DISSERTATION

CARDIOVASCULAR-PROTECTIVE EFFECTS OF BLUEBERRY CONSUMPTION IN POSTMENOPAUSAL WOMEN WITH ABOVE-NORMAL BLOOD PRESSURE

Submitted by

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ABSTRACT

CARDIOVASCULAR-PROTECTIVE EFFECTS OF BLUEBERRY CONSUMPTION IN POSTMENOPAUSAL WOMEN WITH ABOVE-NORMAL BLOOD PRESSURE

Endothelial dysfunction is the first step in atherosclerosis and contributes to its progression, and thus, is central to cardiovascular disease (CVD). It is driven by excessive oxidative stress and inflammation and characterized by impaired endothelium-dependent dilation. Estrogen-deficient postmenopausal women have oxidative stress-mediated suppression of endothelial function that is worsened by high blood pressure. Chronic blueberry consumption may be a beneficial dietary intervention for this population as it has shown to improve vascular function and blood pressure, though some studies have not demonstrated efficacy possibly due to the observed high interindividual variability in response to the intervention. Evidence indicates blueberries improve endothelial function, but studies have not been performed in postmenopausal women. Furthermore, *ex vivo* research has shown that blueberry (poly)phenols and their metabolites can decrease endothelial oxidative stress and inflammation, but whether these mechanisms translate to humans is unclear. The objectives of this dissertation were to 1) examine the efficacy of chronic blueberry consumption to improve endothelial function and blood pressure in estrogen-deficient postmenopausal women with above-normal blood pressure, with a specific focus on identifying mechanisms for improving endothelial function, 2) identify factors that contributed to the efficacy of blueberries as a dietary intervention for improving endothelial function, and 3) explore cellular mechanisms responsible for endothelial function improvements and the anti-atherogenic potential of blueberries. To investigate the aforementioned, we conducted a randomized, double-blind, placebo-controlled clinical trial and assessed endothelial function (measured through flow-mediated dilation (FMD))

and supine brachial blood pressure before and after daily consumption of 22 g of freeze-dried highbush blueberry powder or isocaloric placebo powder for 12 weeks. To examine mechanisms for improved endothelial function, FMD was assessed before and after infusing a supraphysiological dose of the antioxidant ascorbic acid (i.e. vitamin C) and normalized to shear rate area under the curve (FMD/SR_{AUC}). To investigate factors impacting the interindividual variability in the endothelial function responses after the 12 weeks of blueberry consumption, we grouped the blueberry treatment group into responders (≥ +1% unit Δ FMD) and non-responders (< +1% unit Δ FMD) and performed secondary statistical analyses using data produced from the clinical trial. Lastly, to investigate mechanisms for improvements in endothelial function, we used a reverse translational human-to-cell approach leveraging human blood serum collected from participants in the clinical trial to perform *ex vivo* cell culture experiments. Results from the clinical trial showed that daily blueberry consumption significantly improved FMD/SR_{AUC} compared to baseline by 96%. FMD not normalized for shear rate increased by 1.34% though the effects were not statistically significant (but were clinically significant). Improvements in FMD/SR_{AUC} after blueberry consumption were due to reductions in oxidative stress as responses to ascorbic acid infusion were significantly reduced at 12 weeks in the blueberry group compared to baseline, with no changes in the placebo group. There were no major effects on blood pressure, arterial stiffness, endothelial cell protein expression, or other blood biomarkers of cardiovascular health. It was determined that the blueberry intervention was ~50% effective for improving FMD to clinically relevant levels of ≥ +1%, and that responders had decreased cardiovascular health and higher levels of circulating estrogen at baseline compared to non-responders. After 12 weeks of blueberry consumption, responders had reductions in oxidative stress, lower plasma nitrate levels, and higher phosphorylated endothelial nitric oxide synthase protein expression compared to non-responders. Lastly, we cultured HAECs with 15% serum (blueberry and placebo) for 1 h followed by 200 μM hydrogen peroxide (H_2O_2) for 24 h to induce endothelial dysfunction and evaluated the effects of blueberry

(poly)phenol-rich serum on endothelial cell dysfunction and atherosclerosis progression. There were no statistically significant differences on monocyte binding, insulin-stimulated nitric oxide production, or peroxynitrite concentrations between dysfunctional HAECs treated with blueberry and placebo serum from the clinical trial. Collectively, results from these studies indicate that daily blueberry consumption for 12 weeks improves endothelial function in postmenopausal women with above-normal blood pressure through reductions in oxidative stress, and that efficacy (i.e. degree to which postmenopausal women responded to treatment in endothelial function) seems to be dependent on participant characteristics including cardiovascular risk factors and estradiol at baseline. Due to the inconclusive results regarding the *ex vivo* experiment, cellular mechanisms by which blueberry (poly)phenol metabolites impact endothelial function and atherosclerosis progression cannot be determined.

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CHAPTER 1: INTRODUCTION

About 19 million deaths globally were attributed to cardiovascular disease (CVD) in 2022, increasing by 18.7% from 2010.[1] Endothelial dysfunction, characterized by impaired endothelium-dependent dilation of the blood vessels, is an underlying cause for CVD and initial step of atherosclerosis.[2, 3] Oxidative stress and inflammation are two major mechanisms responsible for endothelial dysfunction as they decrease the production and bioavailability of the potent vasodilator nitric oxide (NO), among other adverse effects.[2, 4, 5] Modifiable (e.g. high blood pressure) and non-modifiable (e.g. aging and menopause) risk factors contribute to endothelial dysfunction in part by increasing oxidative stress and inflammation.[6, 7] Specifically, women have progressive impairments in endothelial dysfunction through the menopausal transition due to reductions in estrogen production, and have been demonstrated to have oxidative stress-mediated suppression of endothelial function.[7-10] Estrogen is cardioprotective, as it acts as an antioxidant by scavenging free radicals and promotes endothelial function by binding to estrogen receptors on the endothelial cell to trigger NO production, among other effects.[9, 11, 12] Furthermore, postmenopausal women are at increased risk for having high blood pressure and tend to be the aging population, further contributing to their increased risk for CVD through increased oxidative stress and inflammation.[6, 13-18] Therefore, interventions targeted at reducing oxidative stress and inflammation to improve endothelial function are crucial for reducing CVD risk in postmenopausal women with above-normal blood pressure.

(Poly)phenols are secondary plant metabolites and their consumption has been shown to be beneficial for CVD prevention and management, partly due to their ability to reduce oxidative stress and inflammation.[19-22] Blueberries are rich in (poly)phenols, particularly anthocyanins and phenolic acids, and thus have high potential in this regard.[23, 24] We previously showed in a randomized controlled trial that 22 g of freeze-dried highbush blueberry powder consumed for 8 weeks significantly improved blood pressure, peripheral arterial stiffness (CVD

risk factor known to follow endothelial dysfunction), and plasma NO concentrations in postmenopausal women with above-normal blood pressure.[25] Additional research has shown that chronic blueberry consumption improved endothelial function in healthy men and adults with metabolic syndrome,[26-28] but whether blueberry consumption can improve endothelial function in postmenopausal women with above-normal blood pressure remains unknown. Furthermore, an important factor to consider when investigating the health impacts of diet and nutrition is interindividual variability, as human physiology, metabolism, the gut microbiota, and health status, among other factors, may affect individual's response to dietary interventions.[29-31] Therefore, exploring factors impacting interindividual variability in the efficacy of blueberries is crucial for establishing blueberries as a dietary intervention for improving endothelial function in postmenopausal women with above-normal blood pressure. Lastly, as it remains unclear as to the direct mechanisms by which blueberries improve endothelial function in humans. *Ex vivo* experiments suggest that blueberries can improve attributes of endothelial dysfunction by decreasing vascular oxidative stress and inflammation.[32-37] However, due to the low bioavailability of (poly)phenols in humans after blueberry consumption,[38] the beneficial effects of blueberry consumption on the endothelium may be due to the (poly)phenol metabolites generated by human and gut microbial metabolism. Specifically, blueberry-derived metabolites at physiologically relevant concentrations have reduced monocyte binding and ROS production, while increasing NO production in lipotoxicity- and inflammatory-induced human aortic endothelial cells (HACEs).[33, 37] Yet, whether this is true for an oxidative stress-mediated dysfunctional endothelial cell model remains unknown. Furthermore, such studies used blueberry-derived metabolites that were not generated from human digestion and metabolism,[33, 37] therefore, leaving a gap in the knowledge for understanding the mechanisms associated with improvements in endothelial function in postmenopausal women after chronic blueberry consumption.

The **research objectives** of this dissertation were to 1) examine the efficacy of chronic blueberry consumption to improve endothelial function and blood pressure in estrogen-deficient postmenopausal women with above-normal blood pressure, with a specific focus on identifying mechanisms for improving endothelial function (Chapter 3), 2) identify factors that contributed to the efficacy of blueberries as a dietary intervention for improving endothelial function (Chapter 4), and 3) explore cellular mechanisms responsible for endothelial function improvements and the anti-atherogenic potential of blueberries (Chapter 5).

REFERENCES

1. C. W. Tsao, A. W. Aday, Z. I. Almarzooq, A. Alonso, A. Z. Beaton, M. S. Bittencourt, A. K. Boehme, A. E. Buxton, A. P. Carson, Y. Commodore-Mensah, M. S. V. Elkind, K. R. Evenson, C. Eze-Nliam, J. F. Ferguson, G. Generoso, J. E. Ho, R. Kalani, S. S. Khan, B. M. Kissela, K. L. Knutson, D. A. Levine, T. T. Lewis, J. Liu, M. S. Loop, J. Ma, M. E. Mussolino, S. D. Navaneethan, A. M. Perak, R. Poudel, M. Rezk-Hanna, G. A. Roth, E. B. Schroeder, S. H. Shah, E. L. Thacker, L. B. VanWagner, S. S. Virani, J. H. Voecks, N. Y. Wang, K. Yaffe and S. S. Martin, *Circulation*, 2022, **145**, e153-e639.
2. P. M. Vanhoutte, H. Shimokawa, M. Feletou and E. H. Tang, *Acta Physiol (Oxf)*, 2017, **219**, 22-96.
3. P. Rajendran, T. Rengarajan, J. Thangavel, Y. Nishigaki, D. Sakthisekaran, G. Sethi and I. Nishigaki, *Int J Biol Sci*, 2013, **9**, 1057-1069.
4. K. H. Park and W. J. Park, *J Korean Med Sci*, 2015, **30**, 1213-1225.
5. H. N. Siti, Y. Kamisah and J. Kamsiah, *Vascul Pharmacol*, 2015, **71**, 40-56.
6. S. A. Johnson, N. S. Litwin and D. R. Seals, *J Acad Nutr Diet*, 2019, **119**, 1785-1796.
7. K. L. Moreau and K. L. Hildreth, *Adv Vasc Med*, 2014, **2014**.
8. K. L. Moreau, K. L. Hildreth, A. L. Meditz, K. D. Deane and W. M. Kohrt, *J Clin Endocrinol Metab*, 2012, **97**, 4692-4700.
9. K. L. Moreau, K. L. Hildreth, J. Klawitter, P. Blatchford and W. M. Kohrt, *Geroscience*, 2020, **42**, 1699-1714.
10. E. K. Woolf, J. D. Terwoord, N. S. Litwin, A. R. Vazquez, S. Y. Lee, N. Ghanem, K. A. Michell, B. T. Smith, L. E. Grabos, N. B. Ketelhut, N. P. Bachman, M. E. Smith, M. Le Sayec, S. Rao, C. L. Gentile, T. L. Weir, A. Rodriguez-Mateos, D. R. Seals, F. A. Dinenno and S. A. Johnson, *Food Funct*, 2023, **14**, 2621-2641.

11. Y. B. Somani, J. A. Pawelczyk, M. J. De Souza, P. M. Kris-Etherton and D. N. Proctor, *Am J Physiol Heart Circ Physiol*, 2019, **317**, H395-h404.

12. A. Iorga, C. M. Cunningham, S. Moazeni, G. Ruffenach, S. Umar and M. Eghbali, *Biol Sex Differ*, 2017, **8**, 33.

13. L. L. Yanes and J. F. Reckelhoff, *Am J Hypertens*, 2011, **24**, 740-749.

14. D. Mozaffarian, E. J. Benjamin, A. S. Go, D. K. Arnett, M. J. Blaha, M. Cushman, S. de Ferranti, J. P. Després, H. J. Fullerton, V. J. Howard, M. D. Huffman, S. E. Judd, B. M. Kissela, D. T. Lackland, J. H. Lichtman, L. D. Lisabeth, S. Liu, R. H. Mackey, D. B. Matchar, D. K. McGuire, E. R. Mohler, 3rd, C. S. Moy, P. Muntner, M. E. Mussolino, K. Nasir, R. W. Neumar, G. Nichol, L. Palaniappan, D. K. Pandey, M. J. Reeves, C. J. Rodriguez, P. D. Sorlie, J. Stein, A. Towfighi, T. N. Turan, S. S. Virani, J. Z. Willey, D. Woo, R. W. Yeh and M. B. Turner, *Circulation*, 2015, **131**, e29-322.

15. Y. Brahmbhatt, M. Gupta and S. Hamrahian, *Curr Hypertens Rep*, 2019, **21**, 74.

16. T. D. Giles, G. E. Sander, B. D. Nossaman and P. J. Kadowitz, *J Clin Hypertens (Greenwich)*, 2012, **14**, 198-205.

17. D. R. Seals and L. M. Alexander, *J Appl Physiol (1985)*, 2018, **125**, 1841-1842.

18. D. R. Seals, K. L. Jablonski and A. J. Donato, *Clin Sci (Lond)*, 2011, **120**, 357-375.

19. T. Hussain, B. Tan, Y. Yin, F. Blachier, M. C. Tossou and N. Rahu, *Oxid Med Cell Longev*, 2016, **2016**, 7432797.

20. R. Andriantsitohaina, C. Auger, T. Chataigneau, N. Étienne-Selloum, H. Li, M. C. Martínez, V. B. Schini-Kerth and I. Laher, *Br J Nutr*, 2012, **108**, 1532-1549.

21. M. H. Oak, C. Auger, E. Belcastro, S. H. Park, H. H. Lee and V. B. Schini-Kerth, *Free Radic Biol Med*, 2018, **122**, 161-170.

22. C. C. Tangney and H. E. Rasmussen, *Curr Atheroscler Rep*, 2013, **15**, 324.

23. S. Silva, E. M. Costa, M. Veiga, R. M. Morais, C. Calhau and M. Pintado, *Crit Rev Food Sci Nutr*, 2020, **60**, 181-200.

24. E. Wood, S. Hein, C. Heiss, C. Williams and A. Rodriguez-Mateos, *Food Funct*, 2019, **10**, 7621-7633.

25. S. A. Johnson, A. Figueroa, N. Navaei, A. Wong, R. Kalfon, L. T. Ormsbee, R. G. Feresin, M. L. Elam, S. Hooshmand, M. E. Payton and B. H. Arjmandi, *J Acad Nutr Diet*, 2015, **115**, 369-377.

26. A. J. Stull, K. C. Cash, C. M. Champagne, A. K. Gupta, R. Boston, R. A. Beyl, W. D. Johnson and W. T. Cefalu, *Nutrients*, 2015, **7**, 4107-4123.

27. P. J. Curtis, V. van der Velpen, L. Berends, A. Jennings, M. Feelisch, A. M. Umpleby, M. Evans, B. O. Fernandez, M. S. Meiss, M. Minnion, J. Potter, A. M. Minihane, C. D. Kay, E. B. Rimm and A. Cassidy, *Am J Clin Nutr*, 2019, **109**, 1535-1545.

28. A. Rodriguez-Mateos, G. Istas, L. Boschek, R. P. Feliciano, C. E. Mills, C. Boby, S. Gomez-Alonso, D. Milenkovic and C. Heiss, *J Gerontol A Biol Sci Med Sci*, 2019, **74**, 967-976.

29. C. L. Bush, J. B. Blumberg, A. El-Sohemy, D. M. Minich, J. M. Ordovás, D. G. Reed and V. A. Y. Behm, *J Am Coll Nutr*, 2020, **39**, 5-15.

30. M. T. García-Conesa, K. Chambers, E. Combet, P. Pinto, M. Garcia-Aloy, C. Andrés-Lacueva, S. de Pascual-Teresa, P. Mena, A. Konic Ristic, W. J. Hollands, P. A. Kroon, A. Rodríguez-Mateos, G. Istas, C. A. Kontogiorgis, D. K. Rai, E. R. Gibney, C. Morand, J. C. Espín and A. González-Sarrías, *Int J Mol Sci*, 2018, **19**.

31. D. Milenkovic, C. Morand, A. Cassidy, A. Konic-Ristic, F. Tomás-Barberán, J. M. Ordovas, P. Kroon, R. De Caterina and A. Rodriguez-Mateos, *Adv Nutr*, 2017, **8**, 558-570.

32. J. S. Tang, M. C. M. Vissers, R. F. Anderson, S. Sreebhavan, S. M. Bozonet, A. Scheepens and L. D. Melton, *Mol Nutr Food Res*, 2018, **62**.

33. J. S. Tang, S. M. Bozonet, J. L. McKenzie, R. F. Anderson, L. D. Melton and M. C. M. Vissers, *Mol Nutr Food Res*, 2019, **63**, e1900478.

34. W. Y. Huang, L. Fu, C. Y. Li, L. P. Xu, L. X. Zhang and W. M. Zhang, *J Food Sci*, 2017, **82**, 1239-1246.

35. W.-Y. Huang, X.-N. Wang, J. Wang and Z.-Q. Sui, *Natural Product Communications*, 2018, **13**, 1934578X1801300115.

36. W. Huang, R. P. Hutabarat, Z. Chai, T. Zheng, W. Zhang and D. Li, *Int J Mol Sci*, 2020, **21**.

37. D. Bharat, R. R. M. Cavalcanti, C. Petersen, N. Begaye, B. R. Cutler, M. M. A. Costa, R. Ramos, M. R. Ferreira, Y. Li, L. P. Bharath, E. Toolson, P. Sebahar, R. E. Looper, T. Jalili, N. S. Rajasekaran, Z. Jia, J. D. Symons and P. V. Anandh Babu, *Mol Nutr Food Res*, 2018, **62**.

38. S. Zhong, A. Sandhu, I. Edirisinghe and B. Burton-Freeman, *Mol Nutr Food Res*, 2017, **61**.

CHAPTER 2: BLUEBERRIES AND VASCULAR FUNCTION: REVIEW OF THE EVIDENCE AND POTENTIAL MECHANISMS

Summary

Blueberries are rich in nutrients and (poly)phenols with demonstrated health-protective effects. They are popular to consumers, a major agricultural crop with year-round availability, and have high nutritional quality making them ideal for use in food-based strategies for promoting human health. Accumulating evidence in animals and humans indicates blueberry consumption has cardiovascular-protective benefits. With aging, structural and functional changes to the vasculature occur that can lead to endothelial dysfunction and large elastic arterial stiffening (i.e. vascular dysfunction) which promotes cardiovascular disease (CVD) development. This narrative review synthesizes the evidence on the impact of blueberries on vascular function, including endothelial function and arterial stiffness, and provides insight into underlying mechanisms responsible for clinical effects in humans based on results from cell, animal, and human studies with a focus on oxidative stress, inflammation, and the gut microbiota. Background information on blueberries and vascular function is provided to give context to included studies evaluating the impact of blueberries and their (poly)phenols on vascular function. Acute and chronic blueberry consumption has been shown to improve measures of vascular function in animals and humans. Specifically, evidence indicates that blueberries can improve endothelial function in different healthy and at-risk populations and may modulate arterial stiffness, but that evidence is less certain. Results from cell, animal, and human studies suggest that blueberry consumption improves vascular function through enhancing NO bioavailability and attenuating oxidative stress and inflammation. Limited data in animals suggest the gut microbiome mediates the vascular-protective effects of blueberries; however, there is a paucity of studies in humans. Further research is needed to establish the

clinical efficacy of blueberries to improve vascular function in diverse human populations in a manner that provides mechanistic information. Translation of clinical research to the community/public in a manner that considers feasibility, health equity, community needs, barriers and drivers to consumption, among other factors while evaluating efficacy is needed to establish real-world impacts of blueberry consumption on vascular function and overall cardiovascular health.

Introduction

Cardiovascular disease (CVD) is the leading cause of death in the United States (US), followed by cancer and COVID-19 in 2021.[1] CVD is an umbrella term for disorders affecting the heart and blood vessels, including coronary heart disease, heart attack, stroke, and heart failure.[2] The underlying etiology of CVD is complex and multifactorial, but involves non-modifiable and modifiable risk factors such as age, menopausal status, hypertension (HTN), dyslipidemia, and diet/nutrition, with age being the primary risk factor.[3] Based on National Health and Nutrition Examination Survey (NHANES) 2015-2018 data, CVD prevalence in adults aged 20 years and older was estimated to be 49.2% and found to increase with age.[4] With aging, unfavorable structural and functional changes occur throughout the cardiovascular system contributing to vascular dysfunction which includes endothelial dysfunction and large elastic arterial stiffening.[5] Both endothelial dysfunction and arterial stiffness have been shown to be predictive of CVD and CVD-related events.[6-8] Two main underlying mechanisms of vascular dysfunction include oxidative stress and inflammation, while the gut microbiome has more recently emerged as a major contributing factor.[9-11]

Epidemiological, animal, and clinical studies have shown that consuming (poly)phenol-rich foods may reduce CVD risk through improvements in vascular function.[12-17] (Poly)phenols are secondary plant metabolites necessary for plant health, serving as antioxidants and protecting against biotic and abiotic stressors in plant flowers, leaves, stems, and roots.[18] They

are characterized by their phenolic structures and hydroxyl moieties and are classified as flavonoid and non-flavonoid compounds, with flavonoids having a 15-carbon skeleton consisting of two phenol rings and a heterocyclic ring, and non-flavonoids containing a single phenol ring.[18] Flavonoid subclasses include anthocyanins (ACNs), flavan-3-ols, flavanones, flavones, flavonols, and isoflavones, and non-flavonoid subclasses include lignans, phenolic acids, and stilbenes. Following ingestion and depending on their chemical structure, bioaccessible (poly)phenols are absorbed and metabolized in the upper gastrointestinal (GI) tract and transported to the liver for further metabolism, while unabsorbed (poly)phenols pass to the large intestine for gut microbial metabolism followed by metabolite absorption and transport to the liver. (Poly)phenols and their metabolites have been shown to exert physiological effects in animals and humans through various mechanisms including, but not limited to, attenuating oxidative stress and inflammation, and modulation of the gut environment including the gut microbiota.

Blueberries are rich in nutrients and (poly)phenols and have demonstrated health-protective effects, particularly on the cardiovascular system including vascular function.[12, 17] Studies in animals suggest blueberries exert cardioprotective effects through improving oxidative stress and inflammation, and modulation of the gut microbiota with blueberry consumption.[17, 19] The aim of this narrative review is to synthesize the evidence on the impact of blueberries on vascular function, including endothelial function and arterial stiffness, in animals and humans. An additional aim is to provide insight into underlying mechanisms responsible for clinical effects in humans based on results from cell, animal, and human studies with a focus on oxidative stress, inflammation, and the gut microbiota. Background information on blueberries and vascular function is provided to give context to included studies evaluating the impact of blueberries and their (poly)phenols on vascular function.

Blueberry Components and Metabolism

Blueberries are one of the few fruits native to North America, and the US is the world's largest producer with consumption significantly increasing over the past decade. In fact, consumption per capita has progressively increased and was estimated to exceed 1.79 pounds per person in 2017 and peaking at 2019 (based on most recently available data).[20] Their popularity by consumers, year-round availability, and nutritional quality makes them ideal for use in food-based intervention strategies for preserving vascular function with aging, and thus, reducing CVD risk. Indeed, blueberries are rich in nutrients including dietary fiber, vitamins C and K, and manganese,[21] and non-nutrient bioactive compounds (i.e. (poly)phenols) such as flavonoid compounds like ACNs, proanthocyanidins, flavan-3-ols, and flavonols, and non-flavonoid compounds such as phenolic acids and pterostilbene.[12, 22-24]

While many of the beneficial health effects of blueberries are largely attributed to their (poly)phenols (based on evidence), certain nutrients in blueberries may also protect against CVD through effects on vascular function. For instance, vitamin C has been widely studied and shown to protect against arterial stiffening, as well as lowering blood pressure in older adults.[25] Furthermore, a systematic review and meta-analysis from 44 randomized controlled clinical trials showed that vitamin C supplementation was associated with positive effects on endothelial function, particularly in those with higher CVD risk.[26] Mechanisms for improved vascular health with vitamin C supplementation may include direct free radical scavenging (i.e. reducing oxidative stress) and increased NO production/bioavailability.[26] However, vitamin C supplementation has not been shown to translate to long-term cardiovascular-protective effects, highlighting the benefit of obtaining nutrients through the diet rather than supplementation.[27-29] Indeed, dietary supplementation with vitamins and minerals in general has been associated with little or no benefit in long-term CVD prevention.[29] Vitamin K is another cardiovascular-protective nutrient that has been shown to have positive effects on vascular calcification, a process that can lead to arterial stiffening and elevated blood pressure.[30] The mineral manganese may have

benefits for vascular health and CVD prevention as it is linked with lower inflammation and oxidative stress, and improved antioxidant defense.[31, 32] Lastly, dietary fiber is found in blueberries and has been shown to be a prebiotic (energy source) for gut microbiota, which then in turn can produce key metabolites (e.g. short chain fatty acids, (poly)phenol metabolites) beneficial for improving endothelial function and other aspects of cardiovascular health.[33-35]

With respect to blueberry (poly)phenols, metabolites resulting from gut microbial metabolism and phase I and II metabolism are increasingly being linked to blueberries' health effects rather than the parent compounds themselves which have low bioavailability in the GI tract.[12, 36-38] After (poly)phenol consumption, parent compounds travel to the stomach where a small amount of absorption may take place. Reports are mixed on the absorption of (poly)phenols in the stomach, but specific phenolic acids, quercetin, and ACNs (i.e. (poly)phenols found in blueberries) have been shown to undergo gastric absorption in humans and animals.[39-43] All remaining parent (poly)phenols travel to the small intestine where some undergo deconjugation reactions such as deglycosylation and phase I and II metabolism in enterocytes after absorption, followed by metabolite release into portal circulation for transport to the liver for further phase II metabolism. Many (poly)phenols including ACNs remain intact until they reach the large intestine for gut microbial metabolism, colonocyte phase II metabolism, and absorption into portal circulation for transport to the liver.[37, 38] In fact, less than 1% of parent ACNs are found in urine after blueberry consumption indicating low bioavailability.[12, 43] Following metabolism in the liver, (poly)phenol metabolites enter systemic circulation for travel to target tissues, and then elimination through urine or feces.[12, 37, 38, 44]

Due to this extensive and diverse metabolism, the pharmacokinetic profiles of (poly)phenols are varied. Specifically, blueberry (poly)phenol metabolites have been detected in plasma within the first couple of hours after ingestion (namely catechols, followed by benzoic acids, cinnamic acids, hippuric acids, and flavonols, among others), while gut-derived (poly)phenol metabolites (namely hippuric acids, followed by catechols and benzoic acids,

among others) can peak at later time points following acute and chronic blueberry consumption.[43, 45-47] The detection of blueberry (poly)phenol metabolites in plasma is dependent on the amount of blueberries (and (poly)phenols) consumed and food matrix interactions, among other factors.[43, 46, 48] The contribution of blueberry (poly)phenols and their metabolites (individually, together, and in relation to other nutrient and non-nutrient components) to their vascular-protective effects is not fully understood, but is an area of active investigation within the scientific community. Indeed, several studies have demonstrated acute blueberry consumption improves measures of vascular function, and that improvements are associated with increases in plasma (poly)phenol metabolites.[45-47] Few chronic intervention studies with blueberries have evaluated the relationship between improvements in vascular function and circulating (poly)phenol metabolites.[47, 49, 50] Those that have evaluated this relationship have not established a clear link,[47, 49, 50] potentially due to the high turnover and excretion of (poly)phenol metabolites[51] or other factors.

Blueberries and Vascular Function Evidence Review Methods

PubMed and Google Scholar were used to identify studies examining the impact of blueberry consumption on vascular function. Animal and human research studies were included in the search if the treatment was in the form of whole blueberries, blueberry powder, blueberry extract, and/or blueberry metabolites, and if outcome measures included endothelial function and/or arterial stiffness parameters. Cell culture research studies that assessed mechanisms associated with improvements in vascular function were also included in the search if they were conducted in vascular smooth muscle cells (VSMC) or endothelial cells using any form of blueberry. For this, search terms were as follows: blueberry cardiovascular, blueberry endothelial, blueberry endothelial dysfunction, blueberry endothelial function, blueberry flow-mediated dilation, blueberry reactive hyperemia index, blueberry pulse wave velocity, blueberry augmentation index, blueberry arterial stiffness, blueberry, blueberry human, blueberry human

cardiovascular, blueberry vascular function, blueberry vascular dysfunction, blueberry smooth muscle cell, blueberry endothelial cell, blueberry gut microbiome, blueberry gut microbiome CVD, blueberries gut microbiome, blueberries pulse wave velocity, and blueberries gut microbiota pulse wave velocity. These terms were searched in PubMed using "AND" in between terms as well.

Ten animal studies and 21 human studies assessing the impact of blueberry consumption on vascular function *in vivo* were included. There were 16 cell culture studies assessing mechanisms associated with blueberry consumption and improved vascular function. However, a primary focus of the review is to gain insight into how blueberries improve vascular function in humans, and therefore, only 8 cell studies that included models of (or relevant to) human digestion, absorption, and/or metabolism were included. Included cell culture studies using blueberry (poly)phenol metabolites reported using physiologically relevant concentrations of (poly)phenol metabolites commonly found in human plasma after blueberry consumption. For the purpose of this review, a physiologically relevant dose of metabolites is defined as ≤1 μM (μg/mL or μmol/L) for individual (poly)phenol metabolites, unless physiological concentrations were specified and justified by authors.[43, 45, 47, 52] To our knowledge, all cell, animal, and human studies to date that focused on blueberry consumption and vascular function have been accounted for in the article search and review.

Blueberries and Endothelial (Dys)Function

Endothelial (dys)function overview and methods of assessment

The endothelium, once termed the "wallpaper" of the blood vessels, is a cell monolayer located in the tunica intima of the vasculature that acts as a barrier between blood and the vessel wall.[53] It controls vascular responses to stimuli such as hormones, neurotransmitters, and shear stress.[53] A healthy endothelium is defined by its ability to adequately maintain vascular tone (i.e. the balance between vasoconstriction and vasodilation), as well as to

regulate inflammation, thrombosis, and other aspects of vascular health.[54] Endothelial dysfunction occurs when there is an imbalance, favoring a more vasoconstricted (decreased vasodilation), proinflammatory, prothrombotic, and pro-oxidant state,[54, 55] which is central to the initiation and progression of atherosclerosis, the cause of most CVD.[55]

The main hallmark or clinical manifestation of endothelial dysfunction is impaired endothelium-dependent dilation (i.e. vasodilation) due to the decreased production and/or bioavailability of the endothelium-derived vasodilatory molecule nitric oxide (NO).[5, 56] Stimuli such as shear stress causes production of NO from L-arginine through activation of endothelial NO synthase (eNOS) in the presence of essential cofactors such as tetrahydrobiopterin (BH_4).[56, 57] NO is then released into VSMCs to stimulate vasodilation for increased blood flow to peripheral tissues.[56] NO also has anti-inflammatory and anti-thrombotic properties,[57] therefore, reduced NO bioavailability can promote atherosclerotic CVD development. CVD risk factors such as advanced age, postmenopausal status, high blood pressure, and dyslipidemia can impair NO production and bioavailability, and thus, can promote endothelial dysfunction, atherosclerotic plaque accumulation, and ultimately increase CVD risk.[3, 5, 9, 57-62] Specifically, vascular oxidative stress impairs NO production and bioavailability, and is largely caused by excessive superoxide radical production due to increased NADPH oxidase (NOX) activity, mitochondrial dysfunction, and eNOS uncoupling (i.e. where eNOS produces superoxide instead of NO).[56, 57] Superoxide radicals reduce NO production and bioavailability through several mechanisms including direct NO scavenging thereby rendering it unavailable to function, and eNOS uncoupling.[3, 56, 57] Through a bidirectional relationship with oxidative stress, inflammation also contributes to endothelial dysfunction by inhibiting eNOS and increasing ROS production.[3, 9, 63, 64]

In animals, endothelial function is assessed through analysis of vasoconstriction and vasodilation using pressure myography *ex vivo*, which measures outer and lumen diameters and wall thickness of isolated and pressurized arteries.[65] Though each independent laboratory

utilizes specific and varied techniques (e.g. choice of pharmacological agents used to assess endothelium-dependent mechanisms such as inhibition of enzyme activity, specific vessel type, outcome units of measure, etc.), certain methods are more commonly used. In short, isolated vessels are placed on pressure myography prongs and are re-pressurized. Following phenylephrine (Phe)-induced contraction, increased concentrations of the vasodilator acetylcholine (Ach) are applied to the vessels to measure vessel diameter expansion over a specific time period until max vasodilation occurs and is observed through an imaging microscope system.[65] Results obtained from studies and presented in this review are described as vasoconstriction, vasorelaxation, and vessel sensitivity.

In humans, there are several assessments of endothelium-dependent dilation including non-invasive and invasive methods. For the purpose of this review, only two non-invasive methods used in published studies on blueberries and endothelial function in humans are included, which are ultrasound-assessed brachial artery flow-mediated dilation (FMD) and EndoPAT®-assessed peripheral arterial tonometry (PAT). Both methods assess blood flow in response to a hyperemic stimulus which is reflective of endothelial function, but each assessment has different features. FMD uses a high-resolution ultrasound to assess macrovascular function of conduit arteries by measuring brachial artery diameter change in response to increased blood flow following the release of arterial occlusion.[66] FMD is represented as the change in peak artery diameter in response to increased blood flow. Also, considering shear stress (i.e. the stimulus for vasodilation) varies between individuals and populations, normalizing the FMD response to shear rate (FMD/SR_{AUC}) can provide better insight into endothelium-dependent dilation.[67-69] On the other hand, PAT uses the EndoPAT® device to measure microvascular endothelial function by assessing pulse wave amplitude through the fingertips in response to increased blood flow following the release of arterial occlusion, generating the reactive hyperemia index (RHI) and each participant is served as their own control.[66, 70] FMD is considered the gold standard for non-invasive assessment of

endothelial function in humans, but is highly operator-dependent, challenging, time-consuming to perform/analyze, and requires extensive operator training.[71] On the other hand, PAT has simpler procedures and operator-independency, but may not be directly comparable to FMD as each method assesses endothelial function in different vascular beds.[66, 72] Nonetheless, both methods have been shown to be reflective of endothelial function, validated, and predictive of CVD and related events, and as such, are clinically relevant.[7, 66, 70, 71, 73-77]

Effects of blueberries on endothelial (dys)function: animal studies

Ten studies examined the impact of blueberry consumption on endothelial function in animal models using pressure myography *ex vivo* (**Table 2.1**). Four studies were performed with healthy male rats. First, in thoracic aorta rings of male Sprague-Dawley (SD) rats fed a diet with 8% w/w freeze-dried wild blueberry powder for 13 weeks, a control diet for 13 weeks, or a control diet for 13 weeks followed by a diet with 8% w/w freeze-dried wild blueberry powder for 8 weeks (reverse diet), endothelium-dependent vasoconstriction to Phe decreased in both blueberry diet groups compared to the control group.[78] In another study with male SD rats, 8% w/w freeze-dried wild blueberry powder added to diets for 7 weeks decreased vasoconstriction (to Phe) and vessel sensitivity to Phe and Ach, and increased Ach-induced vasorelaxation in thoracic aorta rings compared to the control group.[79] A study in male SD rats observed that vasoconstriction to Phe decreased in thoracic aorta rings after consuming a diet with 8% w/w freeze-dried wild blueberry powder for 7 weeks compared to a 7-week control diet and a 4-week wild blueberry diet.[80] The same study found that vessel sensitivity to Phe increased in thoracic aortas after the 4-week wild blueberry diet compared to control (with no effects on vasoconstriction), while a decrease in vessel sensitivity to Phe was found after 7 weeks of the wild blueberry diet compared to 7-week control and 4-week wild blueberry diets (potentially due to age-related impacts).[80] Finally, a study assessed vasoconstriction and vasorelaxation in thoracic aorta rings of male Wistar rats fed 2% w/w freeze-dried highbush blueberry powder

added to control chow or high-fat/high-cholesterol diets for 10 weeks and showed decreased vasoconstriction (to Phe) in the blueberry-fed groups compared to control and high-fat/high-cholesterol diets, and increased vasorelaxation (to Ach) in the blueberry plus high-fat/high-cholesterol diet compared to the high-fat/high-cholesterol diet alone.[81]

Three studies were performed with a male rat model of hypertension. One study observed an increase in vasorelaxation and vessel sensitivity to Ach in thoracic aorta rings from male spontaneously hypertensive rats following 7 weeks of consuming a diet with 8% w/w freeze-dried wild blueberry powder compared to the control group.[82] In another study, Phe-induced vasoconstriction decreased while Ach-induced vasorelaxation increased in thoracic aorta rings from male spontaneously hypertensive rats following consumption of a diet with 8% w/w freeze-dried wild blueberry powder for 8 weeks compared to control.[83] A later study examined 8% w/w freeze-dried wild blueberry powder added to the diets of male spontaneously hypertensive rats for 8 weeks and found that Phe-induced vasoconstriction decreased after wild blueberry consumption compared to control.[84]

Three studies were performed with male animal models of obesity and diabetes. It should be noted that evidence indicates endothelium-dependent vasodilator responses are exaggerated while vasoconstrictor responses are impaired in obese Zucker rats, likely due to a compensatory adaptation to preserve blood flow in the presence of oxidative stress and inflammation.[85-90] The first study found that male obese Zucker rats fed a diet with 8% w/w freeze-dried wild blueberry powder for 8 weeks had partial restoration of Phe-induced vasoconstriction compared to obese Zucker rats on a control diet, while lean Zucker rats had no improvements.[91] They also found that vasorelaxation (to Ach) increased in lean rats consuming 8% w/w freeze-dried wild blueberry powder added to the diets for 8 weeks compared to the lean control diet, while there was no change in blueberry-fed obese rats compared to control diet-fed obese rats.[91] Similarly, an additional study using male obese and lean Zucker rats found that 8% w/w freeze-dried wild blueberry powder added to diets for 8 weeks partially restored Phe-

induced vasoconstriction in obese rats compared to the obese control, while vasorelaxation to Ach was increased in lean blueberry-fed rats.[85] Lastly, male obese diabetic *db/db* mice were found to have impaired endothelium-dependent vasorelaxation to Ach relative to control obese mice that was restored by 3.8% w/w freeze-dried wild blueberry powder added to diets for 10 weeks.[19]

Overall, the findings from these animal studies suggest that blueberry consumption improves endothelial function through regulation of vascular tone, though differential effects were observed across studies that may be dependent on the animal model and disease state, animal age, and/or the blueberry intervention (dose and length).

Effects of blueberries on endothelial (dys)function: human studies

Acute blueberry consumption

Nine studies examined the impact of acute blueberry consumption (i.e. ≤ 24 h postprandial time period) on endothelial function in diverse human populations through assessment of RHI and FMD (**Table 2.2**). Five studies evaluated acute effects of blueberries on endothelial function in healthy men. The first study observed no effects on RHI 1 h post consumption of 300 g of whole highbush blueberries partially thawed into a jelly-like mixture, or a control jelly consisting of 20 g of gelatin plus added sugars made to match the consistency and nutrient composition of the blueberry treatment.[92] Another study investigated the time-course and dose-dependent acute effects of freeze-dried wild blueberry powder mixed with water on FMD in healthy males in 2 different randomized controlled trials.[45] Results from the time-course trial showed that 34 g and 57 g of freeze-dried blueberry powder increased FMD 1 and 2 h post consumption compared to baseline and placebo consumption, while FMD post consumption of 80 g of freeze-dried wild blueberry powder was increased compared to baseline and placebo at 1 h and compared to placebo (but not baseline) at 2 h.[45] The 34 g dose increased FMD 6 h post consumption compared to baseline and placebo, while the 54 g dose

increased FMD compared to placebo (but not baseline).[45] Next, the dose-dependent study showed that 14 g, 28 g, and 34 g doses of freeze-dried wild blueberry powder dose-dependently increased FMD 1 h post consumption and plateaued thereafter with no additional benefits observed for 57 g and 80 g doses.[45] Another study conducted in healthy males examined the effects of 34 g of freeze-dried wild blueberry powder in a baked product (processed) or as a drink in water (unprocessed) on FMD.[46] They found that FMD significantly increased at 1, 2, and 6 h post consumption of the baked product containing the wild blueberry powder compared to the control baked product, with maximal increases at 2 h post consumption.[46] The same findings were seen for the unprocessed wild blueberry drink, however, maximal increases in FMD were seen at 1 h instead of 2 h indicating that food matrix might play a role in determining the acute effects of blueberry consumption on endothelial function in healthy males, likely due to differences in (poly)phenol metabolite bioavailability.[46] Furthermore, these results suggest that food processing with wild blueberry powder may not reduce its beneficial effects on endothelial function. Finally, a more recent study examined FMD in healthy men after consumption of 11 g freeze-dried wild blueberry powder mixed in water compared to a control drink, control drink + fiber, control drink + vitamins/minerals, and pure ACNs drink to understand the possible role of ACNs as major bioactives in blueberry.[47] They found that FMD increased at 2 and 6 h post wild blueberry powder and ACNs drink consumption compared to the control drink with no significant differences between the two drinks, and no effects on FMD for the other treatments.[47] These results indicate that 11 g of wild blueberry powder (150 mg ACNs) is the lowest dose needed to acutely improve endothelial function in healthy men, and that effects are largely due to ACN contents.

Three studies examined acute consumption of 300 g of whole fresh highbush blueberries on endothelial function via RHI in smoking and nonsmoking men,[93, 94] and found that RHI decreased in healthy men after smoking and highbush blueberry consumption counteracted these impairments 20 min post consumption.[93] There was also an increase in RHI 120 min post

consumption of whole highbush blueberries in nonsmoking men with peripheral arterial dysfunction (defined as RHI ≤ 1.67) compared to control treatment.[94] However, in smoking men with peripheral arterial dysfunction, RHI increased 120 min after whole highbush blueberry and control treatment consumption compared to smoking alone.[94] Overall, the results of these studies suggest that blueberry consumption may attenuate smoking-induced impairments in endothelial function in healthy men and men with peripheral arterial dysfunction. Lastly, the impact of 26 g freeze-dried highbush blueberry added into a 500 g energy-dense emulsion meal + 50 g of water on FMD in adults with metabolic syndrome was evaluated at baseline (0 h) and 3, 6, and 24 h post consumption, and no effects were observed potentially due to impacts of the meal.[95] Collectively, these studies strongly suggest that acute blueberry consumption improves endothelial function in healthy men, and smoking and non-smoking men with and without peripheral arterial dysfunction. They also suggest that the beneficial impacts of blueberries may be reduced when consumed with a high-fat meal. Further research is needed in diverse populations to establish these acute effects and their generalizability to other healthy sexes and genders, races and ethnicities, and individuals and populations with chronic diseases and/or risk factors.

Chronic blueberry consumption

Eight studies examined the impact of chronic (> 24 h postprandial time period) blueberry consumption on endothelial function in humans through assessment of RHI or FMD (**Table 2.3**). First, 2 studies were performed in healthy men. In the first study, FMD improved after 1 week of consuming 22 g of freeze-dried wild blueberry powder mixed in water with further improvements observed at 2 weeks but plateaued thereafter up to 28 days.[47] In a different subset of healthy men, similar increases in FMD were observed following 28 days of consuming 22 g/day of freeze-dried wild blueberry powder compared to placebo powder, while no acute on chronic improvements in FMD were observed on day 28 (i.e. after consuming a single dose of 22 g of

freeze-dried wild blueberry powder following 28 days of daily wild blueberry powder consumption).[47]

Six studies were performed in populations with cardiometabolic risk factors. One study examined the effects of consuming 25 g/day freeze-dried wild blueberry powder mixed in water for 6 weeks on RHI in heathy men with at least one CVD risk factor, but observed no statistically significant effects.[96] Another study in adults at risk for type 2 diabetes found that 240 mL of wild blueberry juice consumption for 7 days did not lead to statistically significantly improvements in RHI.[97] However, another study in adults with metabolic syndrome observed an increase in RHI compared to control following consumption of 45 g/day of freeze-dried highbush blueberry powder mixed in a yogurt smoothie (12 oz yogurt and skim milk) for 6 weeks.[98] Similarly, in adults with metabolic syndrome, 26 g/day of freeze-dried highbush blueberry powder consumed as drink/smoothie or added to cereals, yogurts, banana toast, or as a salad dressing for 6 months improved FMD compared to the 13 g dose of freeze-dried highbush blueberry powder and the 26 g of placebo powder.[99] In postmenopausal women with above-normal blood pressure, daily consumption of 22 g/day of freeze-dried highbush blueberry powder mixed in water for 12 weeks improved FMD/SR_{AUC} compared to baseline and placebo.[50] Furthermore, there was a clinically (but not statistically) significant increase by +1.34% units in FMD not normalized to shear rate after 12 weeks of blueberry consumption and no change in the placebo group.[50] Finally, healthy older adults had increased FMD following 12 weeks of consuming 26 g/day freeze-dried wild blueberry powder mixed in water compared to placebo.[49]

In conclusion, these studies indicate that chronic blueberry consumption can improve endothelial function in healthy adults and those with CVD risk factors. In particular, evidence suggests daily chronic consumption of at least 22 g/day of freeze-dried blueberry powder for at least 28 days and up to 6 months can improve endothelial function in healthy men and adults with major CVD risk factors (i.e. metabolic syndrome, postmenopausal women with above-normal blood pressure). Whether the different doses and intervention durations can be

generalized beyond each study is not known and requires further investigation. It is possible that higher doses are needed to observe improvements in endothelial function in chronic intervention periods of shorter duration (e.g. 1 to 6 weeks) for populations with CVD and type 2 diabetes risk factors. Dose-dependent and time-course studies in diverse human populations (e.g. sex, gender, race, ethnicity, disease risk or clinical disease, etc.) are needed to further establish the effects of blueberry consumption on endothelial function.

Blueberries and Arterial Stiffness

Arterial stiffness overview and methods of assessment

Arterial stiffness occurs due to functional and structural changes in the tunica media characterized by the loss of arterial elasticity and compliance.[3, 54] With each heartbeat, a pulse wave is generated that travels down the aorta and arterial tree buffering arteries for dilation.[54] Healthy elastic arteries distend during systole and recoil during diastole to facilitate continuous blood flow throughout the body in order to maintain proper blood pressure and flow.[54] However, changes to the arterial wall seen with advancing age and other CVD risk factors result in increased collagen deposition (i.e. fibrosis), elastin fragmentation, and advanced glycation end-product formation which interferes with arterial distensibility and promotes arterial stiffening.[3, 6] With this, arteries are less able to buffer the pulse wave generated by the heart leading to an early arrival of the reflection wave to the aorta resulting in increased systolic blood pressure and an unchanged (or lower) diastolic blood pressure (i.e. isolated systolic HTN), and is considered a robust predictor of CVD risk.[6]

Clinically, arterial stiffness can be assessed non-invasively at various locations along the vascular tree and several assessments exist. For the purpose of this review, only 3 non-invasive methods used in published studies that assessed the impact of blueberries on arterial stiffness are included, which are pulse wave velocity (PWV), augmentation index (AIx), and digital volume pulse (DVP). PWV, or the speed of the forward pressure wave that is propagated

throughout the arterial tree, is commonly used due to its feasibility and high reproducibility, as well as its ability to predict CVD events and mortality.[3, 6, 100] Using a Doppler ultrasound or an applanation tonometer, PWV is calculated as the distance traveled and pulse transit time between 2 arterial sites.[6, 100] A higher PWV value is indicative of arterial stiffness as pulse waves travel faster down the arterial trees in less compliant vessels. The carotid to femoral (cfPWV) arterial segment, also referred to aortic PWV (aPWV), is reflective of aortic stiffness and considered to be the gold standard.[3, 100, 101] Brachial to ankle PWV (baPWV) is also used to assess arterial stiffness but is reflective of both central and peripheral arterial stiffness. Nonetheless, both assessments have been shown to be independent predictors of CVD events.[100-104] PWV is affordable and validated, but there is possible error associated with distance estimation, measurement in individuals with excess body fat or obesity, only allows for regional assessment, and misrepresentation in the acquisition signal.[105]

Another non-invasive but more indirect measure of arterial stiffness is AIx, which is the proportion of central pulse pressure caused by arterial wave reflection and is derived during pulse wave analysis (PWA) assessments. AIx has been shown to predict CVD mortality.[106] PWA, and thus, AIx can be assessed through applanation tonometry, echo tracking, or volumetric displacement using a blood pressure cuff.[3] Its advantages include its noninvasive and simplistic attributes in addition to its validity and reproducibility.[3] However, certain physiological factors such as heart rate, sex, blood pressure, height, and endothelial function can affect the outcome value, which is why it is recommended that AIx also be normalized to heart rate (AIx @75 bpm) and used in conjunction with PWV to assess arterial stiffness.[3] Finally, DVP assesses pulse waveforms through measuring absorption of infrared-light across the finger (i.e. photoplethysmography).[45, 107, 108] DVP is validated, reproducible with low intra-observer variation, and comparable to PWV (Gunarathne et al., 2008).[109]

Effects of blueberries on arterial stiffness: animal studies

No published studies that evaluated the impact of blueberry consumption on arterial stiffness in animals were identified. This is a major gap in the knowledge that would benefit from further investigation, as it may provide insight into the impact of blueberries on arterial stiffness in humans, as well as underlying mechanisms which could support interventions aimed at enhanced efficacy in humans.

Effects of blueberries on arterial stiffness: human studies

Acute blueberry consumption

Six studies examined the effects of acute blueberry consumption (≤ 24 h) on arterial stiffness (**Table 2.2**). In healthy men consuming 34 g, 57 g, and 80 g of freeze-dried wild blueberry powder mixed in water, there were no effects on AIx or DVP at 2, 4, or 6 h post treatment consumption.[45] Three studies analyzed digital AIx (dAIx) and dAIx @75 bpm 20 and 120 min after consuming 300 g whole highbush blueberries in healthy male smokers, and nonsmoking and smoking males with peripheral arterial dysfunction (RHI ≤ 1.67), but saw no effects.[93, 94] Furthermore, in older adults with cognitive decline, no effects were seen on DVP 1 h after consuming ~30 g of freeze-dried highbush blueberry powder mixed in a semi-skimmed milk.[110] Lastly, there was no effect on AIx @75 bpm or cfPWV in adults with metabolic syndrome 3, 6, or 24 h after consuming 26 g of freeze-dried highbush blueberry powder added to 500 g energy-dense emulsion meal + 50 g water.[95] This supports that structural vascular changes occur over time and not acutely.

Chronic blueberry consumption

Eight studies examined chronic blueberry consumption (> 24 h) on arterial stiffness in diverse human populations (**Table 2.3**). Two studies were performed in healthy individuals. In 1 study, daily consumption of 11 g of freeze-dried wild blueberry powder mixed in water for 28

days did not improve AIx or cfPWV in healthy men.[47] In a different study, consuming 160 g of fresh whole highbush blueberries or 20 g freeze-dried blueberry powder daily for 1 week did not improve cfPWV in healthy adults.[111]

Six studies were performed in adults at risk for CVD. In a study in men with at least one CVD risk factor, no effects were observed on AIx or AIx @75 bpm after consuming 25 g freeze-dried wild blueberry powder mixed in water daily for 6 weeks.[96] In another study with sedentary men and postmenopausal women, AIx improved after consuming 36 g of tifblue/rubel blueberry powder daily at evening meals for 6 weeks compared to placebo, with no effects on cfPWV.[112] Similarly, a study in postmenopausal women with above-normal blood pressure found that daily consumption of 22 g of freeze-dried highbush blueberry powder mixed in water for 8 weeks decreased baPWV, but not cfPWV, compared to baseline and placebo.[113] Furthermore, a study evaluating daily consumption of 26 g of freeze-dried highbush blueberry powder consumed as drink/smoothie or added to cereals, yogurts, banana toast, or as a salad dressing for 6 months improved AIx, but not cfPWV, in adults with metabolic syndrome compared to those who consumed 13 g of freeze-dried highbush blueberry powder and placebo powder.[99] A study with postmenopausal women with above-normal blood pressure found that daily consumption of 22 g freeze-dried highbush blueberry mixed in water for 12 weeks had no effects on cfPWV, AIx, or AIx @75 bpm.[50] Finally, healthy older adults had no changes in cfPWV or AIx @75 bpm following 12 weeks of consuming 26 g/day of freeze-dried wild blueberry powder mixed in water.[49]

These findings suggests that chronic blueberry consumption may improve arterial stiffness assessed as AIx and baPWV in sedentary men and postmenopausal women, as well as postmenopausal women with above-normal blood pressure, respectively.[112, 113] Limited studies have assessed chronic blueberry consumption on cfPWV, but existing research has not demonstrated benefits regardless of dose and length of intervention. Further research is needed to understand the effects of blueberry consumption on arterial stiffness. It is possible that

blueberries exert protective effects on peripheral arteries, or that intervention lengths have not allowed for sufficiently capturing structural improvements in the aorta.

Potential Mechanisms by which Blueberries Modulate Vascular Function

Overview of mechanisms contributing to vascular dysfunction

The endothelium is responsible for producing NO, a critical vasodilatory molecule, through activation of eNOS by stimuli such as shear stress or acetylcholine.[114, 115] When the substrate L-arginine is available, eNOS produces NO which can diffuse out of endothelial cells and into VSMCs resulting in activation of soluble guanylyl cyclase (sGC), and thus, formation of cyclic guanylyl monophosphate (cGMP) and initiating vasodilation.[56, 114] Vasodilation also occurs through activation of cyclooxygenase (COX) which metabolizes arachidonic acid to yield prostacyclin (PGI_2). PGI_2 can diffuse out of endothelial cells and into VSMCs triggering activation of cyclic adenosine monophosphate (cAMP), thus, causing vasodilation.[56, 114] COX is also responsible for vasoconstriction, but through the production of vasoconstrictors such as thromboxane instead of vasodilators and under different conditions.[116] Both NO- and COX-related pathways are activated through increased intracellular calcium.

Current evidence indicates that impaired endothelium-dependent dilation (due to endothelial dysfunction) is primarily caused by impairments in NO production and/or bioavailability secondary to excessive oxidative stress and inflammation.[9, 10] The underlying mechanisms contributing to endothelial dysfunction are complex and not fully understood, and this review focuses on mechanisms related to oxidative stress, inflammation, and the gut microbiota. Regarding vascular oxidative stress, the main source of vascular reactive oxygen/nitrogen species (RONS) is NOX,[117] while mitochondrial metabolism and dysfunction and xanthine oxidase 1 (XO-1) are also contributors.[63, 118, 119] With aging and the presence of other CVD risk factors such as HTN, dyslipidemia, and metabolic syndrome, increased RONS production without adequate antioxidant defense (e.g. due to decreased or insufficient

antioxidant enzyme activity including superoxide dismutase (SOD) and heme oxygenase-1 (HO-1)) promotes increased vascular oxidative stress.[9, 63, 119-121] This can lead to eNOS uncoupling through oxidation of its essential cofactor BH_4, rendering eNOS unable to produce NO and instead producing superoxide radicals, further promoting vascular oxidative stress. ROS also reacts directly with NO resulting in decreased NO bioavailability and increased formation of peroxynitrite (i.e. reactive nitrogen species (RNS)) which increases vascular dysfunction.[56] ROS can also stimulate the release of pro-inflammatory molecules (e.g. C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α), cyclooxygenase-2 (COX-2)), which can decrease NO production and bioavailability and enhance vasoconstrictor molecule production.[9, 57, 63, 64, 114, 116, 122] Inflammation also stimulates ROS production perpetuating the vicious cycle of oxidative stress and inflammation contributing to endothelial dysfunction.[3, 9, 63] Monocyte adhesion to endothelial cells also leads to vascular inflammation and atherogenesis, and occurs with endothelial dysfunction.[9, 123, 124] Likewise, oxidative stress and inflammation contribute to arterial stiffening through endothelium-related mechanisms mentioned above.[6, 125] ROS also promote immune cells to release matrix metalloproteinases that are responsible for uncoiled collagen and elastin degradation in the vessel wall leading to vascular fibrosis and VSMC proliferation resulting in structural changes.[125]

Accumulating evidence from epidemiological, animal, and human clinical studies indicate the gut microbiome is an important mediator of vascular function and CVD risk.[11, 126-128] For instance, suppression of the gut microbiome with a broad-spectrum antibiotic cocktail in mice improved age-related vascular dysfunction (i.e. endothelial function and arterial stiffness), and vascular oxidative stress and inflammation.[129] Also, transplantation of an obesity-associated human gut microbiota into germ-free mice led to impaired endothelium-dependent dilation and increased arterial stiffness.[130] Though what is considered to be a “healthy” gut microbiome is complex and not completely understood, it is commonly accepted that a higher microbial diversity, higher abundance of certain health-promoting bacteria such as *Lactobacillus* and

Faecalibacterium prausnitzii, high proportion of Bacteroidetes relative to Firmicutes phyla (i.e. lower Firmicutes/Bacteroidetes ratio), resilience against external pressures, and higher abundance of bacteria that can metabolize complex carbohydrates has been shown to be represented in healthy individuals and those meeting the dietary guidelines.[11, 131, 132] The gut microbiome is responsible for digesting and metabolizing food components such as (poly)phenols and fiber, regulating the immune system, maintaining intestinal homeostasis, protecting against diseases, and producing certain nutrients and bioavailable metabolites important for human health, among numerous other functions.[11, 126, 133, 134] Gut microbial dysbiosis is a compositional change away from what is considered a "healthy" gut microbiome including the loss of beneficial bacteria, expansion of pathobionts, and loss of diversity.[11] Gut microbial dysbiosis is linked with vascular dysfunction due to various mechanisms including, but not limited to, impaired intestinal barrier function resulting in entry of lipopolysaccharide (LPS; gram-negative bacterial wall component) into circulation and toll-like receptor (TLR) signaling which increases production of pro-inflammatory cytokines, free radicals, and trimethylamine N-oxide (TMAO; microbial metabolite of choline) which can promote inflammation, platelet reactivity, and thrombosis.[11, 126-128, 135]

Diet and nutrition are important determinants of oxidative stress, inflammation, and gut microbial composition and function. A typical Western Diet (e.g. high saturated-fat, high-refined carbohydrates, low fruits and vegetables) is linked to gut microbial dysbiosis, oxidative stress, inflammation, arterial stiffness, and endothelial dysfunction.[11, 136, 137] Conversely, diets rich in (poly)phenols can beneficially modulate the gut microbiota, and therefore, may be a promising approach to decrease CVD risk.[138]

NO-related mechanisms for improvements in vascular function with blueberries

Evidence from cell culture studies

One study evaluated the impact of blueberry consumption on biomarkers related to NO. They demonstrated that treatment of human aortic endothelial cells (HAECs) with palmitate suppressed insulin-stimulated NO production but was restored by treatment with physiological levels of synthetic blueberry (poly)phenol metabolites (i.e. benzoic acid-4-sulfate, isovanillic acid-3-sulfate, vanillic acid-sulfate, hydroxy hippuric acid, and hippuric acid).[139] No other physiologically relevant cell culture studies that examined blueberry (poly)phenols and/or their metabolites on mechanistic pathways related to NO were identified except for those evaluating impacts on oxidative stress and inflammation which are described in a later section. This highlights a gap in the literature, and future studies should aim to consider physiologically relevance when designing studies that account for digestion, absorption, and metabolism.

Evidence from animal studies

Ten studies evaluated NO-related mechanisms associated with improvements in vascular function with blueberry consumption in animals (**Table 2.1**). With respect to endothelial function, studies have demonstrated blueberry (poly)phenol metabolites mediate beneficial effects.[78, 81] For instance, in SD, hypertensive, and obese Zucker male rats, vasoconstriction (induced through eNOS inhibition with L-NMMA) was restored following blueberry supplementation compared to respective controls without inhibitors,[79, 80, 82, 83, 91] while vasorelaxation was lower following blueberry consumption (also induced through eNOS inhibition with L-NMMA) compared to the controls,[79, 82, 83, 91] indicating improvements in vascular function were mediated through the eNOS/NO pathway. To support that improved vascular function with blueberry consumption is mediated through the eNOS pathway, male spontaneously hypertensive rats were found to have increased vasoconstriction (induced through GC inhibition) and plasma cGMP following wild blueberry supplementation compared to the control.[84] Furthermore, decreased plasma NO metabolite concentrations were observed in obese male rats after wild blueberry consumption compared to obese controls, while increased

NO metabolite concentrations were observed in lean rats after wild blueberry consumption compared to lean controls suggesting improved NO bioavailability.[85, 91] Interestingly, overproduction of NO coupled with low NO bioavailability due in large part has been observed in pro-inflammatory and oxidative stress-driven conditions including obesity and metabolic syndrome likely due to adaptive compensation, suggesting that a reduction in NO could be beneficial in certain populations.[90, 91] Furthermore, aortic gene expression of eNOS and inducible NOS (iNOS) and plasma NO concentrations were increased in obese Zucker rats compared to lean Zucker rats, and blueberry-fed obese Zucker rats had decreased iNOS gene expression (but not eNOS) compared to obese controls and increased NO concentrations compared to control lean Zucker rats.[85, 91] Overall, these studies support that improvements in endothelial function are mediated through the eNOS/NO pathway.

Evidence from human studies

Seven studies evaluated the impact of blueberry consumption on biomarkers related to NO. Of the human clinical trials that found improved vascular function after blueberry consumption, 1 study found significantly increased NO metabolite concentrations in postmenopausal women with above-normal blood pressure following 8 weeks of daily consumption of 22 g freeze-dried highbush blueberry powder mixed in water (**Table 2.3**).[113] Similarly, another study found significantly increased plasma NO metabolite concentrations after consuming 240 mL of wild blueberry juice for 7 days in adults at risk for type 2 diabetes, even though RHI did not significantly improve (**Table 2.3**).[97] Lastly, plasma cGMP concentrations were increased after consuming 26 g/day of freeze-dried highbush blueberry powder for 6 months in adults with metabolic syndrome, while NO metabolite concentrations were unchanged (**Table 2.3**).[99] Finally, 4 studies measured NO metabolites in relation to vascular function, however, found no statistical differences in NO metabolite concentrations after blueberry consumption.[50, 92, 96, 111] Together, these findings suggest that improvements in endothelial

function in humans may be through the eNOS/NO-pathway. However, further research is needed to understand the extent to which improvements in endothelial function are mediated by NO and the underlying mechanisms related to NO production/bioavailability, particularly in diverse populations that include healthy individuals and those at risk for and/or have chronic diseases that influence the eNOS/NO pathway.

Effects of blueberries on vascular oxidative stress

Evidence from cell culture studies

Three cell culture studies examined the effects of physiologically relevant levels of blueberry (poly)phenols and/or their metabolites commonly found in human plasma after blueberry consumption on endothelial cell-derived oxidative stress. One study performed with healthy human umbilical venous endothelial cells (HUVECs) found that synthetic blueberry (poly)phenol metabolites (i.e. malvidin-3-glucoside (Mv-3-glc) and malvidin-3-galactoside (Mv-3-gal)) independently and combined reduced ROS-producing enzyme XO-1 protein expression and ROS production, and increased antioxidant defense enzyme protein expression of SOD and HO-1 compared to control.[119] Another study observed that treatment of HAECs with palmitate increased NOX4 mRNA expression and ROS production but was attenuated by the addition of synthetic blueberry (poly)phenol metabolites (i.e. benzoic acid-4-sulfate, isovanillic acid-3-sulfate, vanillic acid-sulfate, hydroxy hippuric acid, and hippuric acid).[139] In a different study, the impact of 3 different blueberry (poly)phenol metabolite mixtures on antioxidant defense (i.e. nuclear factor erythroid 2-related factor (Nrf2) and cellular HO-1 protein expression) in HUVECs treated with sub-lethal dosages of hydrogen peroxide (H_2O_2) was evaluated.[140] For that study, (poly)phenol metabolites were first measured over a 24-h period following consumption of a single dose of blueberry juice by 3 healthy humans, and found that 12 metabolites were increased compared to baseline and served as the basis for the metabolite mixtures used (i.e. (1) early mixture/metabolites found in plasma with a $T_{max} \leq 2$ h including 2-

hydroxyhippuric acid, 4-hydroxyhippuric acid, syringic acid, protocatechuic acid, vanillic acid, *trans*-ferulic acid, *p*-coumaric acid); (2) late mixture/compounds found in plasma with a $T_{max} \geq 4$ h including protocatechuic acid, gentisic acid, vanillic acid, *trans*-ferulic acid, *p*-coumaric acid, dihydroferulic acid, dihydrocaffeic acid, dihydro-*m*-coumaric acid, homovanillic acid; and (3) whole mixture containing all of the early and late mixtures metabolites).[140] Results showed that pretreatment with the whole mixture of blueberry (poly)phenol metabolites upregulated Nrf2-regulated antioxidant response proteins including HO-1 and the glutamate-cysteine ligase modifier subunit in HUVECs exposed to 2.5 μM H_2O_2 compared to H_2O_2 control.[140] These studies suggest that blueberries may reduce endothelial cell-derived oxidative stress through modulation of ROS-producing and antioxidant defense enzymes.

Evidence from animal studies

One study examined blueberry consumption on oxidative stress as related to vascular function and showed adding 3.8% w/w freeze-dried blueberry powder to the diets of diabetic *db/*db male mice for 10 weeks reduced NOX4 mRNA expression in vascular endothelial cells and aortic vessel compared to the diabetic control group (**Table 2.1**).[19] No other studies in animals that evaluated vascular oxidative stress were identified, but future studies could support understanding oxidative stress-related mechanisms contributing to improvements in vascular function with blueberry consumption.

Evidence from human studies

Nine studies evaluated the effects of blueberry consumption on oxidative stress as related to vascular function. Acute consumption of 300 g whole highbush blueberries was found to decrease H_2O_2-induced DNA damage in peripheral blood mononuclear cells in healthy men 1 h post blueberry consumption compared to control (**Table 2.2**).[92] Another study observed decreased endogenous oxidized DNA bases and H_2O_2-induced DNA damage in peripheral

blood mononuclear cells from healthy males with at least 1 CVD risk factor after consuming 25 g/day of freeze-dried wild blueberry powder for 6 weeks (**Table 2.3**).[96] In a different study, decreases in neutrophil NOX activity were found 1-2 and 6 h post consumption of 34 g freeze-dried wild blueberry powder in healthy males that were directly associated with improvements in FMD and predicted by plasma (poly)phenol metabolites (vanillic acid, hippuric acid, and homovanillic acid) (**Table 2.2**).[45] A study in postmenopausal women with above-normal blood pressure, a study found that 4 weeks of consuming 22 g/day of freeze-dried highbush blueberry powder led to improvements in 8-hydroxy-2'-deoxyguanosine which is indicative of DNA damage, though levels increased back to baseline at 8 weeks (follow-up study to Johnson *et al.* (2015)), while other circulating biomarkers of oxidative stress and antioxidant defense were not affected by blueberry consumption.[141] In adults with metabolic syndrome, a study found that 6 weeks of consuming 45 g/day of freeze-dried highbush blueberry powder found decreased whole blood superoxide radicals and monocyte ROS (follow-up study to Stull *et al.* (2015)).[142] Finally, a recent study found that FMD/SR_{AUC} increased after acute infusion of a supraphysiological dose of the antioxidant ascorbic acid (vitamin C) in postmenopausal women with above-normal blood pressure at baseline (indicating oxidative stress-mediated suppression of endothelial function), but not after 12 weeks of daily consumption of 22 g freeze-dried highbush blueberry powder (whereas differences stayed trending for the control group) (**Table 2.3**).[50] Additionally, the blueberry group had a reduction in the response to ascorbic acid from baseline to 12 weeks, whereas the placebo group had no change indicating that improvements in endothelial function were mediated by reductions in oxidative stress.[50] Three other studies also measured biomarkers of oxidative stress after blueberry consumption in relation to vascular function, however, did not find improvements.[96, 97, 112] Other human studies have evaluated biomarkers of inflammation and oxidative stress, but were not performed in the context of vascular function and were not included but have been evaluated through a systematic review that indicated results are mixed due to various factors.[143] Nonetheless, circulating biomarkers do

not necessarily reflect vascular oxidative stress, highlighting the need to better understand the impact of blueberries on vascular oxidative relating to vascular function, as well as the mechanisms involved.

Effects of blueberries on vascular inflammation

Evidence from cell culture studies

Six cell culture studies examined the effects of physiologically relevant blueberry (poly)phenol metabolites commonly found in human plasma after blueberry consumption on endothelial-derived inflammation. Specifically, mv-3-glc, mv-3-gal, and mv-3-glc + mv-3-gal decreased supernatant levels of monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule-1 (ICAM-1), and vascular adhesion molecule-1 (VCAM-1), and decreased NFκB translocation and increased IκBα protein expression in HUVECs treated with TNF-α, while significant decreases in VCAM-1 protein expression in cell lysates were found after incubation of mv-3-glc + mv-3-gal in HUVECs treated with TNF-α.[144] They also observed that cell gene expression of MCP-1 and ICAM-1 decreased in TNF-α induced dysfunctional HUVECs after incubating with mal-3-glc and mv-3-glc + mv-3-gal.[144] In a different study, HUVECs were incubated with blueberry (poly)phenol metabolites including gallic acid, syringic acid, and protocatechuic acid (among other parent ACN and phenolic acids), and then monocytes (THP-1) were added with TNF-α.[145] Results showed that gallic acid reduced monocyte adhesion compared to HUVECs treated with TNF-α alone, while syringic acid increased adhesion.[145] Another study showed that incubation of a synthetic blueberry (poly)phenol metabolite mixture (i.e. benzoic acid-4-sulfate, isovanillic acid-3-sulfate, vanillic acid-sulfate, hydroxy hippuric acid, and hippuric acid), as well as a parent ACN mixture, and found the metabolite mixture attenuated palmitate-induced increases in relative mRNA expression of interleukin 8 (IL-8), ICAM-1, VCAM-1, and IκBα, as well as monocyte binding to HAECs.[139] In type 2 diabetic HAECs, a study showed that monocyte (THP-1) adhesion and relative mRNA expressions of IL-

8, VCAM-1, and E-selectin were significantly increased compared to healthy HAECs, but reduced with the addition of a blueberry (poly)phenol metabolite mixture (i.e. benzoic acid-4-sulfate, isovanillic acid-3-sulfate, vanillic acid-sulfate, hydroxy hippuric acid, and hippuric acid).[146] In a different study, monocyte (THP-1) adhesion to healthy HUVECs was stimulated with TNF-α followed by incubation with protocatechuic acid and gallic acid (among other parent ACNs and metabolites), which independently decreased monocyte adhesion to HUVECs, as well as soluble E-selectin in supernatant.[147] Lastly, 3 different blueberry phenolic acid mixtures at physiological levels reflecting early (0-4 h), late (4-24 h), or whole (0-24 h) circulating metabolites post blueberry juice consumption (previously reported in Tang *et al.* (2018)) were used to assess the effects of blueberry (poly)phenol metabolites on monocyte binding and inflammation in TNF-α induced dysfunctional HUVECs.[148] Results showed that 4 h pretreatment with the late mixture, but not early or whole mixtures, decreased monocyte adhesion to HUVECs without affecting endothelial cell surface expression of adhesion molecules.[148] Interestingly, they also observed that exclusion of syringic acid from early and whole mixtures led to reduced monocyte adhesion, while treatment with syringic acid alone had no effects on monocyte adhesion.[148] Overall, these studies suggest that blueberry (poly)phenol metabolites exert anti-inflammatory effects in the vasculature that can attenuate processes involved in atherosclerosis.

Evidence from animal studies

Six studies were identified that examined blueberry consumption on inflammation as related to vascular function in animals (**Table 2.1**). Two studies in male spontaneously hypertensive rats found that inhibition of COX with mefenamic acid *ex vivo* with thoracic aortic rings decreased vasoconstriction after wild blueberry diet consumption compared to control,[82, 83] while another study observed a decrease in vasoconstriction with inhibition of PGI_2 synthase after wild blueberry diet consumption compared to control.[84] Furthermore, male spontaneously

hypertensive and obese Zucker rats were found to have increased plasma 6kPGF$_{1\alpha}$ (COX pathway intermediate) following blueberry diet consumption in several studies demonstrating improvements in endothelial function.[84, 85, 91] Lastly, aortic COX-2 gene and iNOS gene expression decreased in obese Zucker male rats after wild blueberry diet consumption compared to control.[85, 91] Furthermore, obese male Zucker rats that consumed 8% w/w freeze-dried wild blueberry powder for 8 weeks had decreased plasma concentrations of IL-6, TNF-α, and CRP compared to the obese control.[85] Finally, a study found that adding 3.8% w/w freeze-dried blueberry powder to the diets of diabetic *db/db* male mice for 10 weeks decreased serum inflammatory markers (MCP-1/JF, KC/IL-8), decreased aortic gene expression of Iκκβ, KC/IL-8, MCP-1/JE, ICAM-1, and VCAM-1, and decreased carotid endothelial cell gene expression of Iκκβ, KC/IL-8, MCP-1/JE, and VCAM-1 compared to control.[19] Additionally, blueberry (poly)phenol-rich serum from *db/db* mice that consumed wild blueberries suppressed glucose and palmitate-induced monocyte binding to, and KC secretion from, mouse aortic endothelial cells compared to the *db/db* control.[19] Together, these studies suggest that blueberries may improve vascular function through attenuation of inflammation in hypertension, obesity, metabolic syndrome, and diabetes.

Evidence from human studies

Six studies evaluated the effects of blueberry consumption on inflammation as related to vascular function. In postmenopausal women with above-normal, a study found that 4 weeks of consuming 22 g/day freeze-dried highbush blueberry powder led to improvements in 8-hydroxy-2'-deoxyguanosine which is indicative of DNA damage, though levels increased back to baseline at 8 weeks, while other circulating biomarkers of inflammation were unaffected (follow-up study to Johnson *et al.* (2015)).[141] A study performed in adults with metabolic syndrome found that 6 weeks of consuming 45 g/day freeze-dried highbush blueberry powder found decreased monocyte gene expression of TNF-α, IL-6, toll-like receptor 4, as well as serum

granulocyte macrophage colony-stimulating factor and increased myeloid dendritic cells (follow-up study to Stull *et al.* (2015)).[142] A study performed in healthy men found that 28 days of consuming 22 g/day of freeze-dried wild blueberry powder led to differential expression of numerous genes and microRNAs in peripheral blood mononuclear cells that are involved in immune regulation, inflammation, and cell adhesion, among other processes (**Table 2.3**).[47] Lastly, 3 studies also measured biomarkers of inflammation after blueberry consumption in relation to vascular function, however, did not find any statistical significant changes in such biomarkers.[50, 96, 97]

Effects of blueberries on the gut microbiome

Evidence from cell culture studies

No relevant studies using cell culture models related to the gut microbiome and vascular function were identified. *In vitro* fermentation has allowed researchers to investigate the effects of blueberries on the gut microbiome and their potential impacts on human health, and though a limited number of studies have been performed, existing data suggest that blueberries beneficially modulate the gut microbiota.[149-153] Whether such effects translate to beneficial effects on the vasculature remains unknown.

Evidence from animal studies

Only one study in animal models investigate the effects of blueberries on the gut microbiome and vascular health were identified (**Table 2.1**). In diabetic *db/db* male mice fed 3.8% w/w freeze-dried wild blueberry powder for 10 weeks, a study observed trends in changes of microbial alpha diversity indices (Shannon and Simpson) for the blueberry group compared to the *db/db* control, and beta-diversity was significantly different at the phylum and genius level between groups.[19] The blueberry supplementation also significantly increased *Actinobacteria*, *Adlercreutzia, Bifidobacterium,* and *Dorea* abundance compared to *db/db* control.[19]

Evidence from human studies

Studies in humans investigating the effects of blueberries on the gut microbiome in relation to vascular function are limited as only 1 study was identified (**Table 2.3**). In older adults, consumption of 26 g/day of freeze-dried wild blueberry powder for 12 weeks did not significantly alter α or β diversity of the gut microbiome, but increased abundance of *Ruminiclostridium* 9 and *Ruminiclostridium* 5.[49] They also observed a positive correlation between *Cocoprocus* and Family XIII AD03011 with FMD and a negative correlation between *Parabacteroides* and *Ruminococus* UCG.003 with FMD[49]; however, their overall results suggest that improvements in endothelial function were not mediated by the gut microbiome.[49] Other studies have investigated the effects of blueberries on the composition of the gut microbiome but not in the context of vascular function. Briefly, studies in healthy adult men and young/old women indicate that chronic blueberry consumption for 6 weeks increase *Bifidobacterium spp.* and health-associated taxa.[153-155] These data suggest that blueberry consumption may modulate the gut microbiota, but it is unclear as to the extent to which the gut microbiome mediates improvements in vascular function and is a major gap in the field.

Conclusions

Blueberries are rich in nutrients and (poly)phenols with demonstrated cardiovascular-protective effects. They are a widely consumed fruit in the US, a major agricultural crop with year-round availability, and have high nutritional quality and acceptability by consumers, making them ideal for use in food-based strategies for promoting human health including cardiovascular health. Blueberries and their (poly)phenol metabolites have been shown to improve measures of vascular function in animals and humans. Evidence from animal studies indicate that blueberry consumption promotes vasorelaxation and reduces vasoconstriction. Evidence from randomized controlled trials indicates that blueberry consumption can enhance endothelial function acutely in men (healthy men and smokers), and chronically in healthy young and older adults, adults

with metabolic syndrome, and postmenopausal women with above-normal blood pressure. Overall, the data suggest that blueberries improve endothelial function to a greater extent in those with greater CVD risk factors which may also influence the dose needed to observe beneficial effects. Regarding arterial stiffness, the data are less conclusive as no studies assessing arterial stiffness have been performed in animals and limited randomized controlled trials have been performed in humans. However, existing data suggest chronic blueberry consumption for at least 6 weeks may improve certain measures of arterial stiffness, while a longer duration may be needed to observe reductions in aortic arterial stiffness. Results from cell, animal, and human studies suggest that blueberry consumption improves NO bioavailability, likely due to circulating (poly)phenol metabolites and their impact on oxidative stress, thus leading to changes in vascular function. Animal studies suggest a link between the gut microbiota and blueberry-induced improvements in vascular function, though this was not supported in a recent human study. Further research is needed to establish the dose-dependent and time-course clinical efficacy of blueberries to improve endothelial function and arterial stiffness in diverse human populations. Additionally, research aimed at understanding mechanisms contributing to improvements in vascular function is needed, particularly those that are translational and/or reverse translational in nature and can evaluate mechanisms for clinical responses in humans. Finally, translation of clinical research to the community/public in a manner that considers feasibility, health equity, community needs, barriers and drivers to consumption, among other factors while evaluating efficacy is needed to establish real-world impacts.

Tables

Table 2.1. Research studies examining the impact of blueberry consumption on endothelial function in animal models.

Reference & Location	Animal Model	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Possible Mechanistic/ Pathway-related Results	Mechanism/ Pathway Conclusions
Norton *et al.* (2005) *Maine, USA*	Sprague-Dawley male rats (*n* = 30).	Vaso-constriction and vessel sensitivity in thoracic aorta rings.	Freeze-dried WBB powder added to diets at 8% w/w consumed for 13 weeks. Three diet groups: control, WBB, and reverse (consumed control for 13 weeks then switched to WBB diet for 8 weeks).	N/A	↓ vasoconstriction to Phe in BB and reverse diet groups compared to control. No significant effects on other vascular measures.	Diets did not have effects on vasoconstriction nor vessel sensitvity in endothelium-denuded rings.	WBB improves vascular function through the endothelium.
Kalea *et al.* (2009) *Maine, USA*	Sprague-Dawley male rats (*n* = 20).	Vaso-constriction, vaso-relaxation, and vessel sensitivity in	Freeze-dried WBB powder added to diets at 8%	N/A	↓ vasoconstriction to Phe in WBB diet group compared to control.	↑ vasoconstriction in both groups after L-NMMA administration compared to no L-NMMA, with WBB	WBB improves vascular function through the

Table 2.1. Research studies examining the impact of blueberry consumption on endothelial function in animal models.

Reference & Location	Animal Model	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Possible Mechanistic/ Pathway-related Results	Mechanism/ Pathway Conclusions
		thoracic aorta rings.	w/w for 7 weeks. Two diet groups: control and WBB.		↑ vasorelaxation to Ach after WBB diet compared to control. ↓ Vessel sensitivity to Phe and Ach in WBB group compared to control.	group being higher than control. ↓ vasorelaxation in both groups after L-NMMA administration compared to no L-NMMA, with WBB group having less vasorelaxation than control. ↓ vessel sensitivity to Ach in WBB group after L-NMMA as well as MFA administration compared to control group after administration(s).	eNOS/NO pathway.
Kalea *et al.* (2010) *Maine, USA*	Spon-taneously hyper-tensive male rats (*n* = 20).	Vaso-constriction, vaso-relaxation, and vessel sensitivity in thoracic aorta rings.	Freeze-dried WBB powder added to diets at 8% w/w for 7 weeks.	N/A	↑ vasorelaxation to Ach in WBB group compared to control without impacting maximum vasorelaxation force.	↑ vasoconstriction in WBB + L-NMMA compared to control + L-NMMA, as well as compared to WBB and control with no L-NMMA.	WBB improves vascular function through the eNOS/NO and COX pathways.

Table 2.1. Research studies examining the impact of blueberry consumption on endothelial function in animal models.

Reference & Location	Animal Model	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Possible Mechanistic/ Pathway-related Results	Mechanism/ Pathway Conclusions
			Two diet groups: control and WBB.		↑ vessel sensitivity to Ach in WBB group compared to control. No significant effects on other vascular measures.	↓ vasoconstriction in both groups with MFA compared to no MFA, and WBB + MFA had ↓ vasoconstriction compared to control + MFA. ↓ vasorelaxation in both groups after L-NMMA administration compared to no L-NMMA, with WBB + L-NMMA group having greater vasorelaxation than control + L-NMMA. ↑ vasorelaxation in both groups with MFA administration, with control + MFA group having greater vasorelaxation than WBB + MFA.	

Table 2.1. Research studies examining the impact of blueberry consumption on endothelial function in animal models.

Reference & Location	Animal Model	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Possible Mechanistic/ Pathway-related Results	Mechanism/ Pathway Conclusions
						↓ vessel sensitivity to Phe in WBB + L-NMMA as well as WBB + MFA compared to control + L-NMMA groups. ↑ vessel sensitivity to Ach in WBB + L-NMMA compared to control + L-NMMA. ↓ vessel sensitivity to Ach in WBB + MFA compared to control + MFA.	
Kristo *et al.* (2010) *Maine, USA*	Spon-taneously hyper-tensive male rats (*n* = 40).	Vaso-constriction, vaso-relaxation, and vessel sensitivity in thoracic aorta rings.	Freeze-dried WBB powder added to diets at 8% w/w for 8 weeks. Two diet groups: control and WBB.	21 ACNs detected in WBB powder. Peonidin-3-glucoside and malvidin-3-galactoside most abundant making up ~13% of total ACN content	↓ vasoconstriction to Phe in WBB group compared to control. ↑ vasorelaxation in WBB group at lower Ach doses but ↓ at higher Ach doses compared to control.	↑ vasoconstriction in both groups after L-NMMA administration compared to no L-NMMA, but no difference between groups. ↓ vasoconstriction in both groups after MFA administration	WBB improves vascular function through the COX pathway.

Table 2.1. Research studies examining the impact of blueberry consumption on endothelial function in animal models.

Reference & Location	Animal Model	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Possible Mechanistic/ Pathway-related Results	Mechanism/ Pathway Conclusions
				(1.6 ± 0.2 mg/100 mg).		compared to without MFA with ↓ vasoconstriction in WBB + MFA compared to control + MFA ↓ vasorelaxation and vessel sensitivity in both groups after L-NMMA compared to no L-NMMA, with WBB + L-NMMA greater vasorelaxation compared to control + L-NMMA and BB group vessel sensitivity lower than control with L-NMMA. ↑ vasorelaxation in WBB + MFA compared to control + MFA. ↓ vessel sensitivity in control + MFA compared to	

Table 2.1. Research studies examining the impact of blueberry consumption on endothelial function in animal models.

Reference & Location	Animal Model	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Possible Mechanistic/ Pathway-related Results	Mechanism/ Pathway Conclusions
						control without MFA ↑ vessel sensitivity in WBB + MFA compared to control + MFA.	
Del Bo *et al.* (2012) *Maine, USA*	Sprague-Dawley male rats (*n* = 40).	Vaso-constriction and vessel sensitivity in thoracic aorta rings.	Freeze-dried WBB powder added to diets at 8% w/w for 4 or 7 weeks. Four diet groups: control 4-week, WBB 4-week, control 7-week, and WBB 7-week.	21 ACNs detected in blueberry powder. Peonidin-3-glucoside and malvidin-3-galactoside most abundant of total ACNs (1.6 ± 0.2 mg/100 mg), with about 20.7 mg/day of ACNs consumed in WBB groups.	↓ vasoconstriction to Phe for WBB 7-week diet compared to 7-week control and 4-week WBB diets. ↑ vessel sensitivity to Phe for WBB 4-week diet compared to 4-week control diet. ↓ vessel sensitivity to Phe for WBB 7-week diet compared to 7-week control and 4-week WBB diets.	↑ vasoconstriction in all groups after L-NMMA administration compared to controls without L-NMMA. ↑ vasoconstriction for WBB 7-week diet with L-NMMA compared to 7-week control with L-NMMA, WBB 7-week without L-NMMA, and WBB 4-week with L-NMMA. ↓ vessel sensitivity in both 4-week diet groups after L-NMMA compared to without	WBB improves vascular function through eNOS/NO pathway.

Table 2.1. Research studies examining the impact of blueberry consumption on endothelial function in animal models.

Reference & Location	Animal Model	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Possible Mechanistic/ Pathway-related Results	Mechanism/ Pathway Conclusions
						L-NMMA. ↑ vessel sensitivity in both 7-week diets after L-NMMA compared to without L-NMMA 7-week diets, but ↓ compared to their 4-week diet counterparts after L-NMMA.	
Kristo *et al.* (2013) *Maine, USA*	Spon-taneously hyper-tensive male rats (*n* = 20).	Vaso-constriction and vessel sensitivity in thoracic aorta rings.	Freeze-dried WBB powder added to diets at 8% w/w for 8 weeks. Two diet groups: control and WBB.	Total phenolics (gallic acid equivalents) were identified at 4.4% w/w, with chlorogenic acid most abundant (510 mg/100 g). 1.5% w/w ACNs concentration, with malvidin	↓ vasoconstriction to Phe in WBB group compared to control. No significant effects on other vascular measures.	↑ vasoconstriction in both groups after ODQ administration compared to no ODQ, with ↑ vasoconstriction in WBB + ODQ compared to control + ODQ. ↓ vasoconstriction in both groups after TCP administration compared to no TCP but did not change	WBB improves vascular function through NO-sGC-cGMP signalling pathway.

Table 2.1. Research studies examining the impact of blueberry consumption on endothelial function in animal models.

Reference & Location	Animal Model	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Possible Mechanistic/ Pathway-related Results	Mechanism/ Pathway Conclusions
				3-galactoside and peonidin 3-glucoside making up 13% of ACNs content (1.6 ± 0.2 mg/100 mg).		association of WBB response curve to control. ↓ vasoconstriction in WBB + TCP compared to control + TCP. ↓ vasoconstriction in WBB + dazoxiben compared to control + dazoxiben; however, TXA_2 was not associated with WBB improving vascular function since ↓ vasoconstriction in WBB group was seen compared to control regardless of inhibitor administration. ↑ plasma cGMP and $6kPGF_{1\alpha}$	

Table 2.1. Research studies examining the impact of blueberry consumption on endothelial function in animal models.

Reference & Location	Animal Model	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Possible Mechanistic/ Pathway-related Results	Mechanism/ Pathway Conclusions
						in WBB group compared to control.	
Rodriguez-Mateos *et al. (*2013) *Tokushima, Japan and England, UK*	Wistar male rats (*n* = 32).	Vaso-constriction and vaso-relaxation in aorta rings.	Freeze-dried highbush BB powder added to diets at 2% w/w for 10 weeks. Four diet groups: control chow, control chow + BB, high-fat, and high-fat + BB.	Flavonoids (mg/100g diet) in 2% blueberry diets: ACNs 11·80, procyanidins 8·46, procyanidin monomers 0·63, and procyanidin dimers 1·78. Total flavonoids measured were 20·26 mg/100g diet.	↓ vasoconstriction to Phe in control chow + BB group compared to control chow and high-fat group. ↓ vasoconstriction to Phe in high-fat + BB group compared to control chow and high-fat group. ↑ vasorelaxation to Ach in high-fat + BB group compared to high-fat group.	Total flavonoid intake was ~3·85 mg of total flavonoids. ↓ SBP at 8 and 10 weeks in control chow + BB group compared to control chow. ↓ SBP in high-fat + BB group at 10 weeks compared to high-fat control.	Highbush BB improves vascular function through the endothelium.
Vendrame *et al.* (2014) *Maine, USA*	Obese Zucker male rats (*n* = 72).	Vaso-constriction, vaso-relaxation, and vessel sensitivity in aorta rings.	Freeze-dried WBB powder added to diets at 8% w/w for 8 weeks.	Total ACNs was 1.5% w/w, with peonidin-3-glucoside and malvidin-3-galactoside most abundant.	↑ vasoconstriction and ↓ vasorelaxation in obese + WBB group compared to obese control. ↑ vasorelaxation in lean + WBB group	↑ vasoconstriction in obese and lean control groups after MFA or L-NMMA administration compared to controls without inhibitor, with	WBB improves vascular function through COX and eNOS/NO pathways, with

Table 2.1. Research studies examining the impact of blueberry consumption on endothelial function in animal models.

Reference & Location	Animal Model	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Possible Mechanistic/ Pathway-related Results	Mechanism/ Pathway Conclusions
			Four diet groups: lean, lean + WBB, obese, obese + WBB.		compared to lean control. ↑ vessel sensitivty to Ach in lean + WBB and obese control compared to lean control. ↓ vessel sensitivty to Ach in obese + WBB compared to obese control.	obese + WBB + MFA ↑ vasoconstrcition compard to obese control + MFA and a further ↑ in vasoconstrcition in obese + WBB + L-NMMA to the level of lean control. ↓ vasorelaxation in obese + WBB + L-NMMA compared to obese + L-NMMA control. ↑ vessel sesnistivity to Phe in both obese and lean + L-NMMA and MFA compared to obese and lean without inhibitor. ↑ vessel sesnistivity in obese + WBB + L-NMMA compared to obese + WBB	eNOS/NO pathway seeming to be greater.

Table 2.1. Research studies examining the impact of blueberry consumption on endothelial function in animal models.

Reference & Location	Animal Model	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Possible Mechanistic/ Pathway-related Results	Mechanism/ Pathway Conclusions
						and obese control + L-NMMA. ↑ vessel sensitivity in obese + WBB + MFA and obese control + MFA compared to obese + WBB and obese control, respectively. ↓ plasma NO in obese + WBB compared to obese control. ↑ plasma NO in lean + WBB and obese control compared to lean control. ↑ aortic effluent 6kPGF$_{1\alpha}$ in obese + WBB compared to obese control. ↑ iNOS aorta gene expression in obese control and	

Table 2.1. Research studies examining the impact of blueberry consumption on endothelial function in animal models.

Reference & Location	Animal Model	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Possible Mechanistic/ Pathway-related Results	Mechanism/ Pathway Conclusions
						↓ in lean + WBB compared to lean control. ↓ iNOS aorta gene expression in obese + WBB compared to obese control. ↑ COX-2 aorta gene expression in obese control compared to lean control. ↓ COX-2 aorta gene expression in obese + WBB group compared to obese control.	
Klimis-Zacas *et al. (*2016) *Maine, USA*	Obese Zucker male rats (*n* = 72).	Vaso-constriction and vaso-relaxation in thoracic aorta rings.	Freeze-dried WBB powder added to diets at 8% w/w for 8 weeks. Four diet groups:	21 different ACNs were identified in WBB powder: Peonidin-3-glucoside and malvidin-3-galactoside most	↓ vasoconstriction to Phe in obese control compared to lean control. ↑ vasoconstriction to Phe in obese WBB compared to obese control.	↓ IL-6, TNF-α, and CRP in obese WBB group compared to obese control. ↓ NO in obese WBB group compared to obese control.	WBB improves vascular function through eNOS/NO and COX pathways.

Table 2.1. Research studies examining the impact of blueberry consumption on endothelial function in animal models.

Reference & Location	Animal Model	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Possible Mechanistic/ Pathway-related Results	Mechanism/ Pathway Conclusions
			lean control, lean WBB, obese control, and obese WBB.	abundant of total ACNs, and total ACNs content of WBB powder was 1.5% w/w.	↑ vasorelaxation to Ach in lean control compared to obese control.	↑ NO in lean WBB groups compared to lean control. ↑ $6kPGF_{1\alpha}$ in obese WBB group compared to obese control. ↓ aorta gene expression of iNOS in obese and lean WBB group compared to their controls. ↓ aorta gene expression of COX-2 in obese WBB group compared to control.	
Petersen *et al.* (2022) *Utah, USA*	Diabetic *db/*db male mice (*n* = 45).	Vaso-relaxation of mesenteric arteries.	Freeze-dried WBB powder added to diets at 3.8% w/w for 10 weeks.	N/A	↑ Endothelium-dependent vasorelaxation to Ach in WBB group compared to diabetic control.	↓ ICAM-1 and VCAM-1 and monocyte binding to vessel in WBB group compared to diabetic control. ↓ serum inflammatory	WBB improves endothelium-dependent vascular function through reduced NOX4 activity

Table 2.1. Research studies examining the impact of blueberry consumption on endothelial function in animal models.

Reference & Location	Animal Model	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Possible Mechanistic/ Pathway-related Results	Mechanism/ Pathway Conclusions
			Three diet groups: control non-diabetic mice, control diabetic mice, and diabetic mice + WBB.			chemokines, MCP1/JF and KC, in WBB group compared to diabetic control. ↓ monocyte binding and KC secretion in mouse arterial endothelial cells incubated with serum from WBB group compared to diabetic control. ↓ NOX4 and IκkB expression in arterial endothelial cells and aortic vessel in WBB group compared to diabetic control. Gut microbes significantly differed between all groups. Trends in microbiota alpha diversity indices (Shannon and	(and likely reduced oxidative stress), and potentially through reduced inflammation, and changes in the microbiome.

Table 2.1. Research studies examining the impact of blueberry consumption on endothelial function in animal models.

Reference & Location	Animal Model	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Possible Mechanistic/ Pathway-related Results	Mechanism/ Pathway Conclusions
						Simpson) in WBB group compared to diabetic control. Beta-diversity significantly different at the phylum and genius level between all groups. WBB supplementation significantly increased *Actinobacteria* abundance compared to diabetic control. WBB supplementation significantly increased *Adlercreutzia, Bifidobacterium,* and *Dorea* compared to diabetic control. WBB supplementation showed changes in predicted pathways in diabetic mice	

Table 2.1. Research studies examining the impact of blueberry consumption on endothelial function in animal models.

Reference & Location	Animal Model	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Possible Mechanistic/ Pathway-related Results	Mechanism/ Pathway Conclusions
						compared to diabetic control.	

Abbreviations: Ach, acetylcholine (vasodilator); $6kPGF_{1\alpha}$, 6-keto prostaglandin F1; ACNs, anthocyanin; BB, blueberry; CRP, C-reactive protein; COX-2, cyclooxygenase 2; cGMP, cyclic guanosine monophosphate; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase; IκkB, inhibitor Kappa B kinase; ICAM-1, intercellular adhesion molecule 1; IL-6, interleukin 6; NOX4, NADPH oxidase 4; NO, nitric oxide; L-NMMA, L-N^G-monomethyl-arginine (eNOS inhibitor); MFA, mefenamic acid (COX inhibitor); MCP-1, monocyte chemoattractant protein-1; N/A, not available; ODQ, 1H-[1,2,4]oxadiazolo[4,3-α]quinoxalin-1-one (sGC inhibitor); Phe, L-phenylephrine (vasoconstrictor); PDE_5, phosphodiesterase-5; Phe, phenylephrine; PGI_2, prostaglandin I_2; SBP, systolic blood pressure; TXA_2, thromboxane A2, TCP, tranylcypromine (PGI_2 synthase inhibitor); TNF-α, tumor necrosis factor alpha; VCAM-1, vascular cell adhesion molecule 1; WBB, wild blueberry.

Table 2.2. Human clinical trials examining the impact of acute (≤ 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
Del Bo *et al.* (2013) *Milan, Italy*	Randomized, crossover, controlled trial (blinding not specified).	Healthy males (*n* = 10). [2]Age: 20.8 ± 1.6 y BMI 22.5 ± 2.1 kg/m^2; SBP 119.5 ± 8.8 mmHg; DBP 76.5 ± 6.2 mmHg. [2]BB group: SBP 119.5 ± 8.8 mmHg; DBP 76.5 ± 6.2 mmHg; RHI 1.96 ± 0.39. [2]Control group: SBP 122.5 ± 10.4 mmHg; DBP 76.3 ± 4.8 mmHg; RHI 1.94 ± 0.30.	RHI. Measures taken at baseline and 1 h after treatment consumption.	300 g whole highbush BB partially thawed into a gelatinous mixture. Control jelly consisted of 20 g gelatin + sugars to match BB mixed in 200 mL hot water and cooled to solidify to match BB consistency. Each treatment period separated by 10-day washout.	727 mg total phenolic acids, 348.3 mg ACNs, and 90.3 mg chlorogenic acid.	No significant effects on vascular measures.	↓ H_2O_2-induced DNA damage in participant's PBMCs at 1 h, not 2 or 24 h, after BB consumption compared to control.
Rodriguez-Mateos *et al.* (2013) *England, UK*	Randomized, double-blind, crossover, controlled trial.	Healthy males (*n* = 10). [1]Age 27 ± 1.3 y; BMI 25 ± 0.8 kg/m^2; SBP 123 ± 2.3 mmHg;	FMD, PWV, AIx, DVP-SI, and DVP-RI. Measures taken at baseline and	34, 57 and 80 g of freeze-dried WBB powder mixed with 500 mL of low-nitrate water.	34 g WBB powder contains 766 mg total (poly)phenols, 310 mg ACNs, 137 mg	↑ FMD 1 h after WBB consumption (all doses) compared to baseline and placebo.	↑ plasma benzoic acid at baseline and 2 h after 34 g WBB consumption

Table 2.2. Human clinical trials examining the impact of acute (≤ 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
	Time-course trial.	DBP 71 ± 2.1 mmHg; FMD 7.1 ± 0.1%. [1]34 g WBB group: FMD 7.0 ± 0.2%; PWV 5.9 ± 0.2 m/s; AIx 2.3 ± 3.9%; DVP-SI 6.0 ± 0.4 m/s; DVP-RI 67 ± 4%. [1]57 g WBB group: FMD 7.2 ± 0.5%; PWV 5.9 ± 0.2 m/s; AIx 2.3 ± 3.4%; DVP-SI 6.2 ± 0.4 m/s; DVP-RI 78 ± 2%. [1]80 g WBB group: FMD 7.0 ± 0.3%; PWV 5.8 ± 0.2 m/s; AIx 1.4 ± 3.8%; DVP-SI 6.1 ± 0.2 m/s; DVP-RI 70 ± 5%.	2, 4, and 6 h after treatment consumption. FMD was also measured 1 h after treatment consumption.	Placebo drink was matched to the 57 g blueberry drink for macronutrient and micronutrient content. Washout period not specified.	procyanidins, and 273 mg chlorogenic acid. 57 g WBB powder contains 1278 mg total (poly)phenols, 517 mg ACNs, 228 mg procyanidins, and 455 mg chlorogenic acid. 80 g WBB powder contains1791 mg total (poly)phenols, 724 mg ACNs, 320 mg procyanidins, and 637 mg chlorogenic acid.	↑ FMD 2 h after 34 g and 57 g WBB consumption compared to baseline and placebo, while ↑ FMD at 2 h for 80 g dose only compared to placebo. ↑ FMD 6 h after WBB consumption for 34 g dose compared to baseline and placebo, while ↑ FMD at 6 h for 54 g dose compared only to placebo. No significant effects on	compared to placebo. ↑ in 6 plasma (poly)phenol metabolites at increase 1-2 h post 34 g WBB consumption and later increase of 8 metabolites 6 h post 34 g WBB consumption. ↓ neutrophil NOX activity 1-2 and 6 h after 34 g WBB consumption. Δ plasma vanillic acid, hippuric acid, and homovanillic acid predicted ↓ in NOX activity.

Table 2.2. Human clinical trials examining the impact of acute (≤ 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
		[1]Control group: FMD 7.1 ± 0.3%; PWV 5.9 ± 0.4 m/s; AIx 3.1 ± 5.9%; DVP-SI 6.4 ± 0.2 m/s; DVP-RI 69 ± 7%.				other vascular measures.	Signifcant correlation between Δ FMD and NOX activity after 34 g WBB consumption.
Rodriguez-Mateos *et al.* (2013) *England, UK*	Randomized, double-blind, crossover, 6-arm controlled trial. Dose-dependent trial.	Healthy males (*n* = 11). [1]Age 27 ± 1.0 y; BMI 22 ± 0.9 kg/m^2; SBP 120 ± 1.7 mmHg; DBP 65 ± 1.8 mmHg; FMD 6.3 ± 0.2%.	FMD. Measures taken at baseline and 1 h after treatment consumption.	14, 28, 34, 57, and 80 g freeze-dried WBB powder mixed with 500 mL of low-nitrate water. Placebo drink was matched to 57 g WBB drink for macronutrient and micronutrient content. Washout period not specified.	14 g WBB powder contained 319 mg total (poly)phenols, 129 mg ACNs, 57 mg procyanidins, and 114 mg chlorogenic acid. 28 g WBB powder contained 639 mg total (poly)phenols, 258 mg ACNs, 114 mg procyanidins, and 228 mg chlorogenic acid.	↑ FMD at 1 h after WBB consumption up to 34 g WBB and plateaued thereafter for other doses.	No other significant findings.

Table 2.2. Human clinical trials examining the impact of acute (≤ 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
					34 g WBB powder contained 766 mg total (poly)phenols, 310 mg ACNs, 137 mg procyanidins, and 273 mg chlorogenic acid. 57 g WBB powder contained 1278 mg total (poly)phenols, 517 mg ACNs, 228 mg procyanidins, and 455 mg chlorogenic acid. 80 g WBB powder contained 1791 mg total (poly)phenols, 724 mg ACNs, 320 mg procyanidins,		

Table 2.2. Human clinical trials examining the impact of acute (≤ 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
					and 637 mg chlorogenic acid.		
Rodriguez-Mateos *et al.* (2014) *England, UK*	Randomized, crossover, controlled trial.	Healthy males (*n* = 10). [1]Age 27 ± 1 y; BMI 25 ± 0.8; SBP 124 ± 2.6 mmHg; DBP 74 ± 2.5 mmHg; FMD 7.0 ± 0.1%.	FMD. Measures taken at baseline, and 1, 2, 4, and 6 h after treatment consumption.	Baked product using 34 g freeze-dried WBB powder or 34 g unprocessed freeze-dried WBB powder drink or matched control baked product (bun). Each treatment period separated by 1-week washout.	Baked WBB product contained 196 ± 7.7 mg ACNs, 140 ± 7.4 procyanidins, and 221± 10 mg chlorogenic acid. Unprocessed WBB drink contained 339 ± 6.1 mg ACNs, 111 ± 4.1 procyanidins, and 179 ± 1 mg chlorogenic acid.	↑ FMD 1, 2, and 6 h after baked WBB product consumption compared to control bun with max increase at 2 h. ↑ FMD 1, 2, and 6 h after unprocessed WBB drink consumption compared to control bun with max increase at 1 h (instead of 2 h seen with baked product).	At 1-2 h post bun consumption, plasma vanillic and ferulic acids predicted ↑ FMD, while vanillic and benzoic acids predicted ↑ FMD after unprocessed WBB drink consumption. At 4-6 h post unprocessed WBB drink consumption, plasma hippuric, hydroxy-hippuric, and homovanillic acids predicted ↑ FMD after

Table 2.2. Human clinical trials examining the impact of acute (≤ 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
							4-6 h unprocessed WBB drink consumption. *Compared to Control Bun:* At 1-2 h, ↑ in 4 metabolites after baked WBB product and ↑in 6 metabolites after unprocessed BB drink consumption. At 6 h, ↑ in 4 metabolites after baked WBB product and increase in 8 metabolites after unprocessed WBB drink consumption.

Table 2.2. Human clinical trials examining the impact of acute (≤ 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
							Compared to Unprocessed WBB drink: ↑ C_{max} of plasma 3-hydroxy-phenylacetic acid after baked WBB product consumption and ↓ in plasma benzoic and hippuric acids. ↓ AUC 0-6 h plasma benzoic, hippuric, salicyclic, and sinapic acids and ↑ AUC 0-6 h plasma ferulic, hydroxy-hippuric, and 3-hydroxy-phenylacetic acids after

Table 2.2. Human clinical trials examining the impact of acute (≤ 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
							baked WBB product consumption.
Del Bo *et al.* (2014) *Milan, Italy*	Randomized crossover, 3-arm, controlled trial (blinding not specified).	Healthy male smokers, defined as ~15 cigarettes/d (*n* = 16). [1]Age 23.6 ± 0.7 y; BMI 23.0 ± 0.5 kg/m^2; SBP 116.0 ± 1.7 mmHg; DBP 76.1 ± 2.1 mmHg; RHI 2.23 ± 0.07; F-RHI 0.65 ± 0.07; dAIx -8.6 ± 2.0%; dAIX@75 bpm -18.4 ± 2.2%.	RHI, F-RHI, dAIx, and dAIx@75 bpm. Measures were taken at baseline and 20 min after treatment consumption.	300 g whole highbush BB + smoking, control treatment (300 mL water with sugar) + smoking, or smoking alone with no BB or control treatment. Each treatment period separated by 1-week washout.	726.6 mg total phenolic acids, 348.3 mg ACNs, and 90.3 mg chlorogenic acid.	↓ RHI, F-RHI, dAIx, and dAIx@ 75bpm after smoking. Highbush BB consumption counteracted impairments in RHI and F-RHI. No significant effects on other vascular measures.	BB significantly counteracted ↑ SBP from smoking.
Del Bo *et al.* (2017) *Milan, Italy*	Randomized, crossover, 2-arm controlled pilot trial (blinding not specified).	Nonsmoking men with peripheral arterial dysfunction, defined as RHI < 1.67 (*n* = 12).	RHI, dAIx, and dAIx@75 bpm. Measures were taken at baseline	300 g whole highbush BB or control treatment (300 mL water with sugar).	856 mg total phenolic acids, 309 mg ACNs, and 30 mg chlorogenic acid.	↑ RHI after highbush BB consumption in nonsmokers compared to control.	No other significant findings.

Table 2.2. Human clinical trials examining the impact of acute (≤ 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
		[1]Age 24.2 ± 1.2 y; BMI 22.5 ± 1.2 kg/m^2; SBP 116.9 ± 3.2 mmHg; DBP 75.3 ± 2.9 mmHg; RHI 1.41 ± 0.07; dAIx -14.6 ± 2.7%; dAIX@75 bpm -20.0 ± 5.8%.	and 120 min after treatment consumption.	Each treatment period separated by 1-week washout.		No significant effects on other vascular measures.	
Del Bo *et al.* (2017) *Milan, Italy*	Randomized, crossover, 3-arm controlled pilot trial (blinding not specified).	Smoking men with peripheral arterial dysfunction, defined as RHI < 1.67 (*n* = 12). [1]Age 24.5 ± 1.9 y; BMI 22.9 ± 1.1 kg/m^2; SBP 118.2 ± 2.9 mmHg; DBP 75.7 ± 2.7 mmHg; RHI 1.47 ± 0.05; dAIx -12.7 ± 2.5%; dAIx@75 bpm -18.2 ± 5.0%.	RHI, dAIx, and dAIx@75 bpm. Measures were taken at baseline and 120 min after treatment consumption.	300 g whole highbush BB + smoking, control treatment (300 mL water with sugar) + smoking, or smoking alone with no BB or control treatment. Each treatment period separated by 1-week washout.	856 mg total phenolic acids, 309 mg ACNs, and 30 mg chlorogenic acid.	↑ RHI in highbush BB + smoking and control treatment + smoking groups compared to smoking alone. No significant effects on other vascular measures.	No other significant findings.

Table 2.2. Human clinical trials examining the impact of acute (≤ 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
Dodd *et al.* (2019) *Berkshire, UK*	Randomized, crossover, controlled trial (blinding not specified).	Older adults with cognitive decline (*n* = 18). [2]Age 68.72 ± 3.30 y; BMI 25.89 ± 4.46 kg/m^2; SBP 135.02 ± 7.34 mmHg; DBP 78.55 ± 3.38 mmHg.	DVP (secondary outcome). Measures taken at baseline and 1 h after treatment consumption.	Freeze-dried highbush BB powder (~30 g) and control powder mixed in 300 mL semi-skimmed milk. Washout period not specified.	507.79 mg ACNs and 71.03 mg epicatechin oligomers.	No significant effects on vascular measures.	BB treatment trended towards attenuation of ↑ SBP after control treatment.
Rodriguez-Mateos *et al.* (2019) *England, UK*	Randomized, double-blind, crossover, 5-arm controlled trial.	Healthy males (*n* = 5). [2]Age 23 ± 3 y; BMI 24 ± 3 kg/m^2; SBP 124 ± 11 mmHg; DBP 71 ± 9 mmHg.	FMD. Measures taken at baseline and 1, 2, and 6 h after treatment consumption.	5 different drinks consisting of: 1) 11 g freeze-dried WBB powder drink mixed with 500 mL of low-nitrate water, 2) control drink, 3) control drink + fiber, 4) control drink + minerals and vitamins, and 5) 160 mg pure ACNs	WBB drink: 150 mg ACNs and 64 mg chlorogenic acid. ACNs drink: 160 mg ACNs.	↑ FMD at 2 and 6 h post WBB and ACNs drink consumption compared to control drink.	Plasma ferulic acid-4-*O*-sulfate, isoferulic acid-3-*O*-*β*-*D*-glucuronide, dihydroferulic acid, dihydroferulic acid 4-*O*-*β*-*D*-glucuronide, chlorogenic acid, vanillic acid, homovanillic acid, proto-catechuic acid, syringic acid,

Table 2.2. Human clinical trials examining the impact of acute (≤ 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
				drink. Each treatment period separated by 1-week washout.			4-hydroxy-benozic acid, 4-methylgallic acid-3-*O*-sulfate, 1-methyl-pyrogallol-*O*-sulfate, 4-hydroxy-phenyl acetic acid, and quercetin 3-*O*-*β*-*D*-glucuronide all significantly correlated to 2-h FMD in WBB group.
Curtis *et al.* (2022) *Anglia, UK*	Randomized, double-blind, parallel arm, controlled trial.	Adults with metabolic syndrome (*n* = 45). [2]Age 63.2 ± 8.8 y; [2]BMI 31.5 ± 2.9 kg/m^2; [3]SBP 136 (133,140) mmHg; [3]DBP 82.9 (81.0, 84.8) mmHg; [3]FMD	FMD, AIx@75 bpm, and cfPWV. Measures were taken at baseline, and then at 3, 6, and 24 h after consumption.	26 g freeze-dried Highbush BB or 26 g placebo-matched powder. Powder added to 500 g energy-dense	879 mg total phenolic acids and 364 mg ACNs.	No significant effects on vascular measures.	↑ serum and urine ACNs metabolite concentration 24 h after consumption, with greater ↑ serum concentration at 6 and 24 h, and greater ↑ urinary

Table 2.2. Human clinical trials examining the impact of acute (≤ 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
		2.1 (1.6, 2.7)%; [3]AIx@75 bpm 35.7 (33.7, 37.7)%; [3]PWV 11.0 (10.5, 11.4) m/s.		emulsion meal and 50 g water into opaque shaker bottle and consumed by participant within 15 min.			concentration at 6-24 h compared to placebo. ↑ postprandial plasma glucose 1 h and ↓ postprandial plasma glucose and insulin in BB group 3 h compared to placebo. ↑ HDL at 90 min and 6 h in BB group compared to placebo. ↑ apo-A, XL-HDLP, and L-HDLP at 6 h as well as XL-HDLP at 3 h in BB compared to placebo.

Table 2.2. Human clinical trials examining the impact of acute (≤ 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
							↓ TC at 24 h in BB compared to placebo.

[1]Data are presented as mean ± SEM; [2]Data are presented as mean ± SD; [3]Data are presented as mean (ranges).
Abbreviations: ACNs, anthocyanins; Apo-A1, apolipoprotein A-1; AIx, augmentation index; AIx@75 bpm, AIx normalized to heart rate of 75 beats per minute; BB, blueberry; baPWV, brachial-ankle pulse wave velocity; BP, blood pressure; BMI, body mass index; cfPWV, carotid-femoral pulse wave velocity; C_{max}, maximum concentration; DBP, diastolic blood pressure; dAIx, digital augmentation index; DVP-RI, digital volume pulse reflection index; DVP-SI, digital volume pulse stiffness index; FMD, flow-mediated dilation; F-RHI, Framingham reactive hyperemia index; HDL-C, high density lipoprotein-cholesterol; N/A, not available; NO, nitric oxide; PBMC, peripheral blood mononuclear cells; PWA, pulse wave analysis; PWV, pulse wave velocity; RHI, reactive hyperemia index; SBP, systolic blood pressure; XL/L-HDLP, extra-large/large high density lipoprotein particles; TC, total cholesterol; WBB, wild blueberry.

Table 2.3. Human clinical trials examining the impact of chronic (> 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
Riso *et al.* (2013) *Milan, Italy*	Randomized, crossover, controlled trial (blinding not specified).	Healthy males with at least one CVD risk factor (*n* = 18). [2]Age 47.8 ± 9.7 y; BMI 24.8 ± 2.6 kg/m^2; SBP 121 ± 16 mmHg, and DBP 79.4 ± 8.7 mmHg; RHI 1.84 ± 0.46; FRHI 0.32 ± 0.27; AIx 5.22 ± 18.5; AIx@75 bpm 0.0 ± 17.4.	RHI, F-RHI, AIx, and AIx@75 bpm. Measures taken at baseline and after 6 weeks of treatment consumption.	25 g freeze-dried WBB powder or placebo powder containing similar sensory characteristics as WBB powder mixed with 250 mL of water and consumed daily for 6 weeks. Each treatment period separated by 6-week washout.	WBB treatment contained 375 mg ACNs and 127.5 mg chlorogenic acid.	No significant effects on vascular measures.	↓ Endogenous oxidized DNA bases and H_2O_2-induced DNA damage in blood mononuclear cells in WBB group compared to placebo.
McAnulty *et al.* (2014) *North Carolina, USA*	Randomized, double-blind, parallel arm, controlled trial.	Sedentary men and postmenopausal women (*n* = 25). [2]BB group (*n* = 13): age 46.15 ± 11.92 y; BMI 27.8 ± 5.46 kg/m^2; SBP 117.23 ± 7.85	cfPWV and AIx. Measures taken at baseline and after 6 weeks of treatment consumption.	19 g tifblue/rubel BB powder or 19 g placebo powder consumed 2x/day (38 g/day total) each night with evening meal for 6 weeks.	N/A	↓ AIx in BB group compared to placebo. No significant effects on other vascular measures.	↓ aortic systolic pressure in BB group compared to placebo. ↓ DBP in pre-hypertensive participants.

Table 2.3. Human clinical trials examining the impact of chronic (> 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
		mmHg; DBP 74.61 ± 11.46 mmHg; cfPWV 8.4 ± 1.1 $m \cdot s^2$; AIx 18.91 ± 11 m/s^2. [2]Placebo group (*n* = 12): age 39.92 ± 13.38 y; BMI 24.23 ± 3.44 kg/m^2; SBP 113.53 ± 10.39 mmHg; DBP 70.15 ± 8.8 mmHg; cfPWV 8.8 ± 1.9 $m \cdot s^2$; AIx 23.2 ± 7.79 m/s^2.					↑ NK cells in BB group compared to placebo.
Johnson *et al.* (2015) *Florida, USA*	Randomized, double-blind, placebo-controlled, parallel arm, controlled trial.	Postmenopausal women with elevated BP or stage-1 hypertension (*n* = 40). [2]BB group (*n* = 20): age 59.7 ± 4.58 y; BMI 30.1 ± 5.94 kg/m^2; SBP 138 ± 14 mmHg; DBP	baPWV and cfPWV. Measures taken at baseline, 4 weeks, and 8 weeks.	11 g freeze-dried highbush BB powder or 11 g calorie-matched placebo powder mixed in 1 cup (240 mL) of water consumed 2x/day (22 g/day total) for 8 weeks.	BB treatment contained 844.58 mg phenolics and 469.48 mg ACNs.	↓ baPWV in BB group compared to baseline and placebo. No significant effects on other vascular measures.	↓ SBP and DBP in BB group compared to baseline and placebo. ↑ plasma NO in BB group compared to baseline.

Table 2.3. Human clinical trials examining the impact of chronic (> 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
		80 ± 7 mmHg; baPWV 1,498 ± 179 cm/s; cfPWV 1,234 ± 201 cm/s. [2]Placebo group (*n* = 20): age 57.3 ± 4.76 y; BMI 32.7 ± 6.54 kg/m^2; SBP 138 ± 15 mmHg; DBP 78 ± 8 mmHg; baPWV 1,464 ± 194 cm/s; cfPWV 1,233 ± 194 cm/s.					
Stull *et al.* (2015) *Louisiana, USA*	Randomized, double-blind, parallel arm, controlled trial.	Adults with metabolic syndrome (*n* = 44). [1]BB group (*n* = 23): age 55 ± 2 y; BMI 35.2 ± 0.8 kg/m^2; SBP 125.7 ± 2.2 mmHg; DBP 82.7 ± 1.9 mmHg;	RHI. Measures were taken at baseline and after 6 weeks of treatment consumption.	45 g freeze-dried highbush BB powder or placebo-matched powder added in a smoothie containing 12 oz yogurt and skim milk consumed 2x/day (22.5g) for 6 weeks.	BB treatment contained 773.6 mg phenolics and 290.3 mg ACNs.	↑ RHI in BB group compared to control.	No other significant results.

Table 2.3. Human clinical trials examining the impact of chronic (> 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
		RHI 1.94 ± 0.11 (*n* = 22 for RHI). [1]Placebo group (*n* = 21): age 59 ± 2 y; BMI 42.8 ± 1.6 kg/m^2; SBP 125.0 ± 3.2 mmHg; DBP 77.5 ± 1.9 mmHg; RHI 2.34 ± 0.16 (*n* = 18 for RHI).					
Stote *et al.* (2017) *New York, USA*	Randomized, crossover, single-blind, controlled trial.	Adults at risk for type 2 diabetes (*n* = 19). [2]Age 53 ± 6.3 y; BMI 31.4 ± 2.9 kg/m^2; SBP 126.6 ± 14.0 mmHg; DBP 81.7 ± 8.7 mmHg.	RHI. Measures were taken at baseline and after 7 days of treatment consumption.	120 mL WBB juice and 120 mL placebo drink containing similar characteristics to blueberry drink without (poly)phenols consumed 2x/day (240 mL) for 7 days. Each treatment period separated by 8-day washout.	WBB treatment contained 2,138 mg phenolics, and 314 mg ACNs.	No significant effects on vascular measure.	Trend ↓ SBP after WBB treatment. ↑ plasma nitrate and nitrite concentrations after WBB treatment compared to placebo.

Table 2.3. Human clinical trials examining the impact of chronic (> 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
Curtis *et al.* (2019) *Anglia, UK*	Randomized, double-blind, parallel arm, controlled trial.	Adults with metabolic syndrome (*n* = 115). [2]BB group (13 g) (*n* = 37): age 62.6 ± 7.2 y; BMI 31.2 ± 2.6 kg/m^2; [3]SBP 134-138 mmHg; [3]DBP 80.3-83.0 mmHg. [2]BB group (26 g) (*n* = 39): age 63.0 ± 5.9 y; BMI 31.3 ± 3.4 kg/m^2; [3]SBP 134-138 mmHg; [3]DBP 79.6-82.2 mmHg. [2]Placebo group (*n* = 39): age 62.9 ± 8.1 y; BMI 31.1 ± 3.0 kg/m^2; [3]SBP 134-138 mmHg; [3]DBP 79.9-82.5 mmHg.	FMD, AIx, and cfPWV. Measures taken at baseline and after 6 months of treatment consumption.	3 interventions: 1) 26 g freeze-dried highbush BB, 2) 13 g freeze-dried BB and 13 g placebo-matched powder, and 3) 26 g placebo-matched powder consumed as drink/smoothie, or added to cereals/ yogurts, banana toast, or to salads as a dressing 1x/day for 6 months.	26 g BB treatment contained 364 mg ACNs and 879 mg phenolics. 13 g BB treatment contained 182 mg ACNs and 439.5 mg phenolics.	↑ FMD after 26 g BB consumption compared to 13 g BB dose and placebo. ↓ AIx after 26 g BB consumption compared to 13 g dose and placebo. No significant effects on other vascular measures.	↑ mean plasma cGMP concentrations after 26 g BB treatment compared to placebo. ↑ serum and 24-h urine ACNS concentrations after BB treatment in a dose-dependent manner. ↑ HDL-C, ApoA1, and HDL-P in non-statin users after 26 g BB consumption compared to placebo.

Table 2.3. Human clinical trials examining the impact of chronic (> 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
Rodriguez-Mateos *et al.* (2019) *England, UK*	Uncontrolled, single-arm pilot study (blinding not specified).	Healthy males (*n* = 5). [2]Age 24 ± 2 y; BMI 24 ± 3 kg/m^2; SBP 122 ± 7 mmHg; DBP 73 ± 8 mmHg.	FMD. Measures taken at baseline and 7, 14, 21, and 28 days after treatment consumption.	11 g WBB powder consumed 2x/day (22 g total) mixed with 500 mL water for 28 days.	WBB treatment contained 150 mg ACNs, 49 mg flavanol monomers, 31 mg flavanol oligomers, and 64 mg chlorogenic acid.	↑ FMD after 1 week of WBB treatment with further improvement at 2 weeks and plateaued thereafter.	No other related outcomes measured.
Rodriguez-Mateos *et al.* (2019) *England, UK*	Randomized, double-blind, parallel 2-arm, acute-on-chronic, controlled trial.	Healthy males (*n* = 40). [2]Age 33 ± 6 y; BMI 24 ± 3 kg/m^2; SBP 128 ± 10 mmHg; DBP 75 ± 9 mmHg.	FMD, cfPWV, and AIx. Measures taken at baseline and after 28 days of treatment consumption. For the acute-on-chronic study, measures were taken at 0 and 2 h post	11 g WBB powder or placebo-matched powder consumed 2x/day (22 g total) mixed with 500 mL water for 28 days.	WBB treatment contained 150 mg ACNs, 49 mg flavanol monomers, 31 mg flavanol oligomers, and 64 mg chlorogenic acid.	↑ FMD after 28 days of WBB treatment compared to placebo. No further improvement in FMD after acute consumption of WBB treatment on 28th day. No significant effects on	↓ 24-h SBP in WBB group compared to placebo. 21 plasma (poly)phenol metabolites significantly correlated to FMD increase after WBB consumption. 357 genes upregulated and 251 genes downregulated in PBMCs after

Table 2.3. Human clinical trials examining the impact of chronic (> 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
			blueberry consumption on day 28 of intervention.			other vascular measures.	28 days of WBB treatment. 3 miRNA differentially expressed in PBMCs after 28 days of WBB treatment.
Wang *et al.* (2022) *England, UK*	Randomized, crossover, controlled trial.	Healthy adults (n = 37). [2]Age 25.86 ± 6.81; BMI 23.15 ± 3.12. [1]Whole BB group (*n* = 37): SBP 108.727 ± 1.411 mmHg; DBP 64.059 ± 1.440 mmHg; cfPWV 7.630 ± 0.153 m/s. [1]BB powder group (*n* = 37): SBP 110.278 ± 1.711 mmHg; DBP 64.333 ± 1.482 mmHg;	cfPWV. Measures taken at baseline and after 1 week of treatment consumption.	160 g fresh whole highbush BB, 20 g freeze-dried BB powder, and control capsule containing plant-derived fiber micro-crystalline cellulose consumed 1x/day for 1 week. Each treatment period separated by 1-week washout.	Fresh highbush BB contained 220.48 mg/d total (poly)phenols. Freeze-dried BB powder contained 288.43 mg/d total (poly)phenols.	No significant effects on vascular measure.	No other significant results.

Table 2.3. Human clinical trials examining the impact of chronic (> 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
		cfPWV 7.993 ± 0.258 m/s. [1]Placedo group (*n* = 37): SBP 109.314 ± 1.691 mmHg; DBP 63.676 ± 1.395 mmHg; cfPWV 8.265 ± 0.209 m/s.					
Woolf *et al.* (2023) *Colorado, USA*	Randomized, double-blind, parallel arm, controlled trial.	Postmenopausal women with elevated BP or stage-1 hypertension (*n* = 43). [1]BB group (*n* = 22): age 60 ± 1 y; BMI 27.6 ± 1.0 kg/m^2; SBP 130 ± 1 mmHg; DBP 80 ± 1 mmHg; FMD 3.84 ± 1.02%; cfPWV 7.9 ± 0.2 m/s; AIx 36 ± 1%; AIx@75 bpm 29 ±2%.	FMD, FMD/SR$_{AUC}$, cfPWV, AIx, and AIx@75 bpm. Measures taken at baseline and after 12 weeks of treatment consumption.	11 g freeze-dried Highbush BB powder or 11 g placebo powder matched for calories mixed in 1 cup (240 mL) of water consumed 2x/day (22 g/day total) for 12 weeks.	BB treatment contained 766 mg total (poly)phenols and 224 mg ACNs.	↑ FMD/SR$_{AUC}$ after 12 weeks of BB consumption and the Δ from baseline to 12 weeks in FMD/SR$_{AUC}$ ↑ compared to placebo group. FMD statistically non-significant, but a clinically significant,	Supra-physiologic ascorbic acid infusion acutely ↑ FMD/SR$_{AUC}$ in both groups at baseline but significant differences went away at 12 weeks in the BB group while trending differences were still present for placebo group at 12 weeks, and the difference between

Table 2.3. Human clinical trials examining the impact of chronic (> 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
		[1]Placebo group (*n* = 21): age 57.3 ± 4.76 y; BMI 27.8 ± 61.1 kg/m^2; SBP 128 ± 1 mmHg; DBP 82 ± 1 mmHg; FMD 4.69 ± 0.55%; cfPWV 7.4 ± 0.2 m/s; AIx 37 ± 1%; AIx@75 bpm 31 ± 1%.				increase of 1.34% after 12 weeks of BB consumption compared to baseline. No significant effects on other vascular measures.	ascorbic acid and saline FMD/SR_{AUC} ↓ at 12 weeks compared to baseline in the BB group but not for the placebo group. ↑ sum of plasma (poly)phenol metabolites in BB group compared to baseline and placebo at 4, 8, and 12 weeks. Δ from baseline to 4 and 12 weeks in sum of (poly)phenol metabolites ↑ in BB group compared to placebo. ↑ plasma hippuric acid, 3-hydroxy-hippuric,

Table 2.3. Human clinical trials examining the impact of chronic (> 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
							vanillic acid-4-sulfate, protocatechuic acid, and 2,5-dihydroxybenzoic acid in BB group. Δ in 8 plasma (poly)phenol metabolites from baseline to 12 weeks derived from myrcetin, phenyl-propanoic aicd, benzoic acid, cinnamic acid, and phenylacetic acid were correlated with Δ FMD/SR_{AUC} from baseline to 12 weeks in BB group, but not placebo. 1 plasma (poly)phenol

Table 2.3. Human clinical trials examining the impact of chronic (> 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
							metabolite at 12 weeks derived from benzoic acid was correlated to Δ in FMD/SR_{AUC} from baseline to 12 weeks in BB group. Δ p47phox from baseline to 12 weeks was negatively correlated Δ FMD/SR_{AUC} from baseline to 12 weeks, and p-eNOS at 12 weeks was positively correlated to Δ in FMD/SR_{AUC} from baseline to 12 weeks in BB group.
Wood *et al.* (2023) *England, UK*	Randomized, double-blind, parallel arm, controlled trial.	Healthy older adults aged 65-80 y (*n* = 61). [2]BB group (*n* = 32): age 69.44 ±	FMD, cfPWV, and AIx@75 bpm.	26 g freeze-dried WBB powder or 26 g placebo-matched powder mixed	WBB treatment contained 302 mg ACNs and 202.1 mg cholorogenic	↑ FMD after 12 weeks of WBB compared to placebo.	↓ 24-h SBP in WBB group compared to placebo at 12 weeks.

Table 2.3. Human clinical trials examining the impact of chronic (> 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
		3.48 y; BMI 24.57 ± 2.7 kg/m^2; SBP 128.52 ± 11.63 mmHg; DBP 81.05 ± 7.86 mmHg; FMD 3.62 ± 1.53%; cfPWV 7.94 ± 3.07 m/s; AIx@75 bpm 27.8 ± 7.11%. [2]Placebo group (*n* = 29): age 70.76 ± 3.81 y; BMI 23.16 ± 2.59 kg/m^2; SBP 128.36 ± 10.01 mmHg; DBP 79.59 ± 5.59 mmHg; FMD 4.11 ± 1.14%; cfPWV 8.47 ± 2.35 m/s; AIx@75 bpm 29.4 ± 11.0%.	Measures taken at baseline and after 12 weeks of treatment consumption.	in water consumed 1x/day for 12 weeks.	acid.	No significant effects on other vascular measures.	↑ total 24-h urinary (poly)phenol excretion in the WBB group compared to placebo at 12 weeks. ↑ plasma pyrogallol-*O*-sulfate, 2 methyl-pyrogallol-*O*-sulfate, 4-methylcatechol-*O*-sulfate, isoferulic acid, and 4-methylcatechol acid in WBB compared to placebo at 12 weeks. ↓ plasma phenylacetic acid and vanillic acid in plasma from WBB group compared to

Table 2.3. Human clinical trials examining the impact of chronic (> 24 h) blueberry consumption on vascular function.

Reference & Location	Study Design	Participant Baseline Characteristics	Vascular Measures	Blueberry Treatment	Blueberry (Poly)phenol Content	Vascular Results	Other Related Results
							to placebo group at 12 weeks. Δ in 6 plasma (poly)phenol metabolites derived from cinnamic acid, benzoic acid, phenylacetic acid, and hippuric acid from baseline to 12 weeks were correlated to Δ in FMD from baseline to 12 weeks.

[1]Data are presented as mean ± SEM; [2]Data are presented as mean ± SD; [3]Data are presented as ranges.
Abbreviations: ACNs, anthocyanins; ApoA1, apolipoprotein A1; AIx, augmentation index; AIx@ 75bpm, AIx normalized to heart rate of 75 bpm; BB, blueberry; baPWV, brachial-ankle pulse wave velocity; BMI, body mass index; cfPWV, carotid-femoral pulse wave velocity; crPWV, carotid-radial pulse wave velocity; DBP, diastolic blood pressure; FMD, flow-mediated dilation; F-RHI, Framingham reactive hyperemia index; H_2O_2, hydrogen peroxide; HDL-C, high-density lipoprotein cholesterol; HDL, high-density lipoprotein particle concentration; NA, not available; NO, nitric oxide; p47phox, NADPH oxidase/p47; p-eNOS, phosphorylated endothelial nitric oxide synthase; PWV, pulse wave velocity; RHI, reactive hyperemia index; systolic blood pressure, SBP; wild blueberry, WBB.

REFERENCES

1. COVID-19 was third leading cause of death in the United States in both 2020 and 2021, https://www.nih.gov/news-events/news-releases/covid-19-was-third-leading-cause-death-united-states-both-2020-2021).
2. Cardiovascular diseases, https://www.who.int/health-topics/cardiovascular-diseases/#tab=tab_1).
3. S. A. Johnson, N. S. Litwin and D. R. Seals, *J Acad Nutr Diet*, 2019, **119**, 1785-1796.
4. C. W. Tsao, A. W. Aday, Z. I. Almarzooq, A. Alonso, A. Z. Beaton, M. S. Bittencourt, A. K. Boehme, A. E. Buxton, A. P. Carson, Y. Commodore-Mensah, M. S. V. Elkind, K. R. Evenson, C. Eze-Nliam, J. F. Ferguson, G. Generoso, J. E. Ho, R. Kalani, S. S. Khan, B. M. Kissela, K. L. Knutson, D. A. Levine, T. T. Lewis, J. Liu, M. S. Loop, J. Ma, M. E. Mussolino, S. D. Navaneethan, A. M. Perak, R. Poudel, M. Rezk-Hanna, G. A. Roth, E. B. Schroeder, S. H. Shah, E. L. Thacker, L. B. VanWagner, S. S. Virani, J. H. Voecks, N. Y. Wang, K. Yaffe and S. S. Martin, *Circulation*, 2022, **145**, e153-e639.
5. D. R. Seals and L. M. Alexander, *J Appl Physiol (1985)*, 2018, **125**, 1841-1842.
6. J. A. Chirinos, P. Segers, T. Hughes and R. Townsend, *J Am Coll Cardiol*, 2019, **74**, 1237-1263.
7. G. Brevetti, A. Silvestro, V. Schiano and M. Chiariello, *Circulation*, 2003, **108**, 2093-2098.
8. Y. Inaba, J. A. Chen and S. R. Bergmann, *Int J Cardiovasc Imaging*, 2010, **26**, 631-640.
9. Q. N. Dinh, G. R. Drummond, C. G. Sobey and S. Chrissobolis, *Biomed Res Int*, 2014, **2014**, 406960.
10. T. J. Guzik and R. M. Touyz, *Hypertension*, 2017, **70**, 660-667.
11. M. L. Battson, D. M. Lee, T. L. Weir and C. L. Gentile, *J Nutr Biochem*, 2018, **56**, 1-15.

12. E. Wood, S. Hein, C. Heiss, C. Williams and A. Rodriguez-Mateos, *Food Funct*, 2019, **10**, 7621-7633.
13. F. Potì, D. Santi, G. Spaggiari, F. Zimetti and I. Zanotti, *Int J Mol Sci*, 2019, **20**.
14. C. Matsumoto, *Curr Pharm Des*, 2018, **24**, 140-145.
15. R. D. Mendonça, N. C. Carvalho, J. M. Martin-Moreno, A. M. Pimenta, A. C. S. Lopes, A. Gea, M. A. Martinez-Gonzalez and M. Bes-Rastrollo, *Nutr Metab Cardiovasc Dis*, 2019, **29**, 69-78.
16. M. H. Oak, C. Auger, E. Belcastro, S. H. Park, H. H. Lee and V. B. Schini-Kerth, *Free Radic Biol Med*, 2018, **122**, 161-170.
17. B. R. Cutler, C. Petersen and P. V. Anandh Babu, *Mol Nutr Food Res*, 2017, **61**.
18. L. Zhang, Z. Han and D. Granato, *Adv Food Nutr Res*, 2021, **98**, 1-33.
19. C. Petersen, D. Bharat, U. D. Wankhade, J. S. Kim, B. R. Cutler, C. Denetso, S. Gholami, S. Nelson, J. Bigley, A. Johnson, S. V. Chintapalli, B. D. Piccolo, A. K. Satheesh Babu, H. A. Paz, K. Shankar, J. D. Symons and P. V. Anandh Babu, *Mol Nutr Food Res*, 2022, **66**, e2100784.
20. ERS Charts of Note, https://www.ers.usda.gov/data-products/charts-of-note/charts-of-note/?page=2&topicId=b64476d3-203d-4eea-ad5c-59272f480979).
21. Nutrition Facts, https://blueberry.org/health-benefits/nutrition-facts/).
22. C. Li, J. Feng, W. Y. Huang and X. T. An, *J Agric Food Chem*, 2013, **61**, 523-531.
23. J. Li, D. Ruzhi, X. Hua, L. Zhang, F. Lu, T. G. Coursey, S. C. Pflugfelder and D. Q. Li, *Sci Rep*, 2016, **6**, 19408.
24. D. Stevenson, *Journal of Berry Research*, 2012, **2**, 179-189.
25. A. W. Ashor, O. M. Shannon, A. D. Werner, F. Scialo, C. N. Gilliard, K. S. Cassel, C. J. Seal, D. Zheng, J. C. Mathers and M. Siervo, *Clin Nutr*, 2020, **39**, 708-717.
26. A. W. Ashor, J. Lara, J. C. Mathers and M. Siervo, *Atherosclerosis*, 2014, **235**, 9-20.

27. L. Al-Khudairy, N. Flowers, R. Wheelhouse, O. Ghannam, L. Hartley, S. Stranges and K. Rees, *Cochrane Database Syst Rev*, 2017, **3**, Cd011114.

28. J. Zhu, Y. Ling, L. A. Tse, S. Kinra and Y. Li, *Nutr Metab Cardiovasc Dis*, 2021, **31**, 2398-2406.

29. E. A. O'Connor, C. V. Evans, I. Ivlev, M. C. Rushkin, R. G. Thomas, A. Martin and J. S. Lin, *Jama*, 2022, **327**, 2334-2347.

30. A. J. van Ballegooijen and J. W. Beulens, *Curr Nutr Rep*, 2017, **6**, 197-205.

31. O. Meishuo, E. S. Eshak, I. Muraki, R. Cui, K. Shirai, H. Iso and A. Tamakoshi, *J Atheroscler Thromb*, 2022, **29**, 1432-1447.

32. J. K. Kresovich, C. M. Bulka, B. T. Joyce, P. S. Vokonas, J. Schwartz, A. A. Baccarelli, E. A. Hibler and L. Hou, *Biol Trace Elem Res*, 2018, **183**, 49-57.

33. K. Makki, E. C. Deehan, J. Walter and F. Bäckhed, *Cell Host Microbe*, 2018, **23**, 705-715.

34. E. S. Chambers, T. Preston, G. Frost and D. J. Morrison, *Curr Nutr Rep*, 2018, **7**, 198-206.

35. I. Robles-Vera, M. Toral, N. de la Visitación, N. Aguilera-Sánchez, J. M. Redondo and J. Duarte, *Front Physiol*, 2020, **11**, 277.

36. K. Goszcz, G. G. Duthie, D. Stewart, S. J. Leslie and I. L. Megson, *Br J Pharmacol*, 2017, **174**, 1209-1225.

37. J. van Duynhoven, E. E. Vaughan, D. M. Jacobs, R. A. Kemperman, E. J. van Velzen, G. Gross, L. C. Roger, S. Possemiers, A. K. Smilde, J. Doré, J. A. Westerhuis and T. Van de Wiele, *Proc Natl Acad Sci U S A*, 2011, **108 Suppl 1**, 4531-4538.

38. F. Cardona, C. Andrés-Lacueva, S. Tulipani, F. J. Tinahones and M. I. Queipo-Ortuño, *J Nutr Biochem*, 2013, **24**, 1415-1422.

39. Y. Konishi, Z. Zhao and M. Shimizu, *J Agric Food Chem*, 2006, **54**, 7539-7543.

40. V. Crespy, C. Morand, C. Besson, C. Manach, C. Demigne and C. Remesy, *J Agric Food Chem*, 2002, **50**, 618-621.

41. S. Passamonti, U. Vrhovsek, A. Vanzo and F. Mattivi, *FEBS Lett*, 2003, **544**, 210-213.

42. S. Talavéra, C. Felgines, O. Texier, C. Besson, A. Gil-Izquierdo, J. L. Lamaison and C. Rémésy, *J Agric Food Chem*, 2005, **53**, 3902-3908.

43. S. Zhong, A. Sandhu, I. Edirisinghe and B. Burton-Freeman, *Mol Nutr Food Res*, 2017, **61**.

44. W. Kalt, *Molecules*, 2019, **24**.

45. A. Rodriguez-Mateos, C. Rendeiro, T. Bergillos-Meca, S. Tabatabaee, T. W. George, C. Heiss and J. P. Spencer, *Am J Clin Nutr*, 2013, **98**, 1179-1191.

46. A. Rodriguez-Mateos, R. Del Pino-García, T. W. George, A. Vidal-Diez, C. Heiss and J. P. Spencer, *Mol Nutr Food Res*, 2014, **58**, 1952-1961.

47. A. Rodriguez-Mateos, G. Istas, L. Boschek, R. P. Feliciano, C. E. Mills, C. Boby, S. Gomez-Alonso, D. Milenkovic and C. Heiss, *J Gerontol A Biol Sci Med Sci*, 2019, **74**, 967-976.

48. D. P. Cladis, H. Debelo, P. J. Lachcik, M. G. Ferruzzi and C. M. Weaver, *Mol Nutr Food Res*, 2020, **64**, e2000031.

49. E. Wood, S. Hein, R. Mesnage, F. Fernandes, N. Abhayaratne, Y. Xu, Z. Zhang, L. Bell, C. Williams and A. Rodriguez-Mateos, *Am J Clin Nutr*, 2023, DOI: 10.1016/j.ajcnut.2023.03.017.

50. E. K. Woolf, J. D. Terwoord, N. S. Litwin, A. R. Vazquez, S. Y. Lee, N. Ghanem, K. A. Michell, B. T. Smith, L. E. Grabos, N. B. Ketelhut, N. P. Bachman, M. E. Smith, M. Le Sayec, S. Rao, C. L. Gentile, T. L. Weir, A. Rodriguez-Mateos, D. R. Seals, F. A. Dinenno and S. A. Johnson, *Food Funct*, 2023, **14**, 2621-2641.

51. C. E. Iglesias-Aguirre, A. Cortés-Martín, M. Ávila-Gálvez, J. A. Giménez-Bastida, M. V. Selma, A. González-Sarrías and J. C. Espín, *Food Funct*, 2021, **12**, 10324-10355.

52. S. K. Nicholson, G. A. Tucker and J. M. Brameld, *Br J Nutr*, 2010, **103**, 1398-1403.

53. A. Sandoo, J. J. van Zanten, G. S. Metsios, D. Carroll and G. D. Kitas, *Open Cardiovasc Med J*, 2010, **4**, 302-312.

54. A. J. Donato, D. R. Machin and L. A. Lesniewski, *Circ Res*, 2018, **123**, 825-848.

55. P. Theofilis, M. Sagris, E. Oikonomou, A. S. Antonopoulos, G. Siasos, C. Tsioufis and D. Tousoulis, *Biomedicines*, 2021, **9**.

56. T. D. Giles, G. E. Sander, B. D. Nossaman and P. J. Kadowitz, *J Clin Hypertens (Greenwich)*, 2012, **14**, 198-205.

57. P. M. Vanhoutte, H. Shimokawa, M. Feletou and E. H. Tang, *Acta Physiol (Oxf)*, 2017, **219**, 22-96.

58. M. Rizzo, J. Kotur-Stevuljevic, K. Berneis, G. Spinas, G. B. Rini, Z. Jelic-Ivanovic, V. Spasojevic-Kalimanovska and J. Vekic, *Transl Res*, 2009, **153**, 217-223.

59. C. Bleakley, P. K. Hamilton, R. Pumb, M. Harbinson and G. E. McVeigh, *J Clin Hypertens (Greenwich)*, 2015, **17**, 651-654.

60. K. L. Moreau, K. L. Hildreth, A. L. Meditz, K. D. Deane and W. M. Kohrt, *J Clin Endocrinol Metab*, 2012, **97**, 4692-4700.

61. K. L. Moreau and K. L. Hildreth, *Adv Vasc Med*, 2014, **2014**.

62. K. L. Moreau, K. L. Hildreth, J. Klawitter, P. Blatchford and W. M. Kohrt, *Geroscience*, 2020, **42**, 1699-1714.

63. M. A. Incalza, R. D'Oria, A. Natalicchio, S. Perrini, L. Laviola and F. Giorgino, *Vascul Pharmacol*, 2018, **100**, 1-19.

64. H. N. Siti, Y. Kamisah and J. Kamsiah, *Vascul Pharmacol*, 2015, **71**, 40-56.

65. P. F. Lawton, M. D. Lee, C. D. Saunter, J. M. Girkin, J. G. McCarron and C. Wilson, *Front Physiol*, 2019, **10**, 99.

66. A. J. Flammer, T. Anderson, D. S. Celermajer, M. A. Creager, J. Deanfield, P. Ganz, N. M. Hamburg, T. F. Lüscher, M. Shechter, S. Taddei, J. A. Vita and A. Lerman, *Circulation*, 2012, **126**, 753-767.

67. K. E. Pyke, E. M. Dwyer and M. E. Tschakovsky, *J Appl Physiol (1985)*, 2004, **97**, 499-508.

68. J. Padilla, B. D. Johnson, S. C. Newcomer, D. P. Wilhite, T. D. Mickleborough, A. D. Fly, K. J. Mather and J. P. Wallace, *Cardiovasc Ultrasound*, 2008, **6**, 44.

69. J. Padilla, B. D. Johnson, S. C. Newcomer, D. P. Wilhite, T. D. Mickleborough, A. D. Fly, K. J. Mather and J. P. Wallace, *J Vasc Res*, 2009, **46**, 592-600.

70. A. L. Axtell, F. A. Gomari and J. P. Cooke, *J Vis Exp*, 2010, DOI: 10.3791/2167.

71. D. H. J. Thijssen, R. M. Bruno, A. van Mil, S. M. Holder, F. Faita, A. Greyling, P. L. Zock, S. Taddei, J. E. Deanfield, T. Luscher, D. J. Green and L. Ghiadoni, *Eur Heart J*, 2019, **40**, 2534-2547.

72. P. J. Little, C. D. Askew, S. Xu and D. Kamato, *Biomedicines*, 2021, **9**.

73. K. Motozato, Y. Suematsu, K. Norimatsu, T. Kusumoto and S. I. Miura, *J Clin Med Res*, 2020, **12**, 293-299.

74. R. T. Ras, M. T. Streppel, R. Draijer and P. L. Zock, *Int J Cardiol*, 2013, **168**, 344-351.

75. G. Wilk, G. Osmenda, P. Matusik, D. Nowakowski, B. Jasiewicz-Honkisz, A. Ignacak, M. Cześnikiewicz-Guzik and T. J. Guzik, *Pol Arch Med Wewn*, 2013, **123**, 443-452.

76. R. Rubinshtein, J. T. Kuvin, M. Soffler, R. J. Lennon, S. Lavi, R. E. Nelson, G. M. Pumper, L. O. Lerman and A. Lerman, *Eur Heart J*, 2010, **31**, 1142-1148.

77. M. Shechter, S. Matetzky, M. Prasad, O. Goitein, R. Goldkorn, M. Naroditsky, N. Koren-Morag and A. Lerman, *Int J Cardiol*, 2017, **240**, 14-19.

78. C. Norton, A. Z. Kalea, P. D. Harris and D. J. Klimis-Zacas, *J Med Food*, 2005, **8**, 8-13.

79. A. Z. Kalea, K. Clark, D. A. Schuschke and D. J. Klimis-Zacas, *J Med Food*, 2009, **12**, 21-28.

80. C. Del Bo, A. S. Kristo, A. Z. Kalea, S. Ciappellano, P. Riso, M. Porrini and D. Klimis-Zacas, *Nutr Metab Cardiovasc Dis*, 2012, **22**, 127-132.

81. A. Rodriguez-Mateos, A. Ishisaka, K. Mawatari, A. Vidal-Diez, J. P. Spencer and J. Terao, *Br J Nutr*, 2013, **109**, 1746-1754.

82. A. Z. Kalea, K. Clark, D. A. Schuschke, A. S. Kristo and D. J. Klimis-Zacas, *J Nutr Biochem*, 2010, **21**, 14-22.

83. A. S. Kristo, A. Z. Kalea, D. A. Schuschke and D. J. Klimis-Zacas, *J Agric Food Chem*, 2010, **58**, 11600-11605.

84. A. S. Kristo, A. Z. Kalea, D. A. Schuschke and D. Klimis-Zacas, *Int J Food Sci Nutr*, 2013, **64**, 979-987.

85. D. Klimis-Zacas, S. Vendrame and A. S. Kristo, *Journal of Berry Research*, 2016, **6**, 225-236.

86. C. L. Oltman, L. L. Richou, E. P. Davidson, L. J. Coppey, D. D. Lund and M. A. Yorek, *Am J Physiol Heart Circ Physiol*, 2006, **291**, H1780-1787.

87. R. Subramanian and K. M. MacLeod, *Eur J Pharmacol*, 2003, **477**, 143-152.

88. T. J. Andrews, D. W. Laight, E. E. Anggård and M. J. Carrier, *J Pharm Pharmacol*, 2000, **52**, 83-86.

89. S. Moncada and A. Higgs, *N Engl J Med*, 1993, **329**, 2002-2012.

90. V. Mollace, C. Muscoli, E. Masini, S. Cuzzocrea and D. Salvemini, *Pharmacol Rev*, 2005, **57**, 217-252.

91. S. Vendrame, A. S. Kristo, D. A. Schuschke and D. Klimis-Zacas, *Appl Physiol Nutr Metab*, 2014, **39**, 255-261.

92. C. Del Bó, P. Riso, J. Campolo, P. Møller, S. Loft, D. Klimis-Zacas, A. Brambilla, A. Rizzolo and M. Porrini, *Nutr Res*, 2013, **33**, 220-227.

93. C. Del Bo, M. Porrini, D. Fracassetti, J. Campolo, D. Klimis-Zacas and P. Riso, *Food Funct*, 2014, **5**, 3107-3116.

94. C. Del Bo, V. Deon, J. Campolo, C. Lanti, M. Parolini, M. Porrini, D. Klimis-Zacas and P. Riso, *Food Funct*, 2017, **8**, 4108-4117.

95. P. J. Curtis, L. Berends, V. van der Velpen, A. Jennings, L. Haag, P. Chandra, C. D. Kay, E. B. Rimm and A. Cassidy, *Clin Nutr*, 2022, **41**, 165-176.

96. P. Riso, D. Klimis-Zacas, C. Del Bo, D. Martini, J. Campolo, S. Vendrame, P. Møller, S. Loft, R. De Maria and M. Porrini, *Eur J Nutr*, 2013, **52**, 949-961.

97. K. S. Stote, M. I. Sweeney, T. Kean, D. J. Baer, J. A. Novotny, N. L. Shakerley, A. Chandrasekaran, P. M. Carrico, J. A. Melendez and K. T. Gottschall-Pass, *BMC Nutr*, 2017, **3**, 45.

98. A. J. Stull, K. C. Cash, C. M. Champagne, A. K. Gupta, R. Boston, R. A. Beyl, W. D. Johnson and W. T. Cefalu, *Nutrients*, 2015, **7**, 4107-4123.

99. P. J. Curtis, V. van der Velpen, L. Berends, A. Jennings, M. Feelisch, A. M. Umpleby, M. Evans, B. O. Fernandez, M. S. Meiss, M. Minnion, J. Potter, A. M. Minihane, C. D. Kay, E. B. Rimm and A. Cassidy, *Am J Clin Nutr*, 2019, **109**, 1535-1545.

100. J. P. Tsai and B. G. Hsu, *Tzu Chi Med J*, 2021, **33**, 115-121.

101. Q. Zhong, M. J. Hu, Y. J. Cui, L. Liang, M. M. Zhou, Y. W. Yang and F. Huang, *Angiology*, 2018, **69**, 617-629.

102. K. Nagai, S. Shibata, M. Akishita, N. Sudoh, T. Obara, K. Toba and K. Kozaki, *Atherosclerosis*, 2013, **231**, 365-370.

103. H. Tomiyama and K. Shiina, *J Atheroscler Thromb*, 2020, **27**, 621-636.

104. H. L. Kim and S. H. Kim, *Front Cardiovasc Med*, 2019, **6**, 41.

105. T. Pereira, C. Correia and J. Cardoso, *J Med Biol Eng*, 2015, **35**, 555-565.

106. J. H. Janner, N. S. Godtfredsen, S. Ladelund, J. Vestbo and E. Prescott, *Am J Hypertens*, 2010, **23**, 180-185.

107. S. R. Alty, N. Angarita-Jaimes, S. C. Millasseau and P. J. Chowienczyk, *IEEE Trans Biomed Eng*, 2007, **54**, 2268-2275.

108. S. C. Millasseau, R. P. Kelly, J. M. Ritter and P. J. Chowienczyk, *Clin Sci (Lond)*, 2002, **103**, 371-377.

109. A. Gunarathne, J. V. Patel, E. A. Hughes and G. Y. Lip, *Am J Hypertens*, 2008, **21**, 866-872.

110. G. F. Dodd, C. M. Williams, L. T. Butler and J. P. Spencer, *Nutrition and Healthy Aging*, 2019, **5**, 119-132.

111. Y. Wang, J. L. Gallegos, C. Haskell-Ramsay and J. K. Lodge, *Nutrients*, 2022, **14**.

112. L. S. McAnulty, S. R. Collier, M. J. Landram, D. S. Whittaker, S. E. Isaacs, J. M. Klemka, S. L. Cheek, J. C. Arms and S. R. McAnulty, *Nutr Res*, 2014, **34**, 577-584.

113. S. A. Johnson, A. Figueroa, N. Navaei, A. Wong, R. Kalfon, L. T. Ormsbee, R. G. Feresin, M. L. Elam, S. Hooshmand, M. E. Payton and B. H. Arjmandi, *J Acad Nutr Diet*, 2015, **115**, 369-377.

114. M. J. Durand and D. D. Gutterman, *Microcirculation*, 2013, **20**, 239-247.

115. I. Fleming, *Pflugers Arch*, 2010, **459**, 793-806.

116. M. S. Wong and P. M. Vanhoutte, *Acta Pharmacol Sin*, 2010, **31**, 1095-1102.

117. T. Gaziano, K. S. Reddy, F. Paccaud, S. Horton and V. Chaturvedi, *Disease Control Priorities in Developing Countries. 2nd edition*, 2006.

118. J. A. Araujo, M. Zhang and F. Yin, *Front Pharmacol*, 2012, **3**, 119.

119. W. Huang, Y. Zhu, C. Li, Z. Sui and W. Min, *Oxid Med Cell Longev*, 2016, **2016**, 1591803.

120. S. H. Park, S. O. Jeong, H. T. Chung and H. O. Pae, *Plant Foods Hum Nutr*, 2015, **70**, 263-268.

121. T. W. Buford, *Ageing Res Rev*, 2016, **26**, 96-111.

122. M. E. Widlansky, D. T. Price, N. Gokce, R. T. Eberhardt, S. J. Duffy, M. Holbrook, C. Maxwell, J. Palmisano, J. F. Keaney, Jr., J. D. Morrow and J. A. Vita, *Hypertension*, 2003, **42**, 310-315.

123. S. J. Lee, S. E. Baek, M. A. Jang and C. D. Kim, *Exp Mol Med*, 2019, **51**, 1-12.

124. S. Čejková, I. Králová-Lesná and R. Poledne, *Cor et Vasa*, 2016, **58**, e419-e425.

125. A. N. Lyle and U. Raaz, *Arterioscler Thromb Vasc Biol*, 2017, **37**, e1-e11.

126. D. Agnoletti, F. Piani, A. F. G. Cicero and C. Borghi, *J Clin Med*, 2022, **11**.

127. F. H. Karlsson, F. Fåk, I. Nookaew, V. Tremaroli, B. Fagerberg, D. Petranovic, F. Bäckhed and J. Nielsen, *Nat Commun*, 2012, **3**, 1245.

128. M. Witkowski, T. L. Weeks and S. L. Hazen, *Circ Res*, 2020, **127**, 553-570.

129. V. E. Brunt, R. A. Gioscia-Ryan, J. J. Richey, M. C. Zigler, L. M. Cuevas, A. Gonzalez, Y. Vázquez-Baeza, M. L. Battson, A. T. Smithson, A. D. Gilley, G. Ackermann, A. P. Neilson, T. Weir, K. P. Davy, R. Knight and D. R. Seals, *J Physiol*, 2019, **597**, 2361-2378.

130. S. R. J. Trikha, D. M. Lee, K. E. Ecton, S. D. Wrigley, A. R. Vazquez, N. S. Litwin, K. N. Thomas, Y. Wei, M. L. Battson, S. A. Johnson, K. A. Kuhn, S. P. Colgan, C. L. Gentile and T. L. Weir, *Gut Microbes*, 2021, **13**, 1940791.

131. A. D. Baldeon, D. McDonald, A. Gonzalez, R. Knight and H. D. Holscher, *J Nutr*, 2023, DOI: 10.1016/j.tjnut.2023.02.018.

132. L. Narduzzi, V. Agulló, C. Favari, N. Tosi, C. Mignogna, A. Crozier, D. D. Rio and P. Mena, *Microbiome Research Reports*, 2022, **1**, 16.

133. T. Uebanso, T. Shimohata, K. Mawatari and A. Takahashi, *Mol Nutr Food Res*, 2020, **64**, e2000426.

134. A. Vijay and A. M. Valdes, *Eur J Clin Nutr*, 2022, **76**, 489-501.

135. C. Petersen and J. L. Round, *Cell Microbiol*, 2014, **16**, 1024-1033.

136. X. Zhang and P. Gérard, *Comput Struct Biotechnol J*, 2022, **20**, 1528-1540.

137. S. K. Masenga, B. Hamooya, J. Hangoma, V. Hayumbu, L. A. Ertuglu, J. Ishimwe, S. Rahman, M. Saleem, C. L. Laffer, F. Elijovich and A. Kirabo, *J Hum Hypertens*, 2022, **36**, 952-959.

138. T. De Bruyne, B. Steenput, L. Roth, G. R. Y. De Meyer, C. N. D. Santos, K. Valentová, M. Dambrova and N. Hermans, *Nutrients*, 2019, **11**.

139. D. Bharat, R. R. M. Cavalcanti, C. Petersen, N. Begaye, B. R. Cutler, M. M. A. Costa, R. Ramos, M. R. Ferreira, Y. Li, L. P. Bharath, E. Toolson, P. Sebahar, R. E. Looper, T. Jalili, N. S. Rajasekaran, Z. Jia, J. D. Symons and P. V. Anandh Babu, *Mol Nutr Food Res*, 2018, **62**.

140. J. S. Tang, M. C. M. Vissers, R. F. Anderson, S. Sreebhavan, S. M. Bozonet, A. Scheepens and L. D. Melton, *Mol Nutr Food Res*, 2018, **62**.

141. S. A. Johnson, R. G. Feresin, N. Navaei, A. Figueroa, M. L. Elam, N. S. Akhavan, S. Hooshmand, S. Pourafshar, M. E. Payton and B. H. Arjmandi, *Food Funct*, 2017, **8**, 372-380.

142. A. R. Nair, N. Mariappan, A. J. Stull and J. Francis, *Food Funct*, 2017, **8**, 4118-4128.

143. D. Martini, M. Marino, S. Venturi, M. Tucci, D. Klimis-Zacas, P. Riso, M. Porrini and C. Del Bo, *J Nutr Biochem*, 2023, **111**, 109154.

144. W. Y. Huang, Y. M. Liu, J. Wang, X. N. Wang and C. Y. Li, *Molecules*, 2014, **19**, 12827-12841.

145. C. Del Bo, M. Roursgaard, M. Porrini, S. Loft, P. Møller and P. Riso, *Mol Nutr Food Res*, 2016, **60**, 2355-2366.

146. B. R. Cutler, S. Gholami, J. S. Chua, B. Kuberan and P. V. Anandh Babu, *Int J Cardiol*, 2018, **261**, 155-158.

147. C. Del Bo, M. Marino, P. Riso, P. Møller and M. Porrini, *Chem Biol Interact*, 2019, **300**, 49-55.

148. J. S. Tang, S. M. Bozonet, J. L. McKenzie, R. F. Anderson, L. D. Melton and M. C. M. Vissers, *Mol Nutr Food Res*, 2019, **63**, e1900478.

149. J. Correa-Betanzo, E. Allen-Vercoe, J. McDonald, K. Schroeter, M. Corredig and G. Paliyath, *Food Chem*, 2014, **165**, 522-531.

150. Y. Cheng, T. Wu, X. Chu, S. Tang, W. Cao, F. Liang, Y. Fang, S. Pan and X. Xu, *Lwt*, 2020, **125**, 109260.

151. A. Mahnic, J. M. Auchtung, N. Poklar Ulrih, R. A. Britton and M. Rupnik, *Sci Rep*, 2020, **10**, 8358.

152. A. L. Molan, M. A. Lila, J. Mawson and S. De, *World Journal of Microbiology and Biotechnology*, 2009, **25**, 1243-1249.

153. A. Ntemiri, T. S. Ghosh, M. E. Gheller, T. T. T. Tran, J. E. Blum, P. Pellanda, K. Vlckova, M. C. Neto, A. Howell, A. Thalacker-Mercer and P. W. O'Toole, *Nutrients*, 2020, **12**.

154. S. Vendrame, S. Guglielmetti, P. Riso, S. Arioli, D. Klimis-Zacas and M. Porrini, *J Agric Food Chem*, 2011, **59**, 12815-12820.

155. S. Guglielmetti, D. Fracassetti, V. Taverniti, C. Del Bo, S. Vendrame, D. Klimis-Zacas, S. Arioli, P. Riso and M. Porrini, *J Agric Food Chem*, 2013, **61**, 8134-8140.

CHAPTER 3: DAILY BLUEBERRY CONSUMPTION FOR 12 WEEKS IMPROVES ENDOTHELIAL FUNCTION IN POSTMENOPAUSAL WOMEN WITH ABOVE-NORMAL BLOOD PRESSURE THROUGH REDUCTIONS IN OXIDATIVE STRESS: A RANDOMIZED CONTROLLED CLINICAL TRIAL[1]

Summary

Estrogen-deficient postmenopausal women have oxidative stress-mediated suppression of endothelial function that is exacerbated by high blood pressure. Previous research suggests blueberries may improve endothelial function through reductions in oxidative stress, while also exerting other cardiovascular benefits. The objective of this study was to examine the efficacy of blueberries to improve endothelial function and blood pressure in postmenopausal women with above-normal blood pressure, and to identify potential mechanisms for improvements in endothelial function. A randomized, double-blind, placebo-controlled, parallel-arm clinical trial was performed, where postmenopausal women aged 45–65 years with elevated blood pressure or stage 1-hypertension (total n = 43, endothelial function n = 32) consumed 22 g day^{-1} of freeze-dried highbush blueberry powder or placebo powder for 12 weeks. Endothelial function was assessed at baseline and 12 weeks through ultrasound measurement of brachial artery flow-mediated dilation (FMD) normalized to shear rate area under the curve (FMD/SR_{AUC}) before and after intravenous infusion of a supraphysiologic dose of ascorbic acid to evaluate whether FMD improvements were mediated by reduced oxidative stress. Hemodynamics, arterial stiffness, cardiometabolic blood biomarkers, and plasma (poly)phenol metabolites were assessed at baseline and 4, 8, and 12 weeks, and venous endothelial cell protein expression

[1] This is the peer review but unedited manuscript version of the following article: E. K. Woolf, J. D. Terwoord, N. S. Litwin, A. R. Vazquez, S. Y. Lee, N. Ghanem, K. A. Michell, B. T. Smith, L. E. Grabos, N. B. Ketelhut, N. P. Bachman, M. E. Smith, M. Le Sayec, S. Rao, C. L. Gentile, T. L. Weir, A. Rodriguez-Mateos, D. R. Seals, F. A. Dinenno and S. A. Johnson, *Food Funct*, 2023, **14**, 2621-2641.

was assessed at baseline and 12 weeks. Absolute FMD/SR_{AUC} was 96% higher following blueberry consumption compared to baseline ($p < 0.05$) but unchanged in the placebo group ($p > 0.05$), and changes from baseline to 12 weeks were greater in the blueberry group than placebo ($+1.09 \times 10^{-4} \pm 4.12 \times 10^{-5}$ *vs.* $+3.82 \times 10^{-6} \pm 1.59 \times 10^{-5}$, $p < 0.03$, respectively). The FMD/SR_{AUC} response to ascorbic acid infusion was lower ($p < 0.05$) at 12 weeks compared to baseline in the blueberry group with no change in the placebo group ($p > 0.05$). The sum of plasma (poly)phenol metabolites increased at 4, 8, and 12 weeks in the blueberry group compared to baseline and were higher than the placebo group (all $p < 0.05$). Increases in several plasma flavonoid and microbial metabolites were also noted. No major differences were found for blood pressure, arterial stiffness, blood biomarkers, or endothelial cell protein expression following blueberry consumption. These findings suggest daily consumption of freeze-dried blueberry powder for 12 weeks improves endothelial function through reduced oxidative stress in postmenopausal women with above-normal blood pressure. The clinical trial registry number is NCT03370991 (https://clinicaltrials.gov).

Introduction

Cardiovascular disease (CVD) is the leading cause of death in the United States (US) and globally. Each year, there are over 17.9 million CVD-related deaths which makes up approximately 32% of all deaths worldwide.[1, 2] Aging is the primary risk factor for CVD largely due to unfavorable changes to the vasculature, particularly the arteries. Vascular endothelial dysfunction is one major adverse change to the arteries characterized by impaired endothelium-dependent dilation due to an imbalance in endothelium-derived biologically active molecules.[3] The endothelium is crucial for cardiovascular health as it is responsible for maintaining vascular tone through producing nitric oxide (NO) and other vasoactive molecules.[4, 5] NO is a key vasodilatory and vasoprotective molecule,[6] and current knowledge indicates that reduced NO bioavailability secondary to excessive superoxide-driven oxidative stress is central to endothelial

dysfunction.[3, 7-10] Superoxide radicals and other reactive oxygen species (ROS) decrease NO bioavailability by oxidizing the NO-producing enzyme endothelial nitric oxide synthase (eNOS) leading to further superoxide radical production instead of NO. ROS also react directly with NO which further promotes oxidative stress and endothelial dysfunction.[11, 12] Furthermore, ROS can stimulate pro-inflammatory processes, which perpetuates oxidative stress through a vicious cycle and contributes to endothelial dysfunction.[12-15]

Postmenopausal women experience accelerated adverse changes to cardiovascular health, including endothelial dysfunction, which is an independent risk factor and a predictor of CVD events in this population.[16, 17] Indeed, reductions in endothelium-dependent dilation have been observed during the menopausal transition independent of age and other CVD risk factors.[18-21] Estrogen exerts direct and indirect antioxidant and vasodilatory effects, and thus postmenopausal women experience oxidative stress-mediated endothelial dysfunction due to diminished estrogen production.[22-24] Above-normal blood pressure (i.e. elevated blood pressure and hypertension), often present in postmenopausal women, is also a contributor to the increased risk for CVD.[25, 26] Menopause is associated with a 2-fold increase in hypertension, with approximately 75% of postmenopausal women estimated to have hypertension, which worsens endothelial function through mechanisms that include oxidative stress and inflammation.[12, 27] Therefore, intervention strategies targeted at improving endothelial function through reduced oxidative stress and inflammation are important for CVD risk reduction in postmenopausal women, and especially those with above-normal blood pressure.

Lifestyle modification, particularly diet and nutrition, is recommended for CVD risk reduction, and consuming a diet rich in (poly)phenol-rich plant foods is linked to lower CVD risk.[28-30] (Poly)phenols are secondary plant metabolites characterized by their phenolic structures and hydroxyl moieties,[31] and are broken into flavonoids and non-flavonoids. Flavonoids (e.g. anthocyanins) are the most studied (poly)phenols and are found in high concentrations in fruits such as berries.[30, 32] (Poly)phenols and their circulating derivatives of

phase II enzyme and gut microbial metabolism (i.e. metabolites) can reduce oxidative stress and inflammation.[33] For instance, (poly)phenol metabolites can directly interact with free radical-generating enzymes and antioxidant enzymes leading to reductions in oxidative stress.[34-41] Blueberries are especially rich in (poly)phenols, namely anthocyanins and phenolic acids, and accumulating evidence supports their cardiovascular-protective effects, including improvements in endothelial function in healthy adult men, adults with metabolic syndrome, and cigarette smoking men with or without peripheral arterial dysfunction.[42, 43] We previously demonstrated that consuming 22 g freeze-dried blueberry powder (equal to 1 cup fresh blueberries) daily for 8 weeks led to improvements in systolic and diastolic blood pressure and brachial-ankle pulse wave velocity (PWV; a measure of arterial stiffness), and increased plasma concentrations of NO metabolites in postmenopausal women with above-normal blood pressure, suggestive of improvements in endothelial function.[44] Other studies have also observed reductions in blood pressure and measures of arterial stiffness, but the results in this area are mixed.[45]

While promising, the effects of blueberry consumption on endothelial function in postmenopausal women with above-normal blood pressure remains unknown. Furthermore, determining underlying mechanisms is crucial to establish blueberries as a dietary intervention for improving endothelial function, and for identifying ways to increase clinical efficacy. Physiologically relevant cell and animal studies, and limited human studies, suggest that blueberries, their (poly)phenols, and/or resulting metabolites may improve endothelial function through reductions in oxidative stress.[46-48] However, evidence demonstrating that improved endothelial function is directly linked to reductions in oxidative stress following chronic blueberry consumption in humans is needed. Therefore, the purpose of this randomized, double-blind, placebo-controlled, parallel-arm clinical trial was to examine the efficacy of chronic blueberry consumption to improve endothelial function, blood pressure, and other biomarkers of cardiometabolic health in postmenopausal women with above-normal blood pressure, and to gain insight into underlying mechanisms with a specific focus on oxidative stress-related

mechanisms. We hypothesized that daily consumption of 22 g freeze-dried blueberry powder for 12 weeks would improve endothelium-dependent dilation through reductions in oxidative stress, blood pressure, and other cardiometabolic biomarkers in postmenopausal women with above-normal blood pressure.

Methods

Study population and recruitment

Estrogen-deficient postmenopausal women (≥ 1 y free of menses, confirmed with blood measurement of estradiol < 30 pg/mL and follicle-stimulating hormone (FSH) > 30 mIU/mL) aged 45-65 y with elevated blood pressure or stage-1 hypertension (systolic blood pressure 120-139 mmHg and/or diastolic blood pressure 80-89 mmHg) were recruited for the study. Individuals were excluded if they did not meet inclusion criteria, had a body mass index (BMI) < 18.5 or > 40 kg/m^2, presence of clinical disease(s) (i.e. renal, respiratory, thyroid, diabetes, thrombosis, neuropathy, cancer, gastrointestinal, liver, kidney, pancreatic, or cardiovascular), cigarette smoking within past year prior to enrollment, taking > 1 blood pressure medication or taking blood pressure medication for < 3 months, use of sex hormone replacement, lipid-lowering, or phosphodiesterase-5 inhibitor medications, triglycerides (TG) > 350 mg/dL, and/or low-density lipoprotein cholesterol (LDL-C) ≥ 190 mg/dL, body weight change ≥ 3 kg in the 3 months prior to study enrollment or actively trying to lose weight, unwilling to maintain normal eating, drinking, and/or physical activity habits throughout the study, heavy or binge drinking (> 7 drinks/week or > 3 drinks on any given occasion, respectively), and/or have allergies or contraindications to study treatment(s), pharmacological agents, and/or procedures.

Participants were recruited from the greater Fort Collins, Colorado, area through flyer distribution, email, newspaper, Colorado State University (CSU) webpages, direct mailers, and ClinicalTrials.gov between December 2017 and January 2020. Individuals indicated their interest in study participation though email or phone call, and an initial phone screening was

conducted for each interested participant where they were asked specific questions regarding their health history to determine study eligibility prior to their onsite screening visit. The onsite screening visit entailed reading and signing the informed consent form, confirmation of inclusion and exclusion criteria by measuring seated blood pressure in triplicate, calculating BMI, examining blood parameters (blood lipid profiles, estradiol, and FSH), and completing a medical health history questionnaire. Qualified participants were scheduled for their baseline visit. This study was approved by the CSU Institutional Review Board (protocol #2891), conducted in accordance with the Declaration of Helsinki, and is registered at ClinicalTrials.gov as NCT03370991.

Study design

This study was a randomized, double-blind, placebo-controlled, parallel-arm clinical trial. The overall study design, schedule of study visits, and a schematic of study visits for data collection and measurements are presented in **Figure 3.1**. The primary outcomes were brachial artery flow-mediated dilation (FMD; measure of endothelial function) and blood pressure. Secondary outcomes included the impact of intravenous ascorbic acid on oxidative stress-mediated suppression of endothelial function, endothelium-independent dilation, hemodynamics, arterial stiffness, plasma blueberry (poly)phenol metabolites, blood markers of cardiometabolic health, and venous endothelial cell protein expression related to NO production, oxidative stress, and inflammation.

Laboratory measures were taken on 4 different occasions (i.e. study visits) separated by 4 weeks over a 12-week period, between 6:00 am and 11:00 am. All cardiovascular measures were conducted in a supine position, at room temperature, and with dimmed lighting. Participants were asked to be in a fasting state, which consisted of no food and drinks (other than water) for 8 h prior to the study visit, and no dietary supplements or prescription medication use was permitted within 24 h of the study visit. Participants were asked to maintain their usual

diet and physical activity patterns, and to keep fresh and frozen blueberry consumption to ≤ 2 cups/week for the duration of the study (with the exception of the treatment intervention).

Treatment intervention and compliance

Eligible participants (*n* = 48) were randomly assigned to treatments using block randomization stratified by hypertension stage to receive either: 1) 22 g freeze-dried highbush blueberry powder (Tifblue/Rubel 50/50 blend; equivalent to about 1 cup fresh blueberries); or 2) 22 g isocaloric and carbohydrate-matched placebo powder (dextrose, maltodextrin, fructose, and artificial color and flavors) with a similar color, texture, and taste to the blueberry powder. The 22 g dose of blueberry powder contained 87 total kcals, < 1 g fat, 21 g total carbohydrate, < 1 g protein, 726 mg of total (poly)phenols, and 224 mg of anthocyanins, while the 22 g dose of placebo powder contained 83 total kcals, < 1 g fat, 21 g total carbohydrate, < 1 g protein, and was devoid of (poly)phenols. The US Highbush Blueberry Council provided the blueberry and placebo powders. Nutritional analysis of the treatment powders was performed by Medallion Laboratories (Minneapolis, MN, USA) and total (poly)phenol and anthocyanin analyses were performed by International Chemistry Testing (Hopkinton, MA, USA). Treatment powders were packaged in air-tight, opaque 11 g packets to further conceal treatment identity. Participants consumed 11 g of their assigned treatment 2x/day mixed in water in the morning and afternoon/evening, at least 6-8 h apart to decrease the risk for gastrointestinal distress, for 12 weeks.

Treatment compliance was assessed by asking study participants to record the date and time their treatment packets were consumed each day in a daily treatment dosing log, and to document reasons for missing doses (i.e. sick, fell asleep, forgot). Non-compliance was defined as missing ≥ two 11 g doses/week or less than ~86% treatment compliance on average and was calculated using the following equation: ([dosages consumed – missed dosages/dosages required]*100).

Endothelium–dependent and –independent dilation

Brachial artery FMD (endothelium-dependent dilation, *n* = 32) and brachial artery diameter responses to sublingual nitroglycerin (NTG) administration (endothelium-independent dilation, *n* = 20) were determined in a subset of participants as described previously[49-53] and analyzed using commercially available software packages (Brachial Analyzer for Research, Medical Imaging Applications, LLC, Coralville, IA, USA and DATAQ Instruments, OH, USA). Brachial artery diameter and mean blood velocity (MBV) were measured with a 12-MHz linear-array ultrasound probe (Vivid7, GE Healthcare, Wauwatosa, WI, USA) clamped in place ~3-6 cm proximal to the antecubital fossa with a < 60° insonation angle. In brief, to assess FMD, a rapidly inflating blood pressure cuff (D.E. Hokanson, Inc., Bellevue, WA, USA) on the upper forearm (~2 cm distal to the antecubital fossa) was inflated to 250 mmHg to occlude forearm blood flow. Ultrasound images were captured (Vascular Imager, Medical Imaging Applications, LLC, Coralville, IA, USA) at end-diastole for each cardiac cycle at baseline (30 sec), during occlusion (5 min), and post-occlusion (5 min). Shear rate (SR) was calculated for each cardiac cycle as 8 × MBV ÷ diameter, and the SR area under the curve (SR_{AUC}) from cuff release to peak diameter was quantified as an index of the stimulus for FMD.[54] FMD values are expressed as absolute (mm) and relative (%) change from baseline to peak diameter and corrected for shear rate (FMD/SR_{AUC}) to account for inter-individual variability in shear stress.[55-57]

To determine whether daily blueberry consumption improves endothelium-dependent dilation by suppressing oxidative stress, brachial artery FMD was measured before and after a supraphysiologic infusion of the antioxidant ascorbic acid (Mylan Institutional Inc., Rockford, IL, USA). Ascorbic acid was administered intravenously at a dose of 0.06 g/kg fat-free mass[53, 58] over a 20-min period, then brachial artery FMD was reassessed using the aforementioned procedures. Ascorbic acid is a potent antioxidant that scavenges ROS, including superoxide radicals. Thus, this approach has been used to evaluate the extent to which oxidative stress suppresses endothelium-dependent dilation.[53, 58, 59]

Lastly, sublingual NTG (0.4 mg) (Pfizer Inc., New York, NY, USA) was administered to assess endothelium-independent dilation in a subset of study participants (*n* = 20) following a 10 min rest period to reduce carry over effects. Baseline artery diameters were measured, NTG tablet was placed under the participant's tongue, and brachial artery diameters were measured over 8 min to capture peak diameter. NTG values are expressed as absolute (mm) and relative (%) change from baseline to peak diameter.

Hemodynamics and arterial stiffness

Hemodynamics and arterial stiffness were assessed after a 10 min rest in the supine position using the SphygmoCor XCEL (AtCor Medical Inc., Naperville, IL, USA) as previously described. [60, 61] Briefly, supine brachial blood pressure was measured, followed by pulse wave analysis for central aortic parameters (i.e. mean arterial pressure, pulse pressure, heart rate, augmentation index (AIx), AIx normalized to a heart rate of 75 bpm (AIx@75), systolic and diastolic pressure, and aortic pressure).

After blood pressure measurements, carotid-femoral/aortic PWV was measured as previously described.[51, 60] In short, a blood pressure cuff was placed on the upper thigh at the femoral artery and a tonometer was placed on the carotid artery simultaneously to capture pulse waveforms. Three measurements were taken using calipers: 1) distance between carotid artery pulse and sternal notch, 2) sternal notch to the top of the blood pressure cuff, and 3) femoral artery pulse to the top of the blood pressure cuff. The distance traveled between the two sites and the time the waveform traveled was used to automatically calculate carotid-femoral/aortic PWV (distance/time) by the SphygmoCor system. All hemodynamic and arterial stiffness measurements were performed in triplicate.

Blood collection and biomarker analyses

Venous blood was collected using a standard 23-gauge butterfly needle (BD Vacutainer, Becton, Dickson and Company, Franklin Lakes, NJ, USA) or an 18-gauge intravenous catheter (Introcan Safety, Braun Medical Inc., Bethlehem, PA, USA). Blood lipids (high-density lipoprotein cholesterol (HDL-C), LDL-C, total cholesterol (TC), and TG), hemoglobin A1c, FSH, and estradiol were assessed using standard assays at the University of Colorado-Health/Poudre Valley Hospital Lab (Fort Collins, CO, USA). Remaining venous blood was processed for serum and plasma separation using VACUETTE® CAT Serum Sep Clot Activator tubes (Greiner Bio-One North America Inc., Monroe, NC, USA) and BD Vacutainer® K2 EDTA collection tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), respectively and stored at -80°C until analysis. Plasma intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) levels were measured in duplicate using commercially available quantikine ICAM-1 and VCAM-1 enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Bio-Techne brand, Minneapolis, MN, USA). Plasma nitrate/nitrite was measured in duplicate using a commercially available colorimetric assay kit (Cayman Chemical, Ann Arbor, MI, USA).

Chromatography and mass spectrometry analysis of plasma (poly)phenols

The extraction of phenolic metabolites from plasma samples was performed by micro-elution solid phase extraction (µ-SPE) and measured by UPLC-Q-q-Q MS, as per the protocol of a validated method.[62] Briefly, undiluted plasma samples were acidified with 4% phosphoric acid (v:v 1:1). This mix (total volume of 600 µL) was then loaded onto Oasis 96-well reversed-phase HLB (hydrophilic-lipophilic balanced) sorbent µ-SPE plates (Waters, Eschborn, Germany), washed with water and 0.2% acetic acid, and eluted using 90 µL of methanol. Identification and quantification of (poly)phenol metabolites were performed on a SHIMADZU Triple Quadrupole Mass Spectrometer (LCMS8060, SHIMADZU, Kyoto, Japan). Five microliters of the eluded

samples were then injected through a Raptor Biphenyl column 2.1 x 50 mm, 1.8µm (Restek, Bellefonte, USA) with a compatible Raptor Biphenyl Guard Cartridges 5 x 2.1 mm (Restek, Bellefonte, USA) in the UPLC system. A 14-minute gradient followed by a 2-minute equilibration was applied to the run under a flow rate of 0.5 mL/min at 30°C. The identification of metabolites was performed by comparing retention times with authentic standards in corresponding multiple reaction monitoring (MRM) transitions and quantified by calibration curves made from standard mixes using SHIMADZU LabSolutions™ LCMS Software.

Endothelial cell biopsy and protein quantification

Human venous endothelial cell biopsy was performed in a subset of participants (*n* = 24) as previously described.[53, 60, 63-67] Venous endothelial cell protein expression has been shown to correlate with arterial endothelial cell expression[65] and therefore was selected as a proxy for arterial endothelial cells. Briefly, endothelial cells were biopsied from the antecubital vein by inserting 2 sterile 0.025 mm J-shaped guidewires (GuideRight™, Abbott Medical, Plymouth, MN, USA) one at a time into the catheter ~4 cm beyond the catheter tip. Wires were carefully withdrawn, and the distal portion of the wire was immediately transferred to a conical tube containing phosphate buffer saline solution for processing. Isolated endothelial cells were mounted on poly-L-lysine microscope slides, incubated for 5 h at 37°C, and stored at -80°C until immunofluorescence microscopy protein expression analyses.

Previously described methods were used for quantitative immunofluorescence of endothelial cell protein expression with one modification (donkey serum was used in place of goat serum to view and confirm endothelial cells).[60, 65-67] Primary antibodies of interest included manganese superoxide dismutase (MnSOD) (Enzo Life Sciences, Inc., Farmingdale, NY, USA), phosphorylated endothelial nitric oxide synthase (eNOS) (Cell Signaling Technology, Inc., Danvers, MA, USA), phosphorylated nuclear factor kappa B (NFκB) (Cell Signaling Technology, Inc., Danvers, MA, USA), and NADPH oxidase/p47 subunit (MilliporeSigma, Burlington, MA,

USA). Using a microscope (Olympus BX3-CBH Olympus Scientific Solutions Americas Corp., Waltham, MA, USA), cells were imaged by a DP73 digital camera (Olympus Scientific Solutions Americas Corp., Waltham, MA, USA) and automatically analyzed via cellSens Software (Olympus Scientific Solutions Americas Corp., Waltham, MA, USA) to quantify staining intensity. Human umbilical vein endothelial cells (HUVECs) were used as a control for each staining batch to account for between batch staining variability, and values are reported as a ratio of participant endothelial cell protein expression to HUVEC and expressed as arbitrary units (AU).

Anthropometrics, body composition, and energy intake and expenditure

Height without shoes was measured only at screening visits using a standard scale-mounted stadiometer and rounded to the nearest 0.5 cm, and body weight without shoes was measured using a scale and rounded to the nearest kg at all visits (Health o Meter Professional 500KL, Pelstar LLC, Countryside, IL, USA). BMI was calculated as weight (kg) divided by height (m^2). Waist and hip circumferences were measured using a standard measuring tape and rounded to the nearest 0.5 cm, and ratios were calculated by dividing waist circumference by hip circumference. Body composition (fat mass, fat free mass, trunk fat, android fat, and gynoid fat) was assessed using dual-energy x-ray absorptiometry (DEXA) (Hologic, Mississauga, ON, Canada). Self-reported food intake on 3 separate days (2 weekdays and 1 weekend day) was collected prior to each study visit through the Automated Self-Administered 24-hr (ASA) Dietary Assessment Tool (National Institutes for Health, Bethesda, MD, USA) that calculated total calories (kcals) to assess energy intake throughout the study.[68, 69] Physical activity patterns were assessed using a validated 7-day physical activity recall at each study visit to estimate energy expenditure (kcal/day).[70, 71]

Statistical analyses

Collected data were stored electronically using REDCap with double-data entry by two individuals and independent quality review by a third person. Missing data from continuous variables were imputed using the mean values of the outcomes within the groups. Data were evaluated for normality using Shapiro-Wilk tests (SAS Institute Inc., Cary, NC, USA) and log-transformed prior to statistical analysis when appropriate. A linear mixed model approach was used to analyze the data and compare between the treatment groups as well as time with an interaction term, which were set as fixed effects in the model, while participants were set as random effects. Least square differences were calculated for comparisons and the adjustment of the *p*-value was performed using a Tukey-Kramer's correction for multiple comparisons. Univariate analyses were performed with each potential confounder (i.e. age, BMI, hypertension stage, hypertensive medication use, and years since menopause). The variables that met the criteria of a *p*-value of 0.25 were included in the multivariable linear mixed model. A backward elimination process was used to retain the variables that meet the *p*-value of 0.05 to finalize the model for each outcome variable. Spearman correlation analyses were performed to assess the relationship of improvements in endothelial function (FMD/SR_{AUC}) with plasma (poly)phenol metabolites, and endothelial cell protein expression. Endothelial cell protein expression correlation analyses were performed using GraphPad (version 9.4.1, San Diego, CA, USA), and SAS v9.4 (SAS Institute Inc., Cary, NC) was used for all other statistical analyses. Results are presented as mean ± SEM, and a *p*-value of 0.05 was used to determine statistical significance. A *p*-value of less than 0.10 was used to determine a trend for statistical significance.

Results

Study enrollment, attrition, compliance, and baseline characteristics

A detailed schematic of participant flow throughout the study (i.e. CONSORT flow diagram) is provided in **Figure 3.2**. A total of 505 potential participants contacted the laboratory

to express interest in participating in the study and were screened via telephone or email. Of these, 367 individuals were excluded due to not meeting inclusion criteria, subsequently declining to participate, or an inability to contact them, leaving 138 participants who completed an onsite screening visit. Of those, 90 people were excluded for not meeting inclusion criteria or declining to participate. As a result, 48 estrogen-deficient postmenopausal women were enrolled in the study and randomly assigned to an intervention (blueberry n = 24 and placebo n = 24). Throughout the 12-week intervention period, 5 participants dropped from the study due to sickness, medication change, antiseptic allergies, infection, or for unknown reasons, leaving 43 participants who completed the 12-week study (blueberry n = 22 and placebo n = 21). The overall attrition rate for the 12-week intervention study was approximately 10.4% (8.3% in the blueberry group and 12.5% in the placebo group). Overall self-reported treatment compliance was 97%. For the blueberry group, overall self-reported compliance was 97%, and was 98% between baseline and 4 weeks, 98% between 4 and 8 weeks, and 96% between 8 and 12 weeks. For the placebo group, self-reported compliance was 97%, and was 95% between baseline and 4 weeks, 98% between 4 and 8 weeks, and 97% between 8 and 12 weeks. Screening characteristics are presented in **Table 3.1**, and there were no differences (p > 0.05) between groups.

Endothelium–dependent and –independent dilation

Absolute FMD/SR_{AUC} was 96% higher (p< 0.01) at 12 weeks compared to baseline in the blueberry group with no change (p > 0.05) observed in the placebo group (**Figure 3.3A** and **Supplementary Table 3.1** in **Appendix 1**), and the change in FMD/SR_{AUC} from baseline to 12 weeks was higher (p < 0.03) than the placebo group (**Figure 3.3C** and **Supplementary Table 3.1** in **Appendix 1**). Though there were no statistically significant differences (p > 0.05), there was a ~1.34% unit increase in absolute FMD not normalized to SR_{AUC} (35% higher) compared to baseline at 12 weeks in the blueberry group (**Figure 3.3B and 3.3D** and **Supplementary**

Table 3.1 in **Appendix 1**). Individual FMD changes from baseline to 12 weeks are presented in **Supplementary Figure 3.1** in **Appendix 1**. Brachial artery endothelium-independent dilation to sublingual NTG did not change ($p > 0.05$) within or between groups (**Supplementary Table 3.2** in **Appendix 1**), confirming the improvements in endothelial function were endothelium-dependent.

To evaluate oxidative stress-mediated suppression of endothelial function and determine the impact of blueberries on its amelioration, endothelium-dependent dilation was assessed before and following intravenous infusion of the antioxidant ascorbic acid. At baseline (pre-blueberry or -placebo consumption), ascorbic acid administration acutely increased ($p < 0.05$) FMD/SR_{AUC} in both groups (**Figure 3.4A** and **3.4B**). There was an increase ($p < 0.01$) in FMD not normalized to SR_{AUC} in the blueberry group, with trending significance in the placebo group ($p < 0.07$) (**Figure 3.4C and 3.4D**, and **Supplementary Table 3.3** in **Appendix 1**).

After 12 weeks of daily blueberry consumption, ascorbic acid administration did not alter ($p > 0.05$) FMD/SR_{AUC} or FMD not normalized to SR_{AUC} (**Figure 3.4A** and **3.4C**, and **Supplementary Table 3.3** in **Appendix 1**). The placebo group had a strong trend for an increase ($p < 0.08$) in FMD/SR_{AUC} at 12 weeks (**Figure 3.4B** and **Supplementary Table 3.3** in **Appendix 1**), but not FMD not normalized to SR_{AUC} ($p > 0.05$) (**Figure 3.4C and 3.4D**, and **Supplementary Table 3.3** in **Appendix 1**). When assessed as the difference in the FMD/SR_{AUC} response to ascorbic acid vs. saline, the blueberry group had a reduction in the FMD/SR_{AUC} response ($p < 0.04$) and an increase in the SR_{AUC} response ($p < 0.0006$) to ascorbic acid at 12 weeks compared to baseline, while the placebo group had no change ($p > 0.05$) (**Figure 3.4E** and **Supplementary Table 3.3** in **Appendix 1**).

Hemodynamics and arterial stiffness

There were no other significant differences ($p > 0.05$) within or between groups for hemodynamic and arterial stiffness measures except for a significant reduction in aortic pulse

pressure in the blueberry group at 4 weeks compared to baseline ($p < 0.04$) (**Figure 3.5** and **Supplementary Table 3.4** in **Appendix 1**).

Blood biomarkers

Blood biomarker results are presented in **Table 3.2**. Reductions ($p < 0.05$) in TG were observed at 12 weeks compared to baseline in the blueberry group, but while no changes ($p > 0.05$) were noted in the placebo group or for the change from baseline to 12 weeks. Reductions in LDL-C ($p < 0.05$) were noted at 12 weeks compared to baseline in both groups, but not for the change from baseline to 12 weeks ($p > 0.05$). VCAM-1 was increased at 4 weeks ($p < 0.004$) and 8 weeks ($p < 0.008$) compared to baseline in the placebo group, with no changes ($p > 0.05$) in the blueberry group or for the change from baseline. No other effects ($p > 0.05$) were noted.

Plasma (poly)phenol metabolites

A total of 88 plasma (poly)phenol metabolites were detected (**Supplementary Table 3.5** in **Appendix 1**) and the mean sums of (poly)phenol metabolites grouped according to class are presented in **Figure 3.6C**. In the blueberry group, the sum of plasma (poly)phenol metabolite concentrations (i.e. all detected plasma (poly)phenol metabolites) was increased at 4 weeks ($p < 0.0001$), 8 weeks ($p < 0.0001$), and 12 weeks ($p < 0.0001$) compared to baseline, higher than the placebo group at 4 ($p < 0.02$), 8 ($p < 0.02$), and 12 weeks ($p < 0.004$) (**Figure 3.6A** and **Supplementary Table 3.5** in **Appendix 1**), and higher for the change from baseline to 4 ($p < 0.02$) and 12 ($p < 0.05$) weeks than the placebo group (**Figure 3.6A** and **3.6B**, and **Supplementary Table 3.5** in **Appendix 1**).

With regard to individual metabolites (**Figure 3.6** and **Supplemental Table 3.5** in **Appendix 1**), hippuric acid was increased at 4 ($p < 0.0001$), 8 ($p < 0.0001$), and 12 ($p < 0.0001$) weeks in the blueberry group compared to baseline, and compared to the placebo group at 4

weeks ($p < 0.05$), 8 weeks ($p < 0.02$), and 12 weeks ($p < 0.003$) (**Figure 3.6D** and **Supplementary Table 3.5** in **Appendix 1**) and for the change from baseline to 4 ($p < 0.008$), 8 ($p < 0.03$), and 12 ($p < 0.007$) weeks (**Figure 3.6E**). The metabolite 3-hydroxyhippiuric acid was increased at 4 ($p < 0.007$) and 8 ($p < 0.03$) weeks, with a trend for an increase ($p < 0.07$) at 12 weeks compared to baseline in the blueberry group, but not ($p > 0.05$) compared to placebo (**Figure 3.6F** and **Supplementary Table 3.5** in **Appendix 1**) or for the change from baseline (**Figure 3.6G**). In the blueberry group, the metabolite 3-methoxybenzoic acid-4-sulfate (vanillic acid-4-sulfate) was increased at 4 ($p < 0.0001$), 8 ($p < 0.0003$), and 12 ($p < 0.02$) weeks compared to baseline, higher than placebo at 4 weeks ($p<0.04$) (**Figure 3.6H** and **Supplementary Table 3.5** in **Appendix 1**) and for the change from baseline to 4 weeks ($p < 0.03$) (**Figure 3.6I**). The metabolite 3,4-dihydroxybenzoic acid (protocatechuic acid) was increased ($p < 0.04$) at 8 weeks compared to baseline following blueberry consumption (**Figure 3.6J** and **Supplementary Table 3.5** in **Appendix 1**) and was higher ($p < 0.03$) than the placebo group at 8 weeks (**Figure 3.6K**). The metabolite 2,5-dihydroxybenzoic acid was increased at 8 weeks ($p < 0.03$) compared to baseline in the blueberry group and higher ($p < 0.05$) than the placebo group at 12 weeks (**Figure 3.6L** and **Supplementary Table 3.5** in **Appendix 1**), while no differences ($p > 0.05$) were noted for the change from baseline (**Figure 3.6M**). The metabolite 2,3-dihydroxybenzene-1-sulfate was increased at 4 ($p < 0.02$) and 8 ($p < 0.002$) weeks in the blueberry group compared to baseline (**Supplementary Table 3.5** in **Appendix 1**), while no differences ($p > 0.05$) were noted for the change from baseline. The metabolite 3-hydroxy-4-methoxybenzoic acid-5-sulfate was higher at 4 ($p < 0.01$) and 8 ($p < 0.004$) weeks in the blueberry group compared to placebo, and the change from baseline to 8 weeks was higher ($p < 0.05$) than the placebo group (**Supplementary Table 3.5** in **Appendix 1**). The metabolite 2-hydroxybenzene-1-glucuronide was increased ($p < 0.01$) at 8 weeks compared to baseline in the blueberry group and the change from baseline to 4 weeks was higher ($p < 0.02$) than the placebo group (**Supplementary Table 3.5** in **Appendix 1**). The metabolite 2-hydroxy-3-(4'-

hydroxyphenyl)propanoic acid was increased ($p < 0.04$) at 4 weeks compared to baseline in the blueberry group (**Supplementary Table 3.5** in **Appendix 1**). The metabolite 3-(4'-methoxyphenyl)propanoic acid-3'-glucuronide was increased ($p < 0.007$) at 8 weeks compared to baseline in the blueberry group (**Supplementary Table 3.5** in **Appendix 1**). Lastly, myricetin was higher ($p < 0.05$) in the blueberry group at 4 weeks compared to placebo at 4 weeks, and the change from baseline to 4 weeks was higher ($p < 0.04$) than placebo (**Supplementary Table 3.5** in **Appendix 1**).

Correlations between endothelium-dependent dilation and plasma (poly)phenol metabolites

With the exception of flavonols ($p < 0.05$), there were no correlations ($p > 0.05$) observed for the sum of (poly)phenol metabolites, specific metabolite classes, or in the sum of significantly increased metabolites with FMD/SR_{AUC} at 12 weeks in the blueberry group. For correlation analysis for the change from baseline to 12 weeks in the blueberry group (**Figure 3.7**), 3 metabolites had moderate positive correlations with improvements in FMD/SR_{AUC}, specifically myricetin ($p < 0.05$), 4-O-caffeoylquinic acid ($p < 0.05$), and 3-(4'-hydroxyphenyl)propanoic acid-3'-glucuronide ($p < 0.05$). Five metabolites had moderate to moderately strong negative correlations with improvements in FMD/SR_{AUC} (**Figure 3.7**), specifically 5-O-caffeoylquinic acid ($p<0.003$), 3'-hydroxyphenylacetic acid ($p < 0.0007$), 3-(2',4'-dihydroxyphenyl)propanoic acid ($p<0.03$), 3-hydroxy-4-methoxybenzoic acid-5-sulfate ($p < 0.04$), and 2-hydroxy-3-(4'-hydroxyphenyl)propanoic acid ($p < 0.009$). In the placebo group, 5-O-caffeoylquinic acid was positively correlated with the change in FMD/SR_{AUC} ($p < 0.004$), while 3-hydroxybenzoic acid ($p < 0.05$), 4-hydroxybenzoic acid ($p < 0.04$), and 4-hydroxyhippuric acid ($p < 0.03$) were all negatively correlated with the change in FMD/SR_{AUC} (**Figure 3.7**).

For correlation analysis between individual metabolites and FMD/SR_{AUC} at 12 weeks (**Figure 3.7**), 3-hydroxybenzoic acid-4-sulfate was positively correlated in the blueberry group ($p < 0.02$). In the placebo group, 3,5-dihydroxybenzoic acid ($p < 0.02$), 3-hydroxybenzoic acid ($p <$

0.02), and 4-hydroxybenzoic acid ($p < 0.02$) were all negatively correlated with FMD/SR_{AUC}, while only myricetin was positively correlated ($p < 0.009$) (**Figure 3.7**).

Endothelial cell protein expression

No differences ($p > 0.05$) were noted within or between groups at any time point for MnSOD, phosphorylated eNOS, phosphorylated NFκB, or p47phox (NADPH oxidase/p47 subunit) (**Figure 3.8** and **Supplementary Table 3.6** in **Appendix 1**).

Correlations between endothelium-dependent dilation and endothelial cell protein expression

For the change from baseline to 12 weeks, FMD/SR_{AUC} was moderately inversely correlated with NADPH oxidase/p47 subunit protein expression ($p < 0.04$) in the blueberry group but not placebo, and a trend for a moderately positive correlation with phosphorylated NFκB ($p < 0.08$) in the placebo group but not blueberry (**Figure 3.9**). At 12 weeks, FMD/SR_{AUC} had a moderately positive correlation with phosphorylated eNOS protein expression ($p < 0.04$) in the blueberry group but not placebo (**Figure 3.9**). No other correlations ($p > 0.05$) were observed.

Anthropometrics, body composition, energy intake and expenditure

Anthropometrics, body composition, energy intake and expenditure data are presented in **Supplementary Table 3.7** in **Appendix 1**. For anthropometrics, there were no differences ($p > 0.05$) in weight or BMI between or within groups at any time point. For body composition, android fat mass (%) was lower ($p < 0.03$) at 12 weeks compared to baseline in the placebo group but not in the blueberry group ($p > 0.05$), and no other differences ($p > 0.05$) were noted. No differences ($p > 0.05$) within or between groups were noted for energy intake or energy expenditure.

Discussion

In this randomized, double-blind, placebo-controlled, parallel-arm clinical trial, we showed that consuming 22 g/day of freeze-dried highbush blueberry powder for 12 weeks significantly improved endothelial function, but not blood pressure, in postmenopausal women with above-normal blood pressure. These findings are clinically meaningful considering the prevalence of endothelial dysfunction in this population and its central role in CVD pathophysiology.[72] We also found that improvements in endothelial function were due, at least in part, to reductions in oxidative stress, and that plasma (poly)phenol metabolites related to flavonoid and gut microbial metabolism were increased following blueberry consumption, providing insight into physiological mechanisms. Other measures of cardiometabolic health were largely unchanged following blueberry consumption. To our knowledge, this is the first study to assess the effects of blueberry consumption on endothelial function and oxidative stress-mediated suppression of endothelial function in postmenopausal women with above-normal blood pressure.

The finding that daily blueberry consumption for 12 weeks improved macrovascular/conduit artery endothelial function in postmenopausal women with above-normal blood pressure is supported by previous randomized controlled trials performed with healthy adult men, adults with metabolic syndrome, and cigarette smoking men with or without peripheral arterial dysfunction.[45, 73, 74] In our study, we found that endothelium-dependent dilation assessed as FMD corrected for shear stress (i.e. the stimulus for reactive hyperemia) was significantly improved compared to baseline, and the change from baseline to 12 weeks was significantly higher in the blueberry group than placebo. Though changes in FMD (not corrected for shear stress) were not statistically significant in our study, likely due to inter-individual variability in shear stress, a clinically significant increase of 1.34% was observed in the blueberry group compared to baseline. A 1% increase in FMD has been associated with a reduced risk for CVD and related events.[52, 75] Importantly, we observed no changes in

endothelium-independent dilation, confirming that improvements in endothelial function observed were endothelium-dependent. An unexpected finding related to endothelial function was that plasma NO metabolites were unaffected by blueberry consumption, as we and others have demonstrated increases in these metabolites with blueberry consumption previously.[44, 76] However, several investigators have observed no change in plasma NO metabolites following blueberry consumption.[73, 77, 78] In the longest randomized controlled trial to date with blueberries, Curtis et al. observed a 1.45% increase in FMD in middle-aged/older men and women with metabolic syndrome who consumed 26 g freeze-dried highbush blueberry powder daily for 6 months.[73] In that study, circulating concentrations of cyclic guanosine monophosphate (GMP) were increased even though there were not concomitant increases in plasma NO metabolites, suggesting that blueberries exert vasodilatory effects through a NO-mediated mechanism.[73] Rodriguez-Mateos et al.[45] observed a 1.3% increase in FMD following consumption of 22 g freeze-dried lowbush blueberry powder daily for 1 month in healthy young males, but did not measure NO metabolites in their study. Overall, the findings of these 3 independent studies suggest that daily blueberry consumption can improve macrovascular/conduit artery endothelial function in high risk and healthy populations through an endothelium-dependent mechanism likely mediated by NO. Accurately assessing NO status is complex, and a single sampling of blood may not reflect tissue levels. The effects of blueberries on NO production and/or bioavailability, particularly as it relates to measures of endothelial function in humans is unknown at this time but should be evaluated in future randomized controlled trials to better understand mechanisms.

As with many human intervention studies, there was inter-individual variability in the direction and the magnitude of the response to treatment in our study.[79, 80] No studies with blueberries have reported the inter-individual variation in the magnitude and direction of the FMD response, but several have evaluated microvascular endothelial function measured through EndoPAT-assessed reactive hyperemic index (RHI),[74, 76, 77] and inter-individual

variability in responses was reported for a study in healthy males after consuming 25 g of freeze-dried wild blueberry powder daily for 6 weeks,[77] and adults with metabolic syndrome after consuming 45 g of freeze-dried highbush blueberry powder daily for 6 weeks.[74] In the case of FMD, values not corrected for shear stress may reflect conduit artery endothelial function as well as the magnitude of the hyperemic stimulus. To reduce variability and increase the utility of FMD as a measure of endothelial function, normalization of FMD to SR_{AUC} has been validated in numerous research studies and has been recommended for use in research.[56-58] Even with normalization of FMD to shear rate, variability in the direction and magnitude of the response was still noted in the current study. Nonetheless, our finding that daily freeze-dried blueberry consumption at a dose equivalent to about 1 cup (or 148 g) of fresh blueberries improved endothelial function in postmenopausal women with above-normal blood pressure is a clinically significant finding indicative of CVD risk reduction. Progressive impairments in endothelial function have been observed across the menopausal transition, and impairments in endothelial function are predictive of CVD development.[16, 52, 75, 81] Furthermore, sex differences exist for physiological responses to interventions aimed at improving endothelial function such that not all interventions in this population have been successful.[82-85] For instance, endurance exercise training to improve endothelial function in postmenopausal women was only found to be effective in those receiving estrogen replacement therapy, whereas age-matched men consistently responded positively to endurance exercise training.[86-88] The factors contributing to the lack of a response to interventions in postmenopausal women compared to men are not fully understood, but may be due to reduced estrogen receptor and eNOS activation, and oxidative stress. Our findings suggest that daily blueberry consumption may be an effective food-based intervention for reducing CVD risk in a high-risk population of estrogen-deficient postmenopausal women. Research aimed at understanding factors contributing to inter-individual variability in clinical endothelial function responses is needed, as well as ways to improve clinical responses to blueberry consumption.

Postmenopausal women have been demonstrated to have oxidative stress-mediated suppression of endothelium-dependent dilation[23], which is supported by an improvement in endothelium-dependent dilation observed at baseline in both groups following infusion of a supraphysiologic dose of ascorbic acid in our study. At 12 weeks, there were no improvements in endothelium-dependent dilation following ascorbic acid administration in the blueberry group, while there was a strong trend for an improvement in the placebo group. Importantly, the difference in the response to ascorbic acid administration versus saline was significantly reduced from baseline to 12 weeks in the blueberry group, while there was no change in the placebo group. Overall, these data indicate that this population of postmenopausal women with above-normal blood pressure has oxidative stress-mediated suppression of endothelial function that is improved by 12 weeks of daily blueberry consumption. The mechanisms contributing to improvements in oxidative stress-mediated suppression of endothelial function cannot be determined at this time, as included blood and endothelial cell measures related to NO production/bioavailability, oxidative stress, and inflammation were unchanged following blueberry consumption. It is possible that we were unable to detect subtle changes in protein expression and/or that blueberries reduce oxidative stress through alternative mechanisms. Interestingly, significant moderate correlations were observed for FMD/SR_{AUC} such that it was inversely associated with NADPH oxidase protein expression and positively associated with phosphorylated eNOS expression in the blueberry group but not in the placebo group, and inversely associated with phosphorylated NFκB expression in the placebo group but not the blueberry group. These data suggest that improvements in endothelial function with blueberry consumption could be related to eNOS activation and/or NADPH oxidase and NFκB deactivation, but requires further investigation in humans. Rodriguez-Mateos *et al*.[89] previously demonstrated acute blueberry consumption reduced neutrophil NADPH oxidase activity which was associated with increased FMD in healthy men. In diabetic db/db mice, Petersen *et al*.[90] showed that blueberry consumption for 10 weeks improved endothelial function through

reductions in NADPH oxidase 4 gene expression in aortic vessels and vascular endothelial cells. Several investigators have explored the effects of blueberry consumption on biomarkers of oxidative stress and antioxidant defense, and reported results have been mixed.[91] We previously demonstrated reductions in blood concentrations of 8-hydroxydeoxyguanosine, a marker of DNA damage, following 4 weeks of daily consumption of 22 g freeze-dried blueberry powder in postmenopausal women with above-normal blood pressure;[92] however, values returned to baseline levels at 8 weeks and other blood biomarkers of oxidative stress and antioxidant defense were unchanged. Basu *et al*.[93] observed reductions in blood biomarkers of oxidative stress following 8 weeks of 50 g daily blueberry consumption in adults with metabolic syndrome. Studies evaluating oxidative stress in peripheral blood mononuclear cells have observed improvements following chronic daily blueberry consumption, one of which also observed improvements in microvascular endothelial function.[74, 78, 94] As previously highlighted,[92] circulating blood and static biomarkers of oxidative stress and inflammation, including those measured in our studies, may not always respond to dietary interventions in the same way that functional biomarkers do (e.g. evaluation of oxidative stress-mediated suppression of endothelial function, *ex vivo* cytokine release assays in peripheral blood mononuclear cells), and thus may be a factor contributing to discrepant findings in the field. Indeed, daily consumption of 45 g/day of freeze-dried blueberry powder for 6 weeks in adults with metabolic syndrome reduced markers of inflammation in circulating monocytes but not in the blood. The present study has identified reductions in oxidative stress as a mechanism for improvements in endothelial function in postmenopausal women with above-normal blood pressure. Though there are contradictory findings across randomized controlled trials with respect to oxidative stress, the present results and totality of the evidence suggests blueberries can reduce oxidative stress, and that reductions mediate the beneficial effects of blueberries on endothelial function and cardiovascular risk.

With respect to hemodynamic parameters, no significant changes were noted with the exception of a reduction in aortic pulse pressure at 4 weeks compared to baseline in the blueberry group. The finding that daily blueberry consumption did not reduce brachial systolic and/or diastolic blood pressure in the present study was unexpected and contrary to our previous findings demonstrating consumption of 22 g/day freeze-dried blueberry powder for 8 weeks led to statistically and clinically significant reductions in brachial systolic and diastolic blood pressure in postmenopausal women with above-normal blood pressure.[44] However, the body of literature on the antihypertensive effects of blueberries is mixed such that equal numbers of studies have demonstrated benefits and null effects.[44, 45, 73, 74, 76, 77, 95-97] The reason(s) contributing to the discrepancies between our studies cannot be determined at this time. However, one possible contributing factor is the difference in the study population as the present study was performed in Colorado vs. Florida previously,[44] and there could be differences in the health status, physiology, and/or lifestyle factors. Additionally, effects on blood pressure may not have been sufficiently captured through measurement of "office" blood pressure, and future studies should also evaluate "out of office" blood pressure through 24-hr ambulatory blood pressure monitoring and/or home-based blood pressure monitoring. Due to inconsistencies between office and out-of-office blood pressure and the "white coat" effect, it is increasingly being recommended that multiple methods be used.[98-100] Overall, the body of evidence suggest that blueberries can reduce brachial blood pressure, but that factors impact their efficacy (e.g. health status, physiology, and lifestyle) and/or the ability to accurately and consistently detect their effects (e.g. measurement setting, device). Future research is needed to understand the antihypertensive effects of blueberries, particularly large randomized controlled trials that incorporate multiple methods for comprehensive assessment of blood pressure and approaches that will facilitate precision nutrition. Nonetheless, the current findings that endothelial function improved is a clinically meaningful finding considering endothelial dysfunction is a strong predictor of CVD events independent of blood pressure.

Arterial stiffness, as measured through PWV and AIx, has been shown to be a risk factor for CVD and predictive of hypertension progression.[44, 101-103] There were no improvements in measures of arterial stiffness in the present study. We previously found that systemic/peripheral arterial stiffness assessed as brachial-femoral PWV decreased by ~1 m/s^2 following daily consumption of 22 g freeze-dried highbush blueberry powder for 8 weeks in postmenopausal women with above-normal blood pressure,[44] though no changes were observed in carotid-femoral/aortic PWV, suggesting that the current findings are not unexpected. Previously, AIx@75 (but not carotid-femoral/aortic PWV) significantly improved in men and women with metabolic syndrome who consumed 26 g freeze-dried highbush blueberry powder daily for 6 months,[73] and in sedentary men and postmenopausal women who consumed 38 g of freeze-dried highbush blueberry powder daily for 6 weeks,[95] supporting that blueberries may improve systemic/peripheral arterial stiffness. It is possible that the intervention duration in our study was not long enough to observe improvements in carotid-femoral/aortic PWV in our study population, and future longer-term intervention studies are needed.

We found that blueberry consumption led to increases in the sum of all measured plasma (poly)phenol metabolites, and individual metabolites present in blueberries and produced by gut microbial and anthocyanin metabolism. These findings support self-reported compliance data provided by our study participants. Increased overall plasma (poly)phenol metabolite concentrations were largely driven by increases in hippuric acid. Several metabolites were positively and inversely linked to improvements in endothelial function. It is not entirely clear at this time how increases in (poly)phenol metabolites are related to improvements in endothelial function, but many of the increases in individual metabolites noted in our study (e.g. hippuric acid, 3-methoxybenzoic acid-4-sulfate, dihydroxybenzoic acids) are in line with those observed in previously performed chronic intervention trials with blueberries.[45, 73, 104] (Poly)phenols and their metabolites have been shown to modulate endothelial function through mechanisms related to eNOS expression and coupling/uncoupling, NO bioavailability, oxidative

stress, and inflammation.[29, 30, 32] With respect to blueberries, anthocyanin metabolites have been demonstrated to be major mediators of improvements in endothelial function, though other blueberry-derived compounds play a role. (Poly)phenols also serve as prebiotics and the gut microbiota is responsible for metabolizing many (poly)phenols to form bioavailable and bioactive metabolites, suggesting gut microbiome plays an essential role in determining the cardiovascular-protective effects of blueberries. In our study, we observed increases in metabolites of phase II metabolism (i.e. sulfates and glucuronides), which further supports that the gut microbiome plays an important role in blueberry (poly)phenol metabolism. The gut microbiome is being increasingly linked to CVD as a central mediator, though exact mechanisms are complex. Evidence in animal models and limited human studies indicate blueberries modulate the gut microbiota, and emerging evidence suggests beneficial effects on endothelial function may be mediated by the gut microbiota.[90] Research evaluating the impacts on the gut microbiota and the extent to which and how the gut microbiome mediates beneficial health effects of blueberries in humans is needed.

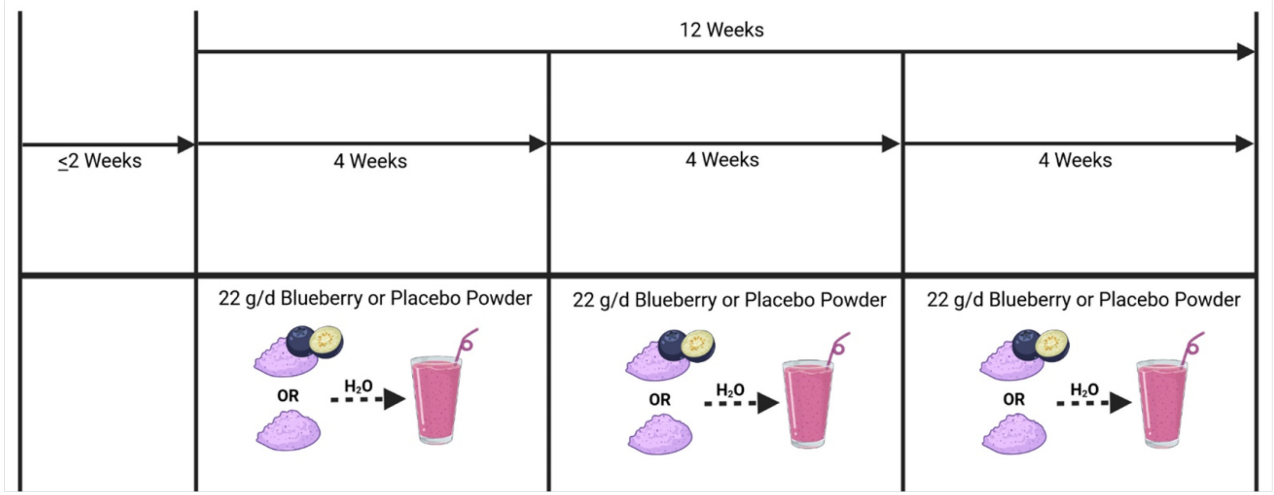
There are several strengths of the present study including the robust randomized, double-blind, placebo-controlled study design and the 12-week length of the dietary intervention. The amount of freeze-dried blueberry powder used in our study is equivalent to about 1 cup of fresh blueberries (or 148 g), which is an achievable dietary dose for daily consumption and supports the translational potential of our findings. We used gold-standard methodologies to directly assess endothelium-dependent and independent-dilation, and to gain mechanistic insight through assessment of oxidative stress-mediated suppression of endothelial function, endothelial cell protein expression, and plasma (poly)phenol metabolites. The inclusion of a high-risk (i.e., above-normal blood pressure) and understudied population of postmenopausal women highlights the translational potential of a food-based intervention for improving cardiovascular health. There are also limitations to our research that have not been previously discussed and are worth mentioning. First, this study was conducted in Northern Colorado and

may not reflect the greater United States and/or international population of postmenopausal women. Unfortunately, the majority of our study participants identified as being White/Caucasian which limits the generalizability to other races and ethnicities. Another limitation is the reduced sample size for some of the analyses, such as endothelial function and endothelial cell biopsy due to various reasons such as the inability to place an intravenous catheter and contraindications to sublingual NTG.

Conclusions

In conclusion, our study showed for the first time that blueberry consumption improved endothelial function in postmenopausal women with above-normal blood pressure, and that improvements were through reductions in oxidative stress. The underlying mechanisms contributing to these results cannot be determined at this time, but our results suggest that improvements may be related to gut-derived circulating (poly)phenol metabolites and endothelial cell protein expression related to NO production, oxidative stress, and inflammation. Future studies evaluating blueberry consumption in humans from diverse populations are needed to provide further insight into physiological mechanisms, and to understand factors contributing to inter-individual variability in clinical responses to support a precision nutrition approach. With respect to blood pressure, the current findings do not suggest that blueberries exert antihypertensive effects in this population. However, considering our previous findings in this population, and the mixed body of evidence surrounding blueberries and blood pressure such that about half demonstrated beneficial effects, further research is needed to better understand the antihypertensive effects of blueberries, and factors that can be modified and/or implemented to improve efficacy.

Figures and Tables



	V1	V2	V3	V4	V5
Informed consent	X				
Confirm eligibility	X				
Blood pressure	X	X	X	X	X
Endothelial function		X			X
Arterial stiffness		X			X
Blood collection	X	X	X	X	X
Endothelial cell biopsy		X			X
Anthropometrics	X	X	X	X	X
Body composition		X			X
Physical activity & diet		X	X	X	X
Treatment distribution		X	X	X	
Treatment compliance			X	X	X

Figure 3.1. Study design and timeline for the 12-week clinical trial.

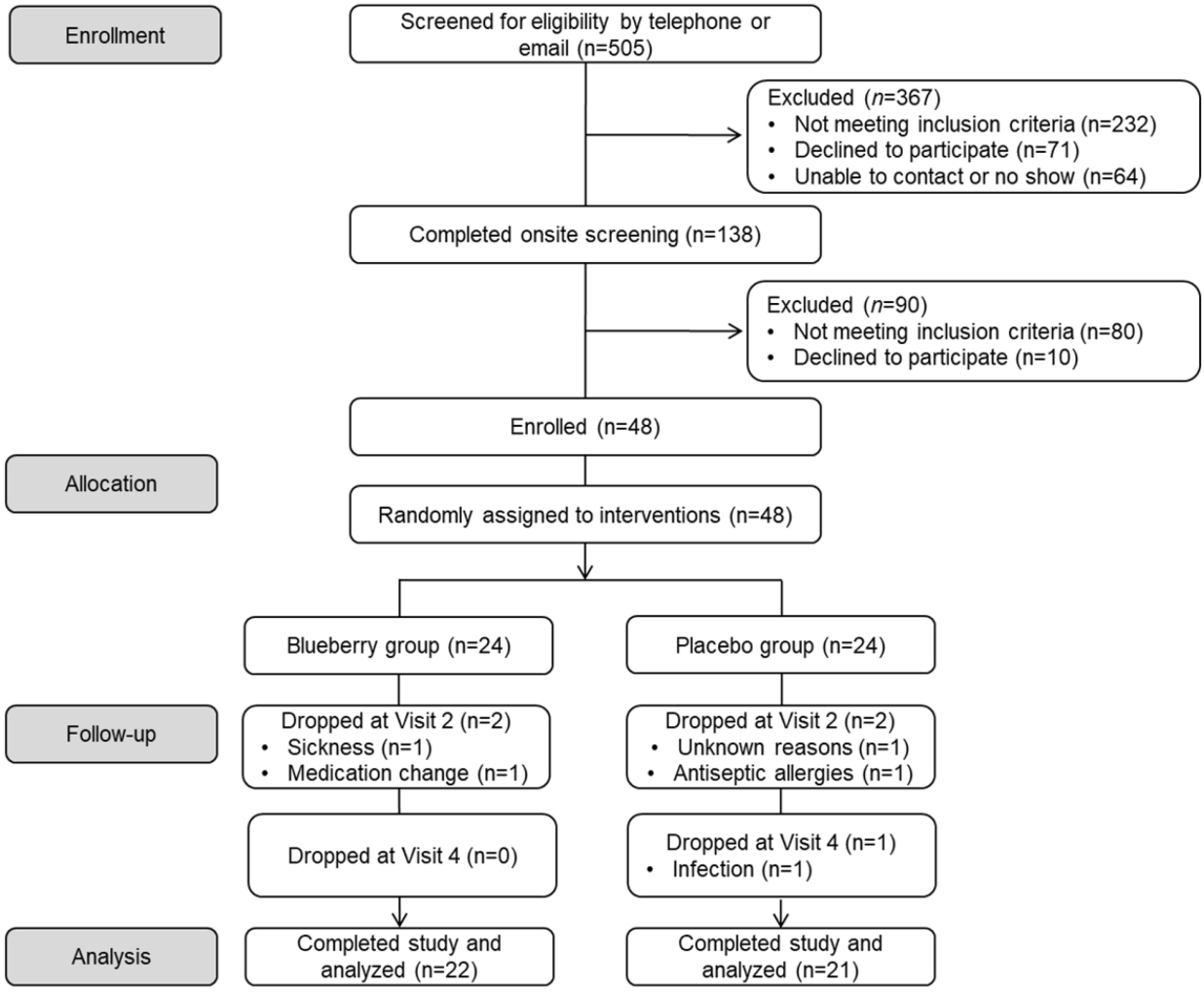


Figure 3.2. CONSORT diagram for the 12-week clinical trial.

Table 3.1. Screening characteristics for postmenopausal women with above-normal blood pressure who completed a 12-week intervention evaluating 22 g freeze-dried highbush blueberry powder vs placebo powder on endothelial function, blood pressure, and other measures of cardiometabolic health.

Variable	Blueberry (*n* = 22)	Placebo (*n* = 21)
Age (yr)	60 ± 1	61 ± 1
Years after menopause	10 ± 1	9 ± 1
Estradiol (pg/mL)	17.0 ± 0.9	17.1 ± 1.0
FSH (mIU/mL)	67.0 ±5.0	67.0 ± 4.8
Height (m)	1.6 ± 0.0	1.6 ± 0.0
Weight (kg)	73.0 ± 3.2	74.6 ± 3.5
BMI (kg/m^2)	27.6 ± 1.0	27.8 ± 1.1
WC (cm)	89 ± 3	89 ± 2
HC (cm)	108 ± 2	107 ± 2
WC:HC	0.82 ± 0.01	0.83 ± 0.01
SBP (mmHg)	130 ± 1	128 ± 1
DBP (mmHg)	80 ± 1	82 ± 1
TG (mg/dL)	121 ± 12	112 ± 14
TC (mg/dL)	214 ± 5	220 ± 6
HDL-C (mg/dL)	58 ± 3	58 ± 3
LDL-C (mg/dL)	132 ± 5	139 ± 5
HgbA1c (%)	5.5 ± 0.1	5.5 ± 0.1
BP med use, n (%)	6 (27)	4 (19)
Race/ethnicity, n (%)		
Non-Hispanic white	22 (100)	20 (95)
Hispanic	0	1 (5)

Data are mean ± SEM (or %). No differences between groups. Abbreviations: BMI, body mass index; BP, blood pressure; DBP, diastolic blood pressure; FSH, follicle-stimulating hormone; HgbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein-cholesterol; HC, hip circumference; LDL-C, low-density lipoprotein-cholesterol; med, medication; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; WC, waist circumference.

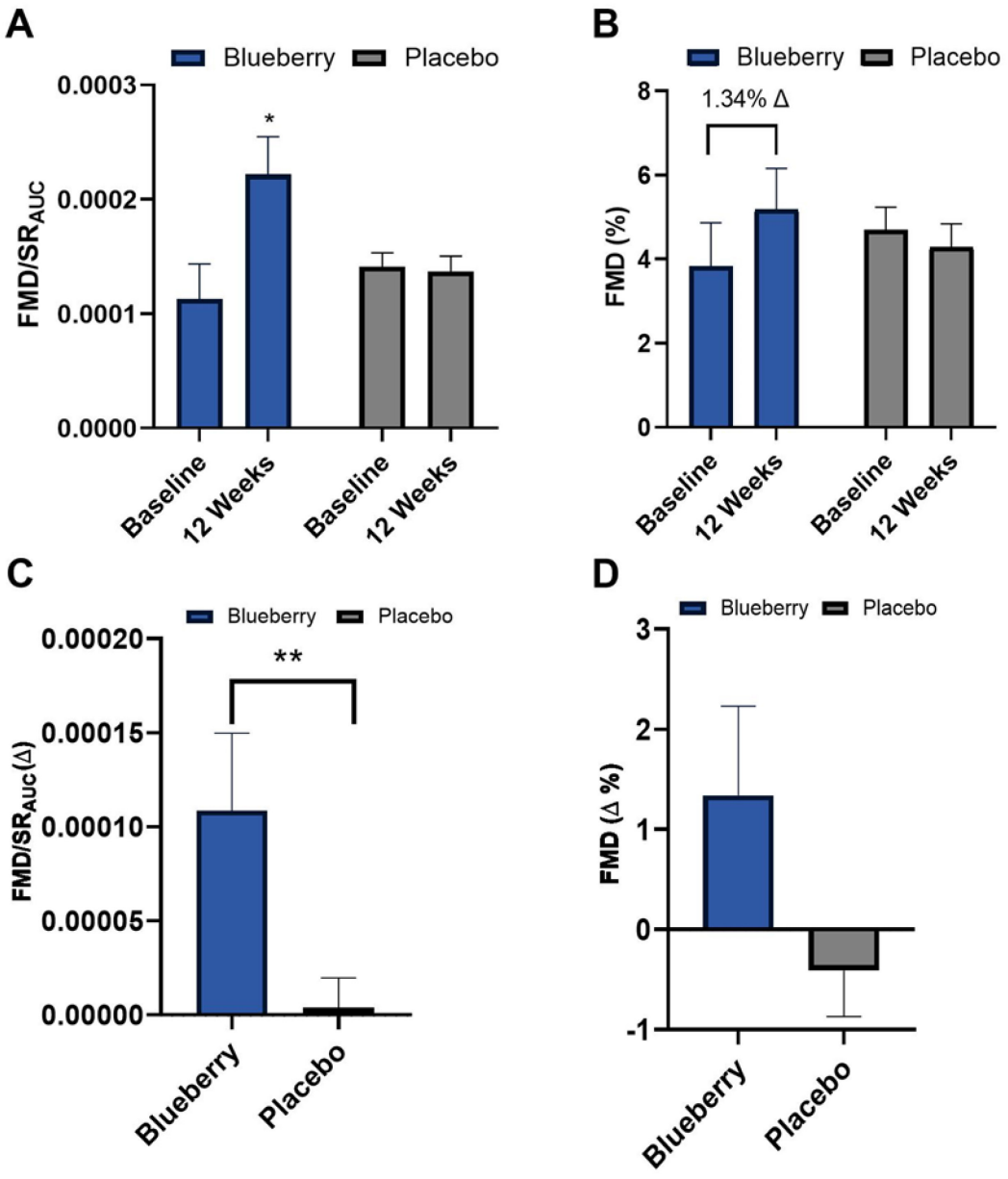


Figure 3.3. (A) Flow-mediated dilation (FMD) normalized to shear rate area under the curve (FMD/SR_{AUC}); (B) FMD%; (C) FMD/SR_{AUC} change from baseline to 12 weeks; (D) FMD% change from baseline to 12 weeks. Data are mean ± SEM. *Different ($p < 0.05$) compared to baseline within group. **Different ($p < 0.05$) between groups.

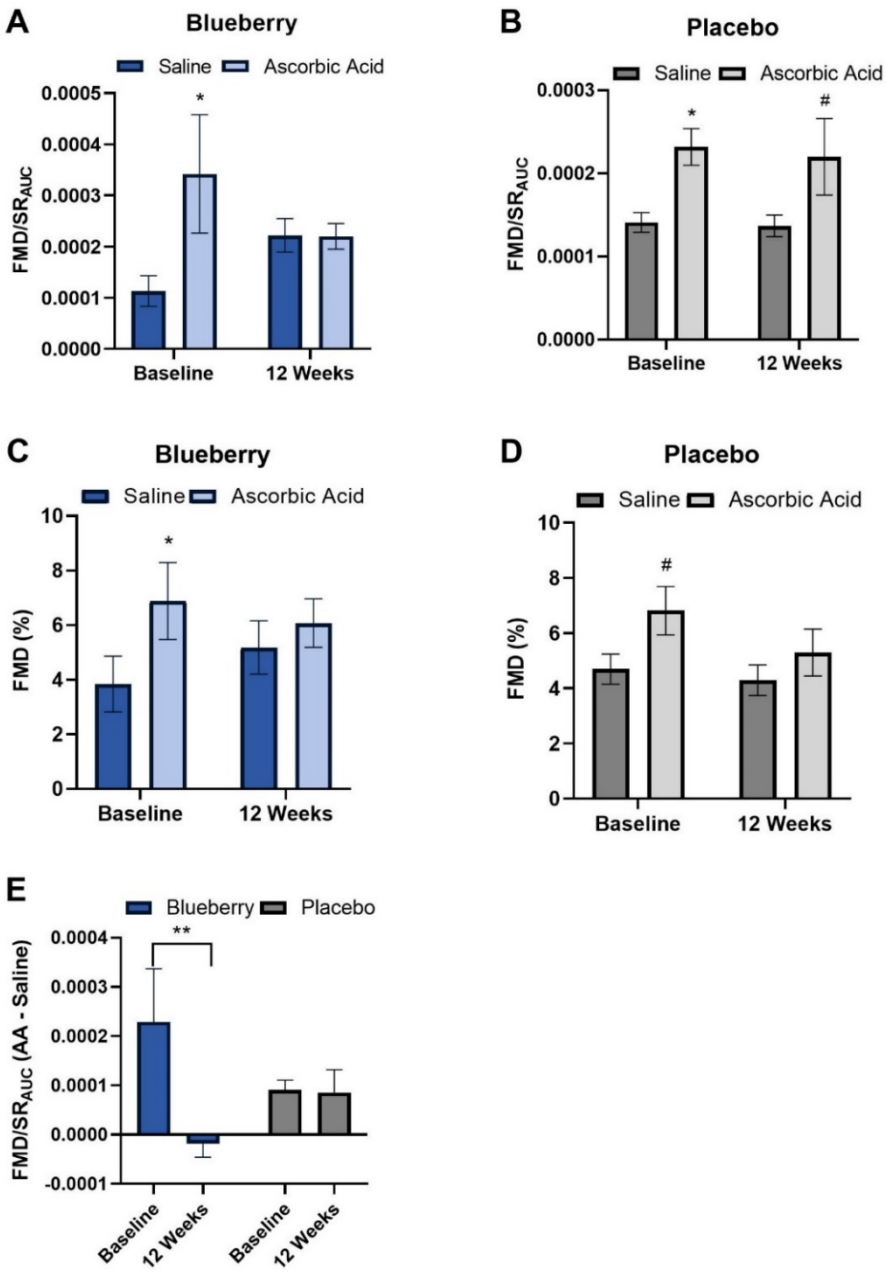


Figure 3.4. (A) FMD/SR_{AUC} during intravenous saline and vitamin C infusion at baseline and after 12 weeks of blueberry consumption; (B) FMD/SR_{AUC} during intravenous saline and vitamin C infusion at baseline and after 12 weeks of placebo consumption; (C) FMD% during intravenous saline and vitamin C infusion at baseline and after 12 weeks of blueberry consumption; (D) FMD% during intravenous saline and vitamin C infusion at baseline and after 12 weeks of placebo consumption; and (E) FMD/SR_{AUC} difference between ascorbic acid (AA) and saline infusion at baseline and after 12 weeks of blueberry and placebo consumption. Data are mean ± SEM. *Different ($p < 0.05$) compared to saline infusion on the same day. #Trend for different ($p < 0.10$) than saline infusion on the same day. **Difference ($p < 0.05$) between time points within group.

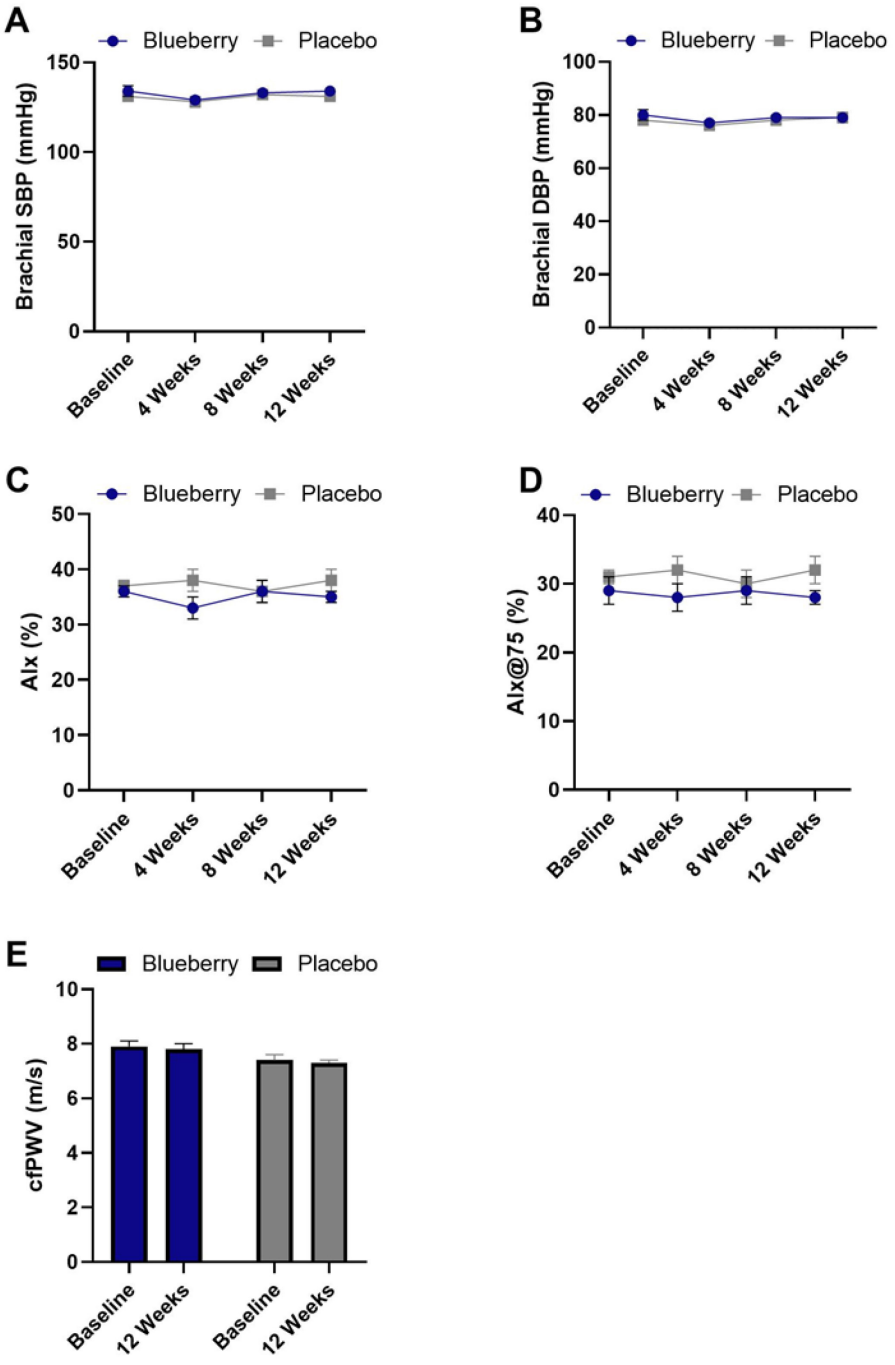


Figure 3.5. Blood pressure and arterial stiffness values throughout the 12 week clinical trial. (A) Brachial systolic blood pressure (SBP) at all time points for blueberry and placebo groups; (B) brachial diastolic blood pressure (DBP) at all time points for blueberry and placebo groups; (C) augmentation index (AIx) at all time points for blueberry and placebo groups; (D) augmentation index normalized to 75 beats per minute (AIx@75) at all time points for blueberry and placebo groups; and (E) carotid-femoral pulse wave velocity (cfPWV) at all time points for blueberry and placebo groups. Data are mean ± SEM. No differences ($p > 0.05$) between or within groups at any time point were noted.

Table 3.2. Blood biomarker concentrations in postmenopausal women with above-normal blood pressure who completed a 12-week intervention evaluating 22 g freeze-dried highbush blueberry powder vs placebo powder on endothelial function, blood pressure, and other measures of cardiometabolic health.

	Blueberry (*n* = 22)	**Placebo (*n* = 21)**
TG (mg/dL)		
Baseline	121±12	114 ± 14
12 Weeks	100 ± 10*	114 ± 18
Δ 0 to 12 Weeks	-21 ± 9	0 ± 9
TC (mg/dL)		
Baseline	214 ± 5	217 ± 5
12 Weeks	198 ± 5	201 ± 7
Δ 0 to 12 Weeks	-17 ± 4	-16 ± 4
HDL-C (mg/dL)		
Baseline	58 ± 3	57 ± 3
12 Weeks	59 ± 4	54 ± 3
Δ 0 to 12 Weeks	1 ± 3**	-3 ± 2
LDL-C (mg/dL)		
Baseline	132 ± 5	137 ± 4
12 Weeks	119 ± 6*	125 ± 5*
Δ 0 to 12 Weeks	-13 ± 5	-12 ± 4
LDL:HDL		
Baseline	2.47 ± 0.20	2.57 ± 0.20
12 Weeks	2.28 ± 0.22	2.50 ± 0.18
Δ 0 to 12 Weeks	-0.19 ± 0.14	-0.07 ± 0.10
HgbA1c (%)		
Baseline	5.5 ± 0.1	5.5 ± 0.1
12 Weeks	5.5 ± 0.1	5.5 ± 0.1
Δ 0 to 12 Weeks	0.0 ± 0.0	0.0 ± 0.0
ICAM-1 (ng/mL)		
Baseline	224.32 ± 16.27	224.88 ± 11.91
4 Weeks	234.42 ± 15.09	275.82 ± 30.07
8 Weeks	219.76 ± 12.60	273.46 ± 15.37
12 Weeks	246.19 ± 13.86	236.62 ± 12.19
Δ 0 to 4 Weeks	10.10 ± 23.29	50.94 ± 34.97
Δ 0 to 8 Weeks	-1.39 ± 21.31	58.58 ± 15.15
Δ 0 to 12 Weeks	21.87 ± 14.19	11.74 ± 18.28
VCAM-1 (ng/mL)		
Baseline	1275.76 ± 123.53	1001.81 ± 97.90
4 Weeks	1675.10 ± 153.41	1549.93 ± 140.86*
8 Weeks	1567.96 ± 179.61	1497.26 ± 122.22*
12 Weeks	1506.62 ± 104.77	1289.09 ± 126.88
Δ 0 to 4 Weeks	399.33 ± 201.69	548.12 ± 192.96
Δ 0 to 8 Weeks	238.92 ± 225.18	495.45 ± 160.61
Δ 0 to 12 Weeks	230.86 ± 107.00	287.28 ± 435.23
Nitrate/nitrite (μM)		
Baseline	9.99 ± 1.13	11.33 ± 1.51
4 Weeks	8.30 ± 1.24	12.37 ± 2.42
8 Weeks	7.93 ± 1.00	10.31 ± 2.01
12 Weeks	8.03 ± 1.31	9.76 ± 1.20

Table 3.2. Blood biomarker concentrations in postmenopausal women with above-normal blood pressure who completed a 12-week intervention evaluating 22 g freeze-dried highbush blueberry powder vs placebo powder on endothelial function, blood pressure, and other measures of cardiometabolic health.

	Blueberry (*n* = 22)	**Placebo (*n* = 21)**
Nitrate/nitrite (μM)		
Δ 0 to 4 Weeks	-1.61 ± 1.09	1.04 ± 1.42
Δ 0 to 8 Weeks	-1.64 ± 1.18	-0.51 ± 1.40
Δ 0 to 12 Weeks	-1.96 ± 1.35	-1.58 ± 1.94

Data are mean ± SEM. *Different ($p < 0.05$) than baseline. **Different ($p < 0.05$) between groups at that time point. Abbreviations: HgbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein-cholesterol, LDL-C, low-density lipoprotein-cholesterol, TC, total cholesterol, TG, triglycerides, ICAM-1, intercellular adhesion molecule-1, VCAM-1, vascular cell adhesion protein-1.

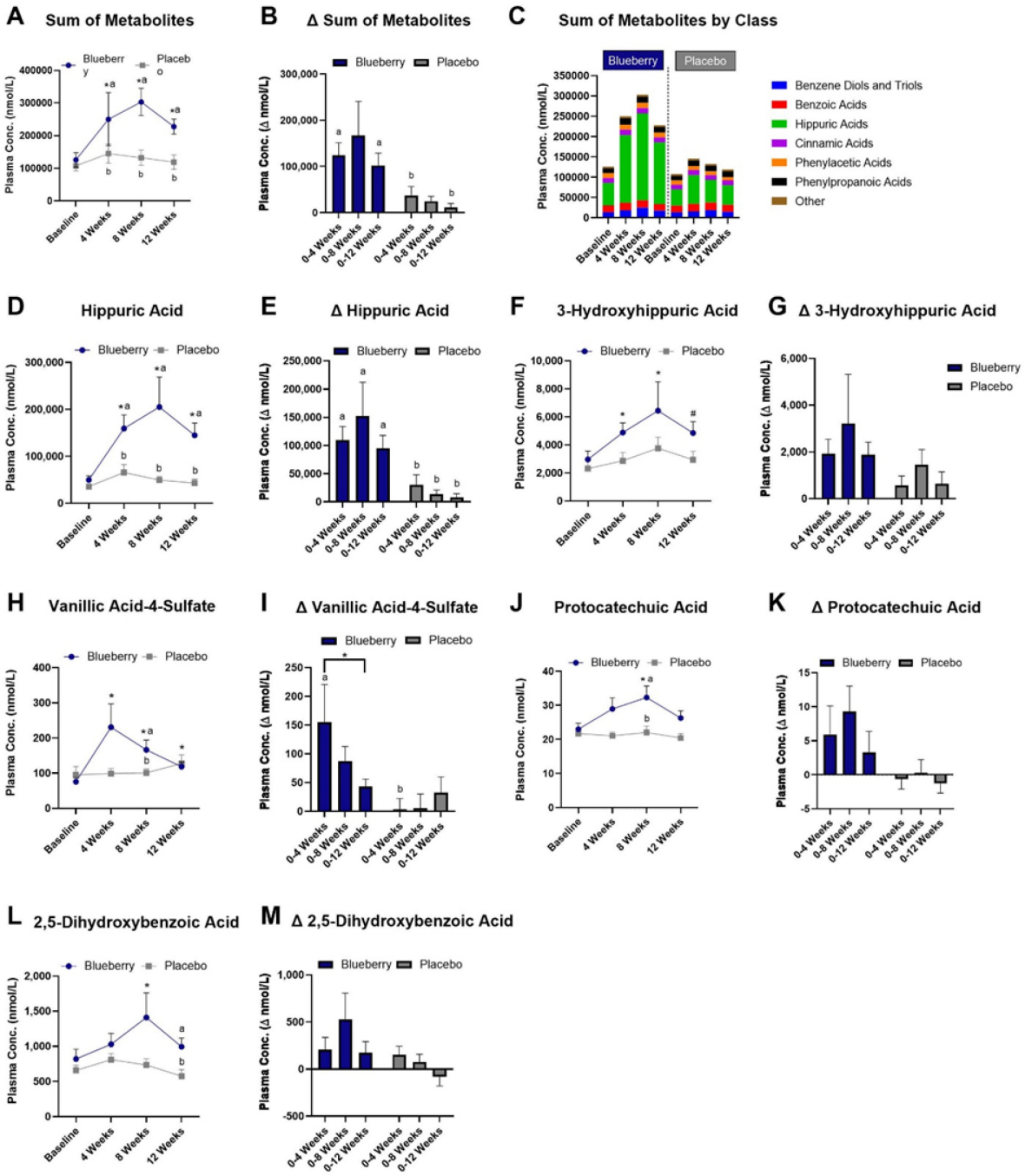


Figure 3.6. (A) sum of (poly)phenol metabolites; (B) change from baseline to 4, 8, and 12 weeks for the sum of (poly)phenol metabolites following blueberry or placebo consumption; (C) mean sum of (poly)phenol metabolites according to class at baseline, 4, 8, and 12 weeks following blueberry or placebo consumption (values are mean); (D) hippuric acid concentrations across all time points; (E) hippuric acid change from baseline to 4, 8, and 12 weeks, (F) 3'hydroxyhippuric acid concentrations across all time points; (G) 3'hydroxyhippuric acid concentrations change from baseline to 4, 8, and 12 weeks; (H) 3-methoxybenzoic acid-4-sulfate (vanillic acid-4-sulfate) concentrations across all time points; (I) 3-methoxybenzoic acid-4-sulfate (vanillic acid-4-sulfate) change from baseline to 4, 8, and 12 weeks; (J) 3,4-

dihydroxybenzoic acid (protocatechuic acid) concentrations across all time points; (K) 3,4-dihydroxybenzoic acid (protocatechuic acid) change from baseline to 4, 8, and 12 weeks; (L) 2,5-Dihydroxybenzoic acid concentrations across all time points; and (M) 2,5-Dihydroxybenzoic acid change from baseline, 4, 8, and 12 weeks. Data are mean ± SEM. *Different ($p < 0.05$) compared to baseline within group. Different letters indicate differences ($p < 0.05$) between groups at that time point.

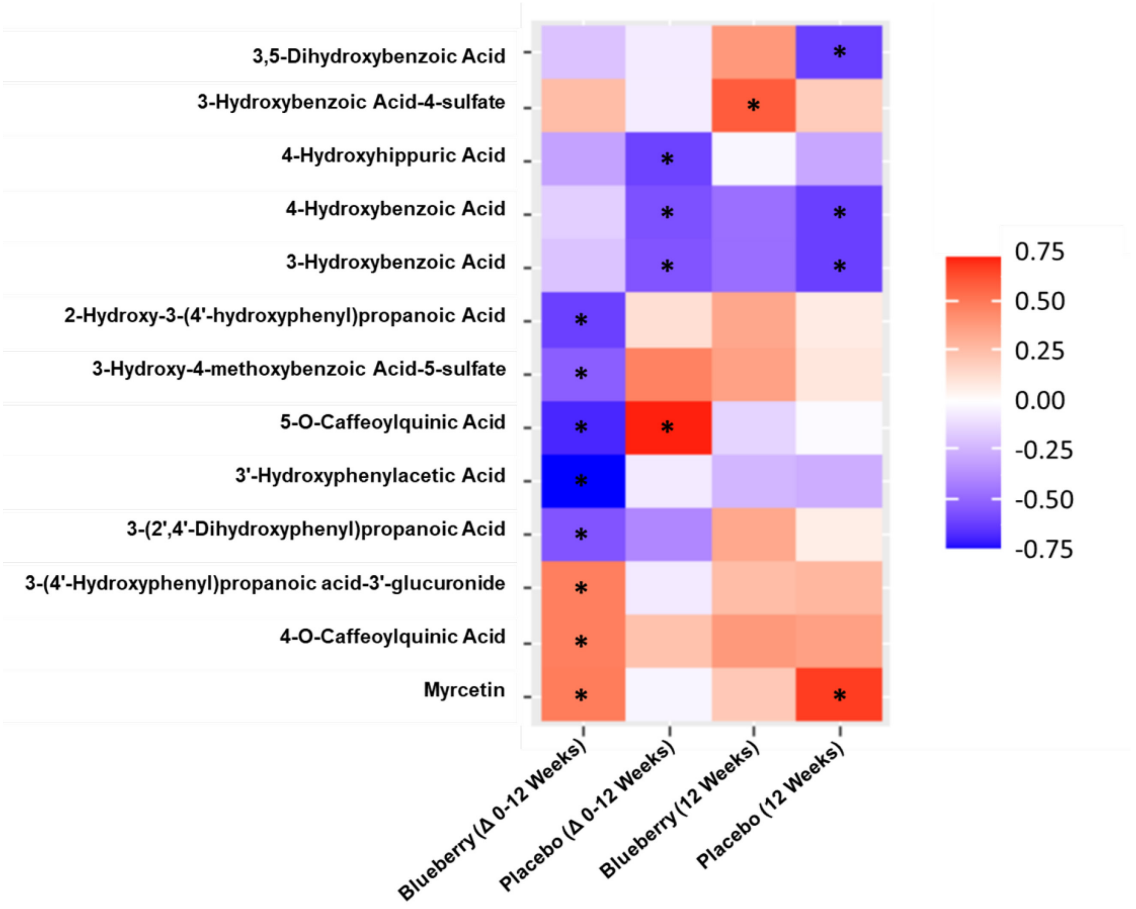


Figure 3.7. Spearman correlations between plasma (poly)phenol metabolites and flow-mediated dilation normalized to shear rate area under the curve for the change from baseline to 12 weeks and the 12-week time point. *Significant correlation ($p < 0.05$).

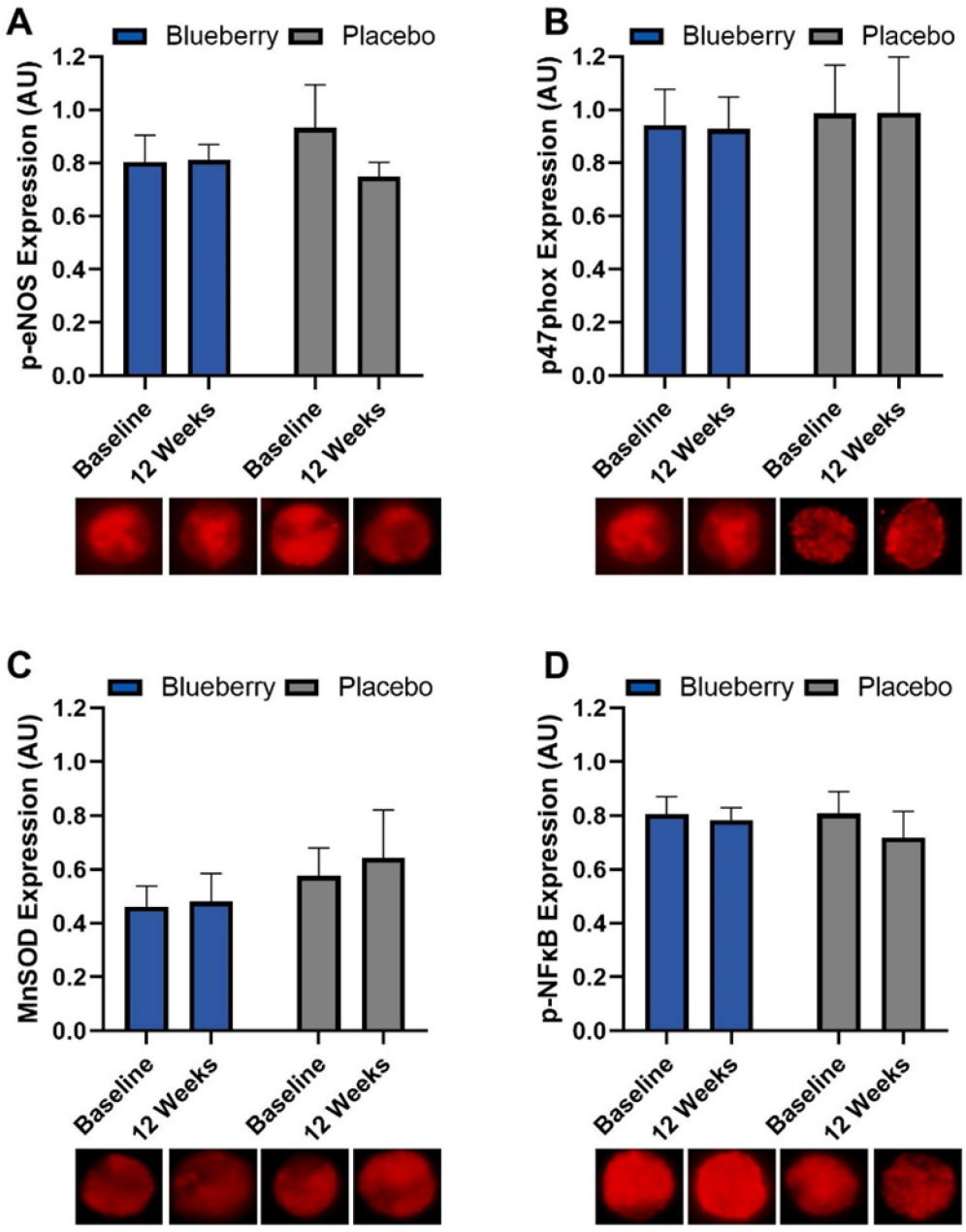


Figure 3.8. Endothelial cell protein expression for (A) phosphorylated endothelial nitric oxide synthase (p-eNOS); (B) NADPH oxidase/p47 (p47phox); (C) manganese superoxide dismutase (MnSOD); and (D) phosphorylated nuclear factor kappa B (p-NFκB). Data are expressed as arbitrary units (AU). Data are mean ± SEM. No differences ($p > 0.05$) were observed.

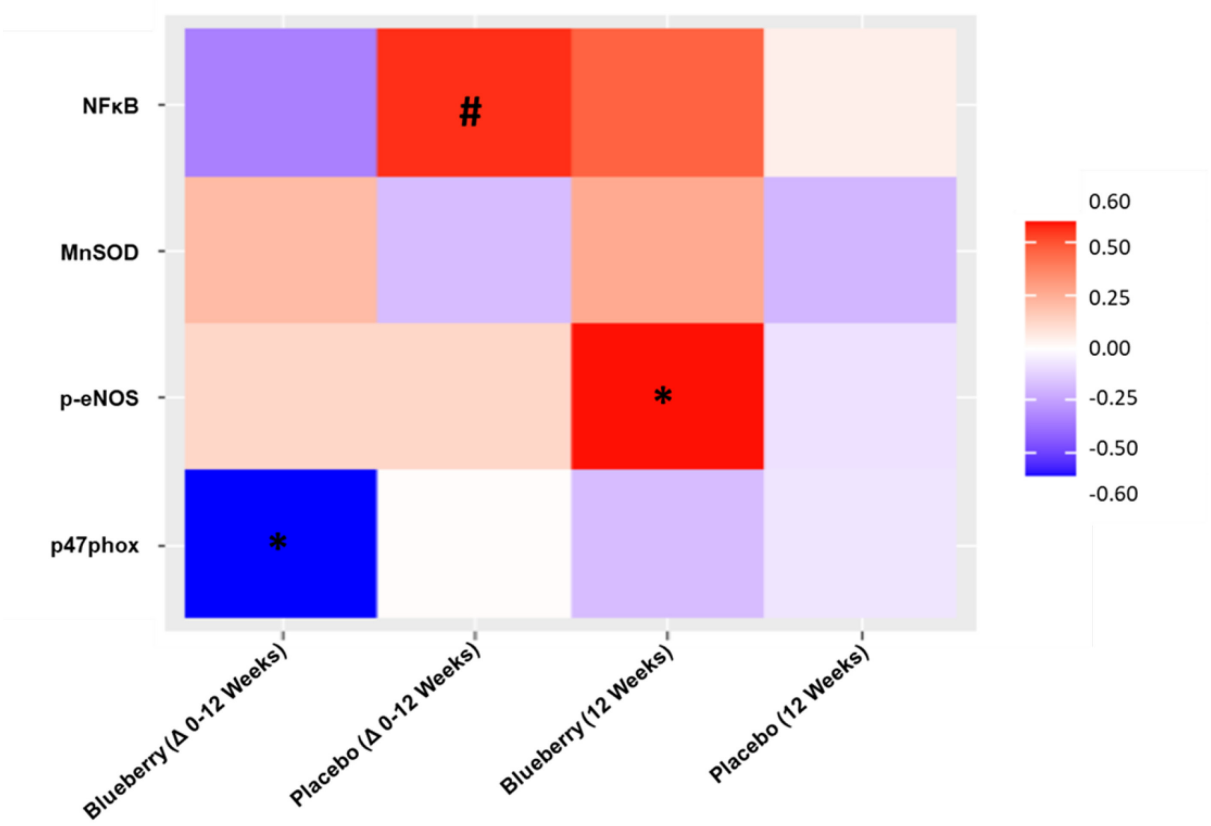


Figure 3.9. Spearman correlations between venous endothelial cell protein expression and flow-mediated dilation normalized to shear rate area under the curve for the change from baseline to 12 weeks and the 12-week time point. *Significant correlation ($p < 0.05$).

REFERENCES

1. X. Ma, O. Laaksonen, J. Zheng, W. Yang, M. Trepanier, H. Kallio and B. Yang, *Food Chem*, 2016, 200, 189-198.
2. W. Yang, O. Laaksonen, H. Kallio and B. Yang, *J Agric Food Chem*, 2016, 64, 1274-1282.
3. D. R. Seals, K. L. Jablonski and A. J. Donato, *Clin Sci (Lond)*, 2011, 120, 357-375.
4. P. M. Vanhoutte, H. Shimokawa, M. Feletou and E. H. Tang, *Acta Physiol (Oxf)*, 2017, 219, 22-96.
5. T. D. Giles, G. E. Sander, B. D. Nossaman and P. J. Kadowitz, *J Clin Hypertens (Greenwich)*, 2012, 14, 198-205.
6. C. Bleakley, P. K. Hamilton, R. Pumb, M. Harbinson and G. E. McVeigh, *J Clin Hypertens (Greenwich)*, 2015, 17, 651-654.
7. H. Cai and D. G. Harrison, *Circ Res*, 2000, 87, 840-844.
8. M. M. Bachschmid, S. Schildknecht, R. Matsui, R. Zee, D. Haeussler, R. A. Cohen, D. Pimental and B. Loo, *Ann Med*, 2013, 45, 17-36.
9. E. G. Lakatta, *Circulation*, 2003, 107, 490-497.
10. C. A. Hamilton, M. J. Brosnan, M. McIntyre, D. Graham and A. F. Dominiczak, *Hypertension*, 2001, 37, 529-534.
11. K. H. Park and W. J. Park, *J Korean Med Sci*, 2015, 30, 1213-1225.
12. Q. N. Dinh, G. R. Drummond, C. G. Sobey and S. Chrissobolis, *Biomed Res Int*, 2014, 2014, 406960.
13. M. A. Incalza, R. D'Oria, A. Natalicchio, S. Perrini, L. Laviola and F. Giorgino, *Vascul Pharmacol*, 2018, 100, 1-19.
14. H. N. Siti, Y. Kamisah and J. Kamsiah, *Vascul Pharmacol*, 2015, 71, 40-56.
15. S. A. Johnson, N. S. Litwin and D. R. Seals, *J Acad Nutr Diet*, 2019, 119, 1785-1796.

16. R. Rossi, A. Nuzzo, G. Origliani and M. G. Modena, *J Am Coll Cardiol*, 2008, 51, 997-1002.

17. M. G. Modena, L. Bonetti, F. Coppi, F. Bursi and R. Rossi, *J Am Coll Cardiol*, 2002, 40, 505-510.

18. K. L. Moreau and K. L. Hildreth, *Adv Vasc Med*, 2014, 2014.

19. K. L. Moreau, K. L. Hildreth, A. L. Meditz, K. D. Deane and W. M. Kohrt, *J Clin Endocrinol Metab*, 2012, 97, 4692-4700.

20. D. S. Celermajer, K. E. Sorensen, D. J. Spiegelhalter, D. Georgakopoulos, J. Robinson and J. E. Deanfield, *J Am Coll Cardiol*, 1994, 24, 471-476.

21. S. Taddei, A. Virdis, L. Ghiadoni, P. Mattei, I. Sudano, G. Bernini, S. Pinto and A. Salvetti, *Hypertension*, 1996, 28, 576-582.

22. Y. B. Somani, J. A. Pawelczyk, M. J. De Souza, P. M. Kris-Etherton and D. N. Proctor, *Am J Physiol Heart Circ Physiol*, 2019, 317, H395-h404.

23. K. L. Moreau, K. L. Hildreth, J. Klawitter, P. Blatchford and W. M. Kohrt, *Geroscience*, 2020, 42, 1699-1714.

24. A. Iorga, C. M. Cunningham, S. Moazeni, G. Ruffenach, S. Umar and M. Eghbali, *Biol Sex Differ*, 2017, 8, 33.

25. D. Mozaffarian, E. J. Benjamin, A. S. Go, D. K. Arnett, M. J. Blaha, M. Cushman, S. de Ferranti, J. P. Després, H. J. Fullerton, V. J. Howard, M. D. Huffman, S. E. Judd, B. M. Kissela, D. T. Lackland, J. H. Lichtman, L. D. Lisabeth, S. Liu, R. H. Mackey, D. B. Matchar, D. K. McGuire, E. R. Mohler, 3rd, C. S. Moy, P. Muntner, M. E. Mussolino, K. Nasir, R. W. Neumar, G. Nichol, L. Palaniappan, D. K. Pandey, M. J. Reeves, C. J. Rodriguez, P. D. Sorlie, J. Stein, A. Towfighi, T. N. Turan, S. S. Virani, J. Z. Willey, D. Woo, R. W. Yeh and M. B. Turner, *Circulation*, 2015, 131, e29-322.

26. L. L. Yanes and J. F. Reckelhoff, *Am J Hypertens*, 2011, 24, 740-749.

27. J. Hsia, K. L. Margolis, C. B. Eaton, N. K. Wenger, M. Allison, L. Wu, A. Z. LaCroix and H. R. Black, *Circulation*, 2007, 115, 855-860.

28. L. Santos, *Eur J Intern Med*, 2022, 97, 18-25.

29. R. Andriantsitohaina, C. Auger, T. Chataigneau, N. Étienne-Selloum, H. Li, M. C. Martínez, V. B. Schini-Kerth and I. Laher, *Br J Nutr*, 2012, 108, 1532-1549.

30. M. H. Oak, C. Auger, E. Belcastro, S. H. Park, H. H. Lee and V. B. Schini-Kerth, *Free Radic Biol Med*, 2018, 122, 161-170.

31. L. Zhang, Z. Han and D. Granato, *Adv Food Nutr Res*, 2021, 98, 1-33.

32. K. B. Pandey and S. I. Rizvi, *Oxid Med Cell Longev*, 2009, 2, 270-278.

33. D. Del Rio, A. Rodriguez-Mateos, J. P. Spencer, M. Tognolini, G. Borges and A. Crozier, *Antioxid Redox Signal*, 2013, 18, 1818-1892.

34. X. Xie, R. Zhao and G. X. Shen, *Int J Mol Sci*, 2012, 13, 15867-15880.

35. M. Edwards, C. Czank, G. M. Woodward, A. Cassidy and C. D. Kay, *J Agric Food Chem*, 2015, 63, 2423-2431.

36. W. Gan, Y. Dang, X. Han, S. Ling, J. Duan, J. Liu and J. W. Xu, *Mol Nutr Food Res*, 2016, 60, 266-277.

37. X. Wu, J. Kang, C. Xie, R. Burris, M. E. Ferguson, T. M. Badger and S. Nagarajan, *J Nutr*, 2010, 140, 1628-1632.

38. J. Li, D. Ruzhi, X. Hua, L. Zhang, F. Lu, T. G. Coursey, S. C. Pflugfelder and D. Q. Li, *Sci Rep*, 2016, 6, 19408.

39. T. Mačičková, J. Pečivová, J. Harmatha, K. Sviteková and R. Nosáľ, *Interdiscip Toxicol*, 2012, 5, 71-75.

40. A. Speciale, S. Anwar, R. Canali, J. Chirafisi, A. Saija, F. Virgili and F. Cimino, *Mol Nutr Food Res*, 2013, 57, 1979-1987.

41. Y. J. Cao, Y. M. Zhang, J. P. Qi, R. Liu, H. Zhang and L. C. He, *Int Immunopharmacol*, 2015, 28, 1018-1025.

42. A. Rodriguez-Mateos, M. Le Sayec, G. Istas and S. A. Johnson, *Berries and Berry Bioactive Compounds in Promoting Health*, 2022, 33, 120.

43. B. R. Cutler, C. Petersen and P. V. Anandh Babu, *Mol Nutr Food Res*, 2017, 61.

44. S. A. Johnson, A. Figueroa, N. Navaei, A. Wong, R. Kalfon, L. T. Ormsbee, R. G. Feresin, M. L. Elam, S. Hooshmand, M. E. Payton and B. H. Arjmandi, *J Acad Nutr Diet*, 2015, 115, 369-377.

45. A. Rodriguez-Mateos, G. Istas, L. Boschek, R. P. Feliciano, C. E. Mills, C. Boby, S. Gomez-Alonso, D. Milenkovic and C. Heiss, *J Gerontol A Biol Sci Med Sci*, 2019, 74, 967-976.

46. W. Huang, Y. Zhu, C. Li, Z. Sui and W. Min, *Oxid Med Cell Longev*, 2016, 2016, 1591803.

47. D. Bharat, R. R. M. Cavalcanti, C. Petersen, N. Begaye, B. R. Cutler, M. M. A. Costa, R. Ramos, M. R. Ferreira, Y. Li, L. P. Bharath, E. Toolson, P. Sebahar, R. E. Looper, T. Jalili, N. S. Rajasekaran, Z. Jia, J. D. Symons and P. V. Anandh Babu, *Mol Nutr Food Res*, 2018, 62.

48. S. H. Park, S. O. Jeong, H. T. Chung and H. O. Pae, *Plant Foods Hum Nutr*, 2015, 70, 263-268.

49. I. Eskurza, L. A. Myerburgh, Z. D. Kahn and D. R. Seals, *J Physiol*, 2005, 568, 1057-1065.

50. P. E. Gates, M. L. Boucher, A. E. Silver, K. D. Monahan and D. R. Seals, *J Appl Physiol (1985)*, 2007, 102, 63-71.

51. N. P. Bachman, J. D. Terwoord, J. C. Richards, B. Braun, C. P. Green, G. J. Luckasen and F. A. Dinenno, *Atherosclerosis*, 2021, 320, 105-111.

52. D. H. J. Thijssen, R. M. Bruno, A. van Mil, S. M. Holder, F. Faita, A. Greyling, P. L. Zock, S. Taddei, J. E. Deanfield, T. Luscher, D. J. Green and L. Ghiadoni, *Eur Heart J*, 2019, 40, 2534-2547.

53. V. E. Brunt, R. A. Gioscia-Ryan, A. G. Casso, N. S. VanDongen, B. P. Ziemba, Z. J. Sapinsley, J. J. Richey, M. C. Zigler, A. P. Neilson, K. P. Davy and D. R. Seals, *Hypertension*, 2020, 76, 101-112.

54. B. A. Parker, T. L. Trehearn and J. R. Meendering, *J Appl Physiol (1985)*, 2009, 107, 1357-1359.

55. K. E. Pyke, E. M. Dwyer and M. E. Tschakovsky, *J Appl Physiol (1985)*, 2004, 97, 499-508.

56. J. Padilla, B. D. Johnson, S. C. Newcomer, D. P. Wilhite, T. D. Mickleborough, A. D. Fly, K. J. Mather and J. P. Wallace, *Cardiovasc Ultrasound*, 2008, 6, 44.

57. J. Padilla, B. D. Johnson, S. C. Newcomer, D. P. Wilhite, T. D. Mickleborough, A. D. Fly, K. J. Mather and J. P. Wallace, *J Vasc Res*, 2009, 46, 592-600.

58. I. Eskurza, K. D. Monahan, J. A. Robinson and D. R. Seals, *J Physiol*, 2004, 556, 315-324.

59. G. L. Pierce, L. A. Lesniewski, B. R. Lawson, S. D. Beske and D. R. Seals, *Circulation*, 2009, 119, 1284-1292.

60. N. S. Litwin, H. J. Van Ark, S. C. Hartley, K. A. Michell, A. R. Vazquez, E. K. Fischer, C. L. Melby, T. L. Weir, Y. Wei, S. Rao, K. L. Hildreth, D. R. Seals, M. J. Pagliassotti and S. A. Johnson, *Curr Dev Nutr*, 2019, 3, nzz113.

61. R. E. Trotter, A. R. Vazquez, D. S. Grubb, K. E. Freedman, L. E. Grabos, S. Jones, C. L. Gentile, C. L. Melby, S. A. Johnson and T. L. Weir, *Benef Microbes*, 2020, 11, 621-630.

62. R. P. Feliciano, E. Mecha, M. R. Bronze and A. Rodriguez-Mateos, *J Chromatogr A*, 2016, 1464, 21-31.

63. I. Eskurza, Z. D. Kahn and D. R. Seals, *J Physiol*, 2006, 571, 661-668.

64. P. C. Colombo, A. W. Ashton, S. Celaj, A. Talreja, J. E. Banchs, N. B. Dubois, M. Marinaccio, S. Malla, J. Lachmann, J. A. Ware and T. H. Le Jemtel, *J Appl Physiol (1985)*, 2002, 92, 1331-1338.

65. A. E. Silver, D. D. Christou, A. J. Donato, S. D. Beske, K. L. Moreau, K. A. Magerko and D. R. Seals, *J Vasc Res*, 2010, 47, 1-8.

66. A. J. Donato, I. Eskurza, A. E. Silver, A. S. Levy, G. L. Pierce, P. E. Gates and D. R. Seals, *Circ Res*, 2007, 100, 1659-1666.

67. A. E. Silver, S. D. Beske, D. D. Christou, A. J. Donato, K. L. Moreau, I. Eskurza, P. E. Gates and D. R. Seals, *Circulation*, 2007, 115, 627-637.

68. A. F. Subar, S. I. Kirkpatrick, B. Mittl, T. P. Zimmerman, F. E. Thompson, C. Bingley, G. Willis, N. G. Islam, T. Baranowski, S. McNutt and N. Potischman, *J Acad Nutr Diet*, 2012, 112, 1134-1137.

69. S. I. Kirkpatrick, F. E. Thompson, A. F. Subar, D. Douglass, T. P. Zimmerman, L. L. Kahle, S. M. George and N. Potischman, *The FASEB Journal*, 2013.

70. J. F. Sallis, W. L. Haskell, P. D. Wood, S. P. Fortmann, T. Rogers, S. N. Blair and R. S. Paffenbarger, Jr., *Am J Epidemiol*, 1985, 121, 91-106.

71. H. A. Hayden-Wade, K. J. Coleman, J. F. Sallis and C. Armstrong, *Med Sci Sports Exerc*, 2003, 35, 801-809.

72. B. Abramson, K. Srivaratharajah, L. Davis and B. Parapid, *Evaluation, and Management of High Blood Pressure in Adults. An Expert Analysis*, 2018.

73. P. J. Curtis, V. van der Velpen, L. Berends, A. Jennings, M. Feelisch, A. M. Umpleby, M. Evans, B. O. Fernandez, M. S. Meiss, M. Minnion, J. Potter, A. M. Minihane, C. D. Kay, E. B. Rimm and A. Cassidy, *Am J Clin Nutr*, 2019, 109, 1535-1545.

74. A. J. Stull, K. C. Cash, C. M. Champagne, A. K. Gupta, R. Boston, R. A. Beyl, W. D. Johnson and W. T. Cefalu, *Nutrients*, 2015, 7, 4107-4123.

75. Y. Inaba, J. A. Chen and S. R. Bergmann, *Int J Cardiovasc Imaging*, 2010, 26, 631-640.

76. K. S. Stote, M. I. Sweeney, T. Kean, D. J. Baer, J. A. Novotny, N. L. Shakerley, A. Chandrasekaran, P. M. Carrico, J. A. Melendez and K. T. Gottschall-Pass, *BMC Nutr*, 2017, 3, 45.

77. P. Riso, D. Klimis-Zacas, C. Del Bo, D. Martini, J. Campolo, S. Vendrame, P. Møller, S. Loft, R. De Maria and M. Porrini, *Eur J Nutr*, 2013, 52, 949-961.

78. C. Del Bó, P. Riso, J. Campolo, P. Møller, S. Loft, D. Klimis-Zacas, A. Brambilla, A. Rizzolo and M. Porrini, *Nutr Res*, 2013, 33, 220-227.

79. D. Milenkovic, C. Morand, A. Cassidy, A. Konic-Ristic, F. Tomás-Barberán, J. M. Ordovas, P. Kroon, R. De Caterina and A. Rodriguez-Mateos, *Adv Nutr*, 2017, 8, 558-570.

80. M. T. García-Conesa, K. Chambers, E. Combet, P. Pinto, M. Garcia-Aloy, C. Andrés-Lacueva, S. de Pascual-Teresa, P. Mena, A. Konic Ristic, W. J. Hollands, P. A. Kroon, A. Rodríguez-Mateos, G. Istas, C. A. Kontogiorgis, D. K. Rai, E. R. Gibney, C. Morand, J. C. Espín and A. González-Sarrías, *Int J Mol Sci*, 2018, 19.

81. R. T. Ras, M. T. Streppel, R. Draijer and P. L. Zock, *Int J Cardiol*, 2013, 168, 344-351.

82. Y. A. Al-Dashti, R. R. Holt, J. G. Carson, C. L. Keen and R. M. Hackman, *J Med Food*, 2019, 22, 982-992.

83. J. R. Santos-Parker, T. R. Strahler, V. M. Vorwald, G. L. Pierce and D. R. Seals, *J Appl Physiol (1985)*, 2017, 122, 11-19.

84. V. Habauzit, M. A. Verny, D. Milenkovic, N. Barber-Chamoux, A. Mazur, C. Dubray and C. Morand, *Am J Clin Nutr*, 2015, 102, 66-74.

85. M. Abshirini, M. Omidian and H. Kord-Varkaneh, *Menopause*, 2020, 27, 1425-1433.

86. C. Ozemek, K. L. Hildreth, P. J. Blatchford, K. J. Hurt, R. Bok, D. R. Seals, W. M. Kohrt and K. L. Moreau, *J Appl Physiol (1985)*, 2020, 128, 739-747.

87. K. L. Moreau, B. L. Stauffer, W. M. Kohrt and D. R. Seals, *J Clin Endocrinol Metab*, 2013, 98, 4507-4515.

88. D. R. Seals, E. E. Nagy and K. L. Moreau, *J Physiol*, 2019, 597, 4901-4914.

89. A. Rodriguez-Mateos, C. Rendeiro, T. Bergillos-Meca, S. Tabatabaee, T. W. George, C. Heiss and J. P. Spencer, *Am J Clin Nutr*, 2013, 98, 1179-1191.

90. C. Petersen, D. Bharat, U. D. Wankhade, J. S. Kim, B. R. Cutler, C. Denetso, S. Gholami, S. Nelson, J. Bigley, A. Johnson, S. V. Chintapalli, B. D. Piccolo, A. K. Satheesh Babu, H. A. Paz, K. Shankar, J. D. Symons and P. V. Anandh Babu, *Mol Nutr Food Res*, 2022, 66, e2100784.

91. D. Martini, M. Marino, S. Venturi, M. Tucci, D. Klimis-Zacas, P. Riso, M. Porrini and C. Del Bo, *J Nutr Biochem*, 2023, 111, 109154.

92. S. A. Johnson, R. G. Feresin, N. Navaei, A. Figueroa, M. L. Elam, N. S. Akhavan, S. Hooshmand, S. Pourafshar, M. E. Payton and B. H. Arjmandi, *Food Funct*, 2017, 8, 372-380.

93. A. Basu, M. Du, M. J. Leyva, K. Sanchez, N. M. Betts, M. Wu, C. E. Aston and T. J. Lyons, *J Nutr*, 2010, 140, 1582-1587.

94. A. R. Nair, N. Mariappan, A. J. Stull and J. Francis, *Food Funct*, 2017, 8, 4118-4128.

95. L. S. McAnulty, S. R. Collier, M. J. Landram, D. S. Whittaker, S. E. Isaacs, J. M. Klemka, S. L. Cheek, J. C. Arms and S. R. McAnulty, *Nutr Res*, 2014, 34, 577-584.

96. S. Vendrame, T. E. Adekeye and D. Klimis-Zacas, *Nutrients*, 2022, 14.

97. Y. Wang, J. L. Gallegos, C. Haskell-Ramsay and J. K. Lodge, *Nutrients*, 2022, 14.

98. K. Asayama, T. Ohkubo and Y. Imai, *J Hum Hypertens*, 2021, DOI: 10.1038/s41371-021-00486-8, 1-9.

99. P. K. Whelton, R. M. Carey, W. S. Aronow, D. E. Casey, Jr., K. J. Collins, C. Dennison Himmelfarb, S. M. DePalma, S. Gidding, K. A. Jamerson, D. W. Jones, E. J. MacLaughlin, P. Muntner, B. Ovbiagele, S. C. Smith, Jr., C. C. Spencer, R. S. Stafford, S. J. Taler, R. J. Thomas, K. A. Williams, Sr., J. D. Williamson and J. T. Wright, Jr., *Hypertension*, 2018, 71, e13-e115.

100. P. K. Whelton, R. M. Carey, G. Mancia, R. Kreutz, J. D. Bundy and B. Williams, *Circulation*, 2022, 146, 868-877.

101. A. N. Lyle and U. Raaz, *Arterioscler Thromb Vasc Biol*, 2017, 37, e1-e11.

102. M. A. Said, R. N. Eppinga, E. Lipsic, N. Verweij and P. van der Harst, *J Am Heart Assoc*, 2018, 7.

103. Q. Zhong, M. J. Hu, Y. J. Cui, L. Liang, M. M. Zhou, Y. W. Yang and F. Huang, *Angiology*, 2018, 69, 617-629.

104. R. P. Feliciano, G. Istas, C. Heiss and A. Rodriguez-Mateos, *Molecules*, 2016, 21.

CHAPTER 4: POTENTIAL FACTORS CONTRIBUTING TO THE INTERINDIVIDUAL VARIABILITY IN ENDOTHELIAL FUNCTION RESPONSE TO CHRONIC BLUEBERRY CONSUMPTION IN POSTMENOPAUSAL WOMEN WITH ABOVE-NORMAL BLOOD PRESSURE

Summary

Evidence indicates oxidative stress and inflammation are major contributors to endothelial dysfunction which is central to cardiovascular disease (CVD) development. Blueberries are a promising dietary intervention for CVD risk prevention, as they are rich in nutrients and polyphenols and have demonstrated CVD-protective effects. We demonstrated that consuming 22 g/day of freeze-dried highbush blueberry powder for 12 weeks by postmenopausal women with above-normal blood pressure improved endothelial function (assessed as brachial artery flow-mediated dilation (FMD)) through reductions in oxidative stress, but there was interindividual variability in the direction and magnitude of the FMD response to blueberry consumption due to unknown factors. The aim of this secondary analysis was to examine potential factors contributing to the efficacy of blueberry consumption to improve FMD. Data collected for the blueberry group (n = 17) was used to assess the impact of study participant characteristics on FMD between responders (n = 8) and non-responders (n = 9). Responders were defined as having a ≥ +1% change in FMD from baseline to 12 weeks. At screening, responders had lower high-density lipoprotein cholesterol (p = 0.008), and higher levels of low-density lipoprotein cholesterol (p = 0.027) and estradiol (p = 0.002), the latter of which was positively correlated with the change in FMD from baseline to 12 weeks (p = 0.002). At baseline, responders had lower FMD (1.80% responder *vs* 5.65% non-responder, p = 0.031), and higher oxidative stress-mediated impaired FMD (p = 0.023) and intracellular adhesion molecule-1 concentrations (p = 0.010) compared to non-responders. Responders improved

more in FMD than non-responders (+4.26% vs -1.25%, $p < 0.001$) from baseline to 12 weeks. At 12 weeks, phosphorylated endothelial nitric oxide synthase expression was higher for responders compared to non-responders ($p = 0.019$). Plasma nitrate concentrations were lower at 4 ($p = 0.048$) and 12 ($p = 0.028$) weeks for responders compared to non-responders. The sum of plasma (poly)phenol metabolites for responders increased at 4 ($p = 0.002$) and 12 ($p = 0.036$) compared to baseline and increased for non-responders at 4 ($p < 0.001$), 8 ($p = 0.006$), and 12 ($p = 0.005$) weeks compared to baseline. Furthermore, differences in 5 individual (poly)phenol metabolite concentrations were noted between groups at various time points ($p < 0.05$). These findings suggest that responders differed in screening/baseline characteristics, such that those with greater impairments in cardiovascular health had worsened endothelial function and higher oxidative stress but greater responses to blueberry consumption.

Introduction

In the United States, 1 out of every 5 deaths is caused by cardiovascular disease (CVD).[1] Endothelial dysfunction, which is characterized by impaired endothelium-dependent dilation, is increasingly being recognized as an early indicator of CVD risk as it precedes and predicts CVD and related events.[2-6] Endothelial cells are located at the inner most lining of blood vessels (i.e. endothelium) and produce vasoactive molecules, such as nitric oxide (NO), to regulate vascular tone and blood flow.[4, 7] NO is a vasodilator with anti-thrombotic, anti-coagulant, and anti-inflammatory properties, therefore, being a crucial molecule for overall vascular health.[3, 4, 7] Endothelial dysfunction occurs when NO production and/or bioavailability is impaired, causing diminished vasodilation and increased vasoconstriction of the blood vessels, which is largely driven by oxidative stress and inflammation.[2, 3, 7-9] Specifically, oxidative stress impairs NO production and bioavailability through uncoupling of endothelial nitric oxide synthase (eNOS) resulting in production of superoxide radicals instead of NO, which can interact with bioavailable NO to form peroxynitrite further promoting oxidative stress.[7, 10] Inflammation

increases ROS production as well, thus, perpetuating the cycle and contributing to endothelial dysfunction.[9-11]

Postmenopausal women have been shown to have progressive impairments in endothelium-dependent dilation across the menopausal transition[12, 13] and oxidative stress-mediated suppression of endothelial function.[8, 14] Estrogen functions as an antioxidant by scavenging free radicals and exerts direct effects on the vasculature by binding to estrogen receptors on endothelial cells stimulating NO production through the eNOS-NO pathway, among other functions.[8, 15, 16] Postmenopausal women are also at risk for above-normal blood pressure, dyslipidemia, and excess body fat or obesity, which have been independently associated with increased oxidative stress and endothelial dysfunction.[3, 17-25] Therefore, interventions that improve endothelial function through reductions in oxidative stress and/or inflammation are needed for postmenopausal women.

Diet and nutrition can aid in CVD risk reduction, as consumption of plant-rich diets have been shown to reduce the risk for cardiovascular mortality and development of CVD by 8.1% and 10.2%, respectively,[26, 27] which may be due in large part to their phytochemical contents.[26] Specifically, (poly)phenols are secondary plant metabolites classified as flavonoids (e.g. anthocyanins) and non-flavonoids (e.g. phenolic acids).[28] Flavonoids are the most diverse and largest group of (poly)phenols and are found in high concentrations in fruits, and evidence indicates their consumption can reduce oxidative stress and inflammation.[29-32] Blueberries are rich in nutrients (e.g. vitamins, minerals, fiber) and non-nutrient compounds like (poly)phenols (e.g. anthocyanins, phenolic acids, pterostilbene).[33, 34] The popularity of blueberries and year-round availability, coupled with their demonstrated health benefits, indicate they can be an effective food-based strategy for preserving endothelial function.[35] Research shows that chronic blueberry consumption can improve endothelial function in healthy males, older adults, adults with metabolic syndrome, and postmenopausal women with above-normal blood pressure.[14, 36-39] Specifically, we recently demonstrated that daily consumption of 22 g freeze-dried highbush

blueberry powder for 12 weeks improved endothelial function measured via flow-mediated dilation (FMD) normalized for shear stress in postmenopausal women with above-normal blood pressure through reductions of oxidative stress in a randomized, double blind, placebo-controlled, parallel-arm clinical trial.[14] These findings are clinically meaningful and provide the first evidence for direct improvements in endothelial function through oxidative stress. However, our study highlighted interindividual variability in the direction and magnitude of FMD responses (range from -4.4% to +8.2% FMD) indicating treatment responders and non-responders.[14] Other researchers have also reported variability in endothelial function responses to chronic blueberry intervention,[37, 39-41] though reasons for such variability were not explored. Evaluation of factors driving efficacy of dietary blueberry consumption for improving endothelial function can facilitate effective interventions and individualized recommendations.

Precision nutrition (i.e. personalized nutrition), the notion that “one size does not fit all”, has been a topic of interest to scientists and practitioners for decades, as it is well-known individuals respond differently to dietary interventions due to the complexity of human physiology and metabolism.[42-44] This is a relatively more recent concept that aims to develop individual nutrition recommendations based on various factors such as biological variables and clinical outcomes, and has emerged as a national priority.[43, 45] Traditional interventional-based research assessing group response provides fundamental information on whether a dietary intervention or treatment is effective at maintaining health and prevention of disease on a large scale. However, there is a need for research to focus on individual responses to such interventions as individuals vary in the magnitude and/or direction in clinical outcomes, including plant-based/polyphenol-rich dietary interventions.[14, 37, 40-42, 44] The purpose to this study was to explore factors contributing to endothelial function responses to 12 weeks of daily blueberry consumption in postmenopausal women with above-normal blood pressure in a secondary analysis of our previously published clinical trial.[14] This study will provide insight into factors driving efficacy of using blueberries as a dietary intervention for reducing CVD risk in

postmenopausal women supporting effective and individualized interventions. We hypothesized that overall disease status (i.e. higher blood lipids, BMI, oxidative stress) at baseline would impact the magnitude of response to the intervention blueberry.

Methods

Study design, participants, and recruitment

A comprehensive and detailed explanation of the study design, participant inclusion/exclusion criteria, and recruitment for the clinical trial has been reported.[14] In short, postmenopausal women aged 45 to 65 years with above-normal blood pressure (120-139 mmHg systolic and/or 80-89 mmHg diastolic) were recruited from the greater area of Fort Collins, Colorado. After initial phone screening, participants were invited to an onsite visit to sign informed consent and confirm eligibility. Forty-eight participants were enrolled in a randomized, double-blind, parallel-arm, placebo-controlled clinical trial assessing daily blueberry consumption on vascular function and blood pressure in postmenopausal women with above-normal blood pressure, and were randomly assigned to consume either 22 g of freeze-dried highbush blueberry powder or an isocaloric placebo powder daily for 12 weeks. Thirty-two participants completed the endothelial function assessments, and of those, 17 were in the blueberry treatment group and are the participants included in this secondary analysis. Participants completed 4 study visits (each separated by 4 weeks), and each visit was conducted between the hours of 6:00 and 11:00 am, and with participants in a fasted state besides water for at least 8 h. Endothelial function and blood pressure were measured in the supine position, at room temperature, and with dimmed lighting. Participants were asked to maintain their current diet and physical activity patterns throughout the duration of the study.

Blueberry intervention

Participants (n = 17) consumed 22 g of freeze-dried highbush blueberry powder daily for 12 weeks, with each dose containing 726 mg total (poly)phenols and 224 mg total anthocyanins.[14] Treatments were packaged in opaque air-tight packets and consumed with 8 oz of water 2x/day, 11 g in the morning and 11 g in the evening 6-8 h apart to reduce possible gastrointestinal distress. The blueberry powder was provided by the US Highbush Blueberry Council and nutrient analyses were performed by Medallion Laboratories (Minneapolis, MN, USA). Overall compliance with the blueberry intervention was reported to be 97%.[14]

Endothelial function assessment

Endothelium-dependent dilation was measured via brachial artery FMD at baseline and 12 weeks.[14] Values for FMD were expressed as the change from baseline to peak diameter ([baseline diameter - peak diameter ÷ baseline diameter] x 100 = FMD %). FMD was also normalized to shear rate (SR) area under the curve (FMD/SR_{AUC}) to account for interindividual variability in shear stress (SR for each cardiac cycle = 8 x mean blood velocity ÷ diameter).

Results from the clinical trial showed that FMD/SR_{AUC} increased at 12 weeks compared to baseline ($p < 0.01$), and the change from baseline to 12 weeks was higher than placebo (+1.09e-4 unit increase for blueberry *vs* +3.82e-6 unit increased for placebo, $p < 0.03$).[14] There was a statistically non-significant, but clinically significant, increase in FMD at 12 weeks compared to baseline in the blueberry group (1.36% unit increase, $p > 0.05$).[14] Therefore, FMD and FMD/SR_{AUC} data were used to determine differences in outcomes between responders and non-responders in the current study. Responders (n = 8) were defined as having ≥ +1% increase in FMD from baseline to 12 weeks, as a +1% increase in FMD has been shown to decrease CVD risk and/or events,[14, 46, 47] and “non-responders” (n = 9) are those with < +1% change in FMD from baseline to 12 weeks.

To assess whether blueberry consumption improved endothelium-dependent dilation through reductions in oxidative stress, brachial artery FMD was also measured before and after 20 min infusion of a supraphysiological dose of the antioxidant ascorbic acid (0.06 g/kg fat-free mass).[14] Results showed that 1) postmenopausal women had oxidative stress-mediated impairments in endothelial function and 2) improvements in endothelial function after blueberry consumption were due to reductions in oxidative stress.[14]

Blood collection and biomarkers

Blood was collected from an antecubital vein and separated for plasma, which was analyzed for estrogen (screening only), lipids, glucose control, and biomarkers of cardiovascular health as previously described.[14] Triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and hemoglobin A1c (HbA1c) were measured at screening and 12 weeks. Intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), nitrate/nitrite, and plasma (poly)phenol metabolites were measured at baseline and 4, 8, and 12 weeks.[14] Results showed that 1) TG concentrations decreased in the blueberry group at 12 weeks compared to baseline (-21 mg/dL, $p < 0.02$), 2) the change from baseline to 12 weeks for HDL-C was higher in the blueberry group compared to placebo (+1 *vs* -3 mg/dL, respectively, $p < 0.05$), and 3) LDL-C was reduced at 12 weeks compared to baseline within both groups (-13 mg/dL, $p < 0.02$ for blueberry and -12 mg/dL, $p < 0.008$ for placebo).[14] Furthermore, VCAM-1 was increased in the placebo group at 4 (+548.12 ng/mL, $p < 0.004$) and 8 weeks (+495.45 ng/dL, $p < 0.008$) compared to baseline, but no differences in ICAM-1 between or within groups were found ($p > 0.05$).[14] Lastly, there were no significant differences for nitrate/nitrite (NO metabolites) between or within groups at any time point ($p > 0.05$),[14] but data were evaluated in this secondary analysis due to the important role NO has on endothelial function.

Plasma (poly)phenol metabolites

(Poly)phenol metabolites were assessed in participant's plasma at baseline, and 4, 8, and 12 weeks via UPLC-Q-q-Q MS as previously described.[14] Results showed the sum of plasma (poly)phenol metabolites in the blueberry group increased at 4 (250,053 nmol/L, $p < 0.0001$), 8 (303,053 nmol/L, $p < 0.0001$), and 12 weeks (227,971 nmol/L, $p < 0.0001$) compared to baseline (125,798 nmol/L) and placebo at each time point.[14] Furthermore, the change from baseline to 4 (+124,255 nmol/L in blueberry *vs* +37,568 nmol/L in placebo, $p < 0.02$) and 12 weeks (+102,172 nmol/L in blueberry *vs* +11,433 nmol/L in placebo, $p < 0.05$) for the sum of plasma (poly)phenol metabolites was higher than the placebo group.[14] Regarding individual metabolites, hippuric acid, 3-hydroxyhippuric acid, vanillic acid-4-sulfate, protocatechuic acid, and 2,5-dihydroxybenzoic acid were increased in plasma at 4, 8, and/or 12 weeks compared to baseline in the blueberry group (all $p < 0.05$).[14] Therefore, data for the sum of plasma (poly)phenol metabolites and the 5 individual (poly)phenol metabolites that were significantly increased were used in the current study.

Endothelial cell biopsy and protein quantification

To explore possible mechanisms in which blueberries improve endothelial function in the clinical trial, venous endothelial cells were biopsied from participants ($n = 13$ for the blueberry group) and protein expression related to NO production/bioavailability was assessed (phosphorylated eNOS (p-eNOS), NADPH oxidase/p47 subunit (NOX), manganese superoxide dismutase (MnSOD), and nuclear-factor kappa B (NFκB)) at baseline and 12 weeks as previously described.[14] No significant differences in protein expression were found in the clinical trial ($p > 0.05$);[14] however, there was an inverse correlation between the change in p47phos (NOX) from baseline to 12 weeks and the change in FMD/SR_{AUC} from baseline to 12 weeks in the blueberry group ($r = -0.61$, $p < 0.04$).[14] Furthermore, there was a positive correlation

between p-eNOS protein expression at 12 weeks and the change in FMD/SR_{AUC} from baseline to 12 weeks in the blueberry group ($r = 0.60$, $p < 0.04$).[14]

Arterial stiffness, blood pressure, fat mass, anthropometrics, and energy expenditure

Arterial stiffness (aortic pulse wave velocity and augmentation index) and fat mass were measured at baseline, while supine brachial blood pressure, weight, waist and hip circumferences (WC and HC, respectively), body mass index (BMI), and energy expenditure were measured at each time point.[14] There were no significant differences in fat mass, arterial stiffness, blood pressure, anthropometrics, or energy expenditure ($p > 0.05$) in the clinical trial.[14] However, these factors were included in the secondary analysis since they are implicated in CVD risk and may impact responses in endothelial function.

Healthy eating index (HEI)

Participants self-reported dietary intake for 3 days (2 weekdays and 1 weekend day) prior to each visit online using Automatic Self-Administered 24-h dietary assessment tool (National Institutes for Health, Bethesda, MD, USA) as previously described.[14] The HEI is a scoring system used to determine overall diet quality, dietary component quality (i.e. fruit intake), and alignment with the Dietary Guidelines for Americans (DGA).[48, 49] For the current study, HEI scores for individual diet components (e.g. whole fruits, whole grains, etc.) and overall diet were calculated (1 - 100%) for each participant across the duration of the study using a pre-determined statistical code (National Institute for Health, National Cancer Institute, Bethesda, MD, USA) using SAS v 9.4 (SAS Institute Inc., Cary, NC, USA). A higher total HEI score percentage indicates higher alignment with DGA.[48]

Statistical analyses

Missing data were imputed using within group means (responders *vs* non-responders) at that time point. Normality was checked by analyzing the histograms and using Shapiro-Wilk test. Data did not meet normality, and therefore, were log (or cube root for change from baseline) transformed prior to statistical tests, in which normality then improved. A repeated measures general linear model (mixed ANOVA) was used to evaluate differences between groups, with time and group as main effects and time*group as an interaction effect. Bonferroni pairwise comparisons was used to determine significant differences within and between groups across the time points. Mann Whitney U test was utilized to determine differences between groups at a single time point or to assess the differences in the change from baseline to 12 weeks of an outcome between groups. Spearman's test was used to examine correlations between an outcome variable at baseline/12 weeks and the change in FMD and FMD/SR_{AUC} from baseline to 12 weeks between groups. SPSS v28.0.1.0 was used for all statistical tests (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± standard error of the mean (SEM). A *p*-value of 0.05 was used to determine statistical significance and 0.10 was used to determine trending statistical significance.

Results

Endothelial function

There was an effect of time and time*group for FMD ($p = 0.035$ and $p = 0.008$, respectively) and FMD/SR_{AUC} ($p = 0.010$ and $p = 0.010$, respectively) between responders and non-responders. FMD was lower at baseline for responders compared to non-responders ($p = 0.031$), with trending differences for FMD/SR_{AUC} ($p = 0.052$) (**Figure 4.1A** and **4.1B**, and **Supplementary Table 4.1** in **Appendix 2**). At 12 weeks, FMD and FMD/SR_{AUC} were higher compared to baseline for responders ($p = 0.002$ and $p = 0.001$, respectively), while no changes were observed for non-responders ($p > 0.05$) (**Figure 4.1A** and **4.1B**, and **Supplementary**

Table 4.1 in **Appendix 2**). Furthermore, at 12 weeks, there were trending differences between groups for FMD/SR_{AUC} ($p = 0.056$), but no differences between groups for FMD ($p > 0.05$) (**Figure 4.1A** and **4.1B,** and **Supplementary Table 4.1** in **Appendix 2**). Lastly, the change in FMD and FMD/SR_{AUC} from baseline to 12 weeks was higher for responders compared to non-responders ($p < 0.001$ and $p = 0.005$, respectively) (**Figure 4.1C** and **4.1D,** and **Supplementary Table 4.1** in **Appendix 2**).

Regarding oxidative stress-mediated impairments in endothelial function at baseline, responders had higher FMD and FMD/SR_{AUC} following acute administration of a supraphysiological dose of ascorbic acid compared to saline ($p = 0.035$ and $p = 0.020$, respectively) (**Figure 4.2A** and **4.2D**, and **Supplementary Table 4.2** in **Appendix 2**), while non-responders did not ($p > 0.05$) (**Figure 4.2B** and **4.2E**, and **Supplementary Table 4.2** in **Appendix 2**). Furthermore, the differences between FMD following ascorbic acid and saline administration at baseline were higher for responders compared to non-responders ($p = 0.023$) (**Figure 4.2C** and **Supplementary Table 4.2** in **Appendix 2**), while no statistically significant differences were observed for FMD/SR_{AUC} between groups at baseline (**Figure 4.2F** and **Supplementary Table 4.2** in **Appendix 2**). However, the difference in FMD/SR_{AUC} (but not FMD, $p > 0.05$) following ascorbic acid *vs* saline administration was lower at 12 weeks compared to baseline for responders ($p = 0.026$), while there was no significant change for non-responders ($p > 0.05$) (**Figure 4.2C and 4.2F** and **Supplementary Table 4.2** in **Appendix 2**).

Screening characteristics

Screening characteristics between responders and non-responders are reported in **Table 4.1**. Estradiol was higher ($p = 0.002$), HDL-C was lower ($p = 0.008$), and LDL-C was higher ($p = 0.027$) for responders than non-responders. No statistically significant differences were noted for other characteristics ($p > 0.05$).

Spearman's correlations for screening characteristics with the change in FMD and FMD/SR_{AUC} from baseline to 12 weeks are presented in **Figure 4.3**. Age had a strong positive correlation with the change in FMD from baseline to 12 weeks for responders ($p = 0.043$) (**Figure 4.3A**). For non-responders, years postmenopausal had a strong positive correlation with the change in FMD ($p = 0.014$), and diastolic blood pressure had a strong negative correlation with the change in FMD ($p = 0.003$) (**Figure 4.3A**). Estradiol was strongly positively correlated with the change in FMD from baseline to 12 weeks for responders ($p = 0.002$), but not for non-responders or for FMD/SR_{AUC} for responders or non-responders (**Figure 4.3A-E**). Besides a trend for a positive moderate correlation between years postmenopausal and the change in FMD/SR_{AUC} from baseline to 12 weeks ($p = 0.097$) for responders, there were no other statistically significant correlations between screening variables and the change in FMD/SR_{AUC} from baseline to 12 weeks for responders or non-responders (**Figure 4.3A**).

Blood biomarkers

There was an effect of time ($p = 0.002$) for TG, with lower TG at 12 weeks compared to baseline for non-responders ($p = 0.008$) and a strong trend for lower TG at 12 weeks compared to baseline for responders ($p = 0.051$) (**Figure 4.4A** and **Supplementary Table 4.3** in **Appendix 2**). An effect of time was also found for TC ($p < 0.001$), where TC at 12 weeks was lower in both responders ($p < 0.001$) and non-responders ($p = 0.002$) compared to baseline (**Figure 4.4B** and **Supplementary Table 4.3** in **Appendix 2**). There was an effect of group for HDL-C ($p = 0.021$), with lower HDL-C at baseline for responders compared to non-responders ($p = 0.011$) and a trend for lower HDL-C at 12 weeks for responders compared non-responders ($p = 0.087$) (**Figure 4.4C** and **Supplementary Table 4.3** in **Appendix 2**). For LDL-C, there was an effect of time ($p = 0.016$) and a trending effect of group*time ($p = 0.094$) (**Supplementary Table 4.3** in **Appendix 2**). Responders had lower LDL-C at 12 weeks compared to baseline (*p*

= 0.007) and higher LDL-C at baseline compared to non-responders (p = 0.032) (**Figure 4.4D** and **Supplementary Table 4.3** in **Appendix 2**).

There were no within or between group differences in VCAM-1 at any time point (**Figure 4.5A** and **Supplementary Table 4.3** in **Appendix 2**); however, there was an effect of group*time for ICAM-1 (p = 0.015) which was higher at baseline (p = 0.010), lower at 8 weeks (p = 0.019, and a trend for higher levels of at 12 weeks (p = 0.094) in responders compared to non-responders, and concentrations were lower for responders at 8 weeks than baseline (p = 0.049) (**Figure 4.5B** and **Supplementary Table 4.3** in **Appendix 2**). Lastly for ICAM-1, there was an effect of group (p = 0.008) and a trending effect of time (p = 0.079) for the change from baseline to 4, 8, and 12 weeks, such that the change from baseline to 4 (p = 0.046) and 8 weeks (p = 0.001) was lower for responders than non-responders, and the change from baseline to 12 weeks was lower than the change from baseline to 8 weeks for responders (p = 0.034) (**Supplementary Table 4.3** in **Appendix 2**). Regarding nitrate/nitrite, there was an effect of group (p = 0.025), where concentrations were lower at 4 and 12 weeks for responders than non-responders (p = 0.048 and p = 0.028, respectively) (**Figure 4.5C** and **Supplementary Table 4.3** in **Appendix 2**).

Spearman's correlations for blood biomarkers with FMD and FMD/SR_{AUC} are presented in **Supplementary Figure 4.1** in **Appendix 2**. There were strong positive correlations for TG and HbA1c at 12 weeks between the change in FMD/SR_{AUC} from baseline to 12 weeks for non-responders (p = 0.036 and p = 0.026, respectively). The change from baseline to 12 weeks in HbA1c was strongly positively correlated to the change from baseline to 12 weeks in FMD/SR_{AUC} for non-responders (p = 0.008). At 12 weeks, VCAM-1 was positively strongly correlated with the change from baseline to 12 weeks in FMD for responders (p = 0.002), while a trend for a negative moderate correlation was noted for non-responders (p = 0.099). At 12 weeks, there was also a trend for a positive moderate correlation between ICAM-1 and the change in FMD/SR_{AUC} from baseline to 12 weeks for responders (p = 0.062).

Plasma (poly)phenol metabolites

There was an effect of time for the sum of plasma (poly)phenol metabolites ($p < 0.001$) where the sum of plasma (poly)phenol metabolites was higher at 4 and 12 weeks compared to baseline for responders ($p = 0.002$ and $p = 0.036$, respectively), and higher at 4, 8, and 12 weeks compared to baseline for non-responders ($p < 0.001$, $p = 0.006$, and $p = 0.005$, respectively) (**Figure 4.6A** and **Supplementary Table 4.4** in **Appendix 2**). There was a trend for a lower sum of plasma (poly)phenol metabolites at 8 weeks for responders compared to non-responders ($p = 0.092$) (**Figure 4.6A** and **Supplementary Table 4.4** in **Appendix 2**). Lastly, there was an effect of time ($p = 0.007$) for the changes from baseline to 4, 8, and 12 weeks in the sum of (poly)phenol metabolites where there was a trend for a lower change from baseline to 12 weeks for the sum of (poly)phenol metabolites compared to the change from baseline to 4 weeks for responders ($p = 0.062$) (**Supplementary Table 4.4** in **Appendix 2**).

Regarding the 5 individual plasma (poly)phenol metabolites that were evaluated, there was an effect of time for all metabolites including hippuric acid ($p < 0.001$), 3-hydroxyhippuric acid ($p = 0.007$), vanillic acid-4-sulfate ($p = 0.015$), protocatechuic acid ($p = 0.049$), and 2,5-dihydroxybenzoic acid ($p = 0.048$), while an effect of time*group for protocatechuic acid was also noted ($p = 0.049$) (**Supplementary Table 4.4** in **Appendix 2**). Hippuric acid was higher at 4 ($p = 0.007$), 8 ($p = 0.007$), and 12 weeks ($p = 0.015$) compared to baseline for non-responders, with a trend for higher concentrations at 4 weeks compared to baseline for responders ($p = 0.090$) (**Figure 4.6B** and **Supplementary Table 4.4** in **Appendix 2**). Responders had higher levels of 3-hydroxyhippuric acid at 4 weeks compared to non-responders ($p = 0.031$) and a trend for higher levels at 4 weeks compared to baseline ($p = 0.071$), while non-responders had higher levels at 8 weeks compared to baseline ($p = 0.047$) and a trend for higher levels at 4 and 12 weeks compared to baseline ($p = 0.054$ and $p = 0.090$, respectively) (**Figure 4.6C** and **Supplementary Table 4.4** in **Appendix 2**). For vanillic acid-4-sulfate at 4 weeks, responders had a trend for higher concentrations compared baseline ($p =$

0.073) and to non-responders (p = 0.079) (**Figure 4.6D** and **Supplementary Table 4.4** in **Appendix 2**). At 8 weeks, there was a trend for higher concentrations of protocatechuic acid for non-responders compared to responders (p = 0.060), but higher compared to baseline for non-responders (p = 0.010) (**Figure 4.6E** and **Supplementary Table 4.4** in **Appendix 2**). Lastly, there was a trend for higher concentrations of 2,5-dihydroxybenzoic acid at 8 weeks for non-responders compared to responders (p = 0.068). (**Figure 4.6F** and **Supplementary Table 4.4** in **Appendix 2**).

When assessing the change of individual metabolites from baseline to 4-, 8-, and 12-week time points (data presented in **Supplementary Table 4.4** in **Appendix 2**), an effect of time for hippuric acid (p = 0.021) observed, though pairwise comparisons showed no significant differences between or within groups. There was an effect of time*group for changes in 3-hydroxyhippuric acid (p = 0.008), with the change from baseline to 4 weeks being greater than the change from baseline to 8 weeks for responders (p = 0.023). There was a trend for an effect of time*group for changes in vanillic acid-4-sulfate (p = 0.083) and an effect of time*group for changes in 2,5-dihydroxybenzoic acid (p = 0.037), though pairwise comparisons showed no significant differences between or within groups. Lastly, there was a trend for an effect of group for protocatechuic acid (p = 0.082), where the change from baseline to 4 weeks was higher for non-responders compared to responders (p = 0.039).

Spearman's correlations for the sum of plasma (poly)phenol metabolites and individual (poly)phenol metabolites with FMD and FMD/SR_{AUC} are presented in **Table 4.2**. The change in the sum of plasma (poly)phenol metabolites from baseline to 12 weeks was positively strongly correlated with the change in FMD from baseline to 12 weeks for responders (p = 0.047) and negatively moderately correlated to the change in FMD/SR_{AUC} from baseline to 12 weeks for non-responders (p = 0.042). Regarding individual metabolites in plasma, 3-hydroxyhippuric acid was inversely strongly correlated at baseline (p = 0.020) and 12 weeks (p = 0.004) to the change in FMD from baseline to 12 weeks for non-responders, while there was a trend for an

inverse moderate correlation between the change in 3-hydroxyhippuric acid from baseline to 12 weeks to the change in FMD from baseline to 12 weeks for non-responders (p = 0.058). Furthermore, there was an inverse strong correlation between the change in 3-hydroxyhippuric acid from baseline to 12 weeks and the change in FMD/SR_{AUC} from baseline to 12 weeks for non-responders (p = 0.036). There was also a trend for an inverse moderate correlation between protocatechuic acid at 12 weeks and the change in FMD from baseline to 12 weeks for responders (p = 0.086). Lastly, the change in 2,5-dihydroxybenzoic acid from baseline to 12 weeks was positively strongly correlated to the change in FMD from baseline to 12 weeks for responders (p = 0.021), and inversely correlated with the change in FMD/SR_{AUC} for non-responders (p = 0.036).

Endothelial cell protein expression

No differences in endothelial cell protein expression were observed between responders and non-responders at baseline (**Figure 4.7** and **Supplementary Table 4.5** in **Appendix 2**). At 12 weeks, there was an effect of group for p-eNOS protein expression (p = 0.019), with responders having higher p-eNOS protein expression at 12 weeks compared to non-responders (p = 0.014) (**Figure 4.7B** and **Supplementary Table 4.5** in **Appendix 2**). Furthermore, there was a trending effect of group for p-NFκB (p = 0.075), however, there were no significant pairwise comparisons (**Supplementary Table 4.5** in **Appendix 2**).

Spearman's correlations for endothelial cell protein expression are presented in **Table 4.3**. Baseline p-eNOS protein expression was strongly inversely correlated with the change in FMD from baseline to 12 weeks for responders (p = 0.004). Baseline p-NFκB protein expression was strongly positively correlated to the change in FMD/SR_{AUC} from baseline to 12 weeks for responders (p = 0.028). Lastly, baseline p47phox (NOX) protein expression was strongly positively correlated with the change in FMD/SR_{AUC} from baseline to 12 weeks for non-responders (p = 0.037).

Arterial stiffness, blood pressure, fat mass, anthropometrics, and energy expenditure

Arterial stiffness, blood pressure, fat mass, anthropometric, and energy expenditure characteristics between responders and non-responders are presented in **Table 4.4**. There was a trending effect of group for diastolic blood pressure ($p = 0.051$), with diastolic blood pressure higher for responders compared to non-responders at 12 weeks ($p = 0.013$). There were no main effects for fat mass ($p > 0.05$), though pairwise comparisons indicated a trend at 12 weeks for responders to have a higher fat mass than non-responders ($p = 0.078$). No differences were observed for arterial stiffness, anthropometrics, or energy expenditure ($p > 0.05$).

Spearman's correlations for arterial stiffness, blood pressure, fat mass, anthropometric, and energy expenditure with the change in FMD and FMD/SR_{AUC} from baseline to 12 weeks are presented in **Supplementary Figure 4.2** in **Appendix 2.** For non-responders, there was a trend for a moderate correlation between augmentation index normalized to 75 beats per minute at 12 weeks and the change in FMD from baseline to 12 weeks ($p = 0.061$), and at baseline with the change in FMD/SR_{AUC} from baseline to 12 weeks ($p = 0.067$). At baseline and 12 weeks, diastolic blood pressure was strongly inversely correlated with the change in FMD from baseline to 12 weeks for non-responders ($p = 0.015$ and $p = 0.004$, respectively), and baseline diastolic blood pressure was strongly inversely correlated with the change in FMD/SR_{AUC} from baseline to 12 weeks for non-responders ($p < 0.001$). Furthermore, a strong positive correlation was found between the change in diastolic blood pressure from baseline to 12 weeks and the change in FMD/SR_{AUC} from baseline to 12 weeks for non-responders ($p = 0.003$). There was a trend for a moderately strong inverse correlation between WC:HC at 12 weeks and the change in FMD/SR_{AUC} from baseline to 12 weeks for responders ($p = 0.058$). Lastly, there was a trend for a moderate correlation between energy expenditure at baseline and the change in FMD from baseline to 12 weeks ($p = 0.099$), but a strong positive correlation between the change in energy expenditure from baseline to 12 weeks and the change in FMD from baseline to 12 weeks for non-responders ($p = 0.016$).

HEI

HEI scores are presented in **Table 4.5**. There were no significant differences in the HEI individual and total scores between responders and non-responders.

Spearman's correlations for the HEI with FMD and FMD/SR_{AUC} are presented in **Figure 4.8**. For the change in FMD from baseline to 12 weeks for responders, there were inverse strong correlations with HEI scores for fatty acids (p = 0.007), saturated fats (p = 0.050), and total HEI score (p = 0.037) and a trend for an inverse strong correlation with the total protein foods score (p = 0.062). For the change in FMD from baseline to 12 weeks for non-responders, there were inverse strong correlations with HEI scores of total fruit (p = 0.021), whole fruit (p = 0.041), sodium (p = 0.042), and total HEI (p = 0.013), and a trend for inverse moderate correlations with scores of total vegetables (p = 0.061) and green and beans (p = 0.059). For the change in FMD/SR_{AUC} from baseline to 12 weeks, there were inverse strong correlations with total vegetables score for responders (p = 0.048) and fatty acids score for non-responders (p = 0.031).

Discussion

We previously demonstrated in a randomized, double-blind, placebo-controlled clinical trial that postmenopausal women with above-normal blood pressure who consumed 22 g of freeze-dried highbush blueberry powder daily for 12 weeks had improvements in endothelial function that were mediated by reductions in oxidative stress.[14] We also observed that individuals responded differently in terms of the direction and magnitude of the change.[14] Results from the current study show that the blueberry intervention was ~50% effective at improving FMD to clinically significant levels (i.e. ≥ 1% increase in FMD from baseline to 12 weeks). One possible reason for differences in efficacy is the degree of endothelial dysfunction present at baseline, as responders had more endothelial dysfunction compared to non-responders (1.80% FMD for responders *vs* 5.56% for non-responders). Even though there are

currently no standard reference ranges for normal endothelial function or endothelial dysfunction measured via FMD, it has been suggested that for a generally healthy population, 'optimal' endothelial function is having a FMD score between 6.5% and 8.8%, impaired function from 3.8% to 6.1%, and < 3.8% is considered pathological.[50] Based on these proposed standards, endothelial function at baseline for responders in our study was in the pathological range while non-responders were in the upper limits for impaired endothelial function. This hypothesis that those with worse baseline endothelial function responded better to blueberry intervention is supported by previous work done in postmenopausal women supplemented with the (poly)phenol resveratrol where participants with worse bone health at baseline had better improvements in bone after the (poly)phenol intervention.[51] Furthermore, it was shown in a meta-analysis that (poly)phenol-based interventions improved FMD to a greater extent in participants with chronic disease compared to healthy individuals.[44] Future studies are needed to understand efficacy of blueberries to improve endothelial function and reduce CVD risk across populations with different degrees of endothelial (dys)function.

One plausible explanation for why responders had greater endothelial dysfunction at baseline could be due to having more vascular oxidative stress. Infusion of a supraphysiological dose of the antioxidant ascorbic acid (i.e. vitamin C) acutely significantly improved responders' FMD and FMD/SR_{AUC} at baseline to the 'optimal' endothelial function range of 6.93% compared to 1.80%. In non-responders, ascorbic acid infusion only acutely improved FMD from 5.65% (saline FMD) to 6.84% at baseline and was not statistically significant. This suggests that non-responders had less oxidative stress-mediated impairments in endothelial function compared to responders. The mechanisms contributing to greater baseline vascular oxidative stress in responders is currently unclear as there were no significant differences between groups at baseline in biopsied venous endothelial enzyme expression that relate to oxidative stress (i.e. eNOS, MnSOD, or NOX), and other mechanisms or indicators of oxidative stress were not measured. Interestingly, endothelial baseline p-eNOS expression was inversely correlated with

the change in FMD from baseline to 12 weeks in responders. This could indicate that less expression of p-eNOS at baseline was associated with greater change in FMD after treatment, which may be related to oxidative stress. Furthermore, HDL-C was significantly lower and LDL-C was significantly higher in responders compared to non-responders at the screening visit. Even though not a direct mechanism for endothelial dysfunction, abnormal blood lipids have been associated with increased production of ROS in the endothelium and impaired endothelial function.[52] Lastly, baseline ICAM-1, a response to inflammatory stimuli,[53] was significantly higher for responders compared to non-responders, while p-NFκB was positively correlated with the change in FMD from baseline to 12 weeks for responders. This indicates that responders might have had more inflammation at baseline, thus, could be a contributing factor for increased oxidative stress since inflammation is known to provoke oxidative stress. Collectively, these findings indicate that responders had more oxidative stress-mediated suppression of endothelial function at baseline, and suggest that baseline factors related to dyslipidemia and inflammation may be contributing factors. Additionally, these findings suggest that those with greater impairments in cardiometabolic health may have greater improvements in endothelial function and vascular oxidative stress with blueberry consumption, but does not exclude that blueberries may exert other health benefits in non-responders.

Regarding endothelial function response to the blueberry intervention, responders significantly improved their FMD by 4.26% after consuming 22 g of freeze-dried highbush blueberry powder for 12 weeks, while non-responders had no change. FMD improvements in responders were due to reductions in vascular oxidative stress as evidenced by FMD and FMD/SR_{AUC} significantly increasing after infusion of a supraphysiologic dose of ascorbic acid at baseline but not at 12 weeks, as well as the fact that the difference between ascorbic acid and saline FMD/SR_{AUC} was significantly reduced at 12 weeks compared to baseline for responders (but not for non-responders). Reductions in vascular oxidative stress at 12 weeks could have contributed to higher p-eNOS expression at 12 weeks in responders compared to non-

responders. This is supported by *ex vivo* research showing that physiological levels of blueberry (poly)phenol metabolites reduced endothelial oxidative stress while increasing eNOS activity.[54-56] Furthermore, there were differential responses in plasma NO metabolites (i.e. nitrate/nitrite) concentrations between responders and non-responders throughout the 12-week blueberry intervention, such that responders had lower concentrations. Measuring NO intermediates (i.e. nitrate and nitrite) can provide insight into plasma NO concentrations, but NO is known to have a short half-life and interacts with superoxide radicals rapidly *in vivo* promoting nitrate formation (Thakor 2010).[57] Interestingly, plasma nitrite was below the threshold of detection in the clinical trial,[14] therefore, such concentrations are more reflective of plasma nitrate. It is possible that reductions in nitrate concentrations in responders compared to non-responders is due to reductions in oxidative stress. This finding is supported by a previous study that when ascorbic acid was infused into blood circulation, it lowered plasma nitrate levels.[57] Furthermore, severely obese children with traditional metabolic risk factors had significantly elevated plasma nitrate concentrations compared to non-obese children and levels were significantly positively correlated to biomarkers of inflammation and oxidative stress.[58] Additionally, studies with animal models of obesity and metabolic syndrome have demonstrated that NO concentrations and endothelium-dependent vasodilator responses are higher compared to those that are healthy, likely due to a compensatory adaptation to preserve blood flow in the presence of oxidative stress and inflammation.[59-61] Collectively, the findings from this study suggests that responders to blueberry consumption improved their endothelial function through reductions in oxidative stress which could be mechanistically related to higher p-eNOS expression and/or reductions in plasma nitrate concentrations but requires further investigation.

Another factor contributing to efficacy of blueberries to improve endothelial function in responders is that responders had higher estradiol concentrations at screening compared to non-responders. In fact, estradiol was positively correlated with the change in FMD from baseline to 12 weeks for responders but not for non-responders, or for FMD/SR_{AUC}. This is

supported by data from studies with exercise interventions indicating efficacy was dependent on estrogen replacement in postmenopausal women.[62, 63] Participants in our study were confirmed to be postmenopausal through assessment of estradiol and follicle-stimulating hormone and there were no differences between groups for age or years postmenopausal, and thus, it is unclear as to why responders had higher estradiol concentrations. In postmenopausal women, estrogen can be produced by adipose tissue;[64] however, there were no significant differences in fat mass or BMI between groups at baseline. Despite this, responders did have non-significantly but numerically higher BMI, fat mass, waist circumference, and hip circumference, indicating that subtle differences in body composition could be a contributing factor. Furthermore, the gut microbiome is implicated in the enterohepatic recirculation of estrogens as conjugated estrogens are either excreted in feces, or they are deconjugated by gut microbiota with β-glucosidase or sulfatase enzymes which allows them to re-enter circulation.[65] Thus, the gut microbiome may also be a contributing factor for increased estradiol but requires investigation.

The sum of plasma (poly)phenol metabolites significantly increased in both groups from baseline to 12 weeks confirming compliance with blueberry intervention. This finding is supported by a recent study demonstrating changes in plasma (poly)phenol metabolites predicted whether participants were in the placebo or blueberry intervention using machine learning algorithms.[39] Interestingly, though (poly)phenolic concentrations were not different between responders and non-responders at 12 weeks with the exception for a trend in differences for 3-hydroxyhippuric acid between groups, several trending significant differences between groups were observed for (poly)phenol metabolites at other time points (i.e. 4 or 8 weeks), such that they were higher in non-responders than responders (besides vanillic acid-4-sulfate being lower for non-responders than responders). It cannot be determined at this time how these findings translate to effects on endothelial function at earlier time points than 12 weeks as FMD was not measured. However, we hypothesize that (poly)phenols might be taken up by endothelial cells and/or other cells and tissues at a faster rate in responders compared to

non-responders due to responders having more vascular oxidative stress. Cell studies have demonstrated that flavonoids (i.e. flavanones and isoflavones) are taken up and metabolized by endothelial cells.[66, 67] Whether increased oxidative stress, uptake by endothelial cells (or other cells and tissues), or other unknown factors are responsible for lower plasma (poly)phenol concentrations in responders than non-responders in our study is not known. Furthermore, it remains unclear as to how circulating (poly)phenol metabolites are involved in improvements in endothelial function observed, and thus, future studies should aim to understand this relationship.

Based on the results from the clinical trial, it was not surprising to find that arterial stiffness, blood pressure, anthropometrics, fat mass, or energy expenditure did not contribute to improvements in FMD for responders. Furthermore, it is currently unclear what the negative correlations between individual diet component HEI scores and the change in FMD and FMD/SR_{AUC} from baseline to 12 weeks indicates. However, regardless of group, the total HEI score is significantly inversely correlated to the change in endothelial function from baseline to 12 weeks, suggesting that a lower quality dietary pattern that is less aligned with the DGA might be associated with higher efficacy of blueberries to improve endothelial function in our study population. This is logical, as it has been shown that diets high refined carbohydrates and saturated fats (i.e. Westernized diet) contribute to more oxidative stress and endothelial dysfunction in humans.[68, 69] It could be interesting for future studies examining blueberry intervention on cardiovascular health to assess how HEI impacts the efficacy of blueberries as a dietary intervention in high-risk populations.

To our knowledge, this is the first study to examine differences in efficacy of blueberries to improve endothelial function highlighting the novelty and strength of the study. This study was exploratory, but provides crucial insight into factors that may determine the clinical efficacy of blueberries as a food-based dietary intervention for postmenopausal women with above-normal blood pressure. Furthermore, data used in the current study are from a well-controlled and

robustly designed clinical trial using the gold standard of FMD to evaluate endothelial function, as well as a supraphysiologic dose of ascorbic acid to assess oxidative stress-mediated suppression of endothelial function and venous endothelial cell biopsy to assess possible mechanisms. Results produced from this study have the potential to provide insight for inclusion/exclusion criteria and study design for future studies assessing (poly)phenol or blueberry intervention(s) on endothelial function in postmenopausal women. Limitations to this study include small sample size, as only 17 participants from the clinical trial in the blueberry group completed endothelial function measurements, therefore, significant differences in various outcomes between groups (e.g. age, anthropometric/body composition measures) could have gone undetected. Another limitation is that the postmenopausal women included in these analyses were all non-Hispanic white and from Northern Colorado, therefore, generalizability is limited. Lastly, some of the findings in this study were based on correlations and correlation does not necessarily mean causation. Therefore, future studies could account for assessing interindividual variability within groups in their study design and power analyses in order to detect significant differences in clinical responses within an intervention group.

Conclusions

Collectively, results from this study suggest that responders had greater impairments in overall cardiometabolic health compared to non-responders. Furthermore, baseline factors such as endothelial function, oxidative stress-mediated suppression of endothelial function, among other factors like circulating estradiol, may contribute to increased efficacy of blueberries to improve endothelial function. Lastly, plasma nitrate concentrations and p-eNOS expression provide insight into potential mechanisms for endothelial function responses and should be explored in future studies. More research is needed to better understand the interindividual variability in endothelial function responses to chronic blueberry consumption in postmenopausal women with above-normal blood pressure, as well as in other populations.

Figures and Tables

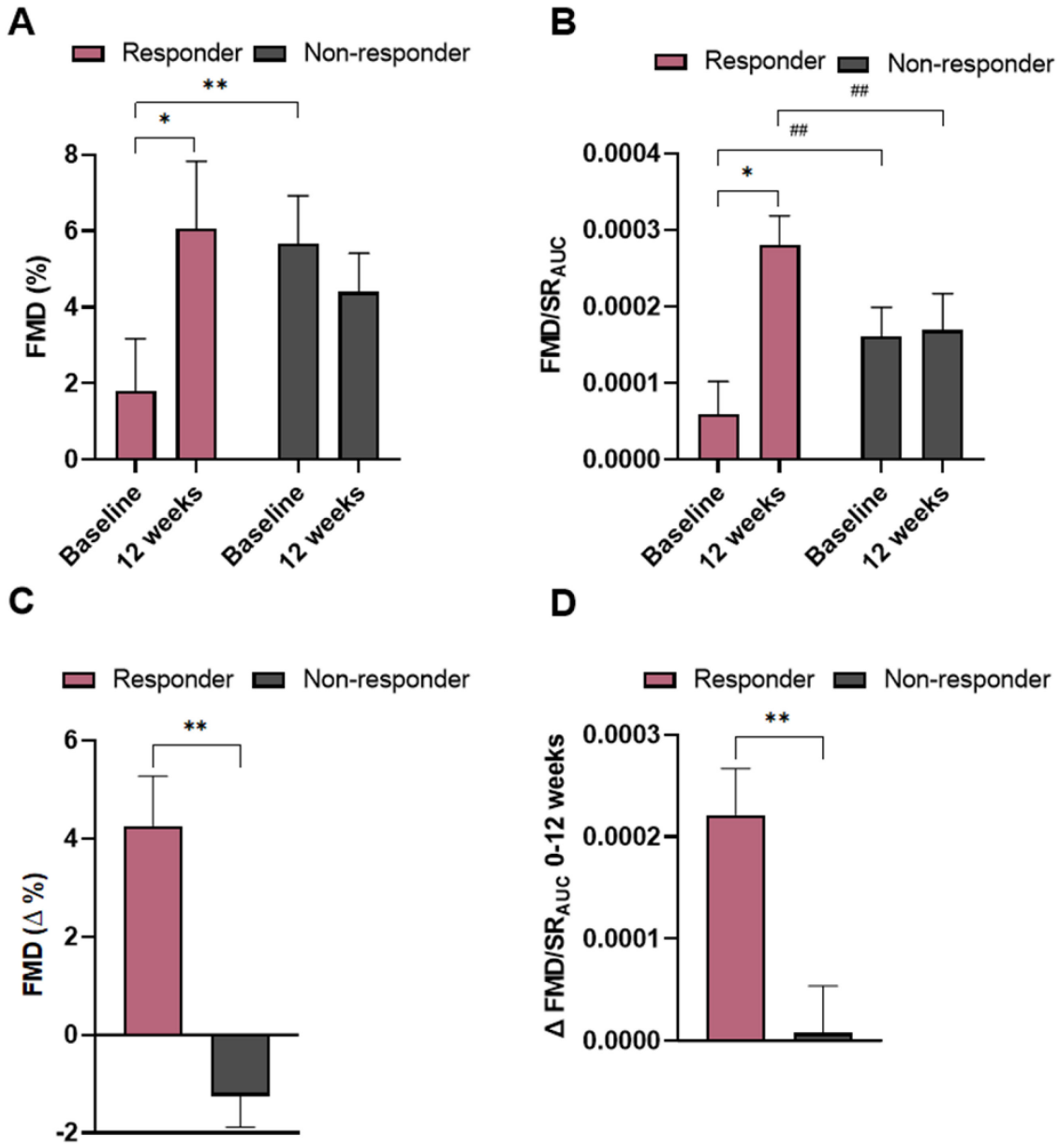


Figure 4.1. (A) Flow-mediated dilation (FMD) between responders (n = 8) and non-responders (n = 9) at baseline and 12 weeks; (B) FMD normalized to shear rate area under the curve (FMD/SR_{AUC}) between responders and non-responders at baseline and 12 weeks; (C) change in FMD (%) from baseline to 12 weeks between responders and non-responders; and (D) change in FMD/SR_{AUC} between responders and non-responders. Data are mean ± SEM. *Different within group compared to baseline ($p < 0.05$). **Different between groups at that time point ($p < 0.05$). ##Trending different between groups at that time point ($p < 0.06$).

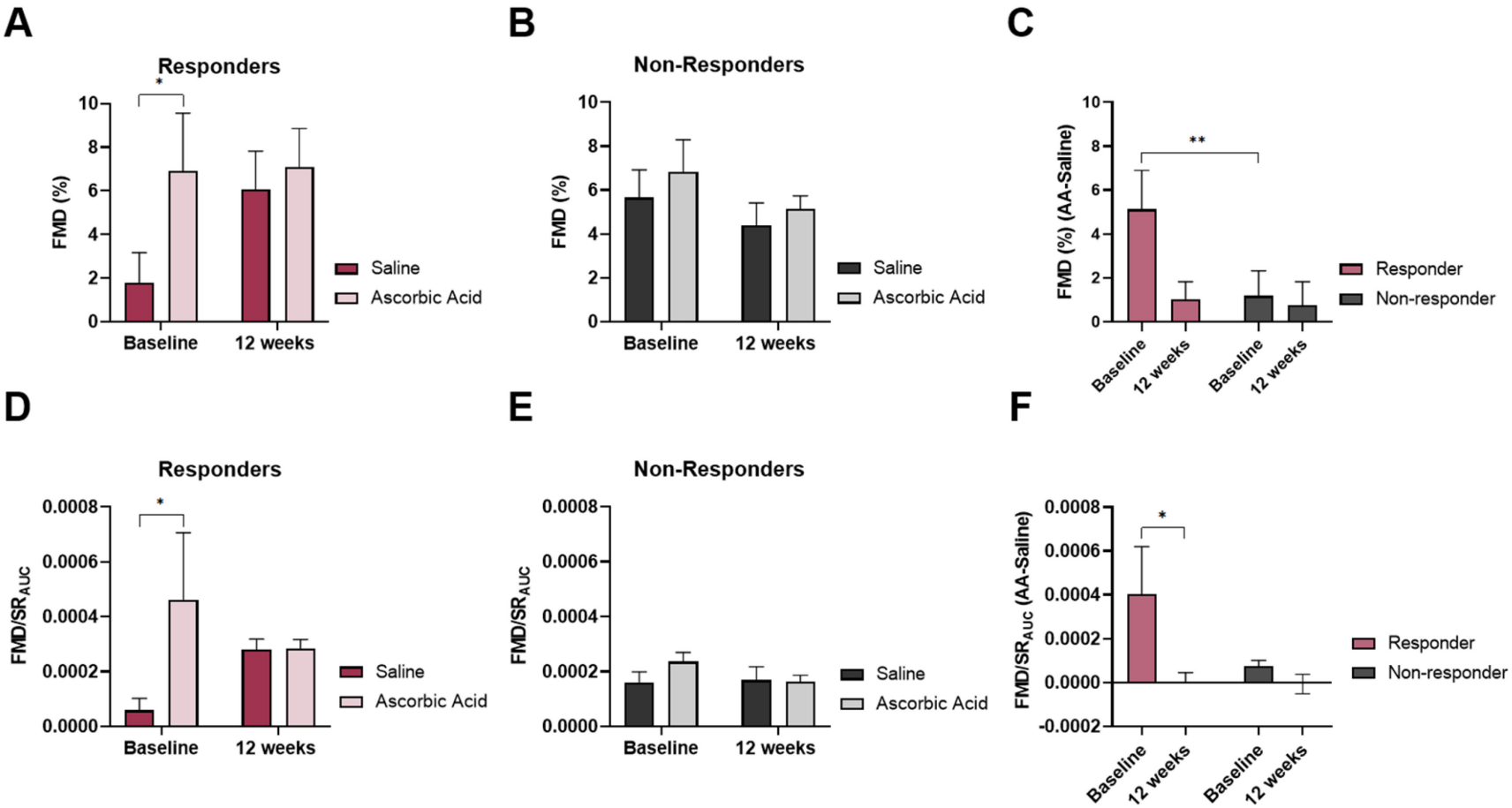


Figure 4.2. (A) Flow-mediated dilation (FMD) between saline and ascorbic acid for responders (n = 8) and (B) non-responders (n = 9) at baseline and 12 weeks; (C) difference in saline and ascorbic acid for FMD between responders and non-responders at baseline and 12 weeks; (D) FMD normalized to shear rate area under the curve (FMD/SR_{AUC}) between saline and ascorbic acid for responders and (E) non-responders at baseline and 12 weeks; and (F) difference in saline and ascorbic acid for FMD/SR_{AUC} between responders and non-responders at baseline and 12 weeks. Data are mean ± SEM. *Different within group compared to baseline ($p < 0.05$). **Different between groups at that time point ($p < 0.05$).

Table 4.1. Screening characteristics for the 17 postmenopausal women who consumed 22 g freeze-dried highbush blueberry powder for 12 weeks and completed the endothelial function measurements.

Variable	Responder (*n* = 8)	Non-responder (*n* = 9)
Age (yr)	61 ± 1	60 ± 1
Years after menopause	11 ± 3	8 ± 2
Estradiol (pg/mL)	19.6 ± 0.9*	14.4 ± 0.9
FSH (mIU/mL)	68.8 ± 7.1	69.4 ± 9.5
Height (m)	1.6 ± 0.0	1.6 ± 0.0
Weight (kg)	76.6 ± 5.6	70.2 ± 5.0
BMI (kg/m^2)	29.2 ± 1.8	26.0 ± 1.8
Fat mass (%)	42.0 ± 1.7	39.6 ± 1.8
WC (cm)	91 ± 3	86 ± 5
HC (cm)	109 ± 4	105 ± 4
WC:HC	0.84 ± 0.02	0.81 ± 0.02
SBP (mmHg)	131 ± 2	129 ± 3
DBP (mmHg)	79 ± 1	81 ± 2
TG (mg/dL)	123 ± 15	122 ± 24
TC (mg/dL)	217 ± 9	213 ± 6
HDL-C (mg/dL)	48 ± 3*	66 ± 6
LDL-C (mg/dL)	144 ± 7*	121 ± 6
HbA1c (%)	5.6 ± 0.1	5.5 ± 0.1
BP med use, n (%)	3 (37.5)	1 (11.1)
Race/ethnicity, n (%)		
Non-Hispanic white	8 (100)	9 (100)

Data are mean ± SEM (or n (%)). *Different between groups ($p < 0.05$). Abbreviations: BMI, body mass index; BP, blood pressure; DBP, diastolic blood pressure; FSH, follicle-stimulating hormone; HgbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein-cholesterol; HC, hip circumference; LDL-C, low-density lipoprotein-cholesterol; med, medication; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; WC, waist circumference.

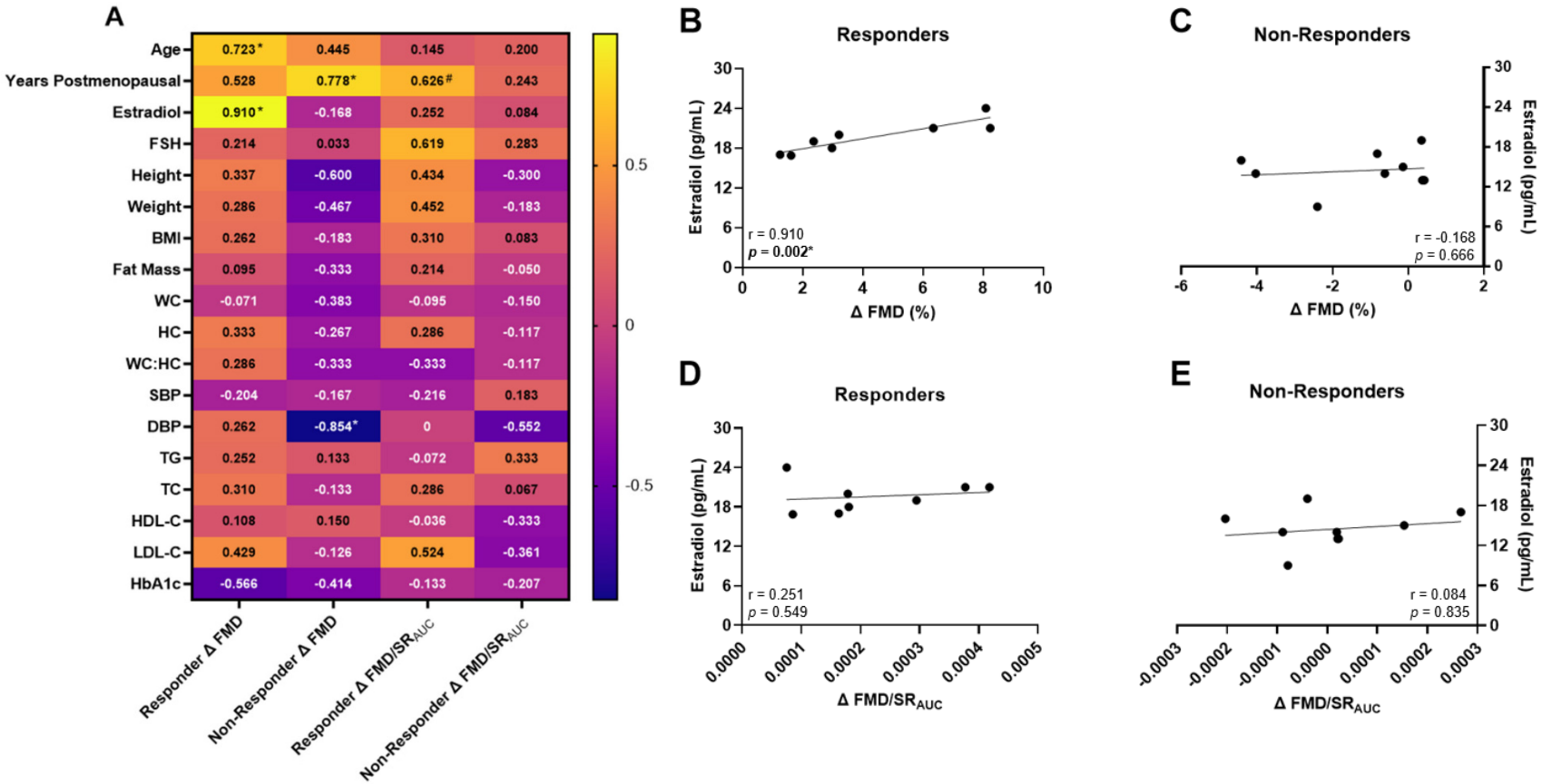


Figure 4.3. (A) Correlations between screening characteristics and the change in flow-mediated dilation (FMD) and the change in FMD normalized to shear rate area under the curve (FMD/SR_{AUC}) from baseline to 12 weeks; (B) correlation between estradiol and the change in FMD from baseline to 12 weeks for responders (*n* = 8) and (C) non-responders *(n* = 9*);* and (D) correlation between estradiol and the change in FMD normalized to shear rate area under the curve (FMD/SR_{AUC} from bassline to 12 weeks for responders and (E) non-responders. Data are Spearman's rho. *Significant correlation ($p < 0.05$). #Trending significant correlation ($p < 0.10$). Abbreviations: BMI, body mass index; BP, blood pressure; DBP, diastolic blood pressure; FSH, follicle-stimulating hormone; HgbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein-cholesterol; HC, hip circumference; LDL-C, low-density lipoprotein-cholesterol; med, medication; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; WC, waist circumference.

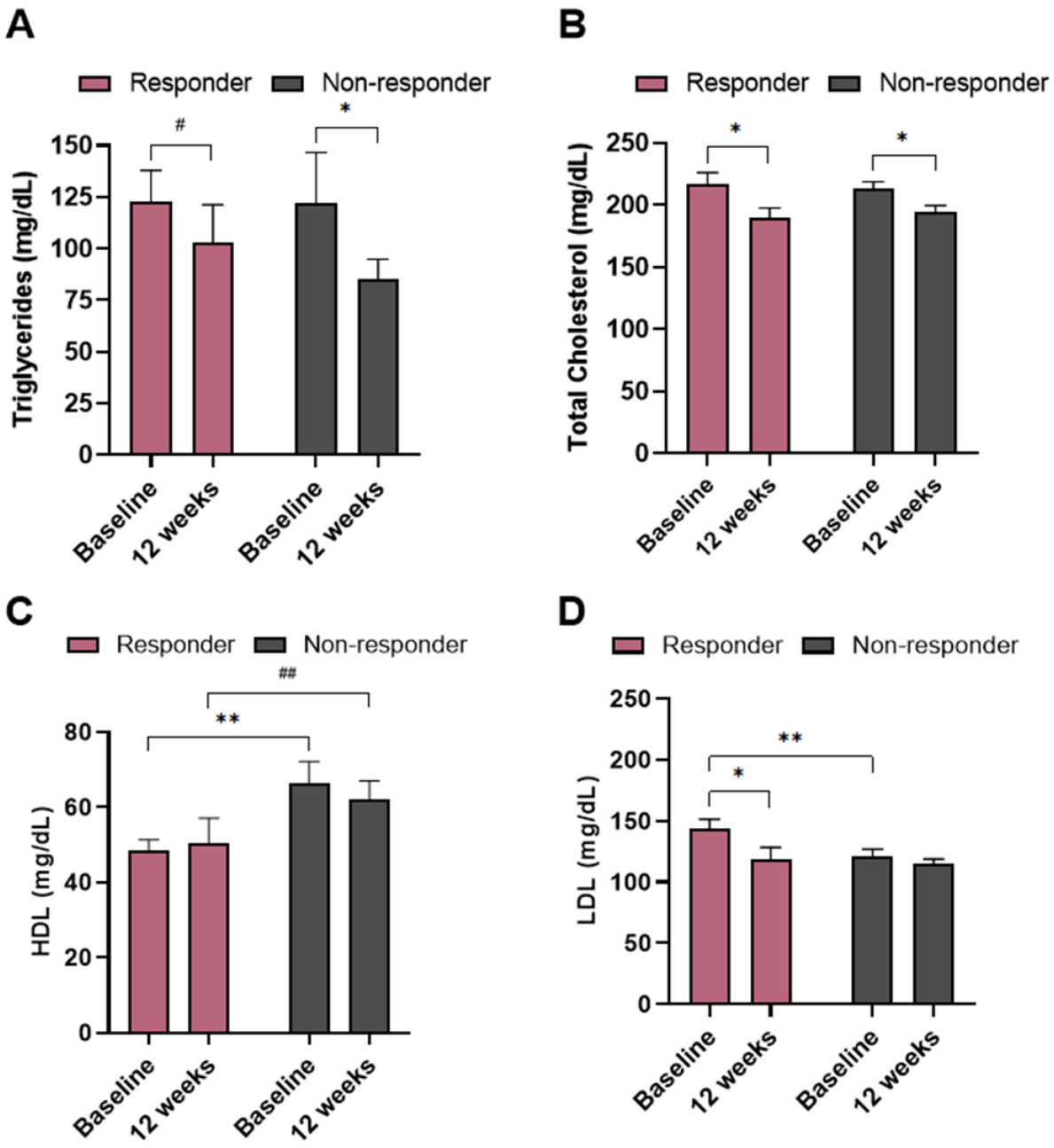


Figure 4.4. (A) Triglyceride (TG) levels for responders (n = 8) and non-responders (n = 9) at baseline and 12 weeks; (B) total cholesterol (TC) levels for responders and non-responders at baseline and 12 weeks; (C) high-density lipoprotein cholesterol (HDL-C) levels for responders and non-responders at baseline and 12 weeks; and (D) low-density lipoprotein cholesterol (LDL-C) levels for responders and non-responders at baseline and 12 weeks. Data are mean ± SEM. *Different within group ($p < 0.05$).**Different between groups at that time point ($p < 0.05$). ##Trending different between groups at that time point ($p < 0.09$).

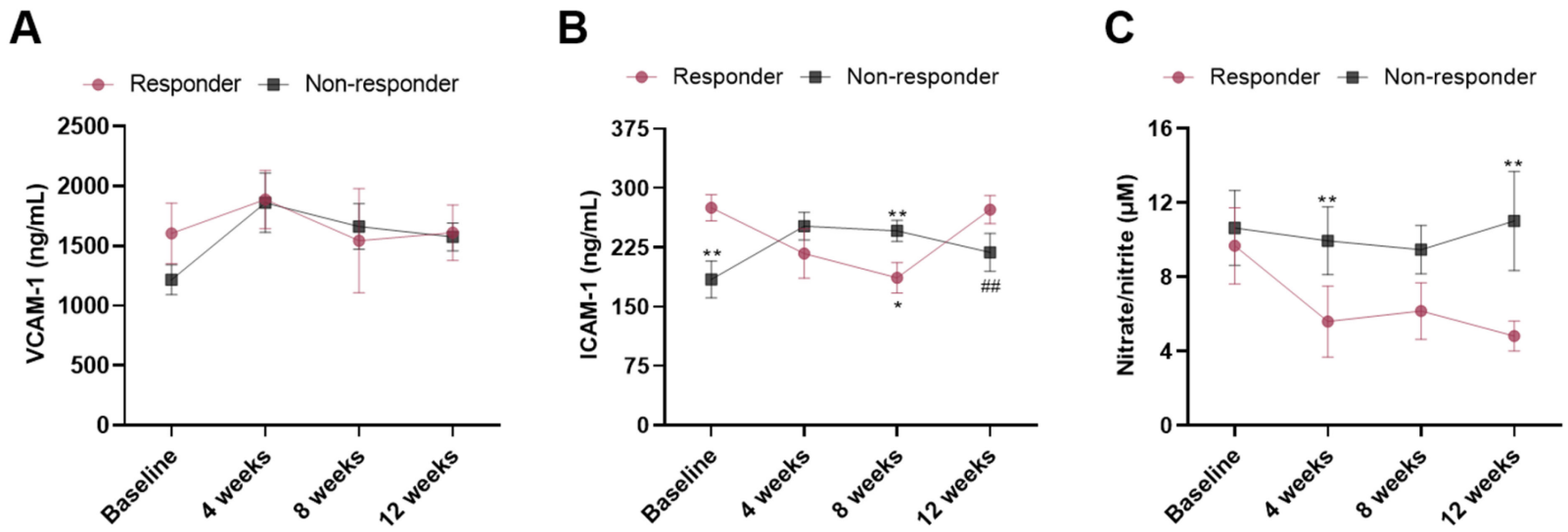


Figure 4.5. (A) Intercellular adhesion molecule 1 (ICAM-1) levels for responders (n = 8) and non-responders (n = 9) at baseline and 4, 8, and 12 weeks; (B) vascular adhesion molecule 1 (VCAM-1) levels for responders and non-responders at baseline and 4, 8, and 12 weeks; and (C) nitrate/nitrite levels for responders and non-responders at baseline and 4, 8, and 12 weeks. Data are mean ± SEM. *Different within group compared to baseline ($p < 0.05$).**Different between groups at that time point ($p < 0.05$). ##Trending different between groups at that time point ($p < 0.10$).

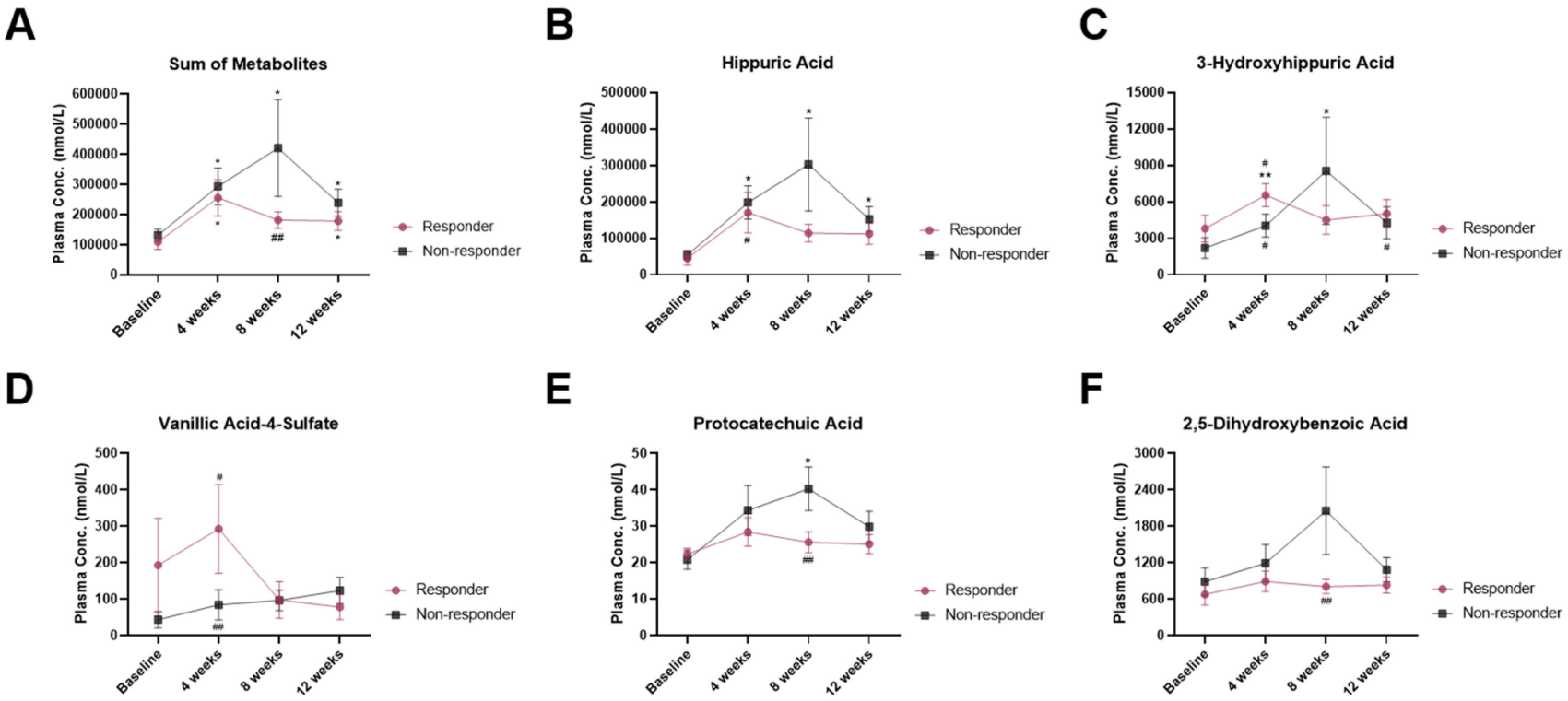


Figure 4.6. (A) Sum of plasma (poly)phenol metabolites concentrations for responders (n = 8) and non-responders (n = 9) at baseline and 4, 8, and 12 weeks; (B) plasma hippuric acid concentrations for responders and non-responders at baseline and 4, 8, and 12 weeks; (C) plasma 3-hydroxyhippuric acid concentrations for responders and non-responders at baseline and 4, 8, and 12 weeks; (D) plasma vanillic acid concentrations for responders and non-responders at baseline and 4, 8, and 12 weeks; (E) plasma protocatechuic acid concentrations for responders and non-responders at baseline and 4, 8, and 12 weeks; and (F) plasma 2,5-dihydroxybenzoic acid concentrations for responders and non-responders at baseline and 4, 8, and 12 weeks. Data are mean ± SEM. *Different within group compared to baseline ($p < 0.05$).**Different between groups at that time point ($p < 0.05$). #Trending different compared to baseline within groups ($p < 0.10$). ##Trending different between groups at that time point ($p < 0.08$).

Table 4.2. Correlations between plasma (poly)phenol metabolites and the change of FMD and FMD/SR_{AUC} from baseline to 12 weeks for the 17 postmenopausal women who consumed 22 g freeze-dried highbush blueberry powder for 12 weeks and completed the endothelial function measurements.

	Δ FMD		**Δ FMD/SR_{AUC}**	
Compound (nmol/L)	**Responder (*n* = 8)**	**Non-responder (*n* = 5)**	**Responder (*n* = 8)**	**Non-responder (*n* = 5)**
Sum of (poly)phenol metabolites				
Baseline	-0.381	-0.267	-0.167	0.250
12 Weeks	-0.048	-0.200	-0.119	-0.133
Δ 0 to 12 Weeks	0.714*	-0.200	0.214	-0.683*
Hippuric Acid				
Baseline	0.190	-0.233	-0.167	0.0367
12 Weeks	0.143	-0.217	-0.048	-0.100
Δ 0 to 12 Weeks	0.524	-0.117	0.143	-0.467
3-Hydroxyhippuric Acid				
Baseline	-0.524	-0.750*	0.000	-0.267
12 Weeks	-0.357	-0.850*	-0.048	-0.467
Δ 0 to 12 Weeks	0.381	-0.650#	0.143	-0.700*
Vanillic Acid-4-Sulfate				
Baseline	-0.327	-0.055	0.464	0.201
12 Weeks	-0.287	-0.285	-0.036	-0.251
Δ 0 to 12 Weeks	0.048	-0.233	-0.381	-0.150
Protocatechuic Acid				
Baseline	-0.048	-0.159	-0.452	0.310
12 Weeks	-0.643#	-0.067	-0.381	-0.417
Δ 0 to 12 Weeks	-0.548	0.017	-0.095	-0.400
2,5-Dihydroxybenzoic Acid				
Baseline	-0.310	0.083	0.000	0.617
12 Weeks	0.333	-0.117	-0.143	0.200
Δ 0 to 12 Weeks	0.786*	-0.367	-0.214	-0.700*

Data are Spearman's rho. *Significant correlation ($p < 0.05$). #Trending significant correlation ($p < 0.09$). Abbreviations: FMD, flow-mediated dilation; FMD/SR_{AUC}; flow-mediated dilation normalized to shear rate area under the curve.

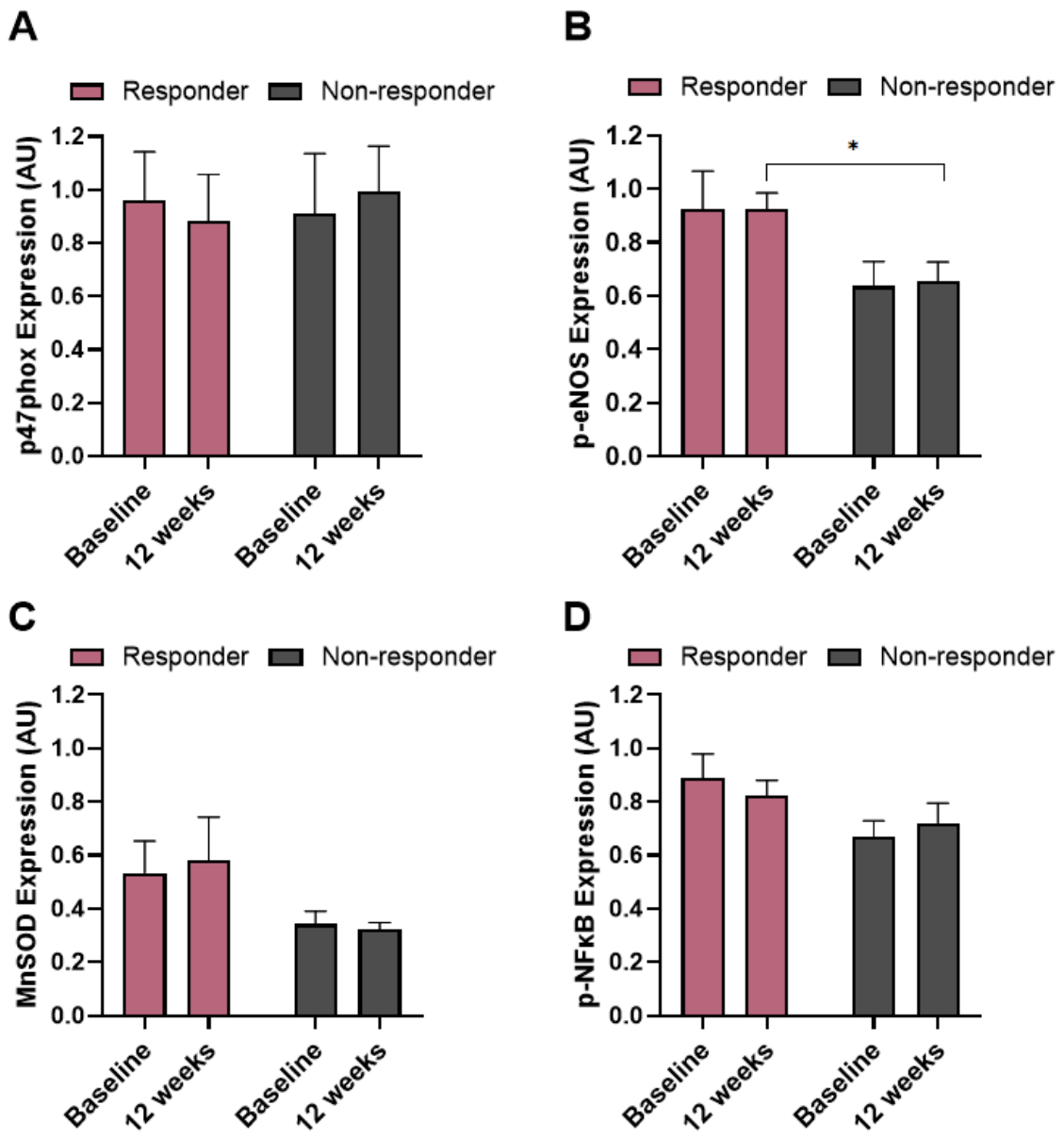


Figure 4.7. Venous endothelial cell protein expression between responder (*n* = 8) and non-responders (*n* = 9) at baseline and 12 weeks. (A) phosphorylated endothelial nitric oxide synthase (p-eNOS); (B) NADPH oxidase/p47 (p47phox); (C) manganese superoxide dismutase (MnSOD); and (D) phosphorylated nuclear factor kappa B (p-NFκB). Data are expressed as arbitrary units (AU). Data are mean ± SEM. *Different between groups at that time point ($p < 0.05$).

Table 4.3. Correlations between venous endothelial cell protein expressions and the change of FMD and FMD/SR_{AUC} from baseline to 12 weeks for the 12 postmenopausal women who consumed 22 g freeze-dried highbush blueberry powder for 12 weeks and completed endothelial cell biopsy.

	Δ FMD		**Δ FMD/SR_{AUC}**	
	Responder (*n* = 8)	**Non-responder (*n* = 5)**	**Responder (*n* = 8)**	**Non-responder (*n* = 5)**
p47phox				
Baseline	0.357	0.800	0.048	0.900*
12 Weeks	-0.167	0.500	-0.405	0.600
Δ 0 to 12 Weeks	-0.690	-0.600	-0.524	-0.800
p-eNOS				
Baseline	-0.881*	0.100	-0.048	0.300
12 Weeks	-0.119	-0.300	0.071	0.100
Δ 0 to 12 Weeks	0.571	-0.600	0.214	-0.700
MnSOD				
Baseline	-0.238	0.000	0.048	0.300
12 Weeks	-0.452	0.100	-0.071	0.200
Δ 0 to 12 Weeks	0.310	0.000	0.476	-0.300
p-NFκB p65 (Ser536)				
Baseline	0.024	-0.100	0.762*	0.000
12 Weeks	0.381	0.700	0.071	0.600
Δ 0 to 12 Weeks	0.405	0.500	-0.452	0.300

Data are Spearman's rho. *Significant correlation ($p < 0.05$). Abbreviations: FMD, flow-mediated dilation; FMD/SR_{AUC}; flow-mediated dilation normalized to shear rate area under the curve; MnSOD, manganese superoxide dismutase; p-eNOS, phosphorylated endothelial cell nitric oxide synthase; p-NFκB, phosphorylated nuclear factor kappa B.

Table 4.4. Arterial stiffness, blood pressure, anthropometrics, fat mass, and energy expenditure values for the 17 postmenopausal women who consumed 22 g freeze-dried highbush blueberry powder for 12 weeks and completed the endothelial function measurements.

	Responder (*n* = 8)	Non-Responder (*n* = 9)
Aortic PWV (m/s)		
Baseline	7.7 ± 0.4	8.0 ± 0.2
12 Weeks	7.4 ± 0.3	7.9 ± 0.2
Δ 0 to 12 Weeks	-0.3 ± 0.4	0.0 ± 0.3
AIx (%)		
Baseline	36 ± 2	36 ± 3
12 Weeks	34 ± 2	37 ± 2
Δ 0 to 12 Weeks	-2 ± 2	1 ± 3
AIx@75 (%)		
Baseline	30 ± 3	28 ± 6
12 Weeks	28 ± 2	29 ± 2
Δ 0 to 12 Weeks	-2 ± 2	1 ± 7
Brachial SBP (mmHg)		
Baseline	137 ± 4	133 ± 3
12 Weeks	138 ± 4	131 ± 2
Δ 0 to 12 Weeks	1 ± 2	-2 ± 3
Brachial DBP (mmHg) [a]		
Baseline	81 ± 2	78 ± 2
12 Weeks	83 ± 2	76 ± 1**
Δ 0 to 12 Weeks	1 ± 2	-2 ± 1
Weight (kg)		
Baseline	76.1 ± 5.6	69.1 ± 4.6
12 Weeks	76.3 ± 5.4	69.0 ± 4.8
Δ 0 to 12 Weeks	0.2 ± 0.3	-0.1 ± 0.4
BMI (kg/m^2)		
Baseline	29.1 ± 1.8	25.7 ± 1.6
12 Weeks	29.2 ± 1.7	25.7 ± 1.6
Δ 0 to 12 Weeks	0.1 ± 0.1	0.0 ± 0.2
WC (cm)		
Baseline	95 ± 4	89 ± 4
12 Weeks	95 ± 4	86 ± 6
Δ 0 to 12 Weeks	0 ± 2	-3 ± 3
HC (cm)		
Baseline	109 ± 4	106 ± 3
12 Weeks	110 ± 4	105 ± 3
Δ 0 to 12 Weeks	1 ± 1	-2 ± 2
WC:HC		
Baseline	0.87 ± 0.02	0.84 ± 0.02
12 Weeks	0.86 ± 0.01	0.82 ± 0.03
Δ 0 to 12 Weeks	-0.01 ± 0.02	-0.02 ± 0.03
Total Fat Mass (%)		
Baseline	42.0 ± 1.7	39.6 ± 1.8
12 Weeks	42.1 ± 1.7	38.0 ± 1.2##
Δ 0 to 12 Weeks	0.1 ± 0.3	-1.5 ± 1.2

Table 4.4. Arterial stiffness, blood pressure, anthropometrics, fat mass, and energy expenditure values for the 17 postmenopausal women who consumed 22 g freeze-dried highbush blueberry powder for 12 weeks and completed the endothelial function measurements.

	Responder (*n* = 8)	**Non-Responder (*n* = 9)**
Average Daily Energy Expenditure (kcal)		
Baseline	2694 ± 235	2411 ± 211
12 Weeks	2631 ± 190	2445 ± 191
∆ 0 to 12 Weeks	-64 ± 92	34 ± 28

Data are mean ± SEM. [a]Trending main effect for group ($p < 0.06$). *Different compared to baseline within group ($p < 0.05$). **Different between groups at that time point ($p < 0.05$). [##]Trending different between groups at that time point ($p < 0.08$). Abbreviations: AIx, augmentation index; AIX@75, augmentation index normalized to 75 beats per minute; BMI, body mass index; DBP, diastolic blood pressure; HC, hip circumference; PWV, pulse wave velocity; SBP, systolic blood pressure; WC:HC, waist to hip circumference ratio.

Table 4.5. Healthy Eating Index (HEI) scores between groups for the 17 postmenopausal women who consumed 22 g freeze-dried highbush blueberry powder for 12 weeks and completed the endothelial function measurements.

	Responder (*n* = 8)	Non-Responder (*n* = 9)	Max Points[48]	Standard for Max Points[48]	Standard for Minimum Points[48]
Total Vegetables	3.8 ± 0.5	3.2 ± 0.6	5	≥1.1 cup equiv. per 1,000 kcal	No vegetables
Greens and Beans	2.7 ± 0.9	3.1 ± 0.8	5	≥0.2 cup equiv. per 1,000 kcal	No greens and beans
Total Fruits	2.6 ± 0.8	2.8 ± 0.6	5	≥0.8 cup equiv. per 1,000 kcal	No fruits
Whole Fruits	2.1 ± 0.8	3.5 ± 0.6	5	≥0.4 cup equiv. per 1,000 kcal	No whole fruits
Whole Grains	2.6 ± 1.4	3.7 ± 1.1	10	≥1.5 oz equiv. per 1,000 kcal	No whole grains
Dairy	5.6 ± 1.4	5.1 ± 0.7	10	≥1.3 cup equiv. per 1,000 kcal	No dairy
Total Protein Foods	4.3 ± 0.5	4.5 ± 0.5	5	≥2.5 oz equiv. per 1,000 kcal	No protein foods
Seafood and Plant Protein	3.7 ± 0.7	3.5 ± 0.7	5	≥0.8 oz equiv. per 1,000 kcal	No seafood or plant proteins
Fatty Acids	6.0 ± 1.5	6.8 ± 1.2	10	(PUFAs + MUFAs)/SFAs ≥2.5	PUFAs + MUFAs)/SFAs ≤1.2
Refined Grains	7.6 ± 0.8	6.8 ± 1.2	10	≤1.8 oz equiv. per 1,000 kcal	≥4.3 oz equiv. per 1,000 kcal
Sodium	5.8 ± 1.2	4.8 ± 1.3	10	≤1.1 gram per 1,000 kcal	≥2.0 grams per 1,000 kcal
Added Sugars	6.7 ± 0.7	7.4 ± 0.9	10	≤6.5% of energy	≥26% of energy
Saturated Fats	5.2 ± 1.6	5.6 ± 1.1	10	≤8% of energy	≥16% of energy
HEI Total Score	58.7 ± 4.9	60.8 ± 6.5	100	--	--

Data are mean ± SEM. No differences between groups ($p > 0.05$). Note: the higher the total HEI score is indicative of a healthier diet.
[48] Abbreviations: Equiv, equivalent; HEI, healthy eating index; kcal, kilocalories; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFA, saturated fatty acids.

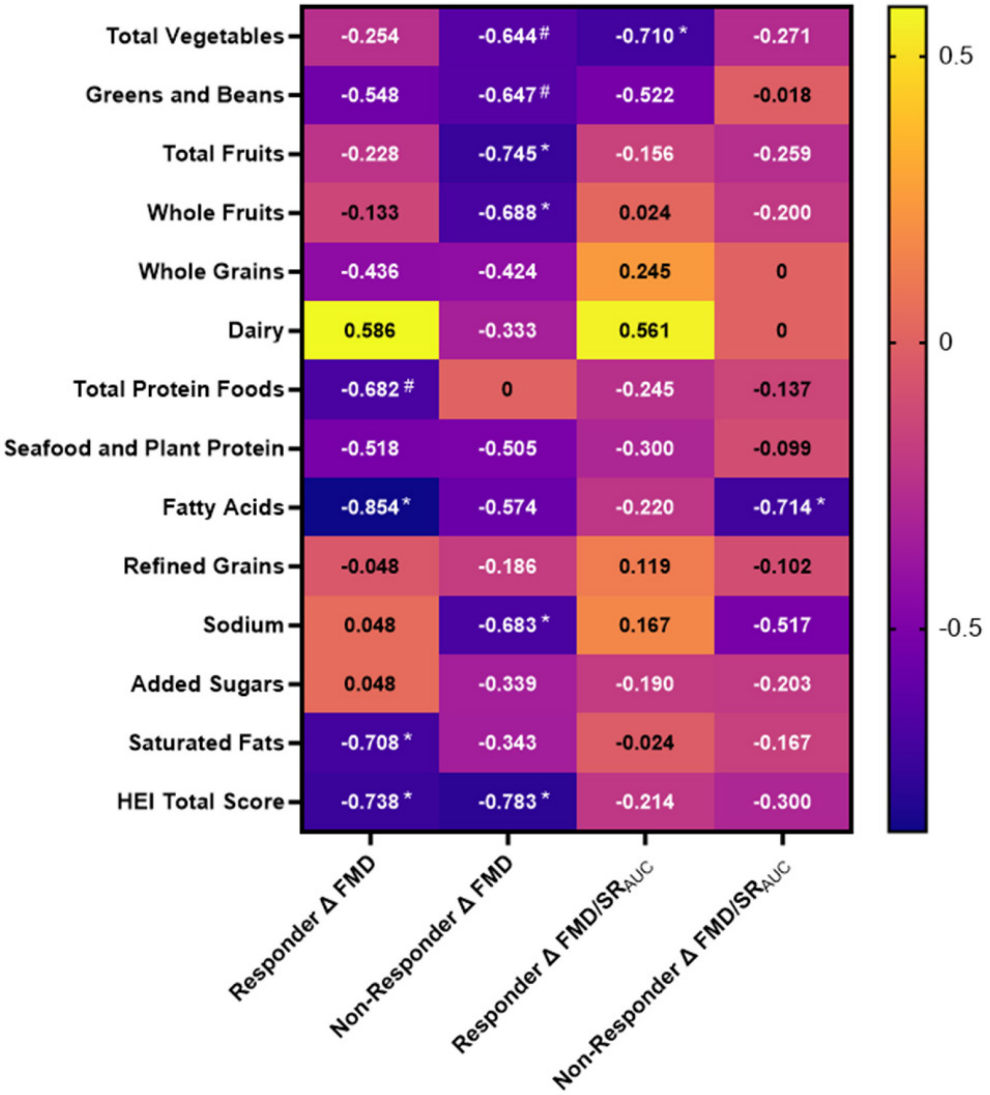


Figure 4.8. Healthy eating index (HEI) scores correlated to the change in flow-mediated dilation (FMD) and the change in FMD normalized to shear rate area under the curve (FMD/SR_{AUC}) from baseline to 12 weeks between responders (n = 8) and non-responders (n = 9). Data are Spearman's Rho. *Significant correlation ($p < 0.05$). #Trending significant correlation ($p < 0.07$). Abbreviations: HEI, healthy eating index.

REFERENCES

1. Heart Disease Facts, https://www.cdc.gov/heartdisease/facts.htm).
2. K. H. Park and W. J. Park, *J Korean Med Sci*, 2015, **30**, 1213-1225.
3. C. Bleakley, P. K. Hamilton, R. Pumb, M. Harbinson and G. E. McVeigh, *J Clin Hypertens (Greenwich)*, 2015, **17**, 651-654.
4. P. M. Vanhoutte, H. Shimokawa, M. Feletou and E. H. Tang, *Acta Physiol (Oxf)*, 2017, **219**, 22-96.
5. S. Jebari-Benslaiman, U. Galicia-García, A. Larrea-Sebal, J. R. Olaetxea, I. Alloza, K. Vandenbroeck, A. Benito-Vicente and C. Martín, *Int J Mol Sci*, 2022, **23**.
6. R. T. Ras, M. T. Streppel, R. Draijer and P. L. Zock, *Int J Cardiol*, 2013, **168**, 344-351.
7. T. D. Giles, G. E. Sander, B. D. Nossaman and P. J. Kadowitz, *J Clin Hypertens (Greenwich)*, 2012, **14**, 198-205.
8. K. L. Moreau, K. L. Hildreth, J. Klawitter, P. Blatchford and W. M. Kohrt, *Geroscience*, 2020, **42**, 1699-1714.
9. H. N. Siti, Y. Kamisah and J. Kamsiah, *Vascul Pharmacol*, 2015, **71**, 40-56.
10. M. A. Incalza, R. D'Oria, A. Natalicchio, S. Perrini, L. Laviola and F. Giorgino, *Vascul Pharmacol*, 2018, **100**, 1-19.
11. Q. N. Dinh, G. R. Drummond, C. G. Sobey and S. Chrissobolis, *Biomed Res Int*, 2014, **2014**, 406960.
12. K. L. Moreau, K. L. Hildreth, A. L. Meditz, K. D. Deane and W. M. Kohrt, *J Clin Endocrinol Metab*, 2012, **97**, 4692-4700.
13. K. L. Moreau and K. L. Hildreth, *Adv Vasc Med*, 2014, **2014**.
14. E. K. Woolf, J. D. Terwoord, N. S. Litwin, A. R. Vazquez, S. Y. Lee, N. Ghanem, K. A. Michell, B. T. Smith, L. E. Grabos, N. B. Ketelhut, N. P. Bachman, M. E. Smith, M. Le

Sayec, S. Rao, C. L. Gentile, T. L. Weir, A. Rodriguez-Mateos, D. R. Seals, F. A. Dinenno and S. A. Johnson, *Food Funct*, 2023, **14**, 2621-2641.

15. Y. B. Somani, J. A. Pawelczyk, M. J. De Souza, P. M. Kris-Etherton and D. N. Proctor, *Am J Physiol Heart Circ Physiol*, 2019, **317**, H395-h404.
16. A. Iorga, C. M. Cunningham, S. Moazeni, G. Ruffenach, S. Umar and M. Eghbali, *Biol Sex Differ*, 2017, **8**, 33.
17. A. Fernández-Sánchez, E. Madrigal-Santillán, M. Bautista, J. Esquivel-Soto, A. Morales-González, C. Esquivel-Chirino, I. Durante-Montiel, G. Sánchez-Rivera, C. Valadez-Vega and J. A. Morales-González, *Int J Mol Sci*, 2011, **12**, 3117-3132.
18. M. Rizzo, J. Kotur-Stevuljevic, K. Berneis, G. Spinas, G. B. Rini, Z. Jelic-Ivanovic, V. Spasojevic-Kalimanovska and J. Vekic, *Transl Res*, 2009, **153**, 217-223.
19. A. Engin, *Adv Exp Med Biol*, 2017, **960**, 345-379.
20. S. R. Davis, C. Castelo-Branco, P. Chedraui, M. A. Lumsden, R. E. Nappi, D. Shah and P. Villaseca, *Climacteric*, 2012, **15**, 419-429.
21. B. A. Phan and P. P. Toth, *Int J Womens Health*, 2014, **6**, 185-194.
22. D. R. Seals, K. L. Jablonski and A. J. Donato, *Clin Sci (Lond)*, 2011, **120**, 357-375.
23. A. Baradaran, H. Nasri and M. Rafieian-Kopaei, *J Res Med Sci*, 2014, **19**, 358-367.
24. D. Mozaffarian, E. J. Benjamin, A. S. Go, D. K. Arnett, M. J. Blaha, M. Cushman, S. de Ferranti, J. P. Després, H. J. Fullerton, V. J. Howard, M. D. Huffman, S. E. Judd, B. M. Kissela, D. T. Lackland, J. H. Lichtman, L. D. Lisabeth, S. Liu, R. H. Mackey, D. B. Matchar, D. K. McGuire, E. R. Mohler, 3rd, C. S. Moy, P. Muntner, M. E. Mussolino, K. Nasir, R. W. Neumar, G. Nichol, L. Palaniappan, D. K. Pandey, M. J. Reeves, C. J. Rodriguez, P. D. Sorlie, J. Stein, A. Towfighi, T. N. Turan, S. S. Virani, J. Z. Willey, D. Woo, R. W. Yeh and M. B. Turner, *Circulation*, 2015, **131**, e29-322.
25. L. L. Yanes and J. F. Reckelhoff, *Am J Hypertens*, 2011, **24**, 740-749.

26. J. Quek, G. Lim, W. H. Lim, C. H. Ng, W. Z. So, J. Toh, X. H. Pan, Y. H. Chin, M. D. Muthiah, S. P. Chan, R. S. Y. Foo, J. Yip, N. Neelakantan, M. F. F. Chong, P. H. Loh and N. W. S. Chew, *Front Cardiovasc Med*, 2021, **8**, 756810.

27. H. Kahleova, S. Levin and N. D. Barnard, *Prog Cardiovasc Dis*, 2018, **61**, 54-61.

28. J. Higbee, P. Solverson, M. Zhu and F. Carbonero, *Food Frontiers*, 2022, **3**, 3-27.

29. M. H. Oak, C. Auger, E. Belcastro, S. H. Park, H. H. Lee and V. B. Schini-Kerth, *Free Radic Biol Med*, 2018, **122**, 161-170.

30. T. Hussain, B. Tan, Y. Yin, F. Blachier, M. C. Tossou and N. Rahu, *Oxid Med Cell Longev*, 2016, **2016**, 7432797.

31. R. Andriantsitohaina, C. Auger, T. Chataigneau, N. Étienne-Selloum, H. Li, M. C. Martínez, V. B. Schini-Kerth and I. Laher, *Br J Nutr*, 2012, **108**, 1532-1549.

32. K. B. Pandey and S. I. Rizvi, *Oxid Med Cell Longev*, 2009, **2**, 270-278.

33. Nutrition Facts, https://blueberry.org/health-benefits/nutrition-facts/).

34. E. Wood, S. Hein, C. Heiss, C. Williams and A. Rodriguez-Mateos, *Food Funct*, 2019, **10**, 7621-7633.

35. ERS Charts of Note, https://www.ers.usda.gov/data-products/charts-of-note/charts-of-note/?page=2&topicId=b64476d3-203d-4eea-ad5c-59272f480979).

36. A. Rodriguez-Mateos, G. Istas, L. Boschek, R. P. Feliciano, C. E. Mills, C. Boby, S. Gomez-Alonso, D. Milenkovic and C. Heiss, *J Gerontol A Biol Sci Med Sci*, 2019, **74**, 967-976.

37. A. J. Stull, K. C. Cash, C. M. Champagne, A. K. Gupta, R. Boston, R. A. Beyl, W. D. Johnson and W. T. Cefalu, *Nutrients*, 2015, **7**, 4107-4123.

38. P. J. Curtis, V. van der Velpen, L. Berends, A. Jennings, M. Feelisch, A. M. Umpleby, M. Evans, B. O. Fernandez, M. S. Meiss, M. Minnion, J. Potter, A. M. Minihane, C. D. Kay, E. B. Rimm and A. Cassidy, *Am J Clin Nutr*, 2019, **109**, 1535-1545.

39. E. Wood, S. Hein, R. Mesnage, F. Fernandes, N. Abhayaratne, Y. Xu, Z. Zhang, L. Bell, C. Williams and A. Rodriguez-Mateos, *Am J Clin Nutr*, 2023, DOI: 10.1016/j.ajcnut.2023.03.017.

40. K. S. Stote, M. I. Sweeney, T. Kean, D. J. Baer, J. A. Novotny, N. L. Shakerley, A. Chandrasekaran, P. M. Carrico, J. A. Melendez and K. T. Gottschall-Pass, *BMC Nutr*, 2017, **3**, 45.

41. P. Riso, D. Klimis-Zacas, C. Del Bo, D. Martini, J. Campolo, S. Vendrame, P. Møller, S. Loft, R. De Maria and M. Porrini, *Eur J Nutr*, 2013, **52**, 949-961.

42. D. Milenkovic, C. Morand, A. Cassidy, A. Konic-Ristic, F. Tomás-Barberán, J. M. Ordovas, P. Kroon, R. De Caterina and A. Rodriguez-Mateos, *Adv Nutr*, 2017, **8**, 558-570.

43. C. L. Bush, J. B. Blumberg, A. El-Sohemy, D. M. Minich, J. M. Ordovás, D. G. Reed and V. A. Y. Behm, *J Am Coll Nutr*, 2020, **39**, 5-15.

44. M. T. García-Conesa, K. Chambers, E. Combet, P. Pinto, M. Garcia-Aloy, C. Andrés-Lacueva, S. de Pascual-Teresa, P. Mena, A. Konic Ristic, W. J. Hollands, P. A. Kroon, A. Rodríguez-Mateos, G. Istas, C. A. Kontogiorgis, D. K. Rai, E. R. Gibney, C. Morand, J. C. Espín and A. González-Sarrías, *Int J Mol Sci*, 2018, **19**.

45. B. Y. Lee, J. M. Ordovás, E. J. Parks, C. A. M. Anderson, A. L. Barabási, S. K. Clinton, K. de la Haye, V. B. Duffy, P. W. Franks, E. M. Ginexi, K. J. Hammond, E. C. Hanlon, M. Hittle, E. Ho, A. L. Horn, R. S. Isaacson, P. L. Mabry, S. Malone, C. K. Martin, J. Mattei, S. N. Meydani, L. M. Nelson, M. L. Neuhouser, B. Parent, N. P. Pronk, H. M. Roche, S. Saria, F. Scheer, E. Segal, M. A. Sevick, T. D. Spector, L. Van Horn, K. A. Varady, V. S. Voruganti and M. F. Martinez, *Am J Clin Nutr*, 2022, **116**, 1877-1900.

46. Y. Inaba, J. A. Chen and S. R. Bergmann, *Int J Cardiovasc Imaging*, 2010, **26**, 631-640.

47. D. H. J. Thijssen, R. M. Bruno, A. van Mil, S. M. Holder, F. Faita, A. Greyling, P. L. Zock, S. Taddei, J. E. Deanfield, T. Luscher, D. J. Green and L. Ghiadoni, *Eur Heart J*, 2019, **40**, 2534-2547.

48. Overview & Background of The Healthy Eating Index, https://epi.grants.cancer.gov/hei/).

49. HEI Scores for Americans, https://www.fns.usda.gov/hei-scores-americans).

50. C. Heiss, A. Rodriguez-Mateos, M. Bapir, S. S. Skene, H. Sies and M. Kelm, *Cardiovasc Res*, 2023, **119**, 283-293.

51. R. H. Wong, J. J. Thaung Zaw, C. J. Xian and P. R. Howe, *J Bone Miner Res*, 2020, **35**, 2121-2131.

52. M. Yamaoka-Tojo, *International Journal of Clinical Cardiology*, 2017, **4**, 1-11.

53. P. G. Frank and M. P. Lisanti, *Am J Physiol Heart Circ Physiol*, 2008, **295**, H926-h927.

54. D. Bharat, R. R. M. Cavalcanti, C. Petersen, N. Begaye, B. R. Cutler, M. M. A. Costa, R. Ramos, M. R. Ferreira, Y. Li, L. P. Bharath, E. Toolson, P. Sebahar, R. E. Looper, T. Jalili, N. S. Rajasekaran, Z. Jia, J. D. Symons and P. V. Anandh Babu, *Mol Nutr Food Res*, 2018, **62**.

55. W. Huang, Y. Zhu, C. Li, Z. Sui and W. Min, *Oxid Med Cell Longev*, 2016, **2016**, 1591803.

56. W. Huang, R. P. Hutabarat, Z. Chai, T. Zheng, W. Zhang and D. Li, *Int J Mol Sci*, 2020, **21**.

57. A. S. Thakor, H. G. Richter, A. D. Kane, C. Dunster, F. J. Kelly, L. Poston and D. A. Giussani, *J Physiol*, 2010, **588**, 4235-4247.

58. P. Codoñer-Franch, S. Tavárez-Alonso, R. Murria-Estal, J. Megías-Vericat, M. Tortajada-Girbés and E. Alonso-Iglesias, *Atherosclerosis*, 2011, **215**, 475-480.

59. D. Klimis-Zacas, S. Vendrame and A. S. Kristo, *Journal of Berry Research*, 2016, **6**, 225-236.

60. S. Vendrame, A. S. Kristo, D. A. Schuschke and D. Klimis-Zacas, *Appl Physiol Nutr Metab*, 2014, **39**, 255-261.

61. V. Mollace, C. Muscoli, E. Masini, S. Cuzzocrea and D. Salvemini, *Pharmacol Rev*, 2005, **57**, 217-252.

62. K. L. Moreau, B. L. Stauffer, W. M. Kohrt and D. R. Seals, *J Clin Endocrinol Metab*, 2013, **98**, 4507-4515.

63. D. R. Seals, E. E. Nagy and K. L. Moreau, *J Physiol*, 2019, **597**, 4901-4914.

64. N. Hetemäki, T. S. Mikkola, M. J. Tikkanen, F. Wang, E. Hämäläinen, U. Turpeinen, M. Haanpää, V. Vihma and H. Savolainen-Peltonen, *J Steroid Biochem Mol Biol*, 2021, **209**, 105849.

65. B. A. Peters, N. Santoro, R. C. Kaplan and Q. Qi, *Int J Womens Health*, 2022, **14**, 1059-1072.

66. N. Toro-Funes, F. J. Morales-Gutiérrez, M. T. Veciana-Nogués, M. C. Vidal-Carou, J. P. Spencer and A. Rodriguez-Mateos, *Food Funct*, 2015, **6**, 98-108.

67. A. Rodriguez-Mateos, N. Toro-Funes, T. Cifuentes-Gomez, M. Cortese-Krott, C. Heiss and J. P. Spencer, *Arch Biochem Biophys*, 2014, **559**, 17-23.

68. I. Edirisinghe and B. M. Burton-Freeman, *Nutrition and Aging*, 2014, **2**, 197-211.

69. B. L. Tan, M. E. Norhaizan and W. P. Liew, *Oxid Med Cell Longev*, 2018, **2018**, 9719584.

CHAPTER 5: EXAMINING THE ATHEROPROTECTIVE EFFECTS OF (POLY)PHENOL METABOLITES IN HUMAN SERUM AFTER CHRONIC BLUEBERRY CONSUMPTION IN POSTMENOPAUSAL WOMEN WITH ABOVE-NORMAL BLOOD PRESSURE

Summary

Blueberry (poly)phenol metabolites may reduce cardiovascular disease (CVD) risk by improving oxidative stress-mediated endothelial dysfunction, which is a central contributor to atherosclerotic CVD. Postmenopausal women develop endothelial dysfunction largely due to decreased estrogen production promoting oxidative stress and inflammation. In a randomized, double-blind, placebo-controlled, parallel-arm clinical trial, 22 g/day of freeze-dried blueberry powder consumption for 12 weeks improved endothelial function reductions of endothelial oxidative stress and increased plasma (poly)phenol metabolites in estrogen-deficient postmenopausal women with above-normal blood pressure; however, cellular mechanisms and broader implications for atherosclerosis are not known. The aim of this study was to use a reverse translational approach from humans to cells to evaluate cellular mechanisms responsible for clinical improvements in endothelial function and the anti-atherogenic potential of blueberries. Human blood serum samples with or without (poly)phenol metabolites (i.e. blueberry and placebo groups) were leveraged from our clinical trial at 12 weeks. Cultured human aortic endothelial cells (HAECs) were incubated with 15% human serum for 1 h followed by 200 μM hydrogen peroxide (H_2O_2) for 24 h. HAECs were incubated with THP-1 cells to assess monocyte binding affinity or assessed for insulin-stimulated nitric oxide (NO) production and peroxynitrite concentrations. There was a strong trend for increased monocyte binding to HAECs with 200 μM H_2O_2 ($p = 0.057$), but 200 μM H_2O_2 had no statistically significant effects on insulin-stimulated NO production or peroxynitrite concentrations ($p > 0.05$). Blueberry serum did not improve monocyte binding or insulin-stimulated NO production in dysfunctional HAECs ($p >$

0.05). Peroxynitrite concentrations were significantly higher in dysfunctional HAECs with the blueberry and placebo serum compared to the controls ($p < 0.001$). This is the first study to examine 1) the effects of 200 μM H_2O_2 on monocyte binding, NO production, and peroxynitrite concentrations in HAECs, and 2) whether 15% human serum containing (poly)phenol metabolites could attenuate adverse effects. Further research is needed to better establish this *ex vivo* cell culture model.

Introduction

About 50% of all deaths in westernized societies are due atherosclerotic cardiovascular disease (CVD), a condition causing narrowing and hardening of the arteries due to lipid accumulation.[1] The first step of atherosclerosis is vascular endothelial dysfunction which is characterized impaired endothelium-dependent dilation. The endothelium is responsible for producing vasoactive substances, such as nitric oxide (NO), to maintain vascular tone and promote blood flow.[2-4] Oxidative stress and inflammation are underlying mechanisms of endothelial dysfunction, due in part to adverse effects on NO production and bioavailability.[5, 6] Oxidative stress occurs when there is an imbalance between reactive oxygen/nitrogen species (ROS/RNS) production and clearance, and inhibits NO production and bioavailability through various mechanisms including endothelial nitric oxide synthase (eNOS) uncoupling resulting in formation of superoxide radicals instead of NO.[3, 7] Superoxide radicals react with available NO causing formation of the RNS peroxynitrite, further contributing to oxidative stress.[3, 7] ROS also upregulate pro-inflammatory pathways triggering monocyte binding to the endothelium (among other processes), thus, promoting atherogenesis.[8] However, NO is protective against such monocyte-endothelial cell interactions and exerts other vascular-protective effects, rendering it an essential molecule for atherosclerotic CVD prevention.[9, 10]

Non-modifiable (e.g. age and menopause) and modifiable (e.g. high blood pressure) risk factors contribute to atherosclerosis, though aging is the primary risk factor for CVD. With aging

there is a decline in physiological and functional processes that contribute to ROS/RNS accumulation.[11, 12] Increased ROS is one mechanism contributing to endothelial dysfunction and increased monocyte adhesion in older adults compared to younger counterparts.[13] Furthermore, accumulating evidence indicates estrogen-deficient postmenopausal women have oxidative stress-mediated suppression of endothelial function.[14-17] Estrogen acts as an antioxidant by scavenging ROS and can bind to estrogen receptors on endothelial cells triggering NO production.[16, 18, 19] Additionally, high blood pressure which increases with age and menopause, is considered the most common modifiable risk factor for CVD and contributes to oxidative stress and endothelial dysfunction.[3, 20-23] Effective lifestyle approaches for increasing NO production/bioavailability and decreasing oxidative stress to improve endothelial function are needed for atherosclerotic CVD prevention in aging individuals, particularly postmenopausal women with elevated or high blood pressure (i.e. above-normal blood pressure).

Consuming diets rich in foods that contain (poly)phenols have been shown to regress atherosclerosis, improve modifiable risk factors (e.g. high blood pressure and cholesterol), and reduce CVD events in humans.[24-27] Adding blueberries to the diet is of particular interest due to their high (poly)phenol content, namely anthocyanins and phenolic acids.[28] We recently demonstrated in a double-blind, parallel-arm, placebo-controlled clinical trial that consuming 22 g of freeze-dried highbush blueberry powder daily for 12 weeks significantly improved endothelial function in postmenopausal women with above-normal blood pressure through reductions in oxidative stress. Furthermore, we observed increases in plasma (poly)phenol metabolite concentrations, many of which were anthocyanin and gut microbial metabolites.[17] However, the direct endothelial cell-related mechanisms contributing to improved endothelial function after chronic blueberry consumption within this study population is currently unknown. Additionally, the atheroprotective effects of blueberry consumption beyond improvements in endothelial function are not known in humans.

Blueberry-derived (poly)phenols have low bioavailability implicating their circulating metabolites derived from phase I and II metabolism and gut microbial metabolism for their atheroprotective effects.[29] *Ex vivo* studies have demonstrated that physiologically relevant concentrations of synthetic blueberry-derived anthocyanin metabolites increased insulin-stimulated NO production, and decreased ROS production and monocyte binding to dysfunctional (i.e. stimulated with palmitate, TNF-α) human-derived endothelial cells.[30, 31] However, whether these results are translatable to oxidative stress-induced dysfunctional human endothelial cells, as well as for blueberry (poly)phenol metabolites found circulating in human serum after consumption has yet to be explored. Therefore, the objective of this study was to establish an *ex vivo* cell culture model of oxidative stress-induced endothelial dysfunction using human aortic endothelial cells (HAECs), hydrogen peroxide (H_2O_2), and human blood serum for examining mechanisms for improved endothelial function and potential atheroprotective effects of blueberry consumption. We hypothesized that (poly)phenol metabolites found in human serum after 12 weeks of daily blueberry consumption would inhibit monocyte binding to dysfunctional HAECs, as well as increase insulin-stimulated NO production and decrease peroxynitrite concentrations.

Methods

Materials and chemicals

HAECs, endothelial basal medium-2 (EBM-2), and endothelial growth medium-2 SingleQuot kit supplements and growth factors were purchased from Lonza (Allendale, NJ, USA). RPMI medium 1640, HyClone® Trypan Blue Solution, phosphate buffer solution (PBS), 0.22 μm membrane filter, and fetal bovine serum (FBS) were purchased from ThermoFisher Scientific (Waltham, MA, USA); TrypLETM Express and calcein-AM were from Life Technologies Corporation (Grand Island, NY, USA); Humulin from Lilly USA, LLC (Indianapolis, IN, USA); human monocyte THP-1 cells from American Type Culture Collection (Manassas, VA,

USA); 4-amino-5-methylamino-2',7'-difluororescein diacetate (DAF-FM) from Invitrogen (Carlsbad, CA, USA); dimethyl sulfoxide (DMSO) and H_2O_2 from Sigma-Aldrich (St. Louis, MO, USA); and peroxynitrite assay kit from Abcam (Cambridge, MA, USA).

Cell culture

HAECs and THP1 cells were cultured as previously described.[30] Briefly, HAECs were cultured in EBM-2 medium supplemented with EGM-2 growth supplements and 2% heat-inactivated FBS. HAECs were passaged at 80-90% confluence, and experiments were conducted at passage 6. THP-1 cells were cultured in RPMI medium 1640 with 10% FBS. All cells were cultured in a 37°C 5% CO2 environment and were grown according to the supplier's protocols.

Human serum containing blueberry metabolites

Human blood serum collected 12 weeks post consumption of 22 g of freeze-dried highbush blueberry powder or isocaloric placebo powder daily by postmenopausal women with above-normal blood pressure was leveraged for the current study.[17] Serum samples were incubated in a water bath at 55°C for 30 min for heat inactivation to destroy the complement activity in the serum. Then, serum samples were sterilized using a 0.22 μm membrane filter before using for the experiment.

Rationale for using H_2O_2 to represent oxidative stress-induced endothelial dysfunction

H_2O_2 is produced by various cells in the human body mainly by reacting with superoxide radicals.[32] At pathological levels, H_2O_2 contributes to oxidative stress and endothelial dysfunction by uncoupling of eNOS and promoting peroxynitrite formation.[33] *Ex vivo* research has used H_2O_2 to induce oxidative stress-mediated dysfunction in HAECs.[34, 35] Specifically, 200 μM H_2O_2 incubated with HAECs for 24 h increased intracellular ROS production.[34] Furthermore,

1,000 μM H_2O_2 incubation for 4 h significantly increased THP-1 (monocyte) binding to HAECs and increased intracellular ROS.[35] In human umbilical venous endothelial cells (HUVECs), incubation of 100 μM H_2O_2 for 1 h and 500 μM H_2O_2 for 4 h significantly decreased NO supernatant concentrations.[36, 37] Collectively, these studies indicate H_2O_2 increases oxidative stress and can be used to induce endothelial dysfunction *in vitro*/*ex vivo*. Therefore, we chose to use H_2O_2 to induce oxidative stress endothelial dysfunction in HAECs to represent the population of postmenopausal women with oxidative stress-mediated suppression of endothelial function in our clinical trial.

Incubating HAECs with serum samples and H_2O_2

In a preliminary experiment, 200 μM, 300 μM, and 500 μM doses of H_2O_2 were incubated with HAECs for 24 h to determine an appropriate H_2O_2 concentration to induce dysfunction without affecting cell viability. Different concentrations of heat inactivated human serums (15%, 25%, and 50%) were also incubated with HAECs (separate from H_2O_2) for 24 h to assess cell viability. Based on these experiments and previous research, 200 μM of H_2O_2 and 15% serum were used for the current study (data not shown).[34, 38]

HAECs cultured in a 96-well plate were used for the experiment. Once 80-90% confluence was reached, HAECs were serum starved for 12 h and refreshed with a complete medium. HAECs were then incubated with 15% sterile heat inactivated human serum for 1 h, followed by incubation of 200 μM H_2O_2 for 24 h.[34]

Monocyte binding to endothelial cells

Calcein-AM-labeled THP-1 cells (1 x 105 cells/100 μl RPMI medium with 1% FBS/well) were used for the monocyte binding assay. HAECs were cultured in 96-well plates and treated with or without human serum for 1 h (FBS for control), and then with or without (control) H_2O_2 for 24 h. The medium was then removed from HAECs, and calcein-AM-labeled THP-1 cells in the

RPMI medium (1 x 105 cells/ well) were added to HAECs and incubated for 30 min in 37°C 5% CO_2. Medium was removed, and the HAECs were gently washed with 100 µL PBS (2 washes) to remove unbound monocytes. Then, 50 µL TrypLETM Express was added to the plates and cells were incubated for 5 min to extract HAECs and THP-1 cells from the plate. Collected cells were diluted with distilled water (1:2) as the fluorescent signal was too high to detect. The binding of monocytes to HAECs were measured by fluorescence using a fluorescent microplate reader (Synergy H1, BioTek Instruments, Inc., Winooski, VT, USA) with excitation and emission set to 496 and 520 nm, respectively.

NO and peroxynitrite assays

DAF-FM was used to detect insulin-stimulated NO production in HAECs as described by the manufacturer's instructions and previously reported.[30, 39] HAECs were cultured in 96-well plates and treated with or without human serum for 1 h (FBS for control), and then with or without (control) H_2O_2 for 24 h. The medium was removed, and HAECs were incubated with 100 nM of insulin for 30 min in 37°C 5% CO_2. Then, the medium was removed from the plate and replaced with serum-free EGM medium. DAF-FM (10 µM) was added to the cells and further incubated for 60 min. Medium was then removed and PBS was added to HAECs. After 30 min incubation, fluorescence was measured using a fluorescent microplate reader (Synergy H1, BioTek Instruments, Inc., Winooski, VT, USA) with excitation and emission set to 495 and 515 nm, respectively.

The peroxynitrite assay protocol was carried out according to Abcam's procedures (Cambridge, MA, USA). Specifically, working solutions were made using sensor green, DMSO, and assay buffer protecting from light. A working solution was added to HAECs (cultured in 96-well plates treated with or without human serum for 1 h (FBS for control), and then with or without (control) H_2O_2 for 24 h. Fluorescence was measured using a fluorescent microplate

reader (Synergy H1, BioTek Instruments, Inc., Winooski, VT, USA) with excitation and emission set to 490 and 530 nm, respectively.

Statistical Analyses

Missing data were imputed using within group means. Normality was checked using histograms and Shapiro-Wilk test. Data that did not meet normality were log transformed prior to statistical tests, in which normality then improved. One-way ANOVA and Tukey's post hoc test was used to determine differences between groups, and data are presented as mean ± standard error (SEM). Since human serum was leveraged from the clinical trial, Spearman's test was utilized to correlate primary outcomes (i.e. monocyte binding affinity, NO production, and peroxynitrite concentrations) to plasma lipids, adhesion molecules, nitrate/nitrite, estradiol, and (poly)phenol metabolites that were measured in plasma at 12 weeks to explore possible relationships to the current *ex vivo* study's outcomes.[17] Data for correlations are presented as Spearman's Rho. SPSS v28.0.1.0 was used for all statistical analyses (SPSS Inc., Chicago, IL, USA). A *p*-value of 0.05 was used to determine significant differences and 0.10 was used to determine a trend for significant differences.

Results

Monocyte binding affinity

There was an effect between groups ($p = 0.048$) with a strong trend for higher monocyte binding to HAECs for control + H_2O_2 compared to control ($p = 0.057$) (**Figure 5.1** and **Supplementary Table 5.1** in **Appendix 3**). Furthermore, there was a trend for lower monocyte binding for control compared to blueberry + H_2O_2 ($p = 0.074$) and placebo + H_2O_2 ($p = 0.077$) (**Figure 5.1** and **Supplementary Table 5.1** in **Appendix 3**).

No significant correlations were found between blood lipids/biomarkers and monocyte binding affinity to HAECs for either the blueberry or placebo serum ($p > 0.05$) (**Table 5.1**).

Similarly, no significant correlations were found between plasma (poly)phenol metabolites and monocyte binding affinity to HAECs for either group ($p > 0.05$) (**Table 5.2**).

NO and peroxynitrite

An effect between groups was found for insulin-stimulated NO production ($p < 0.001$), with control having higher NO production compared to blueberry + insulin + H_2O_2 ($p = 0.014$) and a strong trend for higher NO production compared to placebo + insulin + H_2O_2 ($p = 0.054$) (**Figure 5.2** and **Supplementary Table 5.1** in **Appendix 3**). Control + insulin had higher NO production compared to blueberry + insulin + H_2O_2 and placebo + insulin + H_2O_2 (both $p < 0.001$) (**Figure 5.2** and **Supplementary Table 5.1** in **Appendix 3**).

A moderate positive correlation was found between plasma nitrate/nitrite concentration at 12 weeks and NO production in HAECs for blueberry + insulin + H_2O_2 ($p = 0.010$), as well as a moderate inverse correlation between plasma vanillic acid-4-sulfate concentrations at 12 weeks and NO production in HAECs for blueberry + insulin + H_2O_2 ($p = 0.033$) (**Table 5.1** and **5.2**). Lastly, there was a moderate positive correlation between plasma protocatechuic acid at 12 weeks and NO production in HAECs for placebo + insulin + H_2O_2 ($p = 0.032$) (**Table 5.2**).

An effect between groups was also found for peroxynitrite concentrations ($p < 0.001$) with controls (with and without H_2O_2) having lower peroxynitrite concentrations compared to blueberry + H_2O_2 and placebo + H_2O_2 (both $p < 0.001$) (**Figure 5.3** and **Supplementary Table 5.1** in **Appendix 3**). No significant correlations were found between blood lipids/biomarkers and peroxynitrite concentrations in HAECs for either the blueberry or placebo serum ($p > 0.05$) (**Table 5.1**). Similarly, no significant correlations were found between plasma (poly)phenol metabolites and peroxynitrite concentrations in HAECs for either the blueberry or placebo serum ($p > 0.05$) (**Table 5.2**).

Discussion

To our knowledge, this is the first study to develop a protocol for evaluating the atheroprotective effects of (poly)phenol metabolites in human blood serum on H_2O_2-induced dysfunctional HAECs. Results showed a strong statistical trend for increased monocyte binding after incubating HAECs with 200 μM H_2O_2 for 24 h. A previous study showed that adding 1,000 μM H_2O_2 to HAECs for 4 h significantly increased monocyte binding, therefore, it is possible that HAECs in the current study needed to be incubated with higher amounts of H_2O_2 for a shorter timeframe to see significant increases in monocyte binding.[35] However, results from our preliminary experiment (data not shown) determined that incubating HAECs with 500 μM H_2O_2 for 24 h killed cells, thus, could not be used for these experiments. Nonetheless, the strong trend for increased the monocyte binding to HAECs suggests the potential for using this *ex vivo* model to assess monocyte binding to oxidative stress-induced dysfunctional HAECs. We also found that 200 μM H_2O_2 did not significantly decrease insulin-stimulated NO production or increase peroxynitrite concentrations in HAECs. It has been shown that incubating with 200 μM H_2O_2 for 24 h and 100 μM H_2O_2 for 4 h increased intracellular ROS concentrations in HAECs, but those studies did not measure NO nor peroxynitrite production.[34, 35] However, studies have shown that 100 μM H_2O_2 for 1 h and 500 μM H_2O_2 for 4 h significantly decreased NO concentrations in supernatant from HUVECs.[36, 37] It should be noted that these aforementioned studies measuring the effects of H_2O_2 on NO concentrations did not stimulate the production of NO (e.g. with insulin) and future research could explore whether simulation of NO affects the outcomes. Surprisingly, it seems that *ex vivo* research has not measured peroxynitrite in HAECs after inducing dysfunction with H_2O_2, therefore, this was a highly exploratory experiment and should be tested again for repeatability.

Regarding the effects of blueberry-derived (poly)phenol metabolites on the outcomes of the current study, there were no differences between groups on monocyte binding, insulin-stimulated NO production, or peroxynitrite concentrations in dysfunctional HAECs. It has been

shown that palmitate-induced dysfunctional HAECs treated with physiological concentrations of synthetic blueberry-derived (poly)phenol metabolites for 6 h reduced monocyte (THP-1) binding and increased insulin-stimulated NO production.[30] Another study showed reduced monocyte (peripheral blood mononuclear cells) binding to TNF-α-induced dysfunctional HUVECs incubated with physiological concentrations of blueberry-derived (poly)phenol metabolites for 4 h.[31] However, those studies did not assess monocyte binding affinity to H_2O_2-induced dysfunctional endothelial cells, which is a novel aspect of the present study. Furthermore, the findings of these previous studies suggest that a longer incubation time (i.e. HAECs with serum) may be needed for the (poly)phenol metabolites to enter endothelial cells to exert their effects. In support of this hypothesis, cell culture studies have demonstrated it takes 2 h for flavonoid metabolites to enter endothelial cells.[40, 41] Future research incubating HAECs with serum containing (poly)phenol metabolites for varying amounts of time prior to inducing dysfunction with H_2O_2 is needed to evaluate this hypothesis and determine whether there are differences in monocyte binding affinity, NO production, and/or peroxynitrite concentrations in HAECs.

Surprisingly, treatment with human blood serum (irrespective of blueberry or placebo consumption) and H_2O_2 in HAECs decreased insulin-stimulated NO production and increased peroxynitrite concentrations to a greater extent than H_2O_2 alone. These results suggest that the degree of oxidative stress (and potentially inflammation) might outweigh the protective effects blueberry-derived (poly)phenol metabolites in this *ex vivo* cell culture model. Extracellular ROS including superoxide and H_2O_2, can easily pass through cell membranes, and therefore, stimulate intracellular ROS production and pro-inflammatory pathways, ultimately leading to eNOS uncoupling and decreased NO production.[3, 7, 8, 42-44] Our study population was shown to have oxidative stress-induced endothelial dysfunction, so it is plausible that elevated oxidative stress in human blood serum increased oxidative stress and decreased NO production/bioavailability in the cultured HAECs. However, this cannot be determined at this time since measures of oxidative stress were not assessed in the serum/plasma from the

clinical trial. Furthermore, it could be possible that incubation of HAECs with H_2O_2 and human blood serum could have interfered with insulin-NO signaling in the endothelial cell, and thus, decrease NO production. Regardless, future research is needed to evaluate the impact of using human serum from diverse populations (e.g. different health/disease states) on NO production and peroxynitrite concentrations in this *ex vivo* cell culture model.

Regarding the strengths of the current study, we aimed to establish an *ex vivo* model to evaluate effects and mechanisms of 12 weeks of blueberry consumption by postmenopausal women with above-normal blood pressure on oxidative stress-mediated endothelial cell dysfunction and monocyte binding in HAECs. Lower doses of H_2O_2 to induce endothelial dysfunction were used, as well as human serum containing blueberry-derived (poly)phenol metabolites in order to study clinically and physiologically relevant mechanisms. Results generated from this study provide novel insight for future research. The limitations to this study that have yet to be discussed are worth mentioning. First, Del Bo *et al*. (2016)[45] found no effects on monocyte binding when HUVECs were treated with physiological levels of certain individual blueberry metabolites prior to inducing dysfunction with TNF-α but found decreased monocyte binding to HUVECs when dysfunction was establish prior to incubation with the metabolites (Del Bo 2019).[46] This suggests that blueberry consumption might exert differential effects on the prevention and management of atherosclerosis; however, the current study was designed to evaluate the prevention of atherogenesis (i.e. monocyte binding), and thus, cannot be confirmed at this time. Next, we did not measure levels of (poly)phenols in serum, only in plasma; therefore, we relied on the values of plasma (poly)phenols to reflect serum values. Furthermore, since we used human serum, other factors found in serum (e.g. blood cholesterol, lipids, adhesion molecules, free radicals) could be contributing to the increased monocyte binding, lower NO production, and higher concentrations of peroxynitrite in HACEs incubated with serum. However, correlation analyses indicated no relationship with measured blood biomarkers from the clinical study with the current findings. Another limitation is that the timing of blueberry

consumption *vs* blood draw time was not standardized across participants, so it is possible that individuals had differential circulating concentrations of (poly)phenol metabolites which could have impacted the current results. Finally, this is an *ex* vivo cell culture study that may not reflect *in vivo* mechanisms in humans, and therefore, the results should be interpreted with caution.

Conclusions

Overall, this *ex vivo* study provides crucial insight for future research aiming to explore the effects of oxidative stress on monocyte binding, NO production, and peroxynitrite concentrations in HAECs, and the impact of (poly)phenol-rich foods on attenuating adverse effects. Due to the non-statistically significant findings regarding the *ex vivo* model, future research could either test higher H_2O_2 concentrations and/or longer incubation times with H_2O_2 to determine ideal concentrations and incubation timeframes to observe significant increases in monocyte binding and reductions in insulin-stimulated NO production. Reasons for why H_2O_2 did not affect peroxynitrite levels in HAECs is currently unknown, as it has been demonstrated that H_2O_2 stimulates peroxynitrite production.[33] Lastly, we hypothesize that 1) the amount of time serum was incubated in endothelial cells (1 h) might have been too short in order for (poly)phenols to penetrate into cells, 2) the use of human serum from a population with oxidative stress in combination with adding H_2O_2, and/or 3) incubation of HAECs with serum containing (poly)phenol metabolites prior to establishing H_2O_2-induced dysfunction, may have contributed to the current results.

Figures and Tables

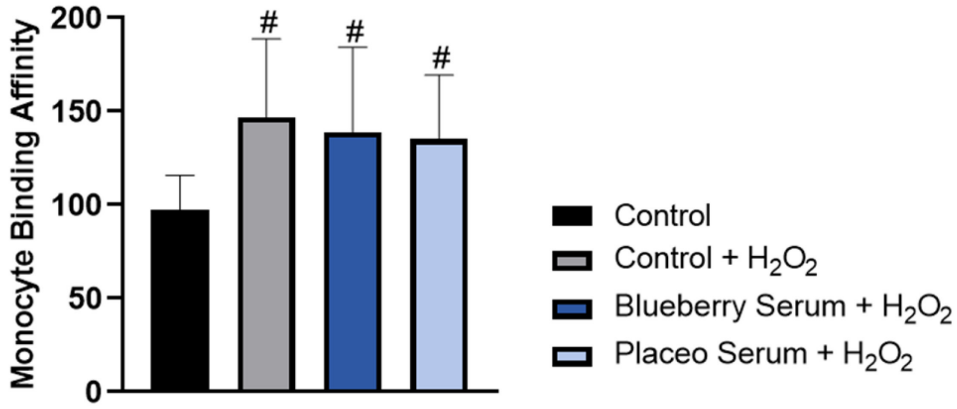


Figure 5.1. Monocyte binding affinity to human aortic endothelial cells (HAECs) treated with blueberry or placebo serum for 1 h and the hydrogen peroxide (H_2O_2) for 24 h. Data are presented as relative fluorescence to control (mean ± SEM). #Trending different compared to control (without H_2O_2) ($p < 0.08$).

Table 5.1. Monocyte (THP-1) binding, insulin-stimulated nitric oxide production, and peroxynitrite concentrations in human aortic endothelial cells correlated to plasma lipids and biomarkers from postmenopausal women with above-normal blood pressure who consumed 22 g freeze-dried highbush blueberry powder or placebo powder for 12 weeks in a randomized clinical trial.

	TG	TC	HDL-C	LDL-C	VCAM-1	ICAM-1	Nitrate/ nitrite	Estradiol
Blueberry + H_2O_2 (*n* = 22)								
Monocyte Binding	-0.107	0.130	0.266	-0.045	0.133	-0.212	-0.021	0.284
Nitric Oxide^	0.187	-0.272	-0.091	-0.249	-0.313	0.338	0.537*	0.138
Peroxynitrite	0.084	0.254	0.023	0.264	-0.161	0.095	0.312	0.108
Placebo + H_2O_2 (*n* = 20)								
Monocyte Binding	-0.029	0.060	0.169	0.053	0.042	-0.053	0.108	-0.142
Nitric Oxide^	0.212	-0.131	-0.193	-0.037	-0.113	-0.179	0.165	-0.039
Peroxynitrite	0.297	0.105	-0.264	0.091	0.171	0.038	0.094	0.027

Data are Spearman's Rho. *Significant correlation ($p < 0.05$). ^Indicates that insulin was also added to blueberry and placebo serum groups for nitric oxide experiment. Abbreviations: HDL-C, high-density lipoprotein-cholesterol, LDL-C, low-density lipoprotein-cholesterol, TC, total cholesterol, TG, triglycerides, ICAM-1, intercellular adhesion molecule-1, VCAM-1, vascular cell adhesion protein-1.

Table 5.2. Monocyte (THP-1) binding, insulin-stimulated nitric oxide production, and peroxynitrite concentrations in human aortic endothelial cells correlated to plasma (poly)phenol metabolites from postmenopausal women with above-normal blood pressure who consumed 22 g freeze-dried highbush blueberry powder or placebo powder for 12 weeks in a randomized clinical trial.

	Sum of Metabolites	Hippuric Acid	3-Hydroxyhippuric Acid	Vanillic Acid-4-Sulfate	Protocatechuic Acid	2,5-Dihydroxy-benzoic Acid
Blueberry + H_2O_2 (*n* = 22)						
Monocyte Binding	-0.135	-0.127	-0.008	0.057	0.165	-0.135
Nitric Oxide	-0.003	0.020	-0.179	-0.475*	-0.232	0.011
Peroxynitrite	0.083	0.104	0.149	0.118	0.030	0.086
Placebo + H_2O_2 (*n* = 20)						
Monocyte Binding	0.132	0.215	0.065	-0.004	-0.290	0.009
Nitric Oxide	0.186	-0.002	-0.056	-0.153	0.481*	0.144
Peroxynitrite	-0.050	-0.170	0.104	0.095	0.056	0.251

Data are Spearman's Rho. *Significant correlation ($p < 0.05$).

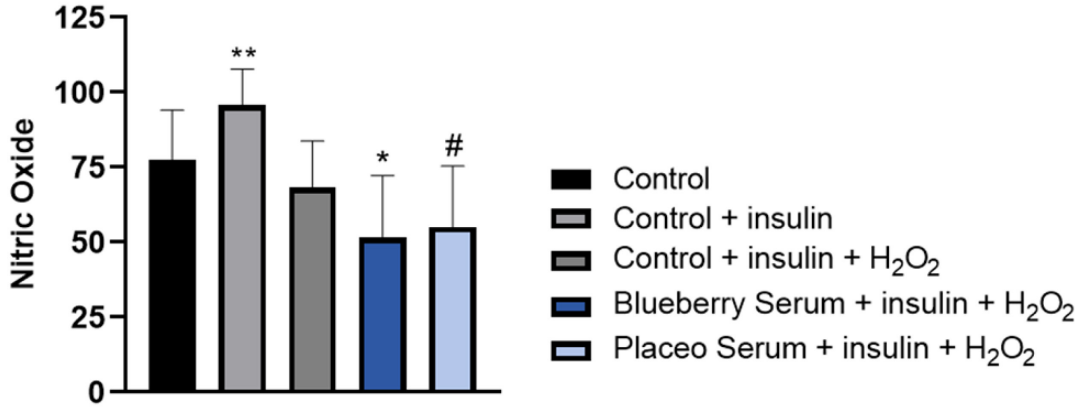


Figure 5.2. Insulin-stimulated nitric oxide (NO) production in human aortic endothelial cells (HAECs) treated with blueberry or placebo serum for 1 h and the hydrogen peroxide (H_2O_2) for 24 h. **Different from blueberry and placebo ($p < 0.001$). Data are presented as relative fluorescence to control (mean ± SEM). *Different from control (without H_2O_2) ($p < 0.05$). #Trending different from control (without H_2O_2) ($p < 0.06$).

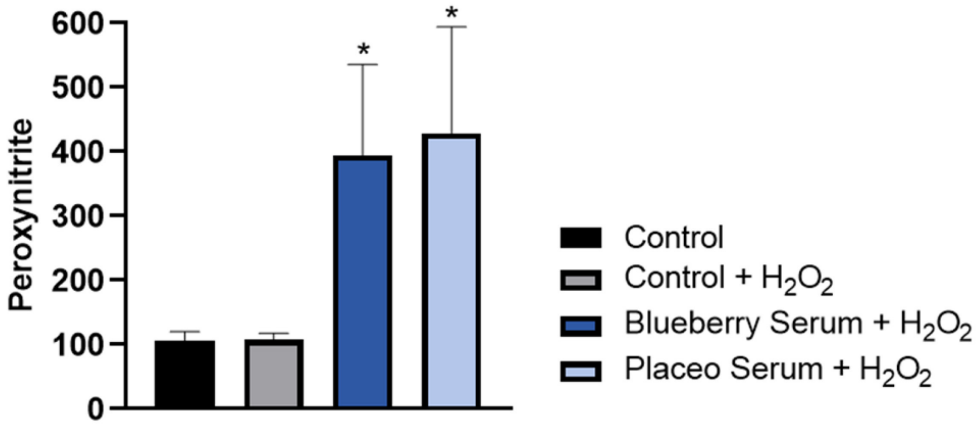


Figure 5.3. Peroxynitrite concentrations in human aortic endothelial cells (HAECs) treated with blueberry or placebo serum for 1 h and the hydrogen peroxide (H_2O_2) for 24 h. Data are presented as relative fluorescence to control (mean ± SEM). *Different from controls with and without H_2O_2 ($p < 0.001$).

REFERENCES

1. R. Pahwa and I. Jialal, in *StatPearls*, StatPearls Publishing Copyright © 2023, StatPearls Publishing LLC., Treasure Island (FL), 2023.
2. C. Bleakley, P. K. Hamilton, R. Pumb, M. Harbinson and G. E. McVeigh, *J Clin Hypertens (Greenwich)*, 2015, **17**, 651-654.
3. T. D. Giles, G. E. Sander, B. D. Nossaman and P. J. Kadowitz, *J Clin Hypertens (Greenwich)*, 2012, **14**, 198-205.
4. P. M. Vanhoutte, H. Shimokawa, M. Feletou and E. H. Tang, *Acta Physiol (Oxf)*, 2017, **219**, 22-96.
5. K. H. Park and W. J. Park, *J Korean Med Sci*, 2015, **30**, 1213-1225.
6. H. N. Siti, Y. Kamisah and J. Kamsiah, *Vascul Pharmacol*, 2015, **71**, 40-56.
7. M. A. Incalza, R. D'Oria, A. Natalicchio, S. Perrini, L. Laviola and F. Giorgino, *Vascul Pharmacol*, 2018, **100**, 1-19.
8. C. M. Steyers, 3rd and F. J. Miller, Jr., *Int J Mol Sci*, 2014, **15**, 11324-11349.
9. P. S. Tsao, B. Wang, R. Buitrago, J. Y. Shyy and J. P. Cooke, *Circulation*, 1997, **96**, 934-940.
10. F. Gao, B. P. Lucke-Wold, X. Li, A. F. Logsdon, L. C. Xu, S. Xu, K. B. LaPenna, H. Wang, M. A. H. Talukder, C. A. Siedlecki, J. D. Huber, C. L. Rosen and P. He, *Front Physiol*, 2017, **8**, 1124.
11. B. J. North and D. A. Sinclair, *Circ Res*, 2012, **110**, 1097-1108.
12. I. Liguori, G. Russo, F. Curcio, G. Bulli, L. Aran, D. Della-Morte, G. Gargiulo, G. Testa, F. Cacciatore, D. Bonaduce and P. Abete, *Clin Interv Aging*, 2018, **13**, 757-772.
13. D. R. Seals, K. L. Jablonski and A. J. Donato, *Clin Sci (Lond)*, 2011, **120**, 357-375.
14. K. L. Moreau, K. L. Hildreth, A. L. Meditz, K. D. Deane and W. M. Kohrt, *J Clin Endocrinol Metab*, 2012, **97**, 4692-4700.

15. K. L. Moreau and K. L. Hildreth, *Adv Vasc Med*, 2014, **2014**.

16. K. L. Moreau, K. L. Hildreth, J. Klawitter, P. Blatchford and W. M. Kohrt, *Geroscience*, 2020, **42**, 1699-1714.

17. E. K. Woolf, J. D. Terwoord, N. S. Litwin, A. R. Vazquez, S. Y. Lee, N. Ghanem, K. A. Michell, B. T. Smith, L. E. Grabos, N. B. Ketelhut, N. P. Bachman, M. E. Smith, M. Le Sayec, S. Rao, C. L. Gentile, T. L. Weir, A. Rodriguez-Mateos, D. R. Seals, F. A. Dinenno and S. A. Johnson, *Food Funct*, 2023, **14**, 2621-2641.

18. Y. B. Somani, J. A. Pawelczyk, M. J. De Souza, P. M. Kris-Etherton and D. N. Proctor, *Am J Physiol Heart Circ Physiol*, 2019, **317**, H395-h404.

19. A. Iorga, C. M. Cunningham, S. Moazeni, G. Ruffenach, S. Umar and M. Eghbali, *Biol Sex Differ*, 2017, **8**, 33.

20. A. V. Poznyak, N. K. Sadykhov, A. G. Kartuesov, E. E. Borisov, A. A. Melnichenko, A. V. Grechko and A. N. Orekhov, *Front Cardiovasc Med*, 2022, **9**, 959285.

21. Q. N. Dinh, G. R. Drummond, C. G. Sobey and S. Chrissobolis, *Biomed Res Int*, 2014, **2014**, 406960.

22. J. Hsia, K. L. Margolis, C. B. Eaton, N. K. Wenger, M. Allison, L. Wu, A. Z. LaCroix and H. R. Black, *Circulation*, 2007, **115**, 855-860.

23. E. Pinto, *Postgrad Med J*, 2007, **83**, 109-114.

24. P. J. Tuso, M. H. Ismail, B. P. Ha and C. Bartolotto, *Perm J*, 2013, **17**, 61-66.

25. P. Tuso, S. R. Stoll and W. W. Li, *Perm J*, 2015, **19**, 62-67.

26. D. Ornish, L. W. Scherwitz, J. H. Billings, S. E. Brown, K. L. Gould, T. A. Merritt, S. Sparler, W. T. Armstrong, T. A. Ports, R. L. Kirkeeide, C. Hogeboom and R. J. Brand, *Jama*, 1998, **280**, 2001-2007.

27. H. Kahleova, S. Levin and N. D. Barnard, *Prog Cardiovasc Dis*, 2018, **61**, 54-61.

28. E. Wood, S. Hein, C. Heiss, C. Williams and A. Rodriguez-Mateos, *Food Funct*, 2019, **10**, 7621-7633.

29. S. Zhong, A. Sandhu, I. Edirisinghe and B. Burton-Freeman, *Mol Nutr Food Res*, 2017, **61**.

30. D. Bharat, R. R. M. Cavalcanti, C. Petersen, N. Begaye, B. R. Cutler, M. M. A. Costa, R. Ramos, M. R. Ferreira, Y. Li, L. P. Bharath, E. Toolson, P. Sebahar, R. E. Looper, T. Jalili, N. S. Rajasekaran, Z. Jia, J. D. Symons and P. V. Anandh Babu, *Mol Nutr Food Res*, 2018, **62**.

31. J. S. Tang, S. M. Bozonet, J. L. McKenzie, R. F. Anderson, L. D. Melton and M. C. M. Vissers, *Mol Nutr Food Res*, 2019, **63**, e1900478.

32. H. J. Forman, A. Bernardo and K. J. Davies, *Arch Biochem Biophys*, 2016, **603**, 48-53.

33. C. H. Coyle and K. N. Kader, *Asaio j*, 2007, **53**, 17-22.

34. C. S. Chen, B. Y. Pan, P. H. Tsai, F. Y. Chen, W. C. Yang and M. Y. Shen, *Int J Mol Sci*, 2021, **22**.

35. D. Shen, L. Tian, T. Shen, H. Sun and P. Liu, *Cell Physiol Biochem*, 2018, **47**, 2261-2277.

36. Y. Sun, X. Hu, G. Hu, C. Xu and H. Jiang, *Biol Pharm Bull*, 2015, **38**, 1134-1141.

37. S. Y. Jeon, M. R. Kim, S. H. Yu, M. J. Kim, K. S. Shim, E. Shin, J. J. Lee and Y. C. Lee, *Prev Nutr Food Sci*, 2020, **25**, 173-183.

38. C. Petersen, D. Bharat, B. R. Cutler, S. Gholami, C. Denetso, J. E. Mueller, J. M. Cho, J. S. Kim, J. D. Symons and P. V. Anandh Babu, *Int J Cardiol*, 2018, **263**, 111-117.

39. L. P. Bharath, R. Mueller, Y. Li, T. Ruan, D. Kunz, R. Goodrich, T. Mills, L. Deeter, A. Sargsyan, P. V. Anandh Babu, T. E. Graham and J. D. Symons, *Can J Physiol Pharmacol*, 2014, **92**, 605-612.

40. N. Toro-Funes, F. J. Morales-Gutiérrez, M. T. Veciana-Nogués, M. C. Vidal-Carou, J. P. Spencer and A. Rodriguez-Mateos, *Food Funct*, 2015, **6**, 98-108.

41. A. Rodriguez-Mateos, N. Toro-Funes, T. Cifuentes-Gomez, M. Cortese-Krott, C. Heiss and J. P. Spencer, *Arch Biochem Biophys*, 2014, **559**, 17-23.

42. R. Bretón-Romero and S. Lamas, *Redox Biol*, 2014, **2**, 529-534.

43. W. H. Park, *Int J Mol Med*, 2013, **31**, 471-476.

44. B. J. Hawkins, M. Madesh, C. J. Kirkpatrick and A. B. Fisher, *Mol Biol Cell*, 2007, **18**, 2002-2012.

45. C. Del Bo, M. Roursgaard, M. Porrini, S. Loft, P. Møller and P. Riso, *Mol Nutr Food Res*, 2016, **60**, 2355-2366.

46. C. Del Bo, M. Marino, P. Riso, P. Møller and M. Porrini, *Chem Biol Interact*, 2019, **300**, 49-55.

CHAPTER 6: SUMMARY AND FUTURE DIRECTIONS

The first objective of this dissertation was to examine the efficacy of chronic blueberry consumption to improve endothelial function and blood pressure in estrogen-deficient postmenopausal women with above-normal blood pressure, with a specific focus on identifying mechanisms for improving endothelial function. Based on previous research,[1-8] we hypothesized that daily consumption of 22 g of freeze-dried highbush blueberry powder for 12 weeks would improve endothelial function through reductions in oxidative stress, as well as blood pressure and other biomarkers of cardiometabolic health in postmenopausal women with above-normal blood pressure. The second objective of this dissertation was to identify factors that contributed to the efficacy of blueberries as a dietary intervention for improving endothelial function and we hypothesized that overall health/disease risk status at baseline would impact the direction and magnitude of the response to blueberry consumption. Lastly, the third objective of the dissertation was to explore cellular mechanisms responsible for endothelial function improvements and the anti-atherogenic potential of blueberries. We hypothesized that blueberry (poly)phenol metabolite-rich human serum collected after 12 weeks of daily blueberry consumption would inhibit monocyte binding to dysfunctional human aortic endothelial cells (HAECs), as well as increase insulin-stimulated NO production and decrease peroxynitrite concentrations.

In agreement with our first hypothesis, we found that daily consumption of 22 g freeze-dried highbush blueberry powder significantly improved flow-mediated dilation (FMD) normalized to shear rate area under the curve (FMD/SR_{AUC}) by 96% through reductions in oxidative stress. We also found that blueberry consumption non-statistically significantly improved FMD (+1.34% units), but such improvements were clinically relevant. Blueberry consumption was also able to significantly increase the sum of plasma (poly)phenol metabolites and individual flavonoid and gut microbial (poly)phenol metabolites, confirming compliance with

the blueberry intervention and indicating an important role of the gut microbiome in blueberry (poly)phenol bioavailability. Furthermore, daily blueberry consumption for 12 weeks did not exert any major effects on blood pressure, arterial stiffness, endothelial cell protein expressions, or other biomarkers of cardiometabolic health. It is unknown why blood pressure did not improve in this population as we have previously demonstrated reductions in blood pressure following 8 weeks of daily blueberry consumption.[2] The body of evidence is mixed regarding chronic blueberry consumption for improving blood pressure;[1-4, 9-14] however, the method of assessment (e.g. office *vs* 24-h blood pressure) might have impacted the results and future research is needed to explore this. However, this is supported by a recent study that found reductions in 24-h ambulatory systolic blood pressure, but not office blood pressure, in healthy older adults after consuming 26 g of freeze-dried wild blueberry powder for 12 weeks.[13] Furthermore, chronic blueberry consumption did not improve carotid to femoral pulse wave velocity (arterial stiffness) in this study population, though this is not surprising as research has yet to demonstrate improvements in this parameter with blueberry consumption.[1-3, 11, 13, 14] It is possible that the duration of the study was not long enough to see changes in central arterial stiffness, which highlights a gap in the knowledge and the need for future research. Lastly, the direct mechanisms for improvements in endothelial function in our study are currently unknown since we did not observe any changes in endothelial cell protein expression after blueberry consumption and other mechanistic assessments were not performed. It is possible that small changes in protein expression were undetected and/or that the study was not powdered enough to observe significant changes, thus, future research is needed to evaluate mechanisms in humans.

Regarding the second objective and hypothesis, we found that ~50% of participants that consumed 22 g of freeze-dried highbush blueberry powder daily for 12 weeks in the clinical trial were able to improve their FMD to clinical relevant levels of ≥ 1% (i.e. responders). We found that responders were in a worse disease risk state compared to non-responders, with

responders with having greater impairments in endothelial function, oxidative stress-mediated suppression of endothelial function, low-density lipoprotein cholesterol, and intercellular adhesion molecule-1, but lower high-density lipoprotein concentrations at baseline compared to non-responders. After 12 weeks of blueberry consumption, responders had reductions in oxidative stress-mediated suppression of endothelial function, lower plasma nitrate levels, and higher phosphorylated endothelial nitric oxide synthase protein expression compared to non-responders supporting possible mechanisms for such reductions. Additionally, estradiol was significantly higher in responders compared to non-responders at baseline and correlated with the FMD change from baseline to 12 weeks for responders, indicating estradiol might be affecting response to treatment as seen in previous interventional studies with postmenopausal women and exercise.[15, 16] Future research might be able to confirm this by testing blueberry consumption in pre- and postmenopausal women, and/or adding estrogen replacement with blueberry consumption to confirm this hypothesis. It is important to highlight that these results were determined based on a secondary analysis of data collected from the original clinical trial. Therefore, future research could further evaluate these findings in a study designed to test the effects of blueberry consumption on endothelial function in various health statuses of postmenopausal women

Lastly, for our third objective and hypothesis, we did not find that blueberry (poly)phenol metabolite-rich human serum collected after 12 weeks of daily blueberry consumption prevented monocyte binding to HAECs. Furthermore, serum from the blueberry group in the clinical trial did not increase nitric oxide production nor decrease peroxynitrite concentrations in dysfunctional HAECs. Therefore, we cannot determine mechanisms by which blueberry consumption improved endothelial function and/or the broader atheroprotective effects of blueberries at this time. However, one possible reason for these null effects is that we used serum collected from a human population known to have increased oxidative stress and inflammation, and other cardiometabolic risk factors. Therefore, other factors in serum could

potentially have impacted the outcomes of this study warranting the need for future research regarding using human serum for *ex vivo* cell culture experiments.

In conclusion, the results of these studies suggest that daily/chronic blueberry consumption is a promising dietary intervention for decreasing cardiovascular disease (CVD) risk in postmenopausal women with above-normal blood pressure, particularly though effects on endothelial function. We found that daily consumption of 22 g freeze-dried highbush blueberry powder for 12 weeks significantly improved endothelial function through reductions of oxidative stress in postmenopausal women with above-normal blood pressure. Furthermore, we determined that the disease risk/health status of the postmenopausal women in the clinical trial impacted the degree to which they responded to the intervention. The exact mechanisms responsible for blueberries improving endothelial function in postmenopausal women were not able to be determined through these research objectives of this dissertation. Future research is needed to fully understand the cardioprotective effects and associated mechanisms for using blueberries at a dietary intervention to reduce CVD risk.

REFERENCES

1. P. J. Curtis, V. van der Velpen, L. Berends, A. Jennings, M. Feelisch, A. M. Umpleby, M. Evans, B. O. Fernandez, M. S. Meiss, M. Minnion, J. Potter, A. M. Minihane, C. D. Kay, E. B. Rimm and A. Cassidy, *Am J Clin Nutr*, 2019, **109**, 1535-1545.
2. S. A. Johnson, A. Figueroa, N. Navaei, A. Wong, R. Kalfon, L. T. Ormsbee, R. G. Feresin, M. L. Elam, S. Hooshmand, M. E. Payton and B. H. Arjmandi, *J Acad Nutr Diet*, 2015, **115**, 369-377.
3. A. Rodriguez-Mateos, G. Istas, L. Boschek, R. P. Feliciano, C. E. Mills, C. Boby, S. Gomez-Alonso, D. Milenkovic and C. Heiss, *J Gerontol A Biol Sci Med Sci*, 2019, **74**, 967-976.
4. A. J. Stull, K. C. Cash, C. M. Champagne, A. K. Gupta, R. Boston, R. A. Beyl, W. D. Johnson and W. T. Cefalu, *Nutrients*, 2015, **7**, 4107-4123.
5. J. S. Tang, M. C. M. Vissers, R. F. Anderson, S. Sreebhavan, S. M. Bozonet, A. Scheepens and L. D. Melton, *Mol Nutr Food Res*, 2018, **62**.
6. S. A. Johnson, R. G. Feresin, N. Navaei, A. Figueroa, M. L. Elam, N. S. Akhavan, S. Hooshmand, S. Pourafshar, M. E. Payton and B. H. Arjmandi, *Food Funct*, 2017, **8**, 372-380.
7. D. Bharat, R. R. M. Cavalcanti, C. Petersen, N. Begaye, B. R. Cutler, M. M. A. Costa, R. Ramos, M. R. Ferreira, Y. Li, L. P. Bharath, E. Toolson, P. Sebahar, R. E. Looper, T. Jalili, N. S. Rajasekaran, Z. Jia, J. D. Symons and P. V. Anandh Babu, *Mol Nutr Food Res*, 2018, **62**.
8. J. S. Tang, S. M. Bozonet, J. L. McKenzie, R. F. Anderson, L. D. Melton and M. C. M. Vissers, *Mol Nutr Food Res*, 2019, **63**, e1900478.

9. K. S. Stote, M. I. Sweeney, T. Kean, D. J. Baer, J. A. Novotny, N. L. Shakerley, A. Chandrasekaran, P. M. Carrico, J. A. Melendez and K. T. Gottschall-Pass, *BMC Nutr*, 2017, **3**, 45.
10. P. Riso, D. Klimis-Zacas, C. Del Bo, D. Martini, J. Campolo, S. Vendrame, P. Møller, S. Loft, R. De Maria and M. Porrini, *Eur J Nutr*, 2013, **52**, 949-961.
11. L. S. McAnulty, S. R. Collier, M. J. Landram, D. S. Whittaker, S. E. Isaacs, J. M. Klemka, S. L. Cheek, J. C. Arms and S. R. McAnulty, *Nutr Res*, 2014, **34**, 577-584.
12. S. Vendrame, T. E. Adekeye and D. Klimis-Zacas, *Nutrients*, 2022, **14**.
13. E. Wood, S. Hein, R. Mesnage, F. Fernandes, N. Abhayaratne, Y. Xu, Z. Zhang, L. Bell, C. Williams and A. Rodriguez-Mateos, *Am J Clin Nutr*, 2023, DOI: 10.1016/j.ajcnut.2023.03.017.
14. Y. Wang, J. L. Gallegos, C. Haskell-Ramsay and J. K. Lodge, *Nutrients*, 2022, **14**.
15. K. L. Moreau, B. L. Stauffer, W. M. Kohrt and D. R. Seals, *J Clin Endocrinol Metab*, 2013, **98**, 4507-4515.
16. D. R. Seals, E. E. Nagy and K. L. Moreau, *J Physiol*, 2019, **597**, 4901-4914.

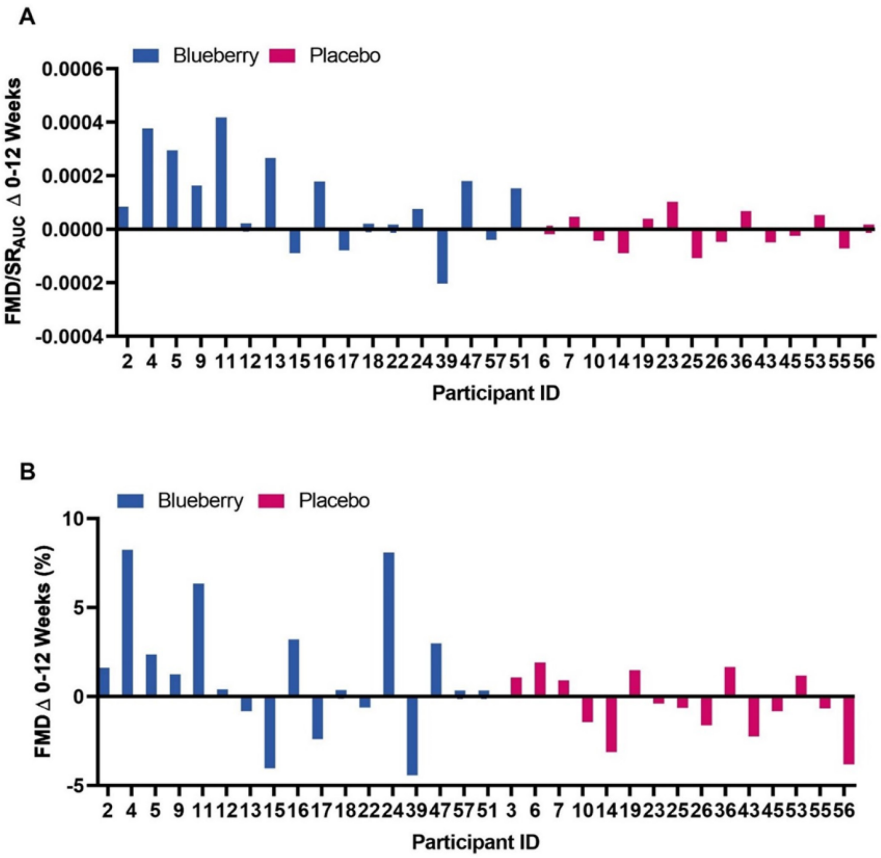
APPENDIX 1: SUPPLEMENTARY DATA FOR CHAPTER 3

Supplementary Figures and **Tables**

Supplementary Table 3.1. Vascular endothelial function of postmenopausal women with above-normal blood pressure who completed a 12-week intervention evaluating 22 g freeze-dried highbush blueberry powder vs placebo powder on endothelial function, blood pressure, and other measures of cardiometabolic health.

	Blueberry (*n* = 17)	**Placebo (*n* = 15)**
Baseline Diameter (mm)		
Baseline	2.81 ± 0.10	2.78 ± 0.10
12 Weeks	2.87 ± 0.10#	2.78 ± 0.08
Δ 0 to 12 Weeks	0.06 ± 0.03	0.00 ± 0.02
Peak Diameter (mm)		
Baseline	2.91 ± 0.10	2.91 ± 0.10
12 Weeks	3.01 ± 0.10*	2.90 ± 0.09
Δ 0 to 12 Weeks	0.10 ± 0.04**	-0.01 ± 0.03
FMD (%)		
Baseline	3.84 ± 1.02	4.69 ± 0.55
12 Weeks	5.18 ± 0.98	4.29 ± 0.55
Δ 0 to 12 Weeks	1.34 ± 0.89	-0.41 ± 0.46
SR_{AUC}		
Baseline	35992 ± 3140	37573 ± 4569
12 Weeks	26241 ± 3271#	32177 ± 3422
Δ 0 to 12 Weeks	-9741 ± 2157	-5395 ± 4692
FMD/SR_{AUC}		
Baseline	1.13e-04 ± 3.03e-05	1.41e-04 ± 1.24e-05
12 Weeks	2.22e-04 ± 3.30e-05*	1.37e-04 ± 1.32e-05
Δ 0 to 12 Weeks	1.09e-04 ± 4.12e-05a	3.82e-06 ± 1.59e05b

Data are mean ± SEM. *Different ($p < 0.05$) than baseline. #Trend for different ($p < 0.1$) than baseline. Different letters indicate a difference ($p < 0.05$) between groups. Abbreviations: FMD, flow-mediated dilation; SR_{AUC}, shear rate area under the curve.



Supplementary Figure 3.1. (A) Individual changes from baseline to 12 weeks for FMD normalized to shear rate area under the curve (FMD/SR_{AUC}); and (B) FMD (not normalized to SR_{AUC}).

Supplementary Table 3.2. Endothelium-independent dilation to sublingual nitroglycerin (NTG) in postmenopausal women with above-normal blood pressure who completed a 12-week intervention evaluating 22 g freeze-dried highbush blueberry powder vs placebo powder on endothelial function, blood pressure, and other measures of cardiometabolic health.

	Blueberry (*n* = 11)	**Placebo (*n* = 9)**
Baseline Diameter (mm)		
Baseline	2.71 ± 0.12	2.74 ± 0.15
12 Weeks	2.77 ± 0.12	2.71 ± 0.13
Δ 0 to 12 Weeks	0.06 ± 0.04	-0.03 ± 0.05
Peak Diameter (mm)		
Baseline	3.60 ± 0.15	3.57 ± 0.15
12 Weeks	3.63 ± 0.15	3.55 ± 0.14
Δ 0 to 12 Weeks	0.04 ± 0.06	-0.02 ± 0.05
Dilation to NTG (%)		
Baseline	33.16 ± 3.07	31.05 ± 4.86
12 Weeks	31.54 ± 4.86	31.43 ± 3.11
Δ 0 to 12 Weeks	-1.62 ± 1.13	0.38 ± 2.97

Data are mean ± SEM. No significant differences. Abbreviations: FMD, flow-mediated dilation; NTG, nitroglycerin.

Supplementary Table 3.3. Vascular endothelial function responses to a supraphysiologic dose of ascorbic acid in postmenopausal women with above-normal blood pressure who completed a 12-week intervention evaluating 22 g freeze-dried highbush blueberry powder vs placebo powder on endothelial function, blood pressure, and other measures of cardiometabolic health.

Blueberry	**Saline (*n* = 17)**	**Ascorbic Acid (*n* = 17)**	**Saline *vs* Ascorbic Acid Difference**
Baseline Diameter (mm)			
Baseline	2.81 ± 0.10	2.72 ± 0.09#	-0.08 ± 0.03
12 Weeks	2.87 ± 0.10	2.69 ± 0.09*	-0.15 ± 0.03
Peak Diameter (mm)			
Baseline	2.91 ± 0.10	2.90 ± 0.09	-0.08 ± 0.03
12 Weeks	3.01 ± 0.10	2.84 ± 0.08*	-0.15 ± 0.03^
FMD (%)			
Baseline	3.84 ± 1.02	6.88 ± 1.41*	3.04 ± 1.11
12 Weeks	5.18 ± 0.98	6.07 ± 0.89	0.69 ± 0.62
SR_{AUC}/Peak			
Baseline	35992 ± 3140	27538 ± 3402#	-8454 ± 2980
12 Weeks	26241 ± 3271	31358 ± 3716	6107 ± 2020**
FMD/SR_{AUC}			
Baseline	1.13e-04 ± 3.03e-05	3.42e-04 ± 1.16e-04*	2.29e-04 ± 3.61e-07
12 Weeks	2.22e-04 ± 3.30e-05	2.20e-04 ± 2.45e-05	-1.86e-05 ± 4.59e-05**
Placebo	**Saline (*n* = 15)**	**Ascorbic Acid (*n* = 15)**	**Saline *vs* Ascorbic Acid Difference**
Baseline Diameter (mm)			
Baseline	2.78 ± 0.10	2.66 ± 0.09*	-0.12 ± 0.03
12 Weeks	2.78 ± 0.08	2.69 ± 0.07*	-0.10 ± 0.02
Peak Diameter (mm)			
Baseline	2.91 ± 0.10	2.84 ± 0.08	-0.07 ± 0.03
12 Weeks	2.90 ± 0.09	2.83 ± 0.08	-0.08 ± 0.04
FMD (%)			
Baseline	4.69 ± 0.55	6.81 ± 1.41#	2.39 ± 0.82
12 Weeks	4.29 ± 0.55	5.29 ± 0.85	1.19 ± 0.97
SR_{AUC}/Peak			
Baseline	37573 ± 4569	32238 ± 3859	-2357 ± 2518
12 Weeks	32177 ± 3422	30139 ± 3149	-1453 ± 1535
FMD/SR_{AUC}			
Baseline	1.41e-04 ± 1.24e-05	2.32e-04 ± 2.16e-05*	9.04e-05 ± 2.00e-05
12 Weeks	1.37e-04 ± 1.32e-05	2.20e-04 ± 4.60e-05#	8.58e-05 ± 4.59e-05

Data are mean ± SEM. *Difference ($p < 0.05$) between saline and ascorbic acid infusion on the same day. #Trend for difference ($p < 0.10$) between saline and ascorbic acid infusion on the same day. **Difference ($p < 0.05$) between baseline and 12 weeks for the difference between saline and ascorbic acid. ^Trend for difference ($p < 0.10$) between baseline and 12 weeks for the difference between saline and ascorbic acid. Abbreviations: FMD, flow-mediated dilation; SR_{AUC}, shear rate area under the curve.

Supplementary Table 3.4. Hemodynamics and arterial stiffness values for postmenopausal women with above-normal blood pressure who completed a 12-week intervention evaluating 22 g freeze-dried highbush blueberry powder *vs* placebo powder on endothelial function, blood pressure, and other measures of cardiometabolic health.

	Blueberry (*n* = 22)	Placebo (*n* = 21)
Brachial SBP (mmHg)		
Baseline	134 ± 3	131 ± 2
4 Weeks	129 ± 2	128 ± 3
8 Weeks	133 ± 2	132 ± 2
12 Weeks	134 ± 2	131 ± 2
Δ 0 to 4 Weeks	-5 ± 2	-3 ± 2
Δ 0 to 8 Weeks	1 ± 4	-1 ± 3
Δ 0 to 12 Weeks	-1 ± 2	-1 ± 1
Brachial DBP (mmHg)		
Baseline	80 ± 2	78 ± 1
4 Weeks	77 ± 1	76 ± 1
8 Weeks	79 ± 1	78 ± 1
12 Weeks	79 ± 1	79 ± 2
Δ 0 to 4 Weeks	-2 ± 1	-1 ± 1
Δ 0 to 8 Weeks	-1 ± 2	0 ± 2
Δ 0 to 12 Weeks	-1 ± 1	1 ± 2
AIx (%)		
Baseline	36 ± 1	37 ± 1
4 Weeks	33 ± 2	38 ± 2
8 Weeks	36 ± 2	36 ± 2
12 Weeks	35 ± 1	38 ± 2
Δ 0 to 4 Weeks	-2 ± 1	1 ± 2
Δ 0 to 8 Weeks	0 ± 2	-1 ± 2
Δ 0 to 12 Weeks	-1 ± 1	1 ± 1
AIx@75 (%)		
Baseline	29 ± 2	31 ± 1
4 Weeks	28 ± 2	32 ± 2
8 Weeks	29 ± 2	30 ± 2
12 Weeks	28 ± 1	32 ± 2
Δ 0 to 4 Weeks	-1 ± 2	1 ± 2
Δ 0 to 8 Weeks	0 ± 2	-1 ± 2
Δ 0 to 12 Weeks	-1 ± 2	0 ± 2
Aortic PWV (m/s)		
Baseline	7.9 ± 0.2	7.4 ± 0.2
12 Weeks	7.8 ± 0.2	7.3 ± 0.1
Δ 0 to 12 Weeks	-0.2 ± 0.2	-0.3 ± 0.2
Aortic SBP (mmHg)		
Baseline	124 ± 2	122 ± 1
4 Weeks	120 ± 2	120 ± 2
8 Weeks	124 ± 2	123 ± 2
12 Weeks	124 ± 2	121 ± 2
Δ 0 to 4 Weeks	-4 ± 2	-2 ± 2
Δ 0 to 8 Weeks	-1 ± 2	2 ± 1
Δ 0 to 12 Weeks	-1 ± 2	0 ± 1

Supplementary Table 3.4. Hemodynamics and arterial stiffness values for postmenopausal women with above-normal blood pressure who completed a 12-week intervention evaluating 22 g freeze-dried highbush blueberry powder *vs* placebo powder on endothelial function, blood pressure, and other measures of cardiometabolic health.

	Blueberry (*n* = 22)	Placebo (*n* = 21)
Aortic DBP (mmHg)		
Baseline	80 ± 2	79 ± 1
4 Weeks	79 ± 1	78 ± 1
8 Weeks	80 ± 1	79 ± 1
12 Weeks	80 ± 1	79 ± 1
Δ 0 to 4 Weeks	-1 ± 1	-2 ± 1
Δ 0 to 8 Weeks	0 ± 1	0 ± 1
Δ 0 to 12 Weeks	-1 ± 1	1 ± 2
Aortic MAP (mmHg)		
Baseline	93 ± 3	95 ± 1
4 Weeks	94 ± 1	93 ± 1
8 Weeks	96 ± 1	95 ± 1
12 Weeks	95 ± 2	94 ± 1
Δ 0 to 4 Weeks	1 ± 3	-1 ± 1
Δ 0 to 8 Weeks	3 ± 2	0 ± 1
Δ 0 to 12 Weeks	1 ± 2	0 ± 2
Aortic Pulse Pressure		
Baseline	47 ± 3	43 ± 1
4 Weeks	41 ± 1*	42 ± 2
8 Weeks	44 ± 1	44 ± 1
12 Weeks	43 ± 1	43 ± 2
Δ 0 to 4 Weeks	-6 ± 3	-1 ± 1
Δ 0 to 8 Weeks	-4 ± 3	1 ± 1
Δ 0 to 12 Weeks	-2 ± 2	0 ± 1
HR (bpm)		
Baseline	61 ± 2	61 ± 2
4 Weeks	63 ± 2	62 ± 2
8 Weeks	61 ± 2	61 ± 2
12 Weeks	61 ± 1	61 ± 1
Δ 0 to 4 Weeks	2 ± 1	2 ± 2
Δ 0 to 8 Weeks	1 ± 1	0 ± 1
Δ 0 to 12 Weeks	0 ± 1	0 ± 2
AP (mmHg)		
Baseline	16 ± 1	16 ± 1
4 Weeks	14 ± 1	16 ± 1
8 Weeks	16 ± 1	16 ± 1
12 Weeks	16 ± 1	16 ± 1
Δ 0 to 4 Weeks	-2 ± 1	0 ± 1
Δ 0 to 8 Weeks	-1 ± 1	0 ± 1
Δ 0 to 12 Weeks	0 ± 1	1 ± 1

Data are mean ± SEM. *Different ($p < 0.05$) than baseline. Abbreviations: AIx, augmentation index; AIx@75, augmentation index normalized to 75 beats per minute; AP, aortic pressure; DBP, diastolic blood pressure; HR, heart rate; MAP, mean arterial pressure; PWV, pulse wave velocity; SBP, systolic blood pressure.

Supplementary Table 3.5. Mean plasma (poly)phenol metabolite concentrations for postmenopausal women with above-normal blood pressure who completed a 12-week intervention evaluating 22 g freeze-dried highbush blueberry powder vs placebo powder on endothelial function, blood pressure, and other measures of cardiometabolic health.

	Blueberry (*n* = 22)				Placebo (*n* = 21)			
Compound (nmol/L)	**Baseline**	**Week 4**	**Week 8**	**Week 12**	**Baseline**	**Week 4**	**Week 8**	**Week 12**
Flavan-3-ols								
(–)-Epicatechin	36.88 ± 6.64	49.67 ± 8.65	47.53 ± 7.8	47.98 ± 10.36	31.09 ± 4.95	38.43 ± 7.58	45.33 ± 7.92	34.2 ± 3.64
(–)-Epicatechin-3'-sulfate	42.69 ± 3.08	55.27 ± 6.71	53.26 ± 8.4	49.88 ± 5.79	40.64 ± 3.99	36.77 ± 3.25	46.73 ± 4.86	48.89 ± 4.94
3'-Methoxy-(–)-epicatechin	721.17 ± 22.46	748.1 ± 23.08	775.6 ± 31.07	750.54 ± 27.81	729.67 ± 23.79	726.65 ± 23.2	725.66 ± 32.31	726.1 ± 21.33
4'-Methoxy-(–)-Epicatechin	852.15 ± 13.1	859.79 ± 9.09	861.29 ± 10.2	847.84 ± 11.27	841.85 ± 12.76	858.29 ± 11.99	846.64 ± 12.33	861.72 ± 10.46
Procyanidin A2	67.13 ± 3.77	69.65 ± 4.27	70.82 ± 2.78	68.5 ± 2.87	64.44 ± 2.06	62.3 ± 1.73	63.65 ± 2.06	67.49 ± 2.81
Flavonols								
Quercetin	13.34 ± 4.43	22.83 ± 7.57	8.41 ± 2.35	9.56 ± 2.42	10.05 ± 1.81	13.11 ± 2.24	32.28 ± 22.64	11.68 ± 2.09
Kaempferol-3-glucuronide	3.41 ± 0.25	3.7 ± 0.27	3.55 ± 0.31	3.53 ± 0.23	3.34 ± 0.25	3.36 ± 0.23	3.21 ± 0.23	3.11 ± 0.23
Myricetin	139.4 ± 22.31	165.49 ± 31.22*	161.62 ± 27.55	151.61 ± 23.05	118.03 ± 5.75	97.27 ± 5.21	111.19 ± 8.21	120.31 ± 6.54
Morin	0.4 ± 0.28	10.02 ± 9.26	0 ± 0	2.41 ± 2.41	0 ± 0	0 ± 0	27.29 ± 27.29	0 ± 0
Benzene diols & triols								
1,2-Dihydroxy-4-methylbenzene	1503.03 ± 209.29	2141.64 ± 311.45	2669.97 ± 748.44	2080.14 ± 341.6	1623.59 ± 191.31	1743.38 ± 192.92	2079.48 ± 400.6	1414.62 ± 191.43
2-Hydroxy-4-methylbenzene-1-sulfate	9486.53 ± 998.76	12470.44 ± 1645.82	18864 ± 7782.74	12468.05 ± 2286.31	10085.21 ± 1024.48	10674.15 ± 898.46	14468.87 ± 3955.65	9026.89 ± 1156.78
2-Hydroxybenzene-1-glucuronide	38.71 ± 15.44	165.79 ± 62.82	239.86 ± 131.97**	68.58 ± 19.57	43.92 ± 19.37	37.36 ± 13.01	82.28 ± 28.43	48.78 ± 15
2,3-Dihydroxybenzene-1-sulfate	113.37 ± 20.24	196.64 ± 33.02**	246.19 ± 55.01**	109.48 ± 10.32	105.71 ± 17.73	125.43 ± 18.05	149.08 ± 21.16	119.49 ± 18.93
2,6-Dihydroxybenzene-1-sulfate	799.51 ± 327.99	710.99 ± 210.3	726.28 ± 172.32	614.55 ± 234.39	408.08 ± 147.5	834.89 ± 366.64	404.23 ± 224.21	810.88 ± 339.62

Supplementary Table 3.5. Mean plasma (poly)phenol metabolite concentrations for postmenopausal women with above-normal blood pressure who completed a 12-week intervention evaluating 22 g freeze-dried highbush blueberry powder vs placebo powder on endothelial function, blood pressure, and other measures of cardiometabolic health.

	Blueberry (*n* = 22)				Placebo (*n* = 21)			
Compound (nmol/L)	**Baseline**	**Week 4**	**Week 8**	**Week 12**	**Baseline**	**Week 4**	**Week 8**	**Week 12**
2-Hydroxy-6-methoxybenzene-1-sulfate	1641.94 ± 564.57	2197.32 ± 538.16	1859.06 ± 376.75	1922.74 ± 499.91	1084.92 ± 312.23	2192.85 ± 842.35	1174.1 ± 541.28	2493.69 ± 1 204.12
3-Hydroxy-2-methoxybenzene-1-sulfate	214.56 ± 72.41	260.67 ± 71.62	266.09 ± 59.66	199.15 ± 73.6	235.7 ± 101	406.62 ± 179.72	296.71 ± 71.75	486.13 ± 194.66
Benzaldehydes								
3,4-Dihydroxybenzaldehyde	5.54 ± 1.03	5.63 ± 0.88	4.76 ± 1.41	5.29 ± 0.98	4.47 ± 0.67	6.25 ± 0.69	5.19 ± 0.71	4.9 ± 0.88
4-Hydroxybenzaldehyde	419.3 ± 32.75	520.2 ± 45.64[#]	459.44 ± 47.69	427.5 ± 37.37	423.22 ± 31.29	499.83 ± 33.59	475.3 ± 30.21	415.6 ± 37.09
4-Hydroxy-3-methoxybenzaldehyde	1680.06 ± 508.74	2199.81 ± 641.53	2148.71 ± 599.72	1989.04 ± 510.64	2179.23 ± 489.26	1821.43 ± 454.71	1840.43 ± 456.91	2244.62 ± 506.42
Benzoic acids								
Benzoic acid	5178.54 ± 1547.39	5126.52 ± 1 503.38	5023.22 ± 1784.53	5584.88 ± 1610.32	6689.47 ± 1499.65	7825.33 ± 1673.13	7902.12 ± 1799.75	6565.77 ± 1463.56
2-Hydroxybenzoic acid	3391.51 ± 1167.92	3177.14 ± 554.36	3373.62 ± 512.47	2582.32 ± 517.92	3236.18 ± 662.33	3170.26 ± 492.84	4008.44 ± 856.15	3620.21 ± 631.85
3-Hydroxybenzoic acid	505.58 ± 299.29	389.87 ± 240.57	428.15 ± 366.39	563.87 ± 396.23	478.3 ± 278.55	240.47 ± 127.98	220.43 ± 119.57	519.4 ± 278.55
4-Hydroxybenzoic acid	312.48 ± 187.13	228.39 ± 142.53	219.51 ± 194.55	395.17 ± 320.47	235.96 ± 160.77	100.31 ± 75.26	127.97 ± 70.75	269.83 ± 152.22
2,3-Dihydroxybenzoic acid	58.73 ± 51.44	206.69 ± 163.52	210.54 ± 168.02	25.38 ± 16.29	42.79 ± 42.79	37.19 ± 37.19	57.41 ± 57.04	35.63 ± 35.63
2,4-Dihydroxybenzoic acid	2517.29 ± 393.79	3066.48 ± 416.26	2329.6 ± 331.28	2150.46 ± 274.95	2147.94 ± 348.93	2431.54 ± 441.62	2286.32 ± 326.44	2359.76 ± 492.05
2,5-Dihydroxybenzoic acid	821.33 ± 136.35	1029.41 ± 154.33	1411.62 ± 348.98**	994.76 ± 124.74*	657.71 ± 72.03	810.89 ± 88.17	733.83 ± 88.04	575.6 ± 92.99
2,6-Dihydroxybenzoic acid	1536.94 ± 235.91	1903.09 ± 256.22	1457.2 ± 205.35	1388.35 ± 180.16	1290.81 ± 202.89	1556.93 ± 277.93	1434.8 ± 217.39	1486.96 ± 309.69

Supplementary Table 3.5. Mean plasma (poly)phenol metabolite concentrations for postmenopausal women with above-normal blood pressure who completed a 12-week intervention evaluating 22 g freeze-dried highbush blueberry powder vs placebo powder on endothelial function, blood pressure, and other measures of cardiometabolic health.

	Blueberry (*n* = 22)				Placebo (*n* = 21)			
Compound (nmol/L)	**Baseline**	**Week 4**	**Week 8**	**Week 12**	**Baseline**	**Week 4**	**Week 8**	**Week 12**
3,4-Dihydroxybenzoic acid	23 ± 1.74	28.93 ± 3.29	32.31 ± 3.35 ±	26.27 ± 2.09	21.71 ± 1.34	21.09 ± 1.03	22.05 ± 1.89	20.45 ± 1.25
3,5-Dihydroxybenzoic acid	306.65 ± 79.72	426.56 ± 125.21	221.36 ± 49.76	302.26 ± 80.02	348.79 ± 181.16	210.1 ± 73.47	279.49 ± 84.27	329 ± 115.11
2,3,4-Trihydroxybenzoic acid	15.94 ± 1.78	20.3 ± 2.99	17.69 ± 3.3	15.36 ± 2.18	14.81 ± 2.19	12.63 ± 1.89	12.8 ± 2.51	12.94 ± 2.08
2-Hydroxy-4-methoxybenzoic acid	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
3-Hydroxybenzoic acid-4-sulfate	1.27 ± 0.76	14.65 ± 6.73	14.56 ± 4.41	6.36 ± 2.01	1.45 ± 0.72	4.4 ± 1.76	12.79 ± 8.69	3.53 ± 1.82
4-Hydroxybenzoic acid-3-sulfate	5 ± 1.99	30.53 ± 12.77	36.14 ± 11.57	13.52 ± 3	7.66 ± 4.68	8.43 ± 4.23	12.8 ± 6.76	6.94 ± 4.37
4-Hydroxybenzoic acid-3-glucuronide	1.75 ± 0.58	3.79 ± 1.22	3.49 ± 1.33	2.79 ± 0.66	1.78 ± 0.56	1.75 ± 0.45	3.93 ± 0.8	1.18 ± 0.38
4-Hydroxy-3,5-dimethoxybenzoic acid	133.53 ± 26.07	166.71 ± 32.15	197.43 ± 50.67	182.98 ± 62.67	152.88 ± 30.05	183.75 ± 39.97	206.01 ± 53.22	146.1 ± 40.91
3,4,5-trihydroxybenzene ethyl ester	1.84 ± 0.23	2.05 ± 0.41	2.07 ± 0.35	2.2 ± 0.48	1.49 ± 0.14	1.54 ± 0.16	1.51 ± 0.17	1.56 ± 0.12
3,4,5-Trihydroxybenzoic acid	1.27 ± 0.33	4.19 ± 1.45	7.4 ± 3.01	3.45 ± 0.92	0.42 ± 0.18	1.9 ± 0.79	0.72 ± 0.31	0.49 ± 0.25
3-Hydroxy-4-methoxybenzoic acid-5-sulfate	270.04 ± 91.16	426.29 ± 189.2*	600.83 ± 341.67*	247.03 ± 76.84	76.54 ± 17.01	329.23 ± 219.97	83.04 ± 31.31	97.26 ± 25.05
4-Hydroxy-3-methoxybenzoic acid	128.48 ± 51.83	205.69 ± 53.62	84.13 ± 23.84	101.94 ± 23.66	69.86 ± 22.56	124.43 ± 42.92	189.21 ± 102.55	256.62 ± 102.73
3-Methoxybenzoic acid-4-sulfate	75.71 ± 11.22	230.85 ± 66.42**	166.41 ± 28.06 ±	118.51 ± 12.83**	95.42 ± 23.64	99.03 ± 14.47	100.89 ± 12.06	127.98 ± 24.14
4-Methoxybenzoic acid-3-sulfate	1763.96 ± 352.28	2358.16 ± 403.46	2578.72 ± 419.56	1881.87 ± 324.71	964.58 ± 153.29	1083.72 ± 240.29	1294.56 ± 244.29	1167.08 ± 191.14

Supplementary Table 3.5. Mean plasma (poly)phenol metabolite concentrations for postmenopausal women with above-normal blood pressure who completed a 12-week intervention evaluating 22 g freeze-dried highbush blueberry powder vs placebo powder on endothelial function, blood pressure, and other measures of cardiometabolic health.

	Blueberry (*n* = 22)				Placebo (*n* = 21)			
Compound (nmol/L)	**Baseline**	**Week 4**	**Week 8**	**Week 12**	**Baseline**	**Week 4**	**Week 8**	**Week 12**
Hippuric acids								
Hippuric acid	49902 ± 8610.51	159259.47 ± 28909.72 ±	205196.88 ± 63762.99 ±	144940.06 ± 26003.74 ±	35799.93 ± 5663.65	65710.24 ± 16822.04	49620.56 ± 7689.91	43238.38 ± 8244.16
2'-Hydroxyhippuric acid	701.57 ± 269.32	570.41 ± 98.89	622.04 ± 93.71	485.59 ± 87.41	543.16 ± 96.02	581.07 ± 80.92	813.35 ± 221.01	617.11 ± 90.64
3'-Hydroxyhippuric acid	2971.78 ± 593.58	4889.13 ± 693.06**	6444.11 ± 2049.88**	4850.0 ± 813.99#	2309.01 ± 683.59	2877.84 ± 588.33	3772.13 ± 783.73	2946.18 ± 602.41
4'-Hydroxyhippuric acid	1304.52 ± 237.83	1629.05 ± 241.9	1546.77 ± 255.12	1238.36 ± 175.78	1037.65 ± 175.71	1279.23 ± 270.57	1560.06 ± 347.1	1389.36 ± 262.08
alpha-hydroxyhippuric acid	0 ± 0	0.45 ± 0.36	0.91 ± 0.52	0.91 ± 0.77	0.55 ± 0.55	0.48 ± 0.29	0 ± 0	0.51 ± 0.51
Cinnamic acids								
Cinnamic acid	10091.3 ± 1482.13	10145.56 ± 1216.63	10411.21 ± 1721.25	9740.43 ± 1156.6	9602.43 ± 596.29	10418.72 ± 691.51	9076.48 ± 655.47	9747.2 ± 590.51
3',4'-Dihydroxycinnamic acid	792.65 ± 87.41	977.56 ± 148.8	902.58 ± 115.48	846.6 ± 118.01	898.03 ± 80.29	895.79 ± 90.28	859.5 ± 68.1	855.8 ± 92.6
3'-Hydroxycinnamic acid-4'-sulfate	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
4'-Hydroxycinnamic acid-3'-sulfate	13.49 ± 5.66	65.83 ± 40.94	77.46 ± 32.11	18.65 ± 6.32	13.49 ± 5	30.46 ± 14.16	30.57 ± 10.75	22.26 ± 12.56
3'-Hydroxycinnamic acid-4'-glucuronide	112.95 ± 25.39	136.86 ± 28.43	129.67 ± 28.68	124.17 ± 27.3	124.54 ± 14.56	127.54 ± 17.16	129.42 ± 15.72	109.25 ± 15.99
4'-Hydroxycinnamic acid-3'-glucuronide	2.37 ± 2.35	3.36 ± 2.1	4.02 ± 2.62	0.86 ± 0.86	0.04 ± 0.04	2.69 ± 1.57	0.4 ± 0.36	1.89 ± 1.42
4'-Hydroxy-3'-methoxycinnamic acid	97.39 ± 28.6	210.94 ± 85.77	149.22 ± 59.47	164.37 ± 73.03	70.05 ± 14.46	93.66 ± 21.84	110.42 ± 22.89	120.92 ± 42.74
3'-Methoxycinnamic acid-4'-sulfate	29.07 ± 29.07	170.75 ± 120.59	111.45 ± 60.8	122.2 ± 95.07	0 ± 0	21.84 ± 11.44	23.52 ± 14.85	38.14 ± 32.43

Supplementary Table 3.5. Mean plasma (poly)phenol metabolite concentrations for postmenopausal women with above-normal blood pressure who completed a 12-week intervention evaluating 22 g freeze-dried highbush blueberry powder vs placebo powder on endothelial function, blood pressure, and other measures of cardiometabolic health.

	Blueberry (*n* = 22)				Placebo (*n* = 21)			
Compound (nmol/L)	**Baseline**	**Week 4**	**Week 8**	**Week 12**	**Baseline**	**Week 4**	**Week 8**	**Week 12**
3'-Methoxycinnamic acid-4'-glucuronide	31.43 ± 9.42	114.46 ± 52.11	132.42 ± 39.03	60.97 ± 14.45	54.39 ± 14.6	88.36 ± 35.57	76.67 ± 14.94	122.46 ± 64.98
3'-Hydroxy-4'-methoxycinnamic acid	194.07 ± 17.1	282.53 ± 55.74	264.15 ± 46.58	232.21 ± 41.82	174.21 ± 14.93	213.03 ± 22.43	221.46 ± 20.03	198.69 ± 28.31
4'-Methoxycinnamic acid-3'-sulfate	10.82 ± 1.12	23.59 ± 5.48	20.71 ± 4.51	27.48 ± 8.54	18.23 ± 3.09	18.99 ± 3.35	22.86 ± 6.77	20.72 ± 5.37
4'-Methoxycinnamic acid-3'-glucuronide	15.13 ± 9.68	33.18 ± 26.48	35.8 ± 17.02	66.37 ± 51.89	5.45 ± 3.59	17.9 ± 12.19	29.4 ± 14.07	12.86 ± 7.06
4-O-Caffeoylquinic acid	2.54 ± 0.35	6.09 ± 3.9	2.39 ± 0.2	2.75 ± 0.34	2.4 ± 0.14	2.48 ± 0.21	2.86 ± 0.32	2.77 ± 0.39
5-O-Caffeoylquinic acid	2.95 ± 0.12	5.45 ± 2.5	5.27 ± 1.42	3.23 ± 0.25	3.77 ± 0.3	3.89 ± 0.72	4.02 ± 0.56	4.16 ± 0.64
3-O-Feruloylquinic acid	2.79 ± 0.21	12.35 ± 8.93	8.87 ± 4.43	3.52 ± 0.3	3.64 ± 0.8	4.12 ± 0.74	3.56 ± 0.43	4.74 ± 1.13
4-O-Feruloylquinic acid	2.82 ± 0.08	23.32 ± 20.43	15.82 ± 12.18	3.08 ± 0.24	2.88 ± 0.15	4.44 ± 1.6	4.02 ± 1.18	3.61 ± 0.66
4'-Hydroxy-3',5'-dimethoxycinnamic acid	18.61 ± 4.61	42.95 ± 17.61	61.72 ± 26.09	18.44 ± 3.48	56.01 ± 14.54	38.55 ± 8.73	41.08 ± 6.7	36.92 ± 7.48
3'-Hydroxycinnamic acid	10.59 ± 2.88	7.98 ± 2.45	10.67 ± 3.53	6.94 ± 3.43	6.47 ± 1.36	9.27 ± 2.13	3.97 ± 1.29	7.23 ± 1.95
4'-Hydroxycinnamic acid	1.53 ± 1.4	3.19 ± 2.62	8.29 ± 6.26	5.38 ± 3.64	0.15 ± 0.1	2.59 ± 1.31	0.59 ± 0.29	2.1 ± 1.3
2'-Hydroxycinnamic acid	742.21 ± 83.53	812 ± 96.73	758.96 ± 104.47	760.47 ± 83.81	735.46 ± 75.77	800.04 ± 71.79	765.59 ± 64.68	767.94 ± 71.26
Cinnamic acid-4'-sulfate	83.03 ± 19.58	115.69 ± 29.3	113.88 ± 34.33	77.92 ± 18.39	56.75 ± 12.38	70.04 ± 13.3	49.3 ± 12.55	51.27 ± 12.71
Cinnamic acid-4'-glucuronide	9.71 ± 5.38	13.66 ± 6.5	26.84 ± 7.48	21.59 ± 8.91	11.13 ± 3.43	14.27 ± 5.4	6.72 ± 2.38	9.82 ± 4.03
Phenylacetic acids								
Phenylacetic acid	3993.51 ± 1048.49	3012.74 ± 946.1	2576.45 ± 633.18	2848.06 ± 744.07	2803.31 ± 794.84	2425.36 ± 668.01	2720.96 ± 458.6	1697.6 ± 393.36

Supplementary Table 3.5. Mean plasma (poly)phenol metabolite concentrations for postmenopausal women with above-normal blood pressure who completed a 12-week intervention evaluating 22 g freeze-dried highbush blueberry powder vs placebo powder on endothelial function, blood pressure, and other measures of cardiometabolic health.

	Blueberry (*n* = 22)				Placebo (*n* = 21)			
Compound (nmol/L)	**Baseline**	**Week 4**	**Week 8**	**Week 12**	**Baseline**	**Week 4**	**Week 8**	**Week 12**
3'-Hydroxyphenylacetic acid	7444.66 ± 622.79	8936.8 ± 1333.29	9575.42 ± 1509.91	8819.77 ± 1006.28	7169.73 ± 506.45	6838.21 ± 558.73	6672.18 ± 561.72	5842.4 ± 542.66
3',4'-Dihidroxyphenylacetic acid	163.15 ± 23.66	220.18 ± 40.62	219.87 ± 36.41	167.95 ± 21.14	130.57 ± 14.04	155.83 ± 13.15	186.4 ± 28.57	154.36 ± 19.85
3'-Methoxyphenylacetic acid-4'-sulfate	151.42 ± 34.32	214.03 ± 67.38	228.29 ± 49.1	185.61 ± 30.26	144.4 ± 20.32	166.51 ± 19.82	137.74 ± 28.27	181.82 ± 39.11
Phenylpropanoic acids								
2-(4'-Hydroxyphenoxy)propanoic acid	12.12 ± 6.6	33.61 ± 12.52	35.44 ± 18.87	33.46 ± 14.4	3.09 ± 3.09	37.66 ± 18.99	4.09 ± 2.78	35.91 ± 34
3-(2'-Hydroxyphenyl)propanoic acid	1354.88 ± 358.48	1782.49 ± 354.11	2146.88 ± 501.84	1787.64 ± 396.06	832.62 ± 325.52	1253.06 ± 350.83	1954.5 ± 412.57[#]	1708.86 ± 397.74
3-(3'-Hydroxyphenyl)propanoic acid	491.02 ± 288.96	1092.42 ± 638.75	804.99 ± 593.89	876.86 ± 456.82	131.97 ± 83.81	1342.84 ± 970.7	648.15 ± 556.16	1571.58 ± 1063.97
3-(2',3'-Dihydroxyphenyl)propanoic acid	14.86 ± 3.53	24.23 ± 8.59	27.21 ± 9.35	15.96 ± 2.68	13.22 ± 1.75	12.93 ± 1.62	14.11 ± 1.54	13.3 ± 1.51
3-(2',4'-Dihydroxyphenyl)propanoic acid	112.05 ± 34.46	188.61 ± 41.73	225.47 ± 85.61	163.84 ± 54.38	104.76 ± 41.2	91.43 ± 34.33	80.41 ± 17.76	109.41 ± 35.63
3-(3',4'-Dihydroxyphenyl)propanoic acid	49.93 ± 4.89	52.16 ± 6.23	60.21 ± 12.66	48.06 ± 3.87	47.75 ± 2.53	47.98 ± 2.47	48.75 ± 2.11	48.48 ± 1.99
3-(3',5'-Dihydroxyphenyl)propanoic acid	149.45 ± 48.54	213.17 ± 53.21	281.03 ± 92.75	186.79 ± 58.85	122.89 ± 43.21	92.58 ± 27.48	102.18 ± 23.16	123.59 ± 37.24

Supplementary Table 3.5. Mean plasma (poly)phenol metabolite concentrations for postmenopausal women with above-normal blood pressure who completed a 12-week intervention evaluating 22 g freeze-dried highbush blueberry powder vs placebo powder on endothelial function, blood pressure, and other measures of cardiometabolic health.

	Blueberry (*n* = 22)				Placebo (*n* = 21)			
Compound (nmol/L)	**Baseline**	**Week 4**	**Week 8**	**Week 12**	**Baseline**	**Week 4**	**Week 8**	**Week 12**
2-Hydroxy-3-(4'-hydroxyphenyl)propanoic acid	8781.25 ± 639.28	10779.37 ± 1011.26**	9922.6 ± 965.4	9190.64 ± 742.1	9008.31 ± 584.62	9690.44 ± 588.98	9240.98 ± 685.18	8880.14 ± 674.67
3-(4'-Hydroxy-3'-methoxyphenyl)propanoic acid	54.19 ± 19.3	143.21 ± 55.34	130.2 ± 56.13	62.92 ± 13.39	80.89 ± 22.39	65.21 ± 19.79	78.69 ± 23.41	55.18 ± 18.47
3-(4'-Hydroxyphenyl)propanoic acid-3'-glucuronide	37.7 ± 10.84	289.4 ± 140.18	74.04 ± 20.1	43.89 ± 13.29	71.79 ± 16.13	144.67 ± 53.93	145.51 ± 44.45	119.36 ± 37.12
3-(4'-Hydroxyphenyl)propanoic acid-3'-sulfate	495.62 ± 63.84	720.37 ± 102.9	826.54 ± 159.08	627.08 ± 95.03	392.07 ± 34.17	480.66 ± 36.9	582.25 ± 85.88	768.31 ± 349.38
3-(3'-Methoxyphenyl)propanoic acid-4'-sulfate	62.17 ± 26.42	131.09 ± 52.54	121.05 ± 46.44**	75.4 ± 21.73	25.77 ± 6.42	46.49 ± 10.6	74.71 ± 25.25**	166.08 ± 110.46**
3-(3'-Methoxyphenyl)propanoic acid-4'-glucuronide	102.29 ± 23.33	201.39 ± 47.96	268.28 ± 105.7	154.27 ± 34.35	64.94 ± 10.57	98.18 ± 18.54	157.33 ± 36.52	200.96 ± 85.72
3-(4'-Methoxyphenyl)propanoic acid-3'-sulfate	244.58 ± 73.4	417.8 ± 114.57	380.29 ± 130.51	299.55 ± 79.13	376.47 ± 104.65	386.55 ± 103.49	349.27 ± 93.39	440.9 ± 108.68
3-(4'-Methoxyphenyl)propanoic acid-3'-glucuronide	26.67 ± 8.15	46.49 ± 9.72	86.18 ± 35.16**	42.69 ± 8.78	58.97 ± 27.89	71.32 ± 24.81	60.06 ± 13.43	116.25 ± 55.22
Valerolactones								
(4R)-5-(3′-hydroxyphenyl)-γ-valerolactone-4′-sulfate	79.12 ± 35.31	97.22 ± 32.22	117.33 ± 40.13	110.96 ± 51.08	41.55 ± 21.55	84.26 ± 38.82	105.25 ± 37.27	47.6 ± 14.68

Supplementary Table 3.5. Mean plasma (poly)phenol metabolite concentrations for postmenopausal women with above-normal blood pressure who completed a 12-week intervention evaluating 22 g freeze-dried highbush blueberry powder vs placebo powder on endothelial function, blood pressure, and other measures of cardiometabolic health.

	Blueberry (*n* = 22)				Placebo (*n* = 21)			
Compound (nmol/L)	**Baseline**	**Week 4**	**Week 8**	**Week 12**	**Baseline**	**Week 4**	**Week 8**	**Week 12**
Sum of Polyphenol Metabolites	125798.37 ± 12727.95	250052.95 ± 34374.48*[a] [#]	303052.73 ± 80146.47*[a]	227970.5 ± 33084.02*[a] [#]	107725.03 ± 6848.71	145293.43 ± 18276.91[b]	132461.22 ± 10352.79	119158.19 ± 10400.79[b]

Data presented as mean ± SEM.
For individual metabolites: *Difference between groups (blueberry vs placebo) at that time point ($p < 0.05$); **Difference ($p < 0.05$) within group compared to baseline; [#]Trend for difference ($p < 0.10$) within group compared to baseline. ± Difference ($p < 0.05$) between groups at that time point and within group compared to baseline.
For Sum of Polyphenol Metabolites: *Differences ($p < 0.05$) within group compared to baseline. Different letters indicate differences ($p < 0.05$) between groups at that time point. [#]Difference ($p < 0.05$) in the change from baseline to 12 weeks between groups at that time point.

Supplementary Table 3.6. Protein expression measured by immunofluorescence microscopy in venous endothelial cells collected from postmenopausal women with above-normal blood pressure who completed a 12-week intervention evaluating 22 g freeze-dried highbush blueberry powder vs placebo powder on endothelial function, blood pressure, and other measures of cardiometabolic health.

	Blueberry (*n* = 13)	**Placebo (*n* = 11)**
p47phox (AU)		
Baseline	0.94 ± 0.14	0.99 ± 0.18
12 Weeks	0.93 ± 0.12	0.99 ± 0.21
Δ 0 to 12 Weeks	-0.01 ± 0.06	0.00 ± 0.09
Phos-eNOS (AU)		
Baseline	0.80 ± 0.10	0.93 ± 0.16
12 Weeks	0.81 ± 0.06	0.75 ± 0.05
Δ 0 to 12 Weeks	0.01 ± 0.10	-0.18 ± 0.12
MnSOD (AU)		
Baseline	0.46 ± 0.08	0.58 ± 0.10
12 Weeks	0.48 ± 0.10	0.64 ± 0.18
Δ 0 to 12 Weeks	0.02 ± 0.07	0.07 ± 0.10
Phos-NFκB p65 (Ser536) (AU)		
Baseline	0.81 ± 0.07	0.81 ± 0.08
12 Weeks	0.78 ± 0.05	0.72 ± 0.10
Δ 0 to 12 Weeks	-0.02 ± 0.07	-0.07 ± 0.06

Data are mean ± SEM. No significant differences. Phos-eNOS, phosphorylated endothelial cell nitric oxide synthase; MnSOD, manganese superoxide dismutase; Phos-NFκB, phosphorylated nuclear factor kappa B.

Supplementary Table 3.7. Anthropometrics, body composition, and energy intake and expenditure of postmenopausal women with above-normal blood pressure who completed a 12-week intervention evaluating 22 g freeze-dried highbush blueberry powder vs placebo powder on endothelial function, blood pressure, and other measures of cardiometabolic health.

	Blueberry (*n* = 22)	**Placebo (*n* = 21)**
Weight (kg)		
Baseline	73.0 ± 3.2	73.6 ± 3.3
4 Weeks	73.3 ± 3.2	74.3 ± 3.5
8 Weeks	73.5 ± 3.3	74.5 ± 3.5
12 Weeks	73.1 ± 3.2	74.7 ± 3.5
Δ 0 to 4 Weeks	0.3 ± 0.2	-0.2 ± 0.2
Δ 0 to 8 Weeks	0.4 ± 0.3	-0.1 ± 0.3
Δ 0 to 12 Weeks	0.0 ± 0.2	0.1 ± 0.3
BMI (kg/m^2)		
Baseline	27.6 ± 1.0	27.7 ± 1.1
4 Weeks	27.7 ± 1.0	27.7 ± 1.1
8 Weeks	27.7 ± 1.0	27.7 ± 1.1
12 Weeks	27.6 ± 1.0	27.8 ± 1.1
Δ 0 to 4 Weeks	0.1 ± 0.1	-0.1 ± 0.1
Δ 0 to 8 Weeks	0.2 ± 0.1	0.0 ± 0.1
Δ 0 to 12 Weeks	0.0 ± 0.1	0.0 ± 0.1
WC (cm)		
Baseline	91 ± 3	88 ± 2
4 Weeks	90 ± 3	88 ± 3
8 Weeks	91 ± 3	92 ± 3
12 Weeks	90 ± 3	91 ± 3
Δ 0 to 4 Weeks	-0.4 ± 1.1	-1.7 ± 1.0
Δ 0 to 8 Weeks	-0.8 ± 1.4	2.0 ± 1.5
Δ 0 to 12 Weeks	0.0 ± 0.9	0.8 ± 1.4
HC (cm)		
Baseline	109 ± 2	107 ± 2
4 Weeks	107 ± 2	108 ± 3
8 Weeks	103 ± 5	108 ± 3
12 Weeks	107 ± 2	107 ± 3
Δ 0 to 4 Weeks	-0.45 ± 0.91	-0.57 ± 0.97
Δ 0 to 8 Weeks	0.50 ± 0.83	-1.26 ± -3.64
Δ 0 to 12 Weeks	-0.15 ± 0.61	-1.55 ± 0.92
Android Fat Mass (%)^		
Baseline	41.59 ± 1.65	37.54 ± 2.26
12 Weeks	39.08 ± 1.76	35.2 ± 2.22*
Δ 0 to 12 Weeks	-2.51 ± 1.05	-2.34 ± 0.68
Gynoid Fat Mass (%)^		
Baseline	43.71 ± 0.87	41.21 ± 1.93
12 Weeks	43.44 ± 0.74	40.74 ± 1.92
Δ 0 to 12 Weeks	-0.27 ± 0.71	-0.48 ± 0.27
Total Fat Mass (%)^		
Baseline	40.72 ± 1.24	38.01 ± 1.78
12 Weeks	39.96 ± 1.11	37.49 ± 1.66
Δ 0 to 12 Weeks	-0.76 ± .67	-0.51 ± 0.42

Supplementary Table 3.7. Anthropometrics, body composition, and energy intake and expenditure of postmenopausal women with above-normal blood pressure who completed a 12-week intervention evaluating 22 g freeze-dried highbush blueberry powder vs placebo powder on endothelial function, blood pressure, and other measures of cardiometabolic health.

	Blueberry (*n* = 22)	**Placebo (*n* = 21)**
Average Daily Energy Intake (kcal)		
Baseline	1717 ± 81	1653 ± 112
4 Weeks	1857 ± 109	1624 ± 133
8 Weeks	1790 ± 100	1607 ± 146
12 Weeks	1685 ± 95	1631 ± 127
Average Daily Energy Expenditure (kcal)		
Baseline	2621 ± 148	2653 ± 140
4 Weeks	2615 ± 146	2651 ± 128
8 Weeks	2699 ± 153	2617 ± 143
12 Weeks	2677 ± 162	2640 ± 138

Data are mean ± SEM. *Different ($p < 0.05$) than baseline. Abbreviations: BMI, body mass index, HC, hip circumference, WC, waist circumference. ^n = 32 for these analyses.

APPENDIX 2: SUPPLEMENTARY DATA FOR CHAPTER 4

Supplementary Figures and **Tables**

Supplementary Table 4.1. Flow-mediated dilation (FMD) results for the 17 postmenopausal women who consumed 22 g freeze-dried highbush blueberry powder for 12 weeks and completed the endothelial function measurements.

	Responder (*n* = 8)	**Non-responder (*n* = 9)**
Baseline Diameter (mm) [a d]		
Baseline	2.88 ± 0.15	2.74 ± 0.14
12 Weeks	2.90 ± 0.16	2.84 ± 0.14*
Δ 0 to 12 Weeks	0.02 ± 0.04	0.10 ± 0.04
Peak Diameter (mm) [a b]		
Baseline	2.92 ± 0.12	2.89 ± 0.16
12 Weeks	3.06 ± 0.15*	2.96 ± 0.15
Δ 0 to 12 Weeks	0.14 ± 0.06	0.07 ± 0.05
FMD (%) [b c]		
Baseline	1.80 ± 1.37	5.65 ± 1.27**
12 Weeks	6.06 ± 1.77*	4.40 ± 1.02
Δ 0 to 12 Weeks	4.26 ± 1.01	-1.25 ± 0.63**
SR_{AUC} [b]		
Baseline	32492.13 ± 4223.78	39103.49 ± 4559.61
12 Weeks	21098.51 ± 4244.94*	30811.26 ± 4566.26[#]
Δ 0 to 12 Weeks	-11393.63 ± 4861.52	-8292.23 ± 4323.64
FMD/SR_{AUC} [b c]		
Baseline	5.92e-5 ± 4.30e-5	1.61e-4 ± 3.77e-5[##]
12 Weeks	2.81e-4 ± 3.75e-5*	1.69e-4 ± 4.78e-5[##]
Δ 0 to 12 Weeks	2.22e-4 ± 4.53e-5	7.86e-6 ± 4.61e-5**

Data are mean ± SEM. [a]Main effect for group. [b]Main effect for time. [c]Main effect for group*time. [d]Trending main effect for time ($p < 0.10$). *Different compared to baseline within group ($p < 0.05$). **Different compared to that time point between groups ($p < 0.05$). [##]Trending different at that time point between groups ($p < 0.06$). Abbreviations: FMD, flow-mediated dilation; FMD/SR_{AUC}; flow-mediated dilation normalized to shear rate area under the curve.

Supplementary Table 4.2. Flow-mediated dilation (FMD) results before and after ascorbic acid infusion for the 17 postmenopausal women who consumed 22 g freeze-dried highbush blueberry powder for 12 weeks and completed the endothelial function measurements.

Responder	**Saline (*n* = 8)**	**Ascorbic Acid (*n* = 8)**	**Saline *vs* Ascorbic Acid Difference**
FMD (%)			
Baseline	1.80 ± 1.37	6.93 ± 2.63*	5.13 ± 1.77[a]
12 Weeks	6.06 ± 1.77	7.09 ± 1.76	1.04 ± 0.79
FMD/SR$_{AUC}$			
Baseline	5.92e-5 ± 4.30e-5	4.62e-4 ± 2.44e-4*	4.03e-4 ± 2.18e-4
12 Weeks	2.81e-4 ± 3.75e-5	2.85e-4 ± 3.22e-5	4.18e-06 ± 4.11e-5**
Non-Responder	**Saline (*n* = 9)**	**Ascorbic Acid (*n* = 9)**	**Saline *vs* Ascorbic Acid Difference**
FMD (%)			
Baseline	5.65 ± 1.27	6.84 ± 1.45	1.19 ± 1.14
12 Weeks	4.40 ± 1.02	5.15 ± 0.58	0.76 ± 1.07
FMD/SR$_{AUC}$			
Baseline	1.61e-4 ± 3.77e-5	2.36e-4 ± 3.41e-5	7.44e-5 ± 2.68e-5
12 Weeks	1.69e-4 ± 4.78e-5	1.62e-4 ± 2.4e-5	-6.92e-6 ± 4.47e-5

Data are mean ± SEM. [a]Different between saline vs ascorbic acid difference between responder and non-responder at that time point. *Different between saline and ascorbic acid within group at that time point ($p < 0.05$). **Different between saline vs ascorbic acid difference compared to baseline within group ($p < 0.05$). Abbreviations: FMD, flow-mediated dilation; FMD/SR$_{AUC}$; flow-mediated dilation normalized to shear rate area under the curve.

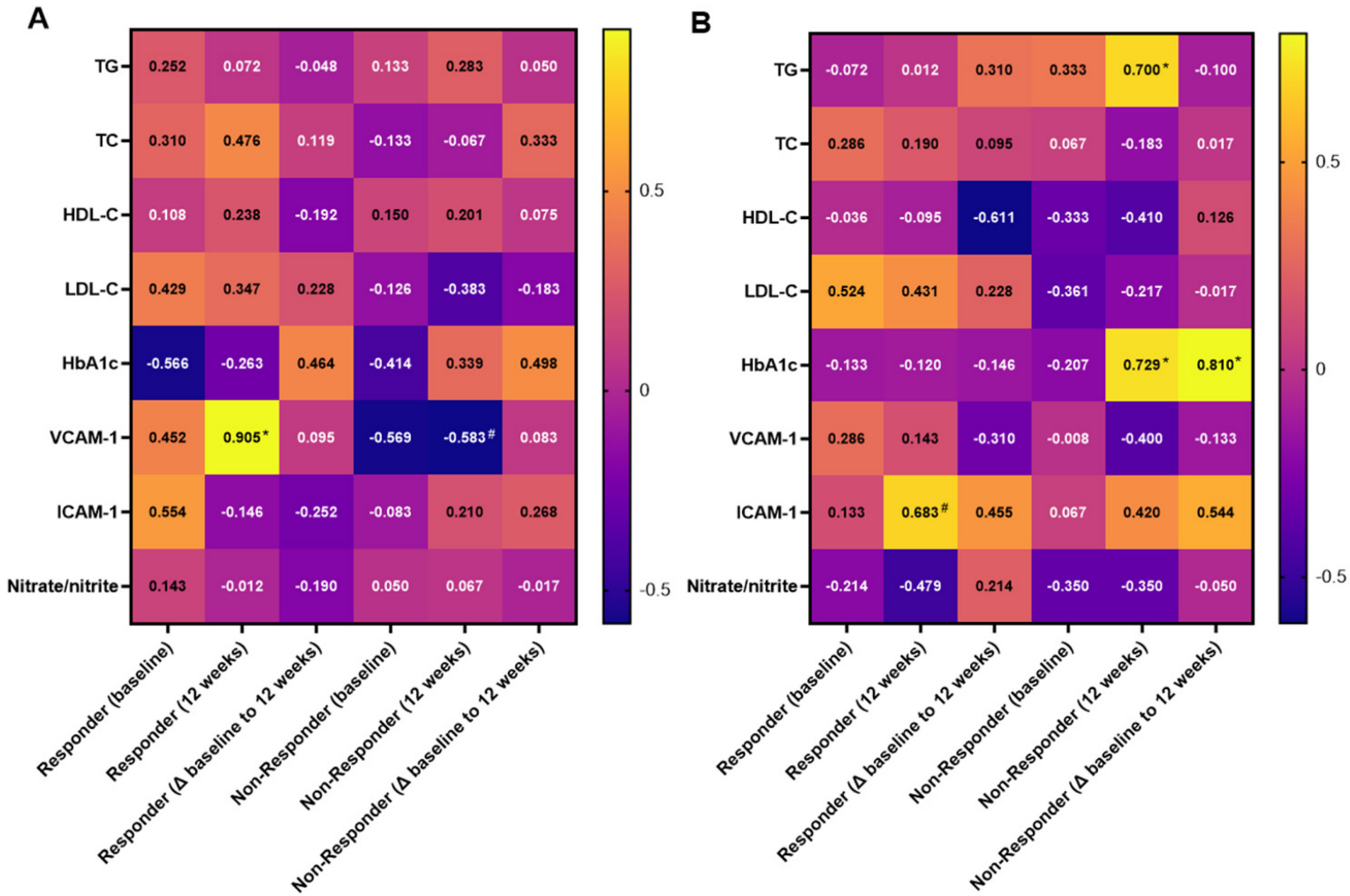
Supplementary Table 4.3. Blood lipids and biomarkers for the 17 postmenopausal women who consumed 22 g freeze-dried highbush blueberry powder for 12 weeks and completed the endothelial function measurements.

	Responder (*n* = 8)	Non-responder (*n* = 9)
TG (mg/dL) [b]		
Screening	122 ± 15	122 ± 24
12 Weeks	103 ± 19[#]	85 ± 10*
Δ 0 to 12 Weeks	-20 ± 10	-37 ± 17
TC (mg/dL) [b]		
Screening	217 ± 9	213 ± 5
12 Weeks	190 ± 8*	194 ± 5*
Δ 0 to 12 Weeks	-28 ± 4	-19 ± 6
HDL-C (mg/dL) [a]		
Screening	48 ± 3	66 ± 6**
12 Weeks	50 ± 7	62 ± 5[##]
Δ 0 to 12 Weeks	2 ± 5	-4 ± 4
LDL-C (mg/dL) [b d]		
Screening	144 ± 7	121 ± 6**
12 Weeks	118 ± 10*	115 ± 3
Δ 0 to 12 Weeks	-26 ± 7	-6 ± 7
HgbA1c (%)		
Screening	5.6 ± 0.1	5.5 ± 0.1
12 Weeks	5.5 ± 0.1	5.5 ± 0.1
Δ 0 to 12 Weeks	-0.1 ± 0.1	0.0 ± 0.1
VCAM-1 (ng/mL)		
Baseline	1606.17 ± 255.56	1216.96 ± 124.77
4 Weeks	1889.70 ± 245.62	1861.90 ± 249.40
8 Weeks	1545.16 ± 435.85	1663.30 ± 191.03
12 Weeks	1612.08 ± 233.78	1575.84 ± 116.53
Δ 0 to 4 Weeks	283.54 ± 408.81	644.93 ± 332.41
Δ 0 to 8 Weeks	-61.00 ± 484.33	446.34 ± 289.90
Δ 0 to 12 Weeks	5.92 ± 237.46	358.88 ± 136.18
ICAM-1 (ng/mL) [c e]		
Baseline	275.15 ± 17	184.36 ± 23.28**
4 Weeks	217.23 ± 31.41	251.74 ± 17.51
8 Weeks	186.36 ± 19.42*	245.85 ± 13.33**
12 Weeks	272.78 ± 17.74	218.57 ± 23.89[##]
Δ 0 to 4 Weeks	-57.91 ± 37.88	67.38 ± 30.32**
Δ 0 to 8 Weeks	-88.79 ± 24.70	61.50 ± 25.45**
Δ 0 to 12 Weeks	-2.37 ± 29.92[&]	34.21 ± 17.49
Nitrate/nitrite (μM) [a]		
Baseline	9.67 ± 2.05	10.36 ± 2.02
4 Weeks	5.59 ± 1.91	9.91 ± 1.84**
8 Weeks	6.16 ± 1.53	9.45 ± 1.29
12 Weeks	4.81 ± 0.81	11.00 ± 2.68**

Supplementary Table 4.3. Blood lipids and biomarkers for the 17 postmenopausal women who consumed 22 g freeze-dried highbush blueberry powder for 12 weeks and completed the endothelial function measurements.

	Responder (*n* = 8)	Non-responder (*n* = 9)
Nitrate/nitrite (μM) [a]		
Δ 0 to 4 Weeks	-4.08 ± 1.87	-0.70 ± 1.62
Δ 0 to 8 Weeks	-3.51 ± 2.45	-1.17 ± 1.57
Δ 0 to 12 Weeks	-4.86 ± 2.42	0.38 ± 2.30

Data are mean ± SEM. [a]Main effect for group ($p < 0.05$). [b]Main effect for time ($p < 0.05$). [c]Main effect for group*time ($p < 0.05$). [d]Trending main effect for group*time ($p < 0.10$). [e]Main effect for group ($p < 0.05$) and trending main effect for time ($p < 0.08$) for the changes in ICAM-1 from baseline to 4, 8, and 12 weeks.*Different compared to baseline within group ($p < 0.05$). **Different between groups at that time point ($p < 0.05$). [#]Trending different compared to baseline within group ($p < 0.09$). [##]Trending differences between groups at that time point ($p < 0.09$). [&]Different compared to the change from baseline to 8 weeks within group ($p < 0.05$). Abbreviations: HgbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein-cholesterol, LDL-C, low-density lipoprotein-cholesterol, TC, total cholesterol, TG, triglycerides, ICAM-1, intercellular adhesion molecule-1, VCAM-1, vascular cell adhesion protein-1.



Supplementary Figure 4.1. Blood biomarkers at baseline, 12 weeks, and the change from baseline to 12 weeks for responders (n = 8) and non-responders (n = 9) correlated with (A) the change in flow-mediated dilation (FMD) and (B) the change FMD normalized to shear rate area under the curve (FMD/SR_{AUC}) from baseline to 12 weeks. Data are Spearman's Rho. *Significant correlation (p < 0.05). #Trending significant correlation (p < 0.10). Abbreviations: HgbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein-cholesterol, LDL-C, low-density lipoprotein-cholesterol, TC, total cholesterol, TG, triglycerides, ICAM-1, intercellular adhesion molecule-1, VCAM-1, vascular cell adhesion protein-1.

Supplementary Table 4.4. Plasma (poly)phenol metabolite concentrations for the 17 postmenopausal women who consumed 22 g freeze-dried highbush blueberry powder for 12 weeks and completed the endothelial function measurements.

Compound (nmol/L)	Responder (*n* = 8)	Non-responder (*n* = 9)
Sum of (poly)phenol metabolites [a ^]		
Baseline	109203.71 ± 24831.93	131983.70 ± 20208.23
4 Weeks	255697.01 ± 60073.86*	293893.40 ± 61105.23*
8 Weeks	181674.00 ± 27088.88	420991.26 ± 161186.94*##
12 weeks	178767.26 ± 31182.66*	239646.60 ± 45154.96*
Δ 0 to 4 Weeks	146493.30 ± 44283.18	161909.69 ± 46401.55
Δ 0 to 8 Weeks	72470.29 ± 25167.92	289007.56 ± 147345.98
Δ 0 to 12 Weeks	69563.55 ± 25592.52%	107662.89 ± 31643.64
Hippuric Acid [a d]		
Baseline	44872.34 ± 18877.47	53737.79 ± 13138.08
4 Weeks	170323.80 ± 55797.31#	198015.97 ± 45588.07*
8 Weeks	114134.05 ± 23843.63	302441.61 ± 127557.71*
12 Weeks	112062.26 ± 29348.70	152605.24 ± 34126.59*
Δ 0 to 4 Weeks	125451.46 ± 42717.35	144278.19 ± 39271.43
Δ 0 to 8 Weeks	69261.72 ± 17647.24	248703.83 ± 121057.72
Δ 0 to 12 Weeks	67189.92 ± 20590.23	98867.45 ± 28536.20
3-Hydroxyhippuric Acid [a e]		
Baseline	3792.44 ± 1098.56	2188.30 ± 855.21
4 Weeks	6539.81 ± 941.93#	4028.44 ± 958.14*#
8 Weeks	4491.73 ± 1182.43	8545.27 ± 4418.02*
12 Weeks	5018.13 ± 1166.76	4262.53 ± 1314.10#
Δ 0 to 4 Weeks	2747.37 ± 877.93	1840.14 ± 1144.20
Δ 0 to 8 Weeks	699.29 ± 1258.94&	6356.97 ± 4452.58
Δ 0 to 12 Weeks	1225.69 ± 837.27	2074.23 ± 837.33
Vanillic Acid-4-Sulfate [a ^^]		
Baseline	193.01 ± 128.66	43.41 ± 22.77
4 Weeks	292.61 ± 122.17#	84.42 ± 41.47##
8 Weeks	97.69 ± 50.32	96.32 ± 28.26
12 weeks	77.98 ± 34.27	123.34 ± 36.30
Δ 0 to 4 Weeks	99.60 ± 49.61	41.01 ± 42.21
Δ 0 to 8 Weeks	-95.32 ± 135.72	52.91 ± 45.43
Δ 0 to 12 Weeks	-115.03 ± 142.86	79.93 ± 50.41
Protocatechuic Acid [a c ^]		
Baseline	22.38 ± 1.59	20.85 ± 2.61
4 Weeks	28.47 ± 3.94	34.39 ± 6.85
8 Weeks	25.66 ± 2.87	40.30 ± 5.98*##
12 Weeks	25.10 ± 2.58	29.91 ± 4.28
Δ 0 to 4 Weeks	6.09 ± 4.44	13.54 ± 7.93&&
Δ 0 to 8 Weeks	3.28 ± 3.80	19.45 ± 5.97
Δ 0 to 12 Weeks	2.72 ± 3.24	9.06 ± 5.83

Supplementary Table 4.4. Plasma (poly)phenol metabolite concentrations for the 17 postmenopausal women who consumed 22 g freeze-dried highbush blueberry powder for 12 weeks and completed the endothelial function measurements.

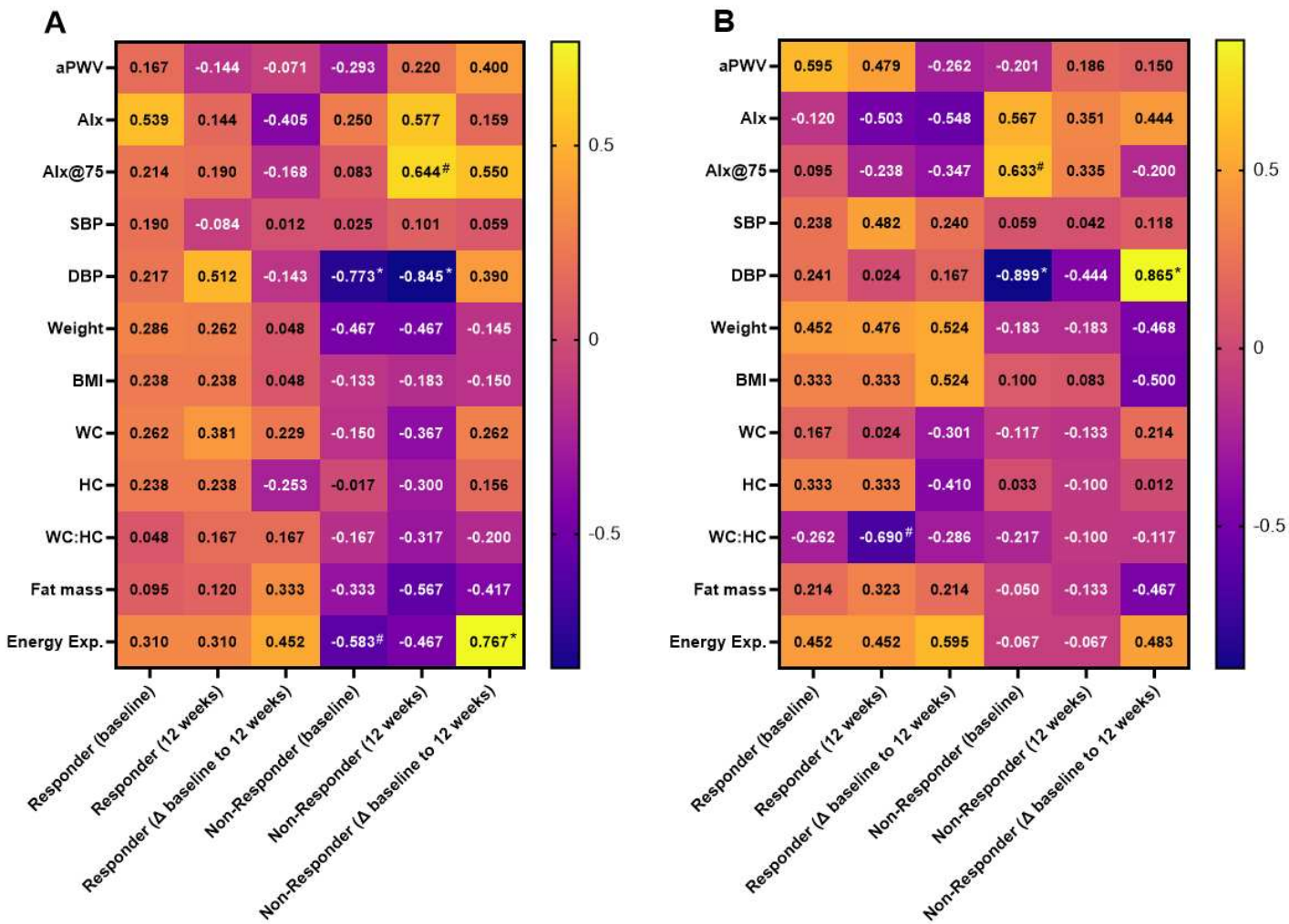
Compound (nmol/L)	Responder (*n* = 8)	Non-responder (*n* = 9)
2,5-Dihydroxybenzoic Acid [a e]		
Baseline	679.48 ± 179.33	887.36 ± 229.33
4 Weeks	891.11 ± 166.75	1190.13 ± 308.81
8 Weeks	807.43 ± 117.30	2055.95 ± 720.14[##]
12 weeks	832.02 ± 131.67	1084.14 ± 199.82
Δ 0 to 4 Weeks	211.63 ± 96.45	302.77 ± 290.81
Δ 0 to 8 Weeks	127.96 ± 172.72	1168.59 ± 584.30
Δ 0 to 12 Weeks	152.55 ± 173.17	196.78 ± 257.13

Data are mean ± SEM. [a]Main effect for group ($p < 0.05$). [b]Main effect for time ($p < 0.05$). [c]Main effect for group*time ($p < 0.05$). [d]Main effect for time for the changes of (poly)phenol metabolites from baseline to 4, 8, and 12 weeks ($p < 0.05$). [e]Main effect for time*group for the changes in (poly)phenol metabolites from baseline to 4, 8, and 12 weeks ($p < 0.05$). [^]Main effect for group for the change in (poly)phenol metabolites from baseline to 4, 8, and 12 weeks ($p < 0.10$). [^^]Main effect for time*group for the change in (poly)phenol metabolites from baseline to 4, 8, and 12 weeks ($p < 0.10$). *Different compared to baseline within group ($p < 0.05$). **Different between groups at that time point ($p < 0.05$). [#]Trending different compared to baseline within group ($p < 0.10$). [##]Trending different between groups at that time point ($p < 0.10$). [%]Trending different compared to the change from baseline to 4 weeks within group ($p < 0.07$). [&]Different compared to the change from baseline to 4 weeks within group ($p < 0.07$). [&&]Different compared to the change from baseline to 4 weeks between groups ($p < 0.05$).

Supplementary Table 4.5. Venous endothelial cell protein expressions for the 13 postmenopausal women who consumed 22 g freeze-dried highbush blueberry powder for 12 weeks and completed endothelial cell biopsy.

	Responder (*n* = 8)	Non-responder (*n* = 5)
p47phox (AU)		
Baseline	0.96 ± 0.18	0.91 ± 0.23
12 Weeks	0.88 ± 0.17	1.00 ± 0.17
Δ 0 to 12 Weeks	-0.08 ± 0.08	0.09 ± 0.08
p-eNOS (AU) [a]		
Baseline	0.92 ± 0.14	0.63 ± 0.09
12 Weeks	0.92 ± 0.06*	0.67 ± 0.07
Δ 0 to 12 Weeks	0.00 ± 0.17	0.02 ± 0.06
MnSOD (AU)		
Baseline	0.53 ± 0.12	0.34 ± 0.05
12 Weeks	0.88 ± 0.16	0.32 ± 0.03
Δ 0 to 12 Weeks	0.05 ± 0.10	-0.02 ± 0.06
p-NFκB p65 (Ser536) (AU) [b]		
Baseline	0.89 ± 0.09	0.67 ± 0.06
12 Weeks	0.82 ± 0.06	0.72 ± 0.07
Δ 0 to 12 Weeks	-0.07 ± 0.09	0.05 ± 0.12

Data are mean ± SEM. [a]Main effect for group ($p < 0.05$). [b]Trending main effect for group ($p < 0.08$).*Different between groups at that time point ($p < 0.05$). Abbreviations: FMD, flow-mediated dilation; FMD/SR_{AUC}; flow-mediated dilation normalized to shear rate area under the curve; MnSOD, manganese superoxide dismutase; p-eNOS, phosphorylated endothelial cell nitric oxide synthase; p-NFκB, phosphorylated nuclear factor kappa B.



Supplementary Figure 4.2. (A) Blood pressure, anthropometrics, and fat mass at baseline, 12 weeks, and the change from baseline to 12 weeks for responders (n = 8) and non-responders (n = 9) correlated with the change in flow-mediated dilation (FMD) from baseline to 12 weeks; and (B) blood pressure, anthropometrics, and fat mass at baseline, 12 weeks, and the change from baseline to 12 weeks for responders and non-responders correlated with the change FMD normalized to shear rate area under the curve (FMD/SR_{AUC}) from baseline to 12 weeks. Data are Spearman's Rho. *Significant correlation ($p < 0.05$). #Trending significant correlation ($p < 0.10$). Abbreviations: aPWV, aortic pulse wave velocity; AIx, augmentation index; AIx@75, augmentation index

normalized to 75 beats per minute; BMI, body mass index; DBP, diastolic blood pressure; Exp, expenditure; HC, hip circumference; SBP, systolic blood pressure; WC:HC, waist to hip circumference ratio.

APPENDIX 3: SUPPLEMENTARY DATA FOR CHAPTER 5

Supplementary Figures and **Tables**

Supplementary Table 5.1. Monocyte (THP-1) binding, insulin-stimulated nitric oxide production, and peroxynitrite concentrations in human aortic endothelial cells.

	Monocyte Binding (AU)	**Nitric Oxide (AU)**	**Peroxynitrite (AU)**
Blueberry + H_2O_2 (*n* = 22)^	139 ± 10$^{\#}$	52 ± 4*	395 ± 30**
Placebo + H_2O_2 (*n* = 20)^	135 ± 8$^{\#}$	55 ± 5$^{\#}$	429 ± 35**
Control (*n* = 8)	97 ± 7	78 ±6	106 ± 5
Control + H_2O_2 (*n* = 8)	147 ± 15$^{\#}$	-	107 ± 4
Control + insulin (*n* = 8)	-	96 ± 4***	-
Control + insulin + H_2O_2 (*n* = 8)	-	68 ± 6	-

Data are presented as relative fluorescence to control (mean ± SEM). ^Indicates that insulin was also added to blueberry and placebo serum groups for nitric oxide experiment. *Different compared to control within group ($p < 0.05$). **Different compared to control and control + H_2O_2 ($p < 0.05$). ***Different compared to blueberry + H_2O_2 and placebo + H_2O_2 ($p < 0.001$). $^{\#}$Trending different compared to control ($p < 0.08$). Abbreviations: AU, arbitrary units; H_2O_2, hydrogen peroxide.