A single portion of blueberry (*Vaccinium corymbosum* L) improves protection against DNA damage but not vascular function in healthy male volunteers

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**ABSTRACT**

It has been suggested that anthocyanin-rich foods may exert antioxidant effects and improve vascular function as demonstrated mainly in vitro and in the animal model. Blueberries are rich sources of anthocyanins and we hypothesized that their intake could improve cell protection against oxidative stress and affect endothelial function in humans. The aim of the study was to investigate the effect of one portion (300 g) of blueberries on selected markers of oxidative stress and antioxidant protection (endogenous and oxidatively induced DNA damage) and of vascular function (changes in peripheral arterial tone and plasma nitric oxide levels) in male subjects. In a randomized cross-over design, separated by a wash out period ten young volunteers received one portion of blueberries ground by blender or one portion of a control jelly. Before and after consumption (at 1, 2, and 24 hours), blood samples were collected and used to evaluate anthocyanin absorption (through mass spectrometry), endogenous and H2O2-induced DNA damage in blood mononuclear cells (through the comet assay), and plasma nitric oxide concentrations (through a fluorometric assay). Peripheral arterial function was assessed by means of Endo-PAT 2000. Blueberries significantly reduced (P < .01) H2O2-induced DNA damage (~18%) 1 hour after blueberry consumption compared to control. No significant differences were observed for endogenous DNA damage, peripheral arterial function and nitric oxide levels after blueberry intake. In conclusion, one portion of blueberries seems sufficient to improve cell antioxidant defense against DNA damage, but further studies are necessary to understand their role on vascular function.

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**Abbreviations:** ACNs, anthocyanins; ANOVA, analysis of variance; BB, blueberries; BMCs, blood mononuclear cells; BMI, body mass index; CJ, control jelly; HDL-C, high-density lipoprotein-cholesterol; BMCs, blood mononuclear cells; BMI, body mass index; FPG, formamidopyrimidine DNA glycosylase; LDL-C, low density lipoprotein-cholesterol; NO, nitric oxide; RHI, reactive hyperemia index; TFA, trifluoroacetic acid; TG, triglycerides; TSC, total serum cholesterol.

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1. Introduction

Blueberries (BB) contain bioactive compounds such as phenolic acids and in particular anthocyanins (ACNs), a group of water-soluble pigments responsible for the blue, red, and purple color of fruits and vegetables [1,2]. Several in vitro and in vivo studies have documented the bioactivity of ACNs, suggesting anti-inflammatory properties, improvement of lipid profiles, modulation of detoxifying enzymes, reduction of blood pressure, and platelet aggregation [2-8]. Some of these biological activities and protective effects can be attributed to their antioxidant activity against reactive oxygen species. In particular, blueberry ACNs have been documented to reduce H$_2$O$_2$-induced reactive oxygen species in endothelial and red blood cells and decrease liver DNA damage in rats [9,10]. Concerning the health effects of berries in human interventions studies, results are still scarce and inconclusive [11]. Duthie et al [12] documented that the intake of 750 mL/d of cranberry juice did not affect endogenous DNA damage, oxidized pyrimidines and H$_2$O$_2$ sensitivity in a group of female volunteers. Ramirez-Tortosa et al. [13] showed no change in baseline DNA strand breaks when volunteers consumed a 200 g of berry dessert (grape, cherry, blackberry, black currant) and raspberry juices for 2 weeks. On the contrary, we documented that 6 weeks of a wild blueberry drink significantly reduced the levels of formamidopyrimidine DNA glycosylase (FPG)-sensitive sites and H$_2$O$_2$-induced DNA damage in subjects with risk factors for cardiovascular diseases [14]. In addition, Wilms et al [15] documented a reduction of the levels of H$_2$O$_2$-induced DNA damage after 4 weeks of supplementation with 1 L/d of a mixture of blueberry and apple juices in healthy female volunteers.

Berries and ACNs are also believed to improve endothelial-dependent vasodilation. Most of the beneficial effects of berries on the modulation of endothelial function derives from in vitro and ex vivo studies [16-20]. We have demonstrated that 7-week consumption of a wild blueberry rich-diet improved the mechanical properties of the aorta in an animal model [20]. In humans, the results are still unconvincing. For example we have recently documented that a 6-week wild blueberry drink intervention did not significantly affect peripheral arterial function determined through the EndoPAT 2000 device in humans [14]. Consequently we hypothesized that, if the modulation of this function is strictly related to the increased ACN circulating levels, the lack of effect may be due to the rapid absorption and elimination of ACNs (generally within the first 3-4 hours). In fact, in our long term study, no ACNs were detectable in plasma following wild blueberry consumption, since blood samples were taken 12 h after the blueberry drink. Thus, we hypothesized that modulation of vascular function may be observed shortly after 1 h from BB intake.

To test this hypothesis, we designed an acute study to investigate the effect of BB both on oxidative stress and vascular function. In particular, we evaluated the effect of a single portion of blueberry (Vaccinium corymbosum) (300 g, providing about 348 mg ACNs) on endogenous FPG-sensitive sites and oxidatively (H$_2$O$_2$)-induced DNA damage (primary end points) with the aim in establishing whether the short-term increase in ACN circulating levels following the intake of blueberry, could affect peripheral arterial function and modulate nitric oxide (NO) plasma levels in a group of healthy volunteers.

2. Methods and materials

2.1. Study subjects

Ten healthy male subjects, 20.8 ± 1.6 years of age, with body mass index (BMI) 22.5 ± 2.1 kg/m$^2$, were recruited from the student population of the University of Milan according to the following inclusion criteria: non-smokers; no history of cardiovascular, diabetes, hepatic, renal, or gastrointestinal diseases; no consumption of any dietary supplement, drug, or medication for at least one month before the beginning of the study. Subjects were selected on the basis of an interview to evaluate their dietary habits and ensure that they were as homogeneous as possible, in particular for fruit and vegetable consumption. This was obtained by means of a food frequency questionnaire previously published and specifically revised to focus on food sources rich in antioxidants [21]. Exclusion criteria were: hypertension (systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg), high total serum cholesterol (TSC) (>5.17 mmol/L), low-high-density lipoprotein cholesterol (HDL-C) (<1.03 mmol/L), high low-density lipoprotein cholesterol (LDL-C) (>3.36 mmol/L), high triglycerides (TG) (>1.69 mmol/L), overweight (BMI ≥ 25 kg/m$^2$). Other exclusion criteria were as follows: high (>5 portions/day) or low (<2 portions/day) intake of fruit and vegetables and alcohol consumption (<3 drinks per week were acceptable). Volunteers who followed a specific diet (e.g. vegetarian, vegan, or macrobiotic) and those who had a specific aversion for blueberry consumption were excluded. All participants gave informed consent and the study was approved by the Ethics Committee of the University of Milan.

2.2. Blueberry and placebo preparation

Blueberries (V corymbosum L “Brigitta”) from a single batch were purchased, sorted and immediately frozen by Individually Quick Freezing technique in a tunnel (Thermolab, Codogno, Italy) and stored at −20°C until use. For the study, BB were partially thawed (3 h at 20°C) and homogenized in a commercial food processor (Moulinex, Paris, France). They were packed in portions of 300 g, thermally sealed under partial vacuum (Minipack-Torre S.P.A., Dalmine, Bergamo, Italy), and stored at −20°C for few days. The evening before the experiment, the BB portions were placed at + 4°C for defrosting. The BB was gelatinous in texture; for this reason, a control jelly (CJ) was utilized as placebo. The CJ was prepared by suspending 20 g of food grade gelatin (Universal, Peru) and adding the same amount of BB sugars (about 27.1 g total, 16.4 g fructose and 10.7 g glucose) in 200 mL of hot water. The CJ containing a food colorant was prepared the day before the experiment and stored at +4°C to solidify.

2.3. Experimental design

Subjects were deprived of ACN-food sources 10 days before experimentation. Volunteers received a complete list of ACN-
rich foods to be avoided; the list included ACN-rich foods such as berry fruits, red wine and red/purple fruits and other colored products. Subjects were randomly divided into 2 groups of 5 subjects each: group 1 was assigned to the sequence BB/wash-out/CJ, whereas group 2 followed the sequence CJ/wash-out/BB. The study was scheduled at different days to avoid interference between withdrawal times and the study of vascular function. Each analysis was separated by 10 days of wash-out period. Lunch and dinner was standardized and subjects were asked to exclude all ACN-containing foods and maintain their regular lifestyle. Moreover, basal levels of peripheral vasoreactivity (RHI, reactive hyperemia index) were measured on a group of fasted volunteers (n = 9) early in the morning, in two different days, to ensure within-subject repeatability. Average data obtained (2.05 ± 0.29 RHI for day 1 and 2.04 ± 0.27 RHI for day 2) did not demonstrate an inter-day effect on vascular function as also recently reported [22].

Thus, for the present study, peripheral arterial function was measured in two consecutive days. Baseline levels were assessed the first day, while the second day peripheral arterial function was evaluated 1h after the intake of BB or CJ. This protocol was chosen to avoid multiple measurements (involving 5-minute arterial occlusion through cuff inflation) in a short time-period, since it could promote vasodilation through NO production [23].

The subjects fasted overnight before the ingestion of one portion of thawed BB (providing 348 mg of ACNs) or CJ (without ACNs). The products were consumed early in the morning and blood was collected by a phlebotomist at time 0 (before the consumption of the products) and 1, 2, and 24 hours after BB or CJ consumption. Samples were drawn into evacuated tubes with heparin as anticoagulant. One day-food records were kept by subjects in each experimental session, 2 days before and 1 day after the intake of the BB product to check compliance to the dietary instructions. Moreover, a direct interview by a registered dietitian was scheduled.

2.4. Sugars, total phenolics, and vitamin C determination in BB

A duplicate sample (50 g) of BB was homogenized and suspended in water, centrifuged at 3000 × g for 1 minute, filtered and injected for the analysis. Glucose and fructose were quantified by ultra-performance liquid chromatography. The LC consisted of an Alliance model 2695 (Waters, Milford, MA, USA) equipped with a model 2996 photodiode array detector (Waters), coupled with mass spectrometry (Micromass, Beverly, MA, USA). The separation was carried out on BEH Amide column (150 × 2.1 mm, 1.7 μm, Waters) at 35°C. Solvents were triethanolamine 0.2% and acetonitrile: triethanolamine at a ratio of 74:26 (v/v). The elution gradient was linear and the amount of triethanolamine was increased from 0% to 35% in 11 minutes at set up flow rate of 0.45 mL min⁻¹. The calibration curve was obtained from 5 mg L⁻¹ to 100 mg L⁻¹ for both sugars. The percentage relative standard deviation was calculated after injecting standard solutions of glucose and fructose at increasing concentration (2 mg L⁻¹, 10 mg L⁻¹ and 50 mg L⁻¹) in quintuplicates. Phenolic compounds were extracted in duplicate from BB by applying a formic acid-water (5:95 v/v) extracting media, according to Brambilla et al. [24]. Total phenolic compounds of the extracts were analyzed by Folin-Ciocalteau assay [25] and expressed as gallic acid equivalents (mg/100g) while chlorogenic acid and individual anthocyanin compounds were analyzed by gradient reverse phase-high-performance liquid chromatography and diode array detection and were quantified by measuring detector response to the commercial standards (Polyphenols Laboratory Sandes, Norway) [24]. All ACN monoglycosides were expressed as cyanidin 3-glucoside equivalents. Vitamin C was extracted and determined by high-performance liquid chromatography analysis as previously described by Riso et al [26].

2.5. Analysis of biochemical parameters

Blood samples were drawn and immediately centrifuged at 1000 × g for 15 minutes for plasma and serum separation and stored at −80°C until analysis. A general laboratory clinical assessment was performed in serum including evaluation of lipid profile (TG, TSC, LDL-C and HDL-C) and glucose. All these parameters were determined using a Cobas 6000 analyzer series (Roche Diagnostics, North America). Plasma concentration of total NO was calculated by measuring the products of oxidation (nitrate and nitrite) by a Fluorometric Assay Kit (Cayman Chemical, Ann Arbor, MI, USA).

2.6. Anthocyanin extraction and analysis in plasma

Two aliquots of plasma (1 mL) were acidified with trifluoroacetic acid (TFA, 1%), vortexed, and centrifuged for 1 min at 4500 × g and the supernatant was stored at −80°C until analysis. Anthocyanins were extracted from plasma using a Micro-Plate solid phase extraction HLB Oasis Cartridge preactivated with methanol (500 μL) and washed with 500 μL acidified water (1% TFA). Plasma (400 μL) was diluted with 140 μL of acidified water (1% TFA) and 60 μL of water containing the Internal Standard (50 ng/mL of cyanidin-3,5-diglucoside). Plasma was vortexed, centrifuged and loaded onto the cartridge.

The samples were drained under gravity and the cartridge washed with acidified water (100 μL; 1% TFA) and 100 μL of water-methanol (80:20 v/v) acidified with TFA (0.1%). The ACNs were eluted from the cartridge using 50 μL of methanol (70%) containing TFA (0.1%). The filtered sample was injected into Ultra performance liquid chromatography-mass spectrometry system for analysis according to a method previously published [27].

2.7. Evaluation of endogenous DNA damage and cell resistance against H₂O₂-induced DNA damage

Blood mononuclear cells (BMcs) were separated from whole blood by density gradient centrifugation [28]. The FPG-sensitive sites (oxidized purines) and cell resistance against H₂O₂ (500 μmol/L, 5 min) induced DNA damage were evaluated by the comet assay as previously described in detail [28,29].
2.8. Evaluation of peripheral arterial function

Endothelial-dependent vasodilation in the small finger arteries was assessed by a non-invasive plethysmographic method (Endo-PAT 2000, Itamar Medical Ltd, Caesarea, Israel) based on the registration of pulsatile blood volume in the fingertips of both hands.

The Endo-PAT equipment consists of two finger-mounted probes, which include a system of inflatable latex air-cushions within a rigid external case; pulsatile volume changes of the fingertip are sensed by a pressure transducer, located at the end of each probe, and transferred to a personal computer where the signal is band pass-filtered (0.3-30 Hz), amplified, displayed, and stored. For the evaluation, subjects were in the supine position and both hands on the same level in a comfortable, thermoneutral environment. Arterial systolic and diastolic blood pressure and heart rate frequency were measured before starting the test. A blood pressure cuff was placed on one upper arm (study arm), while the contralateral arm served as a control (control arm). After a 10-min equilibration period, the blood pressure cuff on the study arm was inflated to 60 mmHg above systolic pressure for 5 min. The cuff was then deflated to induce reactive hyperemia while the signals from both PAT channels (Probe 1 and Probe 2) were recorded by a computer. The RHI, an index of the endothelial-dependent flow-mediated dilation, was derived automatically in an operator independent manner, as the ratio of the average pulse wave amplitude during hyperemia (60-120 s of the post-occlusion period) to the average pulse wave amplitude during baseline in the occluded hand, divided by the same values in the control hand and then multiplied by a baseline correction factor. An RHI value of 1.67 provides a sensitivity of 82% and a specificity of 77% for diagnosing endothelial dysfunction [30].

2.9. Statistical analyses

Sample size has been calculated taking into account the expected variation in the primary endpoint considered as evaluated in our previous study [14]. In particular, ten subjects were calculated to be more than sufficient to evaluate a difference of DNA damage after the wild blueberry drink of 8.6 (SD 0.9), with α = .05 and a statistical power of 80%. This number of subjects is comparable to those used in previous acute studies [31-33] for the evaluation of vascular function modulation (secondary endpoint). Moreover, the “repeated measure” experimental design used, in which each subject acts as its own control, reduces the error variance, thus increasing statistical power.

Statistical analysis was performed by means of STATISTICA software (Statssoft Inc, Tulsa, OK, USA). Data were analyzed by analysis of variance (ANOVA) for repeated measures design. ANOVA with treatment (BB vs CJ) and time (before and after each treatment) as dependent factors was applied to evaluate the effect of BB on the variables under study. Differences were considered significant at P = .05; post-hoc analysis of differences between treatments was assessed by the least significant difference test with P = .05 as level of statistical significance. To evaluate the relationship between variation in ACN plasma levels following BB intake and those in DNA damage, linear correlation analysis was performed. Data are presented as means ± SD.

3. Results

3.1. Composition and characteristic of the blueberry portion

The nutritional composition of BB is reported in Table 1. One portion (300 g) of the BB provided about 27 g of sugars (fructose and glucose), 348 mg of ACNs (malvidin-galactoside, delphinidin-galactoside and malvidin-arabinoside making up more than 50% of the total ACN content), 727 mg of total phenolic acids, 90 mg of chlorogenic acid, and 2.4 mg of vitamin C. The CJ provided the same amount and type of sugars but not of bioactive compounds.

3.2. Baseline characteristics of the subjects

Baseline anthropometric and clinical characteristics of the subjects are reported in Table 2. All data were within the range of normality.

3.3. Plasma concentration of ACNs following BB and CJ intake

Anthocyanins were not detectable in plasma at baseline while a significant (P < .001) increase was observed 1 hour (13.7 ± 10.7 nmol/L) and 2 hours (18.7 ± 6.4 nmol/L) after BB intake. Twenty four hours after BB intake, ACNs were not detected in plasma; CJ intake resulted in undetectable plasma ACNs.

3.4. Effect of BB and CJ intake on the levels of DNA damage in BMCs

Results on DNA damage in BMCs are reported in Table 3. Oxidized purines evaluated through quantification of FPG-sensitive sites were not significantly different following BB or CJ intake. The levels of H2O2-induced DNA damage decreased 1 hour after the BB intake (from 51.7 ± 4.9% to 42.7 ± 8.7%, P ≤ .01), while no effect was observed after CJ (from 53.2 ± 2.8% to 52.0 ± 7.6%, P = .84). However, the protective effect was transient and the level of H2O2-induced DNA damage returned to baseline 2 h after BB consumption. There was no correlation between the decrease in H2O2-induced DNA damage and the increase in ACNs observed at 1 hour.

<table>
<thead>
<tr>
<th>Table 1 – Nutritional composition of BB and CJ</th>
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<tbody>
<tr>
<td><strong>BB</strong></td>
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<tr>
<td>Sugars (g/100 g)</td>
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<tr>
<td>Fructose</td>
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<tr>
<td>Glucose</td>
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<tr>
<td>Total phenolic compounds (mg/100 g)</td>
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<tr>
<td>Chlorogenic acid (mg/100 g)</td>
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</tbody>
</table>

Data are expressed as means ± SD.
Table 2 – Subject characteristics at the beginning of the study

<table>
<thead>
<tr>
<th>Value</th>
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<tbody>
<tr>
<td>Age (y)</td>
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<tr>
<td>Body weight (kg)</td>
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<tr>
<td>BMI (kg/m²)</td>
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<td>Systolic pressure (mmHg)</td>
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<tr>
<td>Diastolic pressure (mmHg)</td>
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<tr>
<td>Heart rate (beat/min)</td>
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<tr>
<td>Glucose (mmol/l)</td>
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<tr>
<td>TG (mmol/l)</td>
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<tr>
<td>TSC (mmol/l)</td>
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<tr>
<td>HDL-C (mmol/l)</td>
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<td>LDL-C (mmol/l)</td>
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</table>

Data (n = 10) are expressed as means ± SD.

3.5. Effect of BB and CJ intake on peripheral arterial function and plasma nitric oxide levels

Peripheral arterial function (reactive hyperemia response), blood pressure, heart rate and plasma NO levels, before and after BB and CJ consumption, are reported in Table 4. According to the repeated measures ANOVA, after either the BB or the CJ intake no significant changes were observed for all the variables under study. The mean percent change in RHI index between the pre- to post-intervention was +0.5% (95% CI: −7.3%, +8.4%) after the BB and −4.5% (95% CI: −13.9%, +6.4%) after the CJ intake. On the whole, a high inter-individual variability was observed in the percent changes of RHI index (Fig).

4. Discussion

Several human studies have demonstrated that the intake of single portions of fruits such as kiwifruits, apples and orange juice was associated with decreased intrinsic levels of oxidatively damaged DNA and increased resistance to H₂O₂-generated DNA damage [34-37]. In the present study, we also documented that the intake of one portion of BB significantly reduced the levels of ex vivo H₂O₂-induced DNA damage in healthy male volunteers as hypothesized. The protective effect was shown 1 h after the consumption of BB but not after 2 h, while no significant effect was observed after CJ intake. The protection against oxidative stress may be related to other bioactives absorbed, apart from ACNs (eg, phenolic acids, vitamin C), acting alone or synergistically. Moreover, these compounds could have indirectly activated signaling mechanisms of defense (eg, antioxidant enzymes through gene expression modulation) [37] even though the effect is not maintained at 2 hours.

No significant effect was observed on oxidized DNA bases after BB or CJ intake. This result as previously reported [34] is not surprising, since the levels of FPG-sensitive sites measured, represent the steady-state levels of oxidatively damaged DNA. In fact, in cultured cells, the repair of FPG-sensitive sites has a half-life of 1 to 5 hours [38-40]. Since the removal of DNA damage is not instantaneous, a long-term supplementation may be required to establish the possibility of in vivo efficacy of BB intake on endogenous levels of oxidatively damaged DNA [34]. In fact, we have recently documented that a 6-week intervention period with a wild BB drink reduced the level of oxidized DNA bases in subjects with cardiovascular risk factors [14].

In addition, beneficial effects have been observed following the intake of one portion of cranberry juice [41], dark chocolate [42,43] or flavonol-rich cocoa drink [31,44], green tea [45,46], or red wine [47] in healthy and unhealthy subjects on endothelial function. The protocols generally used are based on multiple measurements of vasoreactivity (through both flow-mediated dilation and EndoPAT 2000) in a short time-period after the intake of the test products. However, conflicting opinions on this procedure are reported in literature. The International Brachial Artery Reactivity Task Force suggested that multiple measurements may promote vasodilation through NO production [23]. This effect may possibly mask improvement of vascular function due to the intervention (ie, overestimation). In this regard, Liu et al [48] documented a significant increase in the RHI when the PAT was measured at 0.5-hour intervals (for 2.5 hours) indicating a crossover effect, but not at 1-hour intervals (for 4 hours) and 2-hour intervals (for 12 hours) in healthy male subjects. In addition, Forchhammer et al. [49] demonstrated intra-day reproducibility in a group of healthy subjects whose vascular function was measured on four different occasions (in the

Table 3 – Effect of one portion of BB or CJ on background, FPG sensitive sites and H₂O₂-induced strand breaks

<table>
<thead>
<tr>
<th>Value</th>
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<tr>
<td>T 0 h</td>
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<tr>
<td>Background SBs (% DNA in tail, EB)</td>
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<tr>
<td>Net FPG-sensitive sites (% DNA in tail)</td>
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<tr>
<td>Background SBs (% DNA in tail, PBS)</td>
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<tr>
<td>Net H₂O₂-induced DNA damage (% DNA in tail)</td>
</tr>
<tr>
<td>CJ consumption</td>
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<tr>
<td>Background SBs (% DNA in tail, EB)</td>
</tr>
<tr>
<td>Net FPG-sensitive sites (% DNA in tail)</td>
</tr>
<tr>
<td>Background SBs (% DNA in tail, PBS)</td>
</tr>
<tr>
<td>Net H₂O₂-induced DNA damage (% DNA in tail)</td>
</tr>
</tbody>
</table>

Data (n = 10) are expressed as means ± SD.

SBs, strand breaks; PBS, phosphate-buffered saline; EB, endonuclease buffer.

*Significantly different from each other time point in the same row and different with respect to each other time point for the CJ group, P ≤ .01.
morning, before and after lunch and in the afternoon) within the same day. Thus, it seems that the time-period among measurements is an important variable in this type of assessment and it should be seriously considered to avoid crossover effects. In the present study, we measured peripheral arterial function in two consecutive days after demonstration of inter-day reproducibility as demonstrated by others [22]. We failed to demonstrate an effect of BB on peripheral vascular function one reason being that most of the subjects in the present study had RHIs in the normal range (RHI = 1.67). It seems plausible that improvements may be easier demonstrated in subjects with reduced vascular function (eg, elderly or subjects who are at risk of developing cardiovascular diseases) or after vascular function challenges (eg, following smoking or a meal rich in saturated fats).

The lack of the BB effect in modulating vascular function may be also attributed to the length of time between the BB intake and the measurement of peripheral arterial function (1 hour). In fact, more time may be necessary to detect an effect on endothelial function following the exposure to BB and their bioactives. In this regard, Dohadwala et al [41] documented an improvement of vascular function at 2 and 4 hours after the intake of a single portion of cranberry juice.

In the present study, the observations on vascular function are consistent with the non-significant changes in plasma total NO, indicating that the short-term consumption of BB did not exert any changes on this marker. Plasma NO concentration is mainly related to systemic inflammation, whereas the endothelium-derived NO synthase production of NO is a minor contributor to alterations in its plasma concentration. Nevertheless, we cannot exclude that a modulation of NO occurred at the endothelial level without influencing total plasma levels. In fact, some authors reported that the consumption of red wine polyphenols and flavonoids may affect vascular function by increasing the half-life of endothelial NO [50]. Future studies with larger numbers of subjects or with established vascular dysfunction may contribute to our understanding of the beneficial effects of BB consumption on vascular function and modulation of plasma NO levels.

Possible study limitations are the small sample size of healthy subjects considered for the demonstration of an effect on vascular function at one time-point after the ingestion of BB. Although the statistical power increases with the number of subjects, the effect size is not dependent on the sample size. Our results suggest that BB did not produce any short-term protective effects in healthy subjects with uncompromised vascular function but we cannot exclude that improvements in the vascular function occur at later time points than 1 hour after a single BB consumption. In addition, regular BB intake may protect against the development of vascular dysfunction in patients with cardiovascular risk factors.

In conclusion, this study documented that one portion of blueberries (300 g) can improve cell resistance against H2O2-induced DNA damage, and this is in accordance with previous observations with other fruits provided in single portions, thus supporting the importance of consuming vegetable foods regularly.

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