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Blueberries and cardiovascular disease prevention

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Blueberries are a rich source of (poly)phenols, particularly anthocyanins. Epidemiological studies indicate that anthocyanin-rich foods including blueberries are associated with a reduction in the risk of cardiovascular disease. These observational findings are supported by a number of randomized-controlled trials showing improvements in biomarkers of cardiovascular disease risk. The beneficial effects of blueberry (poly)phenols are particularly clear when measuring flow-mediated dilation over various timeframes and study populations. However, other outcomes are less clear, such as effects on blood pressure, arterial stiffness and blood lipid profile. This may be due to the heterogeneity existing in study designs, such as duration of the intervention, and the health status of participants. Longer-term RCTs using gold standard methods in relevant populations which can be translated to the general public are needed to clarify and strengthen the evidence available. While circulating phenolic blueberry metabolites have been linked with improvements in vascular function, the biological activities and mechanisms of action of individual metabolites and their interaction *in vivo* are still unknown. Evaluating the bioactivities of metabolites alone and together, and analysing their structure–activity relationship in well-designed and physiologically relevant experimental and human studies are needed to understand the mechanisms of how these metabolites affect vascular function.

Introduction

The health benefits of blueberries are of particular interest of late due to their high content of phytochemicals known as (poly)phenols (Table 1).[1] Several epidemiological studies suggest that the high (poly)phenol content of blueberries, particularly a subclass known as anthocyanins, may be responsible for their cardiovascular health benefits.[2,3] Recent meta-analyses and systematic reviews of randomised-control trials (RCTs) support that anthocyanin-rich foods such as berries can improve endothelial function, blood pressure and arterial stiffness,[4–7] lower fasting and postprandial glucose, total cholesterol, and LDL-cholesterol,[8–10] and may reduce the risk of developing cardiovascular diseases (CVD) *via* these routes. The aim of the current review is to discuss the current evidence from epidemiological studies and RCTs on the effects of blueberries on CVD risk.

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(Poly)phenol content of blueberries

Blueberries are of particular interest of late as they are high in micronutrients, fibre and (poly)phenols but low in calories (Table 1). Blueberries are one of the most commonly consumed, and most widely studied berries and are a rich source

Table 1 Nutrient composition of blueberries[25,73]

Nutrients	Amount per 100 g fresh weight
Energy (kcal)	57
Carbohydrate	14.5
Fat	0.33
Protein	0.74
Dietary fibre (g)	2.4
Potassium (mg)	77
Fructose (g)	4.97
Total beta carotene (μg)	31
Vitamin C (mg)	9.7
Calcium (mg)	6
Iron (mg)	0.28
Vitamin E (mg)	0.57
Vitamin B1 (mg	0.04
Vitamin B2 (mg)	0.04
Vitamin B6 (mg)	0.05
Phosphorus (mg)	12
Magnesium (mg)	6
Zinc (mg)	0.16
Manganese (mg)	0.336
Niacin (mg)	0.418

of (poly)phenols including anthocyanins, procyanidins, flavonols, and phenolic acids (Fig. 1).[11,12] They differ from other berries such as strawberries and raspberries as they contain different types of (poly)phenols. For example, blueberries do not contain ellagitannins unlike raspberries and strawberries but they are particularly high in anthocyanins, which are red, blue, or purple pigments that play a key role in plant pollination as well as protection from ultra-violet induced damage by absorbing light.[4,13] At least 25 structurally different anthocyanins have been identified in blueberries, including glucosides, galactosides, and arabinosides of five anthocyanidins: delphinidin, malvidin, petunidin, cyanidin and peonidin.[14] The anthocyanin content of blueberries is very variable, typically ranging from 114–487 mg per 100 g fresh weight (FW) of fresh fruit.[15–17] Total flavan-3-ol content range from 33–333 mg per 100 g FW of which most are oligomeric and polymeric forms of B-type proanthocyanidins, which have low bioavailability.[16,18] Unlike cocoa, very little flavan-3-ol monomers are present in blueberry ((+)-catechin and (−)-epicatechin). Flavonols range from 11–39 mg per 100 g FW, and the most abundant phenolic acids are the chlorogenic acids, ranging from 43–114 mg per 100 g FW (Fig. 1).[12,16] There are many factors which will affect the (poly)phenol content of blueberries such as the type of blueberry species, fruit ripeness, size, growing climate, environment, and storage after harvest.[19–21] Cooking, baking, and processing overall can also cause significant changes in the (poly)phenol content of blueberries post-harvest, in particular anthocyanin content can decrease dramatically during storage, juice processing, or when subjected to baking.[22] Factors particularly effecting breakdown of anthocyanins are light and temperature. Therefore, due to large diversity in (poly)phenol content both within and between blueberry species, and due to changes during storage and processing, it is important to measure the (poly)phenol content of each batch of blueberry used, particularly when assessing health benefits in preclinical and clinical studies. Reliable analytical methods for the analysis of (poly)phenols in blueberries should be used, such as liquid/gas chromatography coupled with UV-Visible, electrochemical detection or mass spectrometry, as non-specific methods such as the Folin–Ciocalteu or the pH differential method can lead to big sources of error.[23] The analysis of the major (poly)phenols present in blueberries, including anthocyanins, flavan-3-ols and phenolic acids (chlorogenic and hydroxycinnamic acids) using authentic standards for quantification is recommended for accurate results.[24]

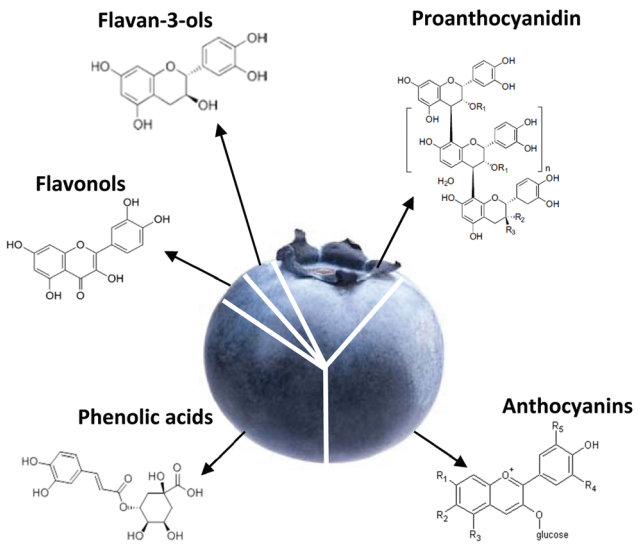

Fig. 1 Main (poly)phenol groups found in blueberries.

Absorption, distribution, metabolism, and excretion (ADME) of blueberry (poly)phenols

In blueberries, the ADME of anthocyanins is especially important to consider as they likely mediate their vascular effects *via* their circulating metabolites.[25] It is known that anthocyanins themselves have a low bioavailability, with less than 1% of the parent compounds being recovered in urine.[26–28] However, novel insights in the last decade have demonstrated that the metabolism of anthocyanins is more complex than previously thought and a large number of phenolic metabolites resulting from the chemical and microbial degradation of anthocyanins in the gastrointestinal tract reach circulation in up to micromolar concentrations. Vitaglione and colleagues (2007) demonstrated extensive degradation of cyanidin-3-*O*-glucoside (C3G) to protocatechuic acid in the small intestine within 2 h of blood orange juice consumption.[29] More recently, a stable isotope-labelled human study in 8 males investigated the concentration of anthocyanins in plasma, urine and faecal samples over 48 h post-consumption of 500 mg ^{13}C isotopically labelled C3G.[30] The total recovery of the ^{13}C dose was 44%, with 5.4% recovered in urine, 6.9% in breath and 32% in faeces. The authors reported a total of 35 ^{13}C-labelled metabolites, including the parent C3G, identified in the samples overall. The analysis showed that the majority of the ^{13}C label were present in the circulation as low molecular weight phenolic metabolites of the parent C3G, such as protocatechuic, vanillic, caffeic, ferulic and phenylacetic acid derivatives.[31] Besides chemical degradation in the gastrointestinal tract, anthocyanins undergo various processes such as phase I and phase II metabolism, microbial metabolism and enterohepatic recirculation, leading to a large number of metabolites being formed at various timepoints over a 48 h period.[31] Enzymes produced by the bacteria in the intestine can hydrolyse glycosides, sulfates and glucuronides, as well as perform complex reactions such as oxidation, demethylation, reduction, decarboxylation and ring fissions to produce smaller catabolites.[32] Therefore, when studying the health effects of anthocyanin-rich food sources, it is important to consider the wide range of metabolites produced as well as their kinetics.

Recent studies using authentic standards and validated methods for analysis have investigated the fate of anthocyanins and other (poly)phenols after blueberry consumption.[33] In healthy men, 19 metabolites increased in plasma 2 h after the consumption of wild blueberry. Those metabolites included benzoic acid, catechol, flavonol, hippuric, and cinnamic acid derivatives, with catechol-*O*-sulfate, benzoic and vanillic acid representing the major changes after 2 h consumption, demonstrating again that anthocyanins are largely transformed into smaller phenolic compounds already in the upper GI tract (Fig. 2A). After daily wild blueberry consumption for one month, hippuric acid was the compound with the highest increase in plasma of volunteers, followed by catechol-*O*-sulfate and 3-hydroxyhippuric acid (Fig. 2B). In this study, the metabolites measured in circulation illustrate that blueberry (poly)phenols are absorbed and extensively metabolised by phase II enzymes and by the gut microbiota. This results in a variety of circulating metabolites that may be responsible for their observed health benefits following blueberry consumption rather than the less bioavailable parent anthocyanins present in blueberries.

There are several important factors that may affect the bioavailability of blueberry (poly)phenols. For example, the absorption of various phenolic metabolites could be increased, unaltered, or decreased when consumed together with other foods rich in proteins, carbohydrates (*i.e.*, fibres), or fats.[19] As well as the food matrix, food processing has also been reported to affect the bioavailability of blueberry (poly)phenols.[22] Another factor to consider is inter-individual differences in (poly)phenol ADME. In the same study previously mentioned,[33] interindividual-variability, expressed as coefficient of variation (CV%), ranged between 40–48% in plasma and 47–54% in urine. Which factors may be responsible for such variability is currently unknown, although some evidence suggest host genetic and epigenetic variations in metabolizing enzymes, differences in gut microbiota composition, sex, age, and dietary habits may play an important role in inter-personal differences.[34]

Epidemiological evidence on anthocyanin and blueberry (poly) phenol consumption and cardiovascular disease risk

Epidemiological evidence provides associations between high intakes of berries and other anthocyanin-rich foods and low incidence of CVD mortality and morbidity.[35–38] Average intakes of blueberries have been reported from the National Health and Nutrition Examination Survey (NHANES), in the U.S., as 0.93 g day^{-1}. This is the equivalent of 3.39 mg day^{-1} of anthocyanins.[17] In the US, blueberries have been reported as the main source of anthocyanins.[39] A meta-analysis of 14 prospective cohort studies, varying between 6 to 28 years follow-ups, reported an association between higher anthocyanin intake and a 11% reduction of future CVD risk, CVD defined as coronary heart disease (CHD), stroke, cardiac arrest, heart failure and sudden death.[2] Supporting these findings, a more recent meta-analysis of 22 prospective studies, also found an 11% reduction in both all-cause mortality and CVD risk in those with high anthocyanin intakes compared with low intakes.[3] Findings from the Nurse's Health Study (NHS), analysing the dietary patterns of 93 600 healthy women, aged 25–45 years, showed a signifi-

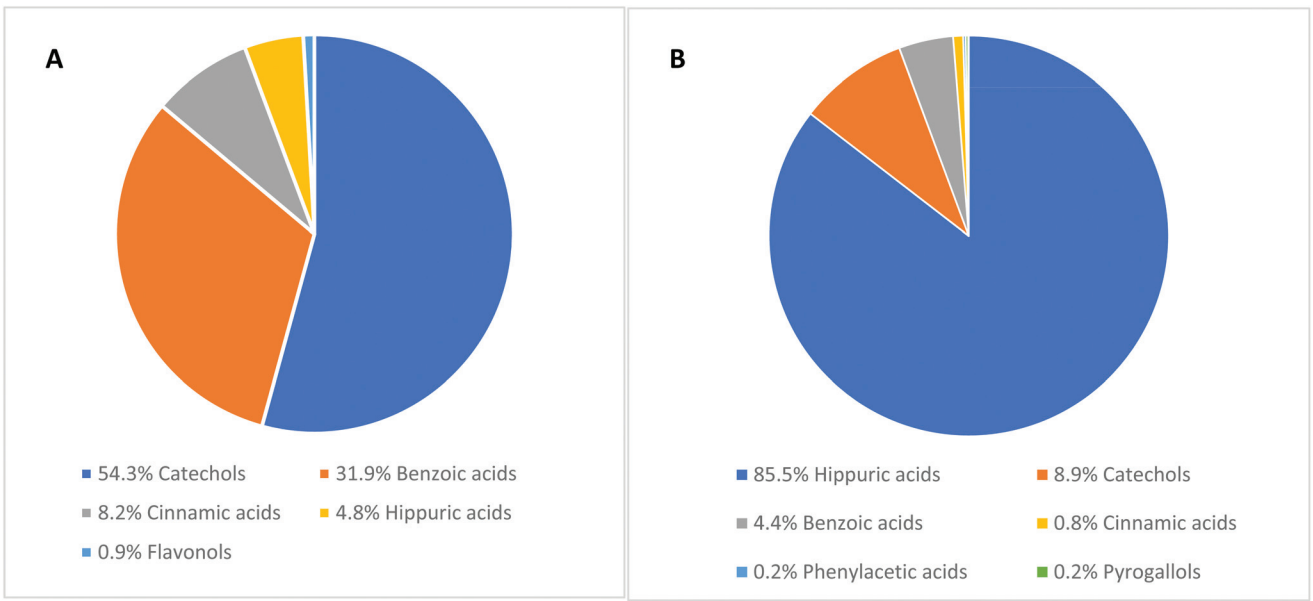

Fig. 2 Percentage of (poly)phenol metabolites main subclasses found in plasma at (A) 2 h and (B) 1 month after blueberry consumption in healthy men.[33]

cant relationship between intake of >3 portions (1 cup per portion) of strawberries and blueberries per week and a lowered risk of myocardial infarction by 34% over an 18-year follow up.[40] Findings from the Kuopio Ischemic Heart Disease Risk Factor Study (KIHD) in 1950 males, with no history of CVD at baseline, showed that men with the highest berry intake (>408 g day^{-1}) compared with the lowest intake (<133 g day^{-1}) had a significantly lower risk of CVD mortality, defined as CHD, stroke, cardiac arrest, heart failure and sudden death,[41] during a 13-year follow up.[37] Data from the *Iowa Women's Health prospective study*, following almost 35 000 post-menopausal women, free of CVD at baseline, for 16 years, found higher anthocyanin intakes were associated with a reduced risk of CHD, total CVD (CHD, stroke, cardiac arrest, heart failure and sudden death),[41] and total mortality by 12%, 9%, and 10%, respectively.[38]

Although promising evidence, there are important limitations in the epidemiological studies conducted so far. The estimation of food consumption or (poly)phenol intake is based on self-reported Food Frequency Questionnaires (FFQ), which are known to have a high degree of bias and inaccuracy.[42] In addition, food composition databases for (poly) phenols such as the Phenol explorer[16] and the USDA database,[43] are still limited to some foods and lack accuracy. As mentioned before, levels of anthocyanins, as well as other flavonoids, in blueberries and other foods will vary depending on the storage, cooking methods and conditions that the food was grown in. One way of overcoming these limitations in the future is the development of validated biomarkers of blueberry/anthocyanin intake, a promising approach in nutritional epidemiology, which is still in its infancy.[44]

Clinical dietary intervention studies on the cardiovascular health benefits of blueberries and anthocyanins

As epidemiological studies investigate the association of habitual dietary intakes with hard outcomes of CVD, they do not provide enough evidence to infer cause-and-effect relationships as several other factors may influence the incidence and progression of CVD. Therefore, the most reliable method to better understand whether blueberry (poly)phenols are cardioprotective is to conduct well-designed RCTs using surrogate markers of CVD risk. There have been several small, short-term human intervention studies investigating the effects of blueberries on validated surrogate markers of CVD risk, namely blood pressure, endothelial function, arterial stiffness, lipids, glucose, and platelet activation. In this section, we will discuss the most relevant human RCTs investigating the effect of blueberries and anthocyanins on at least one of these biomarkers.

Effects on endothelial function

An established marker for the initiation and progression of CVD is endothelial function.[45] A validated measure for endothelial function in humans is flow-mediated dilation (FMD) in the brachial artery using high-resolution ultrasound.[46] Another commonly used marker of endothelial function is endothelial peripheral artery tonometry (endo-PAT), which measures reactive hyperaemia index (RHI) of the fingertips. Previous meta-analyses have demonstrated that a 1% increase in FMD of the brachial artery translates to a 10–13% decreased risk of CVD.[47–49] Several studies have investigated the effects of blueberries on endothelial function (Table 2).[25,50–58] Six of the RCTs used FMD of the brachial artery to assess endothelial function whilst the remaining 7 used endo-PAT.

The RCTs investigating the effect of blueberry on FMD included time- and dose-dependent study designs and reported significant improvements in FMD, in 2 separate groups of young healthy males, at 1, 2, and 6 h post-consumption of a whole freeze-dried wild blueberry drink containing 319, 639, 766, 1278, or 1791 mg total (poly)phenols (129, 258, 310, 517, or 724 mg anthocyanins) equivalent to 100–560 g of fresh wild blueberries.[53] Significant intake-dependent increases in FMD between 1.2 and 2.4% were observed, even at the lowest amount tested (100 g) which is still a relatively large amount only consumed in the highest quartiles of some of the epidemiological studies discussed above. The maximum effect was shown at the equivalent to 240 g of fresh wild blueberries and further amounts did not further increase FMD. Another study, in 10 young healthy males, supported these findings by showing a time-dependant improvement in FMD 1, 2 and 6 h post-consumption of baked blueberry products, and a blueberry drink, both containing 34 g freeze-dried blueberry powder, the baked products contained 196 mg anthocyanins and the drink contained 339 mg.[57] A recent study investigated the effects of daily wild blueberry consumption for 4-weeks on FMD in 40 young healthy males.[25] FMD increased in the wild blueberry group by 1.5% 2 h post-consumption and by 2.3% after 4-weeks daily consumption of 22 g freeze-dried blueberry (300 mg anthocyanins, equivalent to 200 g of fresh blueberries). This suggests that daily consumption of blueberry (poly) phenols can improve FMD in healthy individuals. Supporting these findings in a high CVD risk population, a recent RCT in 115 participants with metabolic syndrome reported a 1.4% increase in FMD following consumption of 26 g freeze-dried blueberries (364 mg anthocyanins, equivalent to 150 g of fresh blueberries) for 6 months.[54] No effect in the group receiving a lower blueberry amount (13 g freeze-dried, 75 g fresh blueberries; 182 mg anthocyanins) was found, suggesting intake dependent effects of long-term blueberry intake on FMD.[54] Therefore, longer-term consumption of large amounts of blueberries can improve FMD significantly in both healthy individuals and those at higher risk of CVD, which could result in a reduction of future CVD risk.

Of the studies using endo-PAT, rather than FMD, as a measure of endothelial function, 3 out of 6 found significant improvements following a blueberry treatment compared with a control. A significant improvement in RHI after consumption of 22.5 g freeze dried blueberry powder (290 mg TP, 1 cup fresh weight blueberries) for 6-weeks was found in men and

Table 2 Table of randomised-controlled trials investigating the effects of blueberries on markers of cardiovascular disease risk

Reference	Study design	Study subjects	Health status of volunteers	Blueberry treatment	ACN (mg for dose)	Control	Duration	Significant findings
Basu *et al.* (2010)[63]	Randomised, single-blinded, parallel, controlled trial	48 men and women (mean age: 50 ± 3)	Metabolic syndrome	50 g freeze-fried BB + vanilla extract or Splenda	742	Water to match the fluid intake in the treatment group (960 ml)	Daily consumption: 8 weeks	BP decreased in blueberry group compared with the control group by −7.8 and −2.5 mmHg respectively. Ox-LDL decrease in blueberry group
Bell *et al.* (2017)[69]	Randomised, double-blind, crossover trial	16 young adults (mean age: 24 ± 5)	Healthy (BMI: 23.7 ± 3.6 kg m^{-2})	Freeze-dried BB: low dose 34 g, low dose 34 g + sugar, high dose 80 g + sugar	310, 310, 724	No-added sugar control or sugar-matched control	Acute: 0, 15, 30, 45, 60, 90, 120 and 150 min	Blueberries extended the postprandial glycaemic response. Blood glucose remained elevated for 2 h in the 724 mg anthocyanin dose and 1.5 h in the 310 mg dose
Blacker *et al.* (2013)[72]	Randomised, crossover, controlled trial	15 men and women (mean age: 22)	Healthy (BMI: 23.6, range: 20–28 kg m^{-2})	5.83 g or 12.5 g freeze-dried BB powder + high-CHO low-fat meal	75 and 161	Sugar and Vitamin C matched + high-CHO low-fat meal	Acute: baseline, 1, 2 and 3 h	Serum ORAC was higher in the high blueberry dose group compared to control up to 2 h postprandially. Serum LO lag time showed a significant trend over 3 h for both blueberry doses
Cheatham *et al.* (2016)[66]	Randomised, double-blind, parallel, controlled trial	113 men and women (mean age: 73 ± 4)	Healthy with MCI	35 g freeze dried BB powder	N/A	Calorie, sugar, taste and colour matched	Daily consumption: 6 months	3 months after consumption serum uric acid decreased in the blueberry group but not after 6 months
Curtis *et al.* (2019)[54]	Randomised, double-blind, parallel, controlled trial	115 men and women (mean age: 63 ± 7	Overweight and obese (≥25 kg m^{-2}) with metabolic syndrome	26 g freeze dried BB powder, or 13 g placebo + 13 g BB powder, or 26 g placebo	364 g, 182 g, 0 g	Isocaloric, carbohydrate-matched control	Daily consumption for 6 months	Improved FMD (+ 1.45%) and reduced augmentation index (−2.24%) following the highest dose of BB, as well as attenuated guanosine monophosphate concentrations.
Del Bo *et al.* (2013)[51]	Randomised, single-blind, crossover, controlled trial	10 males (mean age: 21 ± 2)	Healthy (BMI: 23 ± 2 kg m^{-2})	300 g BB processed: thawed (3 h at 20 °C) and homogenized	348	Control jelly matched for sugars	Acute: 24 hours (measured 1, 2 and 24 h)	Reduction in H_2O_2-induced DNA damage 1 h post consumption of blueberry
Del Bo *et al.* (2014)[56]	Randomised, single-blind, crossover, controlled trial	16 males (mean age: 24 ± 3)	Healthy smokers ~15 cigarettes per day (BMI: 23 ± 2 kg m^{-2}).	300 g BB + smoking	348	Smoking treatment or Smoking + sugar water	Acute: 2 hours (measured 100, 105 and 120 min)	Blueberries counteracted the increase in SBP and attenuated the reduction in RHI that occurs after smoking
Del Bo *et al.* (2017)[52]	Randomised, crossover, controlled trial	24 males (12 smokers, 12 non-smokers. Mean age: 24 ± 1)	Both groups have peripheral arterial dysfunction (RHI < 1.67) (BMI 22.5 ± 1.2 kg m^{-2})	300 g BB with or without smoking	309	Non-smokers: 300 ml sugar water. Smokers: smoking treatment or 300 ml sugar water + smoking	Non-smokers: 0, 100, 120 min. Smokers: 0, 100, 105 and 120 min (smoking at 105 min)	Non-smokers: RHI increased in the blueberry treatment compared to control. Smokers: both control and blueberry treatment improved RHI compared with the smoking only treatment

Table 2 *(Contd.)*

Reference	Study design	Study subjects	Health status of volunteers	Blueberry treatment	ACN (mg for dose)	Control	Duration	Significant findings
Johnson *et al.* (2015)[62]	Randomised, double-blind, parallel, controlled trial	48 postmenopausal women (mean age: blueberry group 60 ± 5; control 57 ± 5)	Pre- and stage-1 hypertensives. BMI: blueberry group 30 ± 6 kg m^{-2}; control 33 ± 7 kg m^{-2}.	22 g freeze-dried BB powder	469	22 g macronutrient-matched control powder	Daily consumption: 8 weeks (measured at 4 and 8 weeks)	BP reduced at 8 weeks in the blueberry group (SBP by −7 mmHg, DBP by −5 mmHg). baPWV reduced in blueberry group but cfPWV did not. SOD increased in both groups
Johnson *et al.* (2017)[74]	Randomised, double-blind, parallel, controlled trial	48 postmenopausal women (mean age: blueberry group 60 ± 5; control 57 ± 5)	Pre- and stage-1 hypertensives BMI: blueberry group 30 ± 6 kg m^{-2}; control 33 ± 7 kg m^{-2}.	22 g freeze-dried BB powder + 240 ml water	469	22 g control powder: sugars, flavourings and colouring, citric acid and silica dioxide + water	Daily consumption for 8 weeks	8-OHdG levels were lower in blueberry compared to control at 4 weeks but not at 8 weeks.
McAnulty *et al.* (2005)[64]	Randomised, parallel, controlled trial	20 men and women (mean age: blueberry group 26 ± 3; control 29 ± 4)	Smokers (min 1 pack per day for >1 year). BMI: blueberry group 30 ± 3 kg m^{-2}; control 29 ± 3 kg m^{-2}.	251 g fresh BB	N/A	Normal dietary habits	Daily consumption: 3 weeks	No change in BP, F2-isoprostane concentration or ACE activity
McAnulty *et al.* (2011)[75]	Randomised, parallel, controlled trial	25 men and women (mean age: blueberry group 31 ± 13; control 33 ± 16)	BMI < 30 kg m^{-2}. No reported CVD or other risk factors.	250 g fresh BB daily then 375 g on the final day, 1 h prior to 2.5 h running	N/A	Normal dietary habits	Daily consumption: 6 weeks	Reduction in plasma F2-isoprostanes, urinary 5-OHMU and significant increases in plasma IL-10 in blueberry group
Nair *et al.* (2017)[76]	Randomised, double-blind, parallel, controlled trial	27 men and women (mean age: blueberry group 55 ± 2, Placebo group 59 ± 3)	Metabolic syndrome (defined by WHO)	22.5 g freeze-dried BB powder + vanilla flavour, fat-free vanilla yogurt, skim milk + water	290.3	Fat-free vanilla yogurt, skim milk, sugar, fibre water and colours	Twice daily consumption: 6 weeks	Blueberry group had lower levels of ROS and superoxide radical levels, elevated myeloid dendritic cells and reduced inflammatory markers (TNFa, TLR4, IL-6) in monocytes
Nyberg *et al.* (2013)[67]	Randomised, cross-over, controlled trial	32 men and women (mean age: 28 ± 7)	Healthy (free from major disease)	150 g BB with exercise	N/A	Exercise without blueberries	4 weeks with running then 4 weeks of minimal exercise	Fasting glucose and HDL-cholesterol increased in the blueberry group. Triglycerides fell in the control group
One-Moore *et al.* (2016)[71]	Randomised, double-blind, crossover, controlled trial	23 men and women (mean age: 30 ± 3)	Healthy (BMI: 21.9 ± 0.4 kg m^{-2})	24.1 g or 48.2 g freeze-dried BB powder with moderately high-fat breakfast	N/A	Macro- and micronutrient matched placebo powder + moderately high-fat breakfast	Acute: samples at baseline and 3.5 h post-consumption	48.2 g blueberry reduced IL-6 compared to the placebo. 24.1 g blueberry decreased LPL-induced secretion of IL-1β compared with placebo
Riso *et al.* (2013)[58]	Randomised, single-blind, crossover, controlled trial	18 males (mean age: 48 ± 10)	BMI: 24.8 ± 2.6 kg m^{-2}. With at least one CVD risk factor (American Heart Association)	25 g freeze-dried wild BB powder + 250 ml water	375	250 ml water + fructose, glucose, citric acid, blueberry flavour and food colouring	Daily consumption: 6 weeks, Washout for crossover: 6 weeks	No change in RHI. Reduction in levels of oxidised DNA and increased resistance to oxidatively induced DNA damage

Table 2 *(Contd.)*

Reference	Study design	Study subjects	Health status of volunteers	Blueberry treatment	ACN (mg for dose)	Control	Duration	Significant findings
Rodriguez-Mateos *et al.* (2019)[25]	Randomised, double-blind, crossover, controlled trial	5 males (mean age: 23 ± 3)	Healthy (BMI: 24 ± 3 kg m^{-2})	Freeze-dried BB powder 11 g (+ water) or pure anthocyanins (160 mg)	150	3 control drinks: a) fructose, (b) fructose + fibre, (c) macro- and micronutrient matched control	1, 2 and 6 h post consumption	Blueberry and anthocyanin treatments increased FMD by 2–3%
Rodriguez-Mateos *et al.* (2019)[25]	Randomised, double-blind, parallel controlled trial	40 males (mean age: 33 ± 6)	Healthy (BMI: 24 ± 3 kg m^{-2})	Freeze-dried BB powder 11 g (+water)	150	Macro- and micronutrient matched control drink (11 g powder)	Twice daily consumption: 4 weeks and baseline *vs.* 2 h at each visit	Blueberry increased FMD by 1.5% after 2 h and by 2.3% after 4 weeks consumption, 24 h SBP reduced by −5.6 mmHg
Rodriguez-Mateos *et al.* (2014)[57]	Randomised, double-blind, crossover, controlled trial	10 males (mean age 27 ± 1)	Healthy (BMI 25 ± 0.8)	Blueberry containing baked product (containing 34 g freeze-dried BB), or 34 g freeze-dried BB	196 mg in the baked product and 339 mg in the unprocessed BB powder	Control matched to baked product	1, 2 4- and 6-hours post-consumption	FMD ↑ at 1, 2 and 6 h (maximum ↑ at 2 h of 2.6%) in the blueberry baked product. The findings were similar in the BB powder, but the FMD ↑ was maximum at 1 h rather than 2. BP was not affected.
Rodriguez-Mateos *et al.* (2013)[53]	Randomised, double-blind, crossover, controlled trial	10 males (mean age: 27 ± 1).	Healthy (BMI: 25 ± 0.8 kg m^{-2})	Freeze-dried BB powder 34, 57 and 80 g (+water).	310, 517 and 724	Macro- and micronutrient matched control drink (to the 57 g dose)	Acute: 24 hours (measured at 0, 1, 2, 4, 6 and 24 h)	FMD increased after 34–80 g blueberry at 1–2 h and 6 h post-consumption. Phenolic metabolites also peaked at 1–2 h and 6 h and neutrophil NADPH oxidase activity decreased
Rodriguez-Mateos *et al.* (2013)[53]	Randomised, double-blind, crossover, controlled trial	11 males (mean age: 27 ± 1)	Healthy (BMI: 22 ± 0.9 kg m^{-2})	Freeze-dried BB powder 14, 28, 34, 57 and 80 g (+water).	129, 258, 310, 517 and 724	Macro- and micronutrient matched control drink (to the 57 g BB dose)	Acute: 1 h	FMD increased (at 1 h) dose dependently up to 34 g blueberry powder then reached a plateau at higher intakes
Stote *et al.* (2017)[50]	Randomised, single-blind, crossover, controlled trial	20 women (mean age: 53 ± 6)	Two risk factors for type 2 diabetes (Canadian Diabetes Association)	240 ml BB juice	314	240 ml placebo: Water, colouring, flavours, sugar	Daily consumption for 1 week	Nitric oxide levels increased in the blueberry group compared to placebo
Stull *et al.* (2010)[68]	Randomised double-blind, parallel, controlled trial	32 men and women (mean age: blueberry group 54 ± 3; control 49 ± 3)	Obese (BMI: 32–45 kg m^{-2}), non-diabetic insulin-resistant	22.5 g freeze-dried BB + light yogurt, skim milk, vanilla flavour and Splenda	334	Light yogurt, skim milk, fibre, sugar, blueberry flavour, red and blue colour.	Twice daily consumption: 6 weeks	Improved insulin sensitivity (hyperinsulinemic-euglycemic clamp) in blueberry group
Stull *et al.* (2015)[55]	Randomised, double-blind, parallel controlled trial	44 men and women (mean age: blueberry group 55 ± 2, placebo group 59 ± 2)	Metabolic syndrome (defined by WHO)	22.5 g freeze dried BB powder + vanilla flavour, light yogurt, skim milk and water	290	Fat-free vanilla yogurt, skim milk, sugar, fibre water and colours	Twice daily consumption: 6 weeks	RHI improved in the blueberry group after adjusting for body fat % and gender

women with metabolic syndrome.[55] Another RCT found that 300 g blueberries (309 mg TP) counteracted the reduction in RHI after smoking a cigarette compared with the control group using young healthy smokers.[56] A similar study in young smokers and non-smokers with low RHI scores (<1.67), found that 300 g of blueberries (309 mg TP) increased RHI 2 h post-consumption in both groups compared with the controls.[52] However, one study investigating 25 g freeze-dried blueberry powder (375 mg anthocyanins) in 18 males, found no effect on RHI 6-weeks post-consumption.[58] Participants in this study had one or more CVD risk factors, such as pre-hypertension (systolic blood pressure and diastolic blood pressure of 120–139 and 80–89 mmHg respectively), high total cholesterol ($\geq$5.17 mmol L^{-1}), low HDL-cholesterol ($\leq$1.09 mmol L^{-1}), high LDL-cholesterol ($\geq$3.36 mmol L^{-1}), overweight (BMI 25–30 kg m^{-2}) and smoking (<10 cigarettes per day).[58] The authors concluded that the lack of results may be due to inter-individual differences in both CVD risk factors and ADME of blueberry (poly)phenols. Another study showing no effect on RHI was conducted in 19 women, with at least 2 risk factors for developing type-2 diabetes.[50] Participants consumed a blueberry juice, containing 314 mg anthocyanins, or a placebo, for one-week in a cross-over RCT with an 8-day wash-out period. In addition, a study investigating the effects of blueberries on endothelial function, in 10 healthy male subjects, found no effect on RHI at 1, 2 and 24 h following 300 g (fresh weight) of blueberry consumption.[51] In summary, 9 RCTs have reported acute and chronic improvements in endothelial function following consumption of 100 to 560 g of blueberries in young healthy individuals as well as individuals with metabolic syndrome and smokers. In contrast, 3 studies, conducted with endo-PAT, did not find any significant differences.

Effects on blood pressure

Arterial hypertension is one of the most important independent risk factors of CVD.[59,60] A recent meta-analysis of 123 studies, with 613 815 participants, concluded that a 10 mmHg reduction in systolic blood pressure (SBP) reduced the risk of major CVD events by 20%.[61] However, the ability of blueberry (poly)phenols to improve blood pressure is less clear as compared to FMD. A meta-analysis of 6 RCTs, investigating the effects of blueberries on blood pressure, with durations ranging from 6–8 weeks, found no significant changes in SBP or diastolic blood pressure (DBP).[7] Johnson *et al.* (2015) conducted a well-controlled RCT investigating the effects of 22 g freeze-dried blueberry powder (469 mg TP) after 8-weeks daily consumption in pre- and stage-1 hypertensive, postmenopausal women.[62] The authors reported a significant reduction in SPB and DBP by 7 mmHg and 5 mmHg, respectively. A more recent RCT investigating daily blueberry consumption (20 g of freeze dried, 200 g fresh) for 4-weeks on ambulatory (24 h) blood pressure in healthy males, found a significant reduction in SBP of 5.6 mmHg, but no significant reduction in DBP.[25] In a single blinded RCT, 8-weeks daily consumption of a freeze-dried blueberry drink (742 mg TP) compared with a control (water), led to a decrease in SBP and DBP of 7.8 mmHg and 2.5 mmHg in men and women with metabolic syndrome.[63]

One study found no significant changes in blood pressure in healthy young smokers following daily consumption of 250 g fresh blueberries for 3-weeks, compared to a control group who had no intervention.[64] Participants were also asked to follow a specific diet which prohibited large amounts of fruit and vegetables. The lack of significant findings may have been due to a short timeframe of the intervention, or possibly due to decreasing the participant's habitual (poly)phenol intake during the study, which may have masked any additional benefits of the blueberry treatment.[64] In addition, the authors did not measure the anthocyanin content of the blueberry, which given as fresh blueberries may not have remained as high as giving the treatment as a freeze-dried powder kept at low temperatures.

Many of the studies investigating the effects of blueberries on blood pressure are over short durations, ranging from acute studies to long-term studies lasting 1–8 weeks (Table 2). Furthermore, only one study investigating the effects of blueberries on blood pressure uses 24 h ambulatory blood pressure, which is considered the gold standard measurement. In summary, more longer-term RCTs using 24 h ambulatory blood pressure measurements are required, to confirm whether blueberry (poly)phenols can improve blood pressure following daily intake.

Effects on arterial stiffness

Arterial stiffness is a validated marker for future CVD risk. Techniques used to measure arterial stiffness include pulse wave velocity (PWV), particularly the carotid-femoral PWV, and augmentation index (AIx). Very few studies have investigated the effect of blueberry consumption on arterial stiffness. A parallel, 8-week blueberry intervention trial in pre- and stage-1 hypertensives, found that following daily blueberry consumption (469 mg TP), brachial-ankle PWV reduced when compared with the control but carotid-femoral PWV did not change.[55] Another study, in participants with metabolic syndrome, showed an improvement Aix (−2.24%) following daily consumption of blueberries (26 g freeze-dried blueberries) for 6-months.[54] However, more recent results showed no improvements in arterial stiffness following both acute and 4-week daily consumption of 22 g freeze-dried blueberries (150 mg anthocyanins) in 40 healthy individuals.[25] This could be due to a relatively short intervention period, and changes in arterial stiffness may take longer than 4-weeks to become apparent.

Effects on blood lipids

High plasma cholesterol, or hypercholesterolemia, is another well-established marker for future CVD risk. A previous meta-analysis of 16 RCTs assessed the effects of *Vaccinium* berries on blood lipids, of which 3 were on blueberries.[65] The authors found no significant effect on blood lipids when combining the blueberry RCTs. More recently, a RCT in 113 healthy men and women with mild-cognitive impairments (MCI) investigated the effects of consuming 35 g freeze-dried blueberry

powder daily for 6-months compared to a control.[66] No differences were found between treatment groups in plasma triglycerides, LDL-cholesterol, HDL-cholesterol, and total cholesterol levels at 3 or 6 months. The authors do not state the anthocyanin content of their treatment. Therefore, the amounts may have been too low to show any effects. A crossover RCT in healthy men and women investigated the effects of 150 g blueberries for 4-weeks combined with exercise, or a control with only exercise.[67] The authors reported a 0.13 mmol L^{-1} increase in HDL-cholesterol in the blueberry group. This study also failed to state the anthocyanin content of the blueberry treatment. A recent RCT conducted over 6-months in 115 participants with metabolic syndrome, showed an improvement in HDL-cholesterol following daily consumption of 26 g freeze-dried blueberry powder compared to placebo.[54] However, this was only seen in non-statin users (excluding n = 44) and was a reasonably small increase of +0.05 mmol L^{-1}. Overall, there is no firm evidence suggesting blueberry consumption can have an effect on the lipid profile, except for 2 RCTs reporting an improvement in HDL-cholesterol.

Effects on blood glucose and insulin sensitivity

Plasma glucose levels and insulin sensitivity are important parameters in identifying pre-diabetes or diabetes. Uncontrolled plasma glucose levels are a major risk factor for future CVD. A small number of studies have investigated the effects of berries on blood glucose and insulin sensitivity with many of these studies finding no effects, this seems to largely depend on the methods used to measure these outcomes.[1] A study in individuals with obesity and insulin resistance (but no diabetes), found a 22% improvement in insulin sensitivity, measured using a hyperinsulinemic-euglycemic clamp which is the gold standard method, following a 6-week daily intake of 22.5 g freeze-dried blueberry (1 cup fresh blueberries).[68] In addition, a recent crossover trial investigated the acute effects of high or low doses of freeze-dried blueberries (containing 310 mg or 724 mg TP) on plasma glucose in healthy young adults.[69] Plasma glucose was determined using a capillary sampling method at baseline and at regular intervals up to 2.5 h postprandial. In the blueberry treatments, postprandial glycaemic response was significantly extended compared to the sugar-matched controls. Therefore, blueberry (poly) phenols may have the potential to slow postprandial glucose response.[70]

No other studies measuring blood glucose or insulin sensitivity have found improvements following blueberry treatments.[50,55,63,67,71,72] The lack of findings on blood glucose or insulin levels following blueberry treatments may be largely due the use of alternative methods to the hyperinsulinemic-euglycemic clamp. For example, most studies use spot fasting

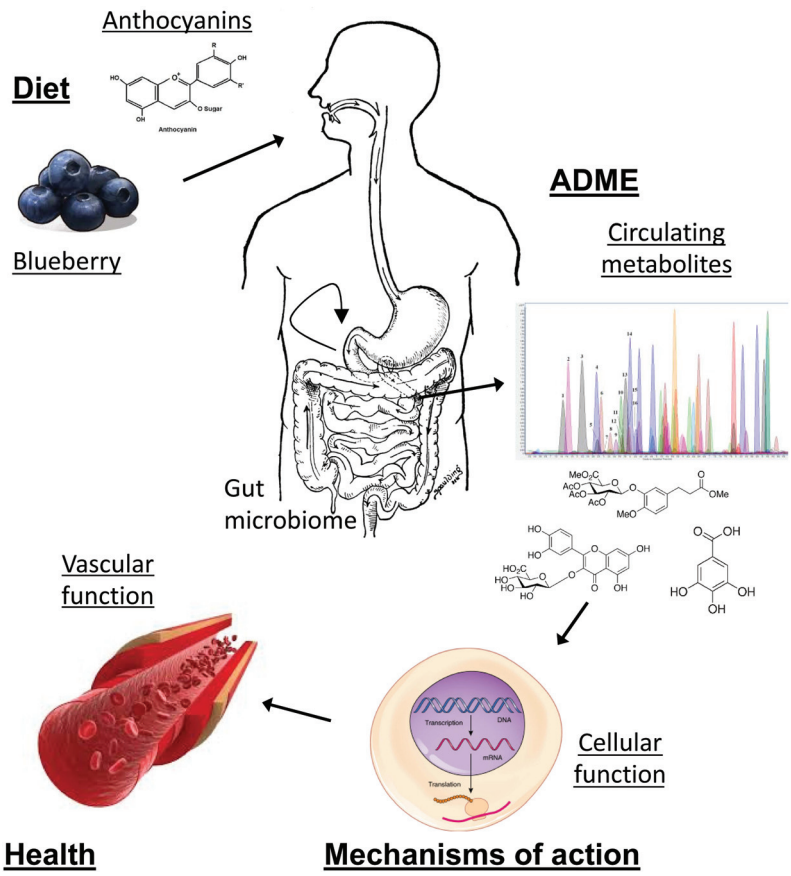

Fig. 3 Concept of how blueberries mediate vascular effects *via* modulation of cellular function by circulating anthocyanin metabolites.

glucose or insulin levels at baseline then at the end of the study. One study used a modified frequently sampled intravenous glucose tolerance test but found no effects following 6-weeks of blueberry consumption.[55] In addition, only one study measured HbA1c levels after 8-weeks blueberry consumption, in 44 individuals with metabolic syndrome, finding no significant difference.[63] This may be due to the short timeframe as HbA1c measures the average blood sugar levels over two to three months.

Mechanisms of action on vascular function

The mechanisms by which blueberries may positively affect vascular function are still largely unknown. Recent evidence, however, suggests that the vascular effects may be mediated by circulating phenolic metabolites derived from blueberry anthocyanins. The dose–response on FMD of isolated anthocyanins and anthocyanins consumed with blueberry are similar with almost identical ED50 values of 131 mg and 119 mg at 2 h after consumption, respectively.[25] Fourteen and 21 of the circulating phenolic metabolites in plasma correlated with FMD increases at 2 h and at 28 days consumption of wild blueberry (150 mg anthocyanins acutely, or 300 mg daily), respectively. Furthermore, data suggest that in humans acute FMD improvements go along with decreased neutrophil NADPH oxidase activity[53] and lead to differential expression (>1.2-fold) of 608 genes and 3 microRNAs, with Mir-181c showing a 13-fold increase in peripheral blood mononuclear cells.[25] The patterns of 13 metabolites were independent predictors of gene expression changes and pathway enrichment analysis revealed significantly modulated biological processes involved in cell adhesion, migration, immune response, and cell differentiation. Importantly, when these metabolite profiles as observed in humans after blueberry consumption were injected into mice targeting similar plasma concentrations FMD significantly increased.[25] This supports that circulating anthocyanin metabolome possesses biological activities and may be responsible for health effects ascribed to blueberry consumption. However, the biological activities and mechanisms of action of individual metabolites and their interaction *in vivo* are unknown (see recent review in ref. 19). Evaluating the bioactivities of metabolites alone and together, analysing their structure–activity relationship in well-designed and physiologically relevant experimental and human studies are needed to understand the mechanisms of how these metabolites affect vascular function (Fig. 3).

Conclusions

Present evidence from both epidemiological and human intervention studies for the cardiovascular health benefits of blueberries is promising. The beneficial effects of blueberries on endothelial function, measured using FMD, are particularly clear. Blueberries may also be beneficial for other surrogate markers of future CVD risk such as blood pressure and HDL-cholesterol. However, in current human intervention studies there is large heterogeneity between the study populations. For example, some studies use healthy individuals and others use individuals with metabolic syndrome or at high risk of CVD, which may be contributing to the differences in findings when investigating their effects on outcomes such as blood pressure. Differences in RCT durations may cause heterogeneity in other outcomes such as lipid profile and arterial stiffness. In addition, more studies are required investigating the effect of blueberries using gold standard techniques for measuring certain CVD risk markers, such as FMD, 24 h ambulatory blood pressure or the hyperinsulinemic-euglycemic clamp. Another factor to consider when conducting RCTs using blueberries is to ensure the intervention product is standardised, and storage is considered to reduce breakdown of unstable anthocyanins. There are very few studies addressing the ADME of blueberry (poly)phenols by measuring the subsequent metabolites found in the plasma, urine or faeces post-consumption, which is essential not only to monitor compliance but also to get mechanistic insights. The current evidence indicates that blueberry (poly)phenols have the potential to improve future cardiovascular health through various well-established surrogate markers.

Conflicts of interest

The authors have no conflicts of interest to declare.

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