HHS Public Access

Author manuscript

J Food Biochem. Author manuscript; available in PMC 2017 August 01.

Published in final edited form as:

J Food Biochem. 2016 August ; 40(4): 404–410. doi:10.1111/jfbc.12226.

Effects of a natural extract of *Aronia Melanocarpa* berry on endothelial cell nitric oxide production

Claudia Elena Varela[1], **Emilie Fromentin**[2], **Marc Roller**[3], **Francisco Villarreal**[1], and **Israel Ramirez-Sanchez**[1,4]

[1]School of Medicine, University of California San Diego, La Jolla, CA,U.S.A

[2]NATUREX DBS LLC., South Hackensack, NJ, U.S.A

[3]NATUREX SA., Avignon Cedex 9, France

[4]Sección de Estudios de Posgrado e Investigación, Escuela Superior de Medicina, Instituto Politécnico Nacional, D.F., Mexico

Abstract

The effects of acute and chronic treatment with Aronia extracts on NO production and endothelial nitric oxide synthase (eNOS) phosphorylation in bovine coronary artery endothelial cells were investigated. Acute time-course and concentration-response experiments were performed to determine the time and concentration at which Aronia induced maximal NO synthesis and eNOS phosphorylation. The findings indicate that relatively low concentrations (0.1 μg/mL) of Aronia extract significantly induced NO synthesis and eNOS phosphorylation after 10 min of treatment. Increased sensitivity of eNOS and a significant increase in NO synthesis resulted from longer-term stimulation with Aronia (48 hr) and an acute re-treatment of the cells (10 min).

PRACTICAL APPLICATIONS—These *in vitro* results may be translated into potential future clinical applications where Aronia extracts may be used for prevention and coadjuvant treatment of cardiovascular diseases via increases in endothelial NO synthesis and related improvements in vascular functions. Given the dose-response effect of Aronia extract *in vitro* and metabolism of polyphenols that occurs in humans, dose-response studies would be necessary to define the optimal daily amount to be consumed.

Keywords

Aronia Melanocarpa; chokeberry; nitric oxide; endothelial cells; vascular

INTRODUCTION

Nitric oxide (NO) is a key modulator of vascular function that, under physiological conditions, is mainly produced by the endothelial nitric oxide synthase (eNOS) isoform (Forstermann *et al.* 1994). The development of endothelial cell dysfunction can impair the

CORRESPONDENCE: Israel Ramirez-Sanchez, Ph. D. Sección de Estudios de Posgrado e Investigación, Escuela Superior de Medicina, Instituto Politécnico Nacional, Plan de San Luis y Díaz Mirón s/n. Colonia Casco de Santo Tomás, Delegación Miguel Hidalgo. C.P. 11340, México, D.F., Mexico. Tel: +52-55-5729-6000, israel.ramirez14@hotmail.com.

physiological production of NO leading to increased susceptibility to cardiovascular diseases (CVD) (Buijsse *et al.* 2006). Therefore, increasing NO bioavailability is recognized as an effective preventive and/or therapeutic approach to reduce the incidence of CVD (Versari *et al.* 2009). Several epidemiological studies have demonstrated that the consumption of polyphenol-rich diets are associated with lower risk of CVD most likely due to their positive impact on endothelial cell function (Vita 2005). Fruits such as berries, vegetables such as olives, red wine, green tea, cocoa products are known to be important sources of polyphenols (Perez-Jimenez *et al.* 2010). Red wine polyphenols and green tea catechins have been proven to induce endothelial cell-dependent relaxations by increasing NO synthesis (Schini-Kerth *et al.* 2010a, Schini-Kerth *et al.* 2010b). Similarly, (−)-epicatechin, a cacao flavonoid, has been shown to directly increase NO levels in human coronary artery endothelial cells (HCAEC) (Ramirez-Sanchez *et al.* 2010). One of the mechanisms responsible for NO increase is the PI3-kinase/Akt-dependent activation of eNOS following the phosphorylation of Ser1177 (an activator site) (Anselm *et al.* 2007, Auger *et al.* 2010, Ndiaye *et al.* 2005, Ramirez-Sanchez *et al.* 2010).

Aronia melanocarpa, also known as black chokeberry, is recognized as having one of the highest content of phenolic compounds among various kinds of berries (Kahkonen *et al.* 2001, Kulling and Rawel 2008, Valcheva-Kuzmanova and Belcheva 2006, Wu *et al.* 2004). Aronia contains high levels of proanthocyanidins, flavanols, anthocyanins (cyanidin glycosides), flavonoids (quercetin glycosides), chlorogenic acids, caffeic acid, triterpenes and fibers (Bermudez-Soto *et al.* 2007, Kokotkiewicz *et al.* 2010). Aronia extracts have been shown to positively impact CVD risk factors such as high blood pressure in patients after myocardial infarction (Naruszewicz *et al.* 2003) and metabolic syndrome (Chrubasik *et al.* 2010). In an animal model of NG-nitro-L-arginine methyl ester (L-NAME) induced hypertension *via* inhibition of eNOS, oral exposure to Aronia ethanolic extract for 8 weeks significantly reduced systolic and diastolic blood pressure (Ciocoiu *et al.* 2013). Moreover, Aronia juice has been proven to induce porcine coronary artery endothelium-dependent relaxations via eNOS activation (Kim *et al.* 2013). However, no studies have examined the acute, dose-dependent effects of concentrated Aronia extracts on coronary artery endothelial cell NO production as well as its sustained actions on eNOS protein and phosphorylation. Therefore, the aim of this study was to evaluate the acute and sustained effects of an Aronia extract on NO production and eNOS phosphorylation levels in cultured bovine coronary artery endothelial cells (BCAEC).

MATERIALS AND METHODS

BCAEC were purchased from Cell Applications, Inc. Dulbecco's Modified Eagle's Media (DMEM), fetal bovine serum (FBS), antibiotic and antimicotic solutions were from Gibco®. DMEM Nonessential Amino Acids and Hank's Balanced Salt Solution (HBSS) phenol red free were from Mediatech, Cellgro Inc. Protease, and phosphatase inhibitor cocktails, dimethyl sulfoxide (DMSO) and bradykinin (BK) were obtained from Sigma Aldrich. β-tubulin, phospho-eNOS (p-eNOS Ser1177) and eNOS primary antibodies, as well as the anti-rabbit horseradish peroxidase (HRP) conjugated secondary antibody were from Cell Signaling Technology. Polyvidinil fluoride (PVDF) transfer membrane was from Millipore.

Enhanced chemiluminescence (ECL) Plus Western Blot detection kit was from Amersham. The nitrite/nitrate fluorometric assay kit was from Cayman Chemical.

Preparation of *Aronia melanocarpa* extract

Aronia extract, reference EK004255, lot number K219/001/D14 used in this study was provided by NATUREX-DBS. It contained 15% anthocyanins and 48.5% of polyphenols, as gallic acid equivalent. The amount of carbohydrates, lipids and proteins (and thus, caloric content) in the extract was negligible.

Cell culture

BCAEC were grown in 10 cm dishes with DMEM complete medium supplemented with 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin and 0.25 μg/mL amphotericin B (medium A). Cells were incubated at 37°C in a humidified incubator with 5% CO_2, 95% air, and used for the experiments on their eighth passage at 80–85% confluence.

Cell treatment

For all experiments, 24 h before treatment, cell media was replaced with DMEM without phenol red plus 0.5% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin and 0.25 μg/mL amphotericin B (medium B). For NO measurements, cells were incubated with Aronia extract (or vehicle) for 10 min at increasing concentrations (0.0001–100 μg/mL). BK (1 μg/mL) was used as a positive control given its known role as an eNOS activator. The extract concentration that yielded highest NO measurement was then used to determine differences in NO production as a function of time. For these experiments, cells were incubated for 5, 10, 15, 30 and 60 min. For longer-term Aronia treatment, cells were incubated with Aronia extract for 48 hr. In a subset of 48 hr experiments, cells were provided an additional (*i.e.* second) dose of Aronia for 10 min to investigate the sustained treatment effects on NO production.

Total protein extraction

Cells were washed three times with cold HBSS without phenol red (5 mL/plate) and lysed in 100 μL of ice cold lysis buffer (1% Triton X-100, 20 mmol/L Tris, 140 mmol/L NaCl, 2 mmol/L ethylenediaminetetraacetic acid, and 0.1% Sodium Dodecyl Sulfate (SDS)) with protease and phosphatase inhibitor cocktails supplemented with 1 mmol/L phenylmethylsulfonyl fluoride, 2 mmol/L Na_3VO_4, and 1 mmol/L NaF. Homogenates were sonicated for 15 min at 4°C, and centrifuged (12,000 *g*) for 15 min to remove cell debris. The total protein concentration was measured in the supernatant using the Bradford method (Ernst and Zor 2010).

NO measurements

In order to evaluate the effect of Aronia on NO production, NO levels were measured in the form of nitrates/nitrites with a fluorescent kit (Cayman Chemical) according to manufacturer instructions using a fluorimeter (FLx800 Bio-Tek Instruments). After 24 hr of incubation with medium B, 5 mL of HBSS without phenol red (supplemented with 400 μM of L-arginine and 1 % of non-essential amino acids) were added to each plate and incubated for 2

h at 37°C in 5 % CO_2/95 % O_2. Following incubation, BCAEC were treated as described above. Medium samples were collected after 10 min. For the NO time-course production experiments, medium samples were collected after the incubation times noted above. Values were normalized to total protein after the cells were scrapped from the plate and lysed as described previously.

Western Blot

The phosphorylation of eNOS at the Ser1177 amino acid residue (p-eNOS) and total eNOS were measured by Western blot. Cells were treated as described above. After treatment, cells were lysed and total protein was extracted. A total of 30 μg of protein was loaded onto a 4–15% SDS polyacrylamide gel, electrotransferred, incubated for 1 hr in blocking solution (5% nonfat dry milk in tris-buffered saline solution plus 0.1% Tween 20 (TBS-T)), and followed by overnight incubation at 4°C with primary antibodies. Primary antibodies were diluted 1:1,000 in TBS-T plus 5% bovine serum albumin. Membranes were washed (3X for 5 min) in TBS-T and incubated 1 h in the presence of HRP-conjugated secondary antibodies diluted 1:10,000 in blocking solution. Membranes were again washed three times in TBS-T, and the immunoblots were developed using an ECL detection kit. The band intensities were digitally quantified and normalized by total amount of eNOS or β-tubulin.

Data analysis

Data was analyzed using Prism 3.0 (GraphPad Software, San Diego, CA). All values are expressed as mean ± standard error of the mean (SE). Statistical analysis of data was performed using one way ANOVA followed by the Tukey's post-hoc test. Data was considered significant when p-values obtained were <0.05.

RESULTS

NO production and eNOS activation by Aronia

As shown in Figure 1, maximal NO production (A) and eNOS activation (B) was achieved with a concentration of 0.1 μg/mL of Aronia extract which was ~75% of that reached with 1 μM BK (as such, all subsequent experiments utilized 0.1 μg/mL Aronia). Changes in the levels of NO production correlated with those observed in p-eNOS. The Aronia extract half maximal effective concentration (EC_{50}) was estimated using NO production levels (Figure 2). EC_{50} numerical values can be found in Table 1. An Aronia extract concentration of 0.001109 μg/mL yielded 50% of maximal NO synthesis.

Time-course of Aronia-induced NO production and eNOS activation

Aronia exposure for 10, 15 and 30 min yielded NO (Figure 3A) and phosphorylated eNOS levels (Figure 3B) statistically higher than those recorded at time 0. The maximal level of NO production and p-eNOS was achieved after 10 min of Aronia stimulation.

Effects of long term treatment

Levels of total eNOS protein were measured and normalized using β-tubulin (Liu and Xu 2006) and results are shown in Figure 4. Levels of total eNOS protein increased (p=0.06)

after 48 hr of Aronia treatment vs. control (Figure 4A). However, re-stimulation (10 min) with Aronia following the 48 hr treatment yielded a significant additional increase in p-eNOS (Figure 4B) and NO levels (Figure 5). p-eNOS values were normalized relative to total eNOS and β-tubulin.

DISCUSSION

Numerous studies have shown that the stimulation of NO production contributes to the cardiovascular health benefits associated with consumption of polyphenol rich products such as red wine, green tea, and cocoa (Ramirez-Sanchez *et al.* 2010, Schini-Kerth *et al.* 2010a, Schini-Kerth *et al.* 2010b). *Aronia melanocarpa* berries have been identified as having one of the highest content of phenolic compounds which characterizes it as a functional food (Kahkonen *et al.* 2001, Kulling and Rawel 2008, Valcheva-Kuzmanova and Belcheva 2006, Wu *et al.* 2004). Studies have utilized Aronia and other polyphenol-rich juices (Anselm *et al.* 2007, Kim *et al.* 2013) leading to similar observations as those in this study. However, the consumption of fruit juices and sugar-sweetened beverages with high caloric content has been associated with increased incidence of metabolic diseases such as Type 2 diabetes (Imamura *et al.* 2015). Therefore, a reasonable alternative to benefit from the nutraceutical potential of polyphenol-rich foods may be to consume extracts in forms such as capsules, tablets or powdered sachet. Furthermore, beyond possible acute effects (e.g. NO mediated vasorelaxation), it is important to ascertain the effects of sustained polyphenol consumption on the NO/eNOS pathway. For example, it has been recognized that statin-induced beneficial effects on the endothelium not only relate to their ability to reduce cholesterol synthesis and atherosclerosis but also by preventing the down regulation of eNOS and increased NO bioavailability (Davignon 2004).

Time-course and concentration-response experiments were performed in BCAEC to determine the optimal parameters at which Aronia stimulation was maximized. Significant increases in p-eNOS were evidenced with Aronia concentrations ranging from 0.001–10 μg/mL with peak effects observed at 0.1 μg/mL. As shown in Figure 1 and 3, these increases parallel the recorded NO levels. Interestingly, the levels of p-eNOS decreased when concentrations reached 1 μg/mL or greater. It is unclear as to what may explain this observation, but it may relate to a feedback control system activated when cells are over-stimulated with such extracts.

Studies that have examined various polyphenol rich sources have demonstrated that peak NO stimulation can be achieved at higher concentrations than those found to be most effective for the Aronia extract (Ndiaye *et al.* 2005, Anselm *et al.* 2007, Ramirez-Sanchez *et al.* 2010). For example, red wine polyphenols (red wine powder) induced significant NO production (~2 fold) at a concentration of 3 μg/mL (or higher) in porcine endothelial cells (Ndiaye *et al.* 2005). A 50% increase in NO levels was noted using the same cells with Concord grape juice at 44 μg/mL (Anselm *et al.* 2007). Studies using pure (−)-epicatechin induced ~3.5-fold increase in NO levels in HCAEC at a concentration of 294 μg/L (1 μM) (Ramirez-Sanchez *et al.* 2010). Therefore, the Aronia extract appears to induce maximal NO synthesis at lower relative concentrations compared to other phenolic extracts and juices; an advantage that could be exploited in the nutraceutical field.

In the time-course experiments, peak NO production and eNOS activation were observed within 10–30 min of Aronia treatment with a maximal NO level achieved at 10 min. This time frame of NO stimulation is similar to those reported for physiological stimulators of eNOS such as BK and estrogens (Chambliss and Shaul 2002, Ignjatovic *et al.* 2004). In line with our results, studies in porcine endothelial cells have reported that Aronia juice (67.3 μg GAE/mL) treatment can induced an approximate linear increase in eNOS phosphorylation after 3 min of treatment with peak effects observed at 30 min (Kim *et al.* 2013). Concord grape juice stimulation yielded a significant increase in p-eNOS levels after 3–30 min of exposure in the same types of cells (Anselm *et al.* 2007). It is worth noting that the levels of p-eNOS after 3–30 min of grape juice exposure seemed to remain unchanged and did not increase in a time-dependent manner as noted after Aronia juice treatment. Studies using rich-phenolic extracts report that p-eNOS levels decreased after peak levels were reached (Madeira *et al.* 2009, Ramirez-Sanchez *et al.* 2010). For example, a significant decrease in p-eNOS levels was observed after 10 min of grape skin extract (300 μg/mL) treatment in porcine endothelial cells (Madeira *et al.* 2009). A similar reduction in NO levels was noted in HCAEC after peak NO was observed at 10–30 min of (−)-epicatechin exposure (Ramirez-Sanchez *et al.* 2010). Although the time-course experiments with Aronia extract yielded a similar trend of NO and p-eNOS levels compared to grape skin and (−)-epicatechin extracts, the concentration utilized in this study was approximately 3 orders of magnitude lower.

No previous studies have reported on the long-term effects of Aronia treatment on eNOS protein levels and a limited number of studies appear to have investigated long term treatment of other rich-phenolic extracts in endothelial cells. Using the most effective concentration of the Aronia extract (0.1 μg/mL) as a treatment for 48 hr, we observed a trend for stimulation of eNOS protein levels (~35% over control) which upon re-treatment of the cells at 10 min led to significant stimulation of NO production and p-eNOS (~1.5 fold). Despite the fact that total protein levels did not substantially increase, greater levels of NO and p-eNOS after 48 hr +10 min of Aronia treatment may indicate greater sensitivity of the enzyme per given stimulus. There are reports on the effects of long-term treatment with polyphenolic rich compounds on eNOS protein and activity levels in endothelial cells (Leikert *et al.* 2002, Takahashi and Nakashima 2012). Leikert *et al.* reported that the stimulation of human umbilical vein endothelial cells for 20 hr with 600 μg/mL red wine polyphenol extract led to approximately 2.1 fold increase in eNOS protein levels and approximately 2 fold in NO production. Similarly, 24 hr treatment with resveratrol (10 mM-1 μM) of HCAEC yielded from 1.5–2.5 fold increase in eNOS protein expression in a concentration-dependent manner. Takahashi and Nakashima also observed substantial time- and concentration-dependent increments of eNOS total protein levels and NO production throughout a 5-day treatment with resveratrol. Approximately, 2.5 fold increase in total eNOS and NO levels were reported after 5 days of resveratrol (50 nM) treatment. However, they did not observe significant increases in phosphorylated eNOS levels with long-term treatment. In contrast, this study observed about a 2.5 fold increase in p-eNOS protein levels following the 48 hr plus 10 min Aronia treatment. Perhaps long-term Aronia exposure prevents the down regulation of eNOS and increases its sensitivity promoting additional NO synthesis, which may have a positive long-lasting preventive effect against CVD.

In conclusion, the results indicate that *Aronia melanocarpa* extract is an effective inducer of NO synthesis in a time- and dose-dependent manner most likely due to its high phenolic content. Long-term exposure to Aronia extract appears to further stimulate NO synthesis which may have important implications in future investigations using the extract.

Acknowledgments

The study was funded by contract with NATUREX-DBS and by NIH R01 support to F. Villarreal (DK0987171)

References

ANSELM E, CHATAIGNEAU M, NDIAYE M, CHATAIGNEAU T, SCHINI-KERTH VB. Grape juice causes endothelium-dependent relaxation via a redox-sensitive Src- and Akt-dependent activation of eNOS. Cardiovasc Res. 2007; 73(2):404–413. [PubMed: 16962569]

AUGER C, KIM JH, CHABERT P, CHAABI M, ANSELM E, LANCIAUX X, LOBSTEIN A, SCHINI-KERTH VB. The EGCg-induced redox-sensitive activation of endothelial nitric oxide synthase and relaxation are critically dependent on hydroxyl moieties. Biochem Biophys Res Commun. 2010; 393(1):162–167. [PubMed: 20117080]

BERMUDEZ-SOTO MJ, TOMAS-BARBERAN FA, GARCIA-CONESA MT. Stability of polyphenols in chokeberry (*Aronia melanocarpa*) subjected to in vitro gastric and pancreatic digestion. Food Chem. 2007; 102(3):865–874.

BUIJSSE B, FESKENS EJ, KOK FJ, KROMHOUT D. Cocoa intake, blood pressure, and cardiovascular mortality: the Zutphen Elderly Study. Arch Intern Med. 2006; 166(4):411–417. [PubMed: 16505260]

CHAMBLISS KL, SHAUL PW. Estrogen modulation of endothelial nitric oxide synthase. Endocr Rev. 2002; 23(5):665–686. [PubMed: 12372846]

CHRUBASIK C, LI G, CHRUBASIK S. The clinical effectiveness of chokeberry: a systematic review. Phytother Res. 2010; 24(8):1107–1114. [PubMed: 20572194]

CIOCOIU M, BADESCU L, MIRON A, BADESCU M. The involvement of a polyphenol-rich extract of black chokeberry in oxidative stress on experimental arterial hypertension. Evid Based Complement Alternat Med. 2013; 2013:912769. [PubMed: 23533529]

DAVIGNON J. Beneficial cardiovascular pleiotropic effects of statins. Circulation. 2004; 109(23 Suppl 1):III39–43. [PubMed: 15198965]

ERNST O, ZOR T. Linearization of the bradford protein assay. J Vis Exp. 2010; (38)

FORSTERMANN U, CLOSS EI, POLLOCK JS, NAKANE M, SCHWARZ P, GATH I, KLEINERT H. Nitric oxide synthase isozymes. Characterization, purification, molecular cloning, and functions. Hypertension. 1994; 23(6 Pt 2):1121–1131. [PubMed: 7515853]

IGNJATOVIC T, STANISAVLJEVIC S, BROVKOVYCH V, SKIDGEL RA, ERDOS EG. Kinin B1 receptors stimulate nitric oxide production in endothelial cells: signaling pathways activated by angiotensin I-converting enzyme inhibitors and peptide ligands. Mol Pharmacol. 2004; 66(5): 1310–1316. [PubMed: 15304551]

IMAMURA F, O'CONNOR L, YE Z, MURSU J, HAYASHINO Y, BHUPATHIRAJU SN, FOROUHI NG. Consumption of sugar sweetened beverages, artificially sweetened beverages, and fruit juice and incidence of type 2 diabetes: systematic review, meta-analysis, and estimation of population attributable fraction. BMJ. 2015; 351:h3576. [PubMed: 26199070]

KAHKONEN MP, HOPIA AI, HEINONEN M. Berry phenolics and their antioxidant activity. J Agric Food Chem. 2001; 49(8):4076–4082. [PubMed: 11513713]

KIM JH, AUGER C, KURITA I, ANSELM E, RIVOARILALA LO, LEE HJ, LEE KW, SCHINI-KERTH VB. Aronia melanocarpa juice, a rich source of polyphenols, induces endothelium-dependent relaxations in porcine coronary arteries via the redox-sensitive activation of endothelial nitric oxide synthase. Nitric Oxide. 2013; 35:54–64. [PubMed: 23973200]

KOKOTKIEWICZ A, JAREMICZ Z, LUCZKIEWICZ M. Aronia plants: a review of traditional use, biological activities, and perspectives for modern medicine. J Med Food. 2010; 13(2):255–269. [PubMed: 20170359]

KULLING SE, RAWEL HM. Chokeberry (Aronia melanocarpa) - A review on the characteristic components and potential health effects. Planta Med. 2008; 74(13):1625–1634. [PubMed: 18937167]

LEIKERT JF, RATHEL TR, WOHLFART P, CHEYNIER V, VOLLMAR AM, DIRSCH VM. Red wine polyphenols enhance endothelial nitric oxide synthase expression and subsequent nitric oxide release from endothelial cells. Circulation. 2002; 106(13):1614–1617. [PubMed: 12270851]

LIU NK, XU XM. beta-tubulin is a more suitable internal control than beta-actin in western blot analysis of spinal cord tissues after traumatic injury. J Neurotrauma. 2006; 23(12):1794–1801. [PubMed: 17184189]

MADEIRA SV, AUGER C, ANSELM E, CHATAIGNEAU M, CHATAIGNEAU T, SOARES DE MOURA R, SCHINI-KERTH VB. eNOS activation induced by a polyphenol-rich grape skin extract in porcine coronary arteries. J Vasc Res. 2009; 46(5):406–416. [PubMed: 19155632]

NARUSZEWICZ M, DANIEWSKI M, LANIEWSKA I, PIKTO-PIETKIEWICZ W, MILLO B, ZAPOLSKA-DOWNAR D. Effect of anthocyanins from chokeberry (Aronia melanocarpa) on blood pressure, inflammatory mediators and cell adhesion molecules in patients with a history of myocardial infarction (MI). Atherosclerosis Supp. 2003; 4(2):143–143.

NDIAYE M, CHATAIGNEAU M, LOBYSHEVA I, CHATAIGNEAU T, SCHINI-KERTH VB. Red wine polyphenol-induced, endothelium-dependent NO-mediated relaxation is due to the redox-sensitive PI3-kinase/Akt-dependent phosphorylation of endothelial NO-synthase in the isolated porcine coronary artery. FASEB J. 2005; 19(3):455–457. [PubMed: 15623569]

PEREZ-JIMENEZ J, NEVEU V, VOS F, SCALBERT A. Identification of the 100 richest dietary sources of polyphenols: an application of the Phenol-Explorer database. Eur J Clin Nutr. 2010; 64(Suppl 3):S112–120. [PubMed: 21045839]

RAMIREZ-SANCHEZ I, MAYA L, CEBALLOS G, VILLARREAL F. (−)-epicatechin activation of endothelial cell endothelial nitric oxide synthase, nitric oxide, and related signaling pathways. Hypertension. 2010; 55(6):1398–1405. [PubMed: 20404222]

SCHINI-KERTH VB, AUGER C, ETIENNE-SELLOUM N, CHATAIGNEAU T. Polyphenol-induced endothelium-dependent relaxations role of NO and EDHF. Adv Pharmacol. 2010a; 60:133–175. [PubMed: 21081218]

SCHINI-KERTH VB, AUGER C, KIM JH, ETIENNE-SELLOUM N, CHATAIGNEAU T. Nutritional improvement of the endothelial control of vascular tone by polyphenols: role of NO and EDHF. Pflugers Arch. 2010b; 459(6):853–862. [PubMed: 20224869]

TAKAHASHI S, NAKASHIMA Y. Repeated and long-term treatment with physiological concentrations of resveratrol promotes NO production in vascular endothelial cells. Br J Nutr. 2012; 107(6):774–780. [PubMed: 21791144]

VALCHEVA-KUZMANOVA SV, BELCHEVA A. Current knowledge of Aronia melanocarpa as a medicinal plant. Folia Med (Plovdiv). 2006; 48(2):11–17. [PubMed: 17408071]

VERSARI D, DAGHINI E, VIRDIS A, GHIADONI L, TADDEI S. Endothelial dysfunction as a target for prevention of cardiovascular disease. Diabetes Care. 2009; 32(Suppl 2):S314–321. [PubMed: 19875572]

VITA JA. Polyphenols and cardiovascular disease: effects on endothelial and platelet function. Am J Clin Nutr. 2005; 81(1 Suppl):292S–297S. [PubMed: 15640493]

WU X, GU L, PRIOR RL, MCKAY S. Characterization of anthocyanins and proanthocyanidins in some cultivars of Ribes, Aronia, and Sambucus and their antioxidant capacity. J Agric Food Chem. 2004; 52(26):7846–7856. [PubMed: 15612766]

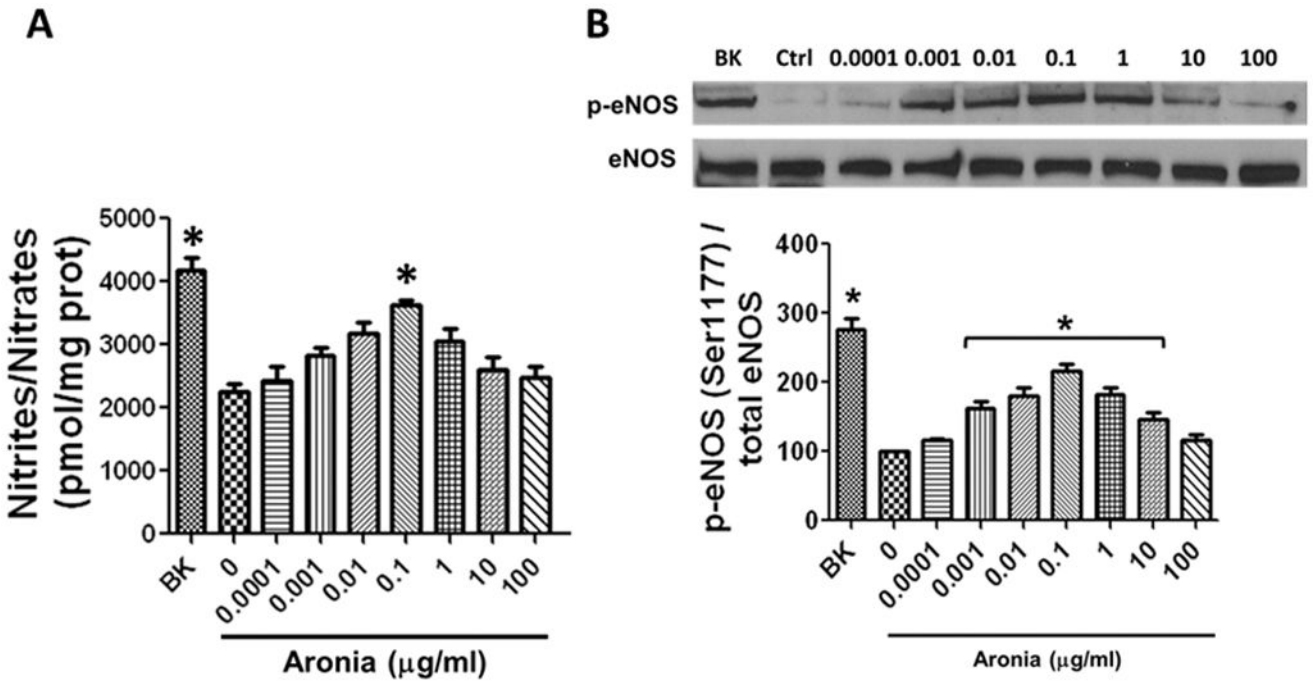

FIGURE 1. CONCENTRATION RESPONSE OF ARONIA-INDUCED NO SYNTHESIS AND ENOS ACTIVATION

A, Aronia concentration-response curve on NO synthesis. NO production after 0.1 μg/mL Aronia treatment showed to be statistically different from basal conditions. B, Concentration-response curve of Aronia-induced eNOS activation. Aronia (.001–10 μg/mL) and Bradykinin treatment significantly increased the phosphorylation of Ser1177 compared to basal level. Normalized basal eNOS activation level was set to 100. Data are expressed as mean ± SE (n=3). *p<0.05 Aronia vs. basal (vehicle only).

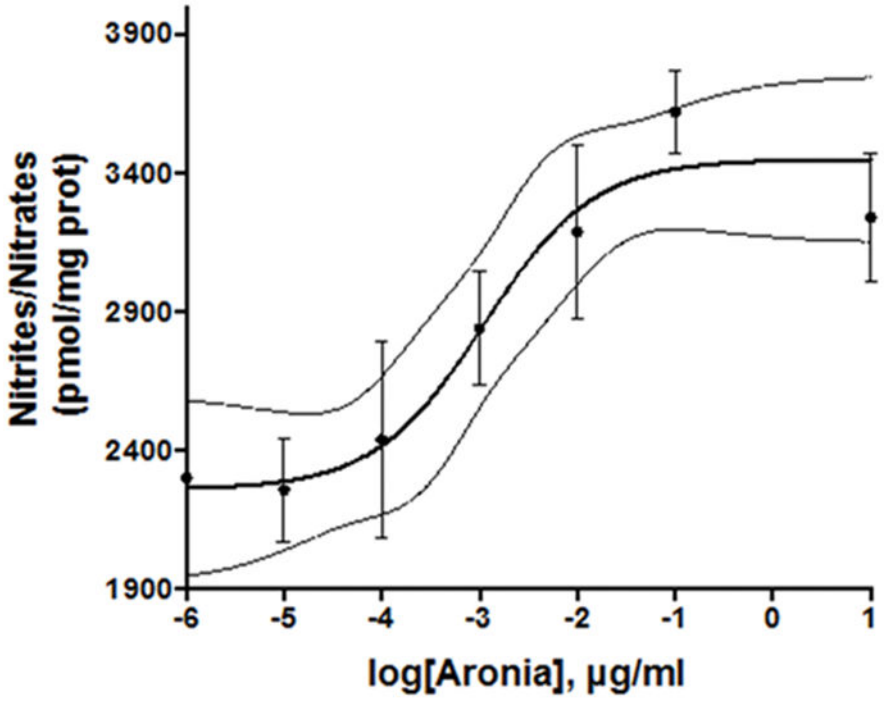

FIGURE 2. HALF MAXIMAL EFFECTIVE CONCENTRATION (EC_{50}) FOR THE STIMULATION OF NO LEVELS BY ARONIA

NO levels (pmol/mg protein) from concentration-response graph (Figure 1A) were used to calculate the EC_{50} value (and confidence intervals) for the Aronia extract. The concentration of Aronia that induced 50% of the maximum NO production level = 0.001109 µg/(n=3).

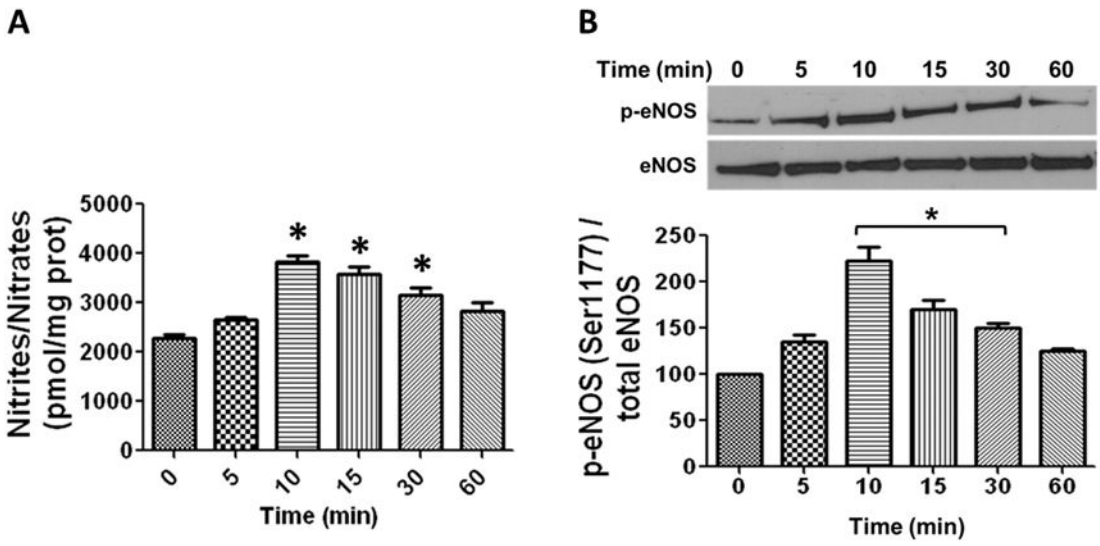

FIGURE 3. TIME-COURSE OF ARONIA-INDUCED STIMULATION OF NO SYNTHESIS AND ENOS ACTIVATION

A, Time-course of NO synthesis by Aronia. B, Time-course of Aronia-induced eNOS activation. Normalized basal level was arbitrarily set to 100. Data are expressed as mean ± SE (n=3). *p<0.05 Aronia vs. basal (time=0).

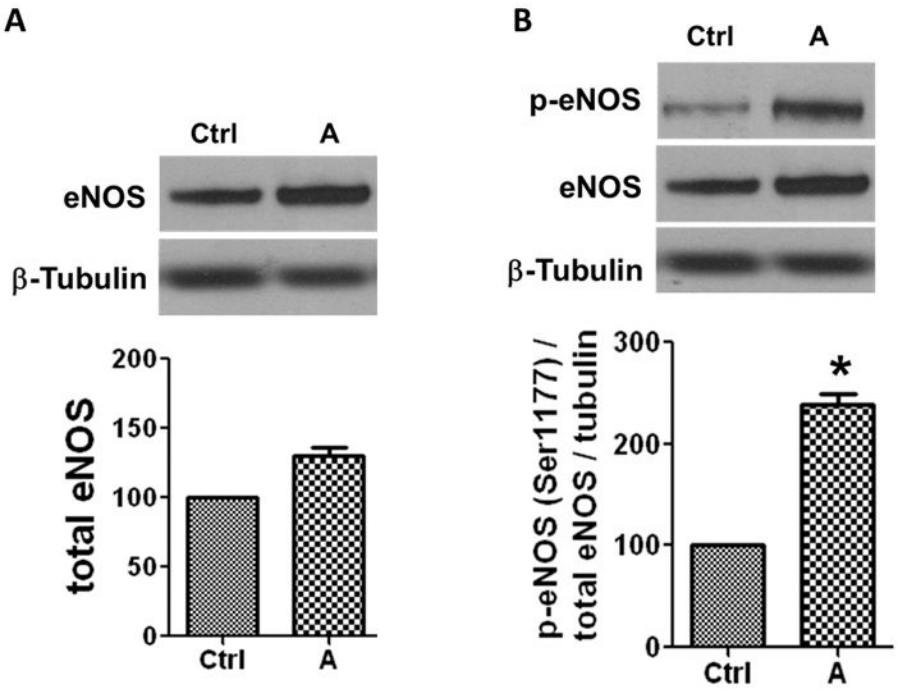

FIGURE 4. NO SYNTHESIS AFTER LONG TERM (48 HR) ARONIA TREATMENT MEASURED VIA ENOS PHOSPHORYLATION

A, Endogenous levels of total eNOS protein measured after 48 hr of Aronia treatment. Compared to control group, total eNOS protein level in the group under 48 hr-Aronia treatment was not significantly increased. B, eNOS phosphorylation detected after 10 min re-treatment with Aronia showing significantly increased levels compared to control group. Control group was maintained under basal conditions. Normalized control protein levels were arbitrarily set to 100. Data are expressed as mean ± SE (n=3). *$p<0.05$ Aronia vs. control (Ctrl = vehicle only).

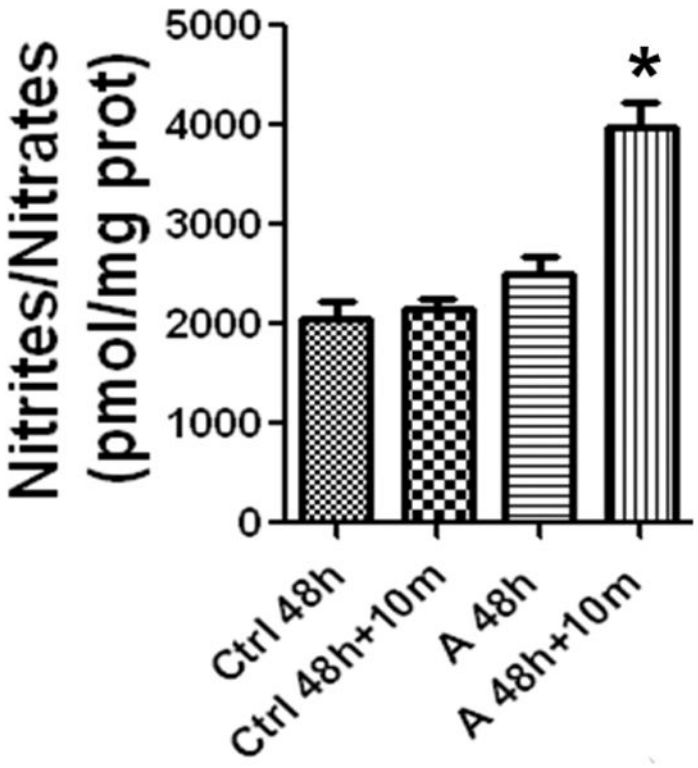

FIGURE 5. NO LEVELS AFTER LONG TERM (48 HR) TREATMENT WITH ARONIA

NO production significantly increased compared to control after long term Aronia exposure followed by acute re-treatment with the extract. Data are expressed in mean ± SE (n=3). *p<0.05 Aronia 48 hr +10 min vs. control.

TABLE 1

NUMERICAL VALUES OF ARONIA EC_{50} CALCULATION.

Best-fit values	
Log EC_{50}	−2.955
Hill Slope	0.7762
EC_{50}	0.001109
Goodness of Fit	
R^2	0.8110