Role of Berries on Vascular Function: A Systematic-Review of Human Intervention Studies

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ABSTRACT

Context Berries are a source of polyphenols with recognised health-promoting activities. ***Objective*** The aim of this systematic review is to provide evidence of short and long-term benefits of berries on vascular function. ***Data sources*** Human intervention studies were collected from PubMed and Scopus databases ***Data extraction*** After selection, 22 randomized-controlled trials were included and analyzed. Most of them were performed in healthy subjects or individuals with cardiovascular risk factors. ***Results*** The overall results seem to suggest a protective role of berries on vascular function even if dependent on time of exposure, type and dose of berry and biomarkers analysed. Flow mediated dilation and reactive hyperaemia index (markers of vascular reactivity) improved following short-term interventions, while pulse wave velocity and augmentation index (markers of arterial stiffness) only after medium-long term studies. ***Conclusions*** In conclusion, the current evidence suggests that berries, at physiological relevant doses, may have a role in the modulation of vascular function and stiffness. High-quality human intervention trials are encouraged in order to strengthen these findings and to better elucidate the mechanisms involved in such modulation.

Keywords: berries; (poly)phenols; endothelial function; vascular function; intervention studies; systematic review

INTRODUCTION

Berries represent a wide group of blue, purple or red small-sized and highly perishable fruits. Blueberry, cranberry, currant, raspberry and blackberry are the most common varieties of berries consumed around the world[1]. Berries are an important source of (poly)phenols, including anthocyanins (ACNs), proanthocyanidins, flavonols, flavones, flavan-3-ols, flavanones, isoflavones, stilbenes, lignans and phenolic acids[2,3].

Berry consumption has been associated with a reduced all-cause mortality[4]. Moreover, in the last few years numerous epidemiological and clinical studies documented the protective effects of berries against many non-communicable chronic diseases, with some focusing on cardiovascular diseases (CVDs)[5–7] which remain the leading causes of death worldwide[8]. The development of CVDs is often accompanied by a decline in vascular health and function. The endothelium represents an important part of the vasculature, by covering the inner surface of the blood vessels, and acting by controlling the flow of nutrients and non-nutrients, the passage of the fluids into the tissues, and the secretion of vasoactive substances, such as the vasodilator nitric oxide (NO) and vasoconstricting molecules like endothelin-1.

Common biomarkers for the evaluation of vascular health include blood pressure (BP), arterial stiffness and vascular reactivity. Vascular reactivity can be assessed through endothelium-dependent (i.e. acetylcholine) or independent (i.e. nitroglycerin) mechanisms[9,10]. The main methods for vascular reactivity assessment are flow-mediated dilation (FMD) and peripheral arterial tonometry/reactive hyperaemia index (PAT/RHI). FMD is considered the gold standard non-invasive ultrasound technique, measuring vasodilation at the level of the brachial artery following a standard occlusion[11,12]. EndoPAT is a plethysmographic technique able to measure pressure changes in the finger tips caused by a 5 min occlusion of the brachial artery[13]. Among other measures, arterial stiffness can be measured through pulse wave velocity (PWV), which directly measures point-to-point pulse wave transit time, and pulse wave analysis (PWA), which uses the pulsatile waveform shape to make assumptions about arterial haemodynamics. The stiffness can be also quantified

through the augmentation index (AIx), defined as the difference between the second and first systolic peak expressed as percentage of the pulse pressure[14].

Some systematic reviews and meta-analyses of observational and randomized controlled trials (RCT) reported a relationship between the consumption of polyphenols and polyphenol-rich foods and modulation of vascular function markers such as AIx, PWV, FMD and RHI[15–17]. Other direct and/or indirect biomarkers of vascular function include serum concentrations of inflammatory markers, adhesion molecules, lipids and lipoproteins, oxidized LDL-C, and clotting factors[18]. A meta-analysis of RCTs, performed by Huang *et al.*[19], has shown that berry consumption may significantly reduce the levels of LDL-C, BP, fasting glucose, and tumour necrosis factor-α (TNF-α) supporting their potential contribution on cardiovascular health.

Based on these premises, the aim of this systematic review is to summarize the research findings of RCTs investigating the effect of berry consumption on markers of vascular function, in order to elucidate their potential role in CV health. The current systematic review exclusively focuses on studies (both acute and chronic interventions) performed with berries and berry products, differentiating it from other recent works.

METHODS

A systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement[20], and included all relevant PRISMA checklist items. A review protocol has not been published and this review has not been registered with any systematic review database.

Eligibility Criteria (Inclusion and Exclusion Criteria)

Studies were included in the present review if they investigated the effect of berry consumption on one or more markers of vascular function in humans. The studies present in literature had to adhere to the following criteria to be considered in the review process: i) to be randomised-controlled trials

that provided results on both acute (i.e. single dose supplementation) or chronic berry consumption and ii) to provide a characterization of the berry polyphenolic content. Conversely, exclusion criteria were: i) the presence of a combination of berries with other foods (because the beneficial effect could not be attributed specifically to berries) and ii) the fact of being published in a language different from English and with no accessible translation. No restrictions for the characteristics of subjects (e.g. age, gender, health condition) were considered.

A more detailed list of criteria for eligibility in this systematic review has been summarized in Supplementary Table 1, by following the PICOS (Population, Intervention, Comparison, Outcome, Study design) format[21].

Search strategy and study selection

A systematic literature search was conducted using PubMed (http://www.ncbi.nlm.nih.gov/pubmed), and Scopus (http://www.scopus.com) databases on December 2017 (updated October 2018). For completeness, searches were augmented by screening the bibliographies of relevant review articles. The search had no limit ranges for year of publication. Three search themes were considered: terms related to berry (e.g. strawberry, blueberry, cranberry) were combined with terms related to outcomes (e.g. pulse wave capacity, flow mediated dilation, arterial stiffness) and population type (e.g. human, volunteers, patients) to identify all potentially relevant literature published (further information on the search strategy in Appendix S1). No language or other restrictions were set in the literature search. The identification process is illustrated in **Figure 1**.

Study selection and data collection process

Two reviewers (MM and DM) independently abstracted data from studies eligible for inclusion. Disagreement between reviewers was resolved through consultation with a third independent reviewer (CDB) to reach a consensus.

The following data were extracted from each study: name of first author, country, registered trial number, sample size at recruitment and enrolment stages, inclusion/exclusion criteria, study design, dietary products used during the interventions, and vascular function outcomes.

Risk of Bias in Individual Studies

Risk of bias in individual studies and across the studies was assessed independently by two review authors (DA and DM) following the criteria of the Cochrane Handbook for Systematic Reviews of Interventions 5.1.0[22]. The following parameters for each component's rating were considered to produce the resulting scores: 1) *Selection Bias.* Sequence generation and allocation concealment; 2) *Performance Bias.* Blinding of participants and personnel; 3) *Detection Bias.* Blinding of outcome assessment; 4) *Attrition Bias.* Incomplete outcome data; 5) *Reporting Bias.* Selective reporting; 6) *Other Bias.* All the scores were assessed as "Low risk of bias", "High risk of bias", or "Unclear risk of bias" if insufficient details about these parameters were reported in the study[22]. All disagreements were resolved by consensus with a third review authors (CDB).

Results

Study selection

The study selection process is shown in **Figure 1**. A total of 880 records were identified from the database search (PubMed and Scopus), while no additional papers were found by hand searching. After removing 179 duplicate articles, 701 studies were screened and 671 were discarded based on title and abstract. After the full-text reading of the remaining 30 eligible papers, a total of 9 records were further excluded were excluded because i) the study had no placebo/control food (n= 3), ii) berries were provided along with other food components (n= 1), iii) the fed products were not fully characterized for their (poly)phenol content (n= 5). At the end of the selection process, 22 RCTs were considered for qualitative analysis.

Study characteristics

The main characteristics of the 22 included studies are reported in Tables 1 and 2. Out of these 22 works, 11 dealt with acute interventions[23–33]and 9 with chronic interventions[34–42], while 2 studies[43,44] investigated the effect of both acute and chronic berry consumption.

The current review summarizes the main findings obtained evaluating the effect of different berries (mainly blueberries, cranberries, strawberries and blackcurrants) on vascular function. The berries were provided as raw fruits, drinks/smoothies, or extracts in capsules. The (poly)phenol content was dependent on the type of berry and on the administered portion (250-300 g for raw fruits, 250-1000 mL/day for drink/smoothie and 600 mg for capsules). The main outcome variables measured were FMD and RHI for the vascular reactivity, and PWV and AIx for the arterial stiffness.

Risk of bias of the studies

Risks of bias within individual studies and across the studies are shown in **Supplementary Figure 1** and **Supplementary Figure 2**, respectively. Results showed the blinding of participants and personnel (performance bias) and blinding of outcome assessment (detection bias) to represent the highest risks of bias.

Acute studies

Table 1 reports the main results obtained in 13 short-term studies[23-33,43,44] performed with berries on FMD (n=5), RHI (n=5) and other markers (n=3) of vascular function. Alqurashi and colleagues[23] have shown that the intake of 200 g of açai smoothie significantly increased FMD at 2 h (+1.4%; p=0.001) and at 6 h (0.8%; p<0.001) post-consumption in healthy overweight men. Similarly, Rodriguez-Mateos *et al.*[32], found that the consumption of 3 pieces/buns of blueberry baked products and/or a blueberry drink (equivalent to 240 g of fresh blueberry) increased FMD after 1, 2, and 6 h from the intake. A significant improvement in FMD occurred after the consumption of the two items, at 1 h for the drink and 2 h for the baked products (up to +2.6%). In another study, the same authors reported that post-acute consumption of 5 cranberry juices (450 mL each) containing different

amounts of (poly)phenols (409, 787, 1238, 1534, and 1910 mg), significantly augmented FMD 1, 2, 4, 6, and 8 h from the intervention[33]. FMD gradually increased in a time- (spiking at 4 h) and dose-dependent manner, with maximum effect after the intake of 1238 mg total polyphenols (about +2.5%) While, Istas *et al.*[29] showed that the intake of two different portions (200 and 400 g) of red raspberries (containing 201 or 403 mg of total polyphenols, respectively) improved FMD at 2 h (+1.6% and +1.2%, respectively) and 24h (+1.0% and +0.7%, respectively) in a group of 10 healthy subjects, so not finding a dose-response relationship.

Regarding RHI, 3 studies reported a significant increase in this outcome measure. Del Bo' *et al.*[27] found that 300 g of blueberries counteracted an impairment in RHI (−4.4 ± 0.8%, $p<0.01$) and improved Framingham (f) RHI (fRHI, +28.3 ± 19.2%, $p<0.0001$) in a group of healthy smokers with normal endothelial function (2 h post consumption). In another study, the authors documented that the same 300 g blueberry portion increased RHI values in smokers (+35.2 ± 7.5%, $p=0.02$) and in non-smokers (+54.8 ± 8.4%, $p=0.01$) with endothelial dysfunction[26]. Finally, Flammer *et al.*[44] observed a significant increase ($p=0.01$) in RHI (from 1.7 ± 0.4 to 2.0 ± 0.6; about +18%) at 1 h after cranberry juice consumption (2x 230 mL) in subjects with peripheral endothelial dysfunction and cardiovascular risk factors.

Conversely, two studies did not report significant effects following short-term interventions with berries. Del Bo' *et al.*[28] showed that a portion of blueberry purée (300g) did not affect vascular reactivity, measured as RHI, after 1 h from the intake in a group of young healthy volunteers with normal peripheral arterial function (RHI>1.67) A similar result was also documented by Jin and colleagues[30], following the intake of 250 mL of blackcurrant juice (20%), in a group of healthy subjects. The investigators measured vascular reactivity by laser Doppler imaging in response to acetylcholine (endothelial dependent) and sodium nitroprusside (endothelial independent), testing the effect after 2 h from the intake of the juice[30].

Regarding PWV and AIx, the studies included in this systematic review failed to observe any significant effect on these markers following berry intervention[25–27,31,33].

Chronic studies

Table 2 shows the results obtained in 11 medium-long term interventions[34-44] providing results on the effect of berries on markers of vascular function. Khan *et al.*[38]showed a significant increase (p=0.022) in FMD (from 5.8±3.1 to 6.9±3.1%; about +19%) after 6-week consumption of blackcurrant juice (1 L/day, providing 815 mg of total polyphenols) in healthy subjects. Similarly, Stull *et al.*[41]reported a significant improvement in RHI after a 6-week intake of two blueberry smoothies (45 g freeze-dried blueberry powder, providing about 800 mg total polyphenol) in subjects with metabolic syndrome. The results showed a greater effect of blueberry *versus* placebo (0.32 ± 0.13 *versus* −0.33 ± 0.14, respectively; p = 0.0023). On the other hand, 4 studies reported no significant effect on RHI or FMD[35,39,43,44]. Djurica *et al.*[43] found that 1-week consumption of 50 g freeze-dried strawberry powder (equivalent to 500 g of fresh strawberries, with pelargonidin-3-glucoside as main phenolic compound) did not improve RHI in overweight/obese adolescents. Flammer *et al.*[44] showed that the intake of cranberry juice (2 × 230 mL/day) over 4 months had no effect on RHI in subjects with peripheral endothelial dysfunction and cardiovascular risk factors. Similarly, Riso *et al.*[39] could not demonstrate an effect on RHI after a 6-week intervention with a wild blueberry drink (250 mL/day, providing 475 mg of anthocyanins) in subjects with cardiovascular risk factors. These results were consistent with Dohadwala *et al.*[35] in which a 4-week cranberry supplementation (480 mL/day, providing 94 mg ACNs and 835 mg total polyphenols) did not affect FMD in subjects with coronary artery disease.

With respect to arterial stiffness, 6 out of 7 studies showed a positive modulation of PWV and AIx following berry intervention. Feresin *et al.*[36] reported that the intake of 240 mL of a strawberry drink (providing 25 g/day of freeze-dried powder, equivalent to approximately 1.5 cups of fresh strawberries) for 8 weeks significantly decreased brachial-artery pulse wave velocity (baPWV) and femoral-artery pulse wave velocity (faPWV) in pre- and stage 1-hypertensive postmenopausal women (−0.73 m s^{-1}, p = 0.03 and −0.55 m s^{-1}, p = 0.02, respectively). Similarly, Johnson *et al.*[37]

(2015) observed a significant reduction in baPWV (from 1,498±179 cm/sec to 1,401±122 cm/sec; at about 6.5%; $p<0.05$) following 8-week consumption of a blueberry drink (providing 22 g/day of freeze-dried blueberry powder) in a comparable population. Dohadwala *et al.*[35] documented a significant reduction in carotid-femoral pulse wave velocity (crPWV; from 8.3 ± 2.3 m/s to 7.8 ±2.2 m/s; at about -6%; P=0.003), but not carotid-radial pulse wave velocity (cfPWV), after a 4-week intake of cranberry juice (480 mL/day) in subjects with coronary artery diseases. Significant findings were also documented for total peripheral resistance (TPR) and AIx. For example, 1-week consumption of New Zealand blackcurrant extract (600 mg/day) reduced TPR (-16%; $p<0.05$) in healthy males, both at rest and during exercise performance[34]. Similar results were also observed following 1-week intake of 6 g/day of New Zealand blackcurrant powder in well trained endurance athletes (TPR, -25%; P=0.003)[42]. Ruel *et al.*[40] observed a decrease in AIx (−10.8% ± 6.4%; $p<0.0001$) following 4-week intervention with cranberry juice (500 mL/day, providing 400 mg of total polyphenols) in obese men, while Riso *et al.*[39] reported no significant effect on AIx after 6-week intervention with wild blueberry drink (250 mL/day, providing 475 mg of anthocyanins) in subjects with cardiovascular risk factors.

Potential risks of bias

The highest risks of bias concerned blinding of participants as well as the blinding of the outcome assessment during the conduction of RCTs. For the latter, very few studies declared the non-blindness, while in most studies personnel blinding was unstated. Allocation has been particularly considered and described in the various studies included in this review. There were high risks of bias when it was impossible to render the control undistinguishable from the berry product. Despite being randomized controlled trials, the randomization processes have been scarcely described in the papers, mainly due to poor or absent explanation of how the randomization was produced. However, it is worth to note that the blindness of the randomization process in dietary interventions is sometimes

248 difficult to achieve, due to the risk of an unbalanced allocation with consequent impact on data
249 reliability.
250
251 **Discussion**
252 There is a clear interest in the exploitation of berries and derived products for their potential role in
253 cardiovascular health, with a specific focus on vascular function. RCTs are considered the gold
254 standard for ascertaining a causal relationship between intervention and effect of a treatment. The
255 effect of polyphenol-rich foods in the modulation of vascular reactivity has been evaluated in several
256 intervention studies but few systematic reviews and meta-analyses summarized their effects. In some
257 cases, the effects were inconsistent for the measured markers of vascular function[45–47]. This
258 discrepancy could be due to the inclusion of studies having heterogeneous characteristics and
259 reporting high risk of bias. Moreover, most of the studies were focused on bioactives and bioactive-
260 rich foods in general, making the specific effects of berries very difficult to be identified. Conversely,
261 the present review exclusively considered studies performed with berries and berry products, selected
262 on the basis of quality criteria, and included only RCTs performed either in acute and chronic
263 interventions. The complete picture obtained through this work points at an improvement in FMD
264 and RHI (markers of vascular reactivity) following acute berry interventions. Some of the studies
265 linked the observed effects to the increase in plasma circulating levels of berry bioactive
266 constituents[27] while others by their circulating gut/liver phenolic metabolites. [29]
267 Only one study showed a dose-response relationship between the intake of berries and vascular
268 reactivity[33] while 4 studies did not report significant effects following berry consumption[24,28,30,43].
269 These results may be due to the characteristics of the studied population (i.e. healthy individuals
270 without specific risk factors and with normal endothelial function at basal levels). Moreover, matrix
271 effect, potentially reducing the availability and/or impact of berry bioactives, small portions (even if
272 more realistic) of berries, and time of evaluation/measurement of vascular function due to the rapid

clearance rate and poor absorbance of polyphenols may all have been diluting factors in the framework of the final evidence.

An additional source of variability among acute studies can be related to the study protocol adopted and the characteristics of the test meals. Some studies provided berries and/or berry products (whole fruits or drink) alone or within/together with a high-fat[23,31] or a high-carbohydrate meal[24], and it is recognized that foods and food matrix may positively or negatively affect polyphenol bioavailability[48]. Moreover, the consumption of high-fat and/or high-carbohydrate meals may transiently increase post-prandial triglycerides and glycaemia with a negative effect on endothelial function[49]. These important variables/aspects could have affected the results obtained in the studies.

Regarding the effects observed in medium-long term interventions, no clear favourable effects of berry products on vascular reactivity markers have been found, in line with the systematic review of Heneghan and coworkers[50] that showed an effect only in 3 out of 7 studies. The discrepancy between short and long-term studies in terms of vascular reactivity (RHI and FMD) is intriguing and may be attributed to the complexity of the mechanisms involved in the maintenance of the vascular system function. For example, Dohadwala and coworkers[35] reported that changes in nitric oxide mediated vascular reactivity can occur rapidly following a dietary intervention and this has been related to the "acute" absorption of food bioactives and/or their metabolites (e.g. able to directly/indirectly affect nitric oxide production). This underlines that necessarily critical factors affecting the evaluation of vascular function include the experimental design (e.g. in terms of timing of measurements), the targeted mechanism (e.g. nitric oxide production), and also the characteristics of the markers used to evaluate vascular reactivity. In fact, the markers available may provide different information depending on the study protocols (acute *vs.* chronic intervention). For example, short term studies can provide information on the direct modulatory effect of the absorbed bioactives (i.e. supporting biological plausibility) conversely, in the long term approach generally the exposure to the food bioactives is absent or limited (due to the active and rapid clearance of phenolic compounds even when consumed regularly). In this context, is not surprising the lack of effect underlined in chronic

299 studies where the measurement is performed about 12 h after the last intake of the bioactives.
300 Moreover, the type of markers used may affect the results depending on the actual targeted
301 measurement.
302 Also the large heterogeneity of the enrolled groups of volunteers among the different RCTs (i.e.
303 healthy subjects, individuals with cardiovascular risk factors or complications), also in terms of
304 vascular function levels, could have affected the results obtained. Moreover, it cannot be excluded
305 that the duration of the intervention was insufficient to exert a beneficial effect involving these
306 specific target groups of population. An additional source of variability can be related to the form and
307 the way through which berries have been provided. Some studies provided berries as raw fruit, others
308 as a beverage, a smoothie, a sweet cake (i.e. muffins), alone or in combination with a meal. Moreover,
309 berries could have been provided in addition to the habitual diet (resulting in an increased energy
310 intake) or as substitutes of other foods normally consumed that are thus being displaced from the diet
311 (isocaloric condition). The lack of the food that has been replaced may be important in determining
312 the final effect on vascular function, although it is quite difficult to ascertain the magnitude.
313 Moreover, the differences in berry administration may have played an important role in the results
314 obtained, since the quality of a meal in terms of energy, macro and micronutrients intake may affect
315 the vascular response. Also, considering that polyphenol intake may represent a confounding factor,
316 subjects were often asked to maintain their usual diet and to refrain from the consumption of berries
317 and other foods throughout the study period. Despite this, only few studies provided data about the
318 actual dietary intake[34,41] and the energy intake during the intervention and between treatments was
319 rather constant. Conversely, no information about the actual intake of polyphenols was provided.
320 Moreover, it is worth noting that the synergistic effects of other coexisting substances in berry foods
321 such as vitamin C[51], fiber[52], potassium[53] and magnesium[54] may play a role in determining the
322 improvement on vascular function.
323 Arterial stiffness has been recognized as a determinant of pulse pressure and elasticity of the
324 blood vessels. The loss of elasticity of the artery walls reduces its compensatory ability to absorb the

pulsatile energy and the wave propagation effects that influence peripheral wave reflection. This inability for compensatory response results in a gradual increase in blood pressure with age, leading to the development of isolated systolic hypertension and cardiovascular risk. Numerous intervention and observational studies have examined the relationship between polyphenols/polyphenol-rich foods and arterial stiffness. In a cross-sectional study, Jennings and colleagues[15] showed that high intake of anthocyanins and flavones were inversely associated with low arterial stiffness (measured as PWV) across extreme quintiles of intake in women. Successively, Lilamand and colleagues[17] assessed the relationship between flavonoids intake and arterial stiffness, measured as PWV, analysing 16 intervention and 2 cross-sectional studies. Four intervention trials reported a significant decrease of arterial stiffness after a flavonoid-based intervention, while the observational studies showed a significant association between high flavonoid consumption and low arterial stiffness. A recent systematic review and meta-analysis of RCTs showed an improvement in arterial stiffness following anthocyanin supplementation[47]. The effects were more evident on PWV after acute intake, while the results on AIx were not univocal following both acute and chronic interventions. In the present systematic review, we documented that short-term interventions with berries failed to modulate PWV and AIx, as well as stiffness and reflation indexes (Table 1), in line, at least in part, with those previous observations. Conversely, medium-long term interventions suggest an improvement of these markers (Table 2), in accordance with results reported in the review of RCTs by Heneghan and coworkers[50]. A potential explanation of the different findings between short and medium/long term trials may be attributed to the type of subjects enrolled and to the duration of the treatment. In fact, the short-term interventions were performed in healthy subjects, and it is plausible that substantial variations in arterial stiffness over a short follow-up are unlikely to be observed in individuals without vascular dysfunction. In addition, also the type of marker analyzed (e.g. PWV *versus* AIx) and its high variability among subjects could have played a crucial role in the obtained results. It is noteworthy that most of the studies did not consider arterial stiffness as a primary outcome, and that the trials

were mostly underpowered for arterial stiffness evaluation. For this very reason, future studies should be specifically designed to ascertain the effect of berries on this specific marker.

Strengths and limitations of the study

Caution should be used when interpreting results or drawing conclusions on vascular effect of berries due to the high heterogeneity among studies in terms of type and dose of berries, administration source (i.e. whole fruit, juice drink or capsules), amount of provided polyphenols, and their bioavailability. Although the inclusion of different types of berries may represent a strength, the different composition of berries in terms of the type and quantity of phenolic compounds may be among the most important factors influencing the *in vivo* effects of berries on vascular function. It is well known that berry fruits contain different anthocyanin profiles[55,56] and, for this reason, it is not always easy to compare study results because most of them differ for the referring standard compounds in anthocyanin intake, *i.e.* cyanidin- and peonidin-derivatives as major ACNs in cranberries, while pelargonidin predominates in strawberries[6]. Another weakness was the lack of good quality information about the bioavailability of anthocyanins and related metabolic products. Actually, after ingestion, anthocyanins metabolic fate is deeply influenced by pH and gut microbiota activities[57]. It is well ascertained that, similarly to other phenolic compounds, anthocyanins have a limited bioavailability, lower than 15%[58]. This is influenced by their interaction with several gut microbial strains and the subsequent phase II metabolism at enterocyte and hepatocyte level, leading to the production of several different metabolites, including phenylpropionic and phenylacetic acids[58–60]. A very limited number of studies provided information about the circulating amount of these metabolites in *in vivo* berry-related studies[61]. Moreover, it is not always easy to link the biological effects of berry consumption to anthocyanin gut microbial derivatives, as they are also the results of the degradation metabolism of several other phenolic compounds[62].

Other critical aspects are represented by the study design (acute *versus* chronic intervention and parallel *versus* crossover design), the duration of the intervention, subjects' characteristics, and sample size. Most of the studies were performed on healthy subjects, so that the inclusion in the

analysis of trials involving volunteers with risk factors or diseases may have increased the heterogeneity of the results, making it difficult to draw any unequivocal conclusion. Moreover, some studies, despite being sufficiently powered, randomized and controlled, were performed in small groups of subjects and, for this reason, the results obtained have to be considered as preliminary and deserve further investigations.

The use of different methods and the lack of standardized procedures and gold standard methodologies for the assessment of vascular function outcomes could be another potential critical point. For example, positioning of the cuff (upper *versus* lower arm), duration of brachial artery occlusion, and timing for the detection of peak hyperaemia still differ among investigators. This information is missing in the papers analysed, but it is clear that the different experimental conditions adopted may have had a role on the modulation of nitric oxide dependent and independent vasodilation mechanisms[63], and affect the results obtained.

Finally, it is important to underline that the search strategy applied in this systematic review excluded other direct and indirect markers of vascular function (e.g. circulating adhesion molecules, cytokines, interleukins, and blood pressure), which, in some studies, have been used to improve the understanding of the obtained results.

Potential mechanisms of action involved in the modulation of vascular function

One of the main hypothesized mechanisms of action of polyphenols consists of the activation of endothelial nitric oxide synthase (eNOS)/NO/cyclic guanosine mono phosphate (cGMP) signalling pathway involved in vasodilation. Once activated, NO stimulates soluble guanylate cyclase in the vascular smooth muscle cells by releasing cGMP, a second messenger, which induces the smooth muscle cells of the vessel to relax[64,65]. In addition, polyphenols have been shown to increase post-prandial release of the active glucagon-like peptide 1, a major intestinal hormone that stimulates glucose-induced insulin secretion from β cells, upregulate endothelial nitric oxide synthase

401 expression and increase endothelial nitric oxide synthase phosphorylation, resulting in improved
402 production of NO and thus endothelium-dependent relaxations[66,67].
403 Beside polyphenols, also insulin response may positively affect vascular function, since the binding
404 to its receptor on endothelial cells seems to activate the eNOS pathway and, thus, the vasodilation
405 process[68]. These processes are usually very fast and have been identified as potential mechanisms of
406 action in the short-term studies.
407 Other putative mechanisms through which polyphenols may affect vasodilation, in the short and
408 medium-long term interventions, involved the regulation of vascular redox signalling. In this regard,
409 berry components may activate nuclear factor E2-related factor 2-antioxidant/xenobiotic response
410 element signalling pathway, which represents the major mechanism in cellular defence against
411 oxidative and electrophilic stress[64]. Furthermore, polyphenols may modulate pro-inflammatory
412 pathways by inhibiting reactive oxygen species and the redox-sensitive transcription of nuclear
413 factor-kappa B, involved in gene expression of several pro-inflammatory cytokines, chemokines,
414 adhesion molecules, inducible nitric oxide synthase, cyclooxygenase 2 and cytosolic phospholipase
415 2, all playing an important role in the regulation of NO production and modulation of vascular
416 function[69].
417
418
419 **CONCLUSIONS**
420 In conclusion, despite the numerous limitations and confounding factors present in the reviewed
421 studies, the overall results of this systematic review seem to suggest a potential positive effect of
422 berries in the modulation of vascular function. In particular, the effects were observed for FMD and
423 RHI in short-term studies and for PWV and AIx in medium-long term ones suggesting that differences
424 in biomarker modulation may depend on the time of exposure to the dietary interventions and/or to
425 the experimental protocol of the study. Future research using appropriate study designs that consider
426 current knowledge gaps and combine the use of different biomarkers are consequently highly

recommended. Further RCTs in different and well characterized target groups of volunteers should be performed in order to strengthen the evidence on the efficacy of such treatments on vascular health and function, and perhaps to shed more light on the mechanisms underneath these effects. In this regards, studies on the structure-activity relationship of berry-polyphenols and/or their metabolic products could help understanding the potential mechanisms through which these compounds interact and positively affect the vascular system. Despite very difficult to estimate, most of the studies have shown an improvement of vascular function for doses of berries higher than 200 g (providing at least 600-700 mg of total polyphenols). This data should be considered indicative and dose- and time-dependent studies would be desirable to better identify the portion of berries (and related polyphenol amount) eliciting a beneficial effect on vasodilation. This information could be useful for the development of new products with vasoactive properties and possibly able to maintain vascular health and reduce the incidence of CVDs, also depending on identified target groups.

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Authors' contribution

DM, DA wrote the first draft of the manuscript. MM with DM made the literature search, reviewed the abstracts of the studies selected, and prepared the tables. CDB acted as a third independent reviewer and improved the manuscript. DDR, PR and MP critically revised the scientific contents and improved the quality of the manuscript.

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Declaration of interest

The authors report no conflicts of interest arising from the present research and its publication.

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FIGURE CAPTIONS

Figure 1. Flowchart of the study selection process

Supplementary Figure 1. Risk of bias summary: review authors' judgments about each risk of bias item for each included study**.**

Supplementary Figure 2. Risk of bias graph: review authors' judgments about each risk of bias item presented as percentages across all included studies.

664 **Table 1.** Characteristics of the study design, population, products, and outcomes of the considered acute intervention studies investigating the effect

665 of berry consumption on one or more markers of endothelial function.

REF.	STUDY DESIGN	STUDY POPULATION	BERRY INTERVENTION	CONTROL/ PLACEBO INTERVENTION	OUTCOME VARIABLES	MAIN FINDINGS
Alqurashi *et al*. (2016)[23], UK	Randomized, crossover, controlled, double-blind	n=23 healthy nonsmoker males (mean age 46 ± 1.9 y; mean BMI 27.6 ± 0.4 kg/m^2)	200 g of açai smoothie (AS, 150 g acai pulp + 50 g banana) Composition per serving: Total Polyphenols 694 mg (493 mg ACN, 173.6 mg GA, 9.6 mg quercetin, 9.3 mg CGA); Total carotenoids: 179.3 mg	200 mg control smoothie (CS) (50 g banana matched for fat) Composition per serving: Total phenolics <10 mg; Total carotenoids: 0 mg;	FMD up to 6 h after consumption	↑FMD at 2 h and 6 h after AS, but not CS consumption
Castro-Acosta *et al*. (2016)[24], UK	Randomized, crossover, controlled, double-blind	n=22 subjects (13 M, mean age 45.4±13.7y, mean BMI 25.5±3.8 kg/m2)	200 mL of three different blackcurrant drinks Composition per 200 mL: Total phenolics: 460, 810 and 1596 mg, respectively (total ACN 131, 322, 599 mg); vitamin C <0.5 mg	Placebo drinks matched for astringency by adding tannins Composition per 200 mL: Total phenolics 207 mg (total ACN 46 mg); vitamin C <0.5 mg	DVP-SI, DVP-RI up to 2h after consumption	=DVP-SI and DVP-RI compared to baseline for all blackcurrant and placebo drinks
Del Bo' *et al*. (2017)[26], Italy	Randomized, crossover, controlled	Study 1: n=12 nonsmokers males with peripheral arterial dysfunction (mean age 24.2±1.2y; mean BMI 22.5±1.2 kg/m^2) Study 2: n=12 smoker males, mean age 24.5±1.9y, mean BMI 22.9±1.1kg/m^2)	Study 1: 300 g of thawed blueberry (BB) Study 2: blueberry treatment + smoking (BS) Composition per serving: Total phenolics 856 mg (309 mg ACN, 30 mg CGA); Vitamin C: 2.4 mg	Study 1: 300 mL of water matched for sugar (C) Study 2: Smoking (S); Smoking + Control treatment (SC) Composition: n.d.	RHI, dAIx, dAIx@75 2h after consumption	Study 1: ↑RHI; =dAIx and dAIx@75 Study 2: ↑RHI; =dAIx and dAIx@75
Del Bo' *et al*. (2014)[27], Italy	Randomized, cross-over, controlled	n=16 healthy male smokers (mean age 23.6±0.7y, mean BMI 23.0±0.5kg/m^2)	300 g BB + smoking Composition per 100 g: Total phenolics 242.4 mg (116.1 mg ACN, 30.1 mg CGA), Vitamin C 0.8 mg	1) smoking 2) 300 mL of water with sugar + smoking Composition per 100 g: Total phenolics: 0 mg; vitamin C 0 mg	RHI, fRHI, dAIx, dAIx@75 2h after consumption	↑RHI and fRHI; =dAIx and dAIx@75

Del Bo' *et al*. (2013)[28], Italy	Randomized, crossover, placebo-controlled	n=10 healthy nonsmoker males (mean age 20.8 ± 1.6y, mean BMI 22.5 ± 2.1 kg/m^2)	300 g of homogenized BB Composition per 100 g: Total phenolics: 242.4mg (30.1mg CGA, 116.1mg ACN); Vitamin C: 0.8 mg	200 g Control Jelly (CJ) (20g of gelatine matched for sugars in 200mL of water) Composition per 100 g: Total phenolics 0 mg; Vitamin C: 0 mg	RHI by EndoPAT 1h after consumption	= RHI after either BB or CJ consumption
Djurica *et al*. (2016)[43], USA	Randomized, crossover, controlled, double-blind	n=25 overweight or obese males (mean age 16 y; mean BMI: not clear)	50 g of freeze-dried strawberry powder (FDSP) Composition per 50 g FDSP: pelargonidin-3-glucoside 198.5 mg, 15.31 mg procyanidin B1, 12.52 mg catechin and other phenolics)	50 g control powder (CP) matched for energy content and sugars Composition per 50 g: Total phenolics 0 mg	RHI &fRHI by PAT 1h after consumption	= RHI and fRHI after either FDSP or CP consumption
Flammer *et al*. (2013)[44], USA	Randomized, placebo-controlled, double-blind, parallel	n=69 subjects with endothelial dysfunction and CV risk factors - Placebo group (n=37, 11M, 2 smokers; mean age 51.4±15.1y, mean BMI 27.2±5.5 kg/m^2) - Cranberry juice (CBJ) group (n=32, 20M, 1 smoker, mean age 44.8±17.5y, mean BMI 27.7±5.9 kg/m^2)	2x230mL CBJ Composition per mL: Total phenolics 1740 µg (151 µg ACN, 2662 µg total proanthocyanidins)	2x230 mL placebo beverage matched for sugars Composition per mL: Total phenolics n.d.	RHI by EndoPAT 1h after consumption	↑ RHI after either CBJ and placebo beverage, no difference between the two groups; =AIx
Istas *et al*. (2018)[29], UK	Randomized, crossover, controlled, double blind	n=10 healthy males (mean age 27±3y, mean BMI 23±2kg/m2)	592 mL of drinks containing 200 or 400 g of frozen raspberries in water. Composition per serving: Total polyphenols 201 and 403 mg (164 and 328 mg ACN), Vitamin C 0.105 g	592 mL of placebo drink matched for micro- and macronutrient to the 400 g raspberry drink	FMD up to 24 h after consumption	↑FMD at 2h after consumption
Jin *et al*. (2011)[30], UK	Randomized, crossover, placebo-controlled, double-blind	n=20 healthy subjects (11 F/9 M, mean age 44.5±13.3y, mean BMI 23.81±2.46 kg/m^2)	250mL of 20% blackcurrant juice (BCJ) Composition per 100 mL: 81.5 mg PAs, 12.2 mg delphinidin, 8.0 mg cyanidin; Vitamin C 10.2 mg	250mL of control drink Composition per 100 mL: <10 mg PAs; Vitamin C: 0 mg	LDI measures of vascular reactivity in response to acetylcholine (endothelial dependent) and sodium nitroprusside (endothelial	=Endothelium dependent and independent vasodilation

					independent) 2h after BCJ consumption	
Richter *et al*. (2017)[31], USA	Randomized, crossover, placebo-controlled	n=30 nonsmokers, overweight or obese subjects (13F, mean age 28.1±2.7y, mean BMI 31.4±0.8kg/m^2; 17M, mean age 28.2±2.0y, mean BMI 31.3±0.6kg/m^2)	40 g FDSP with a high-fat (50 g total fat) meal Composition per 40 g: 158.76 mg pelargonidin-3-glucoside and other phenolics; Vitamin C 229 mg	40 g CP with a high-fat meal Composition per 40 g: Total phenolics not available; Vitamin C 0.196mg	AP, Ai@75, PWV up to 4 h after the meal	↓AP, Ai@75 after both FDSP and Cpcompared to baseline at 2 and 4 h =PWV
Rodriguez-Mateos *et al*. (2016)[33], Germany	Randomized, crossover, controlled, double blind	n=10 healthy males (mean age 24±2y, mean BMI 24±2 kg/m^2)	Five different CBJ Composition per serving: Total polyphenols 409, 787, 1238, 1534, and 1910 mg, respectively (6.8-32.3 mg ACN; 14.5-76.9 mg flavonols; 12.8-59.2 mg PAs)	Control drink matched for macro and micronutrients Composition per serving: Total polyphenols 2.9 mg (2.7 mg PAs)	FMD (%), PWV (m/s), AIx (%) up to 8 h after consumption	↑FMD at 1,2 4 6 and 8 h after consumption (max at 4h) with maximal effects for the drink containing 1238 mg total polyphenols; =AIx and PWV
Rodriguez-Mateos *et al*. (2014)[32], Germany	Randomized, crossover, controlled	n=10 healthy males (mean age 27 ± 1y, mean BMI 25 ± 0.8 kg/m^2)	a) Three baked products containing 34 g BB powder in (BB bun) Composition per bun x3: Total polyphenols: 637 mg (196 mg total ACN, 140 mg total procyanidins, 221 mg CGA) b) 34 g BB powder dissolved in 500 mL water (BB drink) Composition per 500 mL: Total polyphenols: 692 mg (339 mg total ACN, 111 total procyanidins, 179 mg CGA)	Control baked products (control bun) matched for macro and micronutrients Composition: n.a.	FMD up to 6 h after consumption	↑FMD at 1, 2 and 6h after consumption (max at 1h for BB drink and at 2h for BB bun)
Rodriguez-Mateos *et al*. (2013)[25], UK	Two randomized, crossover, controlled, double-blind	Study 1: n=10 healthy males (mean age 27 ± 1.3y, mean BMI 25±0.8 kg/m^2) Study 2: n=11 healthy males (mean age	Study 1: three different BB drinks Composition per serving: total polyphenols 766, 1278, and 1791 mg (310-724 mg ACN; 137-320 mg procyanidin; 273-637 mg	Studies 1 and 2: Control drink matched for macro and micronutrients Composition per serving: Total	Study 1: FMD; PWV; AIx; DVP up to 6 h after consumption Study 2: FMD 1 h after consumption	Study 1: ↑FMD but not at 4 h; =PWV; AIx; DVP; Study 2: ↑FMD dose-dependent to ≤766 mg

	27 ± 1.0y, mean BMI 22±0.9 kg/m^2)	CGA); Vitamin C 4-9.5 mg Study 2: five different BB drinks Composition per serving: total polyphenols 319, 639, 766, 1278, and 1791 mg (129-727 mg ACN; 57-320 mg procyanidin; 114-637 mg CGA); Vitamin C 1.7-9.5 mg	polyphenols 0 mg; Vitamin C: 6.8 mg

Legend: ACN: anthocyanins; AIx: augmentation index; AP: augmentation pressure; AS: açai smoothie; BB: blueberry; BCJ: blackcurrant juice; BMI: body mass index; BS: blueberry + smoking; C: control; CBJ: cranberry juice; CGA: Chlorogenic acid; CJ: control jelly; CP: control powder; CS: control smoothie; CV: cardiovascular; dAIx: digital augmentation index; dAIx@75: dAIx normalized by considering a heart rate of 75 bpm; DVP: digital volume pulse; F: females; FDSP: freeze dried strawberry powder; FMD: flow mediated dilation; fRHI: Framingham reactive hyperaemia index; GA: gallic acid; LDI: laser Doppler imaging; M: males; PAs: phenolic acids; PAT: peripheral arterial tonometry; PWV: pulse wave velocity; RHI: reactive hyperaemia index; RI: reflection index; S: smoking; SC: smoking + control; SI: stiffness index.

674 **Table 2.** Characteristics of the study design, population, products, and outcomes of the considered chronic intervention studies investigating the effect

675 of berry consumption on one or more markers of endothelial function.

REF.	STUDY DESIGN	STUDY POPULATION	DURATION OF INTERVENTION	BERRY INTERVENTION	CONTROL/ PLACEBO INTERVENTION	OUTCOME VARIABLES	MAIN FINDINGS
Cook *et al*. (2017)[34], UK	Randomized, double-blind, crossover	n = 13 healthy males (mean age 25±4 y; mean BMI 25±3 kg/m^2)	1 week	600 mg/day (2 x 300 mg capsule) of New Zealand blackcurrant (NZBC) extract Composition per capsule: 105 mg ACN	600 mg/day (2 × 300 mg capsule) of cellulose Composition: n.d.	Total peripheral resistance	↓total peripheral resistance at rest after NZBC (-25%) and during sustained isometric contraction at 15,30,45,60,90,105 and 120 s
Djurica *et al*. (2016)[43], USA	Randomized, controlled, double-blind, cross-over	n=25 overweight or obese males (mean age 16 y; mean BMI: not clear)	1 week	50 g/day of freeze-dried strawberry powder (FDSP) Composition per 50 g: Pelargonidin-3-glucoside 198.5 mg, 15.31 mg Procyanidin B1, 12.52 mg Catechin and other phenolics)	50 g control powder (CP) matched for energy content and sugars Composition: Total phenolics 0 mg	RHI &fRHI by PAT	= RHI and fRHI after either FDSP or CP consumption
Dohadwala *et al*. (2011)[35], USA	Randomized, double-blind, crossover, placebo-controlled	n=44 subjects with stable coronary artery disease - CBJ first, n=22 (15 M, mean age 61±11y; mean BMI 30±5 kg/m^2) - Placebo (PJ) first, n=22 (15 M, mean age 63±9y, mean BMI 29±4 kg/m^2)	4 weeks	480 mL/day cranberry juice (CBJ) Composition per serving: Total polyphenols 835 mg (94 mg ACN)	480 mL/day PL juice drink matched for calories and sensory characteristics Composition: n.d.	Carotid-radial PWV, carotid-femoral PWV, FMD (%), lnPATratio	↓carotid-femoral PWV after CBJ = FMD (upper arm), lnPATratio, carotid-radial PWV
Feresin *et al*. (2017)[36], USA	Randomized, controlled, double-blind, parallel	n=60 postmenopausal females with pre- or stage-1 hypertension - Control group, n=20 (mean age 58±1y, mean BMI 32.1±0.7kg/m^2) - Intervention group 1, n=20 (mean age 61 ±1y, BMI 31.0±1.0 kg/m^2) -	8 weeks	Intervention group 1: 25 g/day FDSP + 25 g/day of placebo powder Intervention group 2: 50 g/day of FDSP Composition per 25 g FDSP: 99.22 mg pelargonidin-3-glucoside, 7.70 mg procyanidin B1, 6.26 mg catechin and other phenolics	50 g of PL powder Composition: Total phenolics 0 mg	Brachial-ankle and femoral-ankle PWV	↓brachial-ankle PWV and femoral-ankle PWV after 25 g but not 50 g of FDSP. No treatment effect

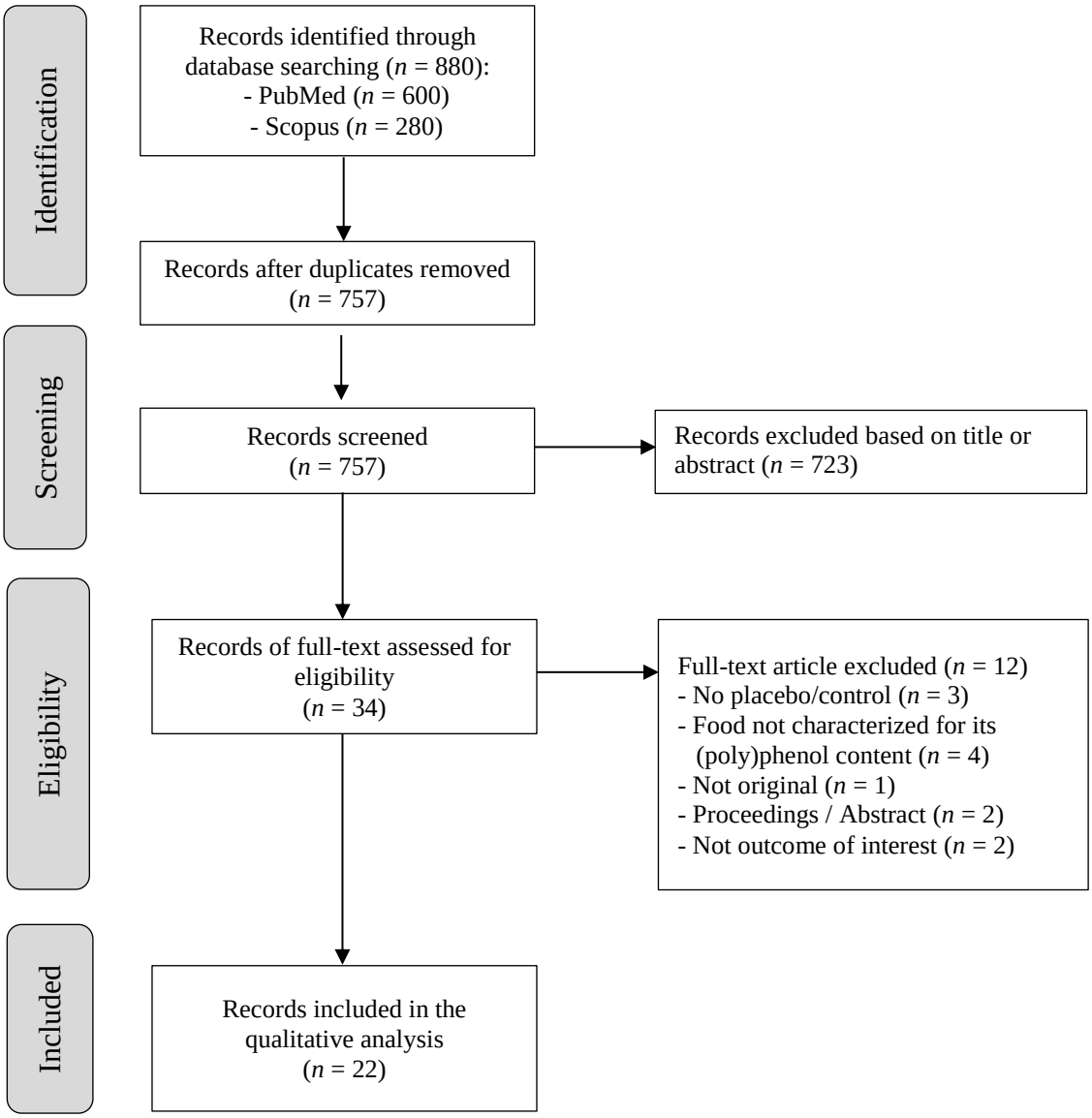
		Intervention group 2, n=20 (mean age 59±1y, mean BMI 32.7±1.1 kg/m^2)					
Flammer *et al*. (2013)[44], USA	Randomized, placebo-controlled, double-blind, parallel	n=69 subjects with endothelial dysfunction and CV risk factors - Placebo group, n=37 (11M, mean age 51.4±15.1y, mean BMI 27.2±5.5 kg/m2) - Cranberry juice (CJ) group, n=32 (20M, mean age 44.8±17.5y, mean BMI 27.7±5.9 kg/m^2)	4 months	2x230mL CBJ Composition per mL: Total phenolics 1740 µg (151 µg total ACN, 2662 µg total proanthocyanidins)	2x230 mL PL beverage matched for sugars Composition: n.d.	RHI	= RHI after either CBJ and PL, no difference between the two groups
Johnson *et al*. (2015)[37], USA	Randomized, controlled, double-blind, parallel	n=48 light smoker subjects with pre-hypertension - Intervention group, n=25 (mean age 59.7±4.58y, mean BMI 30.1±5.94 kg/m^2) -Placebo group, n=23 (mean age 57.3±4.76y, mean BMI 32.7±6.5 kg/m^2)	8 weeks	22 g/day of freeze-dried BB powder Composition per serving: Total phenolics 844.58 mg (469.48 mg ACN), vitamin C 2.27 mg	22g/day of macronutrient-matched CP Composition: Total phenolics 0 mg, vitamin C 0 mg	Carotid-femoral and brachial-ankle PWV	↓brachial-ankle PWV after blueberry but non control =carotid-femoral PWV

Khan *et al*. (2014)[38], UK	Randomized, double-blind, placebo-controlled, parallel	n= 66 healthy subjects - Placebo group, n= 21 (15 M, mean age 51±8y; mean BMI 28.9 ± 6.5kg/m^2) - Intervention group 1, n=22 (15 M, mean age 55±10y, mean BMI 28.4 ± 5.4kg/m^2) - Intervention group 2, n=21 (13 M, mean age 51±11y; mean BMI 29.2 ± 6.9 kg/m^2)	6 weeks	- Intervention group 1: 1 L/day low blackcurrant juice (BCJ, 4 x 250 mL) Composition per 100 mL: Total polyphenols 27.3 mg (4 mg ACN), vitamin C 1.1 mg - Intervention group 2: 1 L/day high BCJ (4 x 250 mL) Composition per 100 mL: Total polyphenols 81.5 mg (14.3 mg), vitamin C 10.2 mg	1 L of flavored water (4x250 mL) Composition: n.d.	FMD	↑FMD after high BCJ, but not after low BCJ, compared to placebo
Riso *et al*. (2013)[39], Italy	Randomized, controlled, crossover	n=18 healthy males with one risk factor for CVD (mean age 47.8±9.7y, mean BMI 24.8±2.6 kg/m^2)	6 weeks	250 mL/day Wild BB drink (25g of BB powder in 250 mL of water) Composition per 25 g powder: 375 mg ACN, 127.5 mg CGA	250 PL drink/day matched for sensory characteristics Composition: n.d.	RHI, fRHI, AIx, AI@75	=RHI, fRHI, AIx, AI@75
Ruel *et al*. (2013)[40], Canada	Randomized, controlled, double-blind crossover	n=35 healthy overweight men (mean age 45±10y, mean BMI 28.3 ± 2.4 kg/m^2)	4 weeks	500 mL/day CBJ (4x125mL) Composition per 500 mL: Total polyphenols 400 mg (20.8 mg ACN, 296 mg proanthocyanidins), vitamin C 128 mg	PL-juice matched for sensory characteristics Composition per 500 mL: Total polyphenols 156 mg (20.8mg ACN, 296 mg proanthocyanidins), vitamin C 128 mg	Resting AIx, AIx salbutamol, AIx GTN, global endothelial function	= resting AIx, AIx salbutamol, AIx GTN and global endothelial function after CBJ compared to placebo, but ↓ within-group resting AIx, AIx salbutamol and global endothelial function after CBJ, and ↓ within-group resting AIx and ↑ within-group AIx salbutamol and AIxGNT in subjects with MetS

Stull *et al*. (2015)[41], USA	Randomized, double-blind, placebo-controlled, parallel	n=44 non-smokers with MetS - Intervention group, n=23 (11 M, mean age 55±2y, mean BMI 35.2±0.8kg/m^2) - Placebo group, n=21 (5 M, mean age 59±2y, mean BMI 36.0±1.1kg/m^2)	6 weeks	Two smoothies /day (2 x 12-oz yogurt and skim milk-based smoothie with 22.5 g of freeze-dried BB powder) Composition per smoothie: Total phenolics 773.6 mg (290.3 mg ACN), vitamin C 2.7 mg	Two smoothies /day (2 x 12-oz yogurt and skim milk-based smoothie without BB powder) Composition per smoothie: Total phenolics n.d, vitamin C 0 mg	RHI	↑RHI after the intervention compared to placebo
Willems *et al*. (2015)[42], UK	Randomized, controlled, double-blind crossover	n=13 triathletes (8 M, mean age 38±8y, mean BMI 23±2 kg/m^2)	1 week	6 g/day Sujon NZBC dissolved in water Composition per serving: 138.6 mg ACN, vitamin C 49 mg	250 mL/day BCJ Composition per serving: 3-4 mg ACN, 32 mg vitamin C	Total peripheral resistance	↓ total peripheral resistance after NZBC compared to control (-16%)

Legend: ACN: anthocyanins; AIx: augmentation index; AIx@75: AIx normalized by considering a heart rate of 75 bpm; BB: blueberry; BCJ: blackcurrant juice; BMI: body mass index; CBJ: cranberry juice; CGA: Chlorogenic acid; CP: control powder; CV: cardiovascular; CVD: cardiovascular disease; F: females; FDSP: freeze dried strawberry powder; FMD: flow mediated dilation; fRHI: Framingham reactive hyperaemia index; GTN: glyceryl trinitrate; lnPAT: natural logarithm of the peripheral arterial tonometry index; M: males; MetS: metabolic syndrome; NZBC: New Zealand blackcurrant; PJ: placebo juice; PL: placebo; PWV: pulse wave velocity; RHI: reactive hyperaemia index.

Figure 1

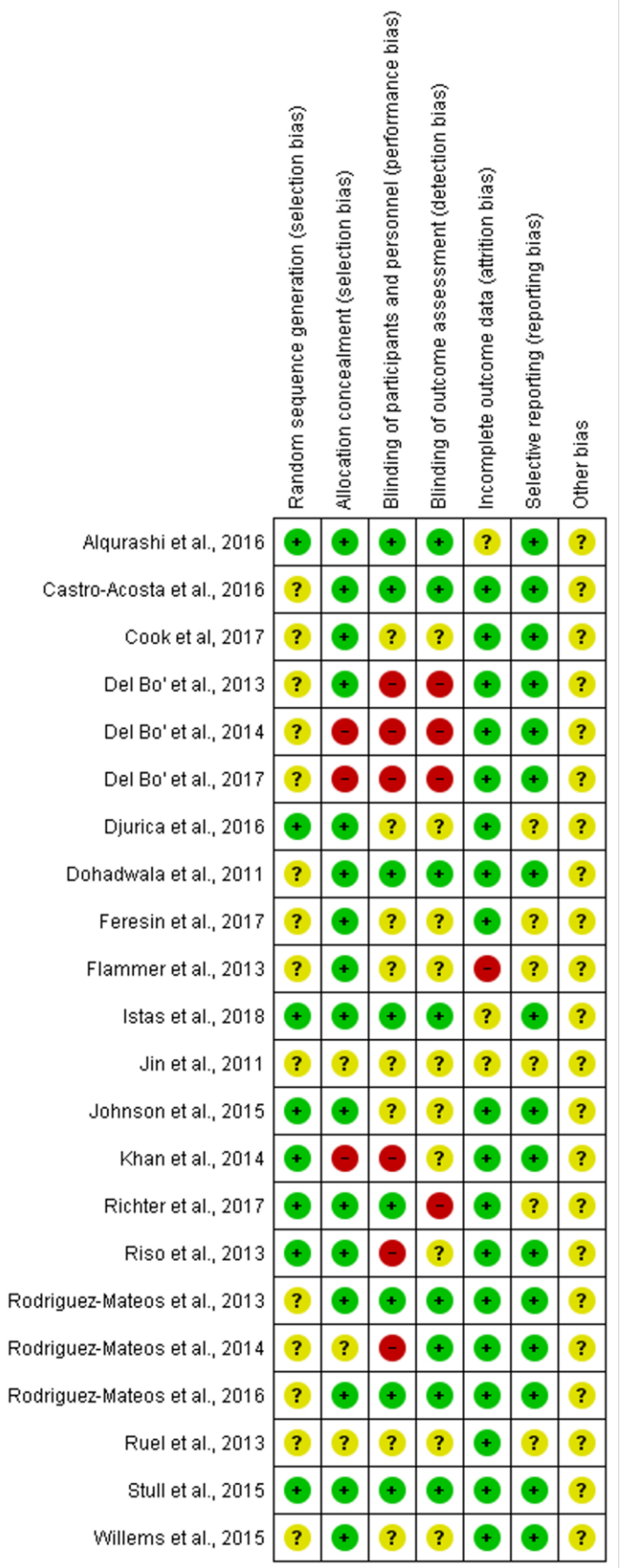

Identification
Records identified through database searching (n = 880):
- PubMed (n = 600)
- Scopus (n = 280)
Records after duplicates removed (n = 757)
Screening
Records screened (n = 757)
Records excluded based on title or abstract (n = 723)
Eligibility
Records of full-text assessed for eligibility (n = 34)
Full-text article excluded (n = 12)
- No placebo/control (n = 3)
- Food not characterized for its (poly)phenol content (n = 4)
- Not original (n = 1)
- Proceedings / Abstract (n = 2)
- Not outcome of interest (n = 2)
Included
Records included in the qualitative analysis (n = 22)

Supplementary Table 1: PICO criteria for the inclusion of the intervention studies[21].

Parameter	Criteria
Population (P)	Not hospitalized children, adolescents or adults, regardless of the age, BMI and health/pathological status.
Intervention (I)	Dietary intervention studies involving the consumption of berries, regardless of the supplied form (raw, juices, supplements, etc.), not in combination with other foods which may overlap the effects.
Comparison (C)	Control group (berries totally or partially excluded, totally or partially substitute with other fruits/supplements).
Outcome (O)	Endothelial dysfunction, such as RHI (Reactive Hyperaemia Index), Aix (Augmentation index), PWV (Pulse Wave Velocity) and FMD (Flow Mediated Dilation).
Study design (S)	Inclusion: Randomized controlled trials Exclusion: Non-randomized controlled trials; Retrospective, prospective, or concurrent cohort studies; Cross sectional studies; Case reports; Editorials

[21.] Lichtenstein AH, Yetley EA, Lau J. Application of systematic review methodology to the field of nutrition. J Nutr. 2008;138:2297-2306

731 Supplementary Figure 1



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734 Supplementary Figure 2

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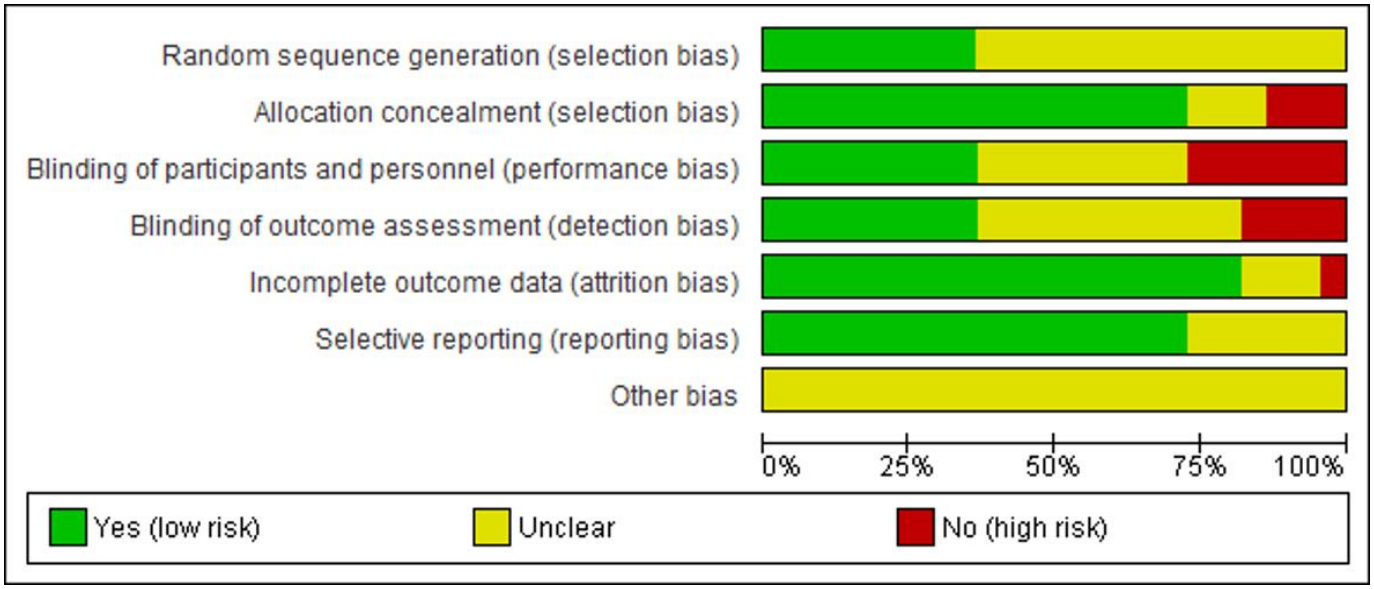

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