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Abstract: The purpose of this work was to investigate whether two-months supplementation with 100% sea buckthorn juice (SBJ) would modify blood lipids, LDL subfractions and other markers of cardiovascular risk in hypercholesterolemic women. A group of 28 hypercholesterolemic non-medicated adult women with a mean age of 50.58 ± 5.76 years consumed 50 mL of 100% SBJ daily for two-months. We observed a significant reduction of body weight (BW; $p < 0.05$), body mass index (BMI; $p < 0.05$), body fat mass (BFM; $p < 0.001$) and visceral fat area (VFA; $p < 0.001$), on the contrary, a significant increase of skeletal muscle mass (SMM; $p < 0.05$) and fat-free mass (FFM; $p < 0.05$). Supplementation with 100% SBJ significantly increased the level of high-density lipoprotein (HDL; $p < 0.05$), reduced the level of low-density lipoprotein (LDL; $p < 0.05$), atherogenic LDL subfractions (LDL_{3-7}; $p < 0.05$) and improved LDL/HDL ratio ($p < 0.001$). After the SBJ consumption, significant reduction of C-reactive protein (CRP; $p < 0.001$), orosomucoid (ORM; $p < 0.001$) and interleukin 6 (IL-6; $p < 0.05$) were found. In conclusion, consumption of SBJ showed an indication of beneficial effects on cardiovascular risk factors in hypercholesterolemic women.

Keywords: berries; sea buckthorn juice; lipid profile; cholesterol; inflammation markers; intervention; anthropometric characteristics

1. Introduction

Cardiovascular diseases (CVD) associated with metabolic disorders, including obesity and diabetes, are the leading cause of death in Europe and worldwide [1–4]. Abnormal lipid profile, such as elevated low-density lipoprotein (LDL) and lower high-density lipoprotein (HDL), are the key indicators of cardiovascular risk [5,6]. Plasma lipoproteins are a heterogeneous population of particles varying in dimension and density and serve for better prognosis of CVD than conventional lipid profiles [7–9]. Low density lipoproteins are composed of large (LDL_1), middle (LDL_2), and small dense particles (sdLDL or LDL_{3-7}) [10,11]. Recent studies suggest that a high density of LDL_{3-7} poses a greater risk for CVD [7,12] because of their greater predisposition to oxidation and higher affinity for the arterial wall than large-buoyant ones [13]. Monitoring and improvement of sdLDL levels could be beneficial for reducing CVD risk [7,14]. Lifestyle and dietary influence the risk of lipid and carbohydrate metabolism disease, changing glucose and cholesterol levels, blood pressure, and body composition [15–18]. Recently, the plant foods including the berries have gained growing interest due to presence of different kinds of nutrients and bioactive compounds with beneficial effects [19–22]. Sea buckthorn (SB) berries, an ancient plant, is a deciduous bush or tree - genus *Hippophae*, family *Elaeagnaceae* [23] with important ecological and economic value [24]. Around 150 species, subspecies,

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and varieties of sea buckthorn have been classified within Eurasia [25], among which *Hippophaë rhamnoides* is the most significant and widespread in Europe [26]. The petite, red and yellow berries from sea buckthorn are an abundant reservoir of many bioactive substances with medicinal and nutritional properties [26–29]. The most important compounds are phytosterols, ascorbic acid, carotenoids, tocopherols and phenolic compounds [30], vitamins, proteins, amino acids, minerals [31], alkaloids, chlorophyll derivatives, amines [32], organic acids [33] and fatty acids [33,34]. Among them, ascorbic acid, tocopherols, carotenoids flavonoids and proanthocyanidins exhibit antioxidant activity [24,27,35,36]. One of the highly regarded features of sea buckthorn is the high content of vitamin C, which is considerably greater than in other favourite fruits [37,38] and lycopene, most active among the carotenoids [39]. The compounds obtained from sea buckthorn possess various beneficial effects such as antioxidative [26], anti-inflammatory [40,41], cardioprotective [42,43] and anticarcinogenic properties [44]. These properties are linked to the weight management, enhancement of lipid and glucose levels, pancreatic revitalisation and lowering of blood pressure [45–47].

The most consumed part of sea buckthorn is the berries [48], from which juice and oil from seeds is most often obtained [25,49,50]. Sea buckthorn juice (SBJ) is a popular drink, rich in proteins, vitamins, organic acids [49,50], which can effectively promote fruit consumption [51–53] and is a serious chance for many to increase the intake of vitamins and other bioactive substances [37,54]. Several studies show that juice, as part of a rational diet, reduces the risk of numerous diseases, such as oncological, neurodegenerative and cardiovascular [52,53,55]. The consumption of SBJ has not yet been systematically investigated, most studies have looked at the effects of sea buckthorn oil or its extracts on the physiological determinants of cardiovascular risk. To date, there is also no study investigating the effect of sea buckthorn bioactive compounds on the distribution of LDL subfractions. Therefore, purpose of this work was to investigate whether two-months supplementation with 100% SBJ would modify blood lipids, LDL subfractions and other markers of cardiovascular risk in hypercholesterolemic women.

2. Materials and Methods

The study was performed at the Institute of Nutrition and Genomics, FAFR, SUA in Nitra from February 2022 until April 2022. The study was approved by the Ethics Committee at the Specialized Hospital St. Zoerardus Zobor, Nitra, Slovak Republic (protocol number 3/101921/2021).

2.1. Study Design

This study was a pre- and post-intervention study involving a total of 51 subjects recruited through health care centers and by advertisements who were screened for eligibility to participate in the study. Of the total registered volunteers, 23 individuals who did not meet the inclusion criteria were excluded (Figure 1).

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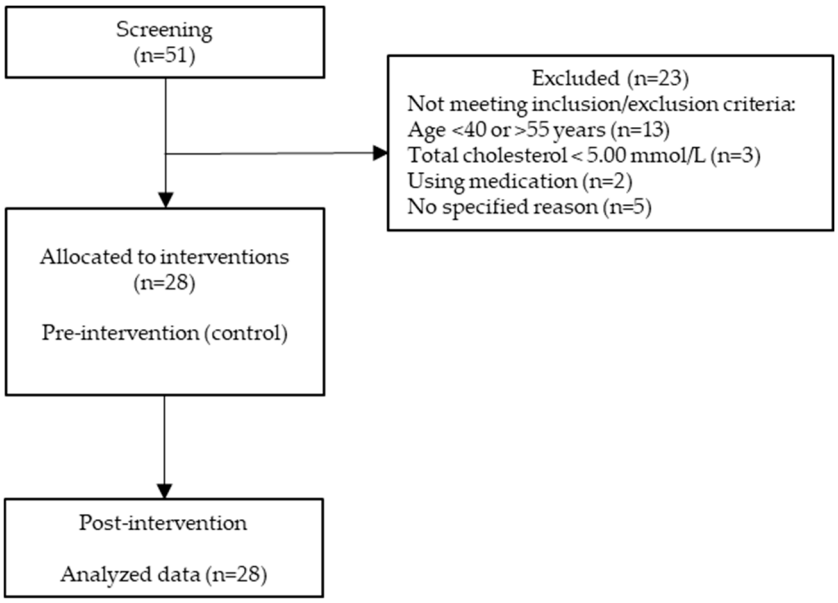

Figure 1. Flow chart showing participants selection and their inclusion in the study.

Twenty-eight non-medicated hypercholesterolemic women with a mean age 50.58 ± 5.76 years were enrolled in the clinical study. Inclusion criteria of volunteers were: willingness to participate in 8-week interventional program, women an age 40–55 years, serum total cholesterol concentration ≥ 5.00 mmol/L; constant body weight (± 3 kg) over the past 3 months, and intake of alcohol ≤ 30 g/day, diet excluding other sources of antioxidants and supplements. Exclusion criteria for participants were: history of cardiovascular disease, use of lipid-regulating medications, liver, kidney and thyroid dysfunction, diabetes, cancer, allergy, regular smoking, alcohol abuse, chronic inflammatory disease, or participation in other intervention trial.

Participants received verbal and written information about the study, and were informed about all risks, benefits and possible discomforts. After providing informed written consent, volunteers underwent standard medical follow-up, including a questionnaire, blood pressure measurement, and standard clinical biochemical blood tests.

2.2. Dietary Intervention

Volunteers were instructed to consume 50 mL of 100% commercial SBJ according to the manufacturer's recommendations daily for 8-weeks period, as a part of their regular diet. The juice was donated by the company ZAMIO Ltd., Trhovište, Slovak Republic. Juice is made by cold pressing from the organic fruits of sea buckthorn (*Hippophaë rhamnoides*), without added sugar or other sweeteners, without dyes and aromas. The product is stabilized by pasteurization, it does not contain chemical preservatives. Study beverages were provided in 700 mL glass bottles and the total amount of juice was from the same batch. Participants were instructed to store bottles in the refrigerator after opening them.

The content of nutrients and some important bioactive substances, as well as the juice antioxidant activity were quantified. The total phenolic content (TPC) was determined according to the Folin-Ciocalteu method using spectropfotometer Shimadzu UV/VIS -1800 [56]. Phenolic compounds were determined by HPLC Agilent 1260 Infinity II (Agilent Technologies GmbH, Waldbronn, Germany) by slightly modified method according to Gabriele et al. [57]. The antioxidant activity of juice was determined using DPPH radical [58]. Vitamin C content was quantified by HPLC system Waters Separations Module 2695 with UV detector 2996. Determination of the content of total carotenoids was carried out using a modified methodology according to Hegedűsová et al. [59] on a JENWAY spectrophotometer (6405 UV/VIS, England). The content of fatty acids in fat (%) was determined using Agilent 6890 A GC (Agilent Technologies, Wilmington, DE, USA). Composition of sea buckthorn juice used in this experiment is presented in Table 1.

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Table 1. Composition of sea buckthorn juice.

Parameter	Units	Quantity	Parameter	Units	Quantity
TPC	mg GAE/g	1.56 ± 0.03	Palmitic acid	(%)	35.91 ± 0.62
Rutin	mg/L	18.26 ± 0.21	Palmitoleic acid	(%)	29.77 ± 0.65
Benzoic acid	mg/L	142.47 ± 1.12	Stearic acid	(%)	0.93 ± 0.08
Caffeic acid	mg/L	7.13 ± 0.34	Oleic acid	(%)	22.01 ± 1.35
Coumaric acid	mg/L	6.23 ± 0.03	Linoleic acid	(%)	2.87 ± 0.01
Ferulic acid	mg/L	18.14 ± 0.21	α-linolenic acid	(%)	0.82 ± 0.01
Myricetin	mg/L	12.28 ± 0.38	Arachidic acid	(%)	0.21 ± 0.01
Resveratrol	mg/L	2.48 ± 0.08	SFA	(%)	3.69 ± 0.01
Neochlorogenic acid	mg/L	1.03 ± 0.06	MUFA	(%)	51.91 ± 0.69
Cryptochlorogenic acid	mg/L	5.53 ± 0.15	PUFA	(%)	37.23 ± 0.54
Vitamin C	mg/100g	385.41 ± 0.38			
Total carotenoids	mg/100g	64.79 ± 5.27			
AA (inhibition of DPPH)	%	42.5 ± 0.43			

Abbreviations: TPC: total phenolic content; GAE: gallic acid equivalents; AA: antioxidant activity; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Participants were instructed to maintain their usual eating habits and lifestyle during the study, including physical activity, but to refrain from consuming dietary supplements (vitamins, minerals, antioxidants and flavonoids). Daily nutrient and energy intakes of the volunteers were calculated from 3-day food records using Mountberry nutritional and fitness software (Wellberry, Ltd., Nitra, Slovak Republic).

We monitored anthropometric characteristics – body weight (BW), waist circumference (WC), waist-hip ratio (WHR), body fat mass (BFM), visceral fat area (VFA), body mass index (BMI), skeletal muscle mass (SMM), fat-free mass (FFM); blood pressure; lipid profile – total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides (TG), lipoprotein subfractions – VLDL, IDL-A, IDL-B, IDL-C, LDL_1, LDL_2, LDL_{3-7}; metabolic and renal markers – alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), bilirubin, urea, creatinine, uric acid, albumin; inflammatory response markers such as C-reactive protein (CRP), interleukin-6 (IL-6), immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM) and orosomucoid (ORM) before starting and after 8 weeks of SBJ consumption.

2.3. Anthropometric Data

Body height was measured using a Tanita WB-300 ambulatory electronic health scale in an upright position, without shoes. Body composition was determined by multi-frequency bioelectrical impedance analysis (MFBIA) - InBody 720 (Biospace Co. Ltd., Seoul, Korea) according to the manufacturer's instructions.

A digital upper arm electronic monitor (Omron M7 Intelli IT, HEM-7361T-EBK, Omron Healthcare, Tokyo, Japan) was used to measure systolic (SBP) and diastolic (DBP) blood pressure in seated subjects. Blood pressure was measured in three repetitions separated by a 2-minute break, and the average values of the three measurements were recorded. Between the intervals of measurements, the participants did not have any physical activity.

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2.4. Blood Sample Collection and Biochemical Analysis

The blood samples for biochemical analysis were collected at two time points: at baseline and after two months of supplementation of SBJ. Venous blood from brachial vein was collected in the morning, between 7 a.m. and 9 a.m., after a 12-hour overnight fasting using EDTA-containing tubes and serum gel by a qualified person. After blood collection, components were separated using centrifuge Hettich® MIKRO 220/220R.

The routine biochemical parameters were measured on the day of sampling. Next serum or plasma were frozen at -80°C for further analyses. The hematological and routine analysis of blood was determined in a biochemical laboratory of Specialized Hospital in Nitra by analyzer BioMajesty JCA-BM6010/C (JEOL Ltd., Tokyo, Japan) using commercial kits DiaSys (Diagnostic Systems GmbH, Holzheim, Germany) according to the instructions of the manufacturer. The LDL level was calculated using the Friedewald equation [60] as TC – HDL – (TG/2.2) in mmol/L.

Blood samples were also used for analysis of LDL subfractions. The LDL subfractions were determined using the analyser Lipoprint® (Quantimetrix corp., Redondo Beach, CA, USA) according to the manufacturer's instructions as previously described [61]. Subfractions LDL_1 and LDL_2 represent large LDL particles, subfractions LDL_{3-7} are small dense LDL. Other fractions are very low density lipoproteins (VLDL) as well as intermediate density lipoproteins (IDL) C, B and A.

2.5. Statistical Analysis

The normality of variables distribution was tested by the Shapiro–Wilk test. Anthropometric assessment and biochemical analysis results of subjects with normal distribution were compared by the paired t-test, and data are expressed as mean values ± standard deviation (SD). The nonparametric Wilcoxon test was used for not normally distributed variables, presented as median (upper-lower quartile). The statistical significance was established at $p < 0.05$. Statistical analysis was carried out using the Statistica Cz version 10 (TIBCO Software, Inc., Palo Alto, CA, USA) and MS Excel 2007 (Microsoft Corporation, Redmond, WA, USA).

3. Results

3.1. Characteristics of Clinical Trial Participants

A group of 28 hypercholesterolemic non-medicated adult women aged between 40 and 55 years with an average age of 50.58 ± 5.76 years participated in this clinical study (Table 2). Average value of total cholesterol was 6.31 ± 0.94 mmol/L. Up to 67.86% of participants had a borderline high (5.00-6.19 mmol/L) and 32.14% high level of total cholesterol (≥ 6.2 mmol/L). According to body mass index (BMI), the study population consisted of 9 participants (25.2%) with obesity (BMI ≥ 30 kg/m^2), 7 overweight (25.0%) participants (BMI 25.0–29.9 kg/m^2) and 12 participants (42.8%) with normal weight (BMI 18.5-24.9 kg/m^2).

Table 2. Baseline parameters of clinical trial group.

Parameter	Mean ± SD	Min.	Max.
Age (year)	50.58 ± 5.76	40	55
BW (kg)	72.89 ± 13.95	49.20	100.80
BMI (kg/m^2)	26.30 ± 5.01	19.56	38.41
TC (mmol/L)	6.02 (6.58-5.66)	5.06	8.33
HDL (mmol/L)	1.70 ± 0.23	1.15	2.01
LDL (mmol/L)	3.86 (4.71-3.43)	2.84	5.83
TG (mmol/L)	0.97 (1.12-0.85)	0.53	3.15
GLU (mmol/L)	4.80 ± 0.35	4.20	5.40
SBP (mm Hg)	127.76 ± 17.40	85	159
DBP (mm Hg)	86.00 ± 10.43	73	110

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Abbreviations: BW: body weight; BMI: body mass index; TC: total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TG: triglycerides; GLU: glucose; SBP: systolic blood pressure; DBP: diastolic blood pressure.

3.2. Effect of Sea Buckthorn Juice Consumption on Anthropometric Characteristics and Blood Pressure

Eight-week consumption of SBJ led to a significant decrease of BW, BMI ($p < 0.05$), BFM, VFA ($p < 0.001$), on the contrary, SMM and FFM were elevated ($p < 0.05$). Consumption of SBJ resulted in a statistically non-significant ($p > 0.05$) decrease of SBP and DBP (Table 3).

Table 3. Effect of 8 weeks SBJ consumption on anthropometric parameters and blood pressure.

Parameter	Baseline	Week 8	*p*-value
BW (kg)	72.89 ± 13.95	72.49 ± 13.98	0.0221
BFM (kg)	25.16 ± 10.37	24.22 ± 10.40	< 0.001
BFM (%)	33.21 ± 8.07	32.07 ± 8.37	< 0.001
BMI (kg/m^2)	26.30 ± 5.01	26.07 ± 4.93	0.0389
VFA (cm^2)	102.31 ± 37.82	98.63 ± 38.55	< 0.001
SMM (kg)	26.20 ± 2.89	26.54 ± 3.01	0.0183
FFM (kg)	47.73 ± 4.92	48.26 ± 5.09	0.0268
SBP (mmHg)	127.96 ± 13.87	127.27 ± 17.23	> 0.05
DBP (mmHg)	85.77 ± 10.29	85.26 ± 8.31	> 0.05

Abbreviations: SBJ: sea buckthorn juice; BW: body weight; BFM: body fat mass; BMI: body mass index; VFA: visceral fat area; SMM: skeletal muscle mass; FFM: fat-free mass. SBP: systolic blood pressure; DBP: diastolic blood pressure.

3.3. Effect of Sea Buckthorn Juice Consumption on Biochemical Characteristics

Sea buckthorn juice consumption was well tolerated by volunteers, with all measurements of metabolic and renal markers remaining within normal physiological ranges after 8 weeks of consumption. After the SBJ consumption, significant reduction of CRP, immunoglobulins (IgA, IgG, IgM), ORM ($p < 0.001$) and IL-6 ($p < 0.05$) were found. Blood serum levels of TC and TG non-significantly increased ($p > 0.05$) after 8 weeks of SBJ consumption. However, supplementation with 100% SBJ significantly increased the level of HDL ($p < 0.01$), decreased the level of LDL ($p < 0.05$) and improved LDL/HDL ratio ($p < 0.001$). The changes of biochemical characteristics of blood serum after 8 weeks of SBJ consumption are shown in Table 4.

Table 4. Effect of 8 weeks SBJ consumption on biochemical characteristics.

Parameter	Baseline	Week 8	*p*-value
ALT (μkat/L)	0.24 (0.20-0.32)	0.27 (0.22-0.36)	> 0.05
AST (μkat/L)	0.31 (0.27-0.36)	0.32 (0.27-0.38)	> 0.05
GGT (μkat/L)	0.28 (0.24-0.34)	0.28 (0.23-0.38)	> 0.05
Bilirubin (μmol/L)	8.60 (7.05-11.40)	8.55 (6.55-10.72)	> 0.05
Urea (mmol/L)	4.68 ± 1.29	4.75 ± 1.22	> 0.05
Creatinine (μmol/L)	65.90 ± 9.31	68.30 ± 8.70	> 0.05
Uric acid (μmol/L)	275.29 ± 61.05	284.71± 67.07	> 0.05
Albumin (g/L)	49.11 ± 2.62	48.65 ± 2.68	> 0.05
CRP (mg/L)	4.40 (4.00-5.70)	4.05 (3.50-5.05)	< 0.001
IL-6 (ng/L)	7.84 ± 0.95	7.36 ± 0.79	0.0387
ORM (g/L)	0.86 ± 0.21	0.55 ± 0.18	< 0.001
IgA (g/L)	1.70 ± 0.62	1.55 ± 0.62	< 0.001
IgG (g/L)	10.97 ± 2.78	10.43 ± 2.62	< 0.001
IgM (g/L)	1.22 ± 0.54	1.12 ± 0.52	< 0.001
TC (mmol/L)	6.02 (6.58-5.66)	6.06 (6.85-5.23)	> 0.05

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HDL (mmol/L)	1.70 ± 0.23	1.77 ± 0.28	0.0051
LDL (mmol/L)	3.86 (4.71-3.43)	3.73 (4.60-3.31)	0.0335
TG (mmol/L)	0.97 (1.12-0.85)	1.07 (1.33-0.77)	> 0.05
LDL/HDL ratio	2.44 ± 0.57	2.31 ± 0.58	0.0070

Abbreviations: ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma glutamyl transferase; CRP: C-reactive protein; IL-6: interleukin 6; ORM: orosomucoid; IgA: immunoglobulin A; IgG: immunoglobulin G; IgM: immunoglobulin M; TC: total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TG: triglycerides.

3.4. Effect of Sea Buckthorn Juice Consumption on LDL Subfractions

Of all study participants, we detected atherogenic $LDL_{3–7}$ subfractions at baseline in eight volunteers, after nutritional intervention in four of them. After 8 weeks of SBJ consumption, there were significant increase of IDL-A ($p < 0.01$), IDL-B ($p < 0.05$) and significant decrease of $LDL_{3–7}$ ($p < 0.05$). Table 5 shows changes in lipoprotein subfractions after nutritional intervention in probands with the presence of atherogenic LDL subfractions ($LDL_{3–7}$).

Table 5. Effect of 8 weeks SBJ consumption on lipoprotein subfractions (mmol/L) and LDL particle size (nm) (n=8).

Parameter	Baseline	Week 8	*p*-value
TC	5.98 (7.42-5.85)	6.35 (7.14-5.96)	NS
LDL	3.21 (4.64-3.18)	3.93 (4.59-3.62)	NS
HDL	1.62 ± 0.32	1.49 ± 0.33	NS
TG	1.49 (2.26-1.01)	1.44 (1.86-1.14)	NS
VLDL	1.14 ± 0.35	0.99 ± 0.21	NS
IDL-A	0.59 ± 0,25	0.87 ± 0.30	0.0058
IDL-B	0.36 (0.52-0.29)	0.50 (0.68-0.42)	0.0241
IDL-C	0.53 (0.64-0.33)	0.66 (0.72-0.56)	NS
LDL_1	1.21 ± 0.58	1.34 ± 0.39	NS
LDL_2	0.79 ± 0.26	0.52 ± 0.32	NS
LDL_{3-7}	0.13 (0.36-0.08)	0 (0.04-0)	0.0321
Mean LDL particle size	267.17 ± 5.27	271.67 ± 4.13	0.0053

Abbreviations: TC: total cholesterol; LDL: low-density lipoprotein; HDL: high- density lipoprotein; TG: triglycerides; VLDL: very low-density lipoprotein; IDL: intermediate density lipoprotein.

Atherogenic lipoprotein phenotype (pattern B), characterized by a marked dominance of $LDL_{3–7}$ was determined in four volunteers at baseline. After the eight-week intervention, one woman improved and her lipoprotein profile was classified as non-atherogenic (pattern A). Figure 2 shows the typical lipoprotein profile of a woman with phenotype B and its improvement after consumption of SBJ.

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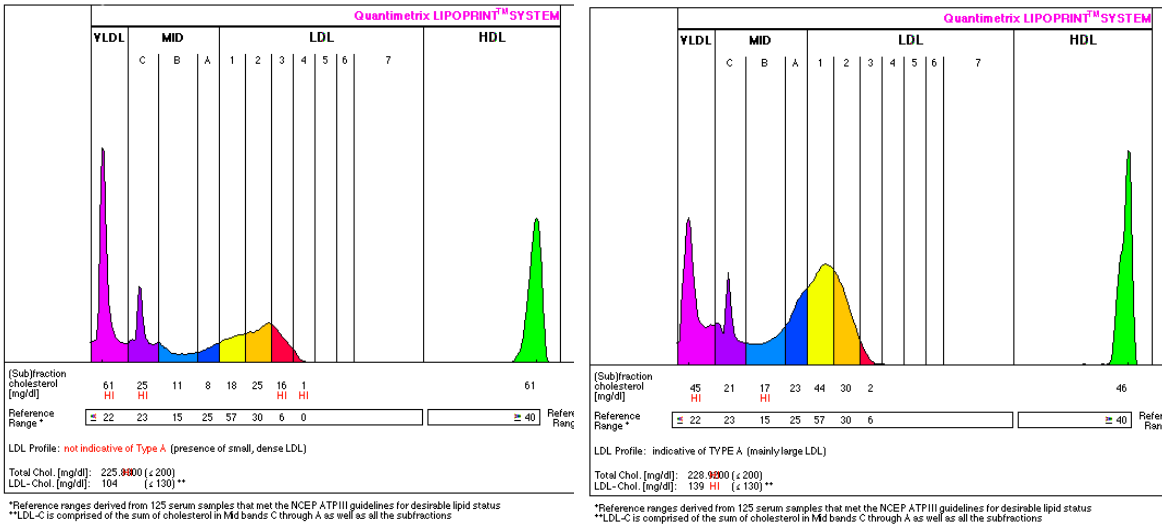

(**a**) (**b**)

Figure 2. Typical electrophoretograms of LDL subfractions at baseline (a) and after consumption (b) of SBJ analysed using the Lipoprint system. Atherogenic subfractions $LDL_{3–7}$ are red; the large (less atherogenic subfractions) $LDL_{1–2}$ are yellow.

4. Discussion

We monitored the effect of two- month supplementation with sea buckthorn juice on cardiovascular risk factors such as blood lipids, LDL subfractions, anthropometric characteristics and inflammatory markers in hypercholesterolemic women. We found a significant improvement in lipid profile which is also associated with decrease of atherogenic subfractions $LDL_{3–7}$, low-density lipoprotein, selected inflammatory markers and an increase in high-density lipoprotein.

Study participants consumed daily 50 mL of 100% organic pasteurized SBJ without additives for 8 weeks as part of their normal diet. Despite the fact that SBJ is characterized by a bitter and sour taste, all study participants did not have a problem with its consumption.

The studies regarding the favorable effects of oil and juice from sea buckthorn in the prevention of cardiovascular diseases suggest antiatherogenic, hypocholesterolemic and antiaggregation effects [62–64] primarily due to high phenolic compound content acting in synergy with unsaturated fatty acid and vitamin C [26,65]. Vitamin C and lycopene are two important antioxidants found in high amounts in fruit [26]. Sea buckthorn is a unique vitamin C source, the lowest value in the literature is 80.58 mg vitamin C/100 g fresh fruit [38]. For example, Tiitinen et al. [66] reported 128-1300 mg/100 mL of SBJ, which is much higher value than in fruits naturally rich in vitamin C - lemons, oranges [27] or kiwifruit [67]. The main identified constituents of the consumed SBJ are vitamin C (385.41 mg/100g), carotenoids (64.79 mg/100g), benzoic acid (142.47 mg/L), ferulic acid (18.14 mg/L) and others. It is famous that cardiovascular diseases are very often linked to obesity [68], which can be influenced by the consumption of plant sources (fruits and vegetables) rich in antioxidant-active phytochemicals [69–72]. Some low-calorie juices have the potential to prevent metabolic diseases, which is also useful for healthy cardiovascular system. In an animal study, Wu et al. [73] observed that mulberry and blueberry juice reduced BW and TC in C57BL/6 mice with high fat diet. *Ad libitum* intake of plum and peach juice inhibited BW gain in obese Zucker and lean rats [74], and mice receiving green juice had considerably less weight gain than mice drinking water [75]. On the contrary, Dupak et al. [76] found that after 3 months of sea buckthorn supplementation, BW non-significantly change in Zucker diabetic fatty (ZDF) rats versus the control group. Analyzing the dynamic of body composition during the 8 weeks of supplementation with SBJ, we can observe a statistically significant decrease in BW, BMI (($p < 0.05$) and VFA, BFM ($p < 0.001$). In the treatment of obesity, it is necessary to reduce weight. However, some treatment procedures may cause a decrease in muscle mass [77], in our study taking sea buckthorn juice for 8 weeks caused a significant increase of SMM and FFM ($p < 0.05$). Studies by Lehtonen et al. [78] and Larmo et al. [79] confirmed the positive effect of sea

buckthorn on overweight and obesity in women. Several studies have investigated fruit and fruit juice consumption and their impact on blood pressure [80]. For example, Huang et al. [81] found a significant decrease in SBP after berry consumption, which may be as a result of bioactive compounds - polyphenols, vitamins, minerals intake [80]. In our study, consumption of sea buckthorn juice led to non-significant decrease of blood pressure ($p > 0.05$).

The effect of sea buckthorn juice consumption on determinants of cardiovascular health has not yet been extensively studied. The most studies looking at the effect of berries, oil or extracts [82–84]. In a study conducted by Sayegh et al. [83] consumption of sea buckthorn berries improved the serum lipid profile of individuals at higher cardiovascular risk. Similarly, Guo et al. [85] demonstrated that SBJ intake had a beneficial effect on clinically relevant blood lipids (TC and TG) in hypercholesterolemic subjects, particularly over a short period (< 2 months). However, other studies have not observed a positive effect of sea buckthorn consumption in persons at cardiovascular health [79,86]. HDL is known to be a predictor of cardiovascular risk [87], while LDL contributes to atheroma formation [88]. Studies have shown that intake of fruits, vegetables, legumes, fish, nuts, and olive oil could increase the level of HDL [89], which can remove cholesterol from cells and atheroma [88]. Our results showed that consumption of 50 mL SBJ significantly decreased LDL and increased HDL ($p < 0.05$) in subjects with hypercholesterolemia. A 20% increase of HDL without affecting LDL and TG levels as a result of SBJ consumption in healthy volunteers is also reported by Eccleston et al. [90]. Johansson et al. [91] did not observed changes in HDL after sea buckthorn oil intervention. Based on data from prospective studies, TG are a risk factor for cardiovascular diseases independent of HDL [92]. The present study also determined that SBJ consumption did not affect TC and TG levels ($p > 0.05$). The results of Yang [93] showed that 4-week treatment with dried *Hippophaë* emulsion reduced blood TC by 19.2%, arteriosclerosis index [(TC-HDL)/HDL] by 28.2% and increased HDL by 18.1%. LDL and HDL levels are routinely measured in clinical practice to screen individuals for CVD risk [94], however LDL/ HDL ratio is a better marker for CVD risk than individual indicators [95,96]. The SBJ intake improved the LDL/HDL ratio ($p < 0.05$). Also Habanova et al. [97] observed an improvement of the LDL/HDL ratio after intake of apple/berry juice.

Clinical results have shown that sdLDL can be better biomarker of cardiovascular risk than conventional lipid profile screening [7,98,99]. Several methods have been designed to identify and quantify LDL fractions [100]. In our study the Lipoprint LDL System was used to identify and quantify LDL subfractions and mean LDL particle size. Currently, there are few studies investigating the effect of bioactive natural substances of fruits on sdLDL levels. There is evidence that sdLDL levels were significantly decreased as a result of consumption of freeze-dried strawberry beverage [101], freeze-dried strawberries [102], berries/apple mixed juice [103], apple/berry juice [97]. In the study of Zunino et al. [104] strawberry powder significantly increased LDL particle size. In another study [105] consumption of freeze-dried grape powder did not change LDL particle size and level of sdLDL. Our work is the first study that investigates the effect of bioactive sea buckthorn compounds on the distribution of LDL subfractions. In this study, the presence of atherogenic LDL subfractions (LDL_{3-7}) was quantified in eight women at baseline and only in four at the end of the trial. After 8 weeks of SBJ consumption, there were significant differences in the IDL-A ($p < 0.01$), IDL-B ($p < 0.05$) and LDL_{3-7} ($p < 0.05$). Small dense low-density lipoprotein has a greater atherogenic potential compare to other LDL subfractions [106–108]. Similarly, large VLDL particles are associated with an increased risk of atherosclerosis [109,110], their concentration was non-significantly reduced in our study. Zitnanova et al. [111] confirmed the protective function of IDL-A in the atherogenic process, in this study IDL-A significantly increased ($p < 0.01$). We also found a non-significant increase in the LDL_1 subfraction ($p > 0.05$), whose atheroprotective role was confirmed by Zitnanova et al. [111] and Oravec et al. [112]. Lipoprint analysis showed that an atherogenic lipoprotein phenotype (pattern B) was determined in four women, and one woman changed to non-atherogenic lipoprotein profile (pattern A) after the juice consumption.

Increasing evidence shows that one of the major conditions associated with increased morbidity and mortality from cardiovascular disease is chronic inflammation [113,114]. Inflammation in the atherosclerotic process is mainly caused by excessive production of nuclear factor kappa B, C-reactive

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protein (CRP), interleukin-6 (IL-6), IL-18, tumor necrosis factor alpha (TNF-α), and other inflammatory markers [115,116]. Some diet with natural antioxidants could contribute to the suppression of chronic diseases associated with inflammation, oxidative stress, and development of atherosclerosis [117]. Reducing effects of berries on inflammatory indicators have been examined in clinical trials [54,78,97,118–121]. Li et al. [122] confirmed the anti-inflammatory effects of tomato juices containing lycopene in cardiovascular system. The results of other studies showed that CRP, TNF-α and IL-6 were significantly reduced after consumption of a new food product containing mandarin juice [123] and red orange juice in subjects at cardiovascular risk [124]. Our results show that SBJ consumption led to a significant decrease of inflammatory markers, especially CRP ($p < 0.001$) and IL-6 ($p < 0.05$). The anti-inflammatory effect of the fruit is probably due to the synergistic mechanisms of flavonoids and vitamin C [125–128].

A limitation of this study is the absence of a control group consuming a placebo. The production of placebo juice for the control group, which is very similar in color and taste to sea buckthorn juice, was very difficult. Considering this fact, we decided to conduct a study with an 8-week intervention period, without changing eating habits, in which the participants were their own controls and the changes in their parameters were evaluated. The relatively small number of volunteers and the short duration of our intervention study are also limitations of this study. Further larger studies with larger numbers of participants and longer intervention durations are needed to further investigate the extent to which sea buckthorn juice may affect lipid profiles, LDL subfractions, and other factors of cardiovascular health.

5. Conclusions

Recently, a number of clinical trials have demonstrated specific biological functions of phytonutrients from fruits that may have beneficial effects in the prevention and/or treatment of cardiovascular disease. The purpose of our trial was to investigate whether two-months supplementation with 100% sea buckthorn juice would modify blood lipids, LDL subfractions and other markers of cardiovascular risk in hypercholesterolemic women. The consumption of sea buckthorn juice with a high contents of bioactive substances such as phenolic compounds, vitamin C and carotenoids led to a positive modulation of the HDL, LDL, LDL subfractions and LDL/HDL ratio. Findings also indicate that the phytonutrients of sea buckthorn juice may modulate body composition and inflammatory markers. The results obtained in this study support the hypothesis that the daily SBJ consumption for a period of 8 weeks shows the possible prevention of cardiovascular disease risk factors in women with elevated total cholesterol in a non-pharmacological way.

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Informed Consent Statement: A written informed consent was obtained from all participants involved in the study.

Data Availability Statement: All data sets related to the results of this study are available from the primary author on request.

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