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Freeze-dried bilberry (*Vaccinium myrtillus*) dietary supplement improves walking distance and lipids after myocardial infarction: an open-label randomized clinical trial

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ARTICLE INFO

Article history:
Received 2 November 2018
Revised 9 November 2018
Accepted 13 November 2018

Keywords:
Anthocyanins
Bilberries
Cholesterol
Exercise test
Inflammation
Myocardial Infarction

ABSTRACT

Bilberries, *Vaccinium myrtillus*, have a high content of phenolic compounds including anthocyanins, which could provide cardiometabolic health benefits following acute myocardial infarction (AMI). We hypothesized that standard medical therapy supplemented with freeze-dried bilberry after AMI would have a more beneficial effect on cardiovascular risk markers and exercise capacity than medical therapy alone. Patients were allocated in a 1:1 ratio within 24 hours of percutaneous coronary intervention in an 8-week trial either to *V myrtillus* powder (40 g/d, equivalent to 480 g fresh bilberries) and standard medical therapy or to a control group receiving standard medical therapy alone. High-sensitivity C-reactive protein and exercise capacity measured with the 6-minute walk test were the primary biochemical and clinical end points, respectively. Fifty subjects completed the study. No statistically significant difference in high-sensitivity C-reactive protein was detected between groups. The mean 6-minute walk test distance increased significantly more in the bilberry group compared to the control group: mean difference 38 m at follow-up (95% confidence interval 14-62, $P = .003$). Ex vivo oxidized low-density lipoprotein was significantly lowered in the bilberry group compared to control, geometric mean ratio 0.80 (95% confidence interval 0.66-0.96, $P = .017$), whereas total cholesterol and low-density lipoprotein cholesterol did not differ significantly between groups. Anthocyanin-derived metabolites in blood increased significantly in the bilberry group

Abbreviations: 6MWT, 6-minute walk test; AC, anthocyanin; AMI, acute myocardial infarction; CI, confidence interval; CVD, cardiovascular disease; gmean, geometric mean; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; IQR, interquartile range; LDL, low-density lipoprotein; NSTEMI, non–ST-segment elevation myocardial infarction; PCI, percutaneous coronary intervention; STEMI, ST-segment elevation myocardial infarction.

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https://doi.org/10.1016/j.nutres.2018.11.008

during the intervention and were different after 8 weeks between the bilberry group and control. Findings in the present study suggest that bilberries may have clinically relevant beneficial effects following AMI; a larger, double-blind clinical trial is warranted to confirm this.

1. Introduction

Bilberries contain vitamin C, vitamin K, folic acid, carotenoids, and dietary fiber in varying proportions depending on species [1]. They are also rich in anthocyanins (ACs), a flavonoid subclass which is responsible for the red-blue color of bilberries, and have been the subject of research with respect to their health benefits properties [2,3]. It has been suggested that consumption of flavonoids, and in particular ACs, found in berries may reduce the risk of death from cardiovascular disease (CVD) [4-7] potentially due to beneficial effects on platelet function [8], increased high-density lipoprotein (HDL) cholesterol [6], inflammation [9], and lowering of blood pressure [8,10]. Bilberries also contain other phenolic compounds such as cinnamic and benzoic acids and flavonols which also have been associated with beneficial outcomes [11].

Plants commonly known as blueberry (*Vaccinium corymbosum, V ashei,* and *V angustifolium*) and bilberry (*V myrtillus*) are often considered the same. Despite similarities, the berries differ in taste, pulp color, and nutritional content. The dominant ACs differ, and the quantity of ACs in bilberries is more than twice that in blueberries [12]. The AC content seems to be higher in wild plants compared to cultivated [13,14]. In vitro studies and studies in healthy subjects have demonstrated cardioprotective effects of bilberry [9,15,16]. The ACs in bilberries show antioxidant properties in vitro in vascular endothelium and vascular smooth muscle even at low concentrations [17]; however, the biological relevance of direct antioxidant effects of ACs for cardiovascular health in humans is not established [18]. ACs are further reported to reduce inflammation in people with metabolic syndrome [9] and those at increased risk for CVD [15]. The antioxidative and anti-inflammatory effects of ACs from bilberries could improve recovery from exercise [19-21] and potentially affect vasoactive properties that could increase aerobic exercise performance [22,23].

Evidence that dietary factors can prevent and combat CVD is accumulating, and strong evidence supports a positive effect of Mediterranean diets [24]. Similarly, an accumulating number of studies show similar benefits of a healthy Nordic diet, where Nordic berries such as bilberries have typically been included [25-28]. Clinical research of nutritional supplement intervention following acute disease is scarce. We designed an open-label randomized clinical trial to test the efficacy of bilberry *V myrtillus* supplement initiated within 24 hours of percutaneous coronary intervention (PCI) for acute myocardial infarction (AMI). We hypothesized that standard medical therapy supplemented with freeze-dried bilberry after AMI would have a more beneficial effect on cardiovascular risk markers and the outcome of a 6-minute walk test (6MWT) than medical therapy alone.

2. Methods and materials

2.1. Study objective and design

The "Bilberry as a Dietary Supplement after Myocardial Infarction" (BEARSMART) trial was a prospective, open-label, randomized, controlled clinical trial stratified for type of infarction and sex. From June 2014 to May 2015, subjects were recruited from patients referred to Örebro University Hospital for PCI after ST-segment elevation myocardial infarction (STEMI) or non–ST-segment elevation myocardial infarction (NSTEMI). Following informed consent, individuals were allocated in a 1:1 ratio within each stratum to bilberry supplementation or to no dietary intervention, in addition to standard medical therapy. Clinical assessment was conducted, with data and blood samples collected at baseline and after 8 weeks. The trial was approved by the regional ethics review board in Uppsala, Sweden (Drn: 2013/311) and registered in a clinical trials registry (ClinicalTrials.gov identifier: NCT01958034).

2.2. Patient inclusion and exclusion criteria

STEMI was defined as chest pain suggestive of myocardial ischemia for at least 30 minutes before hospital admission, time from onset of symptoms of less than 24 hours, and an electrocardiogram with new ST-segment elevation in 2 or more contiguous leads of ≥0.2 mV in leads V2-V3 and/or ≥0.1 mV in other leads, or a probable new-onset left bundle-branch block. *NSTEMI* was defined as a combination of onset of symptoms such as central chest pain or aggravated angina pectoris, with or without electrocardiographic changes, with ST-segment depression or an inverted T wave and a rise of troponin I above the hospital-established margin of 0.070 μg/L. Five months into the study, high-sensitivity analysis of troponin I was introduced, and the established margin was changed to 0.016 μg/L for females and to 0.032 μg/L for males. Exclusion criteria were emergency coronary artery bypass grafting; inability to provide informed consent; age <18 years; daily intake, or the intent to initiate daily intake, of bilberry in some form (fresh berries, powder, soup, etc); or previous randomization in the BEARSMART trial. After providing written informed consent, patients who fulfilled inclusion criteria with no exclusion criteria were randomized according to a computer-generated random-number sequence.

2.3. Patient instructions

Subjects randomized to bilberry supplementation were instructed to use a dedicated 25-mL spoon, corresponding to ~13.3 g, to measure a dose of dehydrated bilberry to take with meals 3 times a day, for a total of 40 g of powder per day, equaling

Table 1 – Anthocyanins in *V myrtillus* powder

ACs	Mean (mg/g) (SD)
Peonidin-3-galactoside	3.0 ± 0.01
Peonidin-3-glucoside	0.00
Cyanidin-3-galactoside	3.11 ± 0.04
Cyanidin-3-glucoside	0.00
Cyanidin-3-arabinoside	1.20 ± 0.01
Delphinidin-3-galactoside	3.68 ± 0.03
Delphinidin-3-glucoside	0.16 ± 0.01
Pelargonidin-3-galactoside	0.87 ± 0.01
Pelargonidin-3-glucoside	0.01 ± 0.01
Petunidin-3-galactoside	4.7 ± 0.02
Malvidin-3-galactoside	6.26 ± 0.04

approximately 480 g of fresh berries. The powder (Immune, Skellefteå, Sweden, http://www.immun.se/) contained several ACs (Table 1) and other phenolic compounds reported before [29]. Nutritional value of the powder was as follows (per 100 g): energy 377 kcal/1600 kJ, protein 5 g, carbohydrates 82 g (sugars 30 g, fructose 19 g, glucose 11 g, sucrose <2 g), dietary fiber 25 g (insoluble 19 g, soluble 6 g), fat 4 g, and ACs 2250 mg. Subjects were instructed to take the powder uncooked, mixed with food or beverage. A three-times-per-day intake was considered appropriate given the relatively short metabolic half-life of ACs in blood [30]. The bilberry intervention was initiated within 24 hours of PCI, and the trial continued for 8 weeks. Subjects in the control group were instructed not to consume bilberry or blueberry.

2.4. *Examinations and blood tests*

An overview of examinations carried out during the study is shown in Fig. 1. All patients underwent the following examinations at baseline and at 8 weeks:

2.4.1. *Six-minute walk test*

The purpose of a 6MWT was to assess exercise capacity by measuring the distance the subject was able to walk in 6 minutes on a hard, flat surface [31]. Tests were carried out by qualified physiotherapists blinded to the type of study intervention.

2.4.2. *Transthoracic echocardiography (CX50; Philips, Bothell, WA, USA)*

At baseline, left ventricular systolic function, expressed as global ejection fraction in percent, was evaluated by echocardiography by the discharging physician. The procedure was repeated after the 8-week. intervention by an experienced echocardiography technician blinded to results of the initial examinations. The physician and the technician were blinded to the type of intervention. Two-dimensional biplane Simpson method was used for ejection fraction assessment.

2.4.3. *Heart rate and blood pressure*

Heart rate and blood pressure were measured at baseline and after the 8-week. intervention by an automatic sphygmomanometer after 15 minutes of rest.

2.4.4. *Analyses of blood samples*

Venous blood was drawn from the arm after 10 minutes of rest by an experienced nurse. Inflammatory markers, lipid profile, glycosylated hemoglobin (HbA1c), and heart function markers were analyzed at the accredited Clinical Chemical Laboratory at Örebro University Hospital, Sweden. Blood samples were centrifuged immediately and stored at –70°C until further analyses.

Analysis of phenolic compounds in berry powder and in blood samples was conducted to assess compliance on the presumption that the concentrations of selected phenolic compounds would be higher in the bilberry group. AC-derived metabolites in blood associated with bilberry consumption were analyzed by a validated method combining liquid chromatography with mass spectrometry and authentic standards as previously described [32]. Total antioxidant activity of serum was evaluated at the Department of Ecology, Physiology, and Ethology, Hubert Curien Pluridisciplinary Institut, University of Strasbourg, France, using the Radicaux Libre Kit biological test based on free radical-induced hemolysis (M Prost, Spiral/Kirial International, Strasbourg, France) [33-36]. Standard whole blood (Biomérieux, Marcy l'Etoile, France), with and without the presence of diluted serum samples, was exposed to organic free radicals produced at 37°C under air atmosphere from thermal decomposition of 2,2'azobis (2-amidinopropane) dihydrochloride. Hemolysis was recorded in a 96-well microplate reader by measuring the optical density decay (KRL Reader, Spiral/Kirial International). Results were expressed as the time required to reach 50% of maximum hemolysis (half-hemolysis time, T1/2 in minutes), an indicator of blood resistance to free radical attack. The *antioxidant activity of serum* was defined as the percent serum-mediated increase of the half-hemolysis time

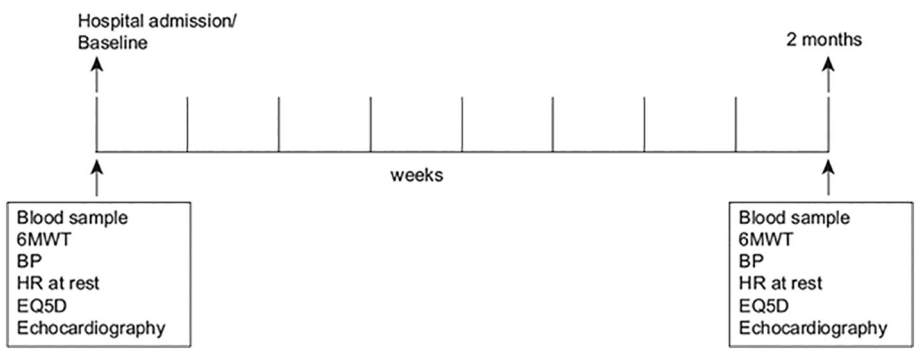

Fig. 1 – A schematic of examinations carried out during the course of the study. BP, blood pressure; HR, heart rate.

of standard blood alone. This antioxidant activity was standardized in equivalent mmol Trolox per liter of serum. Analysis of serum-oxidized low-density lipoprotein (LDL) was performed at Uppsala University Hospital, Sweden, with sandwich enzyme-linked immunosorbent assay (Mercodia, Uppsala, Sweden) according to manufacturer's instructions. In an initial incubation step, oxidized LDL reacted with antioxidized LDL antibodies bound to a microtitration well. After washing, a peroxidase-conjugated anti–human apolipoprotein B antibody recognizes the oxidized LDL bound to the solid phase. A substrate solution was subsequently added. The reaction was stopped by adding acid, and the absorbance was measured in a SpectraMax 250 (Molecular Devices, Sunnyvale, CA, USA). The total coefficient of the assay was approximately 5%. The assay was performed blinded, without knowledge of the clinical diagnosis or randomization. Analysis of high-sensitivity C-reactive protein (hs-CRP) (Advia 1800; Siemens, Munich, Germany), total cholesterol (Vitros; Ortho, Rochester, NY, USA), HDL cholesterol (Vitros; Ortho), LDL cholesterol (Vitros; Ortho), triglycerides (Vitros; Ortho), HbA1c (Tosoh, Rochester, NY, USA), brain natriuretic peptide (Architecht; Abbott, Lake Forest, IL, USA), troponin I (Architecht; Abbott, Lake Forest, IL, USA), and high-sensitivity troponin I (Architecht; Abbott, Lake Forest, IL, USA) was performed at Örebro University Hospital, Örebro, Sweden, according to manufacturers' instructions.

2.4.5. *Quality of life questionnaire*

To evaluate quality of life, subjects were asked to complete a European Quality of Life-5 Dimensions (EQ5D) questionnaire [37] at baseline and at 8 weeks.

2.5. *End points and definitions*

Our primary biochemical end point was differences between groups in level of hs-CRP, as a marker of inflammation after the 8-week. intervention. Secondary biochemical end points were lipid levels, oxidative stress markers, and heart function indicators after 8 weeks. of intervention. Our primary clinical end point was the 6MWT outcome, whereas secondary clinical end points included left ventricular systolic function, resting blood pressure, resting heart rate, and EQ5D outcome.

2.6. *Statistics*

We based sample size calculation primarily on the findings of Karlsen et al [15], a clinical study of *V myrtillus* and CVD risk where consumption of bilberry juice for 4 weeks reduced hs-CRP by 23% compared to baseline median change of –0.30 mg/L, whereas, in a placebo group, an increase of 15% (median change 0.15 mg/L) was seen ($P = .027$). Assuming comparable effects among our subjects and a standard deviation of hs-CRP of 0.5 mg/L, we needed to study 21 patients in the bilberry group and 21 patients in the control group to be able to reject the null hypothesis that the experimental and control treatments were identical, with a probability (power) of .80. The type I error probability associated with testing the null hypothesis was 0.05 (http://clincalc.com/Stats/SampleSize.aspx). To control for dropouts, we aimed to include 25 subjects in each group.

Clinical, biochemical, and compliance outcome variables were evaluated with a random intercept linear mixed model, a model that is similar to a 2-way analysis of variance for repeated measurements. Study groups, time (pre, post), and the interaction groups*time were fixed factors. The model estimated marginal mean differences of outcome between pre and post within the study groups, and changes in mean differences between the study groups, all reported with 95% confidence intervals (CIs). Log10 transformation was used for outcome variables that showed better fit to the normal distribution assumption after transformation. These variables are reported with geometric means and interquartile range (IQR) and geometric mean ratios with 95% CI. A geometric mean ratio of 1 implies no mean difference between the study groups (or pre to post), a geometric mean ratio of 1.5 implies a 50% higher mean, whereas a geometric mean ratio of 0.9 implies a 10% lower mean in bilberry compared to control group. For compliance variables with values that fell below the limit of detection, an estimated concentration of 50% of the detection limit was used. As some of the compliance outcome variables had many values below the limit of detection, these results should be interpreted with caution. A P value lower than .05 was considered statistically significant. All analyses were performed with the use of IBM SPSS Statistics 22 (Armonk, NY, USA).

3. Results

Of 60 candidate patients, 50 (42 male) of median age 68 years (IQR 61-72) completed the study (Fig. 2). Five patients declined participation, 4 withdrew consent, and 1 patient randomized to intervention was excluded after stating that he had not consumed the bilberry powder. Results were analyzed according to a modified intention-to-treat analysis that included all patients who consumed bilberry powder at least once (active arm) and all patients allocated to no intervention.

The baseline characteristics were well balanced as shown in Table 2. The mean atorvastatin dose in the bilberry group (excluding 1 patient receiving simvastatin) was 75.8 ± 14.1 mg at baseline and 76.7 ± 11.1 mg at 8 weeks. The control group received 79.2 ± 13.9 mg at baseline and 74.2 ± 21.2 mg at 8 weeks. The biochemical outcomes are presented in Table 3. The primary biochemical outcome, hs-CRP, evaluated on log scale, was not significantly different between groups after the 8-week intervention: geometric mean (gmean) ratio 1.07 (95% CI 0.52-2.18). However, the bilberry group had a significantly lower gmean at 8 weeks compared to baseline, 0.40 (95% CI 0.24-0.66), and the control group, 0.38 (95% CI 0.23-0.62). No differences were found in total cholesterol (mean difference –0.6, 95% CI –1.4 to 0.3, $P = .17$) or LDL cholesterol (gmean ratio 0.80, 95% CI 0.63-1.03, $P = .08$) between study groups, but significant reductions in both total and LDL cholesterol were found from baseline to after 8 weeks of intervention in the bilberry group (Table 3). The oxidized LDL was significantly lowered (~20%) in the bilberry compared to control group: gmean ratio 0.80, 95% CI 0.66-0.96, $P = .017$. Other biochemical outcome variables, including antioxidant activity, changed numerically in favor of bilberry administration, but differences were not statistically significant (Table 3).

In the bilberry group, the mean distance covered in the 6MWT increased significantly from 439 ± 115 m at baseline to 520 ± 136 m at 8 weeks, which was significantly greater

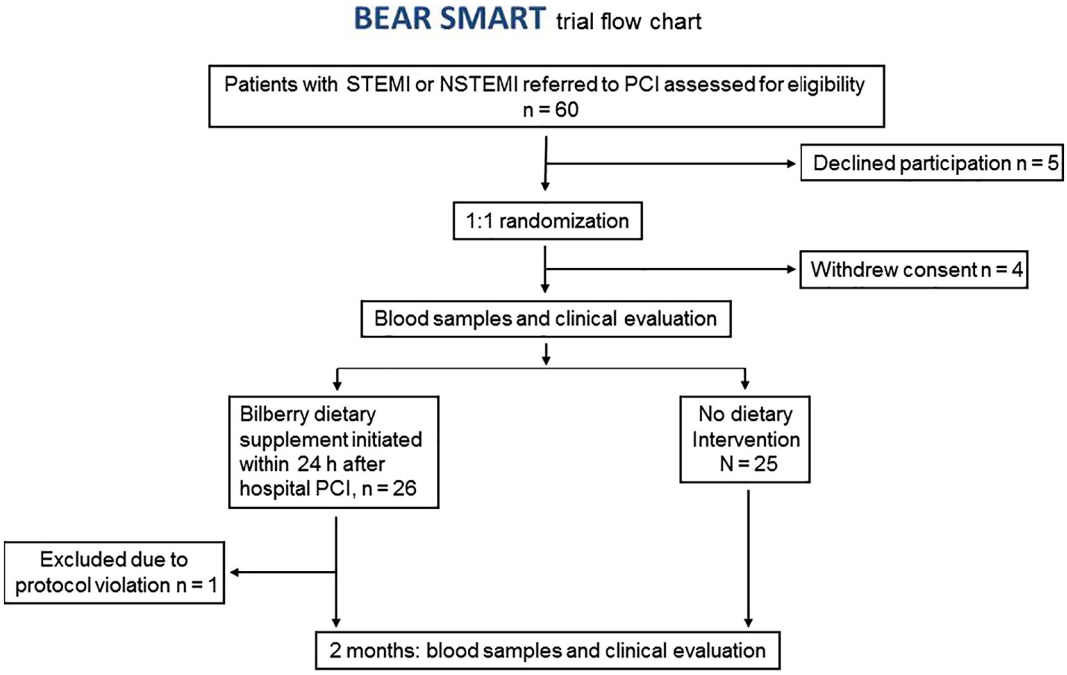

Fig. 2 – Study design.

increase than found in the control group, baseline 475 ± 103 m to 522 ± 108 m, resulting in a mean difference between groups of 38 m (95% CI 14-62, $P = .003$) (Table 4). No other clinical parameters changed significantly in bilberry compared to control group.

Measures of AC-derived metabolites showed a significantly higher increase of gallic acid, vanillic acid-4-O-sulfate, p-coumaric acid, and caffeic acid 4-β-D-glucuronide in the bilberry compared to the control group. A higher increase in measures of gallic acid, vanillic acid-4-O-sulfate, 3-hydroxyphenyl acetic acid, p-coumaric acid, caffeic acid 3-β-D-glucuronide, and caffeic acid 4-β-D-glucuronide was detected in the plasma after the 8-week intervention in the bilberry group compared to baseline (Supplemental Table S1). No adverse events were reported or observed with bilberry supplementation.

Table 2 – Baseline characteristics of subjects

		Bilberry	Control
n		25	25
Age, y	median (IQR)	66 (62-71)	68 (62-74)
Female sex	n (%)	5 (20.0)	3 (12.0)
Diabetes mellitus	n (%)	6 (24.0)	6 (24.0)
Current smoker	n (%)	4 (16.0)	5 (20.0)
Ex-smoker	n (%)	9 (36.0)	6 (24)
BMI <25	n (%)	6 (24.0)	7 (28.0)
BMI ≥25	n (%)	19 (76.0)	18 (72.0)
Previous MI	n (%)	5 (20.0)	5 (20.0)
Previous PCI	n (%)	7 (28.0)	9 (36.0)
Previous CABG	n (%)	0 (0.0)	1 (4.0)
STEMI	n (%)	14 (56.0)	14 (56.0)
NSTEMI	n (%)	11 (44.0)	11 (44.0)
β-Blocker	n (%)	24 (96,0)	22 (88.0)
Ticagrelor	n (%)	23 (92.0)	21 (84.0)
Clopidogrel	n (%)	2 (8.0)	4 (16.0)
ASA	n (%)	24 (96.0)	25 (100.0)
ACE inhibitor/ARB	n (%)	22 (88.0)	22 (88.0)
NSAID	n (%)	2 (8.0)	0 (0.0)
Statin	n (%)	25 (100)	25 (100)

ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; ASA, acetylsalicylic acid; BMI, body mass index; CABG, coronary artery bypass graft; MI, myocardial infarction; NSAID, nonsteroidal anti-inflammatory drug.

4. Discussion

Although the primary biochemical end point, hs-CRP, did not change with bilberry compared to control group, we found a clinically relevant significant effect on the secondary end point of oxidized LDL. We found a significant improvement in our primary clinical end point, 6MWT, with significantly improved walking distance after bilberry compared to control. To the best of our knowledge, an improvement in exercise capacity with berry consumption in patients with AMI has not previously been reported. A study on beetroot juice showed increased submaximal endurance in older patients with heart failure [38]. Our findings for the biochemical outcomes were in partial agreement with results of a recent meta-analysis of clinical trials of bilberry supplements in mostly healthy humans that demonstrated reduction in LDL and increase in HDL cholesterol [39]. Oxidized LDL, embedded in arteriosclerotic plaques [40], has been reported to decrease after supplementation with delphinidin [41], an AC found in bilberry [42]. Oxidized LDL is associated with all stages of atherosclerosis and to comorbidities linked with CVD, such as diabetes mellitus, hypertension, and obesity [43]. Therefore, we speculate that bilberry could have a role in supplementing medical therapy.

Table 3 – Biochemical measurements of subjects

	Bilberry					Control					Bilberry vs control	
	Baseline		8 wk		8 wk vs baseline	Baseline		8 wk		8 wk vs baseline		
	n	Gmean (IQR)	n	Gmean (IQR)	Gmean ratio [a] (95% CI)	n	Gmean (IQR)	n	Gmean (IQR)	Gmean ratio [a] (95% CI)	Gmean ratio [b] (95% CI)	P
hs-CRP, mg/L	25	3.9 (1.6-13.6)	25	1.6 (0.8-3.1)	0.40 (0.24-0.66)	25	3.5 (1.6-7.8)	25	1.3 (0.6-2.6)	0.38 (0.23-0.62)	1.07 (0.52-2.18)	.90
BNP, ng/L	24	145 (81-278)	24	59 (28-102)	0.41 (0.27-0.62)	25	173 (82-330)	25	88 (38-158)	0.51 (0.34-0.76)	0.81 (0.46-1.44)	.47
LDL cholesterol, mmol/L	24	3.2 (2.6-3.8)	24	1.7 (1.5-1.9)	0.53 (0.45-0.64)	25	2.9 (2.1-4.0)	25	1.9 (1.5-2.4)	0.67 (0.56-0.80)	0.80 (0.63-1.03)	.08
HDL cholesterol, mmol/L	24	1.1 (1.0-1.4)	24	1.1 (0.8-1.3)	0.98 (0.88-1.07)	25	1.1 (0.9-1.4)	25	1.1 (0.9-1.3)	1.00 (0.91-1.09)	0.98 (0.85-1.12)	.72
Triglycerides, mmol/L	24	1.6 (1.1-2.1)	24	1.6 (1.2-2.0)	1.00 (0.82-1.23)	25	1.4 (1.0-1.8)	25	1.6 (1.0-2.7)	1.13 (0.93-1.37)	0.89 (0.67-1.17)	.40
HbA1C, mmol/mol	25	43.0 (38.5-45.0)	25	42.9 (38.5-45.5)	1.00 (0.96-1.03)	25	40.6 (35.5-44.5)	25	41.0 (37.0-44.5)	1.01 (0.98-1.04)	0.99 (0.94-1.04)	.60
Oxidized LDL, mU/L/1000	25	44.4 (39.6-53.3)	24	29.2 (24.9-33.3)	0.65 (0.58-0.75)	22	42.5 (31.5-55.7)	25	35.3 (31.5-40.0)	0.81 (0.72-0.94)	0.80 (0.66-0.96)	.017
	n	Mean ± SD	n	Mean ± SD	Mean difference [c] (95% CI)	n	Mean ± SD	n	Mean ± SD	Mean difference [c] (95% CI)	Mean difference [d] (95% CI)	P
Total cholesterol, mmol/L	24	5.2 ± 1.1	24	3.4 ± 0.8	−1.8 (−2.3 to −1.2)	25	5.1 ± 1.6	25	3.9 ± 0.8	−1.2 (−1.8 to −0.6)	−0.6 (−1.4 to 0.3)	.17
Antioxidative activity, %	25	24.0 ± 3.6	25	26.1 ± 3.8	2.1 (1.2-2.9)	25	24.3 ± 3.5	25	25.6 ± 3.3	1.4 (0.5-2.3)	0.7 (−0.5 to 1.9)	.27
Antioxidative activity, mmol Trolox/L	25	2.8 ± 0.4	25	3.0 ± 0.4	0.24 (0.14-0.34)	22	2.8 ± 0.4	25	3.0 ± 0.4	0.17 (0.06-0.27)	0.07 (−0.06 to 0.21)	.29

BNP, brain natriuretic peptide.

[a] A gmean ratio less than 1 indicates a lower mean at 8 weeks than at baseline.

[b] A gmean ratio less than 1 indicates a greater mean decrease from baseline to 8 weeks in the bilberry group compared to control.

[c] A mean difference less than 0 indicates a lower mean at 8 weeks than at baseline.

[d] A mean difference less than 0 indicates a greater mean decrease from baseline to 8 weeks in the bilberry group compared to control.

Table 4 – Clinical outcome variables of subjects

	Bilberry					Control					Bilberry vs control	
	Baseline		8 wk		8 wk vs baseline	Baseline		8 wk		8 wk vs baseline		
	n	Mean ± SD	n	Mean ± SD	Mean difference (95% CI)	n	Mean ± SD	n	Mean ± SD	Mean difference (95% CI)	Mean difference (95% CI)	*P*
6MWT, m	25	439 ± 115	24	520 ± 136	82 (64-98)	25	475 ± 103	24	522 ± 108	44 (27-61)	38 (14-62)	.003
LVEF, %	24	53 ± 10	25	55 ± 10	2.7 (–1.7 to 7.1)	25	54 ± 8	25	53 ± 7	–1.2 (–5.6 to 3.2)	3.9 (–2.3 to 10.1)	.22
Heart rate, beat/min	25	70 ± 13	25	66 ± 8	–3.7 (–8.3 to 0.9)	25	66 ± 13	25	64 ± 10	–1.8 (–6.4 to 2.8)	–2.0 (–8.5 to 4.5)	.55
Systolic BP, mm Hg	25	134 ± 20	25	131 ± 17	–2.7 (–12.4 to 7.1)	25	131 ± 24	25	134 ± 25	3.1 (–6.6 to 12.9)	–5.8 (–19.6 to 8.0)	.40
Diastolic BP, mm Hg	25	77 ± 9	25	72 ± 8	–4.0 (–9.1 to 1.0)	25	76 ± 12	25	75 ± 11	–1.2 (–6.3 to 3.8)	–2.8 (–9.9 to 4.4)	.43
Weight, kg	25	89.2 ± 17.0	23	89.4 ± 16.9	0.8 (–0.2 to 1.8)	25	82.8 ± 9.3	23	83.4 ± 9.6	0.5 (–0.5 to 1.4)	0.3 (–1.1 to 1.7)	.63
BMI, kg/m^2	25	28.2 ± 8.1	23	28.1 ± 8.1	0.2 (–0.1 to 0.6)	25	27.1 ± 2.8	23	27.2 ± 2.8	0.1 (–0.2 to 0.5)	0.1 (–0.3 to 0.5)	.63
EQ5D index[a]	24	0.80 ± 0.16	24	0.82 ± 0.18	0.01 (–0.05 to 0.08)	24	0.80 ± 0.14	23	0.84 ± 0.16	0.04 (–0.03 to 0.05)	–0.03 (–0.12 to 0.06)	.56

BP, blood pressure; LVEF, left ventricular ejection fraction.

[a] The EQ5D index ranges from 0 to 1, with a higher score indicating higher quality of life.

4.1. *Clinical implications*

All patients in this study were treated with statins (3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors). The bilberry intervention was associated with a statistically significant reduction in oxidized LDL cholesterol, but total cholesterol and LDL cholesterol showed nonsignificant difference from standard therapy. Antioxidant capacity did not differ between groups. However, the lipid-lowering effect of bilberry could be partly explained by the high quantity of ACs [12]. We found significant correlations between LDL cholesterol and oxidized LDL with levels of ACs after the 8-week intervention, indicating a possible dose-response relationship with cholesterol levels and AC in bilberries (see online supplement Table S1). High quantity of AC has been reported to affect mitochondria [44] and activate AMP-activated protein kinase, which leads to phosphorylation of acetyl-CoA carboxylase and thereby an increased fatty acid oxidation [45]. In addition to ACs, other flavanol compounds in bilberries having pleiotropic effects might contribute to cholesterol lowering [46,47]. Hs-CRP did not differ between groups at 8 weeks, although some dietary flavonoids have been associated with a lower concentration of inflammatory biomarkers [48]. Our patient population showed a high inflammatory burden at baseline, and the natural course of the disease, aided by statin treatment, may have reduced the hs-CRP to a level beyond which bilberry had no effect. ACs may increase exercise tolerance and could partly explain improvement in 6MWT in the intervention group [49,50].

4.2. *Strengths and limitations*

In contrast to most previous studies using healthy individuals, we initiated intervention with bilberry in a well-defined patient group following AMI. Bilberry administration was initiated within 24 hours of PCI at a vulnerable and critical clinical phase [51,52]. This is an important study strength. The study was originally designed as a randomized, double-blind, placebo-controlled trial. However, at enrolment of 8 subjects, 3 complained of gastrointestinal distress. The placebo powder (Gold Coast Ingredients, Inc, Los Angeles, CA, USA; blueberry flavoring EU0548) was identified as the source of the problem, and the study was terminated and decoded. The study was redesigned as open label with a nondietary intervention arm and no placebo. After ethics approval, the study was reinitiated with new subjects. No results from the initial double-blind trial were included in this report. We acknowledge that an open-label design is methodologically weaker, a factor for which we attempted to control by including biochemical assessment of compliance. Our study was small, and significant findings were in secondary outcome variables. Because patients were aware of their treatment allocation, our primary clinical outcome, 6MWT, was prone to ascertainment bias; thus, the results of this test should be interpreted with caution.

4.3. *Conclusions*

We found no effect of an 8-week treatment of bilberry powder as a supplement to standard therapy post AMI on the primary biochemical end point, hs-CRP, but we found a significant improvement in 6MWT. Moreover, we found that blood lipid profiles were altered within the bilberry group of a magnitude that could potentially translate into a relevant reduction in future CVD events in a better powered study. Larger placebo-controlled studies addressing hard clinical end points are needed to determine whether bilberry, *V myrtillus*, derivatives have practical use in the treatment of CVD.

Acknowledgment

We would like to thank project assistant Lotta Mazouch and research nurses Johan Josefsson, Annika Eriksson, and Ingela Östman for their excellent help in all aspects of the study. Also, a great thank you to Kerstin Angergård and Marie Hellmark, the physiotherapists responsible for the 6MWT. A special thanks to Prof Anders Larsson, Department of Medical Sciences, Uppsala University, for organizing analysis of oxidized LDL cholesterol. We would also like to thank the *V myrtillus* provider, Immun Skellefteå, Sweden, for collaboration. The study was supported by a grant from the Örebro University Hospital Research Foundation. The authors have no conflict of interest to declare.

Appendix A. Supplemental materials

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nutres.2018.11.008.

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