DRINKING OF CHOKEBERRY JUICE FROM THE ECOLOGICAL FARM DZIĘCIOŁOWO AND DISTENSIBILITY OF BRACHIAL ARTERY IN MEN WITH MILD HYPERCHOLESTEROLEMIA

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Abstract: The aim of the study was to estimate the influence of drinking chokeberry juice on the endothelial function in men with mild hypercholesterolemia. It was shown that chronic treatment with flavonoids improves vascular function and reduces cardiovascular remodelling by increasing NO release from endothelial cells. A group of 35 men diagnosed with mild hypercholesterolemia (mean age: 53.9 ± 5.8 years), with no earlier pharmacological treatment, were enrolled to the study. In all men, assessment of endothelial function, and serum lipids level were carried out at four time points: at the beginning of the studies, after 6 weeks of regular drinking of chokeberry juice, after 6 weeks without drinking the juice, then after repeated 6 weeks of drinking chokeberry juice. During the study, significant decreases in serum total cholesterol, LDL cholesterol and triglycerides levels were observed. A statistically significant increase in serum nitric oxide (NO) concentration and in the flow mediated dilatation (FMD) were observed. At the beginning, FMD ≥ 7% was present in 13 of 35 subjects (37.1%). After 6 weeks of regular chokeberry juice drinking, FMD ≥ 7% was present in 29 of 35 subjects (82.9%). However, after 6 weeks abstaining from drinking the juice and repeated exposure to 6 weeks drinking of chokeberry juice, FMD ≥ 7% was present in all studied subjects. Regular drinking of chokeberry juice has a beneficial effect on endothelial function and lipid metabolism in men with mild hypercholesterolemia.

INTRODUCTION

Nutritional biologically active substances influence the metabolism of dietary protein, carbohydrates and lipids, modifying organ and tissue function. These effects can occur in the cardiovascular system as important protective mechanisms. An anti-sclerotic effect of polyphenols, especially of the anthocyanins contained in them, results from their hypolipemic [2, 8, 9], hypoglycemic [8, 10], antioxidant [3, 4, 6, 7, 11], and anti-inflammatory [5] properties. Apart from their metabolic activity, anthocyanin-mediated functional changes were observed in the vascular wall. Decreases in systolic and diastolic blood pressures were shown in middle-aged men [8], hypertensive patients

[6], and in patients diagnosed with metabolic syndrome [2]. It was shown that chronic flavonoid treatment improves vascular function and reduces cardiovascular remodeling by an increased NO release from endothelial cells [1]. In this way, flavonoids can reduce cardiovascular risk, thus providing compounds valuable in cardiovascular prevention.

Multiple polyphenols and anthocyanins in particular are contained in the fruits of *Aronia melanocarpa*, a common plant in Europe and North America. The strongly coloured and pungently-flavored juice is widely used for juice and wine production. Due to its acrid taste, it effectively inhibits thirst, but it can also irritate the mucosa of the gastrointestinal tract. Recently, numerous observations indicate that *Aronia melanocarpa* fruit juice seems to be a suitable natural product for an anti-atherosclerotic diet.

The first stage of atherosclerosis involves dysfunction of endothelium. The normally functioning endothelium retains blood fluid within the vascular bed and controls vascular tone. One of the methods used to evaluate endothelial function is testing of brachial artery dilatation response to passive hyperemia (FMD, flow mediated dilatation). This non-invasive method is easily reproduced and applied. Hyperemia may increase flow by as much as 20%. The decreased FMD values are associated with the classic atherosclerosis risk factors, such as hyperlipidemia, hypertension, diabetes mellitus, smoking, high body mass index, postmenopausal period, and older age. The FMD level is influenced by many factors, such as baseline artery diameter (smaller arteries feature a relatively higher capacity to dilate), site and duration of occlusive compression of the brachial artery, and type of meals consumed (lipid-rich meals impair FMD more than carbohydrate-rich meals).

However, no reference FMD values have been defined, and mean FMD differs widely between studies, overlapping between populations. In some studies, a value of 5%, 7%, 8% or even 10% is considered normal. Additionally, it must be remembered that FMD may fluctuate by as much as 20–25% if measured on consecutive days.

The test of artery dilation response represents an indirect index of the capacity of endothelial cells to release nitric oxide. Nitric oxide, synthesized from L-arginine, is one of the main compounds released by the endothelium. This substance is a vasodilator and inhibitor of platelet aggregation and has an important protective role in preventing vasospasm and thrombus formation.

The aim of this study was to estimate the influence of chokeberry juice consumption on the endothelial function by assessment of brachial artery relaxation, and determination of serum nitric oxide level in men with mild hypercholesterolemia.

MATERIALS AND METHODS

A group of 35 men diagnosed with the mild hypercholesterolemia (the total serum cholesterol was slightly higher than 200 mg/dl), with no earlier pharmacological treatment, were enrolled to the study. Mean age of the studied subjects was 53.9 ± 5.8 years, mean height 177.2 ± 6.8 cm, mean weight 86.2 ± 10.8 kg, and mean body mass index (BMI) 27.4 ± 2.9 kg/m^2. Mean total blood cholesterol concentration in the study group was 252.1 ± 59.3 mg/dl, mean concentration of LDL cholesterol was 160.9 ± 50.8 mg/dl, mean concentration of HDL cholesterol was 49.5 ± 11.8 mg/dl, and mean concentration of triglycerides amounted to 207.9 ± 123.6 mg/dl. All men were occupationally active, in general led a healthy lifestyle with regular physical efforts, they did not smoke cigarettes and did not abuse alcohol. All of the patients had optimum or high normal values of blood pressure.

In all the men, assessment of functional and biochemical endothelial function, and serum lipids level were carried out at four time points: at the beginning (I), after 6 weeks of regular chokeberry juice drinking (II), after 6 weeks abstaining from drinking of the juice (III), then after 6 weeks of repeated exposure to chokeberry juice drinking (IV). During the study, the participants did not changed neither their life style nor diet. They did not declare consumption of any other products rich in vitamins A and E in their diet.

Written informed consent was obtained from all volunteers taking part in the study. The study was approved by the Local Ethics Committee, No. KB – 1016/2003.

The 100% chokeberry juice obtained naturally from the Dzięciołowo ecological farm (A. M. Lech, Poland) was drunk in the amount of 250 ml per 24 hours. The content of phenolic compounds in the juice (mg/100ml) was as follows [6]: neochlorogenic acid 49.21; chlorogenic acid 45.50; (-)epicatechin 1.48; p-coumaric acid derivative 0.40; polymeric procyanidins 293.38; quercetin 3-rutinoside 1.68; quercetin 3-galactoside 2.83; quercetin 3-glucoside 2.25; quercetin 3-vicianoside 1.15; quercetin 3-robinobioside 1.17; eriodietyol 3-7-glucouronide 7.86; cyanidin 3-galactoside 12.49; cyanidin 3-glucoside 0.71; cyanidin 3-arabinoside 5.12; cyanidin 3-xyloside 0.59; cyanidin 0.22; total 426.04.

Laboratory tests on fasting venous blood were carried out four times. Blood lipid levels were assessed with the use of routine methods. Concentrations of total cholesterol, low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were measured using enzymatic assay (Spinreact). Nitric oxide concentration (NO) was measured by colorimetric nitrite/nitrate method (NO_2^-/NO_3^-) using kits of R&D Systems (Minneapolis, USA). Because NO released from cells rapidly auto-oxidizes to yield nitrite (NO_2^-), which interacts with hemoglobin turning to nitrate (NO_3^-), it was measured indirectly by determination of nitrite/nitrate.

The assessment of brachial artery relaxation was performed in standard conditions: the same time of day (between 08:00–09:00 am), after an overnight fast in a room with constant air humidity at 23°C with the subjects in the study in a supine position after a 10-minut rest. A blood

pressure cuff was placed around the arm. Brachial artery diameter was visualized 5–10 cm above the elbow on B-mode imaging using an ALOKA device equipped with a 7.5 MHz linear transducer. Baseline flow hemodynamic parameters and maximal arterial lumen diameter in diastole (B1) were measured. The blood pressure cuff was inflated to 50 mmHg above systolic pressure for 5 minutes. The image of the artery was recorded continuously until 5 minutes after the cuff deflation. The cuff was then released, and arterial lumen diameter was measured two minutes after deflating the cuff, during diastole (B2). Change in brachial artery diameter (BAD), and flow mediated dilatation (FMD) were calculated according to the formulas: change in BAD: B2–B1 (mm), and FMD: $[(B2-B1)/B1] \times 100\%$.

Statistical analysis was performed with the use of Statistica PL 6.0 programme (Stat Soft, Poland). Means (x) and standard deviations (SD) were given. Distribution of the variables was checked with W-Shapiro-Wilk test. ANOVA Friedman test was then used because the variables manifested a non-parametric distribution. Statistically significant differences between the means were estimated using a *post-hoc* test. Values of $p < 0.05$ were accepted as indicating statistical significance.

RESULTS

During the study, significant decreases in the levels of serum total cholesterol (I *vs.* III: $p<0.05$; I *vs.* IV: $p<0.01$), LDL cholesterol (I *vs.* IV: $p<0.05$), and triglyceride (I *vs.* II: $p<0.05$; I *vs.* III: $p<0.01$; I *vs.* IV: $p<0.01$) were observed. HDL cholesterol concentration was similar in all measurements (Tab. 1).

A statistically significant increase in serum nitric oxide concentration was noted during the study (I *vs.* IV: $p<0.01$; II *vs.* IV: $p<0.05$) (Tab. 1).

Mean value of brachial artery diameter, brachial artery diameter after occlusion, and change in brachial artery

Table 2. Baseline brachial artery diameter (B1), brachial artery diameter after occlusion (B2), change in brachial artery diameter (B2-B1), and flow mediated dilatation (FMD) in the study group (n = 35 men) at the beginning of study (I), after 6 weeks of regular chokeberry juice drinking (II), and then after 6 weeks abstaining from juice drinking (III), and a repeated 6 weeks of chokeberry juice drinking (IV).

	Baseline BAD (B1) [mg/dl]	BAD after occlusion (B2) [mg/dl]	Change in BAD (B2–B1) [mm]	FMD [%]
I	3.81 ± 0.62	4.06 ± 0.65	0.25 ± 0.06	6.54 ± 1.30
II	3.92 ± 0.55	4.29 ± 0.62	0.37 ± 0.13	9.56 ± 3.18
III	3.90 ± 0.51	4.33 ± 0.55	0.42 ± 0.06	10.83 ± 1.35
IV	3.89 ± 0.58	4.32 ± 0.63	0.43 ± 0.07	11.04 ± 1.44
p	ns	ns	I *vs.* II: p<0.001	I *vs.* II: p<0.001
			I *vs.* III: p<0.001	I *vs.* III: p<0.001
			I *vs.* IV: p<0.001	I *vs.* IV: p<0.001
			II *vs.* III: p<0.001	II *vs.* III: p<0.05
			II *vs.* IV: p<0.001	II *vs.* IV: p<0.01

BAD = brachial artery diameter

diameter are shown in Table 2. During the study, a significant increase in FMD (I *vs.* II: $p<0.001$; I *vs.* III: $p<0.001$; I *vs.* IV: $p<0.001$; II *vs.* III: $p<0.05$; II *vs.* IV: $p<0.01$) was observed (Tab. 2).

According to an earlier published study, we accepted the FMD value $\geq 7\%$ to represent the standard. At the beginning, FMD $\geq 7\%$ (with the mean value of 6.54%) was present in 13 of 35 subjects (37.1%). After 6 weeks of regular chokeberry juice drinking, FMD $\geq 7\%$ (with mean value of 9.56%) was present in 29 of 35 subjects (82.9%). However, after 6 weeks abstaining from juice drinking, and then repeated exposure to 6 weeks of chokeberry juice drinking, FMD $\geq 7\%$ (mean value 10.83% and 11.04%, respectively) was present in all study subjects.

Table 1. Serum lipids and nitric oxide (NO) concentration in the study group (n = 35 men) at the beginning of study (I), after 6 weeks of regular chokeberry juice drinking (II), then after 6 weeks abstaining from drinking the juice (III), and after 6 weeks of a repeated exposure to chokeberry juice drinking (IV).

	Total cholesterol [mg/dl]	LDL cholesterol [mg/dl]	HDL cholesterol [mg/dl]	Triglycerides [mg/dl]	NO [ng/dl]
I	252.1 ± 59.3	160.9 ± 50.8	49.5 ± 11.8	207.9 ± 123.6	2.26 ± 1.04
II	240.7 ± 35.3	163.8 ± 69.4	47.8 ± 9.9	155.7 ± 42.4	2.42 ± 1.54
III	227.1 ± 54.9	142.9 ± 54.0	49.8 ± 11.7	144.6 ± 57.2	2.68 ± 1.78
V	221.9 ± 39.0	143.3 ± 69.9	50.7 ± 12.3	138.7 ± 47.2	3.47 ± 2.46
p	I *vs.* III: p<0.05	I *vs.* IV: p<0.05	ns	I *vs.* II: p<0.05	I *vs.* IV: p<0.01
	I *vs.* IV: p<0.01			I *vs.* III: p<0.01	II *vs.* IV: p<0.05
				I *vs.* IV: p<0.01	

DISCUSSION

The men participating in this study had at least three cardiovascular risk factors. The mild hypercholesterolemia was one of the most frequent abnormalities. The association of male sex and overweight, significantly increases the probability of stroke, infarct or death. The prophylaxis of such vascular disasters poses the problem of selecting appropriate pharmacological or non-pharmacological treatment. In practice, recommendations concerning the appropriate diet are often not observed by the patients. On the other hand, there is a significant lack of easily-accessible and inexpensive food products which are effective in the reduction of mild hyperlipemia.

In this study, the men with mild hypercholesterolemia were not treated with pharmacotherapy and kept their formerly practiced diet. In these conditions, drinking chokeberry juice for 6 weeks, and then after a 6-week pause, drinking the juice again for 6 weeks, significantly decreased serum lipid concentrations. A significant decline in the serum total- and LDL cholesterol levels, as well as triglycerides, took place after the second round of treatment. In comparison to basal values, these lipids concentrations remained lower after a 6 week break in drinking the juice. The involvement of LDL cholesterol and of triglycerides in the development and progression of atherosclerosis is one of the best proven pathogenetic mechanisms in modern medicine. A positive correlation between LDL cholesterol or triglycerides and the risk of cardiovascular events was observed in many large-scale population studies. The advantages of reducing these lipids levels have been proved in many interventional studies. The hypolipemic effect of chokeberry juice was gradual and relatively long-lasting.

Simultaneously, chokeberry juice drinking progressively increased the serum nitric oxide level and FMD value during the study. Both of them indicate an improvement in endothelial function, induced by chokeberry juice. A similar effect of chokeberry juice on the cardiovascular system has been shown in other studies. Broncel *et al.* indicated a significant decrease in blood pressure, serum total cholesterol, LDL cholesterol, triglycerides level, and plasma endothelin-1 concentration after two months therapy with anthocyanins from *Aronia melanocarpa* [2]. In rats with hyperlipemia, Valcheva-Kuzmanova *et al.* observed an anti-hyperlipemic effect, and no adverse effects within the wall of the aorta during drinking of chokeberry juice [9].

Endothelial dysfunction observed in hypercholesterolemic subjects is an early marker of arteriosclerotic lesions. It develops due to a reduced bioavailability of nitric oxide and/or enhanced effect of oxidatively modified LDL in the vascular wall. However, in our study, no linear correlations between NO, LDL and FMD values could be detected.

The common mechanism of the advantageous metabolic and functional changes may result from the strong antioxidant effect of flavonoids, contained in chokeberry juice. It is known that *Aronia melanocarpa* fruit juice is rich in anthocyanins, having a high oxygen radical absorbing capacity [4, 7]. This property can explain the anti-inflammatory potential and anti-thrombotic activity, as well as the improvement of endothelial function under the effect of the tested juice.

CONCLUSIONS

Regular drinking of chokeberry juice has a beneficial effect on endothelial function and lipid metabolism in men with mild hypercholesterolemia. This study showed a significant role of drinking *Aronia melanocarpa* fruit juice in the induction of reduced cardiovascular risk, as well as in improvement of vasodilatory potential.

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