

Polyphenol-rich cranberry juice has a neutral effect on endothelial function but decreases the fraction of osteocalcin-expressing endothelial progenitor cells

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Abstract

Purpose Cranberry juice (CJ) contains a remarkably high concentration of polyphenols, considered to be beneficial for cardiovascular and bone health. The current double-blind, randomized study was designed to test whether daily consumption of double-strength Ocean Spray light CJ (2×230 ml) over 4 months has beneficial effects on vascular function and on endothelial progenitor cells (EPCs) carrying the osteoblastic marker osteocalcin in particular. **Methods** A total of 84 participants (49.5 ± 16.2 years) with peripheral endothelial dysfunction and cardiovascular risk factors were enrolled in this double-blind, randomized, controlled trial (69 completed the 4-month protocol—32 in the CJ group and 37 in the placebo group, respectively). Vascular responses to reactive hyperemia were measured non-invasively by peripheral arterial tonometry (EndoPAT). Peripheral blood mononuclear cells were stained for EPC markers, as well as osteocalcin, and counted by flow cytometry.

Results Baseline characteristics were similar in both groups. The effect of CJ on peripheral endothelial function and on circulating EPC counts ($CD34^+/CD133^+/KDR^+$) did not change during the study. A high percentage of EPCs expressed osteocalcin ($59.4 \pm 35.7\%$). CJ, as compared to placebo, induced a decrease in the fraction of EPCs expressing osteocalcin (-8.64 ± 48.98 and $19.13 \pm 46.11\%$, respectively, $p = 0.019$). Systemic levels of the adhesion marker ICAM correlated significantly with the number of EPCs expressing osteocalcin.

Conclusions The study demonstrated that long-term supplementation of polyphenol-rich CJ did not improve peripheral endothelial function. However, the decrease in the fraction of osteocalcin+ EPCs suggests a potential beneficial effect of polyphenol-rich CJ.

Keywords Endothelial function · Cranberry · Polyphenols · Endothelial progenitor cells · Osteocalcin

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Introduction

Epidemiologically, consumption of food rich in fruit and vegetables is inversely correlated with all-cause mortality and ischemic heart disease [1] and might as well contribute to bone health [2]. However, the mechanisms responsible for these effects are still unclear and likely multi-factorial. Recent research has identified several natural dietary components, which may be particularly beneficial, including the phytochemical polyphenols that are commonly found in the human diet. Cranberries have the highest total phenol content among the commonly consumed fruits in the American diet [3], which could make them especially pertinent as a supplement to Western diet.

Although prospective randomized studies evaluating morbidity and mortality with polyphenol-rich foods are lacking, many small prospective studies, especially with cocoa [4], demonstrate improvement in important cardiovascular surrogates of vascular health such as endothelial function [5]. Endothelial dysfunction, as a marker for vascular injury, is associated with atherosclerosis [6, 7], involving continuous vascular injury and repair, partly mediated by endothelial progenitor cells (EPCs) [8]. We recently reported that coronary endothelial dysfunction is characterized by an increased proportion of EPCs carrying the osteoblastic marker osteocalcin (OCN), which may mediate abnormal repair and vascular calcification. Interestingly, these circulating osteoblastic EPCs that are increased in patients with early and severe coronary atherosclerosis are believed to contribute to vascular calcification as opposed to initiating normal vascular repair [9].

Interestingly, there is an evidence for a link between bone metabolism and the vasculature as women with osteoporosis or with high bone turnover have increased cardiovascular mortality [10], and polyphenols, as found in high concentration in cranberry juice, are associated with bone health in a Scottish perimenopausal population [11]. Polyphenols have been shown to be protective for bone loss in middle-aged female rats [12] and are able to mitigate bone loss induced by inflammation in rats [13]. Furthermore, around 20% of human peripheral blood CD34⁺ cells express mRNA for OCN and when infused into immunocompromised rats, those cells localize to fracture sites and differentiate into endothelial cells and osteoblasts [14], pointing out the potentially important interplay between vascular function and bone homeostasis.

Thus, we aimed to study the impact of polyphenol-rich cranberry juice—as compared to placebo juice—on vascular function as well as on EPCs, OCN-positive EPCs in particular, in patients with risk factors for atherosclerosis and endothelial dysfunction.

Participants and methods

Participants

Participants over the age of 18 years with endothelial dysfunction and cardiovascular risk factors or known cardiovascular disease regardless of previous history of cardiovascular events were included in this randomized double-blind, placebo-controlled, investigator-initiated trial. Patients were recruited from the Division of Cardiology at the Mayo Clinic in Rochester, MN, USA, as well as an institutional Classifieds advertisement seeking research participants. Patients underwent peripheral endothelial function testing with an EndoPAT device [7].

Patients with presumably normal endothelial function (an arbitrary RH-PAT score over >2.5 was used as a cutoff; see below) and/or uncontrolled hypertension with blood pressure >180/100 were excluded from the study. Other exclusion criteria were history of renal or liver failure and relevant food allergies. Informed consent was obtained and signed from all participants. The study was approved by the local ethics committee and was done in accordance with the Declaration of Helsinki in 1975 and as revised in 1983.

Experimental protocol

All patients were instructed to fast at least 4 h prior to their appointment and refrain from caffeine-containing products and smoking for at least 24 h prior to the study. Examinations were always performed in the morning. After a short clinical examination including measurement of blood pressure and body weight, baseline measurement with EndoPAT was taken and blood samples were drawn and processed immediately. Participants were then randomized to receive either double-strength light cranberry juice Ocean Spray[®] cocktail twice a day (2 × 230 ml) for 4 months or isocaloric placebo beverage (see below). Measurements were taken at baseline (time of randomization) and 45 min after the intake of the first dose of cranberry juice or placebo to assess their short-term effects. Measurements were also taken after 4 months to assess long-term effects. Patients were contacted by phone and/or e-mail at 1 and 3 months to assess compliance and any changes in medications or symptoms. Participants were sent home with a box of juice (12 bottles) after their baseline appointment. The remainder of the boxes were shipped to their home address over the next months.

Study cranberry juice and control

For the study, double-strength Ocean Spray[®] light cranberry juice cocktail (54% cranberry juice) was used. Other ingredients include filtered water, fructose and sucralose. Overall sugar content was as follows: 4.9 mg/ml fructose; 15.3 mg/ml glucose; and 0.6 mg/ml sucrose. Titratable acidity was 10.7 mg/ml. Total phenolic content was 1,740 µg/ml; total anthocyanin content was 151 µg/ml; and total proanthocyanidin content was 2,662 µg/ml. Ocean Spray also provided a placebo juice beverage, an isocaloric formulation mimicking the flavor and color of the cranberry beverage without any cranberry ingredients: Overall sugar content was as follows: fructose 7.1 mg/ml; glucose 17.4 mg/ml; and sucrose 0.6 mg/ml. No detectable phenolics, anthocyanins and proanthocyanidins were found. Data were provided from Ocean Spray. Both beverages came in identical containers.

Endothelial function assessment

Studies were performed in a designated quiet, temperature-controlled and uniformly lit room. Peripheral arterial tonometry (PAT) signals were obtained using the EndoPAT 2000 device (Itamar Medical Inc. Ltd, Caesarea, Israel.), which has been used previously to assess peripheral arterial tone in other populations [7].

Endothelial function was measured by a reactive hyperemia-peripheral arterial tonometry index (RH-PAT index) as previously described [7]. In brief, after a 5-min baseline measurement, a blood pressure cuff on one arm was inflated to 60 mmHg above baseline systolic blood pressure, or at least 200 mmHg for 5 min. Occlusion of pulsatile arterial flow was confirmed via the PAT tracing. After 5 min, the cuff was deflated and the PAT tracing recorded for an additional 6 min. The ratio of the PAT signal after cuff release compared to baseline (RH-PAT index) was calculated through a computer algorithm automatically normalizing for baseline signal and indexed to the contra-lateral arm. Reactive hyperemia responses were recorded at baseline, 1 h after the first intake of cranberry juice or placebo and after the four-month treatment period.

Endothelial progenitor cells

Peripheral blood mononuclear cells were isolated using a Ficoll density gradient and stained using the following fluorescent conjugated antibodies: CD34-PerCP Cy 5.5 (Beckton-Dickinson), CD133-phycoerythrin (PE) (Miltenyi Biotec GmbH), kinase insert domain receptor KDR-APC (R&D Systems) and the appropriate isotype controls, as previously described [9]. In addition, co-staining for osteoblast marker osteocalcin [OCN+] was performed using anti-human OCN (Santa Cruz) and a fluorescein isothiocyanate (FITC) secondary antibody (Jackson ImmunoResearch). Cell fluorescence was measured immediately after staining (Becton–Dickinson, FACS Calibur), and data were analyzed using CellQuest software (Becton–Dickinson). A total of 150,000 events were counted, and final data were obtained within the lymphocyte gate, and EPCs were defined as CD34⁺/CD133⁺/KDR⁺ cells.

Blood tests

Blood measurements of lipids, inflammatory markers and oxidative stress markers were taken at baseline, 1 h after the first intake of cranberry juice or placebo and at 4 months as described previously. Inflammatory markers measured included hsCRP, IL-6, soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1) and TNF- α . Endogenous oxidative stress markers measured included oxidized

LDL (oxLDL). Standard lipid profiles (total cholesterol, triglycerides (TG), HDL) were also assessed.

Statistical analysis

Non-normally distributed data are presented as median [interquartile (25–75th percentile) range], and normally distributed variables are presented as mean \pm standard deviation (SD). Differences between the two randomized groups were analyzed using Person's chi-squared test, the Wilcoxon signed-rank test and unpaired *t* test as appropriate. Bivariate correlations were done using Spearman's correlation coefficients. Statistical significance was accepted at $p < 0.05$. A department statistician who was blinded to patient allocation assisted with data analysis. Analyses were performed using SAS and SPSS, respectively.

Results

Clinical characteristics

Seven hundred and eighty-seven participants were screened and 84 participants were qualified and randomized for the study; 69 were able to complete the 4-month protocol as well as pre- and post-treatment measurements. Baseline characteristics of the study population are shown in Table 1. No significant difference was found between the two groups with the exception of a higher percentage of men in the cranberry group. Medication use was not different between the two groups. Systolic and diastolic blood pressures were well within the normal range in this population (Table 2).

Laboratory parameters

We found significant correlations between BMI and metabolic markers: BMI correlated with hsCRP ($n = 58$, $R = 0.34$, $p = 0.01$), TG ($n = 58$, $R = 0.46$, $p < 0.001$), HDL ($n = 58$, $R = -0.51$, $p < 0.001$) and IL-6 ($n = 58$, $R = 0.48$, $p < 0.001$). However, both cranberry juice and placebo juice had no immediate or long-term effects on metabolic, inflammatory markers (C-reactive protein) or oxidative stress markers, including isoprostanes and oxidized LDL (Table 3).

Endothelial function

EndoPAT RHI was similar in both groups at baseline. In the short term (acutely 1 h after consuming cranberry juice or placebo), RHI increased significantly from 1.7 ± 0.4 to 2.0 ± 0.6 , $p = 0.01$ after cranberry juice and from 1.7 ± 0.4 to 2.0 ± 0.5 , $p = 0.06$ after placebo,

Table 1 Baseline characteristics

| | Placebo | Cranberry juice | <i>p</i> value |
|--|-------------|-----------------|----------------|
| Age | 51.4 ± 15.1 | 44.8 ± 17.5 | 0.06 |
| Men, no. (%) | 11 (25%) | 20 (48%) | 0.029 |
| Body mass index | 27.2 ± 5.5 | 27.7 ± 5.9 | 0.70 |
| LVEF, % | 61.3 ± 5.3 | 63.0 ± 6.5 | 0.50 |
| Family history of CAD, no. (%) | 15 (34%) | 16 (39%) | 0.69 |
| Hyperlipidemia, no. (%) | 24 (55%) | 19 (46%) | 0.75 |
| Smoking, no. (%) | 2 (5%) | 1 (2%) | 0.58 |
| Hypertension currently drug treated, no. (%) | 11 (25%) | 12 (29%) | 0.66 |
| Diabetes mellitus, no. (%) | 2 (5%) | 0 (0%) | 0.17 |
| Obesity (BMI ≥30), no. (%) | 12 (27%) | 8 (20%) | 0.40 |
| Arrhythmia (afib/SVT/VT), no. (%) | 5 (11%) | 2 (5%) | 0.28 |
| Valve disease, no. (%) | 1 (2%) | 1 (2%) | 0.96 |
| Cerebral vascular disease, no. (%) | 1 (2%) | 0 (0%) | 0.33 |
| Previous MI, no. (%) | 0 (0%) | 1 (2%) | 0.30 |
| PTCA/atherectomy/stent (Angioplasty), no. (%) | 0 (0%) | 1 (2%) | 0.30 |
| CABG surgery (Coronary artery bypass graft), no. (%) | 0 (0%) | 1 (2%) | 0.30 |
| ACE inhibitor, no. (%) | 4 (9%) | 3 (8%) | 0.77 |
| Aspirin, no. (%) | 14 (33%) | 9 (23%) | 0.31 |
| Calcium blocker, no. (%) | 1 (2%) | 4 (10%) | 0.14 |
| Statins, no. (%) | 11 (25%) | 11 (28%) | 0.79 |
| Omega-3 FA, no. (%) | 10 (23%) | 6 (15%) | 0.34 |
| Any vitamin (multi/C/E), no. (%) | 24 (56%) | 18 (45%) | 0.32 |

LVEF left ventricular ejection fraction, *CAD* coronary artery disease, *afib* atrial fibrillation, *SVT* supraventricular tachycardia, *MI* myocardial infarction, *PTCA* percutaneous transluminal coronary angioplasty, *ACE* angiotensin-converting enzyme, *FA* fatty acids

respectively (Fig. 1; Table 2). However, there was no difference between the two groups ($p = 0.87$ for inter-group changes). Similarly, there was no long-term effect of cranberry juice on peripheral finger-tip endothelial function after 4 months (1.7 ± 0.3 after cranberry juice and 1.8 ± 0.6 after placebo, $p = 0.37$, Fig. 1).

Endothelial progenitor cells

EPC measurements at baseline and after 4 month were taken in 69 patients ($n = 32$ in the CJ and 37 in the placebo group, respectively). Overall, we found 21 [8/42] EPC ($CD34^+/CD133^+/KDR^+$) counts per 100,000 gated lymphocytes in the cranberry group and 15 [4, 36] in the placebo group. There was no significant change during the course of the study in either group. EPC number at baseline was correlated with both body mass index and HDL ($n = 68$, $r = -0.24$, $p = 0.03$ and $n = 57$, $r = 0.27$, $p < 0.05$, respectively).

Furthermore, in this patient group with endothelial dysfunction and several cardiovascular risk factors, a high percentage of the EPCs co-expressed OCN surface markers ($59 \pm 36\%$). Importantly, cranberry juice decreased the percentage of OCN-positive EPCs after 4 month, compared to placebo (-8.64 ± 48.98 as compared to 19.13 ± 46.11 , $p = 0.019$, Fig. 2).

Circulating levels of sICAM correlated with EPCs co-expressing OCN at baseline ($n = 55$, $r = 0.33$, $p = 0.02$).

Discussion

In this double-blind, placebo-controlled study in patients with endothelial dysfunction or concurrent cardiovascular risk factors, we demonstrate a potentially beneficial effect of cranberry juice on the phenotype of circulating osteocalcin-positive EPCs. However, there was no significant improvement in peripheral endothelial function after daily consumption of cranberry juice for 4 months. This study demonstrates a potential differential effect of polyphenol-rich food on the characteristics of EPCs that may mediate vascular repair.

While we found significant effects of cranberry juice on the expression of osteocalcin-positive EPCs, we were not able to demonstrate improvement in peripheral endothelial function. The reasons might be manifold, but a likely reason is that the effect on endothelial function may be time dependent, and our study is too short to demonstrate this effect. However, other potential explanations may be speculative. Most studies with polyphenol-rich food showed significant effects on endothelial function in the short term (hours), when polyphenol plasma concentrations

Table 2 Effect of the study interventions on endothelial function and hemodynamic measures

| | Baseline placebo | Baseline cranberry | <i>p</i> | Acute placebo | Acute cranberry | <i>p</i> | 4 month placebo | 4 month cranberry | <i>p</i> | Delta placebo 4-month baseline | Delta cranberry 4-month baseline | <i>p</i> |
|-----|------------------|--------------------|----------|---------------|-----------------|----------|-----------------|-------------------|----------|--------------------------------|----------------------------------|----------|
| RHI | 1.7 ± 0.4 | 1.7 ± 0.4 | 0.95 | 2.0 ± 0.5 | 2.0 ± 0.6 | 0.99 | 1.8 ± 0.6 | 1.7 ± 0.3 | 0.37 | 0.1 ± 0.5 | −0.0 ± 0.3 | 0.32 |
| SBP | 116.8 ± 15.2 | 115.1 ± 14.0 | 0.59 | 117.7 ± 16.8 | 112.9 ± 23.3 | 0.31 | 115.3 ± 16.6 | 109.8 ± 21.6 | 0.23 | −2.9 ± 10.8 | −4.3 ± 23.8 | 0.77 |
| DBP | 71.5 ± 8.6 | 70.2 ± 9.6 | 0.52 | 71.9 ± 10.2 | 69.9 ± 9.8 | 0.40 | 68.8 ± 8.6 | 70.5 ± 8.6 | 0.44 | −2.8 ± 8.0 | 0.5 ± 5.7 | 0.06 |
| PP | 45.7 ± 12.1 | 44.8 ± 11.7 | 0.72 | 45.8 ± 14.0 | 45.6 ± 13.9 | 0.96 | 46.5 ± 12.4 | 42.8 ± 12.7 | 0.23 | −0.2 ± 9.6 | −1.2 ± 8.5 | 0.65 |
| HR | 67.5 ± 9.4 | 66.1 ± 10.9 | 0.53 | 67.3 ± 8.5 | 67.7 ± 9.9 | 0.86 | 66.5 ± 9.3 | 68.6 ± 11.0 | 0.40 | −0.3 ± 7.0 | 3.2 ± 10.0 | 0.09 |
| AI | 18.1 ± 19.8 | 10.7 ± 18.5 | 0.11 | 17.3 ± 24.0 | 11.9 ± 21.4 | 0.33 | 17.9 ± 17.6 | 12.5 ± 19.0 | 0.23 | −0.2 ± 12.1 | 1.8 ± 13.9 | 0.53 |

RHI reactive hyperemia index, SBP systolic blood pressure (mmHg), DBP diastolic blood pressure (mmHg), PP pulse pressure (mmHg), HR heart rate (bpm), AI augmentation index (measured via EndoPAT)

are high, rather than in the long term likely because of the fast metabolic turnover of polyphenols, with a half-life of about only 1–2 h [15]. However, only few studies, most of them conducted with polyphenol-rich cocoa, were able to demonstrate sustained improvement in endothelial function in the longer term such as in patients with coronary artery disease [16]. In line with our findings, a recent study in patients with coronary artery disease demonstrated that long-term supplementation with cranberry juice had no impact on endothelial function [17].

Moreover, our patients have several cardiovascular risk factors and were treated for hypertension, hyperlipidemia and other conditions with medications known to have a positive impact on endothelial function, which might have obscured the effect of cranberry juice on the endothelium. In addition, we measured endothelial function with a technology, which mainly assesses the microcirculation and provides distinct information compared to the flow-mediated vasodilation technique by ultrasound in the brachial artery [18]. Importantly, our results are in agreement with Dohadwala et al. [17], which have shown similar neutral results of the same cranberry juice in patients with coronary artery disease and more pronounced endothelial dysfunction at baseline.

The observed increase in reactive hyperemia index after 45 min in both groups is intriguing. In retrospect, the measurements might have been taken too close to the baseline visit, especially as a recent study reported a tendency to an increased response to reactive hyperemia shortly after a first measurement [19]. The fact that no significant difference between both groups has been seen might be due to the short period between cranberry juice ingestion and measurements. Recent studies, do, however, demonstrate that cranberry juice polyphenols (of the same cranberry juice brand) are bioavailable in humans with a peak concentration 1–3 h after consumption [15]. Both the cranberry juice and placebo beverage contain some amount of glucose and fructose, potentially obscuring a differential effect on endothelial function as postprandial hyperglycemia is associated with decreased endothelial function [20].

Bone marrow-derived EPCs that contribute to the maintenance of vascular health and several lifestyle interventions, as well as statin therapy, have been shown to improve EPC mobilization [21]. In our study, we found a rather low cell count of circulating EPCs, probably typical for our study population, which was characterized by endothelial dysfunction or multiple cardiovascular risk factors. However, after 4 months of cranberry juice intake, we did not observe significant changes in EPC numbers, similar to other studies with polyphenol-rich food [22], where others showed modest increases in EPC number [23, 24]. However, data on the specific effect of cranberry juice on EPCs were unknown so far.

Table 3 Effect of study interventions on laboratory parameters

| | Baseline placebo | Baseline cranberry | <i>p</i> | Acute placebo | Acute cranberry | <i>p</i> |
|-------------|----------------------|----------------------|----------|--------------------------------|----------------------------------|----------|
| hsCRP | 0.1 (0.1, 0.2) | 0.1 (0.0, 0.3) | 0.70 | 0.1 (0.1, 0.2) | 0.1 (0.0, 0.3) | 0.40 |
| VCAM | 544.0 (483.0, 666.0) | 534.5 (432.5, 623.0) | 0.38 | 548.0 (458.0, 654.0) | 538.0 (431.0, 583.0) | 0.35 |
| ICAM | 193.5 (156.5, 215.5) | 189.0 (159.0, 213.0) | 0.85 | 173.0 (155.0, 220.0) | 172.0 (156.0, 213.0) | 0.83 |
| IL-6 | 1.1 (0.9, 1.5) | 1.2 (0.6, 1.5) | 0.49 | 1.1 (0.9, 1.5) | 1.2 (0.6, 1.5) | 0.42 |
| TNF-alpha | 1.1 (0.7, 1.3) | 1.3 (0.6, 1.5) | 0.53 | 1.1 (0.8, 1.7) | 1.1 (0.8, 1.3) | 0.78 |
| oxLDL | 2.7 (2.0, 4.3) | 3.2 (1.8, 16.7) | 0.35 | 2.8 (2.0, 4.8) | 3.0 (1.7, 16.6) | 0.63 |
| Cholesterol | 186.0 (165.0, 210.0) | 180.5 (152.0, 213.0) | 0.61 | 191.0 (160.0, 207.0) | 180.0 (146.0, 204.0) | 0.43 |
| HDL | 51.0 (38.0, 59.0) | 45.5 (35.0, 62.0) | 0.90 | 50.0 (37.0, 56.0) | 45.0 (36.0, 62.0) | 0.91 |
| TG | 93.0 (83.0, 138.0) | 104.5 (62.0, 143.0) | 0.97 | 97.0 (82.0, 144.0) | 104.0 (66.0, 142.0) | 0.79 |
| | 4-month placebo | 4-month cranberry | <i>p</i> | Delta placebo 4-month baseline | Delta cranberry 4-month baseline | <i>p</i> |
| hsCRP | 0.1 (0.1, 0.2) | 0.1 (0.0, 0.3) | 0.77 | 5.8 (−20.2, 47.9) | 21.1 (−6.2, 81.9) | 0.21 |
| VCAM | 546.0 (502.0, 726.0) | 550.0 (469.0, 664.0) | 0.45 | 2.4 (−1.2, 7.4) | 2.5 (−2.5, 9.8) | 0.79 |
| ICAM | 191.0 (172.0, 210.0) | 199.0 (174.0, 228.0) | 0.33 | 1.0 (−8.9, 6.8) | 2.1 (−1.4, 10.8) | 0.35 |
| IL-6 | 1.2 (0.9, 1.9) | 1.4 (1.0, 1.7) | 0.72 | 0.0 (−18.2, 60.0) | 16.7 (−6.7, 83.3) | 0.29 |
| TNF-alpha | 1.0 (0.7, 1.3) | 1.1 (0.8, 1.3) | 0.39 | 0.0 (−40.5, 12.8) | −5.4 (−15.4, 13.3) | 0.55 |
| oxLDL | 2.3 (1.9, 3.4) | 3.1 (1.8, 18.2) | 0.16 | −0.1 (−0.4, 0.1) | 0 (−0.2, 0.1) | 0.21 |
| Cholesterol | 197.0 (167.0, 217.0) | 179.0 (157.0, 220.0) | 0.39 | 5.3 (0.0, 9.5) | 3.8 (−0.9, 7.2) | 0.44 |
| HDL | 52.0 (44.0, 57.0) | 48.0 (36.0, 63.0) | 0.54 | 4.7 (−5.1, 15.6) | 0.0 (−6.7, 4.2) | 0.12 |
| TG | 95.0 (76.0, 132.0) | 87.0 (68.0, 166.0) | 0.65 | −9.0 (−24.4, 14.5) | 6.5 (−12.6, 24.7) | 0.09 |

hsCRP high sensitivity C-reactive protein (mg/l), VCAM vascular cell adhesion molecule, (ng/ml) ICAM inter-cellular adhesion molecule, (ng/ml) IL-6 Interleukin-6 (pg/ml), TNF tumor necrosis factor (pg/ml), oxLDL oxidized low-density lipoprotein, HDL high-density lipoprotein (mg/dl), TG triglyceride (mg/dl)

Interestingly, chronic administration of cranberry juice significantly decreased the percentage of EPCs co-expressing osteoblastic cell markers; a subgroup of EPCs recently suggested playing an important role in the process of atherosclerosis [9, 25]. In patients with coronary atherosclerosis, as compared to participants with normal endothelial function and no structural coronary artery disease, a higher percentage of EPCs co-expressed the OCN

and served as a peripheral marker of early atherosclerosis and outperformed the commonly used systemic marker hsCRP [9]. In addition, CD34⁺ cells were able to form mineralized deposits in cell culture [9], and around 20% of human peripheral blood CD34⁺ cells do express mRNA for OCN, and when infused into immunocompromised rats, those cells localize to fracture sites and differentiate to

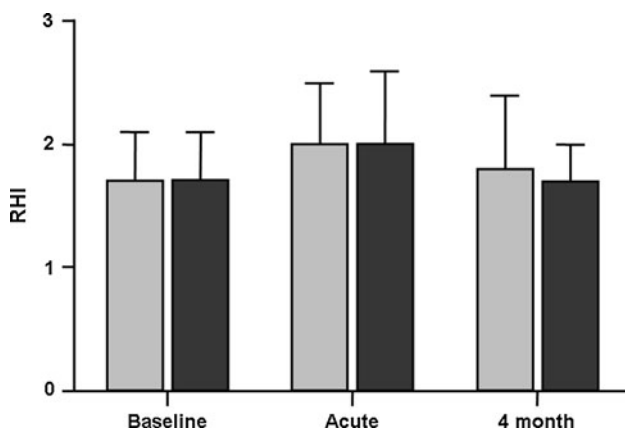


Fig. 1 EndoPAT reactive hyperemia index (RHI) at baseline, shortly after ingestion of the cranberry juice (acute) and after 4 month. Grey Placebo, Black Cranberry, *p* non-significant

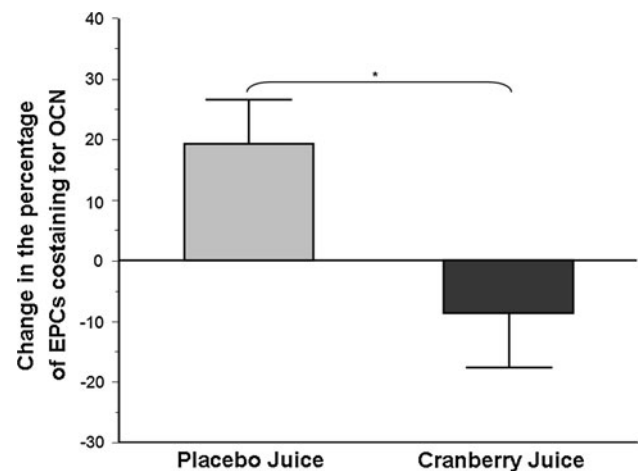


Fig. 2 Absolute changes in the percentage of intermediate EPCs, which are co-staining for OCN. **p* = 0.019. Error bars show standard error

endothelial cells and osteoblasts [14]. In a further study, we demonstrated that in patients with early coronary atherosclerosis, circulating EPCs with an osteoblastic phenotype are retained within the coronary circulation, suggesting their functional role in the vascular repair process [25]. Thus, vascular injury is likely associated with the activation of osteoblastic genes by EPCs, especially as considering the overlap between endothelial and osteoblastic lineages [26]. However, there is currently lack of studies demonstrating the ability of any intervention to change the expression of the EPCs.

In light of these studies, our findings of the high percentage of EPCs co-staining with osteocalcin are particularly interesting. The percentage of EPCs co-staining for osteocalcin in patients with peripheral endothelial dysfunction was similar to our recent study in patients with coronary endothelial dysfunction or overt cardiovascular disease [9]. In this study, we were able to show that a simple food intervention over 4 months with cranberry juice may have a different, presumably positive effect on EPC co-staining for the OCN. Theoretically, this dietary intervention could therefore influence the dynamic process of atherosclerosis and might expand knowledge about the healthy effects of polyphenol-rich fruits and vegetables in general.

Moreover, this finding might be especially interesting in the context of high bone turnover states, especially as green tea polyphenols have been shown to be protective for bone loss in middle-aged female rats [12] and are able to mitigate bone loss induced by inflammation in rats [13]. Thus, the effect of cranberry juice on potentially osteogenic EPCs in our study might highlight a possible link between the alterations in the bones and vascular calcifications.

Of interest is our finding that EPCs co-staining with OCN correlate with ICAM, an endothelial cell adhesion molecule that plays an important role in vascular inflammation and atherosclerosis by facilitating the attachment of monocytes to the endothelium, especially in atherosclerosis-prone areas [27]. Recently, cranberry juice has been linked with anti-inflammatory and antioxidative properties [28], and interestingly, cranberry juice is able to prevent urinary tract infections through the ability of the cranberry polyphenols to inhibit the adhesion of bacteria [29]. Whether similar mechanisms might also play a role in preventing mononuclear cell adhesion to the atherosclerotic wall by ICAM is unknown and highly speculative, but should be further evaluated. A recent study (although without a placebo control group) demonstrated lower sICAM levels after cranberry juice intake [30], an effect not supported by our findings.

There are some limitations in our study. Although in this study we report a differential effect of cranberry juice on the co-expression of OCN in EPCs with flow cytometry,

we do not demonstrate whether this change has functional implications in cell culture. Another limitation might be the lack of polyphenol measurements in blood plasma after cranberry juice ingestion. Recent studies, however, used the same cranberry juice brand and documented bioavailability of cranberry polyphenols in serum after ingestion [15]. Our study was well matched between the groups, except for gender with a higher proportion of women in the CJ group. However, this unlikely affected our results, because subgroup analysis revealed the same impact of CJ on endothelial function and EPCs in women as in men.

In conclusion, although endothelial function remains unaffected, our study demonstrates for the first time a potentially beneficial differential effect of CJ on osteoblastic EPCs, which are linked to the development of atherosclerotic lesions. This preliminary finding is of particular interest as it might partly explain the proposed beneficial effect of CJ and other polyphenol-rich nutrition on cardiovascular health.

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Conflict of interest AL is on the advisory board of Itamar Medical, and the other authors report no actual or potential conflicts of interest in connection with this study.

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