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Original Article

Effect of 8-weeks intensive lifestyle intervention on LDL and HDL subfractions

Boris Bajer[a], Žofia Rádiková[a], Andrea Havranová[a], Ingrid Žitňanová[b], Miroslav Vlček[a], Richard Imrich[a], Peter Sabaka[c], Matej Bendžala[c], Adela Penesová[a,*]

[a] Institute of Clinical and Translational Research, Biomedical Research Center, Slovak Academy of Sciences, Bratislava, Slovakia
[b] Institute of Medical Chemistry, Biochemistry and Clinical Biochemistry, Faculty of Medicine, Comenius University Bratislava, Slovakia
[c] Department of Infectology and Geographic Medicine, Faculty of Medicine, Comenius University Bratislava, Slovakia

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ABSTRACT

Objective: Atherogenic dyslipidemia is a cardinal feature of obesity and the metabolic syndrome, which increases the risk of cardiovascular diseases. Many interventional studies, describing the influence of weight loss on cardiometabolic risks, are bariatric surgery studies. The aim of our study was to analyze the effect of intensive lifestyle changes on LDL- and HDL-cholesterol subfractions and cardiometabolic risk factors in obese subjects.

Methods: A group of 41 patients with obesity (11M/30F; 44.1 ± 12.4 years; BMI 30.2 ± 6.3 kg/m^2) participated in an 8-week weight loss interventional program (NCT02325804), consisting of caloric intake reduced by 30% and physical activity (150 min/week). Insulin sensitivity was evaluated according to the homeostasis model assessment of insulin resistance (HOMA-IR) and physical fitness was measured using bicycle ergometry. Lipid subfractions were measured using the Lipoprint system (Quantimetrix Corp., CA, USA).

Results: After the intervention, body weight was reduced by 5.4 ± 4.5 kg, as well as body fat mass and waist circumference. Physical fitness improved, systolic and diastolic blood pressure as well as heart rate decreased after the intervention. Insulin sensitivity improved after the intervention. Total, LDL, HDL cholesterol, as well as triglycerides decreased after the intervention. Regarding the lipoprotein subfractions, LDL2 and small HDL subfractions decreased, while others have not changed.

Conclusion: Eight weeks of diet and physical activity intervention led to weight and fat mass loss and induced improvement of insulin sensitivity, as well as atheroprotective changes of lipid profile. However, the weight loss associated changes in cholesterol subfractions as cardiovascular risk biomarkers deserve further studies.

Introduction

Obesity is an emerging public health issue due to its worldwide prevalence and its association with the risk of development of chronic non-communicable diseases [1]. In Slovak adults according to waist circumference, central obesity was present in 37.3% of males and 41.8% of females between the years 2009–2012 [2]. Obesity is considered an independent risk factor of cardiovascular diseases (CVD), diabetes mellitus type 2, dyslipidemia, and metabolic syndrome (MS) [3]. Obesity induces changes in the structure and function of the cardiovascular (CV) system to adapt to increased body weight. Other indirect effects of obesity are mediated by risk factors such as hypertension, insulin resistance (IR), hyperglycemia, and dyslipidemia [4,5]. Dyslipi-

Abbreviations: BMI, Body Mass Index; BPdia, diastolic blood pressure; BPsys, systolic blood pressure; CV, Cardiovascular; CVD, Cardiovascular disease; ECG, Electrocardiography; EDTA, Ethylenediaminetetraacetic acid; HC, Hip circumference; HDL-c, High-density lipoprotein cholesterol; IDF, International Diabetes Federation; IDL, Intermediate-density lipoprotein cholesterol; IGT, Impaired glucose tolerance; HOMA-IR, Insulin resistance homeostatic model assessment; LDL-c, Low-density lipoprotein cholesterol; LAGB, Laparoscopic adjustable gastric banding; MET, Metabolic Energy Turnover; mmHg, Millimeters of mercury; MS, Metabolic syndrome; PA, physical activity; RYGB, Roux-en-Y Gastric Bypass; SAS, Slovak Academy of Sciences; SPSS, Statistical Package for Social Sciences; T-chol, total cholesterol; TG, Triglyceride; TSH, Thyroid stimulating hormone; VLDL, very low-density lipoprotein; VO2, max Maximal oxygen consumption; WC, waist circumference; WHR, Waist to hip ratio; WL, Weight loss.

* Corresponding author.
E-mail address: adela.penesova@savba.sk (A. Penesová).

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demia means elevated levels of low–density lipoproteins (LDL-c), small very low–density lipoproteins (VLDL) and intermediate-density lipoproteins (IDL), triglyceride (TG), and low serum levels of high-density lipoprotein cholesterol (HDL-c) and its lipoprotein subfractions, which correlate with an increased risk for CVD [4,6].

The LDL-c and HDL-c are heterogeneous populations of particles that differ in quality and/or size. Recent research found out that low HDL-c and/or high LDL-levels per se may not be responsible for the increased risk of CVD [7]. Levels of different subclasses of HDL-c and LDL-c are more useful determinants of pro- or anti-atherogenic lipid profile than their total circulating levels. There have been identified different LDL subfractions, with different biological effects and therefore also different atherogenic potential. The most atherogenic subfractions are small dense LDL subfractions 3–7. Their role in the pathogenesis of coronary atherosclerosis was confirmed [8]. It is very well known that small dense LDL subfractions penetrate easier into the arterial wall, remain longer in the circulation, have lower affinity for LDL receptors, increased susceptibility to oxidative stress and therefore are more atherogenic than the large LDL particles [9]. However, medium size LDL particles, or LDL2 subfraction by the Lipoprint system, have also been identified to be independently associated with the increased risk of premature myocardial infarction [10].

The HDL lipoproteins represent a protective part of plasma lipoproteins; three major HDL subfractions were identified: large HDL (HDL1–HDL3), intermediate HDL (HDL4–HDL7) and small (HDL8–HDL10) HDL subclasses [8]. Results of studies, where large and intermediate HDL are considered anti-atherogenic subclasses, while the small HDL subfraction is assumed to display atherogenic properties, are inconsistent and widely discussed [11]. Increased level of the small HDL subclass was detected in patients with CVD with an atherogenic lipoprotein phenotype B, as well as in patients with lower extremity artery disease [8,11]. Subjects in studies with increased cardiovascular risk showed lower levels of large atheroprotective HDL subclasses and higher levels of the small proatherogenic ones [7]. Patients with coronary artery disease had dyslipidemia with typically increased levels of small HDL and decreased levels of large HDL subfractions [7]. Majority of the studies showed that large HDL subfractions have more protective effect than small HDL, however these data are not consistent [7,8].

Weight loss, in fact, is an important goal and has proved to be beneficial in preventing health risks related to obesity [12]. Dietary restriction and increased physical activity still remain the main and essential cornerstones of lifestyle intervention in the treatment of obesity, with the goal to reduce the risk of developing CVD and diabetes mellitus type 2 in individuals with obesity [12,13]. It is well known that higher cardiorespiratory fitness is an important protective factor against developing hypertension or dyslipidemia [14].

Data in literature about the influence of losing weight on the HDL and LDL particles, are inconsistent. However, these data are based on one lifestyle interventional study [15] and studies where weight loss was reached through bariatric surgery [16–18]. A meta-analysis [19] indicates lower LDL-c 1 year after the surgery (either in subjects provided with sleeve gastrectomy or gastric banding), and these results were comparable to control patients without surgery. Recent study with bariatric surgery (the laparoscopic adjustable gastric banding (LAGB)) showed that weight loss (approximately 14%) led to antiatherogenic changes in lipid profile (small HDL decreased and larger HDL increased) [18].

Even the smallest weight loss (5–10 %), but still within the obesity range, might have a positive effect on CVD risk [12]. However, comprehensive data, regarding the effect of weight loss after the life style intervention (diet and/or physical activity) on the HDL or LDL subfractions are still missing.

Therefore, the aim of our study was to clarify the impact of weight loss, achieved by 8-weeks of intensive life style modification through diet and physical activity on lipoprotein spectrum, with the special concentration on HDL and LDL subfractions, and on the interaction between HDL, LDL subfractions with other cardiometabolic risk factors (insulin resistance and physical fitness).

Material and methods

Study design and participants

The data were obtained in longitudinal prospective study with the intervention focused on weight loss, registered on ClinicalTrials.gov under No: NCT02325804, conducted in Biomedical Research Center, in Bratislava, Slovakia. The project was approved by the Ethic committee of Bratislava Self-Governing Region No.05239/2016/HF. This study was executed in conformity with the principles from the Declaration of Helsinki for experiments involving human beings. After reading the written informed consent and after the explanation of the particular steps of the study and discussion with investigators, the signed informed consent was obtained from all subjects before participating in the study.

Subjects

We studied nonsmoking Caucasian volunteers with central obesity (men and women) with higher amount of body fat (men ≥25%, women ≥28%). We enrolled 52 subjects and 41 of them completed the study (4 patients did not meet inclusion and exclusion criteria, 7 did not finish the intervention; reasons for a dropout: pregnancy, inability to stick to the diet or physical activity (PA), or family issues). Participants were recruited using advertisements and from the registry of our outpatient clinic for internal medicine and diabetes. Potential participants first had a phone screening to ascertain, whether they meet the criteria.

The volunteers had to meet the **inclusion criteria:** (1) age 18–60 years, (2) increased amount of body fat% (men ≥ 25%, women ≥ 28%); (3) sedentary lifestyle (assessed using Lagerros questionnaire), (4) willingness to participate in an 8-week interventional program. **Exclusion criteria** included: (1) an inability to give informed consent; (2) chronic diseases (diabetes on insulin, rheumatic, metabolic, pulmonary, chronic infectious disease, chronic kidney disease, CVD, use of hypolipidemics, corticosteroids, etc.); (3) active liver disease; (4) Thyroid stimulating hormone (TSH) concentration higher/lower that the normal values (reference range); (5) anemia with hemoglobin < 95 g/l; (6) malignancies, (7) recent major trauma or surgery that would interfere with participation; (8) pregnancy or plans to become pregnant during the study; (9) breastfeeding; (10) tobacco, alcohol or drug addiction.

Study protocol

Volunteers were asked to stay 12 h fasting and to avoid intensive exercise 24 h before the examination. Examination started in the morning at 08:00 a.m. in the outpatient clinic of internal medicine and diabetes at the Institute of Clinical and Translational Research, Biomedical Research Center, Slovak Academy of Sciences, Bratislava. First we took medical and personal history, thereafter we measured the body weight, height, body fat percentage, amount of fat mass, fat-free mass using the bioelectrical impedance (Omron 511BF, OMRON HEALTHCARE Co., Ltd. Kyoto, Japan). Waist and hip circumference were measured too. The body mass index (BMI) and waist to hip ratio (WHR) were calculated. Blood pressure was measured on the arm during at least 5 min of rest (Dinamap Vital Sign Monitor, model 845 XT, Criticon X, Inc., Tampa, FL, USA). The cubital vein was cannulated (Terumo Europe N.V., Leuven, Belgium). Blood

was drawn in fasting state into polyethylene tubes with Ethylenediaminetetraacetic acid (EDTA) as the anticoagulant and immediately cooled in ice and into polyethylene tubes without anticoagulant (to obtain serum). After centrifugation at 4 °C, all plasma and serum aliquots were stored at –70 °C until assayed.

After 30 min of rest all subjects underwent measurement of **resting metabolic rate (RMR)** and metabolic substrate preference (RQ) using indirect calorimetry in the fasting state (Ergostik, Geratherm-Respiratory, Germany). Thereafter, study participants underwent standard **2-h oral glucose tolerance test** to determine glucose tolerance.

Biochemical analysis

All biochemical parameters (except lipoprotein subfractions) were measured in certified hospital laboratory (SYNLAB Bratislava, Slovakia) using autoanalyzer Beckman Coulter AU (Beckman Coulter, Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA). Serum insulin concentrations were measured using Chemiluminescent Microparticle Immunoassay (CMIA; ARCHITECT Insulin: Abbott Laboratories Diagnostics Division Abbott Park, IL 60064 USA). Serum glucose levels were measured by spectrophotometry method using Beckman Coulter AU analyzers. Index of insulin resistance HOMA-IR was calculated from fasting glucose and insulin concentrations. Fasting serum total cholesterol (T-chol), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, and triglyceride (TG) levels were measured with enzymatic color assay for quantitative determination using an autoanalyzer Beckman Coulter AU (Beckman Coulter, Inc., 250 S. Kraemer Blvd. Brea, CA 92821, USA). Thyroid stimulating hormone (TSH) and free thyroxine (fT4) were measured by immunoanalysis method ARCHITECT using CMIA Technology.

Atherogenic Index of Plasma (**AIP**) is a logarithmically transformed ratio of molar concentrations of triglycerides to HDL-cholesterol.

HDL and LDL subpopulations were separated and quantified using a commercial kit Lipoprint® kit (Quantimetrix Corp., (Redondo Beach, CA, USA), approved by Food and Drug Administration (FDA) as a diagnostic tool. This method allows to separate: the LDL subfractions (1 and 2 are large LDL and 3–7 subfractions are small dense LDL), the HDL subfractions (large, intermediate, and small), very low-density lipoprotein (VLDL) fractions, as well as the intermediate-density lipoprotein (IDL) C, B and A.

Physical activity level (PAL) at baseline was assessed by the Slovak version of the Lagerros questionnaire [20], from which the energy expenditure was estimated thereafter. Physical activity was estimated during a standard week day using a questionnaire with nine physical activity steps with gradually rising physical activity according to intensity. Each level exemplifies a typical activity and represents a value expressed as a multiple of Metabolic Energy Turnover (MET). The MET values were designed to the nine different levels were 0.9, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0 and 8.0. Volunteers were instructed to report the time spent on each intensity level during an average day and night, hence total physical activity time should add up to 24 h and allow for an estimate of MET*hours per day (METh/day). The total physical activity score was calculated as the sum of the individual level activities [20].

VO_2 max measurement

Maximal oxygen consumption (VO2 max), an indicator of cadiorespiratory fitness was measured using the ramp protocol [21] for cycle ergospirometer (PowerCube, Ganshorn Medizin Electronic GmbH, Germany). The testing started with a three-minute warm-up without load, then the continuous increase ramp was set to achieve the predicted maximum load after 10 min of exercise. The test was finished when the subject could not continue to exercise at required load level or due to medical contraindication (dizziness, pain, systolic blood pressure above 200 mmHg, Electrocardiography (ECG) markers of cardiac ischemia, significant presence of arrhythmias). Blood pressure was measured before the test started and then in 2 min intervals. The fitness level was assessed as amount of oxygen consumption per minute per kg of body weight at the time of maximally achieved load. Anaerobic threshold was determined by the V-slope method.

Intervention

The subjects had to undergo an 8-week weight loss intervention program, including caloric intake reduced by 30% of weight maintenance calories and 150 min per week of moderate to intensive aerobic exercise. The investigator provided detailed instructions and counseling with each participant individually about life style changes– personalized plan of physical activity and each volunteer obtained individualized nutritional plan made using software PLANEAT (www.planeat.io). Nutritional plan contains general instructions how to choose the right foodstuff (groceries), about healthy choices of macronutrients as well as 15–20 different options for breakfast, lunch, dinner as well as tea time food individually made according to calories, food intolerance, allergies, etc. Food plan contains also recipes, educational graphs with macronutrients calories, glycemic index, glycemic load, amount of cholesterol, as well as a shopping list.

The subjects had to exercise using endurance (100−120 min/week) and resistance training (50−30 min/week). Our patients received individual training plan and guidance on increasing their level of physical activity. Endurance exercise (such as walking, jogging, aerobic ball games) was recommended as a way to increase aerobic capacity and improve cardiorespiratory fitness. Our patients had part of the training also a exercise with free weights or traditional muscle building equipment, or progressive, individually tailored, circuit-type resistance-training sessions with the aim of improving the functional capacity and strength of the large muscle groups. They were instructed to perform a moderate to high number of repetitions and to take a break of 15–60 seconds between the stations on the circuit. They were instructed to perform a moderate to high (6–15) number of repetitions. More than 1/2 of our subjects exercised under the supervision of an experienced trainer (B. Bajer, MD), or at different fitness centers according the individual training plan supervised by the staff.

Statistical analyses

For statistical analysis we used the Statistic Program (SPSS, version 22.0, Chicago, IL, USA). The parametric variables are presented as mean ± standard deviation and the non-parametric variables as median (interquartile range). We used the Wilcoxon Signed Ranks Test and the paired Student's t-test to evaluate the effect of intervention. To evaluate relationships between data sets we used Spearman's rank correlation. A p value lower than 0.05 was considered statistically significant.

Results

Forty-one patients (30F/11M, mean age 44 ± 12 yrs.; F: 44 ± 12, M: 45 ± 10 years) completed the study. At baseline we diagnosed 1 patient with impaired fasting glucose, 4 patients with impaired glucose tolerance (IGT), and 4 patients with postprandial glucose concentration higher than 11.0 mmol/l. After the intervention fasting or postprandial glucose levels decreased, one patient remained type 2 diabetic patient, however with decreased postprandial

Table 1
Clinical and anthropometrical parameters before and after 8 weeks of intervention. BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist to hip circumference ratio; BPsys, systolic blood pressure; BPdia, diastolic blood pressure; TSH, thyroid stimulating hormone; fT4, free thyroxine; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TG, triglycerides. Values are presented as mean ± standard deviation or median (25th–75th percentile), if data were non-parametric.

	Baseline	After 8. weeks	p=
Age (yrs.)	44 ± 12	–	–
Gender (M/F)	11/30	–	–
BMI (kg/m^2)	30.9 ± 6.9	28.7 ± 6.4	<0.001
WC (cm)	98 ± 17	91 ± 14	<0.001
HC (cm)	110 ± 12	106 ± 11	<0.001
WHR	0.89 ± 0,10	0.85 ± 009	<0.001
Body fat %	35.6 ± 7.0	29.9 ± 7.5	<0.001
Fat mass (kg)	31.2 ± 11.2	25.0 ± 10.8	<0.001
Fat free mass (kg)	56.1 ± 15.2	56.6 ± 13.7	ns.
BPsys (mmHg)	126 ± 14	117 ± 19	<0.001
BPdia (mmHg)	72 (68-80)	70 (67–79)	0.03
TSH (mIU/l)	2.01 ± 1.28	1.67 ± 0.79	0.03
fT4 (pmol/l)	13.5 ± 2.9	13.5 ± 2.4	ns.
Heart rate (1/min)	74 ± 13	70 ± 12	0.03
Total cholesterol (mmol/l)	4.92 (4.18–5.73)	4.32 (3.93–5.52)	0.006
LDL cholesterol (mmol/l)	3.11 (2.55–3.83)	2.77 (2.39–3.54)	0.004
HDL cholesterol (mmol/l)	1.47 ± 0.38	1.40 ± 0.32	0.03
TG (mmol/l)	0.94 (0.62–1.44)	0.80 (0.56–1.16)	0.03

glycemia by approximately 50% and in 2 patients remained IGT. According to the International Diabetes Federation (IDF) diagnostic criteria for metabolic syndrome (MS) [22], 8 patients displayed at least 3 features of MS. On average patients spent 74 ± 11% of the day in sedentary behaviors, only 5 patients spent less than 60% of the day in sedentary behaviors. Therefore majority (88%) of our patients can be considered as sedentary [23]. All subjects were clinically euthyroid, baseline TSH was in normal range, and after the intervention TSH significantly decreased (p = 0.04; Table 1), and fT4 remained stable (p = 0.87; Table 1).

Average weight loss was 5.4 ± 4.5 kg (range: 0–14.5 kg; p = 0.0006). Body fat percentage, as well as fat mass, waist and hip circumference, as well as WHR significantly decreased (p < 0.001 for all parameters). However, amount of lean body mass remained stable (p = 0.45, Table 1).

Measurement of physical fitness (VO_2max) was terminated earlier (before reaching exhaustion) in 4 patients due to dizziness, or high blood pressure, or due to arrhythmias during the test. Physical fitness significantly improved after the intervention (as measured by VO_2 max: 25.5 ± 5.8 vs. 28.6 ± 5.2 ml.kg^{-1}. min^{-1}, p ≤ 0.001).

REE was an average 1642 ± 485 before the intervention and 1638 ± 448 kcal. 24h^{-1} after the intervention, without significant changes. Metabolic substrate preference (RQ = VCO_2 / VO_2) was an average 0.84 ± 0.08 before and 0.81 ± 0.06 after the intervention (p = 0.03).

Systolic, as well as diastolic blood pressure, and heart rate significantly decreased after intervention, respectively (p < 0.001, p = 0.03; p = 0.03, Table 1).

Fasting serum glucose decreased after the intervention (4.57 (3.90–4.65) vs. 4.10 (3.70–4.50) mmol/l; p = 0.05), as well as fasting plasma insulin decreased significantly (7.03 (4.65–9.7) vs. 4.9 (3.60–6.95) μIU/ml; p = 0.02). Insulin resistance index HOMA-IR significantly decreased after the intervention (1.30 (0.90–1.95) vs. 0.85(0.64–1.40); p = 0.01).

Total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides significantly decreased after intervention, respectively (p = 0.02, p = 0.02, p = 0.02, p = 0.03; Fig. 1).

AIP had tendency to decrease after the intervention, however it did not reach statistical significance (–0.17 ± 0.30 vs. –0.21 ± 0.26; p = 0.08).

When we looked at the LDL subfractions, only LDL2 subfractions significantly decreased (p = 0.007; Fig. 2). In 90% of patients LDL3-7 concentrations were undetectable (only 4 patients before and only 2 patients after the intervention had measurable levels, therefore it cannot be further analyzed).

From HDL subfractions only small HDL decreased significantly (p = 0.002, Fig. 2), while large and intermediate HDL remained stable. Other parameters, as VLDL, IDLA, IDLB, IDLC, LDL1, LDL3-7 did not changed after intervention (Fig. 2).

Correlations

Change of body weight positively correlated with the change of LDL-c level (r = 0.329, p = 0.036), as well as with the change in TG levels (r = 0.382, p = 0.013), however not with the change in LDL2 lipoprotein subfractions (r = 0.04, p = 0.665). Change in TG levels negatively correlated with baseline BMI (r = –0.384, p = 0.013).

Change in VO_2max correlated negatively with LDL-c cholesterol level (r = –0.400, p = 0.02), but not with a change of HDL-c (r = –0.215, p = 0.190).Change in VO_2max had a tendency to be associated with a change in body weight, however it did not reach the statistical significance (r = –0.312, p = 0.07; Fig. 3).

Discussion

In our study we found that weight loss achieved by eight weeks of life style intervention– diet and physical activity - in subjects with central obesity led to the improvement of insulin sensitivity, decreased blood pressure, decreased total LDL-c and TG concentration and last but not least to the improvement of the lipid profile by altering the lipoprotein fractions composition.

The composition of LDL-c and HDL-c lipoprotein fractions described as the concentration of its subfractions, appears to be a more appropriate CVD risk marker than the total concentration of LDL or HDL [24]. Regarding the LDL subclasses, we demonstrated reduction in concentration of medium LDL particles (LDL 2). There is substantial body of evidence that concentration of small dense LDL particles (LDL 3–7) is positively associated with risk of coronary heart disease [24,25]. However, medium LDL or LDL2 subfractions have been also identified as independent predictor of myocardial infarction and are believed to be highly atherogenic [10]. Reduction of medium LDL that we observed represents the shift of LDL fraction composition towards larger LDL particles. However, it is not clear if this change can be considered atheroprotective, it seems that LDL2 subfraction has double role in the atherogenic process depending on the phenotype of individuals [26]. That is in concordance with the decrease of TG concentration since it correlates negatively with LDL particle size [27]. In HDL class of particles, we found significant reduction in concentration of small HDL subclasses.

To our knowledge, linear electrophoresis using Lipoprint® Lipoprotein Subfractions analytic system was used in only 3 studies mostly dealing with the effect of weight loss after bariatric surgery on the HDL and LDL subclasses. Coimbra et al. [18] reported that bariatric surgery in patients with BMI > 50 kg/m^2 led to atheroprotective changes in lipid profile, increased amount of larger HDL and decreased small HDL, which are less protective subfractions. In another study, Kjellmo et al. [17] found decreased LDL-c and large LDL subfractions, and increased HDL-c and large HDL subfractions. However, they were not able to detect any effect on medium LDL or small LDL subfractions one year after the surgical intervention. These results are in contrast with our data. In another study [16], following morbidly obese patients one year after Roux-en-Y gastric bypass, no significant changes in LDL subfractions were observed. On the other hand, a significant shift towards antiatherogenic larger HDL2, without any significant changes in total HDL-c levels, and

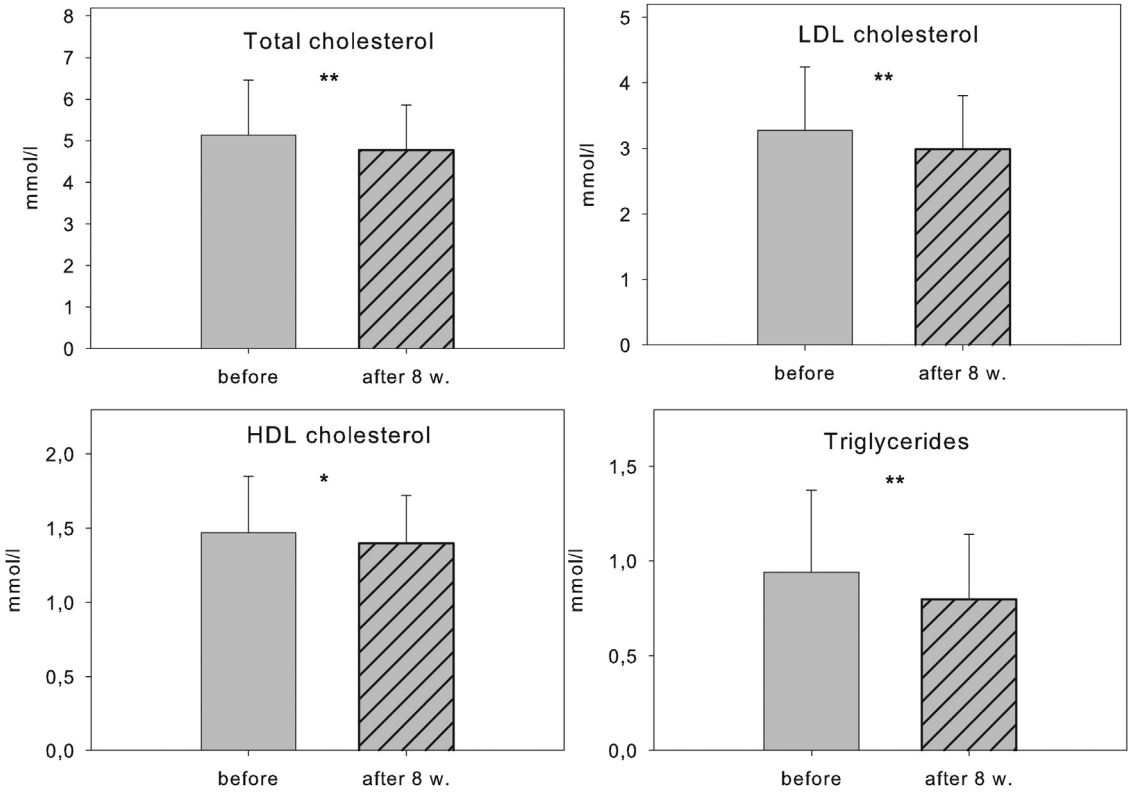

Fig. 1. Lipid profile: Total cholesterol, HDL and LDL cholesterol, triglycerides before and after 8 weeks of intervention. HDL, high-density lipoprotein, LDL, low-density lipoprotein, TG, triglycerides. *p < 0.05; **p < 0.01.

reduction of the oxidized LDL, thus lowering of the lipoprotein atherogenicity was observed.

In another interventional study in patients with metabolic syndrome with 3 weeks intensive program (physical exercise 90 min/day and diet –500 kCal/day) and 1 year follow-up, the authors showed significant improvements in lipoprotein subfractions (increase in LDL size, decrease of small dense LDL, increase of large HDL, decrease of small HDL) after the first 3 weeks of the program. However, after a 12-months follow-up period, the values failed to reach the control group and trended to their baseline levels, but still remained better than before the intervention. Only large HDL increased throughout the 1 year follow-up period [15]. This study in obese patients suggests that the greatest impact of weight loss intervention on lipoprotein subfractions may manifest shortly after beginning of intervention. HDL-c has atheroprotective properties that are primarily ascribed to its important role in reverse cholesterol transport and blockading of macrophage foam cell formation. Cholesterol is removed from macrophages and other cells in the vessel wall and transported to the liver; this leads to reduced LDL accumulation, and helps to prevent atherosclerosis. Therefore HDL-c contributes to reducing the CVD risk [28]. HDL cholesterol had been long time considered as a primary therapeutic target for lowering the risk of a CVD event. The effective HDL-raising drugs were developed (e.g. Cholesteryl ester transfer protein (CETP) inhibitors), however many clinical trials showed that even when the HDL increased, this change in lipid profile did not lead to reduced numbers of CVD events. Nowadays the HDL-raising drugs are not recommended in guidelines for treatment of dyslipidemia [29]. Only lifestyle changes that increase HDL-C are also associated with reduced risk of CVD [29]. However these facts highlight the complexity of HDL particle metabolism, structure, and function.

Weight loss after more invasive bariatric surgeries are linked with lowering of LDL-c [30], that is not present with restrictive bariatric surgery or lifestyle changes [31,32]. Some studies showed that weight loss after lifestyle intervention, may not be enough to reduce LDL-c levels and LDL particles, however for reducing CVD risk it is important to have less oxidized LDL particles. The effect of weight loss after bariatric surgery seems to be able to increase HDL-c levels and mend HDL subpopulations profile onto atheroprotection. In our study we have not found decreased HDL-c levels after lifestyle intervention. Our patients with obesity have reached median >7% of weight loss and showed indeed lower LDL2 and small HDL subfractions. It seems, that weight contributes mainly to the quality of LDL-c and, therefore decreases the CVD risk.

The different techniques used for evaluation of LDL and HDL subclasses as well as their different terminology and characterization, may be responsible for the conflicting outcomes on the effect of weight loss on lipoproteins, not to mention the different types and duration of the intervention strategies, the participating subjects with different age, grade of obesity, comorbidities, etc. [33]. Sedentary lifestyle and lack of physical activity contribute to the increase prevalence of chronic non-communicable diseases [34], with increasing evidence that the risk of those diseases is related to the degree of inactivity. It is clear that physical activity is not the one and only positive factor with favorable health effects, even diminishing the time spent with sedentary activities may reduce the development of chronic diseases [34]. Subjects practicing any kind of physical activity (light, moderate, vigorous) had lower risk for CVD mortality, regardless of their cardiometabolic risk factors [13]. Even 20 min exercise performed for only 4 days by sedentary subjects might have a positive effect on fasting as well as postprandial lipoprotein profile, decrease of small HDL and LDL2 subfractions [35]. These findings are in agreement with our study. Decrease in HDL cholesterol, neither in small HDL subfractions in our study was

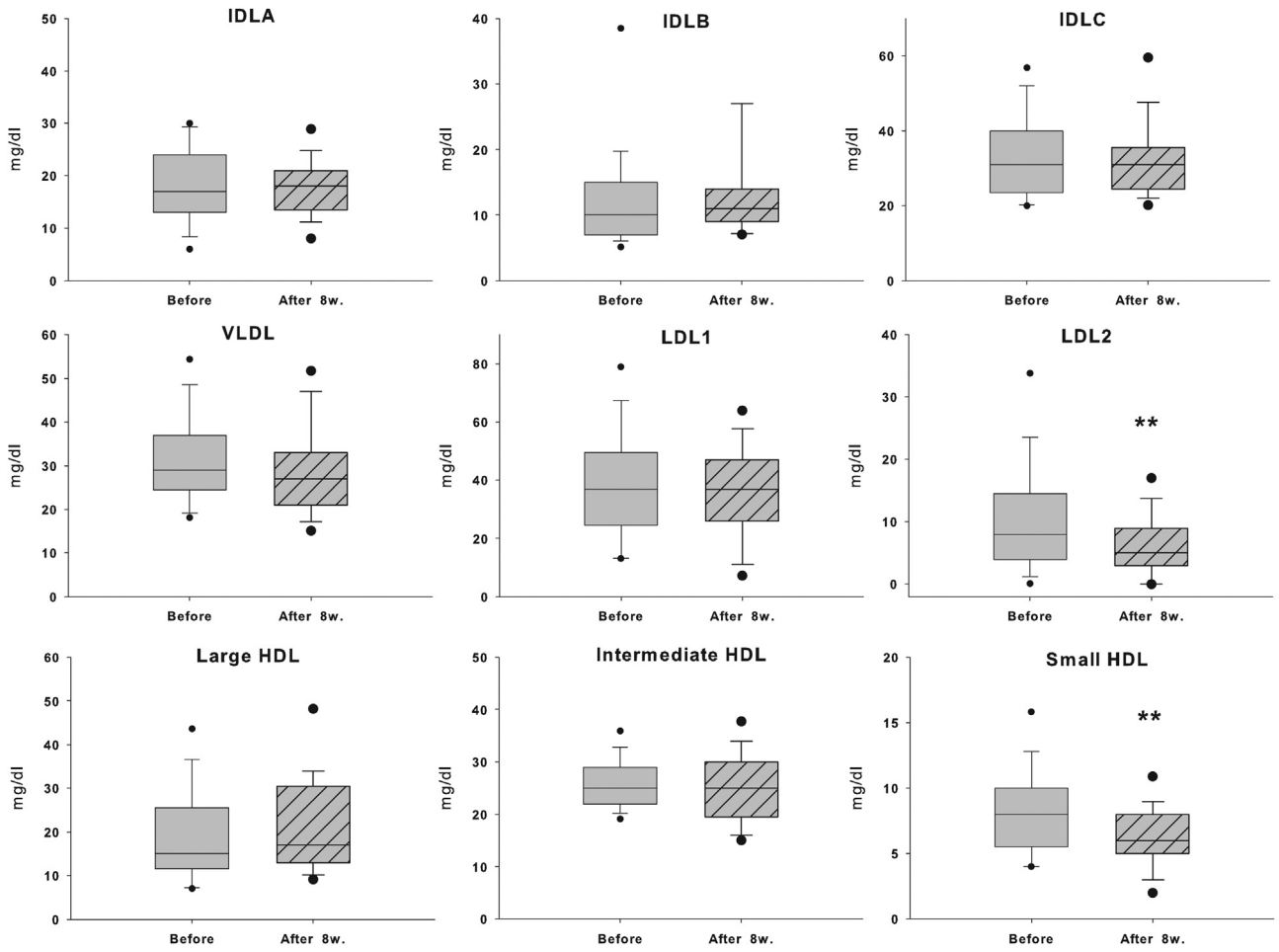

Fig. 2. Lipoprotein subfractions before and after 8 weeks of intervention. HDL (high-density lipoproteins), LDL (low-density lipoproteins), VLDL (very low-density lipoproteins), IDL (intermediate density lipoproteins). Results are presented as box-and whisker plots; the box is the range between the lower and upper quartile, the horizontal line is the median, and the whiskers are the minimum and maximum, the dots are outliers. $^{**}p < 0.01$.

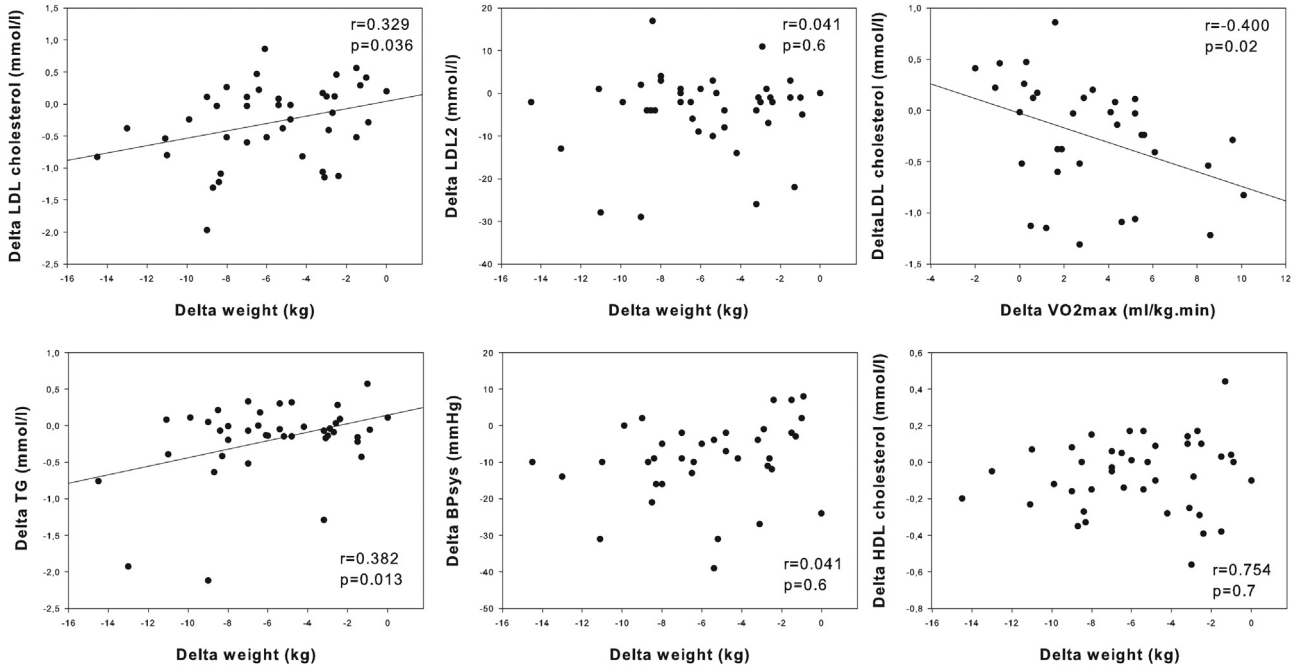

Fig. 3. Changes in LDL and LDL2 subfraction in association with changes of body weight or physical fitness (VO2max). HDL (high-density lipoprotein), LDL (low-density lipoprotein), TG (triglycerides), BPsys (systolic blood pressure), VO_2max- maximal oxygen consumption.

not associated with body weight reduction nor with the physical fitness improvement. Results of metaanalysis consisting of 170,000 participants show that regular physical activity increases HDL- c without changes in LDL-c and TG, and may theoretically even compensate the increase in LDL- c and TG [36]. It has been proven that independently of aerobic exercise, resistance exercise of even less than 1 h/week, was related to decreased risk of hypercholesterolemia and metabolic syndrome. Therefore resistance exercise along with aerobic training, as performed by volunteers in our study, should be recommended for individuals'physical activities for prevention of metabolic syndrome and related cardiovascular complications [37,38]. Moderate regular exercise without dietary modifications does not lead to satisfying weight loss. However, even without achieving significant weight loss, exercise alone has cardiometabolic benefits in improving insulin sensitivity, preventing weight gain and promoting weight maintenance [39].

The major limitation of our study is the small number of participants. Further bigger clinical trials with life style intervention are necessary to investigate the effect of weight loss on lipoprotein size, composition and functionality.

Second limitation is the lack of reference method to measure lipoprotein subfractions, and therefore results from different studies reveal inconsistent conclusions.

Majority of our study subjects (76%) were middle aged women, which are in fact highly motivated to change their body weight. Another limitation might be a fact, that we did not measure the exact amount of physical activity during intervention. We also did not objectively measure the particular domains of sedentary behavior (to sit at work, watch TV, play video games, read, etc.). There is a need to combine the self-report data from patients with objective measurements of different sedentary activities in future studies [34].

Conclusions

Our data shows that 8-weeks of lifestyle intervention, diet and increased physical activity favored a reduction in LDL and TG as well as in small HDL subfraction. Moreover, we observed that weight loss had a beneficial impact on insulin sensitivity, blood pressure, heart rate as well as physical fitness, thus led to improvement of many CVD risk factors. Decrease of concentration in small HDL subfraction together with decrease in LDL and TG might be seen as beneficial and atheroprotective. However, whether lipoprotein subfractions will be a useful biomarker of atherogenesis or a cardiovascular risk factor will require further studies.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AP, BB, MV have participated in study conception and design; ZR, MV, RI, AH and AP have been responsible for acquisition of data, analysis and interpretation of data; BB, ZR, MV, RI and AP have been involved in drafting the paper; AP, MV, IZ, PS, MB and ZR revised the final draft of manuscript for important intellectual content. All authors read and approved the manuscript.

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