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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 H_2O_2-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 ($P < .01$) H_2O_2-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.

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; 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_2O_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_2O_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_2O_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_2O_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 evidence 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_2O_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-