Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Natural Resources and Agricultural Sciences
Department of Food Science

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Antocyaniner och deras effekt på blodtryck

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Abbreviations

BP	blood pressure
CAD	coronary artery disease
CVD	cardiovascular disease
DBP	diastolic blood pressure
eNOS	endothelial nitric oxide synthase
FMD	flow mediated dilution
MS	metabolic syndrome
NO	nitric oxide
NOS	nitric oxide synthase
PWV	pulse wave velocity
SBP	systolic blood pressure

Abstract

Flavonoids, naturally occurring secondary metabolites in plants, have been associated with positive effects on high blood pressure (hypertension) which is one of the most important risk factors for cardiovascular disease. Anthocyanins, a subgroup of flavonoids, have in more recent years gained particular attention for its effects on blood pressure.

The aim of this literature review was to investigate the scientific evidence for potential beneficial effects of anthocyanins on hypertension and identify possible mechanisms for such effects.

Long term epidemiological studies indicate that anthocyanins do have positive effects on blood pressure as well as maintaining normal vascular function. Several randomized controlled trials (RCTs) have also observed beneficial effects of anthocyanins but there are also several studies which have not observed any effects at all. Mechanistic animal studies indicate that vaso-relaxing properties from the anthocyanins may be responsible for the effects on blood pressure through the upregulation of nitric oxide synthesis, (NOS).

In conclusion, some studies indicate positive effects on blood pressure owed to anthocyanins, but more studies are needed to prove cause-effect. The observed vaso-relaxing effects are most likely due to the activity of NO which seem to be influenced by the metabolites derived from anthocyanins.

Keywords: anthocyanidins, anthocyanins, cardiovascular health, hypertension, blood pressure, NO

Sammanfattning

Flavonoider, naturligt förekommande sekundära metaboliter hos växter, har visat sig ha positiva effekter på högt blodtryck (hypertoni), en av de viktigaste riskfaktorerna för kardiovaskulära sjukdomar. Effekterna har särskilt kopplats till en undergrupp av flavonoiderna; antocyaniner.

Syftet med denna litteraturstudie var att undersöka om antocyaniner har positiva effekter på hypertoni och vilka mekanismer som kan ligga bakom effekterna.

Epidemiologiska långtidsstudier har ofta påvisat positiva effekter av antocyaniner på blodtryck och kärlfunktion, men kausalitet kan ej säkerställas genom denna typ av studier. Flera randomiserade, kontrollerade studier har påvisat positiva effekter medan andra studier inte uppvisat några effekter alls. Mekanistiska djurstudier ger stöd för att effekterna beror på en ökad produktion av kväveoxid syntas.

Sammanfattningsvis visar sammanställningen att det finns studier som observerat blodtryckssänkande effekter av antocyaniner, men att fler studier behövs för att säkerställa kausalitet. Mycket tyder på att observerade vasorelaxerande effekter beror på aktivitet från NO som kan påverkas positivt av antocyaninmetabolitet.

Nyckelord: antocyanidiner, antocyaniner, kardiovaskulär sjukdom, hypertoni, blodtryck, NO

1. Introduction

A high intake of fruits and vegetables has been strongly associated with beneficial effects on several diseases such diabetes and cardiovascular diseases (CVDs) (Wang *et al.*, 2016; Turati *et al.*, 2015). Besides the high nutritional value in fruits and vegetables, several studies have concluded that flavonoids, secondary metabolites of plants, have positive effects on the regulation of blood pressure (Lajous *et al.*, 2016; Barona *et al.*, 2012; Jennings *et al.*, 2012; Cassidy *et al.*, 2011; Zhu *et al.*, 2011; Erlund *et al.*, 2008). Elevated blood pressure (hypertension) is one of the most common risk factors for developing cardiovascular diseases, the leading cause of death worldwide. Preventing development of hypertension involves physical activity and a balanced diet (WHO, 2014).

In recent years, anthocyanins have gained more attention and have been associated with lowering of blood pressure (Lajous *et al.*, 2016). Anthocyanins are flavonoids which are responsible for the blue, red and violet colors of plants. The widely distribution of anthocyanins in berries have led to the use of berries as the antocyanin source in many randomized controlled trials (RCTs). Most of the observed beneficial effects of anthocyanins have been derived from long term epidemiological studies which are observational studies (Lajous *et al.*, 2016; Jennings *et al.*, 2012; Cassidy *et al.*, 2011). Results from RCTs are mixed (Zhu *et al.*, 2011; Basu *et al.*, 2010a; Basu *et al.*, 2010b; Stull *et al.*, 2010).

This literature review aimed to investigate if there is a scientific support for a positive effect of anthocyanins on blood pressure and hypertension. The aim was further to identify potential mechanisms for such an effect. Both epidemiological and RCT studies were included in the survey as well as some animal studies for further understanding of the mechanisms behind the effects. A short summary of the findings on the bioavailability of anthocyanins and possible mechanisms of action is also included.

2. Hypertension

Cardiovascular disease, CVD, is rated as the number one cause for deaths worldwide and includes a number of dysfunctionalities of the heart and blood vessels. Presence of behavioral risk factors, such as use of tobacco, unhealthy diets and inactivity, may in turn lead to medical risk factors. Raised blood glucose, raised blood pressure (hypertension) and raised blood lipids are all associated with the risk of strokes and heart attacks (WHO, 2016).

Hypertension (Systolic blood pressure (SBP)/Diastolic blood pressure (DBP) ≥140/90 mmHg) has been identified in 22% of the adult population (age >18) globally, and is therefore one of the leading risk factors for CVD. In 2010, hypertension was estimated to be responsible for the death of 9.4 million people (WHO, 2014). Raised blood pressure puts the blood vessels under stress and may lead to weakening and rupture of the vessels. Hypertension may also lead to narrowing of the vessels and the risk of blocking the passage by blood clots or fats increases (WHO, 2015).

Hypertension has been associated with endothelial dysfunction (Vita, 2005) and the clinical symptoms of CVD risk factors are related to loss of endothelial control of vascular homeostasis (Widlansky *et al.*, 2003). Endothelial antiatherosclerotic and antithrombotic properties are reduced during endothelium dysfunction which demonstrates the importance of the endothelium (Perticone *et al.*, 2001). If hypertension causes endothelial dysfunction or vice versa is yet to be established but the available data seem to suggest that they reinforce each other (Dharmashankar & Widlansky, 2010).

The epithelium maintains normal vascular tone by producing vasodilators and vasoconstrictors that act locally. One of the vasodilators is nitric oxide (NO) which also inhibits the adherence of leukocytes to the endothelial walls thus prevents platelet adhesion and aggregation (Ignarro *et al.*, 1987). Studies have observed a heavy reduction of the effects of the regulation mechanism when the effects of NO were inhibited (Madrid *et al.*, 1997; Cowley *et al.*, 1992). Blockage of NO synthase in rats lead to the development of hypertension, indicating that blood pressure is regulated by the endothelial-derived production of NO (Dananberg *et al.*, 1993).The epithelium also controls the fluidity of the blood as well as the coagulation process by producing factors that ex. regulates platelet activity. CVD risk factors induces a chronic inflammatory process which decreases vasodilators and anti-thrombotic factors while vasoconstrictors and pro-thrombotic products are increased (Widlansky *et al.*, 2003). Vascular disorders such as hypertension impairs the vasomotor functions of the

endothelium thus the vascular regulation via NO loses its efficiency (Lazzè *et al.*, 2006). The mechanism involves the decrease of NO production and responsiveness of the vascular smoot muscle cells to NO (Lüscher, 1992) which implements endothelial dysfunction (Perticone *et al.*, 2001).

Hypertension seems partly to be induced and aggravated by excessive reactive oxygen species which can be generated by NADPH oxidase, mitochondria, endothelial derived NO synthase and some other cellular sources (Kizhakekuttu & Widlansky, 2010). Reactive oxygen species can in turn decrease the production of NO by damaging the endothelium or chemically decrease the activity of NO (Wallace, 2011) .

3. Flavonoids

Flavonoids are polyphenolic compounds with putative health properties. They may be divided into subclasses based on their structure, oxidation state and subgroups of the oxygenated ring; flavonols, flavones, flavanones, flavan-3-ols and anthocyanins (Figure 1). More than 5000 flavonoids have been identified in plants; vegetables, fruits and grains. Flavonoids bound to sugar molecules are called flavonoid glycosides and the ones not bound to sugar are referred to as aglycones. Contents of flavonoids in plants depend on the variety, climate, agricultural practice, maturity at harvest and post-harvest conditions (Coultate, 2009)(Erdman *et al.*, 2007).

Several flavonoids have been associated with a reduced risk of CVDs in numerous studies (Hollman *et al.*, 2010; Vita, 2005; Geleijnse *et al.*, 2002; Rein *et al.*, 2000; Knekt *et al.*, 1996) and lowering of blood pressure and vasorelaxing effects (Barona *et al.*, 2012).

3.1 Anthocyanins

Anthocyanins are water soluble polyphenolic pigments responsible for the pink, red, violet and blue colors in flowers, fruits and vegetables. They are flavonoids widely distributed in nature, found in the vacuoles of plant cells. Anthocyanins are the glycosidic form of anthocyanidins of which only six different ones are found in nature; pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvinidin (Figure 2). The six anthocyanidin aglycones give rise to numerous anthocyanins by different glycosylation and acylation patterns. When glycosylated, the sugar residue is always found at position 3, often together with a glucose at position 5 or sometimes at position 7, 3’ or 4’ (Figure 1). The most common sugar residues are glucose, galactose, rhamnose and arabinose (Coultate, 2009).

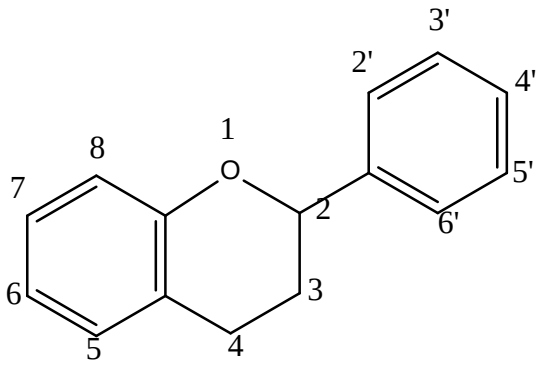
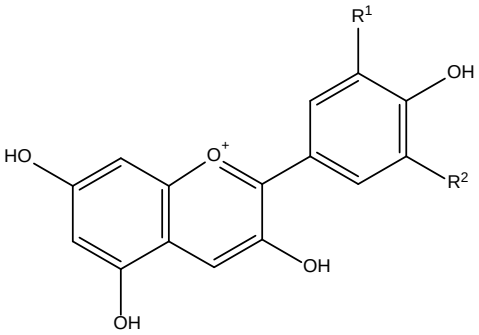


Figure 1. The basic structure of flavonoids and anthocyanins (Redrawn from Coultate, 2009)

The maximum absorbance of anthocyanidins is just above 500 nm which gives rise to a red color in the visible spectrum. The addition of the different side groups of the anthocyanidins shown in Figure 2 moves the visible color towards purple and blue. The full spectrum of the colors associated to anthocyanidins demands changes is pH and presence of other compounds which either enhances or weakens the intensity in color. At low pH, anthocyanidins are primarily found in the form of a flavylium ion (Figure 2) which has a red color. With increasing pH, the flavylium ion loses a proton and a colorless molecule, carbinol pseudo-base, is formed instead. With higher pH the flavylium ion transforms into a non-ionized quinoidal base which gives rise to a purple color. After addition loss of another proton an ionized quinoidal base, which have a deep blue color, is formed (Coultate, 2009).



Anthocyanidin	**R^1**	**R^2**
Pelargonidin	H	H
Cyanidin	OH	H
Peonidin	OCH_3	H
Delphinidin	OH	OH
Petunidin	OCH_3	OH
Malvinidin	OCH_3	OCH_3

Figure 2. The basic structure of the flavylium ion and the sidegroups of the six antocyanidins found in nature (redrawn from Coultate, 2009).

Anthocyanins are synthesized by the plant as a part of the plants chemical defense. They are involved in the protection of the plant from invasive insects and herbivores but also attracts pollinators. They have also been shown to be involved in the UV-protection of the plant. Flavonoids over all have demonstrated antioxidant properties by being able to scavenge different oxidizing species such as superoxide anions and hydroxyl- and peroxy radicals (Harborne & Williams, 2000; Chalker-Scott, 1999).

Anthocyanins, with cyanidin being the most common aglycone (Coultate, 2009), are mainly found in fresh fruits, berries and vegetables, such as black currents, grapes, bilberries (Swedish National food agency, 2016) but also in some cereals, red wine and some leaf and root vegetables (Erdman *et al.*, 2007). It has been estimated that Americans consume about 12.5 mg anthocyanins per day (Wu *et al.*, 2006) but estimations of intake vary widely between studies as can be seen in Table 1, where mean intake varies from 0.2-71±43 mg/day. The content of anthocyanins varies greatly between different food items which is reflected in the large variations in

intakes. In gooseberries the concentration of anthocyanins have been estimated to 0.7 mg/100 g fresh weight compared with chokeberries which have been estimated to contain a concentration of 1480 mg anthocyanins/100 g fresh weight (Wu *et al.*, 2006).

3.2 Bioavailability

The absorption pathway of anthocyanins seems to deviate compared to the pathway of other flavonoids (Fang, 2014; Manach *et al.*, 2005). In rats anthocyanins have been shown to be efficiently and rapid absorbed intact in the stomach as glycosides and found short thereafter in bile as intact molecules and metabolites (Talavéra *et al.*, 2003). However, anthocyanins have been reported to be one of the least absorbed polyphenols (Manach *et al.*, 2005). Only 0.19±0.02 % of orally ingested blackberry anthocyanins (91.2 % cyanidin-3-glucoside) was identified in rat urine 24 hours post consumption. Intact blackberry anthocyanins, methylated- and glucurono-conjugated derivatives were all detected in the urine. In the plasma, blackberry anthocyanins, methylated forms of cyanidin, monoglucuronide derivatives as well as aglycones were detected. Mainly blackberry anthocyanins were detected in the stomach and no metabolites were found, indicating that the anthocyanins are absorbed intact. The jejunum was found to be the most anthocyanin rich area with blackberry anthocyanins and traces of methylated- and aglycone forms and glucurono-conjugated derivatives (Talavéra *et al.*, 2005).

A review by Manach *et al.* (2005) concluded that anthocyanin metabolites appears 1.5 (0.75-4) hours after consumption in the human plasma and 2.5 hours in urine. After supplementations ranging from 150 mg-2 g, the measured concentrations in plasma appeared to be very low (10-50 nmol/L) and even lower in urine (0.004-0.1%). The observed poor absorption of anthocyanins may be due to the rapid metabolism and the fact that all of its metabolites have not yet been analyzed and therefore the results may be underreported (Manach *et al.*, 2005). A more recent study observed a much higher recovery of the anthocyanins in urine and plasma, more in line with the recovery percentages of other flavonoids (Czank *et al.*, 2013). The observed poor absorption may also be due to the sensitivity anthocyanins exhibits towards pH-changes. After consumption of 200 g strawberries, mostly containing pelargonidin-3-glucoside, a recovery of 1.8 ±0.29%, mainly of glucuro- and sulfoconjugates, was recovered in human urine samples. More than 2/3 was excreted after 4 hours. Acidification of the urine samples lead to the disappearance of two out of six peaks identified by HPLC. In addition, one peak decreased and another peak

increased indicating the instability of some of the metabolites (Felgines *et al.*, 2003).

The low absorption and excretion of anthocyanins was also observed in elderly women consuming elderberry extract and blueberries. Excretions of 0.077 % within four hours respective 0.004 % within six hours were observed. The anthocyanins were excreted in their original form, methylated and glucuronide conjugates (Wu *et al.*, 2002).

Additional hydroxyl- and methoxy groups of anthocyanidins, as well as anthocyanin glucosides adds to the stability of the molecule (Fleschhut *et al.*, 2006). This may be one of the explanations for the many different results yielded in studies which have examined metabolites derived from food matrixes compared with pure anthocyanins. The parent anthocyanin is subsequently often recovered in very small concentrations (Ludwig *et al.*, 2015; de Ferrars *et al.*, 2014).

Phenolic acids seem to be the major stable metabolites derived from anthocyanins (Fleschhut *et al.*, 2006; Keppler & Humpf, 2005). Phenolic acids, mainly hippuric acid, was indeed found in human urine 120 min after blackcurrant consumption. Phenolic acids were also detected in plasma, peaking at 30 and 180 minutes after consumption (Jin *et al.*, 2011). The fast recovery in plasma also indicates the possibility for pH-initiated degradation of the anthocyanins in the early gastrointestinal tract (Ludwig *et al.*, 2015).

Some studies also indicate the impact colon microbiota may have on the degradation of anthocyanins into different metabolites. Microbial degradation of ^{13}C-labeled cyanidin-3-glucoside into hydroxybensoic acid in humans was observed in one study (de Ferrars *et al.*, 2014) and the recovery of phenylacetic acid in human plasma 6 hours post supplementation of raspberry anthocyanins, indicated both microbial degradation and absorption in the colon (Ludwig *et al.*, 2015).

4. The effects of anthocyanins on blood pressure

4.1 Epidemiological studies

Several observational studies have investigated the association between different flavonoid subclasses, including anthocyanins, and CVD risk factors, such as hypertension and elevated blood pressure. Such studies are summarized for comparison in Table 1 and information regarding participants, sources and doses of anthocyanins used and observed results on hypertension related factors are shown.

In large population based studies following women of 25-55 years (Nurses' Health Study I and II) and men of 40-75 years (Health Professionals Follow-Up Study) during 14 years, anthocyanins were found to be strongly associated with a reduced risk of hypertension. Pooled results from all three cohorts suggested an 8 % reduced risk for developing hypertension among participants with the highest intake of anthocyanin rich food compared to the group with the lowest intake. Intake of other subclasses of flavonoids was not associated with hypertension. The fact that some anthocyanins can act as inhibitors of the endothelial NADPH oxidase, an enzyme that limits the bioactivity of NO in the blood vessels was suggested as a mechanism behind the observation (Cassidy *et al.*, 2011).

Another large cohort study investigated the association between flavonoid intake and CVD mortality in men and women of 70 respective 69 years of age during in average 7 years of follow up. The conclusion from this study was that some flavonoid classes, including anthocyanins, were associated with a lower risk of CVD. The study also observed that participants with higher flavonoid intake were, amongst other factors, also less likely to have a history of hypertension (McCullough *et al.*, 2012).

A recent study conducted by Lajous *et al.* (2016) on healthy French women aged 45-58 years observed an inverse association between anthocyanin intake and hypertension. About 9 % lower incident of hypertension was found in the lowest antocyanin intake quantile compared to the highest quintile, after adjustment for other lifestyle factors. However, in contrast with Cassidy *et al.* (2011), this study also found inverse association between total flavonoid intake and hypertension incidence (Lajous *et al.*, 2016).

Significant inverse association between anthocyanin intake and CVD mortality was also observed in a prospective study on postmenopausal women of age 55-69 years. The inverse association was however not specific for hypertension since CVD mortality was the investigated endpoint of the study (Mink *et al.*, 2007).

Jennings *et al*. (2012) conducted a study in women aged 18-75 years and observed that anthocyanins were associated with a major impact on blood pressure. The total amount of consumed flavonoids did not influence arterial stiffness, central blood pressure or atherosclerosis. However, the total amount of anthocyanins was associated with a lowered peripheral and central systolic blood pressure and mean arterial pressure significantly. The study concluded that anthocyanins most probably have a protective role in regulation of blood pressure and arterial stiffness (Jennings *et al.*, 2012).

In conclusion, when it comes to epidemiological studies, intake of anthocyanins seems to be associated with beneficial effects on cardiovascular disease, hypertension included. Most studies have been conducted on female participants of higher age and anthocyanin intake varied widely.

Table 1. Summary of epidemiological cohort studies investigating association between anthocyanin intake from different foods and risk of hypertension or mortality due to cardiovascular diseases.
♀=women; ♂=men

Subjects (n) and conditions	**Average follow-up time**	**Anthocyanin food source**	**Anthocyanin dose (mg/d)**	**Association**	**Reference**
Healthy n (♀)=87242+46672 n (♂)=23043	14 years	Berries	12.5-15.2	Inverse	(Cassidy *et al.*, 2011)
Healthy n (♀)=60289 n (♂)=38180	7 years	Berries, wine	3.8 (<5.5)-13.7 (11.5-16.6)	Inverse only for mortality caused by cardiovascular diseases	(McCullough *et al.*, 2012)
Healthy n (♀)=40574	14 years	Fruits, wine	71 ±43 (39 ± 19.9 – 94 ±52.6)	Inverse	(Lajous *et al.*, 2016)
Postmenopausal n (♀)=34489	16 years	Berries, fruits, wine, bran, chocolate	0.2 (0.01-1040)	Inverse only for mortality caused by cardiovascular diseases	(Mink *et al.*, 2007)
(♀)	11 years	Fruits, wine, berries	17.7±14.9 (8.4-23.6)	Inverse	(Jennings *et al.*, 2012)

4.2 Randomized controlled trials

Some RCTs have found positive effects of anthocyanins on blood pressure (Table 2) and some have not (Table 3). The anthocyanins were often administered as different berry extracts, such as freeze-dried powder, juices and purees. The participants included most often had some kind of condition related to cardiovascular diseases such as metabolic syndrome or diabetes.

Dohadwala *et al.* (2011) investigated the effects of cranberry juice on vascular function in patients with stable coronary artery disease (CAD) with a mean age of 62 ± 8 years both in an acute pilot study and in a crossover study. In the acute pilot study brachial artery flow-mediated dilution (FMD - a method of measuring endothelial function) was significantly improved after 4 hours respective 2 and 4 hours. However, no cumulative effects of juice consumption were observed in the crossover study for brachial artery flow-mediated dilution, blood pressure or endothelial function. Decrease in arterial stiffness was observed as reduced carotid-femoral PWV (Dohadwala *et al.*, 2011).

Intake of anthocyanin-rich blueberries was shown to decrease blood pressure in obese participants aged 50.0 ± 3.0 years. The blood pressure had improved significantly compared to the control group (Basu *et al.*, 2010a). Another study found similar results when lower blood pressure after consumption of anthocyanin-rich berries was observed in unmedicated,

middle-aged participants with elevated risk of CVD. The effects was however only observed in the participants which already possessed elevated baseline BP ranging from 133.3-184.7 mmHg (Erlund *et al.*, 2008).

A more recent study by Rodriguez-Mateos *et al*., (2013) also investigated the impact of blueberry polyphenols on the endothelial function in healthy men of 18-40 years. The metabolites from the polyphenols improved the vascular function greatly, most probably due to that they were able to decrease NADPH oxidase activity. The increase of FMD reached the maximum value after 1-2 hours after consumption of 766 mg polyphenols of which 310 mg was anthocyanins. The increase of FMD after 6 hours was associated with an increase of metabolites derived from anthocyanins and chlorogenic acids (Rodriguez-Mateos *et al.*, 2013).

Zhu *et al*. (2011) investigated the effects of purified anthocyanins derived from blueberries and blackcurrant on endothelial function in a long-term and a short-term study in hypercholesterolemic patients aged 40-55 years. After 12 weeks of supplementation, SBP had decreased significantly and FMD was increased compared to baseline and placebo. The short-term intervention observed increased concentrations of plasma deliphinidin-3-O-β-glucosides and cyanidin-3-O-β-glucosides (metabolites of anthocyanins) 1 hour after supplementation. All in all, the anthocyanins improved endothelial-dependent vasodilation (FMD) significantly and the mechanism was thought to include the activation of the NO-cAMP signaling pathway (Zhu *et al.*, 2011).

Table 2. Summary of results from randomized controlled trials where statistically significant improvements (+) of blood pressure (BP), arterial stiffness measured as pulse wave velocity (PWV) or endothelial function measured by flow mediated dilution (FMD) have been observed.
♂=men; sex not mentioned= both women and men or not mentioned in study

Subjects (n) and conditions	**Duration of intervention**	**Anthocyanins source**	**Dose (mg/d)**	**Berries (g)**	**BP**	**Arterial stiffness/Endothelial function**	**Reference**
Coronary artery disease n=44	4 weeks	Cranberry (juice)	94			+ (PWV)	(Dohadwala *et al.*, 2011)
Metabolic syndrome n=48	8 weeks	Blueberry (freeze dried)	742	350	+		(Basu *et al.*, 2010a)
Increased risk of cardiovascular disease n=72	8 weeks	Bilberry Lingonberry (nectar) Blackcurrant/ strawberry (purée) Chokeberry/ Raspberry (juice)	515	100 + 1 drink	+		(Erlund *et al.*, 2008)
n (♂) =21	6 hours	Blueberry	310	240		+ (FMD)	(Rodriguez-Mateos *et al.*, 2013)
Hypercholesterolemic n=150	12 weeks	Pure	320		+	+ (FMD)	(Zhu *et al.*, 2011)
n=12	4 hours						

There are also several RCT studies that have not observed any change in blood pressure or any difference compared with control after an intervention with polyphenol rich diet (Del Bo′ *et al.*, 2013; Riso *et al.*, 2012; Stull *et al.*, 2010; McAnulty *et al.*, 2005). A summary of some of these studies are presented in Table 3.

When healthy young men aged 20.8 ± 1.6 years consumed blueberries in an acute study conducted by Del Bo´ *et al*. (2013), no effects on peripheral arterial function or BP was observed. The failure to demonstrate an effect on peripheral arterial function was thought to be due to the fact that the subjects were all healthy. The effects may be easier demonstrated in subjects with reduced vascular function. The measurement may also have been carried out to soon after intake (1 hour) (Del Bo′ *et al.*, 2013) since

maximum concentration of effective metabolites in plasma may appear later (Manach *et al.*, 2005).

Failure to demonstrate significant decrease in BP and peripheral arterial function have also occurred in studies where older men aged 47.8 ± 9.7 years with one risk factor for CVD consumed freeze-dried blueberry powder. The 6 weeks intervention study included a large number of individuals with differences in CVD-risk factors and a large inter-individual variation was seen in the subjects as half of the group did in fact have improved values (Riso *et al.*, 2012).

In obese, insulin resistant men and women with normal blood pressure no alteration in BP was found after consumption of anthocyanin rich blueberry powder for 6 weeks. It is however unclear how the BP was measured since the endpoint of the study was to measure insulin sensitivity (Stull *et al.*, 2010). After 3 weeks of consumption of 250 g blueberries/d, alterations in BP was not observed in chronic smokers either (McAnulty *et al.*, 2005).

Adults with three of five symptoms of metabolic syndrome consumed cranberry juice for 8 weeks. No significant change of BP was observed in the study (Basu *et al.*, 2011). A similar study where subjects aged 47.0 ± 3.0 years with metabolic syndrome consumed freeze-dried strawberries instead for 8 weeks did not either observe any change in BP (Basu *et al.*, 2010b).

Healthy men aged 35-51 years, with high BP (>140/90 mmHg) ingested pure anthocyanins for 4 weeks in a double-blind crossover study and alteration in BP was not observed after treatment (Hassellund *et al.*, 2012).

In a double-blind crossover acute meal study, healthy subjects aged 44.55 ± 13.34 years consumed blackcurrant juice or placebo. No significant change in BP was observed between juice and placebo (Jin *et al.*, 2011).

Table 3. Studies where no positive effects on blood pressure or vascular function was observed after anthocyanin intake.

♂= men; sex not mentioned= both women and men or not mentioned in study

Subjects (n) condition	**Duration of intervention**	**Anthocyanin source**	**Dose mg/d**	**Reference**
n (♂)=10	24 hours	Blueberry (frozen)	348	(Del Bo′ *et al.*, 2013)
Risk factor for cardiovascular disease n=18	6 weeks	Blueberry (freeze-dried)	375	(Riso *et al.*, 2012)
Obese, insulin resistant n=32	6 weeks	Blueberry (freeze-dried)	668	(Stull *et al.*, 2010)
Chronic smokers n=20	3 weeks	Blueberry		(McAnulty *et al.*, 2005)
Metabolic syndrome n=36	8 weeks	Cranberry (juice)	24.8	(Basu *et al.*, 2011)
Metabolic syndrome n=27	8 weeks	Strawberry (freeze-dried)	154.0	(Basu *et al.*, 2010b)
Healthy n (♂)=31	4 weeks	Pure	640	(Hassellund *et al.*, 2012)
Healthy n=20	24 hours	Blackcurrant (juice)	~50 (only delphinidin and cyanidin)	(Jin *et al.*, 2011)

4.3 Animal and *in vitro* experiments

Animal and *in vitro* model studies have been conducted to study potential mechanism behind blood pressure reduction caused by anthocyanins. Diebolt *et al*. (2001) observed increased endothelial NO activity in aorta vessels from rats when red wine polyphenols, including anthocyanins, were given. The study provided clear evidence that the red wine polyphenols could lower blood pressure under short-term oral administration. The blood pressure decreased without any changes in heart rate in the animals. The decrease of blood pressure was thought to be due to the increase of endothelial NO activity (Diebolt *et al.*, 2001).

When different fractions of red wine polyphenolic compounds were added to norepinephrine precontracted rat aorta, the activity of endothelium-dependent vasorelaxation of anthocyanins were comparable with the original red wine polyphenolic compounds. The red wine powder contained 64 mg anthocyanins/g red wine powder and the vasorelaxing effects were observed between concentrations of 10^{-4}-10^{-3} g/L. The results clearly stated that the anthocyanin fraction of red wine polyphenols was responsible for

the vasorelaxing effects and were comparable with the original red wine polyphenols (Andriambeloson *et al.*, 1998).

A study examining the effect of cyanidin-3-glucoside on eNOS in bovine artery endothelial cells found anthocyanins to be associated with the upregulation eNOS. The results contribute to the evidence for the effects of anthocyanins participating in the maintaining of the blood pressure and improvement of dysfunctional endothelia via increased NO production (Xu *et al.*, 2004). Similar findings were observed when human umbelicial vein endothelial cells were treated for 24 hours with the two anthocyanidins delphinidin and cyanidin. Increased protein levels of human eNOS was observed *in vitro*, especially for delphinidin (Lazzè *et al.*, 2006).

Newer studies seem to focus more on the effects of the metabolites rather than the intact anthocyanins since evidence for the vaso-relaxing effects points in the direction of the metabolites (Rodriguez-Mateos *et al.*, 2013).

5. Mechanism of action

Some studies have attempted to suggest possible mechanistic pathways for the observed effects of anthocyanins on blood pressure. Following ingestion of anthocyanins, Zhu *et al*. (2011) observed improved FMD correlated with increased levels of cGMP in serum, used as an indicator of NO activity. The activation of the NO-cGMP signaling pathway therefore seem to be involved in the mechanism of action for the endothelial dependent vasorelaxation observed after anthocyanin supplementation (Zhu *et al.*, 2011). Improvement of FMD has also been shown to correlate with increasing plasma metabolites derived from anthocyanins in a study from Rodriguez-Mateos *et al*. (2013). Both increase in FMD and plasma metabolites was observed to peak 1-2 hours respective 6 hours after intake of blueberry polyphenols. In line with the increase of FMD and phenolic acids (hippuric-, vanillic- and homovanillic acids) a decrease of NADPH oxidase was observed suggesting a potential mechanism of action in which the bioavailability of NO increases as a results from the inhibition of NADPH oxidase. Indeed, all three phenolic acids have structural similarities with a known NADPH oxidase inhibitor; apocynin (Rodriguez-Mateos *et al.*, 2013).

A major anthocyanin metabolite, protocatechuic acid, have been observed both *in vitro* and *in vivo* to reduce monocyte adhesion to the endothelium in ApoE-deficient mice by inhibiting the expression of two adhesion molecules. Reduced activation of NF-κB, an important transcriptional regulator for the two adhesion molecules was also observed *in vitro*. All in all, these findings suggested that these major metabolites of anthocyanins have positive inverse effects on the formation on atherosclerotic lesions in apoE-deficient mice (Wang *et al.*, 2010). Results from RCTs with healthy humans support the inhibitory effects of anthocyanins on NF-κB (Karlsen *et al.*, 2007). The positive effects on the atherosclerotic lesions from the anthocyanin metabolite supports a functional epithelium, a crucial factor when considering maintenance of normal blood pressure.

Considering the low bioavailability of anthocyanins, the evidence from epidemiological studies and RCTs together with the potential mechanisms behind the effects observed both in animals and humans *in vitro* and *in vivo* all in all suggest that the lowering in blood pressure most probably is caused by anthocyanin metabolites rather than the intact anthocyanins themselves.

6. Discussion and Conclusion

Foods contain a mixture of flavonoids and it is likely that observed effects on blood pressure may be due to a specific anthocyanin or its derivatives and are aided by other subclasses of flavonoids (Cassidy *et al.*, 2011). Consumers of high mean intake of flavonoids generally consume more fruits, vegetables and less trans- and saturated fats (McCullough *et al.*, 2012) which may influence results from epidemiological studies. Several participants in the study by Basu *et al.* (2010a) experienced gastrointestinal discomfort and problems when consuming the freeze dried blueberry powder, probably due to the increased amount of fiber consumption (Basu *et al.*, 2010a). The association between lower blood pressure and consumption of some anthocyanin-rich food sources may therefore to some extent also depend on the fiber intake, since fiber has been found to correlate with lower risk of CVD (Mink *et al.*, 2007). It is therefore possible that overall healthier diets and lifestyle may have residual-confounded the results of observed associations with blood pressure in some epidemiological studies, despite extensive adjustment for confounders.

The use of different anthocyanin databases provides different estimations of anthocyanin contents in foods which leads to lower precision in the intake estimations and systematic differences between studies using different databases. Moreover, the use of different statistical models adjusting for different confounding factors across studies, may further have contributed to differences in results in some epidemiological studies – to different degree. Problems like these makes comparison between studies complex. Future studies should therefore aim to use more similar study designs and statistical models.

Despite somewhat different results, many epidemiological studies show a beneficial inverse association between anthocyanin intake and blood pressure. However, cause and effect cannot be inferred from these studies. More double blinded, RCTs should be conducted with intakes of pure anthocyanins instead of mixtures of polyphenols, which contains substances that may interference with the results. Studies using pure, labelled anthocyanins would make it possible to identify the metabolites and their interaction with other substances and may contribute to a better understanding of the role anthocyanins play in hypertension as well. However, the possible inhibitory effects of excessive concentrations should be evaluated further before using pure anthocyanins in future study models.

Regarding recovery studies of anthocyanins, there seem to be a lack of detection of all metabolites with the current available techniques, even though ^{13}C-labeling is used (de Ferrars *et al.*, 2014). The possibility for

chemical binding between anthocyanins and their metabolites and proteins may obstruct the detection of all metabolites in plasma and urine samples. This obstacle may be overcome by the continued widespread use of labeled anthocyanins (Wallace, 2011).

In conclusion, there seem to be positive effects of anthocyanins on BP mainly in older subjects with already elevated risk of hypertension. However, additional RCTs using pure anthocyanins are needed to establish the cause and effect as well as dose-response relationships. The mechanisms of actions are not fully understood, but there are some evidence suggesting upregulation/activation of NO as a result of the metabolites derived from anthocyanins. More studies conducted on both animals and humans *in vivo* as well as *in vitro* with labeled anthocyanins and their metabolites may provide a possibility to establish cause and effect relationship for specific metabolites on the role of NO in anthocyanin induced lowering of blood pressure.

7. References

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