Role of berries in vascular function: a systematic review of human intervention studies

Daniela Martini, Mirko Marino, Donato Angelino, Cristian Del Bo’, Daniele Del Rio, Patrizia Riso, and Marisa Porrini

Context: Berries are a source of polyphenols with recognized health-promoting activities. Several studies suggest that consumption of berries may improve vascular function. Objective: The aim of this systematic review is to provide evidence of short- and long-term benefits of berries on outcomes of vascular function. Data Sources: Human intervention studies were collected from PubMed and Scopus databases. Study Selection: Studies were eligible if they investigated the effects of acute or chronic berry consumption on one or more markers of vascular function in humans and provided a characterization of the berry polyphenolic content. Only randomized controlled trials were included, and studies were excluded if berries were combined with other foods. Data Extraction: After selection, 22 randomized controlled trials were included and analyzed, most of which were performed in healthy individuals or patients with cardiovascular risk factors. Results: The overall results seem to suggest a protective role of berries in vascular function, likely dependent on the time of exposure, the type and dose of berry, and the biomarkers analyzed. Flow-mediated dilation and reactive hyperemia index (markers of vascular reactivity) improved following short-term interventions, while pulse wave velocity and augmentation index (markers of arterial stiffness) improved only after medium- to long-term intervention. Conclusions: 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.

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. Berries are an important source of phenols and polyphenols, including anthocyanins, proanthocyanidins, flavonols, flavones, flavan-3-ols, flavanones, isoflavones, stilbenes, lignans, and phenolic acids. Berry consumption has been associated with reduced all-cause mortality. Moreover, in recent years, numerous epidemiological and clinical studies have documented the protective effects of berries against many noncommunicable chronic diseases, with some focusing on cardiovascular disease, which remains
the leading cause of death worldwide. The development of cardiovascular disease is often accompanied by a decline in vascular health and function. The endothelium, which covers the inner surface of the blood vessels, is an important part of the vasculature. It controls the flow of nutrients and non-nutrients, the passage of fluids into the tissues, and the secretion of vasoactive substances, such as vasoconstricting molecules like endothelin-1 and the vasodilator nitric oxide.

Common biomarkers for the evaluation of vascular health include blood pressure, arterial stiffness, and vascular reactivity. Vascular reactivity can be assessed through endothelium-dependent or -independent mechanisms, using acetylcholine or nitroglycerin, respectively. The main methods used to assess vascular reactivity are flow-mediated dilation and peripheral arterial tonometry–reactive hyperemia index (RHI). Flow-mediated dilation, considered the gold standard noninvasive ultrasound technique, measures vasodilation at the brachial artery following a standard occlusion. EndoPAT is a plethysmographic technique used to measure pressure changes in the finger tips caused by a 5-minute occlusion of the brachial artery. Pulse wave velocity (PWV), which directly measures point-to-point pulse wave transit time, and pulse wave analysis, which uses the pulsatile waveform shape to make assumptions about arterial hemodynamics, can be used to measure arterial stiffness. Stiffness can be also quantified through the augmentation index, defined as the difference between the second and first systolic peak, expressed as percentage of the pulse pressure.

Some systematic reviews and meta-analyses of observational and randomized controlled trials (RCTs) have reported a relationship between the consumption of polyphenols and polyphenol-rich foods and the modulation of vascular function markers such as augmentation index, PWV, flow-mediated dilation, and RHI. Other direct or indirect biomarkers of vascular function include serum concentrations of inflammatory markers, adhesion molecules, lipids and lipoproteins, oxidized low-density lipoprotein cholesterol, and clotting factors. A meta-analysis of RCTs, performed by Huang et al., has shown that berry consumption may significantly reduce the levels of low-density lipoprotein cholesterol, blood pressure, fasting glucose, and tumor necrosis factor-α, supporting the potential contribution of berries to cardiovascular health.

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 the potential role of berries in cardiovascular health. The current systematic review focuses exclusively on studies (both acute and chronic interventions) performed with berries and berry products, thus differentiating it from other recent works.

**METHODS**

A systematic review was conducted according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement and included all relevant PRISMA checklist items (see Table S1 in the Supporting Information online). A review protocol has not been published, and this review has not been registered with any systematic review database.

**Eligibility criteria**

Studies were eligible for the present review if they investigated the effect of berry consumption on one or more markers of vascular function in humans. To be included, studies had to be RCTs of either acute (ie, single-dose supplementation) or chronic berry consumption and provide a characterization of the berry polyphenolic content. Studies were excluded if berries were combined with other foods (because any beneficial effect could not be attributed specifically to berries) or if the language of publication was other than English, with no accessible translation. There were no restrictions pertaining to the characteristics of study participants (eg, age, sex, health condition).

A more detailed list of eligibility criteria, developed by following the PICOS (Population, Intervention, Comparison, Outcome, Study design) format, is summarized in Table 1.

**Search strategy and study selection**

A systematic literature search was conducted using the PubMed and Scopus databases in December 2017 (updated search conducted in October 2018). To ensure completeness, searches were augmented by screening the bibliographies of relevant review articles.

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**Table 1 PICO-S table for inclusion of studies**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Inclusion criteria</th>
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<tbody>
<tr>
<td>Population</td>
<td>Not hospitalized children, adolescents, or adults of any age, body mass index, or health/pathological status</td>
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<tr>
<td>Intervention</td>
<td>Dietary intervention studies involving the consumption of berries, regardless of the form supplied (raw, juices, supplements, etc), not in combination with other foods that may have overlapping effects</td>
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<tr>
<td>Comparison</td>
<td>Control group (berries totally or partially excluded from diet, or berries totally or partially substituted with other fruits or supplements)</td>
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<tr>
<td>Outcome</td>
<td>Endothelial dysfunction, as determined by reactive hyperemia index, augmentation index, pulse wave velocity, or flow-mediated dilation</td>
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<tr>
<td>Study design</td>
<td>Randomized controlled trials</td>
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</table>
The search had no limit ranges for the year of publication. Three search themes were considered: terms related to berry (eg, strawberry, blueberry, cranberry) were combined with terms related to outcomes (eg, PWV, flow-mediated dilation, arterial stiffness) and population type (eg, human, volunteers, patients) to identify all potentially relevant literature published (for further information on the search strategy, see Appendix S1 in the Supporting Information online). No language or other restrictions were applied in the literature search. The literature identification process, based on the PRISMA statement, is illustrated in Figure 1.

**Study selection and data collection process**

Two authors (M.M. and D.M.) independently abstracted data from studies eligible for inclusion. Disagreement between reviewers was resolved through consultation with a third independent reviewer (C.D.B.) 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 enrollment stages, inclusion and 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 2 authors (D.A. and D.M.) following the criteria of the Cochrane Handbook for Systematic Reviews of Interventions, version 5.1.0. The following components of the risk of bias were rated, using the parameters (shown in parentheses) 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); and (6) other bias. All scores were assessed as indicating a low risk of bias, a high risk of bias, or, if insufficient details about these parameters were reported in the study, an unclear risk of bias. All disagreements were resolved by consensus with a third author (C.D.B.).

**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), and no additional records were found by hand searching. After removing 123 duplicate articles, 757 studies were screened and 723 were excluded on the basis of title and abstract. After the full-text reading of the remaining 34 eligible papers, another 12 records were excluded for the following reasons: (1) the study had no placebo or control food (n = 12), (2) the study had no outcome of interest (n = 2), (3) the products consumed were not fully characterized for their (poly)phenol content (n = 4), (4) proceedings (n = 2), and (5) not original (n = 1). At the end of the selection process, 22 RCTs were included in the qualitative analysis.

**Study characteristics**

The main characteristics of the 22 included studies are reported in Table 2 and Table 3. Eleven of these studies investigated acute interventions,44,45 and 9 chronic interventions,35–43 while 24,45 examined the effects of both acute and chronic consumption of berries.

The current review summarizes the main findings on 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 both the type of berry and the amount administered (250–300 g for raw fruits, 250–1000 mL/d for drinks/smoothies, and 600 mg for capsules). The main outcome variables were vascular reactivity, assessed by measuring flow-mediated dilation and RHI, and arterial stiffness, assessed by measuring PWV and the augmentation index.

**Risk of bias of the studies**

Risks of bias within individual studies and across the studies are shown in Figures S1 and S2 in the Supporting Information online, respectively. The results show the lack of blinding of participants and personnel (performance bias) and the lack of blinding of outcome assessment (detection bias) to represent the highest risks of bias.

**Acute studies**

Table 2 reports the main results obtained in 13 short-term studies of berry interventions33–44 that used flow-mediated dilation (n = 5), RHI (n = 5), and other markers (n = 3) to assess vascular function. Alqurashi et al24 showed that the intake of 200 g of açai smoothie significantly increased flow-mediated dilation at 2 hours (+ 1.4%; P = 0.001) and at 6 hours (+ 0.8%; P < 0.001) post consumption in healthy overweight men. Similarly, Rodriguez-Mateos et al33 found that the consumption of 3 pieces (buns) of baked blueberry-containing products and/or a blueberry drink (containing the equivalent of 240 g of fresh blueberries) increased flow-mediated dilation at 1, 2, and 6 hours post consumption. A significant improvement in flow-mediated dilation occurred after consumption of either of the 2 items, at 1 hour for the drink and at 2 hours for the baked products (up to + 2.6%). In another study, the same authors reported that acute consumption of 5 servings (450 mL each) of cranberry juices containing different amounts of (poly)phenols (409 mg, 787 mg, 1238 mg, 1534 mg, and 1910 mg, respectively) resulted in significantly augmented flow-mediated dilation at 1, 2, 4, 6, and 8 hours post intervention.34 Flow-mediated dilation increased gradually, both time dependently (spiking at 4 hours) and dose dependently, with the maximum effect (about + 2.5%) observed after an intake of 1238 mg of total polyphenols.

While Istas et al30 showed that the intake of 2 different portions (200 g and 400 g) of red raspberries (containing 201 mg or 403 mg of total polyphenols, respectively) improved flow-mediated dilation in 10 healthy individuals at 2 hours (+ 1.6% and + 1.2%, respectively) and 24 hours (+ 1.0% and + 0.7%, respectively), there was no difference between the 2 doses and, thus, no dose-response relationship.

Three studies reported a significant increase in the RHI outcome measure. Del Bo’ et al28 found that 300 g of blueberries counteracted an impairment in RHI (−4.4 ± 0.8%; P < 0.01) and improved Framingham RHI (+ 28.3 ± 19.2%; P < 0.0001) in a group of healthy smokers with normal endothelial function (2 hours post consumption). In another study, the authors documented that the same 300-g blueberry portion increased RHI values in both smokers (+ 35.2 ± 7.5%; P = 0.02) and nonsmokers (+ 54.8 ± 8.4%; P = 0.01) with endothelial dysfunction.27 Finally, Flammer et al45 observed
<table>
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<tr>
<th>Reference, country</th>
<th>Study design</th>
<th>Study population</th>
<th>Berry intervention</th>
<th>Control or placebo intervention</th>
<th>Outcome variables</th>
<th>Main findings</th>
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<tr>
<td>Alqurashi et al (2016), 24 UK</td>
<td>Randomized, crossover, controlled, double-blind</td>
<td>n = 23 healthy non-smoker males (mean age 46±1.9 y; mean BMI 27.6±0.4 kg/m²)</td>
<td>200 g of açai smoothie (150 g of açai pulp + 50 g of banana) Composition per serving: total polyphenols, 694 mg (493 mg of ACNs, 173.6 mg of gallic acid, 9.6 mg of quercetin, 9.3 mg of CGA); total carotenoids, 179.3 mg</td>
<td>200 mg of control smoothie (50 g of banana matched for fat) Composition per serving: total polyphenols, &lt; 10 mg; total carotenoids, 0 mg</td>
<td>FMD up to 6 h after consumption</td>
<td>Increased FMD at 2 h and 6 h after açai smoothie consumption, but not after control smoothie consumption</td>
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<tr>
<td>Castro-Acosta et al (2016), 25 UK</td>
<td>Randomized, crossover, controlled, double-blind</td>
<td>n = 22 participants (13 males, 9 females; mean age 45.4±13.7 y; mean BMI 25.5±3.8 kg/m²)</td>
<td>200 mL of 3 different blackcurrant drinks Composition per 200 mL: total phenolics, 460 mg, 810 mg, and 1596 mg, respectively (total ACNs, 131 mg, 322 mg, and 599 mg); vitamin C, &lt; 0.5 mg</td>
<td>Placebo drinks matched for astrignency by adding tannins Composition per 200 mL: total phenolics, 207 mg (46 mg of ACNs); vitamin C, &lt; 0.5 mg</td>
<td>DVP stiffness index and DVP reflection up to 2 h after consumption</td>
<td>Unchanged DVP stiffness index and DVP reflection index compared with baseline for all blackcurrant and placebo drinks</td>
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<tr>
<td>Del Bo’ et al (2017), 27 Italy</td>
<td>Randomized, crossover, controlled</td>
<td>Study 1: n = 12 non-smoker males with peripheral arterial dysfunction (mean age 24.2±1.2 y; mean BMI 22.5±1.2 kg/m²) Study 2: n = 12 males who smoked (mean age 24.5±1.9 y; mean BMI 22.9±1.1 kg/m²)</td>
<td>Study 1: 300 g of thawed blueberries Composition per serving: total phenolics, 856 mg (309 mg of ACNs, 30 mg of CGA); vitamin C, 2.4 mg 300 g of blueberries + smoking Composition per 100 g: total phenolics, 242.4 mg (116.1 mg of ACNs, 30.1 mg of CGA); vitamin C, 0.8 mg</td>
<td>Study 1: Only smoking Study arm 2: 300 mL of water with sugar + smoking Composition per 100 g: total phenolics, 0 mg; vitamin C, 0 mg</td>
<td>RHI, dAIx, and dAIx@75 2 h after consumption</td>
<td>Study 1: increased RHI; unchanged dAIx and dAIx@75 Study 2: increased RHI; unchanged dAIx and dAIx@75</td>
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<tr>
<td>Del Bo’ et al (2014), 28 Italy</td>
<td>Randomized, crossover, controlled</td>
<td>n = 16 healthy males who smoked (mean age 23.6±0.7 y; mean BMI 23.0±0.5 kg/m²)</td>
<td>Composition per serving: total phenolics, 39 mg of ACNs, 30 mg of CGA); vitamin C, 0.8 mg</td>
<td>Composition: ND</td>
<td>RHI, fRHI, dAIx, and dAIx@75 2 h after consumption</td>
<td>Increased RHI and fRHI; unchanged dAIx and dAIx@75</td>
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<tr>
<td>Reference, country</td>
<td>Study design</td>
<td>Study population</td>
<td>Berry intervention</td>
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<tr>
<td>Del Bo’ et al (2013), Italy</td>
<td>Randomized, cross-over, placebo-controlled</td>
<td>n = 10 healthy non-smoker males (mean age 20.8 ± 1.6 y; mean BMI 22.5 ± 2.1 kg/m²)</td>
<td>300 g of homogenized blueberries Composition per 100 g: total phenolics, 242.4 mg (30.1 mg of CGA, 116.1 mg of ACNs); vitamin C, 0.8 mg</td>
<td>200 g of control jelly (20 g of gelatin matched for sugars in 200 mL of water) Composition per 100 g: total phenolics, 0 mg; vitamin C, 0 mg</td>
<td>RHI by EndoPAT 1 h after consumption</td>
<td>Unchanged RHI after either blueberry or control jelly consumption</td>
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<td>Djurica et al (2016), USA</td>
<td>Randomized, cross-over, controlled, double-blind</td>
<td>n = 25 overweight or obese males (mean age 16 y; mean BMI not clear)</td>
<td>50 g of freeze-dried strawberry powder Composition per 50 g of freeze-dried strawberry powder: 198.5 mg of pelargonidin-3-glucoside, 15.31 mg of procyanidin B1, 12.52 mg of catechin and other phenolics</td>
<td>50 g of control powder matched for energy content and sugars Composition per 50 g: total phenolics, 0 mg</td>
<td>RHI and fRHI by peripheral arterial tonometry 1 h after consumption</td>
<td>Unchanged RHI and fRHI after consumption of either freeze-dried strawberry powder or control powder</td>
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<td>Flammer et al (2013), USA</td>
<td>Randomized, placebo-controlled, double-blind, parallel</td>
<td>n = 69 individuals with endothelial dysfunction and cardiovascular risk factors Placebo group (n = 37; 11 males, 2 of whom smoked; mean age 51.4 ± 15.1 y, mean BMI 27.2 ± 5.5 kg/m²) Cranberry juice group (n = 32; 20 males, 1 of whom smoked; mean age 44.8 ± 17.5 y; mean BMI 27.7 ± 5.9 kg/m²)</td>
<td>2 × 230 mL of cranberry juice Composition per milliliter: total phenolics, 1740 µg (151 µg of ACNs, 2662 µg of total proanthocyanidins)</td>
<td>Placebo beverage matched for sugars, 2 × 230 mL Composition per milliliter: total phenolics, ND</td>
<td>RHI by EndoPAT 1 h after consumption</td>
<td>Increased RHI after either cranberry juice or placebo beverage, no difference between the 2 groups; unchanged Aix</td>
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<td>Reference, country</td>
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<td>Istas et al (2018), UK</td>
<td>Randomized, cross-over, controlled, double blind</td>
<td>$n = 10$ healthy males (mean age $27 \pm 3$ y; mean BMI $23 \pm 2$ kg/m$^2$)</td>
<td>592 mL of drinks containing 200 g or 400 g of frozen raspberries in water Composition per serving: total polyphenols, 201 mg and 403 mg (164 mg and 328 mg of ACNs); vitamin C, 0.105 g</td>
<td>592 mL of placebo drink matched for micro- and macronutrients to a drink containing 400 g of raspberries</td>
<td>FMD up to 24 h after consumption</td>
<td>Increased FMD at 2 h after consumption</td>
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<td>Jin et al (2011), UK</td>
<td>Randomized, cross-over, placebo-controlled, double-blind</td>
<td>$n = 20$ healthy individuals (11 females, 9 males; mean age $44.5 \pm 13.3$ y; mean BMI $23.81 \pm 2.46$ kg/m$^2$)</td>
<td>250 mL of 20% blackcurrant juice Composition per 100 mL: 81.5 mg of phenolic acids, 12.2 mg of delphinidin, 8.0 mg of cyanidin, 10.2 mg of vitamin C</td>
<td>250 mL of control drink Composition per 100 mL: phenolic acids, &lt; 10 mg; vitamin C, 0 mg</td>
<td>LDI measures of vascular reactivity in response to acetylcholine (endothelial dependent) and sodium nitroprusside (endothelial independent) 2 h after blackcurrant juice consumption</td>
<td>Unchanged endothelium-dependent and independent vasodilation</td>
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<td>Richter et al (2017), USA</td>
<td>Randomized, cross-over, placebo-controlled</td>
<td>$n = 30$ nonsmoking overweight or obese individuals (13 females; mean age $28.1 \pm 2.7$ y; mean BMI $31.4 \pm 0.8$ kg/m$^2$; 17 males; mean age $28.2 \pm 2.0$ y; mean BMI $31.3 \pm 0.6$ kg/m$^2$)</td>
<td>40 g of freeze-dried strawberry powder with a high-fat (50 g of total fat) meal Composition per 40 g: 158.76 mg of pelargonidin-3-glucoside and other phenolics, 229 mg of vitamin C</td>
<td>40 g of control powder with a high-fat meal Composition per 40 g: total phenolics, NA; vitamin C, 0.196 mg</td>
<td>Augmentation pressure, Ai@75 and PWV up to 4 h after the meal</td>
<td>Decreased augmentation pressure and Ai@75 after both freeze-dried strawberry powder and control powder compared with baseline at 2 h and 4 h; unchanged PWV</td>
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<td>Rodriguez-Mateos et al (2016), Germany</td>
<td>Randomized, cross-over, controlled, double-blind</td>
<td>$n = 10$ healthy males (mean age $24 \pm 2$ y; mean BMI $24 \pm 2$ kg/m$^2$)</td>
<td>5 different cranberry juices Composition per serving: total polyphenols, 409 mg, 787 mg, 1238 mg, 1534 mg, and 1910 mg, respectively (6.8–32.3 mg of ACNs, 14.5–76.9 mg of flavonols, 12.8–59.2 mg of phenolic acids)</td>
<td>Control drink matched for macro- and micronutrients Composition per serving: total polyphenols, 2.9 mg (2.7 mg of phenolic acids)</td>
<td>FMD (%), PWV (m/s), and AiX (%) up to 8 h after consumption</td>
<td>Increased FMD at 1, 2, 4, 6, and 8 h after consumption (maximum at 4 h), with maximal effects observed with the drink containing 1238 mg of total polyphenols; unchanged AiX and PWV</td>
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<table>
<thead>
<tr>
<th>Reference, country</th>
<th>Study design</th>
<th>Study population</th>
<th>Berry intervention</th>
<th>Control or placebo intervention</th>
<th>Outcome variables</th>
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<tr>
<td><strong>Rodriguez-Mateos et al (2014), Germany</strong>&lt;sup&gt;23&lt;/sup&gt;</td>
<td>Randomized, cross-over, controlled</td>
<td>n = 10 healthy males (mean age 27 ± 1 y; mean BMI 25.0 ± 0.8 kg/m²)</td>
<td>Study arm A: 3 baked products containing 34 g of blueberry powder in a blueberry bun Composition per 3 buns (intervention amount): 637 mg of total polyphenols (196 mg of total ACNs, 140 mg of total procyanidins, 221 mg of CGA) Study arm B: 34 g of blueberry powder dissolved in 500 mL of water (blueberry drink) Composition per 500 mL: 692 mg of total polyphenols (339 mg of total ACNs, 111 mg of total procyanidins, 179 mg of CGA)</td>
<td>Control: baked products (control bun) matched for macro- and micronutrients Composition: NA</td>
<td>FMD up to 6 h after consumption</td>
<td>Increased FMD at 1, 2, and 6 h after consumption (maximum at 1 h for blueberry drink and at 2 h for blueberry bun)</td>
</tr>
<tr>
<td><strong>Rodriguez-Mateos et al (2013), UK</strong>&lt;sup&gt;26&lt;/sup&gt;</td>
<td>2 randomized, cross-over, controlled, double-blind studies</td>
<td>Study 1: n = 10 healthy males (mean age 27.0 ± 1.3 y; mean BMI 25.0 ± 0.8 kg/m²) Study 2: n = 11 healthy males (mean age 27 ± 1 y; mean BMI 22.0 ± 0.9 kg/m²)</td>
<td>Study 1: 3 different blueberry drinks Composition per serving: total polyphenols, 766 mg, 1278 mg, and 1791 mg (310–724 mg of ACNs, 137–320 mg of procyanidin, 273–637 mg of CGA); vitamin C, 4.0–9.5 mg Study 2: 5 different blueberry drinks Composition per serving: total polyphenols, 319 mg, 639 mg, 766 mg, 1278 mg, and 1791 mg (129–727 mg of ACNs, 57–320 mg of procyanidin, 114–637 mg of CGA); vitamin C, 1.7–9.5 mg</td>
<td>Studies 1 and 2: control drink matched for macro- and micronutrients Composition per serving: total polyphenols, 0 mg; vitamin C, 6.8 mg</td>
<td>Study 1: FMD, PWV, AIx, and DVP up to 6 h after consumption Study 2: FMD 1 h after consumption</td>
<td>Study 1: increased FMD at 1-2 h and 6 h, but not 4 h, unchanged PWV, AIx, and DVP Study 2: FMD increased dose-dependently, to ≤ 766 mg</td>
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</table>

**Abbreviations:** ACN, anthocyanin; AIx, augmentation index; BMI, body mass index; CGA, chlorogenic acid; dAIx, digital augmentation index; dAIx@75, dAIx normalized by considering a heart rate of 75 bpm; DVP, digital volume pulse; FMD, flow-mediated dilation; fRHI, Framingham reactive hyperemia index; LDI, laser Doppler imaging; m/s, meters per second; NA, not available; ND, not determined; PWV, pulse wave velocity; RHI, reactive hyperemia index.
Table 3 Characteristics of the chronic intervention studies investigating the effects of berry consumption on one or more markers of endothelial function

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study design</th>
<th>Study population</th>
<th>Duration of intervention</th>
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<tr>
<td>Cook et al (2017),35</td>
<td>Randomized, crossover, double-blind</td>
<td>[n = 13] healthy males (mean age (25 \pm 4) y; mean BMI (25 \pm 3) kg/m(^2))</td>
<td>1 wk</td>
<td>NZBC extract, 600 mg/d (2 × 300-mg capsule)</td>
<td>600 mg (2 × 300-mg capsule) cellulose per day</td>
<td>Total peripheral resistance</td>
<td>Decreased total peripheral resistance at rest after NZBC (−25%) and during sustained isometric contraction at 15, 30, 45, 60, 90, 105, and 120 s</td>
</tr>
<tr>
<td>Djurica et al (2016),44</td>
<td>USA</td>
<td>[n = 25] overweight or obese males (mean age (16) y; mean BMI not clear)</td>
<td>1 wk</td>
<td>Freeze-dried strawberry powder, 50 g/d</td>
<td>50 g of control powder, matched for energy content and sugars</td>
<td>RHI and fRHI by peripheral arterial tonometry</td>
<td>Unchanged RHI and fRHI after either freeze-dried strawberry powder or control powder consumption</td>
</tr>
<tr>
<td>Dohadwala et al (2011),36</td>
<td>USA</td>
<td>[n = 44] patients with stable coronary artery disease</td>
<td>4 wk</td>
<td>Cranberry juice, 480 mL/d</td>
<td>Placebo juice drink, 480 mL/d, matched for calories and sensory characteristics</td>
<td>Carotid-radial PWV, carotid-femoral PWV, FMD (%), and InPAT ratio</td>
<td>Decreased carotid-femoral PWV after cranberry juice. Unchanged FMD (upper arm), InPAT ratio, and carotid-radial PWV</td>
</tr>
<tr>
<td>Feresin et al (2017),37</td>
<td>Parallel, controlled, double-blind</td>
<td>[n = 60] postmenopausal females with prehypertension or stage 1 hypertension</td>
<td>8 wk</td>
<td>Intervention group 1: freeze-dried strawberry powder, 25 g/d</td>
<td>50 g of placebo powder, Composition: total phenolics, 0 mg</td>
<td>Brachial-ankle PWV and femoral-ankle PWV</td>
<td>Decreased brachial-ankle PWV and femoral-ankle PWV after 25 g but not 50 g of freeze-dried strawberry powder. No treatment effect observed</td>
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<tr>
<th>Reference</th>
<th>Study design</th>
<th>Study population</th>
<th>Duration of intervention</th>
<th>Berry intervention</th>
<th>Control or placebo intervention</th>
<th>Outcome variables</th>
<th>Main findings</th>
</tr>
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<tr>
<td>Flammer et al (2013),45 USA</td>
<td>Randomized, parallel, placebo-controlled, double-blind</td>
<td>n = 69 patients with endothelial dysfunction and CVD risk factors Placebo group, n = 37 (11 males, 26 females; mean age 51.4 ± 15.1 y; mean BMI 27.2 ± 5.5 kg/m²)</td>
<td>4 mo</td>
<td>Cranberry juice, 2 × 230 mL Composition per milliliter: total phenolics, 1740 µg (151 µg of total ACNs, 2662 µg of total proanthocyanidins)</td>
<td>2 × 230 mL of placebo beverage, matched for sugars Composition: ND</td>
<td>RHI</td>
<td>Unchanged RHI after either cranberry juice or placebo; no difference between the 2 groups</td>
</tr>
<tr>
<td>Johnson et al (2015),38 USA</td>
<td>Randomized, parallel, controlled, double-blind</td>
<td>n = 48 individuals with prehypertension who were light smokers Intervention group, n = 25 (mean age 59.7 ± 4.58 y; mean BMI 30.1 ± 5.94 kg/m²) Placebo group, n = 23 (mean age 57.30 ± 4.76 y; mean BMI 32.7 ± 6.5 kg/m²)</td>
<td>8 wk</td>
<td>Freeze-dried blueberry powder, 22 g/d Composition per serving: total phenolics, 844.58 mg (469.48 mg of ACNs); vitamin C, 2.27 mg</td>
<td>Macronutrient-matched control powder, 22 g/d Composition: total phenolics, 0 mg; vitamin C, 0 mg</td>
<td>Carotid-femoral PWV and brachial-ankle PWV</td>
<td>Decreased brachial-ankle PWV after blueberries but not after control. Unchanged carotid-femoral PWV</td>
</tr>
<tr>
<td>Khan et al (2014),39 UK</td>
<td>Randomized, parallel, placebo-controlled, double-blind</td>
<td>n = 66 healthy participants Placebo group, n = 21 (15 males, 6 females; mean age 51 ± 8 y; mean BMI 28.9 ± 6.5 kg/m²) Intervention group 1, n = 22 (15 males, 7 females; mean age 55 ± 10 y; mean BMI 28.4 ± 5.4 kg/m²) Intervention group 2, n = 21 (13 males, 8 females; mean age 51 ± 11 y; mean BMI 29.2 ± 6.9 kg/m²)</td>
<td>6 wk</td>
<td>Intervention group 1: 1 L of 6.4% (ie, “low”) blackcurrant juice per day (4 × 250 mL) Composition per 100 mL: total polyphenols, 27.3 mg (4 mg of ACNs); vitamin C, 1.1 mg Intervention group 2: 1 L of 20% (ie, “high”) blackcurrant juice per day (4 × 250 mL) Composition per 100 mL: total polyphenols, 81.5 mg (14.3 mg of ACNs); vitamin C, 10.2 mg</td>
<td>1 L of flavored water (4 × 250 mL) Composition: ND</td>
<td>FMD</td>
<td>Increased FMD after high blackcurrant juice but not after low blackcurrant juice compared with placebo</td>
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<tr>
<th>Reference</th>
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<th>Outcome variables</th>
<th>Main findings</th>
</tr>
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<tbody>
<tr>
<td>Riso et al (2013),40 Italy</td>
<td>Randomized, crossover, controlled</td>
<td>n = 18 healthy males with 1 risk factor for CVD (mean age 47.8 ± 9.7 y; mean BMI 24.8 ± 2.6 kg/m²)</td>
<td>6 wk</td>
<td>Wild blueberry drink (25 g of blueberry powder in 250 mL of water), 250 mL/d Composition per 25 g of powder: 375 mg of ACNs, 127.5 mg of chlorogenic acid</td>
<td>Placebo drink, 250/d, matched for sensory characteristics Composition: ND</td>
<td>RHI, fRHI, Alx, and Al@75</td>
<td>Unchanged RHI, fRHI, Alx, and Al@75</td>
</tr>
<tr>
<td>Ruel et al (2013),41 Canada</td>
<td>Randomized, crossover, controlled, double-blind</td>
<td>n = 35 healthy overweight men (mean age 45 ± 10 y; mean BMI 28.3 ± 2.4 kg/m²)</td>
<td>4 wk</td>
<td>Cranberry juice, 500 mL/d (4 × 125 mL) Composition per 500 mL: total polyphenols, 400 mg (20.8 mg of ACNs, 296 mg of proanthocyanidins); vitamin C, 128 mg</td>
<td>Placebo juice, matched for sensory characteristics Composition per 500 mL: total polyphenols, 156 mg (20.8 mg of ACNs, 296 mg of proanthocyanidins); vitamin C, 128 mg</td>
<td>Resting Alx, Alx responses to salbutamol, Alx responses to glyceryl trinitrate, and global endothelial function</td>
<td>Unchanged resting Alx, Alx responses to salbutamol and glyceryl trinitrate, and global endothelial function after cranberry juice compared with placebo, but decreased within-group resting Alx, Alx response to salbutamol, and global endothelial function after cranberry juice, and decreased within-group resting Alx and increased within-group Alx responses to salbutamol and glyceryl trinitrate after cranberry juice in patients with MetS</td>
</tr>
<tr>
<td>Stull et al (2015),42 USA</td>
<td>Randomized, parallel, placebo-controlled, double-blind</td>
<td>n = 44 patients with MetS who were nonsmokers Intervention group, n = 23 (11 males, 12 females; mean age 55 ± 2 y; mean BMI 35.2 ± 0.8 kg/m²) Placebo group, n = 21 (5 males, 16 females; mean age 59 ± 2 y; mean BMI 36.0 ± 1.1 kg/m²)</td>
<td>6 wk</td>
<td>2 smoothies per day (2 × 12-oz yogurt and skim milk–based smoothie with 22.5 g of freeze-dried blueberry powder) Composition per smoothie: total phenolics, 773.6 mg (290.3 mg of ACNs); vitamin C, 2.7 mg</td>
<td>2 smoothies per day (2 × 12-oz yogurt and skim milk–based smoothie without blueberry powder) Composition per smoothie: total phenolics, ND; vitamin C, 0 mg</td>
<td>RHI</td>
<td>Increased RHI after intervention compared with placebo</td>
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a significant increase \((P = 0.01)\) in RHI (from 1.7\% ± 0.4 \% to 2.0\% ± 0.6\%; about +18\%) at 1 hour after cranberry juice consumption (230 mL, twice daily) in patients with peripheral endothelial dysfunction and cardiovascular risk factors.

Conversely, 2 studies did not report significant effects following short-term interventions with berries. Del Bo’ et al.\(^{29}\) showed that a portion of blueberry purée (300 g) did not affect vascular reactivity, measured as RHI, 1 hour after intake in a group of young healthy volunteers with normal peripheral arterial function (RHI > 1.67). A similar result was also documented by Jin et al.\(^{31}\) following the intake of 250 mL of a 20% blackcurrant juice drink in a group of healthy individuals. Using laser Doppler imaging, the investigators measured vascular reactivity to acetylcholine (endothelial dependent) and sodium nitroprusside (endothelial independent), testing the effect 2 hours after intake of the juice.\(^{31}\)

None of the 5 studies included in this systematic review that measured PWV or augmentation index following berry intervention\(^{26–28,32,34}\) reported any significant effect on these outcomes.

### Chronic studies

Table 3 shows the results obtained in 11 medium- to long-term interventions\(^{34–44}\) that investigated the effect of berries on markers of vascular function. Khan et al.\(^{39}\) reported a significant increase \((P = 0.022)\) in flow-mediated dilation (from 5.8\% ± 3.1\% to 6.9\% ± 3.1\%; about +19\%) in healthy individuals who consumed blackcurrant juice (1 L/d, providing 815 mg of total polyphenols) for 6 weeks. Similarly, Stull et al.\(^{42}\) reported a significant improvement in RHI after a 6-week intake of 2 blueberry smoothies (45 g of freeze-dried blueberry powder, providing about 800 mg of total polyphenols) in patients with metabolic syndrome. The results showed a greater effect of blueberry vs placebo (RHI, 0.32 ± 0.13 vs –0.33 ± 0.14, respectively; \(P = 0.0023\)). On the other hand, in 4 studies, no significant effect on RHI or flow-mediated dilation was reported.\(^{36,40,44,45}\) Djurica et al.\(^{44}\) found that a 1-week consumption of 50 g of freeze-dried strawberry powder (equivalent to 500 g of fresh strawberries, with pelargonidin-3-glucoside as the main phenolic compound) did not improve RHI in overweight or obese adolescents. Flammer et al.\(^{45}\) showed that the intake of cranberry juice (230 mL, twice daily) over 4 months had no effect on RHI in patients with peripheral endothelial dysfunction and cardiovascular risk factors. Similarly, Riso et al.\(^{40}\) could not demonstrate an effect on RHI after a 6-week intervention with a wild blueberry–based drink (250 mL/d, providing 475 mg of anthocyanins) in individuals with cardiovascular risk factors. These results were consistent with
those reported by Dohadwala et al, who found that a 4-week intervention with cranberry juice (480 mL/d, providing 94 mg of anthocyanins and 835 mg of total polyphenols) did not affect flow-mediated dilation in patients with coronary artery disease.

With respect to arterial stiffness, 6 of 7 studies showed a positive modulation of PWV and augmentation index following an intervention with berries. Feresin et al reported that daily intake of 240 mL of a strawberry drink (providing 25 g of freeze-dried powder, equivalent to approximately 1.5 cups of fresh strawberries) for 8 weeks significantly decreased brachial artery PWV and femoral artery PWV in postmenopausal women with prehypertension or stage 1 hypertension (− 0.73 m/s, P = 0.03, and − 0.55 m/s, P = 0.02, respectively). Similarly, Johnson et al observed a significant reduction in carotid-femoral PWV (from 1498 ± 179 cm/s to 1401 ± 122 cm/s at about 6.5%; P < 0.05) following an 8-week consumption of a blueberry drink (providing 22 g of freeze-dried blueberry powder per day) in a comparable population. Dohadwala et al documented a significant reduction in carotid-femoral PWV (from 8.3 ± 2.3 m/s to 7.8 ± 2.2 m/s, ie, a decrease of about 6%; P = 0.003) but not in carotid-radial PWV after a 4-week intervention with cranberry juice (480 mL/d) in patients with coronary artery disease. Significant findings were also documented for total peripheral resistance and augmentation index. For example, a 1-week consumption of New Zealand blackcurrant extract (600 mg/d) reduced total peripheral resistance (− 16%; P < 0.05) in healthy males, both at rest and during exercise performance. Similar results were also observed following a 1-week intake of 6 g of New Zealand blackcurrant powder per day in well-trained endurance athletes (total peripheral resistance, − 25%; P = 0.003). Ruel et al observed a decrease in augmentation index (−10.8% ± 6.4%; P < 0.0001) following a 4-week intervention with cranberry juice (500 mL/d, providing 400 mg of total polyphenols) in obese men, while Riso et al reported no significant effect on augmentation index after a 6-week intervention with a wild blueberry–based drink (250 mL/d, providing 475 mg of anthocyanins) in individuals with cardiovascular risk factors.

**Potential risks of bias**

The factors associated with the highest risks of bias were the blinding of participants and the blinding of the outcome assessment during RCTs. For the latter, very few studies declared the lack of blinding, with most studies not stating whether blinding of personnel was implemented. Allocation was frequently 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 product undistinguishable from the berry product. Although all studies included in this review were RCTs, most provided only a poor description, if any, of the randomization methods used. However, it is worth noting that blinding of the randomization process in dietary interventions is sometimes difficult to achieve because of the risk of an unbalanced allocation, which can have a consequent effect on data reliability.

**DISCUSSION**

There is a clear interest in the exploitation of berries and berry-derived products for their potential role in cardiovascular health, with a specific focus on the effects of berries on vascular function. Randomized controlled trials are considered the gold standard for ascertaining a causal relationship between an intervention and its effect. The effect of polyphenol-rich foods in the modulation of vascular reactivity has been evaluated in several intervention studies, but few systematic reviews and meta-analyses have summarized the results of these studies. In some cases, the markers of vascular function that were measured showed inconsistent effects. This could be due to the inclusion of studies with heterogeneous characteristics or a high risk of bias. Moreover, most of the studies focused on bioactive compounds and bioactive-rich foods in general, making the specific effects of berries very difficult to identify. Conversely, the present review exclusively considered studies performed with berries and berry products; studies were selected on the basis of quality criteria, and only RCTs in which either acute or chronic interventions were investigated were included. Overall, the results of this review indicate an improvement in flow-mediated dilation and RHI (markers of vascular reactivity) following acute interventions with berries. In some studies, the observed effects were linked to an increase in plasma circulating levels of berry bioactive constituents, while in others, the findings were attributed to increased circulating phenolic metabolites following metabolism of phenolic compounds in the gut or liver.

Only 1 study showed a dose-response relationship between the intake of berries and vascular reactivity, while 4 studies did not report significant effects following berry consumption. These results may be attributable to the characteristics of the population studied (ie, healthy individuals without specific risk factors and with normal endothelial function at basal levels). Moreover, a number of other factors may have affected the results obtained, including a matrix effect, which could potentially reduce the availability or the impact of bioactive compounds in berries; a small
portion size (even if more realistic) of berries; and the time of evaluation or measurement of vascular function, since polyphenols have a rapid clearance rate and are poorly absorbed.

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

Regarding the effects observed in medium- to long-term interventions, no clear favorable effects of berry products on vascular reactivity markers have been found, which is in line with the systematic review of Heneghan et al that showed an effect in only 3 of 7 studies. The discrepancy between short- and long-term studies in terms of vascular reactivity (measured by RHI and flow-mediated dilation) is intriguing and may be attributed to the complexity of the mechanisms involved in the maintenance of vascular system function. For example, Dohadwala et al reported that changes in nitric oxide-mediated vascular reactivity can occur quickly following a dietary intervention, and this has been related to the rapid absorption of bioactive food components or their metabolites or both (eg, bioactive food components are able to directly or indirectly affect nitric oxide production). This highlights the critical factors that affect the evaluation of vascular function, including the experimental design (eg, in terms of the timing of measurements), the targeted mechanism (eg, nitric oxide production), and the characteristics of the markers used to evaluate vascular reactivity. In fact, the different markers available may provide different information, depending on the study protocol (acute vs chronic intervention). For example, short-term studies can provide information on the direct modulatory effect of the bioactive compounds absorbed (ie, thereby supporting biological plausibility). Conversely, in long-term studies, the exposure to bioactive food compounds is generally absent or limited (owing to the active and rapid clearance of phenolic compounds, even when consumed regularly). In this context, the lack of effect observed in chronic studies, where measurements are taken approximately 12 hours after the last intake of the bioactive compounds, it not surprising. Moreover, the type of markers used may affect the results, depending on the actual targeted measurement.

The large heterogeneity of the enrolled groups of volunteers in the different RCTs (ie, healthy study participants, individuals with cardiovascular risk factors or complications), along with heterogeneity in terms of vascular function, could have affected the results obtained. Moreover, it cannot be excluded that the duration of the intervention was insufficient to exert a beneficial effect in these specific target populations. An additional source of variability might be related to both the form and the manner in which berries were provided. Some studies provided berries as raw fruit, while others provided berries as part of a beverage, a smoothie, or a sweet cake (ie, muffins), alone or in combination with a meal. Moreover, berries may have been provided in addition to the habitual diet (resulting in an increased energy intake) or as substitutes for other foods normally consumed that are thus being displaced from the diet (isocaloric condition). The lack of the food that is replaced under these conditions may be important in determining the precise effect of the intervention on vascular function, although the magnitude of such an effect is difficult to determine. Moreover, differences in the manner of berry administration may have played an important role in the results obtained, since the quality of a meal—in terms of intake of energy, macronutrients, and micronutrients—may affect the vascular response. Furthermore, since polyphenol intake may represent a confounding factor, individuals were often asked to maintain their usual diet and to refrain from consuming berries and other foods during the study period. Despite this, only a few studies provided data on dietary intake, and in these studies the energy intake during the intervention and between treatments was rather constant. Conversely, no information about the actual intake of polyphenols was provided.

Moreover, it is worth noting that the synergistic effects of other substances in berry foods, such as vitamin C, fiber, potassium, and magnesium, may affect the improvement in vascular function. Arterial stiffness has been recognized as a determinant of pulse pressure and elasticity of the blood vessels. The loss of elasticity of the arterial walls reduces the compensatory ability of the walls 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 et al showed that high intakes of anthocyanins and flavones were inversely...
associated with low arterial stiffness (measured as PWV) across extreme quintiles of intake in women. Successively, Lilamand et al.\textsuperscript{17} assessed the relationship between flavonoid intake and arterial stiffness, measured as PWV, analyzing 16 intervention and 2 cross-sectional studies. Four intervention trials reported a significant decrease in 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.\textsuperscript{48} The effects on PWV were more evident after acute intake, while the effects on augmentation index following both acute and chronic interventions were not univocal.

The present review shows that short-term interventions with berries failed to modulate PWV, augmentation index, or stiffness and reflation indexes (Table 2), in line, at least in part, with previous observations. Conversely, results of medium- to long-term interventions suggest an improvement in these markers (Table 3), in accordance with results reported in the review of RCTs by Heneghan et al.\textsuperscript{51} The difference in findings between short-term and medium- to long-term trials may be related to the type of participants enrolled or the duration of treatment. In fact, the short-term interventions were performed in healthy individuals, and it is plausible that substantial variations in arterial stiffness over a short follow-up period are unlikely to be observed in individuals without vascular dysfunction. In addition, the type of marker analyzed (eg, PWV vs augmentation index) and the high between-individual variability could have played a crucial role in the results obtained. It is noteworthy that most studies did not consider arterial stiffness as a primary outcome and that the trials were underpowered for evaluation of arterial stiffness. For this reason, future studies should be specifically designed to ascertain the effect of berries on arterial stiffness.

**Strengths and limitations**

Caution should be used when interpreting results or drawing conclusions about the effect of berries on vascular function, as high heterogeneity among studies was found in terms of the type and dose of berries, the manner of berry administration (ie, as whole fruit, juice drink, or capsules), and the amount and bioavailability of polyphenols provided. Although the inclusion of different types of berries may represent a strength of this review, 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 have different anthocyanin profiles,\textsuperscript{56,57} which can hinder the comparison of study results because anthocyanin intake can vary depending on the berry consumed. For example, cyanidin and pelargonidin derivatives are present as the major anthocyanins in cranberries, while pelargonidin derivatives predominate in strawberries.\textsuperscript{6} Another weakness of this review is the lack of high-quality information about the bioavailability of anthocyanins and related metabolic products. The metabolic fate of anthocyanins after ingestion is deeply influenced by the pH and microbiota of the gut.\textsuperscript{58} It is well documented that anthocyanins, like other phenolic compounds, have a limited bioavailability, ie, less than 15%.\textsuperscript{59} This is influenced by their interaction with several gut microbial strains and the subsequent phase II metabolism in entocytes and hepatocytes, which leads to the production of several different metabolites, including phenylpropionic and phenylacetic acids.\textsuperscript{59–61} Very few in vivo intervention studies have provided information about circulating amounts of these metabolites following berry consumption.\textsuperscript{62} Moreover, it is not always easy to link the biological effects of berry consumption to microbial derivatives of anthocyanins in the gut, as several other phenolic compounds present in berries are also metabolized in the gut.\textsuperscript{63}

Other potential limitations include the study design (acute vs chronic interventions and parallel vs crossover designs), the duration of the intervention, participants' characteristics, and sample size. Most of the studies were performed in healthy individuals. The inclusion of these studies 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 participants. The results of such studies must be considered preliminary and deserve further investigation.

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 limitation. For example, the position of the cuff (upper vs lower arm), the duration of brachial artery occlusion, and the timing of the detection of peak hyperemia still differ between investigators. This information is missing from the studies included in this review, but it is clear that different experimental conditions may have had a role in the modulation of nitric oxide–dependent and –independent vasodilation mechanisms,\textsuperscript{64} thereby affecting the results obtained.

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

**Potential mechanisms of action involved in the modulation of vascular function**

One of the main hypothesized mechanisms of action of polyphenols is the activation of the endothelial nitric oxide synthase and cyclic guanosine monophosphate signaling pathway involved in vasodilation. Once activated, nitric oxide stimulates soluble guanylate cyclase in the vascular smooth muscle cells by releasing cyclic guanosine monophosphate, a second messenger, which induces the smooth muscle cells of the blood vessel to relax.65,66 In addition, polyphenols have been shown to increase the postprandial release of the active glucagon-like peptide 1, a major intestinal hormone that stimulates glucose-induced insulin secretion from β cells, upregulates endothelial nitric oxide synthase expression, and increases endothelial nitric oxide synthase phosphorylation, resulting in improved production of nitric oxide and, thus, endothelium-dependent relaxation.67,68

Besides polyphenols, insulin response may also positively affect vascular function through activation of the endothelial nitric oxide synthase pathway and, thus, vasodilation.69 These processes are usually very fast and were identified as potential mechanisms of action in the short-term studies.

Other putative mechanisms through which polyphenols may affect vasodilation in short-term and medium- to long-term interventions involve the regulation of vascular redox signaling. For example, berry components may activate the nuclear factor E2-related factor 2 (Nrf2) antioxidant or xenobiotic response element signaling pathway, which represents the major mechanism in cellular defense against oxidative and electrophilic stress.65 Furthermore, polyphenols may modulate proinflammatory pathways by inhibiting reactive oxygen species and the redox-sensitive transcription of nuclear factor κB, involved in the gene expression of inducible nitric oxide synthase, cyclooxygenase 2, cytosolic phospholipase 2, and several proinflammatory cytokines, chemokines, and adhesion molecules, all of which play an important role in the regulation of nitric oxide production and the modulation of vascular function.70

**CONCLUSION**

Despite the limitations and confounding factors present in the reviewed studies, the results of this systematic review seem to suggest several potential positive effects of berries on vascular function. In particular, effects on flow-mediated dilation and RHI in short-term studies and on PWV and augmentation index in medium- to long-term studies were observed, suggesting that differences in biomarker modulation may depend on the time of exposure to the dietary intervention, the experimental protocol of the study, or both.

Future research using appropriate study designs that consider current knowledge gaps and combine the use of different biomarkers are highly recommended. Further RCTs in different, well-characterized target populations should be performed to strengthen the evidence of the efficacy of such treatments on vascular health and function, and perhaps to shed more light on the mechanisms underlying the effects observed thus far. For example, studies on the relationship between the structure and the activity of berry polyphenols or their metabolic products could help elucidate the potential mechanisms through which these compounds interact and positively affect the vascular system. Although it is difficult to estimate clinically efficacious doses of berries, most studies have shown an improvement in vascular function with doses higher than 200 g/d (providing at least 600–700 mg of total polyphenols). This data should be considered indicative only. Dose- and time-dependent studies are needed to better identify the amount of berries (and related polyphenols) that elicit a beneficial effect on vasodilation. The results of such studies could be useful for the development of new products with vasoactive properties that might help to maintain vascular health and reduce the incidence of cardiovascular disease in certain identified target groups.

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Author contributions. D.M. and D.A. wrote the first draft of the manuscript. M.M. and D.M. conducted the literature search, reviewed the abstracts of the studies selected, and prepared the tables. C.D.B. acted as a third independent reviewer and improved the manuscript. D.D.R., P.R., and M.P. critically revised the scientific content and improved the quality of the manuscript.

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Declaration of interest. The authors have no relevant interests to declare.

Supporting Information
The following Supporting Information is available through the online version of this article at the publisher’s website.

Table S1 PRISMA checklist

Figure S1 Risk of bias for each item assessed in each of the included studies

Figure S2 Graph showing risk of bias for each item assessed, presented as a percentage across all included studies combined

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