Assessment of Delphinol® on Measures of Vascular Function in Men and Women

Jacquelyn Pence[1], Roddy Morris[1], Ivo Pischel[2], Richard J. Bloomer[1]

*[1]Center for Nutraceutical and Dietary Supplement Research, College of Health Sciences, University of Memphis, Memphis, TN, USA.

[2]Centre for Pharmacognosy and Phytotherapy, UCL School of Pharmacy, University of London, 29-39 Brunswick Square, London WC1N 1AX, UK.

RESEARCH

Please cite this paper as: Pence J, Morris R, Pischel I, Bloomer RJ. Assessment of Delphinol® on measures of vascular function in men and women. Journal of Food & Nutritional Sciences [2021] 3(2): 18-30.

***Corresponding Author:**

Richard J. Bloomer, PhD

Center for Nutraceutical and Dietary Supplement Research, College of Health Sciences, University of Memphis, Memphis, TN, USA, Tel: 901-678-5638; E-mail: rbloomer@memphis.edu

ABSTRACT

Antioxidant agents have been reported to impact measures of vascular health. **Methods:** Using a crossover design, 10 men and women ingested a botanical agent known as Delphinol® (an extract of maqui berries) at a dosage of 60mg, 120mg, and 180mg for one week, with a one-week washout period between each dose. Before and following the intervention periods, the perfusion index (PI) was measured after 5 minutes of brachial artery occlusion, while blood nitrate/nitrite was measured before and following occlusion. **Results:** No statistically significant differences were noted between conditions for nitrate/nitrite or for the change in PI from pre- to post-occlusion ($p>0.05$). However, there was a ~20% increase in the PI from pre- to post-occlusion for the 180mg condition, with much less of an increase noted for the other doses. **Conclusion:** A lower dosage of Delphinol® does not influence the PI following brachial artery occlusion; however, a dosage of 180mg taken for one week may have an impact on vascular function, as indicated by the PI. Future studies involving a longer time course of treatment may be needed to more fully elucidate the impact of Delphinol® on vascular function.

Keywords: Nitrate, vascular, antioxidants, maqui berries.

LIST OF ABBREVIATIONS

Perfusion index (PI)

Vascular reactivity index (VRI)

Electrocardiogram (ECG)

Analysis of variance (ANOVA)

Cardiovascular disease (CVD)

Low-density lipoprotein (LDL)

Flow-mediated dilation (FMD)

Pulse wave velocity (PWV)

Oxidized LDL (oxLDL)

High-density lipoprotein (HDL)

INTRODUCTION

Antioxidant compounds have been well-studied in relation to surrogate measures of overall health, with a particular impact on the function of blood vessels [1]. Delphinol® is a botanical agent (an extract of maqui berries) with antioxidant properties primarily owing to its high

anthocyanin content [2]. Delphinol® has excellent bioavailability, as reported recently [3]. Acute and chronic anthocyanin consumption has demonstrated the ability to improve vascular reactivity assessed via flow-mediated dilation and pulse wave velocity (PWV), across a range of dosages [4]. Antioxidant compounds have also been reported to favorably impact nitric oxide, often estimated using the surrogate measure of blood nitrate/nitrite.

In a previous study using Delphinol® at various dosages, this agent significantly and dose-dependently improved glucose homeostasis acutely [5], with noted lowering of blood glucose and lipids [6]. The mechanism of action for this effect appears related to a novel sodium glucose co-transporter inhibition [7]. This favorable effect on glucose and lipid metabolism may be associated with the protection of vascular function [8]. A dosage of 450mg of Delphinol® daily has resulted in improvements in oxidative stress markers—another factor that can potentially contribute to vascular homeostasis [9]. Together, these prior studies suggest Delphinol® may influence vascular function; however, no studies have directly determined this in human subjects.

Though similar human studies exist demonstrating anthocyanin consumption to improve vascular function [10], none to date have investigated different doses of a maqui extract on vascular function. The aim of this pilot study was to determine the impact of Delphinol® at varying dosages on vascular function assessed using the vascular reactivity index (VRI), perfusion index (PI), as well as blood nitrate/nitrite concentrations, in men and women.

MATERIALS AND METHODS

A total of 5 men and 5 women were enrolled and completed this study. All procedures were approved by the University of Memphis Institutional Review Board for Human Subjects Research (protocol FY-2020-66). Subjects were required to be between 18 and 35 years old, non-tobacco users, not obese (body mass index under 30 kg/m^2), with no history of diabetes, cardiovascular disease (including electrocardiogram [ECG] abnormalities), or neurological disease, willing to refrain from fruit, alcohol, and caffeine within 48 hours of each test day, willing to refrain from strenuous exercise for 24 hours prior to each test day, without active infection of any kind, and if female, not pregnant. Subject descriptive characteristics are presented in Table 1. Subjects were paid $200 for their full participation.

Screening visit

During the initial visit to the laboratory, subjects completed the informed consent form, health history, medication and dietary supplement usage, and physical activity questionnaires. Subjects' heart rate and blood pressure, height, weight, waist, and hip circumference were measured. Subjects were screened for normal ECG tracings and glucose measurements. To confirm non-pregnancy, females were provided with a urine pregnancy test kit, escorted to a private restroom (within the lab), and asked to perform the test. Eligible subjects were scheduled for bi-weekly testing visits after screening was completed.

Independent Variable

The botanical agent known as Delphinol® was delivered in a capsule at a daily dosage of 60mg, 120mg, and 180mg of the maqui berry extract powder, for a period of one week, in addition to a placebo (cellulose). A random order, cross-over design with a one-week washout period between conditions was used. A contract manufacturer produced the capsules in accordance with Good Manufacturing Practices. The capsules were similar in appearance and provided in unlabeled bottles. Subjects reported to the lab in a 10-hour fasted state. Women started the first round of treatment during week one of the menstrual cycle. The total subject involvement was approximately 8 weeks.

Dietary Intake and Physical Activity

Subjects followed their usual activity patterns over the course of the study period but refrained from strenuous activity for the 24 hours preceding each lab test day. Dietary

intake was similar over the entire study period. However, subjects consumed the same standard prepackaged meals during the day prior to each test day. These included meal replacement drinks (Orgain; Irvine, CA), food bars (Clif Builder; Emeryville, CA), and nuts (Emerald; S-L Snacks National LLC, Irvine, CA). Each subject received an allotment of these items, based on preference (e.g., 3 meal replacement drinks, 4 food bars, 2 packs of nuts). They were then given the same items and volume for subsequent visits; following the same food plan for the days prior to all lab test days. No other food should have been consumed during the day prior to each lab visit, other than what was provided to subjects by the investigators.

Study Test Visits

During the first test visit (baseline), resting heart rate and blood pressure were measured following a 20-minute rest, and 6 mL of blood was collected in a Hematology K3 EDTA Evacuated Tube (Greiner Bio-One; Monroe, North Carolina) to quantify nitrate/nitrite (see corresponding section below for details). Vascular function was then assessed using PI and VRI (see vascular function section below for details). Subjects were randomly assigned to one of the four conditions: 60mg, 120mg, or180mg Delphinol®, or cellulose placebo which they took while in the lab at the end of the visit and at home daily until their next test visit.

On day 8, subjects returned for their 2nd test visit. Resting heart rate and blood pressure were obtained following a 20-minute rest, and approximately 6mL blood was collected. Subjects then ingested the daily dose of their assigned condition. Ninety minutes after ingesting the condition, blood pressure and heart rate were reassessed and approximately 6 mL of blood was collected. Vascular function was again assessed using PI and VRI. After the 2nd visit, subjects underwent a 7-day washout period before starting one of the remaining conditions, randomly assigned. The subsequent three visits followed this same pattern.

Nitrate/Nitrite quantification

After each collection, blood samples were immediately placed in a refrigerated centrifuge (4°C), and the plasma was separated by centrifugation at 1,500g for 15 minutes. The plasma was then stored immediately at −80°C until analyzed for nitrate/nitrite, using commercially available assay kits (kit 780001; Cayman Chemical, Ann Arbor, MI, USA). After conversion of nitrate to nitrite using nitrate reductase, Greiss reagent was added to the sample, which converts nitrite into a deep purple azo compound. The absorbance of this azo chromophore was then detected photometrically at 540 nm using a BioTek Instruments Powerwave 340 microplate reader (BioTek Instruments, Winooski, Vermont, USA), and unknown sample values were determined using a nitrite standard curve, via software (BioTek Gen5™). Assays were performed in duplicate, and the coefficient of variation was found to be 7.6%.

Vascular Function

Vascular function was monitored 90 min post ingestion of supplement using a Vendys II unit (Endothelix; Houston, TX). Subjects were in the seated position with their hands resting on an insulated lap pad. A pressure cuff was placed around their upper right arm. A neck heating blanket was used as necessary to achieve a fingertip temperature between 30° to 33°C. Temperature probes were placed on the lap pad (for measuring ambient temperature) and subject's left and right first fingers to measure changes in fingertip temperature, which the Vendys II used in calculating VRI. Blood pressure and heart rate were measured, and the average PI was measured on subject's right index finger by a Masimo MightySat® pulse oximeter (Masimo, Irvine, CA) and recorded every minute for 5 minutes prior to occlusion. The pressure cuff was then inflated to 35 mmHg above their systolic blood pressure. The blood flow was occluded for 5 min and then the pressure was released. The average PI was then recorded for each min for 5 min post-occlusion. The averages over the 5 min periods were taken and used in the further analysis.

Data Analysis

The data are presented as mean ± SD. Most data were analyzed using a 5 condition x 2 time analysis of variance (ANOVA). A one-way ANOVA was used for change in perfusion index between pre-occlusion and post-occlusion. Analyses were performed using JMP software (SAS, Cary, NC) and statistical significance was set at $p \leq 0.05$.

RESULTS

Overview

All 10 subjects successfully completed the study. None of these subjects reported a problem with capsule consumption and all conditions were well tolerated. As the response was similar for both men and women, data were analyzed collectively and presented as such.

Heart Rate and Blood Pressure

There were no significant main effects for heart rate (condition; p=0.86, time; p=0.16, or condition x time interactions; p=0.54), systolic blood pressure (condition; p=0.74, time; p=0.66, or condition x time interactions; p=0.87), or diastolic blood pressure (condition; p=0.52, time; p=0.67, or condition x time interactions; p=0.49). Values remained relatively stable across time and were not different between conditions. Data for heart rate are presented in Figure 1, while those for systolic and diastolic blood pressure are presented in Figure 2 and 3, respectively.

Nitrate/Nitrite

The nitrate/nitrite measurements for one subject were significantly and consistently higher than for all other subjects; hence, data for this subject were excluded from the analysis. No condition; p=0.91, time; p=0.23, or condition x interactions; p=0.91 were noted for nitrate/nitrite levels. Values remained relatively stable across time and were not different between conditions. Data for nitrate/nitrite are presented in Figure 4.

PI

No significant differences between conditions were detected in the change in PI from pre- to post-occlusion between conditions (p>0.05). However, there was a ~20% increase from pre- to post-occlusion for the 180mg condition (p=0.40), with much less increase noted for the other conditions. Data for the change in PI from pre-occlusion to post-occlusion are presented in Figure 5.

VRI

A very high degree of variability in VRI values was observed in the study and was therefore not included in the data analysis. However, a visual of the overall results is shown in Figure 6.

DISCUSSION

Anthocyanins are polyphenolic flavonoids contributing to the red, blue, and purple pigment of many fruits, vegetables, and flowers and are of medicinal interest for their anti-oxidant and anti-inflammatory properties [11]. The benefits of anthocyanins are being explored for numerous inflammatory diseases including Alzheimer's, diabetes, cardiovascular disease (CVD), obesity, and Parkinson's Disease [11–15]. One way that anthocyanins contribute to disease prevention is by reducing oxidative stress through anti-oxidation or scavenging free-radicals, which damage cells and cause inflammation. The types (e.g., cyanidins, delphinidins, malvidins, pelargonidins, peonidins and petunidins) and amounts of anthocyanins present within botanicals vary largely. Overall and coworkers characterized the anthocyanin profile within six different berries and demonstrated that the berries resulted in different metabolic effects [16]. Therefore, it is important not to generalize the benefits observed for one particular berry.

Consumption of anthocyanin- and anthocyanidin-rich foods have been negatively correlated to the risk of myocardial infarction in women and non-fatal myocardial infarction in men [17–19]. Flavonoids have been shown to

have cardiovascular benefits, including in meta-analyses specific to anthocyanin interventions [4,19–23]. In particular, anthocyanins improved levels of low-density lipoprotein (LDL) cholesterol in individuals at risk for or having cardiovascular disease [22,23]. Improvements to fasting glucose and Hemoglobin A1c were also observed [23]. As far as direct measures to changes in vascular function, both acute and chronic anthocyanin consumption has been shown to improve vascular reactivity assessed via flow-mediated dilation and pulse wave velocity across a range of dosages [4].

Maqui berries are the fruit of the Aristotelia chilensis tree from South America and are rich in anthocyanin and other polyphenols [24]. Like other sources of anthocyanin, maqui berries have been shown to have anti-inflammatory, anti-oxidant and cardio-protective properties [24]. Maqui berries contain approximately 137.6 mg of total anthocyanins per 100 g fruit, primarily consisting of delphinidins and cyanidins [2]. One study in rats found that maqui berry extracts protected against heart damage following acute ischemia and reperfusion by lowering lipid oxidation as quantified by thiobarbituric acid reactive substances [25].

In the present study, an encapsulated maqui berry extract standardized to contain 35% of total anthocyanins and 25% delphinidins, called Delphinol®, was used to evaluate vascular benefits within healthy men and women. Delphinol® has excellent bioavailability with both elevations in Delphinidin-3-O-glucoside and Cyanidin-3-O-sambubioside and their breakdown products observed during the hours following ingestion [3].

Delphinol® has previously been shown to induce physiological changes that lend support to the hypothesis that the supplement could aid vascular health. In past studies, Delphinol® significantly and dose-dependently improved glucose homeostasis acutely, with noted lowering of blood glucose and lipids, potentially by inhibiting a novel sodium glucose cotransporter [5–7]. Further, a dose of 450mg Delphinol® per day for four weeks resulted in improvements in oxidative stress markers, another factor that can potentially contribute to vascular homeostasis [9]. The current study sought to further elucidate the effects of acute ingestion of low dosages of Delphinol® on vascular function through various parameters associated with vascular health: blood pressure, heart rate, changes in perfusion following acute ischemia, as well as nitrate/nitrite.

Meta-analyses of the role of polyphenols and flavonoids on blood pressure did not find significant changes to blood pressure, while certain specific polyphenolic sources such as high-flavanol containing cocoa or olive oil were beneficial to blood pressure [26,27]. Similarly, meta-analyses on the effects of anthocyanins on blood pressure found no significant benefit [22,28]. Most of these studies involved higher concentrations of anthocyanins (7.35-640 mg/day) with much longer interventions than used in the present study (e.g., 3-24 weeks). As noted in the Results section, our data agree with the above, as we found no significant changes to either systolic or diastolic blood pressure for any of the three dosages used in our study. Additionally, no changes to heart rate were observed.

Vascular function was assessed by differences in VRI calculated by finger temperature rebound following a 5 min occlusion and changes in perfusion index from a pulse-oximeter following occlusion. High variability was present within subjects' VRI values, possibly due to variations in testing conditions between visits, including room temperature and initial fingertip temperatures. We therefore did not analyze these data. No differences of statistical significance were detected in the perfusion indices pre-occlusion or in the change in PI between pre- and post-occlusion for any dosage. However, there was an approximate 20% increase from pre- to post-occlusion for the 180mg condition. Due to the high degree of variability across subjects, the increase was not of statistical significance, but may have clinical relevance and deserves further study. Perhaps a larger sample size and reduced variability would have resulted in a different outcome. It is known that the PI is characterized by high skewness and

high inter-individual variability but may be helpful monitoring the trend within a given subject [29–31]. While there were no observed changes in PI, it must be noted that subjects were young and healthy, with very good vascular function. Therefore, there may have been little room for improvement in the noted measures. Moreover, the dose and timeframe of treatment may have been too low/short in order to detect potential improvements.

A meta-analysis of previous studies including both healthy and unhealthy populations, has shown that acute and chronic anthocyanin supplementation led to higher flow-mediated dilation (FMD), while acute anthocyanin supplementation only led to improved pulse-wave velocity [4]. The studies included in the analysis varied greatly in both anthocyanin doses (1.34-724 mg per dose) and interventions lengths (1 hr-6 months). Only a small number of studies involved healthy individuals with healthy BMIs. Rodriguez-Mateos et al. observed a dose dependent response in FMD to anthocyanins (129-724 mg anthocyanin from blueberry drink) one hour after acute ingestion in healthy men and improvements to FMD at 1, 2, and 6 hours for 310 and 517 mg anthocyanin, but no change in pulse wave velocity (PWV) was observed [10]. In a separate study, healthy men received acute doses of cranberry juice containing relatively low doses of anthocyanin (6.8 - 32.3 mg per dose) but high in polyphenols (409 - 1910 mg per dose) and found changes in FMD over the 8 hour data collection period with different doses [32]. Two separate studies, one using 127 mg anthocyanin daily for 4 weeks and a second using 274 mg anthocyanin for 6 weeks, found no significant changes in PWV within healthy individuals [33,34]. Therefore, not all vascular function measures have been reported to be altered when assessed in a healthy population. Additionally, many of the prior studies used a much higher dose of anthocyanin than used in the present study, which contained approximately 21-63 mg anthocyanin per dose. A higher dosage may, therefore, lead to changes in vascular function that were not observed in the present study.

Nitric oxide is an important vascular regulator that is protective against cardiovascular diseases [35]. Previous in vitro studies with endothelial cells, as well as in vivo study of aged rats, found anthocyanin and anthocyanin containing extracts upregulated the synthesis of nitric oxide and endothelial nitric oxide synthase in endothelial cells and rat aortas [36–38]. As nitric oxide is difficult to measure, it is often estimated using the surrogate measure of blood nitrate/nitrite [39,40]. In the present study, no differences were observed either at 0 or 90 minutes for any of the conditions. Again, this could be due to the low dosage of anthocyanin used, or the short timeframe of treatment, as previous studies have noted increased nitrates/nitrites with supplementation at high dosages of ingredients or following many weeks of treatment [40,41].

As a pilot study, the findings of the current study were limited by the small sample size. Previous studies found significant benefits of Delphinol®, including lowering fasting glucose at 1 hr following a single low dose of Delphinol®, as well as improvements to lipid levels [5,6]. Although the same Delphinol® dosages were employed in the present study as in these previous studies, the subject populations differed; these previous studies observed pre-diabetic subjects who may respond differently than the healthy individuals involved in the present study. Similarly, Davinelli et al. found decreases in oxidized LDL (oxLDL) using a higher dosage (450 mg daily Delphinol®) for a 4-week intervention in obese smokers. Additionally, while Alvarado et al. observed high-density lipoprotein (HDL) increased over baseline for the duration of a 3 month study with 180 mg Delphinol® daily, decreases in LDL were not observed until 3 months of intervention [6]. A higher dose may be required to induce improvements to vascular health markers within a healthy population, or if Delphinol® only affects glucose, changes might not be seen at any dosage within the healthy population. Similarly, a longer intervention may be required to induce vascular changes. Many studies that observed cardiovascular benefits employed higher dosages of anthocyanins over longer intervals [4,22,23,42]. Higher dosages of Delphinol® over

longer interventions may therefore demonstrate additional improvements to vascular health. Additionally, the study used relatively young, healthy, and active adults without known cardiovascular disease. Future studies should focus on individuals at risk of, or with diagnosed cardiovascular disease, as these individuals may experience greater changes within vascular function measures.

CONCLUSION

Acute Delphinol® supplementation at concentrations equal to or less than 180 mg did not lead to statistically significant benefits to vascular health (blood pressure, PI, and nitrate/nitrite levels) within a small sample of healthy, young adults. Although not meeting criteria for statistical significance, the 180mg condition did result in an increase in the PI of approximately 20%. Future studies of Delphinol® should include a larger sample size, and focus on individuals at risk of CVD, as there may be a greater chance of benefits. Such studies may also use higher dosages over longer periods, as well as other methods such as flow-mediated dilation and pulse wave velocity, to evaluate vascular function.

AUTHOR CONTRIBUTIONS

JP was responsible for coordinating subject recruitment and scheduling, data collection, data entry, and manuscript preparation. RM assisted in data collection. IP assisted in the study design and manuscript preparation. RJB was responsible for the study design, data analysis, and manuscript preparation.

ACKNOWLEDGMENTS

Funding for this work was provided in part by Anklam Extrakt GmbH and the University of Memphis.

CONFLICTS OF INTEREST

No author declares a conflict of interest related to this work. IP has worked as a consultant to Anklam Extrakt GmbH. The sponsor had no role in the execution of the study, or in the interpretation of the data.

REFERENCES

1. Sinha, N., Dabla, P.K. Oxidative Stress and Antioxidants in Hypertension-a Current Review. Curr. Hypertens. Rev. 2015, 11, 132–142.

2. Escribano-Bailón, M.T., Alcalde-Eon, C., Muñoz, O., Rivas-Gonzalo, J.C., Santos-Buelga, C. Anthocyanins in Berries of Maqui (Aristotelia Chilensis (Mol.) Stuntz). Phytochem. Anal. PCA 2006, 17, 8–14.

3. Schön, C., Wacker, R., Micka, A., Steudle, J., Lang, S., Bonnländer, B. Bioavailability Study of Maqui Berry Extract in Healthy Subjects. Nutrients 2018, 10.

4. Fairlie-Jones, L., Davison, K., Fromentin, E., Hill, A.M. The Effect of Anthocyanin-Rich Foods or Extracts on Vascular Function in Adults: A Systematic Review and Meta-Analysis of Randomised Controlled Trials. Nutrients 2017, 9.

5. Alvarado, J.L., Leschot, A., Olivera-Nappa, Á., Salgado, A.-M., Rioseco, H., Lyon, C., Vigil, P. Delphinidin-Rich Maqui Berry Extract (Delphinol®) Lowers Fasting and Postprandial Glycemia and Insulinemia in Prediabetic Individuals during Oral Glucose Tolerance Tests. BioMed Res. Int. 2016, 2016, 9070537.

6. Alvarado, J., Schoenlau, F., Leschot, A., Salgad, A.M., Vigil Portales, P. Delphinol® Standardized Maqui Berry Extract Significantly Lowers Blood Glucose and Improves Blood Lipid Profile in Prediabetic Individuals in Three-Month Clinical Trial. Panminerva Med. 2016, 58, 1–6.

7. Hidalgo, J., Flores, C., Hidalgo, M.A., Perez, M., Yañez, A., Quiñones, L., Caceres, D.D., Burgos, R.A. Delphinol® Standardized Maqui Berry Extract Reduces Postprandial Blood Glucose Increase in Individuals with Impaired Glucose Regulation by Novel Mechanism of Sodium Glucose Cotransporter Inhibition. Panminerva Med. 2014, 56, 1–7.

8. Loader, J., Montero, D., Lorenzen, C., Watts, R., Méziat, C., Reboul, C., Stewart, S., Walther, G. Acute Hyperglycemia Impairs Vascular Function in Healthy and Cardiometabolic Diseased Subjects: Systematic Review and Meta-Analysis. Arterioscler. Thromb. Vasc. Biol. 2015, 35, 2060–2072.

9. Davinelli, S., Bertoglio, J.C., Zarrelli, A., Pina, R., Scapagnini, G. A Randomized Clinical Trial Evaluating the Efficacy of an Anthocyanin-Maqui Berry Extract (Delphinol®) on Oxidative Stress Biomarkers. J. Am. Coll. Nutr. 2015, 34 Suppl 1, 28–33.

10. Rodriguez-Mateos, A., Rendeiro, C., Bergillos-Meca, T., Tabatabaee, S., George, T.W., Heiss, C., Spencer, J.P. Intake and

Time Dependence of Blueberry Flavonoid-Induced Improvements in Vascular Function: A Randomized, Controlled, Double-Blind, Crossover Intervention Study with Mechanistic Insights into Biological Activity. Am. J. Clin. Nutr. 2013, 98, 1179–1191.

11. Khoo, H.E., Azlan, A., Tang, S.T., Lim, S.M. Anthocyanidins and Anthocyanins: Colored Pigments as Food, Pharmaceutical Ingredients, and the Potential Health Benefits. Food Nutr. Res. 2017, 61.

12. Igwe, E.O., Charlton, K.E., Probst, Y.C., Kent, K., Netzel, M.E. A Systematic Literature Review of the Effect of Anthocyanins on Gut Microbiota Populations. J. Hum. Nutr. Diet. 2019, 32, 53–62.

13. Lin, B.-W., Gong, C.-C., Song, H.-F., Cui, Y.-Y. Effects of Anthocyanins on the Prevention and Treatment of Cancer. Br. J. Pharmacol. 2017, 174, 1226–1243.

14. Lee, Y.-M., Yoon, Y., Yoon, H., Park, H.-M., Song, S., Yeum, K.-J. Dietary Anthocyanins against Obesity and Inflammation. Nutrients 2017, 9.

15. Reis, J.F., Monteiro, V.V.S., de Souza Gomes, R., do Carmo, M.M., da Costa, G.V., Ribera, P.C., Monteiro, M.C. Action Mechanism and Cardiovascular Effect of Anthocyanins: A Systematic Review of Animal and Human Studies. J. Transl. Med. 2016, 14, 315.

16. Overall, J., Bonney, S.A., Wilson, M., Beermann, A., Grace, M.H., Esposito, D., Lila, M.A., Komarnytsky, S. Metabolic Effects of Berries with Structurally Diverse Anthocyanins. Int. J. Mol. Sci. 2017, 18.

17. Cassidy Aedín, Mukamal Kenneth J., Liu Lydia, Franz Mary, Eliassen A. Heather, Rimm Eric B. High Anthocyanin Intake Is Associated With a Reduced Risk of Myocardial Infarction in Young and Middle-Aged Women. Circulation 2013, 127, 188–196.

18. Cassidy, A., Bertoia, M., Chiuve, S., Flint, A., Forman, J., Rimm, E.B. Habitual Intake of Anthocyanins and Flavanones and Risk of Cardiovascular Disease in Men. Am. J. Clin. Nutr. 2016, 104, 587–594.

19. McCullough, M.L., Peterson, J.J., Patel, R., Jacques, P.F., Shah, R., Dwyer, J.T. Flavonoid Intake and Cardiovascular Disease Mortality in a Prospective Cohort of US Adults. Am. J. Clin. Nutr. 2012, 95, 454–464.

20. Hooper, L., Kroon, P.A., Rimm, E.B., Cohn, J.S., Harvey, I., Le Cornu, K.A., Ryder, J.J., Hall, W.L., Cassidy, A. Flavonoids, Flavonoid-Rich Foods, and Cardiovascular Risk: A Meta-Analysis of Randomized Controlled Trials. Am. J. Clin. Nutr. 2008, 88, 38–50.

21. Wang, X., Ouyang, Y.Y., Liu, J., Zhao, G. Flavonoid Intake and Risk of CVD: A Systematic Review and Meta-Analysis of Prospective Cohort Studies. Br. J. Nutr. 2014, 111, 1–11.

22. Wallace, T.C., Slavin, M., Frankenfeld, C.L. Systematic Review of Anthocyanins and Markers of Cardiovascular Disease. Nutrients 2016, 8.

23. Huang, H., Chen, G., Liao, D., Zhu, Y., Xue, X. Effects of Berries Consumption on Cardiovascular Risk Factors: A Meta-Analysis with Trial Sequential Analysis of Randomized Controlled Trials. Sci. Rep. 2016, 6, 23625.

24. Masoodi, H., Villaño, D., Zafrilla, P. A Comprehensive Review on Fruit Aristotelia Chilensis (Maqui) for Modern Health: Towards a Better Understanding. Food Funct. 2019, 10, 3057–3067.

25. Céspedes, C.L., El-Hafidi, M., Pavon, N., Alarcon, J. Antioxidant and Cardioprotective Activities of Phenolic Extracts from Fruits of Chilean Blackberry Aristotelia Chilensis (Elaeocarpaceae), Maqui. Food Chem. 2008, 107, 820–829.

26. Amiot, M.J., Riva, C., Vinet, A. Effects of Dietary Polyphenols on Metabolic Syndrome Features in Humans: A Systematic Review. Obes. Rev. Off. J. Int. Assoc. Study Obes. 2016, 17, 573–586.

27. Ellwood, L., Torun, G., Bahar, Z., Fernandez, R. Effects of Flavonoid-Rich Fruits on Hypertension in Adults: A Systematic Review. JBI Database Syst. Rev. Implement. Rep. 2019, 17, 2075–2105.

28. Zhu, Y., Bo, Y., Wang, X., Lu, W., Wang, X., Han, Z., Qiu, C. The Effect of Anthocyanins on Blood Pressure: A PRISMA-Compliant Meta-Analysis of Randomized Clinical Trials. Medicine (Baltimore) 2016, 95, e3380.

29. Hasanin, A., Mukhtar, A., Nassar, H. Perfusion Indices Revisited. J. Intensive Care 2017, 5, 24.

30. Pinto Lima, A., Beelen, P., Bakker, J. Use of a Peripheral Perfusion Index Derived from the Pulse Oximetry Signal as a Noninvasive Indicator of Perfusion. Crit. Care Med. 2002, 30, 1210–1213.

31. Okada, H., Tanaka, M., Yasuda, T., Kamitani, T., Norikae, H., Fujita, T., Nishi, T., Oyamada, H., Yamane, T., Fukui, M. The Perfusion Index Is a Useful Screening Tool for Peripheral Artery Disease. Heart Vessels 2019, 34, 583–589, doi:10.1007/s00380-018-1276-4.

32. Rodriguez-Mateos, A., Feliciano, R.P., Boeres, A., Weber, T., dos Santos, C.N., Ventura, M.R., Heiss, C. Cranberry (Poly)Phenol Metabolites Correlate with Improvements in Vascular Function: A Double-Blind, Randomized, Controlled, Dose-Response, Crossover Study. Mol. Nutr. Food Res. 2016, 60, 2130–2140, doi:10.1002/mnfr.201600250.

33. Lynn, A., Mathew, S., Moore, C.T., Russell, J., Robinson, E., Soumpasi, V., Barker, M.E. Effect of a Tart Cherry Juice Supplement on Arterial Stiffness and Inflammation in Healthy Adults: A Randomised Controlled Trial. Plant Foods Hum. Nutr. 2014, 69, 122–127, doi:10.1007/s11130-014-0409-x.

34. Lynn, A., Hamadeh, H., Leung, W.C., Russell, J.M., Barker, M.E. Effects of Pomegranate Juice Supplementation on Pulse Wave Velocity and Blood Pressure in Healthy Young and Middle-Aged Men and Women. Plant Foods Hum. Nutr. 2012, 67, 309–314, doi:10.1007/s11130-012-0295-z.

35. Naseem, K.M. The Role of Nitric Oxide in Cardiovascular Diseases. Mol. Aspects Med. 2005, 26, 33–65, doi:10.1016/j.mam.2004.09.003.

36. Xu Jin-Wen, Ikeda Katsumi, Yamori Yukio Upregulation of Endothelial Nitric Oxide Synthase by Cyanidin-3-Glucoside, a Typical Anthocyanin Pigment. Hypertension 2004, 44, 217–222, doi:10.1161/01.HYP.0000135868.38343.c6.

37. Edirisinghe, I., Banaszewski, K., Cappozzo, J., McCarthy, D., Burton-Freeman, B.M. Effect of Black Currant Anthocyanins on the Activation of Endothelial Nitric Oxide Synthase (ENOS) in Vitro in Human Endothelial Cells. J. Agric. Food Chem. 2011, 59, 8616–8624, doi:10.1021/jf201116y.

38. Lee, G.-H., Hoang, T.-H., Jung, E.-S., Jung, S.-J., Han, S.-K., Chung, M.-J., Chae, S.-W., Chae, H.-J. Anthocyanins Attenuate Endothelial Dysfunction through Regulation of Uncoupling of Nitric Oxide Synthase in Aged Rats. Aging Cell 2020, 19, e13279, doi:https://doi.org/10.1111/acel.13279.

39. Möller, M.N., Rios, N., Trujillo, M., Radi, R., Denicola, A., Alvarez, B. Detection and Quantification of Nitric Oxide–Derived Oxidants in Biological Systems. J. Biol. Chem. 2019, 294, 14776–14802.

40. Bloomer, R.J., Tschume, L.C., Smith, W.A. Glycine Propionyl-L-Carnitine Modulates Lipid Peroxidation and Nitric Oxide in Human Subjects. Int. J. Vitam. Nutr. Res. 2009, 79, 131–141.

41. Bloomer, R.J., Butawan, M., Pigg, B., Martin, K.R. Acute Ingestion of A Novel Nitrate-Rich Dietary Supplement Significantly Increases Plasma Nitrate/Nitrite in Physically Active Men and Women. Nutrients 2020, 12.

42. Kimble, R., Keane, K.M., Lodge, J.K., Howatson, G. Dietary Intake of Anthocyanins and Risk of Cardiovascular Disease: A Systematic Review and Meta-Analysis of Prospective Cohort Studies. Crit. Rev. Food Sci. Nutr. 2019, 59, 3032–3043.

PEER REVIEW

Not commissioned. Externally peer reviewed.

TABLES

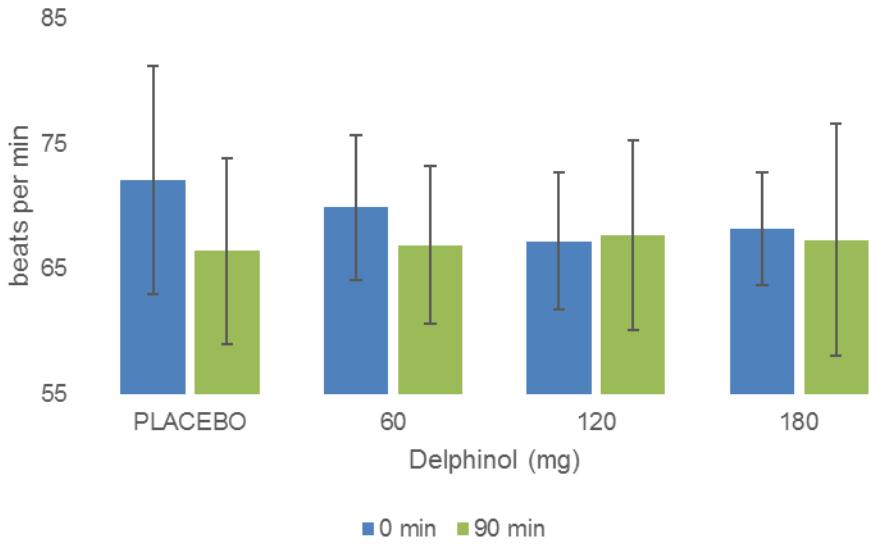
Table 1. Characteristics of men and women.

Variable	Men (n=5)	Women (n=5)
Age (years)	22.2±1.1	23.0±3.5
Height (cm)	178.5±5.0	162.2±5.2
Weight (kg)	76.4±5.6	58.3±6.1
BMI (kg/m^2)	24.0±1.2	22.2±2.9
Waist Circumference (cm)	80±4.6	69.2±4.7
Hip Circumference (cm)	99.8±1.1	93.4±4.2
Waist:Hip	0.74±0.05	0.80±0.02
Resting HR (bpm)	63.0±7.8	74.6±9.1
Resting SBP (mm Hg)	121.8±7.8	119.0±9.1
Resting DBP (mm Hg)	71.8±5.3	75.0±1.9

Values are Mean ± SD

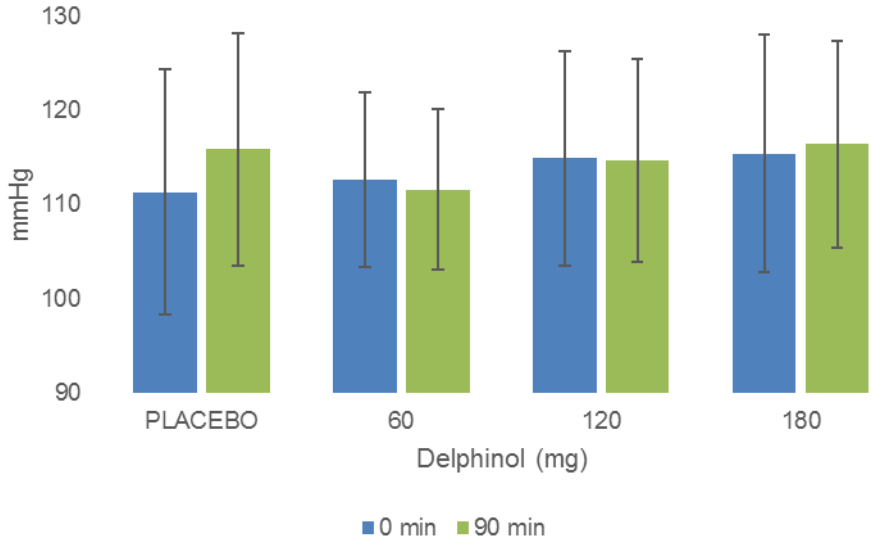
FIGURES

Figure 1. Heart rate response of 10 men and women ingesting different doses of Delphinol®.



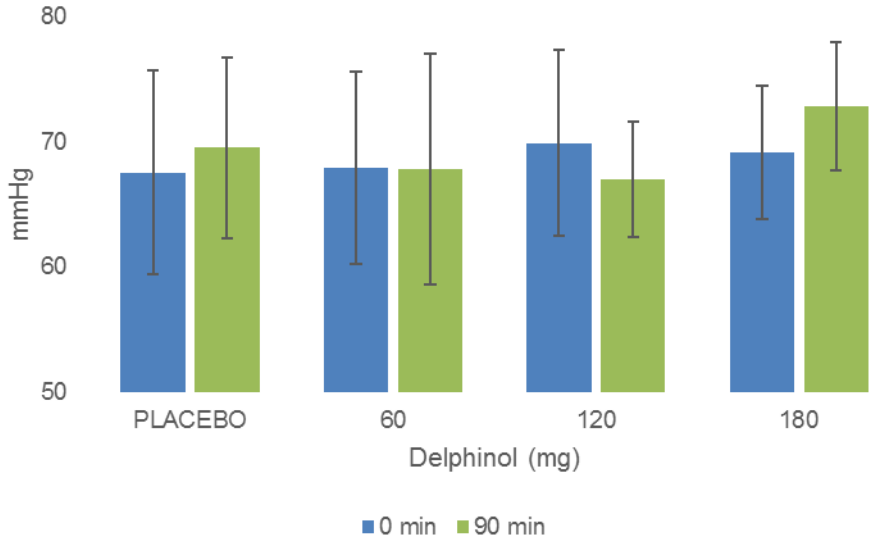
Values are mean ± SD
No differences of statistical significance were noted (p>0.05)

Figure 2. Systolic blood pressure response of 10 men and women ingesting different doses of Delphinol®



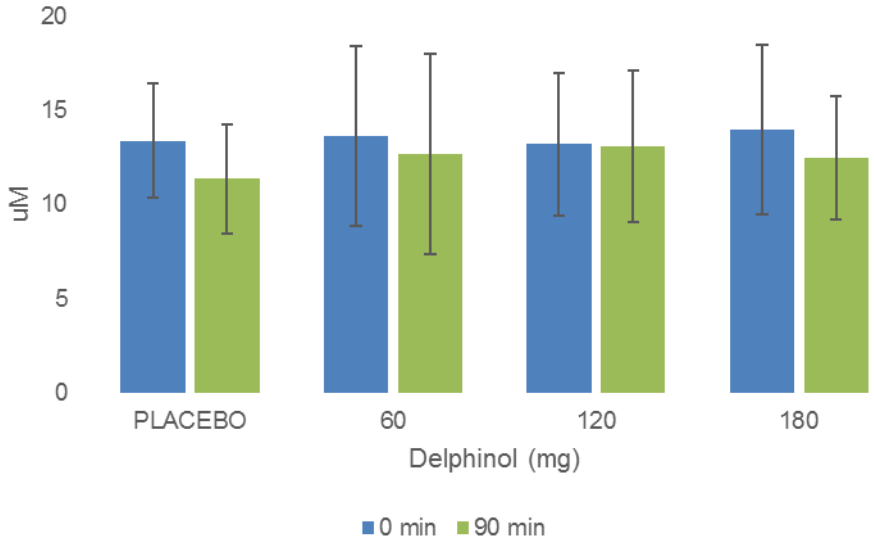
Values are mean ± SD
No differences of statistical significance were noted (p>0.05)

Figure 3. Diastolic blood pressure response of 10 men and women ingesting different doses of Delphinol®



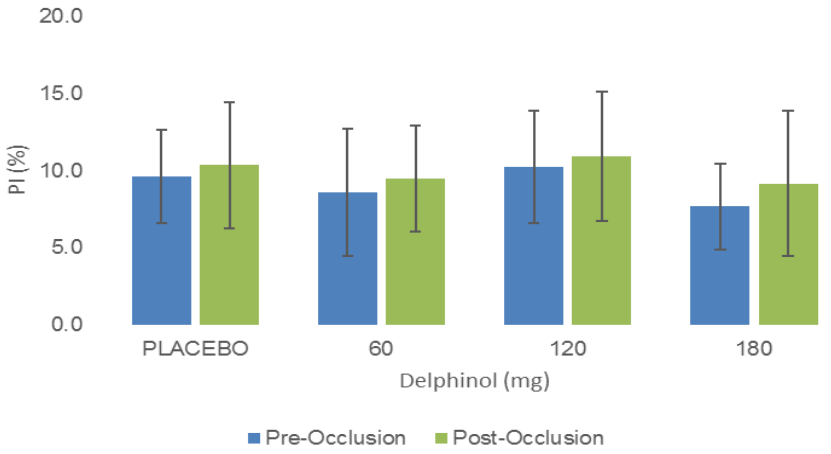
Values are mean ± SD
No differences of statistical significance were noted (p>0.05)

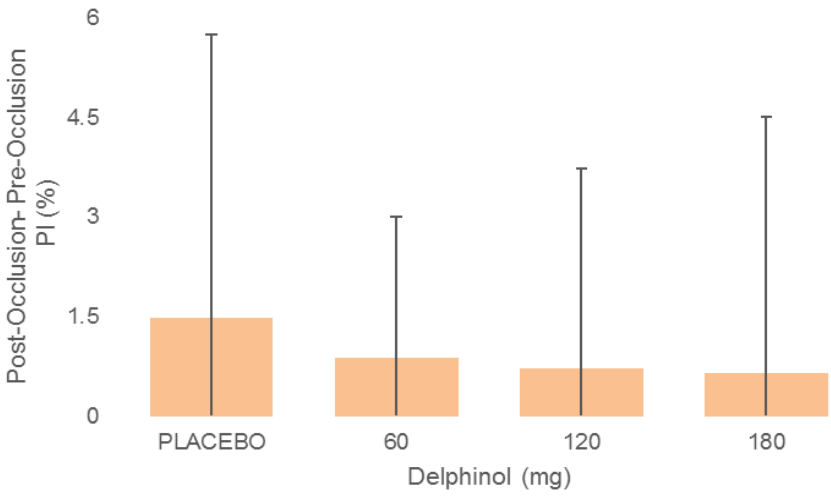
Figure 4. Nitrate/nitrite response of 9 men and women ingesting different doses of Delphinol®



Values are mean ± SD
No differences of statistical significance were noted (p>0.05)

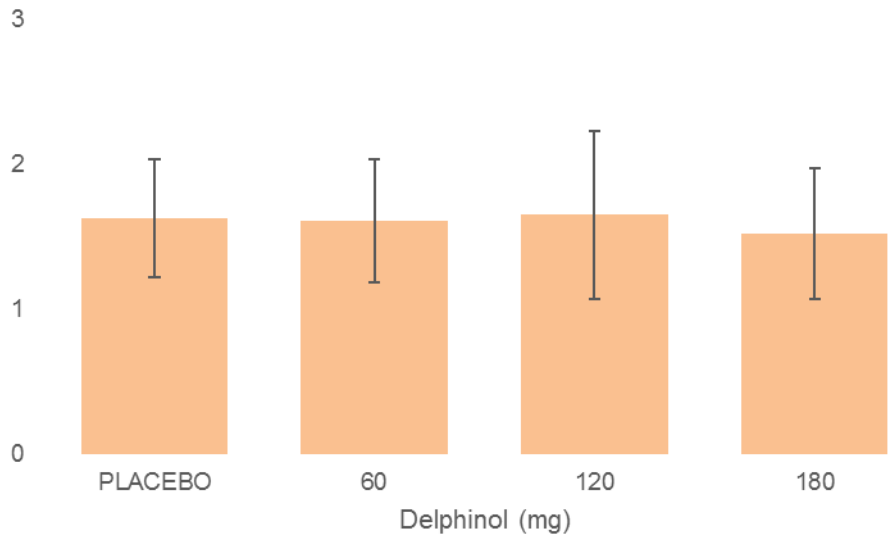
Figure 5. Perfusion Index of 10 men and women ingesting different doses of Delphinol®





Values are mean ± SD
No differences of statistical significance were noted (p>0.05)

Figure 6. Vascular Reactivity Index of 10 men and women ingesting different doses of Delphinol®



Values are mean ± SD
No differences of statistical significance were noted ($p>0.05$)