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Clinical Study

Sea Buckthorn Pulp Oil Treatment Prevents Atherosclerosis in Obese Children

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This study aimed to determine the effects of sea buckthorn pulp oil treatment (800 mg/day for 60 days) on inflammatory, systemic oxidative/reductive status and endothelial function, in obese children. A total of 41 obese children (10–18 years old) and thirty controls were enrolled. The measurements were done before and after pulp oil administration. Ultrasounds were used for flow-mediated dilatation (FMD) and carotid artery intima-media thickness (IMT) measurements and colorimetric and ELISA methods for biochemical parameters. In the obese children versus the lean ones, increased oxidative stress (high malonyldialdehyde, high respiratory burst (RB)), low antioxidant defence (low blood glutathione, low TEAC), inflammatory status (high CRP, ceruloplasmin), adipocytokines disturbance (low adiponectin and high leptin), insulin resistance (high fasting C peptide), low FMD ($P < 0.001$), and high IMT ($P < 0.01$) were measured. Treatment reduced total cholesterol ($P < 0.03$), triglycerides ($P < 0.01$), RB ($P < 0.03$), leptin ($P < 0.049$), ceruloplasmin ($P < 0.01$), fasting C peptide ($P < 0.01$), blood pressure ($P < 0.01$), and IMT ($P < 0.03$). In conclusion, sea buckthorn pulp oil treatment prevents atherosclerosis by lowering triglyceridemia, cholesterolemia, and blood pressure (strong effects) and by reducing oxidative stress, inflammation, and insulin resistance (weak effects).

1. Introduction

According to research, the number of children who are clinically obese has raised threefold in the past 30 years [1]. The increase in prevalence and severity of obesity in children is concerning, as is progression to type 2 diabetes and cardiovascular diseases in adulthood [2].

Obesity is associated with chronic low-grade inflammation and insulin resistance [3]. In obesity, high plasma levels of leptin, TNF-α, IL-6, and other proinflammatory adipocytokines, together with increased plasma concentrations of fatty acids, mediate insulin resistance. Inflammatory pathways to insulin resistance include several serine/threonine kinases members and oxidative stress [4, 5]. Leptin seems to promote atherogenesis by stimulating vascular smooth muscle hypertrophy, inflammation, and oxidative stress [6]. But what happens with leptin in obese children has remained unknown [7].

While leptin is considered a biomarker of vascular dysfunction, adiponectin improves endothelial cells function. Adiponectin may protect the endothelium, via its insulin-sensitizing, antiatherogenic, anti-inflammatory, and antioxidant properties [8]. Recent studies on childhood obesity and adiponectin have demonstrated the contribution of hypoadiponectinemia to low-grade systemic chronic inflammatory state [9], to proatherogenic effect [10], and to high blood pressure [11].

Many sources of oxidative stress exist in obesity: hyperglycemia, hyperleptinemia, increased tissue lipid levels, inadequate antioxidant defenses, increased rates of free radical formation, enzymatic sources within the endothelium, and chronic inflammation [12–14]. Monocytes by means of their respiratory burst (NADPH oxidase activity) are an important source of free radicals that promote multiple atherogenic pathways in the vascular wall [15]. A disturbed oxidative/reductive state could play a role in the initiation

of cardiometabolic diseases such as atherosclerosis. The glutathione system can serve as an early marker of metabolic perturbations leading to cardiometabolic diseases [16–18].

The statements mentioned above are important to describe the complex metabolic misbalances present, both in childhood obesity and adulthood obesity, but in obese children there are still controversies about pathogenic mechanisms [11, 19].

Endothelial dysfunction has been found to be present in the early stages of atherosclerosis. An indicator of subclinical atherosclerotic disease is endothelial vasodilator dysfunction, assessed by brachial artery flow-mediated dilation (FMD). Impaired brachial FMD is related to the prevalence and extent of coronary atherosclerosis and predicts cardiovascular events [20]. The dilatation response with increased blood flow is mainly mediated by nitric oxide released from arterial endothelial cells [21].

The common carotid artery intima-media thickness (C-IMT) measured by ultrasound imaging is also a marker of preclinical atherosclerosis. C-IMT values predict the likelihood of cardiovascular events in adults [22].

Because severe childhood obesity is associated with oxidative stress, providing antioxidant food supplements in addition to a hypocaloric diet is important for the treatment of obese children [18]. It was demonstrated that daily consumption of diets rich in natural antioxidants may improve endothelial function in adolescents with metabolic syndrome [23]. Clinical and animal studies demonstrated that sea buckthorn pulp oil has antioxidant, anti-inflammatory, and hypolipidemic effects [24, 25]. Cell culture studies for studying pulp oil effects have not been done yet [25]. In clinical studies, adverse effects of sea buckthorn supplements have been rare (like gastrointestinal upset) and even doses of 2.5–10 mL/kg of sea buckthorn CO_2 extracted seed oil in experimental studies was safe [25].

The pulp oil composition depends on the processing and origin of the berries [26, 27].

The sea buckthorn pulp oil is rich in tocopherols and tocotrienols (up to 180 mg/100 g), vitamin K, carotenoids (up to 350 mg/100 g), fatty acids (67% unsaturated), and phytosterols (up to 2.9 g/100 g). The best natural source for palmitoleic acid, an essential fatty acid, is sea buckthorn pulp oil. Also, this oil provides a 1 : 1 ratio of omega-3/omega-6 and so has a lot of medical uses [28].

We designed this study to determine the effect of sea buckthorn (*Hippophae rhamnoides*, subspecies *carpatica*) pulp oil treatment in obese children. We evaluate the inflammatory and oxidative stress status in relation to disturbed metabolism in obese children and adolescents. We focused on two adipocytokines with opposed effects (leptin *versus* adiponectin) on endothelial function and estimate the effect of treatment by using FMD and IMT values.

2. Materials and Methods

2.1. Study Subjects. A total of 41 overweight children (22 boys and 19 girls) with a mean age of 13.32 ± 4.6 years and 30 healthy children and adolescents (16 boys and 14 girls) with normal weight were enrolled. Children under medications or those with chronic disease (endocrine disease, hereditary disease, or systemic inflammation) were excluded. All subjects were nonsmokers. The analytic evaluations were performed before and immediately after sea buckthorn pulp oil (Hofigal product) treatment (800 mg/day for 60 days), in obese children. The children took the drug *bis in diem*. All participants were instructed not to change their lifestyle (dietary and drinking habits, physical activity) during the whole study and not to take any additional medication including vitamin supplements. The study was carried out between June and September 2012. In the first two months a placebo was given both to obese children and to control subjects. At the end of this time, blood samples were collected. In the next two months the dietary supplement was administered 2 × 1, after meals, only to obese children. A washout period was not considered necessary. The general practitioner was involved in monitoring the treatment by maintaining contact with the parents by phone. None of the subjects gave up taking part in the study. The study protocol was approved by the Ethical Commission of "Carol Davila" University of Medicine, Bucharest, and a written informed consent was obtained from each parent.

2.2. Clinical Characteristics. Anthropometric measurements, body mass index (BMI), waist circumference (WC), and hip circumference (HC), were assessed. BMI was calculated as body weight (kg) divided by square height (m^2). Overweight is defined as a BMI at or above the 85th percentile and lower than the 95th percentile. Obesity is defined as a BMI at or above the 95th percentile for children of the same age and gender [29]. Obese group was formed by overweight and obese children. Waist circumference (WC) was measured at the midway between the lower rib and the iliac crest, and hip circumference was measured at the widest part at the gluteal region. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice at the right arm after a 10-minute rest, in the supine position using an automated sphygmomanometer.

2.3. Biochemical Measurements. Blood samples were taken after an overnight fast. Standard enzymatic methods were used for measuring levels of serum total cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-C). Total analytical variability, expressed as coefficient of variation (CV) was 2%, 1.9%, and 0.9%, respectively. Low-density lipoprotein-cholesterol (LDL-C) concentration was calculated by the Friedewald formula [30]. Metabolic markers, fasting serum glucose and uric acid, were measured by colorimetric methods. Enzymes activities for alanine aminotransferase (ALT) and for γ-Glutamyl transferase (γ-GT) were performed by standard methods using an automatic analyzer Hitachi and kits from DiaSys (Germany). Leptin and adiponectin were determined by using ELISA (EIA-2395, EIA-2935 kits, DRG Instruments GmbH, Germany). The intra-assay reproducibility was 5.95–6.91%, and interassay reproducibility was 8.66–11.55%. C peptide was measured by using EIA-1293 kit, DRG Instruments GmbH, Germany. The intra-assay reproducibility was 5.13–6.70%, and inter assay reproducibility was 8.38–9.92%.

Oxidative stress status was evaluated by measuring monocyte respiratory burst (RB or NADPH oxidase activity), total plasma antioxidant capacity (TEAC), plasma malondialdehyde (MDA), erythrocyte glutathione peroxidase activity (GPx), and total blood glutathione (GSH).

Isolation of Peripheral Blood Mononuclear Cells (PBMCs) and Respiratory Burst (RB). PBMCs were isolated by density centrifugation on Ficoll-Paque Plus (1.0077 g/mL). After centrifugation at 630 g for 30 min, the mononuclear cells (PBMCs) were collected, washed twice, and resuspended in 1 mL PBS. Cell viability by Trypan Blue exclusion was ≥90%. The ability to produce a respiratory burst was monitored by lucigenin-enhanced chemiluminescence [31]. In short, to PBMC (0.3×10^6 cells) resuspended in phosphate-buffered saline, dark-adapted lucigenin (final concentration 0.143 μmol/L) was added. After monitoring spontaneous chemiluminescence for 15 min, the respiratory burst was initiated by adding 100 μL of phorbol myristate acetate (final concentration 5.4 μmol/L), and the maximum chemiluminescence peak was recorded (Luminometer TD 20/20, Turner Designs). Chemiluminescence production was expressed as the relative chemiluminescence units (RLU). Total plasma antioxidant capacity (TEAC) was evaluated by the ABTS decolorization assay, CV 1.6%. The etalonation curve is done with TROLOX and the results are expressed in mmol/liter TROLOX [32, 33]. ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), potassium persulfate, and TROLOX (6-hydroxy-2,5, 7,8-teramethylchromane-2-carboxylic acid) were purchased from Sigma Chemical Co., St. Louis, MO, USA.

Plasma total malonyldialdehyde (CV 13.8%) was analyzed by pretreating plasma samples with thiobarbituric acid in orthophosphoric acid containing butylhydroxytoluene as antioxidant. The pink-colored product was measured spectrophotometrically at 532 nm [34].

Reduced glutathione (GSH) in whole blood was measured by a colorimetric method using Ellman's reagent, CV 9.8% [35]. The GPx activity of red blood cell hemolysate was assessed by using the Ransel kit (Randox, Antrim, United Kingdom; CV was 14.5%).

2.4. Ultrasound Measurements. Subjects were studied under identical conditions by the ultrasound systems equipped with vascular software for two-dimensional (2D) imaging, color and spectral Doppler Mindray DC 3, an internal electrocardiogram (ECG) monitor, and a high-frequency vascular transducer. A linear array transducer with a minimum frequency of 10 MHz, attached to a high-quality mainframe ultrasound system, is used to acquire images with sufficient resolution for subsequent analysis. On the study day, the subjects were asked to refrain from caffeine, anti-inflammatory drugs, and herbal supplements for ≥12 h before testing. All subjects gave their written, informed consent to participate. The brachial artery was identified at 5 cm proximal to the transient bifurcation. The brachial artery diameter was measured on longitudinal images, between lumen-intima interfaces of the near (anterior) wall and far (posterior) wall, at the same time in the cardiac cycle, during end diastole, which was identified at the onset of the R wave.

To obtain maximum diameter, we stimulated blood flow in the brachial artery with the use of a sphygmomanometer, which was placed antecubital, at the forearm. Its arm cuff was inflated to more 50 mmHg above systolic blood pressure, for 5 minutes. After the cuff was deflated, the maximal increase in diameter occurred approximately 45–60 seconds after release of the occlusive cuff.

The children were allowed at least 10 minutes of rest after reactive hyperemia. We measured again the baseline diameter. Then we administrated one tablet, sublingual, of nitroglycerin of 0.4 mg. Peak vasodilation occurred 3 to 4 minutes after nitroglycerin administration. Images were continuously recorded during this time. The maximum diameter was the maximum mean diameter observed at 3 to 4 minutes after nitroglycerin administration [36]. After measurements of FMD (flow-mediated dilation) and NMD (nitroglycerin-mediated dilation), the ratio FMD/NMD was calculated. In the laboratory, two independent investigators performed the measurements. The intraobserver and interobserver variations for FMD are 6.5% and 9.3%, respectively. A possible limitation of our study is that we did not normalize FMD for the peak shear rate.

Carotid artery IMT measurements were done according to a predetermined, standardized scanning protocol [37]. A minimum of 4 measurements of the common carotid artery were taken, and the intraobserver and interobserver variations are 1.9% and 2.8%, respectively. Carotid arterial wall segments were assessed in a longitudinal view, strictly perpendicular to the ultrasound beam. We used depth of focus 30 mm, frame rate 25 Hz, and log gain compensation 60 db. Gain settings were adjusted to obtain a symmetrical brightness on the near and far walls, with a clearly identified double-line pattern (media-adventitia interface and intima-lumen interface). The optimal measurement of IMT was obtained during end diastole by automatic cine loop detection. We measured IMT on the far wall of the left common carotid artery at least 5 mm below its bifurcation. We obtained mean IMT values averaged across 10 mm length of a straight arterial segment.

2.5. Statistical Analysis. Results were expressed as mean ± standard deviation (SD)/standard error of the mean (SEM) for quantitative variables. Data were analyzed using the statistical package SPSS version 16.0. Differences between groups were analyzed using Student's t-test, and two-tailed P values <0.05 were considered as statistically significant. Differences between groups were calculated using two-way ANOVA with Bonferroni post hoc tests. Pearson correlations were applied to identify the relationship between the various parameters.

3. Results

Body mass index (BMI), waist circumference (WC), hip circumference (HC), systolic blood pressure (SBP), and diastolic blood pressure (DBP) were higher in the obese children versus the lean ones (Table 1). SBP, DBP, and waist circumference were reduced after 60-day intake of 800 mg/day pulp oil sea buckthorn (Table 1).

Table 1: Clinical parameters.

Parameters	Control group	Obese before treatment	Obese after treatment	P	P''
Age (years)	13.5 ± 4.8	13.32 ± 4.6	13.4 ± 4.7	Ns	Ns
Sex (F/M)	14/16	19/22	19/22	Ns	Ns
Weight (kg)	44.82 ± 10	68.76 ± 16.02	65.9 ± 15.1	<0.001	Ns
Height (m)	1.54 ± 0.11	1.52 ± 0.05	1.53 ± 0.06	Ns	Ns
BMI (kg/m^2)	18.81 ± 1.7	30.2 ± 2.4	29.7 ± 3.1	<0.001	Ns
WC (cm)	67.4 ± 6.6	91.37 ± 16.75	90.03 ± 9.2	<0.001	$P < 0.02$
HC (cm)	83.46 ± 7.1	99.25 ± 15.5	99.07 ± 10.5	<0.001	Ns
SBP mmHg	97.3 ± 5.2	110.92 ± 8.78	104.33 ± 5.08	<0.001	<0.01
DBP mmHg	59.25 ± 2.6	72.58 ± 4.9	67.03 ± 5.3	<0.001	<0.01

BMI: body mass index; WC: waist circumference; HC: hip circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; P value represents the t-test result of comparison of obese *versus* control groups, and P'' value represents the t-test result of comparison variables in the obese group before treatment and after treatment.

There were statistically significant differences between control and obese groups regarding plasma levels of triglycerides, total cholesterol, uric acid, albumin/globulin ratio, albumin, adiponectin, leptin, and C-peptide (Table 2). Biochemical parameters, triglycerides, total cholesterol, C-peptide, and leptin, were significantly decreased while albumin/globulin ratio and albumin were significantly increased after 60-day intake of 800 mg/day pulp oil sea buckthorn (Table 2).

The markers of subclinical atherosclerosis were modified in the obese children versus control (IMT was higher, and FMD was lower). After treatment, IMT was decreased (Table 3). The nitroglycerin-mediated dilation (NMD) was 12.66% ± 1.01 in the obese children, and the ratio between FMD and NMD in this group was 0.89. For the lean children, NMD was 14.33 ± 0.5, and the ratio between FMD and NMD is 76.75.

Correlations ($P < 0.05$) of the two markers of preatherosclerosis (IMT and FMD) with different plasma parameters are shown in Table 4.

Relations ($P < 0.05$) of the two markers for endothelial function (adiponectin and leptin) with different plasma parameters are shown in Table 5.

In the obese children, the antioxidants: total blood glutathione, and total plasma antioxidant activity (TEAC), were decreased, while oxidative stress markers: MDA (malondialdehyde) and monocyte NADPH oxidase (RB), were increased (Table 6). The erythrocyte glutathione peroxidase activity (GPx) was increased in the obese versus the lean children.

Puberty influences the plasma values of the parameters measured in this study. A possible limitation of our study is that most of the subjects are at puberty, and gender is important.

4. Discussion

Obesity in children has been independently correlated to endothelial dysfunction, inflammation, and oxidative stress markers, major risk factors for atherosclerosis [38–40].

Sea buckthorn flavonoids are the berry part most studied for the beneficial effects in preventing cardiovascular diseases. The clinical and animal studies on cardiovascular effects of sea buckthorn seed or pulp oil are few [41–43]. In these clinical studies it was demonstrated that intake of sea buckthorn seed and pulp oil did not influence triglyceridemia but increased HDL-C level [41], decreased the level of vascular cell adhesion molecule [42], and inhibited platelet aggregation [43].

In hypercholesterolemic rabbits, CO_2 extracted sea buckthorn seed oil had significant antiatherogenic and cardioprotective activity, improving the plasma lipid profile [44]. On a spontaneous model obese type 2 diabetes rats, researchers demonstrated that palmitoleic reduced hyperglycemia and hypertriglyceridemia. Downregulation of proinflammatory gene expressions and decreased hepatic lipid accumulation improved insulin sensitivity in studied rats [45].

In our study, treatment with sea buckthorn pulp oil reduced the total plasma cholesterol, the apoB/apoAI ratio, and plasma triglycerides. An improvement should be noted of HDL-C and LDL-C levels, but the effect was weak. The composition of sea buckthorn pulp oil, rich in monounsaturated fatty acids (MUFA), can justify this result. Kris-Etherton et al. demonstrated that a high-MUFA, cholesterol-lowering diet may be preferable to a low-fat diet because of more favorable effects on the cardiovascular disease risk profile [46]. Also, phytosterols are the major constituents of the unsaponifiable fraction of sea buckthorn oils and have important effects in lowering plasma cholesterol level [25].

Fasting C peptide is known as a surrogate marker of insulin resistance and reflects the endocrine secretory reserve of the pancreas. In this study, sea buckthorn pulp oil treatment, by improving the plasma fasting C peptide level, may have beneficial effects, reducing insulin resistance. This effect may be due both to palmitoleic acid and to antioxidant vitamins in which sea buckthorn pulp oil is rich. There are some arguments. It was demonstrated that palmitoleic acid reduces muscle insulin resistance and prevents beta-cell apoptosis [45]. Also, vitamin E improved oxidative stress and insulin sensitivity in overweight subjects [47, 48].

Fasting insulin levels are generally increased in obese adolescents. It is known that insulin stimulates vascular smooth muscle growth [49]. According to this statement and also due to the increased sympathetic tone [19] it is not amazing that

Table 2: Plasma variables.

Parameters	Control group	Obese before treatment	Obese after treatment	P	P''
Cholesterol mg/dL	148.4 ± 16	179.25 ± 28.27	163.34 ± 34.5	<0.02	<0.03
Triglycerides mg/dL	68.4 ± 28.7	114.25 ± 49.4	86.9 ± 33.5	<0.001	<0.01
HDL-C mg/dL	50.37 ± 12.9	46.38 ± 13.5	50.31 ± 12.44	<0.05	Ns
LDL-C mg/dL	83.7 ± 7.7	104.23 ± 30.4	97.25 ± 13.5	Ns	Ns
apoB/apoAI	0.43 ± 0.04	0.58 ± 0.2	0.55 ± 0.3	<0.01	Ns
Uric Acid mg/dL	4.48 ± 0.5	5.82 ± 1.45	5.89 ± 0.7	<0.002	Ns
GGT (UI/L)	13.85 ± 1	16.27 ± 5.8	18.7 ± 4.9	Ns	Ns
Glycemia mg/dL	85.2 ± 3.4	86.25 ± 6.8	84.15 ± 7.1	Ns	Ns
ALT (UI/L)	13.07 ± 2	18.9 ± 9.1	22.05 ± 8.3	<0.05	Ns
Creatinine mg/dL	0.80 ± 0.15	0.77 ± 0.07	0.75 ± 0.1	Ns	Ns
Bilirubin mg/dL	0.55 ± 0.29	0.48 ± 0.29	0.56 ± 0.33	Ns	Ns
Leptin ng/mL	2.16 ± 1.3	19.12 ± 12.3	11.64 ± 7.8	<0.001	<0.01
Adiponectin ng/mL	15.3 ± 1.2	8.19 ± 1.06	9.22 ± 1.09	<0.03	Ns
C peptide ng/mL	1.08 ± 0.4	1.92 ± 1.23	1.45 ± 0.9	<0.03	<0.049
Fibrinogen g/L	3.23 ± 0.12	3.26 ± 0.27	3.20 ± 0.22	Ns	Ns
CRP mg/dL	0.95 ± 0.5	2.3 ± 2.10	2.02 ± 1.7	<0.04	Ns
Ceruloplasmin mg/dL	29.82 ± 3	33.99 ± 4.45	29.02 ± 4.33	<0.02	<0.01
Albumin g/dL	4.21 ± 0.4	3.98 ± 0.6	4.17 ± 0.6	<0.002	<0.008

HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol; TG: triglycerides; CRP: C reactive protein; ALT: alanine amino-transferase; γ-Glutamyl transferase (γ-GT); P value represents the t-test result of comparison of the obese group versus control group, and P'' value represents the t-test result of comparison variables in the obese group before treatment and after treatment.

Table 3: Markers of preatherosclerosis.

Parameters	Control group	Obese before treatment	Obese after treatment	P	P''
IMT (mm)	0.41 ± 0.03	0.50 ± 0.08	0.46 ± 0.06	<0.01	$P < 0.03$
FMD %	18.64 ± 0.81	10.89 ± 1.84	11.17 ± 1.98	<0.0001	Ns

IMT: carotid artery intima-media thickness; FMD: flow-mediated dilation; P value represents the t-test result of comparison of obese versus control groups, and P'' value represents the t-test result of comparison variables in the obese group before treatment and after treatment.

Table 4: Correlations for IMT and FMT.

Correlations	Weight (kg)	BMI (kg/m^2)	Waist circumference (cm)	Fasting C peptide (ng/mL)	CRP (mg/dL)	Leptin (ng/mL)	Adiponectin (ng/mL)	apoB/apoA	DBP (mmHg)
IMT (mm)	0.60	0.57	0.56	0.36	0.36	0.40	−0.36	0.39	0.36
FMD %	−0.38	−0.58	−0.51	−0.38	—	—	0.38	−0.36	−0.36

Table 5: Correlations for adiponectin and leptin.

Correlations	Weight (kg)	BMI (kg/m^2)	Waist circumference (mg/dL)	Fasting C peptide (ng/mL)	HDL-C (mg/dL)	DBP (mmHg)	apoB/apoAI	apoA g/L
Leptin (ng/mL)	0.47	0.62	0.57	—	—	0.40	—	—
Adiponectin (ng/mL)	−0.52	−0.45	−0.49	−0.36	0.56	−0.52	−0.50	0.56

obese children had higher blood pressure (both systolic and diastolic) levels than the lean children. The treatment used in this study reduced significantly the blood pressure. Moreover, it is known that the enzyme nitric oxide synthase (NOS) is an insulin dependent enzyme and so, it becomes impaired with insulin resistance, resulting in modified vascular tone [50].

In our study, the obese children had much lower values for FMD (flow-mediated dilation), a marker for early atherosclerosis. FMD was correlated negatively with anthropometric parameters, with apoB/apoAI ratio, with fasting C peptide, and with diastolic blood pressure and positively with adiponectin. Although sea buckthorn treatment reduced

Table 6: Redox plasmatic profiles in the studied groups.

Parameters	Control group	Obese children	Obese children after treatment	P	P''
TEAC (mM Trolox)	1.68 ± 0.24	1.48 ± 0.16	1.52 ± 0.16	<0.02	Ns
NADPH oxidase (RLU)	0.40 ± 0.02	0.62 ± 0.05	0.45 ± 0.04	<0.01	<0.03
Total blood glutathione (micromol/g Hb)	5.19 ± 0.9	4.24 ± 1.29	4.43 ± 1.54	<0.05	Ns
GPx (UI/L)	37.7 ± 12.9	52.38 ± 23.5	47.86 ± 19.44	<0.03	Ns
MDA (mmol/liter)	0.27 ± 0.08	0.36 ± 0.12	0.38 ± 0.1	<0.05	Ns

P value represents the t-test result of comparison of obese *versus* control groups, and P'' value represents the t-test result of comparison variables in the obese group before treatment and after treatment.

blood pressure and fasting C peptide level, the FMD values were not improved significantly after treatment.

The other marker of early atherosclerosis, the IMT, was higher in the obese children *versus* the lean ones. This parameter was positively correlated with anthropometric parameters, apoB/apoAI ratio, fasting C peptide, leptin, CRP, and diastolic blood pressure and negatively with adiponectin. The associations of IMT with these markers highlight the implication of different pathogenic mechanisms in atherosclerosis, in childhood obesity. Our results are in agreement with other studies done in obese children [51, 52].

In our study, the plasma inflammatory markers (CRP, leptin, and ceruloplasmin) were significantly increased in the obese children, while fibrinogen was not modified compared to the levels in the lean children. Treatment had a weak anti-inflammatory effect by reducing leptin and ceruloplasmin. In the literature, a few studies on obese children have shown increased plasma levels of inflammatory markers, such as leptin, IL-6, CRP, and fibrinogen [5, 53]. It was suggested that insulin resistance may precede the development of CRP elevation in the evolution of the metabolic syndrome [54, 55]. The anti-inflammatory effect of sea buckthorn must be proved by more clinical studies. In the presence, in a double blind, randomized, and placebo-controlled trial done on healthy volunteers, sea buckthorn frozen puree intake, 28 g for 90 days *versus* placebo, decreased the serum CRP concentrations significantly [56]. In a clinical study done in dry eyes patients, sea buckthorn oil did not affect the serum concentrations of inflammation markers [25]. In aged rats, sea buckthorn fruit oil, by raising serum AMPc, regulates antiinflammatory properties [57].

As we mentioned, leptin was high and adiponectin was low in the obese children *versus* the control. Adiponectin plasma level was not influenced by the treatment, but leptin was lowered. It is known that leptin induces release of other cytokines such as TNF-alpha or IL-6 by acting on monocytes. Moreover it leads to increased proliferation and differentiation of monocytes. Acting on neutrophils, leptin leads to an increase of CD11b expression as well increased neutrophil chemotaxis and oxidative burst [58]. So, we measured the respiratory burst (monocyte NADPH oxidase activity) in the obese children, and the level was increased *versus* the control. We did not find a correlation between plasma leptin and respiratory burst (RB), but we calculated an inverse correlation between total blood glutathione and RB ($r = -0.45$). This relation together with the modified oxidative stress markers convinced us to consider that a systemic prooxidant status is created in the obese children by a double pathogenic mechanism: low blood antioxidant defence (low total blood glutathione, low TEAC) and high oxidative stress (increased RB, increased MDA). This imbalance could contribute to the endothelial dysfunction in the obese children.

Even though there are only few studies on systemic oxidative stress in childhood obesity, most of them demonstrated increased lipid peroxidation, estimated by MDA [18, 59] and low TEAC [60] in obese children and adolescents. Sea buckthorn pulp oil can decrease systemic oxidative stress. It was demonstrated that administration of sea buckthorn pulp oil in patients with gastric ulcers lowered the increased levels of plasma MDA and increased the lower levels of tocopherols [61]. In another clinical study, a dietary supplement containing blueberry and sea buckthorn concentrate increased blood antioxidant capacity in type 1 diabetic children [62]. In vitro study demonstrated that the antioxidant activity of sea buckthorn fruits extracts can be attributed to its property of capturing free radicals [63].

The low total blood glutathione concentration and high GPx activity in the obese children *versus* the lean ones are in agreement with other studies [16, 17]. These authors consider that a modification in the blood glutathione system can be an early biomarker of chronic oxidative stress that can be an early step in the development of cardiometabolic complications. They suggest also that greater GPx activity might be a compensatory response to the enhanced peroxide production, which supports the idea of oxidative stress. In this study GPx activity was negatively correlated with total blood glutathione ($r = -0.47$) and with HDL-C ($r = -0.70$). The first correlation may suggest that GPx is active to metabolize peroxides (H_2O_2), hence the glutathione is consumed, and the second one reflects the relation between oxidative stress and low HDL-C.

Albumin represents a very abundant and important circulating antioxidant known to protect endothelial cell from oxidative injury [59]. In the present study, plasma albumin was lower in the obese group, and treatment increased significantly its level.

It is thought that the evaluation of oxidative status may allow for the identification of patients at an increased risk of complications. Decreasing the levels of chronic inflammation and oxidative stress in childhood may decrease cardiovascular morbidity and mortality in adulthood [64].

Providing foods with high antioxidant/anti-inflammatory capacity, in addition to a hypocaloric diet, is crucial for the treatment of obese children. Because of its unique composition, [65] and of lack of side effects [25], sea buckthorn oil has great future as a food supplement used in preventing obesity complications. Nowadays, it is not known if beneficial changes revert back to normal if the sea buckthorn supplement is stopped.

In this study, treatment with sea buckthorn pulp oil (800 mg/day, 60 days) did not modify significantly the markers of antioxidant defence (TEAC, GPx, and total blood GSH) or the level of lipid peroxidation (MDA) in the obese children but reduced respiratory burst. By lowering NADPH oxidase activity in monocytes, sea buckthorn pulp oil treatment reduces the formation of the free oxygen radicals (the anion superoxide) and so may improve atherosclerosis by reducing macrophage-mediated inflammation.

In conclusion, treatment with sea buckthorn pulp oil (800 mg/day, 60 days) influences the blood levels of different variables, known as markers of different pathogenic pathways of atherosclerosis. The treatment lowered inflammation, blood pressure levels, improved plasma lipid profile, and reduced respiratory burst. The strong argument that sea buckthorn pulp oil prevents atherosclerosis is by lowering IMT values.

Disclosure

None of the authors of the paper have any direct financial relation with the commercial identities mentioned in the paper.

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