanins
could be their phyto-oestrogenic effects, with clear implications for human subjects. Finally, it is interesting to note that berry-derived polyphenols may be active in different and specific brain regions. Morris water maze tests showed that strawberry supplementation to high-energy and charge particles irradiated – rats partially overcame spatial deficits as animals were better able to retain place information – behaviour associated with the a hippocampus. A blueberry supplementation, however, seemed to improve reversal learning, a behaviour more dependent on intact striatal function [163].

**Anticarcinogenic activity**

*In vitro* studies

Cancer-protective effects of cyanidin glucosides have been demonstrated in studies employing cancer cell lines [164] including apoptotic effects via G2/M growth cycle arrest. Anthocyanin-rich extracts from berries and grapes and several pure anthocyanins and anthocyanidins have exhibited proapoptotic effects in multiple cell types *in vitro* [165-167]. Cell cycle arrest and apoptosis have been demonstrated in mutated cells exposed to cherry anthocyanins [168, 169] and in human oral (KB, CAL27), colon (HT-29, HCT116, SW480, SW620), and prostate (RWPE-1, RWPE-2, 22Rv1) cancer cell lines treated with cranberry extracts [170]. The anthocyanidins inhibited proliferation of human cancer cell lines AGS (stomach), HCT-116 (colon), MCF-7 (breast), NCI H460 (lung), and SF-268 (central nervous system) [171]. Malvidin exhibited a potent antiproliferative effect on AGS cells. The malvidin-induced inhibition of proliferation was accompanied by the arrest of AGS cells at the G0/G1 phase. The occurrence of apoptosis induced by malvidin was confirmed by morphological and biochemical features, including apoptotic body formation, loss of mitochondrial membrane potential, elevation of the Bax: Bcl-2 ratio, caspase 3 activation, and PARP proteolysis [169]. Delphinidin showed G2/M phase cell cycle arrest, apoptosis, and inhibition of NF-κB signaling in 22Rnu1 cells. Delphinidin treatment of human prostate cancer LNCaP, C4-2, 22Rnu1, and PC3 cells resulted in a dose-dependent inhibition of cell growth without showing any substantial effect on normal human prostate epithelial cells. The induction of apoptosis by delphinidin was mediated via activation of caspases [172]. Delphinidin also inhibited VEGF-stimulated human umbilical endothelial cell migration and proliferation and neovascularisation *in vivo* in a chorioallantoic membrane model [173]. Finally, 3,4-dihydroxybenzoic acid was reported to be a strong apoptosis inducer in gastric adenocarcinoma cells [174]. Cyanidin may also promote cellular differentiation and thus reduce the risk for malignant transformation [175].
Cancer metastasis refers to the spread of cancer cells from the primary neoplasm to distant sites, where secondary tumors are formed, and is the major cause of death from cancer. The metastatic cascade includes cell-cell attachment, tissue-barrier degradation, migration, invasion, cell-matrix adhesion and angiogenesis. The available scientific evidence indicates that anthocyanin exert extensive *in vitro* anti-invasive and *in vivo* anti-metastatic activities. In lung cancer, the treatment of cyanidin 3-glucoside and cyanidin 3-rutinoside, which are isolated from mulberry, dose dependently inhibited the migration and invasion of A549 cells and also decreased MMP-2 and uPA and enhanced TIMP-2 and PAI. Moreover, the transcription factors NFkB and AP-1 were also repressed [176]. By attenuating the phosphorylation of ERK and the activation of AP-1, peonidin 3-glucoside significantly inhibited the invasion, motility, and expression of MMP-2/MMP-9 and uPA in H1299 cells [177]. Anthocyanins inhibit metastasis through regulation of MMP-2 and MMP-9 also in B16-F1 cells, and it modulates the expression levels of Ras, PI3K, phospho-Akt, and NF-κB [178]. In breast cancer, treatment of HGF-stimulated MCF-10A cells with delphinidin decreased the expression of Met and the phosphorylation of FAK and Src; induction of the paxillin, Gab-1, GRB-2, Ras–ERK MAPKs, and PI3K/Akt/ mTOR/p70S6K pathways was also inactivated by delphinidin. Moreover, the blockage of HGF-mediated activation of NF-κB and STAT3 and translocation of PKC were also observed in delphinidin-treated MCF-10A cells [179]. Cyanidin 3-glucoside attenuated ethanol-induced migration, invasion, and cell–matrix adhesion and inhibited ethanol stimulated phosphorylation of ErbB2, cSrc, FAK, and p130Cas of high ErbB2-expressing breast cancer BT474, MDA-MB-231, and MCF-7ErbB2 cells [180]. In prostate cancer, anthocyanin-enriched fractions from blueberry (Vaccinium angustifolium) downregulate MMP-2/MMP-9 and upregulate TIMP-1/TIMP-2 in DU145 cells by modulating PKC and MAPK pathways [181, 182]. The inhibitory effects of anthocyanins on motility and invasion of HCT-116 human colon carcinoma cells were associated with the suppression of claudin (a tight junction-related protein) and the inhibition of MMP-2/MMP-9 through p38MAPK and PI3K/Akt pathways [183]. In oral and cervical cancer, the invasion of SCC-4 cells and HeLa cells were diminished by the treatment of peonidin 3-glucoside and cyaniding 3-glucoside [184]. In fibrosarcoma, delphinidin slightly inhibited the activities of MMP-2/MMP-9, which might responsible, in part, for the inhibition of invasion in HT-1080 cells [185]. In glioblastoma, Lamy [186] revealed that the aglycons of the most abundant anthocyanins in fruits, including cyanidin, delphinidin, and petunidin, act as potent inhibitors for the migration of glioblastoma U-87 cells.
**In vivo studies**

Anthocyanin-rich extracts from bilberry, chokeberry and grape were fed for 14 weeks to male rats treated with a colon carcinogen, azoxymethane [187]. The number and multiplicity of colonic aberrant crypt foci, colonic cell proliferation, urinary levels of oxidative DNA damage and expression of cyclo-oxygenase genes were measured as biomarkers of colon cancer. The lower levels of these specific biomarkers in treated rats with respect to controls suggest a protective role of berry extracts in colon carcinogenesis and indicate multiple mechanisms of action. Black raspberries were also able to suppress the development of N-nitrosomethylbenzylamine- induced tumours in the rat oesophagus when administered as either a 5% freeze-dried powder, an anthocyanin-rich fraction or an ethanol-based organic solvent-soluble extract [188]. Grape juice inhibited mammary adenocarcinoma multiplicity compared with that in controls through the inhibition of DNA synthesis [189]. It was reported that anthocyanin-rich red grape extract containing oenocyanin interferes with intestinal adenoma development in the Apc(Min) mouse. The development of adenoma was found to be reduced by oenocyanin-induced modulation of Akt in small intestinal adenomas [190].

Using a mouse model of colorectal cancer, a multiple regime feeding trial was conducted to assess the role of cherry bioactive food components in reducing cancer risk. Mice were fed one of the following: 1) a cherry diet, 2) anthocyanins, 3) cyanidin, 4) control diet or 5) control diet with added sulindac (an anti-inflammatory agent) to determine their effects on tumor development [45]. Results suggested that mice assigned to any of the three test diets showed significantly fewer and smaller volume cecal tumors, but not colonic tumors, than control or sulindac supplemented mice, suggesting that the bioactivity of cyanidin may be responsible for the site specific inhibition of cecal tumors.

**Conclusions**

Anthocyanins assumed in a diet rich in fruits and vegetables are associated with a decreased risk of inflammation-related chronic diseases. However, evidence that anthocyanins are safe, multtargeted, efficacious, and affordable demands further investigations. It remains unknown what amount of anthocyanins are needed and for how long time and whether it is better to consume food with anthocyanins or if supplements will suffice as well. Most pharmacologic effects are presumed following preclinical investigation, and more additional clinical trials are needed to further strengthen hypothesis for
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therapeutic efficacy. Most chronic diseases incubate for 20–30 years before they manifest, therefore structuring such clinical trials will be difficult.

Anthocyanin from fruits and vegetables are absorbed to varying degrees in the human body. As the evidence of therapeutic effects of anthocyanins continues to accumulate, it is becoming more important to understand the nature of absorption and metabolism in vivo. Conventionally, the bioavailability of anthocyanins was thought to be very low. Several studies hypothesize a potential for substantial absorption, but absorption is hypothesized as disappearance of anthocyanin from the test system. Conversely, it is possible that anthocyanin is transformed into molecular structures that are not detected by the current analytical methods, rather than being absorbed. More research is needed to fully understand which molecules are effective and what mechanisms are involved in their action. There is a large number of compounds that potentially could be formed from the degradation of anthocyanins in the GIT. A comprehensive study of anthocyanin transformation products present in the GIT would be a valuable contribution towards the understanding of anthocyanin bioactivity. Thanks to advancement in analytical technologies, the metabolic pathways of anthocyanins can now be properly mapped to provide information on the roles and significance of metabolites. These products may contribute to the health effect of anthocyanins either directly in the GIT or after absorption from the colon. As a result of the biotransformation by intestinal microflora, some of these compounds can reach mM concentrations in faecal water. Since microbial catabolites may be present at many sites of the body in higher concentration than the parent compound, it is proposed that at least a part of the biological activities ascribed to anthocyanins are due to their colonic catabolites. The amount of anthocyanin metabolites formed in the gastrointestinal tract may therefore be explored as a measure to evaluate anthocyanin efficacy. However, the link between functional doses of anthocyanin intake and metabolite concentrations remains poorly understood.

Although anthocyanins are typically consumed as part of a meal, there is little information on how their bioavailability is affected by other components in the diet. In all likelihood, it is the synergy among bioactive fruits and vegetable components (ascorbic acid, carotenoids, and other flavonoids) that results in the health-promoting effects realized from consuming the whole fruit. To obtain a better understanding of effects of anthocyanins as drugs, it is necessary to characterize their properties at the molecular level, and it is necessary to take into account the effects of co-existing compound species in extracts. It is critical that the mechanistic research findings be further substantiated through the implementation of well
designed human feeding studies using fruits produced, harvested, stored, and distributed under standardized conditions as both pre-harvest and post-harvest conditions can significantly affect the concentrations of bioactive food component. Greater understanding of these processes will enable the development of new food products, both fresh and manufactured, with greater therapeutic efficacy.

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 are produced by currently unestablished chemical properties beyond the antioxidant capacity of the molecules. The molecular basis for anthocyanans pharmacological activity includes the regulation of plethora of mechanisms mainly involved in: (1) suppression of the inflammatory response through targeting the PI3K/Akt and NF-κB pathways, (2) reduction of diabetes incidence through modulation of insulin sensitivity and glucose utilization, (3) protection from cardiovascular diseases by exerting (i) antihypertensive and endothelium-protective activity through targeting the Akt/eNOS and ACE pathways, (ii) antiatherogenic activity through targeting NF-κB mediated ICAM expression; (4) neuroprotection through amelioration of oxidative stress and neuroinflammation, (5) growth/differentiation control and tumor suppression by exerting (i) cell cycle arrest and induction of apoptosis through the JNK/p38 MAPK mediated caspase activation, (ii) anticancerogenic activity through targeting the HGF signaling pathways, (iii) tumor anti-invasive activity through targeting the VEGF signaling pathway. The estrogen-like activity of anthocyanans could be utilized in cancer and hormone-replacement therapy.

The collected data provide a concise insight into molecular mechanisms of protective and therapeutic activities of anthocyanans in various pathological conditions. Activities may not be attributed solely to their antioxidant activity but also to direct blockage of signaling pathways. Structure-activity analysis reveals that the number of hydroxyl groups and presence of sugar moiety are crucial for their specific modulatory actions. Nevertheless, much remains to be elucidated before a comprehensive understanding of the effects of anthocyanans and related functions emerges.

In conclusion, with the aim to establish whether these compounds are really capable to influence positively the incidence and progression of many chronic diseases, a great deal of work in several areas is still necessary. This includes: 1) epidemiological studies to evaluate the relationship between anthocyanans rich food consumption and incidence of given pathologies; 2)
feeding studies of anthocyanins to rats and to human healthy subjects to unravel their main catabolites, to clarify their fate within the body and to understand the main sites of absorption; 3) studies to understand the interaction between anthocyanins and colon microflora; 4) analysis of factors affecting bioavailability, including interaction with other dietary compounds; 5) identifying anthocyanins catabolites able to cross the blood–brain barrier; 6) studies to analyze metabolites at low concentrations by means of more sensitive high-resolution analytical techniques such as mass spectrometry analysis or immunoassays; 7) *in vitro* studies to fully characterise the bioactivity of anthocyanins and anthocyanins catabolites generated *in vivo*.

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