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Cranberry (poly)phenol metabolites correlate with improvements in vascular function: a double-blind, randomized, controlled, dose-response, crossover study

Ana Rodriguez-Mateos[1], Rodrigo P Feliciano[1], Albert Boeres[1], Timon Weber[1], Claudia Nunes dos Santos[2,3], M. Rita Ventura[2] and Christian Heiss[1]

[1]Division of Cardiology, Pulmonology, and Vascular Medicine, Medical Faculty, University Düsseldorf, 40225, Düsseldorf, Germany; [2]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Avenida da República, EAN, 2781-901 Oeiras, Portugal; [3] Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras.

Correspondence: Division of Cardiology, Pulmonology and Vascular Medicine; e-mail: ana.rodriguez-mateos@med.uni-duesseldorf.de; phone: +492118115893; Fax: +492118115493.

Abbreviations used: Augmentation Index, AIX; brachial artery, BA; cardiovascular disease, CVD; coronary artery disease, CAD; diastolic blood pressure, DBP; flow-mediated dilation, FMD; pulse wave velocity, PWV; randomized controlled trial, RCT; systolic blood pressure, SBP; total (poly)phenols, TP.

Key words: Cranberry, endothelial function, primary prevention, (poly)phenols, metabolites

Abstract

Scope: Cranberries are rich in potentially bioactive (poly)phenols. The aim of this work was to investigate whether cranberry juice intake can improve vascular function in healthy men in a dose- and time-dependent manner, and to understand which of the circulating (poly)phenol metabolites correlate with vascular effects.

Methods and results: A double-blind randomized controlled crossover trial was conducted in 10 healthy males. Flow-mediated dilation (FMD), blood pressure, pulse wave velocity and augmentation index were investigated at baseline, 1, 2, 4, 6, and 8h post-consumption of cranberry juices containing 409, 787, 1238, 1534, and 1910 mg of total cranberry (poly)phenols (TP), and a control drink. Plasma (poly)phenol metabolites were analyzed by UPLC-Q-TOF MS using authentic standards. We observed dose-dependent increases in FMD at 1, 2, 4, 6, and 8h with a peak at 4h and maximal effects with juice containing 1238 mg TP. A total of 60 metabolites were quantified in plasma after cranberry consumption. Twelve (poly)phenol metabolites significantly correlated with the increases in FMD, including ferulic and caffeic acid sulfates, quercetin-3-*O*-ß-D-glucuronide and a γ-valerolactone sulfate.

Conclusion: (Poly)phenols in cranberry juice can improve vascular function in healthy males and this is linked to the presence of specific newly identified plasma metabolites.

Introduction

Accumulating evidence from epidemiological and human intervention studies indicates that the cardiovascular health benefits of diets rich in berries are in part related to their (poly)phenol content [1]. Cranberries, in particular, are rich in proanthocyanidins, anthocyanins, and phenolic acids [2, 3].

A limited number of double blind randomized controlled trials (RCT) have investigated the effects of cranberry (poly)phenols on clinically relevant and accredited markers of vascular function, such as endothelial function, blood pressure, and arterial stiffness with mixed results [4-9]. Importantly, the study populations were mainly subjects at increased cardiovascular risk or manifest cardiovascular disease. Currently, it is unknown whether cranberry consumption can improve vascular function in healthy individuals and, if so, at which intake amount and which role (poly)phenols may play in this.

Therefore, the major aim of this work was to investigate the time course and dose-dependent effects of cranberry (poly)phenols on endothelial function in healthy individuals, as determined by flow-mediated vasodilation (FMD; primary endpoint) in the context of important vascular determinants thereof (secondary endpoints) including blood pressure, pulse wave velocity (PWV), and aortic augmentation index (AIX) after acute consumption of cranberry juice with increasing amounts of (poly)phenols. The second major aim was to quantify novel cranberry-derived (poly)phenol metabolites in plasma (tertiary endpoints) to link with vascular effects.

SUBJECTS AND METHODS

Intervention study subjects

Ten healthy male volunteers from 18 to 35 years were recruited from the University of Duesseldorf and surrounding area. Health was ascertained by a routine clinical physical exam and specific cardiovascular history performed by a cardiovascular specialist (cardiology/vascular medicine). Manifest cardiovascular disease including coronary artery disease, cerebrovascular disease and peripheral artery disease, diabetes mellitus, acute inflammation, terminal renal failure, malignancies, and heart rhythm other than sinus were exclusion criteria. The study flow is represented in **Figure 1A**.

Study Design

A 6-arm randomized, double blind, crossover, controlled intervention trial was conducted, where volunteers were asked to consume a cranberry juice drink or a macro- and micro-nutrient isocaloric control drink. FMD, peripheral blood pressure measurements and blood samples were taken before (baseline) and at 1, 2, 4, 6, and 8 h post-acute consumption of each of the 6 intervention juice drinks (450 mL) on 6 different days separated by 1 week washout. PWV, AIX and central blood pressure were measured at 0, 1.5, 4, 6 and 8 hours post-consumption (**Figure 1B**).

Volunteers were instructed not to alter their usual dietary or fluid intake. Those selected for the study were asked to refrain from the following for 72 h prior to, and during, the study: consumption of polyphenol-rich foods including fruits, vegetables, cocoa, chocolate, coffee, tea and wine, intake of nitrate rich foods: leafy green vegetables and beetroot, participating in vigorous exercise (> 3 x 20 min/week) and consuming more than 168 g of alcohol (any form) per

week. Volunteers were also asked not to eat anthocyanin-rich foods such as berries or red wine for one week before starting (run-in) and until the completion of the study. Compliance to the diet and lifestyle restrictions was determined via 24 h-dietary recalls and via interview. Written informed consent was obtained from all subjects prior to their participation in the study.

The primary end point was an improvement of endothelial vasodilator function as measured by FMD using high-resolution ultrasound. Secondary endpoints were improvements in key determinants of vascular function and included decreases in PWV, AIX, and blood pressure (peripheral and central) as determined automatically by a blood pressure monitoring system and applanation tonometry (Sphygmocor). Tertiary endpoints include the quantification of plasma cranberry-derived (poly)phenols and was subsequently correlated with the primary endpoint. During the study day, a low (poly)phenol meal was giving together with the test drink, and no other food or drink was allowed until after 8 hours post-consumption, except for water *ad libitum*.

Office blood pressure was measured three times after 10 min of rest using an automated clinical digital sphygmomanometer (Dynamap, Tampa, FL, USA) with appropriately sized cuff placed around the upper arm at heart level.

A qualified researcher enrolled participants on the study. Participants and researchers administering interventions and assessing study outcomes were blinded to the interventions. An independent researcher generated the random allocation to treatment sequence (using a Williams design) and implemented the allocation sequence. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the University of Duesseldorf Research Ethics Committee (ref: 14-012). The

study was also registered with the National Institutes of Health (NIH)-randomized trial records held on the NIH ClinicalTrials.gov website (NCT02517775). This study was conducted at the University of Duesseldorf from January to September 2015.

Cranberry and control drinks

Ocean Spray supplied the cranberry and control drinks. Volunteers were asked to consume a cranberry juice drink containing 409, 787, 1238, 1534, and 1910 mg of total polyphenols (TP) (equivalent to 25, 48, 76, 94, and 117% concentrated cranberry juice) or a macro- and micro-nutrient isocaloric control drink which was indistinguishable in color and taste to the cranberry juice test drinks and contained 3 mg of TP. The 6 intervention drinks consisted of a total volume of 450 mL from cranberry juice and control drink. The (poly)phenol content of all test drinks is shown in **Table 1** and the nutritional composition of control and the most concentrated drink in **Table 2**.

Ultrasound measurements of arterial function and pulse wave velocity

FMD was measured as previously described [10]. Briefly, the diameter and flow velocity of the brachial artery (BA) was measured using a 12 MHz transducer (Vivid I, GE, Frankfurt, Germany) and automatic edge-detection software (Brachial Analyzer, Medical Imaging Applications, Iowa City, IA, USA) yielding standard deviations of mean differences between repeated measurements of less than 1%. BA diameter was measured approximately 2 cm proximal to the elbow. Reactive hyperemia was induced by 5 min of distal lower arm with a sphygmomanometric cuff inflated to 250 mm Hg. After cuff deflation (0 sec), 20, 40, 60, and 80 sec, the diameter was assessed and FMD calculated as maximal relative diameter gain relative to baseline. The FMD was expressed as $(diameter_{max}\text{-}diameter_{baseline})/diameter_{baseline} * 100$.

Central blood pressure parameters including AIX and PWV were measured by applanation tonometry using the SphygmoCor® system (AtCor Medical, West Ryde, Australia). Via a transfer function, the pressure waveform of the ascending aorta was synthesized. PWV was determined from measurements taken at the carotid and femoral artery as previously described [11].

Biochemical analyses

The blood samples collected in EDTA/heparin tubes were spun (1700 x g; 15 min; 4ºC) immediately after collection. Samples for (poly)phenol analysis were spiked with 2% formic acid. All samples were aliquoted and frozen at -80°C until analysis. All clinical chemistry parameters including total, LDL and HDL-cholesterol, tryglycerides (enzymatic photometric assay; RocheDiagnostics), HbA1c, glucose (hexokinase assay) and whole blood count (flow cytometry; Sysmex) were measured using standard techniques by the Institute for Clinical Chemistry, University Hospital Duesseldorf, Germany.

UPLC-Q-TOF MS analysis of plasma (poly)phenols

The identification and quantification of plasma (poly)phenol metabolites in plasma after cranberry juice consumption was performed as previously described using micro-elution solid phase extraction followed by UPLC-Q-TOF MS [12].

Materials

All (poly)phenol metabolites (sulfates and glucuronides) were obtained from Toronto Research Chemicals (Toronto, Canada), except kaempferol-3-*O*-ß-D-glucuronide which was obtained from Extrasynthese (Genay, France). 1-Methylpyrogallol-*O*-sulfate, 2-methylpyrogallol-*O*-sulfate, 4-methylcatechol-*O*-sulfate, 4-methylgallic-3-*O*-sulfate , catechol-*O*-sulfate , pyrogallol-

148 *O*-1-sulfate , pyrogallol-*O*-2-sulfate and vanillic acid-4-*O*-sulfate were kindly provided by Dr
149 Claudia Nunes dos Santos and Dr Rita Ventura and their synthesis has been described elsewhere
150 [13]. All the polyphenol and phenolic acid aglycones were obtained from Sigma-Aldrich Co.
151 (Steinheim, Germany) and 2-, 3- and 4-hydroxyhippuric acids were purchased from Enamine
152 (Kiev, Ukraine). Acetic acid was from Carl Roth (Karlsruhe, Germany) and Oasis HLB µElution
153 plates were from Waters (Eschborn, Germany). Milli-Q system (Merck KGaA, Darmstadt,
154 Germany) ultra pure water was used. Unless otherwise stated, all chemicals and reagents were
155 obtained from Sigma-Aldrich Co. (Steinheim, Germany).

156 **Power calculation and statistical analysis**

157 Power calculations were performed for the primary endpoint, change in FMD response. Power
158 was based on the intra-individual variability of the operator who performed the FMD analysis
159 (5% CV, SD=0.3). At 0.8 power, a 0.05 significance level and a mean FMD of 7.2%, the number
160 of subjects required to detect a difference of 0.3% in the response of matched pairs in a crossover
161 study is 10. This number is consistent with other studies carried out with similar endpoints and
162 study design [14-16]. The characteristics of the study population are expressed as mean values
163 and standard deviations. Results are presented as mean values and their standard error of means,
164 and differences between responses are presented as mean values and 95 % confidence intervals.
165 Differences in the outcome variables were compared by one-way ANOVA using Tukey post-hoc
166 test. Data not normally distributed were compared with Wilcoxon test. Statistical analysis was
167 performed with GraphPad Prism (version 6.00, GraphPad software, CA, US), and JMP Pro
168 (version 11.0.0; SAS institute Inc., NC, US). Correlations are presented as Pearson's r.
169

RESULTS

Baseline characteristics of the study population and tolerance of intervention: The baseline characteristics of young healthy non-obese males were all within normal limits (**Table 3**). The cardiovascular risk at baseline (10 year CAD risk) was low (1.0±0.5%), according to the Framingham risk score. All drinks were well tolerated by all subjects and no adverse events were reported.

Dose- and time-dependent improvements in vascular function following cranberry juice consumption

The single consumption of the test drinks containing between 409 and 1910 mg of TP but not the control drink led to a time-dependent increase in the primary endpoint, FMD. As depicted in **Figure 2**, FMD gradually increased after consumption of the cranberry juices with a maximum at 4h. FMD increased dose-dependently with maximal effects seen after drink containing 1 238 mg TP. The median effective dose (ED_{50}), was 436 mg (95% CI 226, 841 mg). When comparing changes in FMD after consumption of the cranberry juice drinks (409-1910 mg TP) with changes in FMD after consumption of the control drink, significantly greater improvements were observed at 1, 2, 4, 6, and 8 hours post-consumption of the juices containing 1910 and 1534 mg TP (p=0.0048, 0.0022, 0.0006, 0.0002, and 0.0080 for 1910 mg TP and p=0.0006, 0.0007, 0.0017, 0.0062 and 0.0247 for 1534 mg TP, respectively); at 1, 2, 4, and 6 h for the juice containing 1238 mg TP (p=0.0455, 0.0173, 0.0140, 0.0058, respectively); at 1, 2, 6 and 8 h for the drink containing 787 mg TP (p=0.0017, 0.0173, 0.0113, 0.0452, respectively), and at 2 h for the drink containing 409 mg TP (p=0.0048) (**Figure 2A**).

The area under the curve of FMD % versus time after consumption of the cranberry juices was significantly higher (p=0.0257, 0.0312, 0.0046, 0.0028 for 787, 1238, 1534 and 1910 mg TP, respectively) than after consumption of the control drink except for the lower dose (409 mg TP) (**Figure 2B**).

No significant results were observed when comparing changes in blood pressure, PWV or AIX after consumption of the cranberry drinks with changes after consumption of the control drink (data not shown). However, a significant decrease in central systolic blood pressure (CSBP) was observed at 6 h after consumption of the drink containing the largest amount of TP (1910 mg TP) when compared to baseline (97 ± 1.6 mm Hg versus 107 ± 2.6 mm Hg, p=0.0351). Central diastolic blood pressure and office blood pressure did not significantly change after any intervention. A significant decrease in AIX with respect to baseline was also observed at 1.5 and 6 h post-consumption of the cranberry drink containing 1534 mg TP (-14 ± 3.2%, -13 ± 2.9% versus -4.2 ± 2.5%, p=0.0306 and 0.0483, respectively), and after 4 and 6 h post-consumption of drink containing 409 mg TP (-15 ± 3.4%, -16 ± 3.5% versus -6.1 ± 2.1%, p=0.0341 and 0.0342, respectively).

Identification and quantification of novel phenolic metabolites after cranberry juice consumption

Using authentic standards, we have recently reported the identification and quantification of 60 (poly)phenol metabolites in the plasma of the volunteers participating in the study, with 43 of them being reported for the first time after consumption of cranberry juice [12]. Most metabolites were conjugated and non-conjugated phenolic acid compounds, with only 3 of them being flavonoid derivatives (kaempferol, kaempferol-3-*O*-ß-D-glucuronide, and quercetin-3-*O*-ß-

D-glucuronide). Many of the individual compounds were absorbed in a dose-dependent manner, so the plasma concentration increased with increasing concentration of the cranberry juices (**Figure 3**).

Novel cranberry phenolic metabolites correlate with vascular effects

In order to link the circulating metabolites with vascular effects, we performed a correlation analysis with the increases in FMD at 1, 2, 4, 6, and 8 h as the dependent variable and all metabolites as independent variables. This analysis showed 12 phenolic metabolites that could predict the vascular effects (**Table 4**). Seven of them were cinnamic acid derivatives (caffeic acid, caffeic acid 4-*O*-ß-D-glucuronide, dihydro caffeic acid 3-*O*-sulfate, ferulic acid 4-*O*-sulfate, dihydroferulic acid 4-*O*-sulfate, dihydro isoferulic acid 3-*O*-sulfate, cinnamic acid), and 3 of them were benzoic acid derivatives (vanillic acid-4-*O*-sulfate, homovanillic acid sulfate, 4-methylgallic-3-*O*-sulfate) (**Figure 4**). Quercetin 3-*O*-β-D-glucuronide was the only conjugated flavonoid that correlated with FMD. A proanthocyanidin/flavan-3-ol-derived metabolite, (4R)-5-(3'-hydroxyphenyl)-γ-valerolactone-4'-*O*-sulfate, also correlated with FMD. Six metabolites correlated with the changes in FMD after 1 h post consumption, 9 metabolites correlated with 2 h values, 7 with 4 h values, 8 with 6 h values, and 6 with 8 h values (Table 4). The AUC of the concentration of all these metabolites in plasma correlated with the AUC of the FMD responses between 0 and 8 h (Pearson $r = 0.510$, p-value <0.0001).

Discussion

To our knowledge this is the first study that reports improvements in endothelial function after cranberry juice consumption in healthy individuals. Only one study has tested the effect of cranberry on flow-mediated dilation in patients with CAD [5, 6] and with mixed results. The authors observed a significant improvement in FMD (1%) at 4 h after acute cranberry juice consumption (835 mg TP, 94 mg anthocyanins) [5]. In the same study, the authors showed in these CAD patients that daily cranberry juice consumption over 4 weeks did not confer any type of chronic effect i.e. FMD increase that is still present after an overnight fast. Our present study supports that indeed cranberry juice can induce strong immediate improvements in FMD of up to 2.6% in healthy subjects over several hours after consumption. Results from recent meta-analyses have demonstrated that an increase in FMD of 1% translates into a decrease in CVD risk of 10-13% [17-19]. Therefore, the average increase of 2% after cranberry juice consumption observed in the present study could be interpreted as a decrease of 20% in the risk of CVD. This, however, implies that the improvements are maintained over time potentially requiring regular repetitive consumptions of cranberry juice and/or other foods containing bioactives. Future long-term studies will determine whether positive effects persist with chronic consumption and if this indeed leads to health benefits in primary prevention and whether this is also true in a broader segment of the health population (generalizability).

The current data present a basic understanding of cranberry (poly)phenol pharmacodynamics that was so far unknown. The FMD improvements observed after cranberry consumption followed a non-linear dose-dependency, with the linear part of the response curve after consumption of the cranberry juices containing 409 to 1238 mg TP and plateauing at higher intake amounts. This is in agreement with previous work from our group, where the dose-dependent effects of blueberry

(poly)phenol consumption were investigated [16]. The polyphenol intake needed to achieve half-maximal effects (ED50) was found to be 482 mg, which is remarkably similar to the present study (436 mg TP), despite significant differences in the polyphenol profile of the test products. The tested blueberries had a higher content of anthocyanins and chlorogenic acid than the cranberry juices, which in turn had a higher proanthocyanidin content. Consistent with this, a meta-analysis has reported a non-linear dose-response for FMD after consumption of other polyphenol rich foods, where the FMD response increased in magnitude with increasing doses only in foods containing lower than 1,000 mg of total flavonoids and procyanidins per day [20]. While there is a clear intake-dependence with regards to total (poly)phenols, the relative contribution of individual compounds is less clear.

In order to evaluate the role of cranberry (poly)phenols on vascular function, we investigated the plasma levels of phenolic metabolites after cranberry consumption. Up to now, only few studies have investigated the bioavailability of cranberry (poly)phenols [21-26], and none of them had used authentic standards of phase II metabolites for quantification. We have recently demonstrated the presence of 60 cranberry-derived phenolic metabolites in human plasma after cranberry juice consumption, 43 of them novel [12]. In a correlation analysis, we now investigated which of them could explain the observed vascular effects. Of these, 12 phenolic compounds were found to correlate with the vascular response, of which only one was a flavonoid conjugate, coming from the flavonols present in cranberry (quercetin-3-*O*-ß-D-glucuronide). The valerolactone sulfate is very likely to derive from proanthocyanidins and flavan-3-ols, as it has been reported after consumption of proanthocyanidin-rich foods such as cocoa flavanols and tea [27-29]. The other ones were non-specific phenolic metabolites (cinnamic, benzoic and phenylacetic acid derivatives) that could originate from any of the

(poly)phenols present in the cranberry juice, for example by direct absorption of hydroxycinnamic acids, breakdown of anthocyanins, or gut microbial metabolism of proanthocyanidins. When calculating the AUC of all those metabolites together, a significant correlation was also found with the AUC of the FMD response. Thus, our results indicate that several (poly)phenols present in cranberry juice may lead to a complex profile of candidate bioactives in plasma that were so far not in the focus of research. These candidate bioactives may synergistically contribute to the improvements in endothelial function after cranberry juice consumption. This, however, needs to be shown in future studies establishing causality and identifying the potential underlying mechanism(s) of action also keeping in mind that other nutrients or bioactives present in cranberry may contribute to the observed vascular effects. With regards to these future studies, the observed concentrations of compounds being associated with vascular effects, which ranged between low nM and low µM [12], need to be taken into considerations and used for evaluation.

Although the mechanism(s) of action of (poly)phenols in the vascular system have not yet been elucidated, the improvements in endothelial function observed here are likely to involve an increase in nitric oxide (NO) bioavailability, as it is known that FMD is at least partially, NO mediated [30, 31]. We have recently shown that blueberry (poly)phenol metabolites inhibited NADPH oxidase activity and correlated with FMD improvements and plasma phenolic metabolites [16], so it is possible that plasma cranberry phenolic metabolites improved NO bioavailability via inhibition of NADPH oxidase activity. Indeed, most of the metabolites that correlated with the FMD, such as ferulic, caffeic, vanillic acid derivatives or quercetin glucuronide have structural homologies to the pharmacologic NADPH oxidase inhibitor apocynin [32] and have been proposed as potent NAPDH oxidase inhibitors in endothelial cells [33, 34].

However, most metabolites which correlated with vascular responses in the present study have not been subjected to mechanistic studies up until now because they are not commercially available. It was recently shown that both quercetin and its major metabolites, including quercetin glucuronide, are able to confer an acute endothelial protective effect via activation of AMPK pathway, which can induce endothelial nitric oxide synthase (eNOS) activation, and, therefore, NO production [35]. It is also likely that other potential mechanisms of action may play a role in the vascular effects of (poly)phenols, such as regulation of heme oxigenase-1 and Nrf2 signaling [36].

Conclusion

(Poly)phenols in cranberry juice can improve vascular function in healthy males and this is linked to the presence of specific newly identified plasma metabolites. The nutritional relevance of our findings is underscored by the fact that significant and dose-dependent improvements in endothelial functions were seen even after the consumption of widely available single (25% concentrated) and double strength (48% concentrated) cranberry juices.

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Conflict of Interest: The funders of this study had no input on the design, implementation, analysis or interpretation of the data. We declare that we received by way of a gift the experimental drinks from Ocean Spray. There are no other conflicts of interest the authors wish to declare.

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Table 1. (Poly)phenol content of the cranberry and placebo drinks, expressed in mg/450 mL (single dose).

	Control drink	409 mg TP	787 mg TP	1238 mg TP	1534 mg TP	1910 mg TP
Phenolic acids	**2.7**	**12.8**	**24.5**	**35.3**	**48.6**	**59.2**
Benzoic acid	2.3	4.5	7.8	11.1	15.5	17.1
Salicylic acid (2-hydroxybenzoic acid)	0.0	0.0	0.1	0.1	0.2	0.3
Protocatechuic acid (3,4-dihydroxybenzoic acid)	0.0	0.4	1.1	1.8	2.4	3.1
Gallic acid (3,4,5-trihydroxybenzoic acid)	0.0	0.0	0.1	0.1	0.2	0.2
Vanillic acid (4-hydroxy-3-methoxybenzoic acid)	0.0	0.4	1.0	1.1	1.7	3.2
t-Cinnamic acid	0.3	0.6	1.0	1.8	2.1	2.6
p-Coumaric acid (4-hydroxycinnamic acid)	0.0	3.5	6.9	9.6	13.3	16.2
Caffeic acid (3,4-dihydroxycinnamic acid)	0.0	0.6	1.2	1.9	2.5	3.1
Ferulic acid (3-methoxy-4-hydroxycinnamic acid)	0.0	0.0	0.0	0.3	0.5	0.6
Chlorogenic acid	0.0	2.6	5.2	7.5	10.3	12.8
Flavan-3-ols	**0.0**	**2.5**	**5.0**	**6.8**	**10.1**	**12.3**
Catechin	0.0	0.2	0.5	0.8	1.2	1.5
Epicatechin	0.0	2.2	4.5	6.0	8.9	10.8
Flavonols	**0.2**	**14.5**	**31.3**	**48.9**	**62.8**	**76.9**
Quercetin	0.0	4.0	8.5	15.5	18.2	20.5
Quercitrin (Quercetin-3-*O*-rhamnoside)	0.0	2.2	4.6	6.5	8.1	10.7
Hyperoside (Quercetin-3-*O*-galactoside)	0.0	2.0	3.9	5.5	7.2	9.4
Myricetin	0.2	4.0	8.2	11.2	14.7	18.4
Myricetrin (Myricetin-3-*O*-rhamnoside)	0.0	1.6	3.2	4.8	6.4	7.4
Myricetin-3-*O*-galactoside	0.0	0.7	2.9	5.4	8.2	10.4
Anthocyanins	**0.0**	**6.8**	**16.2**	**23.2**	**26.3**	**32.3**
Cyanidin-3-arabinoside	0.0	3.7	6.8	9.5	12.1	14.7
Cyanidin-3-galactoside	0.0	0.0	1.7	2.4	2.9	3.6
Cyanidin-3-glucoside	0.0	0.0	0.0	0.0	0.0	0.0
Peonidin-3-arabinoside	0.0	2.0	2.1	3.6	7.0	8.4
Peonidin-3-galactoside	0.0	1.1	2.0	2.9	3.3	4.5
Peonidin-3-glucoside	0.0	0.0	0.0	4.8	1.0	1.1
Proanthocyanidins						
BL-DMAC	0.0	124.8	242.5	278.2	420.9	485.5
OSC-DMAC	0.0	372.6	710.5	1124.0	1386.3	1729.1
Total Phenolics (Folin method)	0.0	180.0	517.5	778.5	1318.5	1521.0
Total sum (poly)phenols	**2.9**	**409.0**	**787.5**	**1238.1**	**1534.1**	**1909.9**
% Cranberry juice	0.0	25.1	48.2	75.8	94.0	117.0

Table 2. Nutritional analysis of the control drink and the most concentrated cranberry juice (1910 mg TP) per serving size (450 mL).

	Control drink	**Drink 1910 mg TP**
Energy (kcal)	208	200
Energy from fat (kcal)	<4.72	<4.72
Total fat (g)	<0.009	<0.009
Total carbohydrates (g)	50.9	49.5
Total dietary fiber (g)	<3.54	<3.54
Total sugars (g)	42	35
Protein (g)	0.90	0.75
Vitamin C (mg)	<4.7	<4.7
Moisture (g)	419	421

Table 3: Baseline clinical characteristics study population (n=10).

	Mean ± SD
Age (years)	24 ± 2
Weight (kg)	79 ± 8
BMI (kg/m^2)	24 ± 2
Total cholesterol (mg/dL)	149 ± 33
LDL cholesterol (mg/dL)	90 ± 31
HDL cholesterol (mg/dL)	49 ± 7
Triglycerides (mg/dL)	66 ± 17
Glucose (mg/dL)	88 ± 5
GOT (U/L)	24 ± 5
GPT (U/L)	22 ± 6
γ-GT (U/L)	19 ± 7
Uric acid (mg/dL)	6 ± 1
Creatinine (mg/dL)	1.0 ± 0.1
Bilirubin (mg/dL)	0.5 ± 0.3
Heart rate (bpm)	60 ± 8
Systolic blood pressure (mm Hg)	119 ± 8
Diastolic blood pressure (mm Hg)	66 ± 10
FMD (%)	6.0 ± 1.4
Pulse wave velocity (m/s)	5.3 ± 0.8
Augmentation Index (%)	-4.3 ± 14

Table 4: Significant correlations ($p<0.05$) between plasma cranberry-derived (poly)phenol metabolites and changes in FMD at different timepoints after consumption of cranberry juice with respect to baseline at 0 h (n=10). Correlations correspond to Pearson's r. *$p<0.05$; $^{\#}p<0.01$; $^{\S}p<0.001$.

	Pearson's r				
Metabolites correlating with ΔFMD at respective timepoints	**1h**	**2h**	**4h**	**6h**	**8h**
(4*R*)-5-(3'-hydroxyphenyl)-γ-valerolactone-4'-*O*-sulfate			0.325*		0.345*
4-Methylgallic acid-3-*O*-sulfate	0.297*	0.460$^{\#}$	0.332*		
Caffeic acid			0.682§*		0.337*
Caffeic acid 4-*O*-ß-D-glucuronide		0.320*			
Cinnamic acid		0.303*			
Dihydro caffeic acid 3-*O*-sulfate				0.395$^{\#}$	0.470§
Dihydro ferulic acid 4-*O*-sulfate				0.405$^{\#}$	0.477§
Dihydro isoferulic acid 3-*O*-sulfate	0.367$^{\#}$	0.397$^{\#}$	0.338*	0.371$^{\#}$	0.471§
Ferulic acid 4-*O*-sulfate	0.408$^{\#}$	0.437$^{\#}$	0.362$^{\#}$	0.369$^{\#}$	0.461§
Homovanillic acid sulfate	0.378$^{\#}$	0.428$^{\#}$	0.430$^{\#}$	0.455$^{\#}$	0.339*
Quercetin-3-*O*-ß-D-glucuronide	0.275*	0.423$^{\#}$	0.308*	0.371$^{\#}$	
Vanillic acid-4-O-sulfate	0.387$^{\#}$	0.494§	0.364$^{\#}$	0.347*	

Figure captions

Figure 1: A) Study flow (n=10) and B) study design

Figure 2. A) Changes in flow-mediated dilation (FMD) respect to baseline and B) changes in areas under the curve of the FMD response over time after consumption of the cranberry juice drinks containing 409, 787, 1,238, 1,534, and 1,910 mg of total (poly)phenols and the control drink (n=10).*$p<0.05$ significantly different from control.

Figure 3. Examples of time-course of (poly)phenols plasma concentrations after consumption of cranberry juice drinks containing 409, 787, 1,238, 1,534, and 1,910 mg of total (poly)phenols and a control drink (n=10):, (A) ferulic acid 4-*O*-sulfate, (B) vanillic acid-4-*O*-sulfate, (C) (4*R*)-5-(3'-hydroxyphenyl)-γ-valerolactone-4'-*O*-sulfate, (D) 4-methylgallic acid-3-*O*-sulfate, (E) quercetin-3-*O*-β-D-glucuronide and F) caffeic acid -4-*O*-ß-D-glucuronide. Symbols are means, and error bars correspond to SEM.

Figure 4. Structures of plasma (poly)phenol metabolites that correlated with changes in FMD after cranberry juice consumption.