HAROKOPIO UNIVERSITY

SCHOOL OF HEALTH SCIENCE AND EDUCATION

DEPARTMENT OF NUTRITION AND DIETETICS

APPLIED NUTRITION AND DIETETICS

CLINICAL NUTRITION

Effect of 8 weeks of aerobic training with or without aronia juice consumption on cardiovascular risk factors and physical self-description of young healthy women

Master thesis

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δηλώνω υπεύθυνα ότι:

1) Είμαι ο κάτοχος των πνευματικών δικαιωμάτων της πρωτότυπης αυτής εργασίας και από όσο γνωρίζω η εργασία μου δε συκοφαντεί πρόσωπα, ούτε προσβάλει τα πνευματικά δικαιώματα τρίτων.

2) Αποδέχομαι ότι η ΒΚΠ μπορεί, χωρίς να αλλάξει το περιεχόμενο της εργασίας μου, να τη διαθέσει σε ηλεκτρονική μορφή μέσα από τη ψηφιακή Βιβλιοθήκη της, να την αντιγράψει σε οποιοδήποτε μέσο ή/και σε οποιοδήποτε μορφότυπο καθώς και να κρατά περισσότερα από ένα αντίγραφα για λόγους συντήρησης και ασφάλειας.

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ABSTRACT

Background: Evidence suggests that young women are prone to unhealthy eating habits, sedentarism, eating disorders and body image distortion, some being cardiovascular disease (CVD) risk factors and others impairing life quality.

Aim: To examine whether 8 weeks of aerobic dance (3 times a week for 60 min), alone or combined with daily ingestion of antioxidant-rich aronia juice is beneficial for CVD risk factors and physical self-concept (PSC) in young sedentary healthy women.

Methods: Randomized controlled intervention clinical trial was performed. Recruitment took place through advertisements on social networks and word of mouth. Exclusion criteria were: smoking, regular physical activity for more than 1,5 hour a week, medication or weight loss treatment, diagnosis of cardiovascular or metabolic disease, recent surgery, blood donation, pregnancy or lactation. Of 55 participants screened for eligibility, 43 healthy sedentary non-obese young women, aged 19-29 years, were finally included and randomly assigned to one of three 8-week treatments: 1) Body Combat aerobic training with daily aronia juice (100 ml) consumption (BCA group) 2) Body Combat aerobic training only (BC group) and 3) no treatment (control group (CON)). Thirty eight participants completed the study and included in the final analysis. Drop-out rate was 12% (n=5). Anthropometric, total and segmental body composition biompedance measurements and blood sampling were obtained after an overnight fast and 3-hour abstinence from water intake or recent training, both pre- and post-intervention. General demographic, dietary (food frequency questionnaire and 24 hrs recall) and physical self-description (PSDQ) questionnaires were handed out at the same occasions.

Results: Between and within the group analysis revealed no significant differences in the majority of the anthropometric, total or segmental body composition, biochemical or hematological variables examined. Only left leg fat percentage (LL-F%), visceral fat rating, lactate dehydrogenase, erythrocytes blood count (together with several hematological variables related to it) and averaged scores of PSDQ scales Health Condition, Endurance and Fatness differed significantly between the groups due to the intervention. Within the group analysis revealed that in BCA group all PSDQ scales improved significantly, while the BC group improved on 8 out of 11 examined scales and dimensions. Control group improved only in the General Physical Condition dimension. All groups significantly decreased body fat mass, fat percentage of lower limbs, hemoglobin, mean corpuscular hemoglobin concentration and

increased fat free mass, total muscle mass, body water content, muscle mass of lower limbs and basic metabolic rate. Central body muscle mass improved significantly in both exercising groups but not in the control group.
Conclusion: Intervention improved visceral fat rating, LL-F% and several components of young women's PSC. Its potential to prevent CVD remains to be proven in further analyses with a closer monitoring of the dietary intake, menstrual cycle and training intensity.

Keywords: Body Combat, body composition, aronia, women, physical self-concept

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ABBREVIATION LIST

ACSM	American College of Sports Medicine
AHA	American Heart Association
ALT	Alanine transferase
ANOVA	Analysis of variance
AST	Aspartate transferase
BC	Body Combat training intervention group
BCA	Body Combat training and aronia juice consumption intervention group
BF%	Total body fat percentage
BFM	Total body fat mass
BM	Bone mass
BW%	Body water percentage
BWM	Body water mass
CB-F%	Central body fat percentage
CB-MM	Central body muscle mass
CENM	Center of Research Excellence in Nutrition and Metabolism
CHD	Coronary heart disease
CON	Control group of participants
CVD	Cardiovascular diseases
FC	Folded change
FFM	Fat-free mass
FM	Fat mass
Gluc	Fasting blood glucose
GP	General practitioner
GRA	Granulocytes
HbA1c	Glycosylated hemoglobin
HCT	hematocrit
HDL-C	High density lipoprotein cholesterol
HGB	hemoglobin
HRmax	Maximum heart rate for age
LA- F%	Left arm fat percentage
LA-MM	Left arm muscle mass

LDH	Lactate dehydrogenase
LDL-C	Low density lipoprotein cholesterol
LE	Leukocytes
LL-F%	Left leg fat percentage
LL-MM	Left leg muscle mass
LYM	Lymphocytes
MCHC	Mean corpuscular hemoglobin concentration
METs	Metabolic equivalents
MM	Muscle mass
MON	monocytes
NAFLD	Nonalcoholic fatty liver disease
NHANES	National Health and Nutrition Examination Survey
PA	Physical activity
PSC	Physical self-concept
PSDQ	Physical self-description questionnaire
PSPP	Physical self-perception profile instrument
Q	quartile
RA- F%	Right arm fat percentage
RA-MM	Right arm muscle mass
RBC	Erythrocytes (red blood cell) count
RDW	Red blood cells distribution width
RL- F%	Right leg fat percentage
RL-MM	Right leg muscle mass
SD	Standard deviation
TE	Total energy intake
TNFα	Tumor necrosis factor alpha
T2DM	Type 2 diabetes mellitus
TC	Total blood cholesterol
TG	Fasting blood triglycerides
VO_{2max}	Maximum oxygen consumption capacity
WHO	World Health Organization

1. INTRODUCTION

1.1. NON-COMMUNICABLE DISEASE RISK IN YOUNG ADULTS

Non-communicable diseases are major cause of mortality and morbidity in developed and developing countries nowadays. In Serbia, Southeastern European country in transition, non-communicable diseases account for two thirds of all deaths. Out of these, 53% of deaths are caused by cardiovascular impairments, followed by various cancers (1). Data from National Health and Nutrition Examination Survey (NHANES) 2003-2006 in United States (2) reveal the presence of metabolic syndrome (defined as a constellation of several risk factors: abdominal obesity, dyslipidemia, hyperglycemia, and high blood pressure) in 20% of young males and 16% of young females, pointing to an increased risk for its development over time.

Since the origin of non-communicable diseases is multifactorial, with age, gender and genetics being non-modifiable risk factors, and lifestyle factors such as smoking, physical activity, nutrition, body weight and body fat being modifiable risk factors, a lot can be done with proper and timely interventions. This could possibly slow down or even reverse the negative trend, revealed by the data from local and international epidemiological and clinical studies which show that the majority of young adults does not comply with the official Guidelines for healthy eating and physical activity (3-5).

Young adults in Serbia, especially those on University level spend on average 6 hours a day sedentarily (6) and most of them, especially females do not engage in regular physical activity, therefore failing to fulfill the minimum recommendations (3-5, 7), of at least 150 min of moderate or 60-75 min of vigorous physical activity weekly. Neither their nutrition patterns comply with the World Health Organization (WHO) recommendations for sufficient intake of fruit, vegetables and carbohydrates. This is not surprising, having in mind that only every second inhabitant of Serbia walks 30 minutes a day (45,8% of women vs. 54,9% men, according to the 2014 annual report of Institute of Public Health of Serbia (6)). These findings are consistent with the data for the U.S. population in 2005, reported by the American Colleague of Sports Medicine (ACSM) and American Heart Association (AHA) in 2007 (7), where only 50,7% of men and 47,9% of women meet the recommended levels of physical activity. Moreover, local data suggest that only every second Serbian woman consumes fruit every day. Only 5,3% of Serbian women engage in organized recreational physical activities for at least 90 minutes a week (6).

Notably, Serbian Public Health Survey 2006 showed that the greatest increase in obesity is observed at the age 18– 29 years during the transition from adolescence to adulthood and therefore a group of Serbian authors (8) decided to conduct a cross-sectional study which showed that being overweight and having irregular macronutrient composition of diet, with higher fat intake and lower fruit intake is more often in young adults living alone and preparing their meals on their own or eating in college cantina, than in those eating at parental home. In addition, fruit intake was found to be inversely related to the body fat %. The latter had mean value of 27% and slightly exceeded the average for that age (9). Another group of Serbian authors (10), analyzed obesity prevalence in student population of Novi Sad in 2012 and classified 21% of their sample as overweight.

It should also be noted, that transition from high school to university or working environment is followed (at least in Serbia) by the exclusion of compulsory hours of physical education, increased workload and duties, more time spent in sedentary activities and lack of free time for leisure physical activities. Thus, young adulthood may be a challenging period for body weight, body composition and food choices (8), leading at the same time to a higher risk of distorted body image and general impairment of physical and psychological health (11) in susceptible individuals. Absence of sufficient physical activity, insufficient ingestion of fruit, development of obesity and increased body fat could thus predispose young women for the cardiovascular disease onset in adulthood (12) or impair their current body image (13-15).

1.2. PHYSICAL ACTIVITY HEALTH BENEFITS AND ADHERENCE THEORIES

1.2.1. PHYSICAL ACTIVITY AND CARDIOMETABOLIC AND PSYCHOSOCIAL BENEFITS

There is a compelling body of evidence suggesting beneficial effects of regular physical activity on the overall health and well-being (4, 5). ACSM, 2014 (4) reports of strong or moderate-strength evidence for dose- response relationship between physical activity and: all-cause mortality, cardiovascular health (coronary vascular disease (CVD) or coronary artery disease (CAD), hypertension, stroke), metabolic health (type 2 diabetes mellitus (T2DM), metabolic syndrome), energy balance, weight loss, weight maintenance, abdominal obesity, muscle and bone health (osteoporosis), functional health, breast and colon cancers as well as mental health (depression and distress) along with improvement of cognitive function.

Regarding cardiovascular disease risk factors, literature suggest: reducing systolic blood pressure (SBP), diastolic blood pressure (DBP), increasing serum levels of high density lipoprotein cholesterol (HDL-C), decreasing serum triglycerides (TG), reducing body fat percentage (BF %), intra-abdominal fat, improving glucose tolerance, reducing platelet activation and aggregation as crucial for decreasing mortality, morbidity and lower incidence rates for CVD, CAD, stroke, T2DM and metabolic syndrome (4, 5, 16).

A meta-analysis (17) which included randomized controlled trials from the period of 1995-2003– with aerobic exercise (with average intensity of 69% VO_{2max}) lasting for at least 8 weeks and with the main outcome being impact on blood lipids and lipoproteins in women (≥ 18 years old, BMI=25,5 kg/m^2) also found significant reduction in total cholesterol (TC) by 2%, low density lipoprotein cholesterol (LDL-C) by 3%, TG by 5%, whereas HDL-C increased by 3%. Secondary outcome was statistically significant decrease in both body weight and BMI by approximately 2%, whereas body fat reduction was approximately 4%.

Evidence also exists for potential increase in antioxidant capacity or reduced inflammation when performing exercise regularly. In particular, a group of authors (18) found reduction in systemic oxidative stress after 4 months of aerobic exercise, but only in young women within the highest quartile of baseline levels of F2 isoprostanes, as indicators of lipid peroxidation. Reduced inflammation was also documented in several other studies (16, 19, 20).

Eventually, recently published reports by the ACSM and the British Association for Sports and Exercise Science (4, 5), as well as reports published in preceding decades (21-23) point out beneficial effects of exercise on mental health. While the former underline decrease in anxiety and depression (21-23) together with increase in feeling of well-being, the latter additionally suggest improvement in self-esteem (21-23), physical self-satisfaction and body image, sleep quality (21), reduction of aggression and youth delinquency (23), better adherence to alcohol abstinence (22), improved tolerance and stress coping (21), as well as reduction in coronary prone (Type A) behavior (22). This further implies that exercise may diminish cardiovascular risk (21, 22) not only via physiological but also via psychological risk factor improvement. The aforementioned psychosocial benefits of regular exercise may lead to ultimate goals of each individual: longevity, overall satisfaction and greater life quality.

In addition to the above mentioned reviews, metanalysis, reports, intervention studies and population based recommendations which all focus on the regularly performed exercise, there are review (24) and intervention studies (25-27) that documented the beneficial effects even when one bout of exercise is applied. The latter is capable of lowering the blood pressure, thus causing a “post-exercise hypotension”(PEH), which can persist for up to 16h (24). Evidence was also presented that a single exercise session causes improvement in lipid and lipoprotein profiles, namely, acute increase in HDL-C and acute decrease in TG. As for TG, this transient decrease can persist for up to 48-72h following the exercise session. Nevertheless, the acute effect of exercise on TG and HDL-C appears to depend on overall energy expenditure (24-27). Evidence suggests that exercise affects TG kinetics in women in a manner different than in men and that it cannot be reproduced by an equivalent diet-induced energy deficit. Moreover, authors speculate that an acute improvement in insulin sensitivity and glucose homeostasis may depend on exercise intensity and is elicited with the exercise nearing 70% of VO_{2max} (24).

1.2.2. PHYSICAL ACTIVITY AND BODY COMPOSITION

In addition to increased aerobic capacity (measured as VO_{2peak}) and increased energy expenditure, many of the functional body benefits of physical activity (PA) are expressed through more favorable body composition (16) of regularly active individuals. Increase in the proportion of muscle mass (being metabolically more active than fat) associated with regular exercise is not of surprise, since physical activity triggers the increase of GLUT4 glucose transporter expression and its translocation to outer membrane layer of muscle cells (24) which together with improved insulin sensitivity could enhance “anabolic reactions” through facilitated influx of nutrients necessary for the building of the muscle mass. In the same time, while fat depots supply the body with the necessary energy during prolonged exercise, their extent may become smaller over time.

Cross-sectional studies (28, 29) in a large sample of adults aged 18 to 98 years, found that physically active individuals were more likely to have low body fat mass index (BFMI) and less likely to have high BFMI than their sedentary counterparts. Also, they were less likely to have abnormal (low or very high) fat-free mass index (FFMI). Another cross-sectional study (30) which specifically focused on young females (as we do) found statistically significantly lower

BF% in the regularly exercising group (18±1,6% vs. 23±4,0%) than in their sedentary counterparts (mean ages of 18 and 19 years, respectively).

When previously found (28, 29) association of body composition with activity level was followed up longitudinally (31) conclusion was met that physically active individuals were less likely to gain body fat (BF) when gaining weight and more likely to lose BF when losing weight (at least at 1 year of follow-up). This positive inverse correlation between physical activity (PA) and BF content remains significant after 1 year, but not after 3 years of follow-up.

1.2.3. PHYSICAL ACTIVITY AND OXIDATIVE STRESS

When addressing potential risks of PA in young adults, the ACSM 2014 (4) only mentioned sudden cardiac events and injuries. However, since the incidence of sudden cardiac arrest is regarded low in young adults, being caused in most cases by inherited disorders, physical exercise is generally regarded safe for healthy young individuals. In spite of all the benefit of exercise, chronic exercise represents also a form of oxidative stress. Namely, increased metabolic rate, 10- to 20-fold increased oxygen uptake and its consumption by muscle fibers as well as increased temperature and decreased pH during exercise could altogether favor the production of free radicals. It is speculated that in high intensity, strenuous, prolonged physical activity the production of reactive oxygen species (ROS) could exceed body’s endogenous antioxidant capacity (32). At the same time, mitochondrial ROS production in the muscle during exercise is programmed and required for the main signaling pathways for muscle adaptation to exercise, as reported in a review published in 2015 (33). Authors of this report point out the potential increase in antioxidant capacity by regular non-exhaustive exercising. In line with this, the previously mentioned study (18) discovered a reduction in systemic oxidative stress after 4 months of aerobic exercise in young women with baseline elevated levels of F2 isoprostanes. However, when it comes to increase in intensity or training volume, additional antioxidant ingestion is to be considered and that is the reason why we decided to combine aronia juice rich in antioxidants with an increase in training volume in our previously sedentary sample.

1.2.4. PHYSICAL ACTIVITY ADHERENCE – THEORIES AND DETERMINANTS

Evidence suggests that, although the health-related benefits of physical activity may be widely known, the compliance and adherence to physical activity is still low (6, 7). The ACSM (4) reports that even 50% of those individuals enrolled in physical activity programs will drop out during first six months. Epidemiological studies and studies exploring attitudes toward exercise showed that young adult females engage less in physical activity, have less positive attitudes toward physical activity and develop negative body image more often than males (11). Negative body image can be a barrier to participation in physical activity, eventually leading females to experience less joy and health and psychosocial benefits of physical activity than males.

Therefore, physical activity programs, especially those designed for females should include as many reinforcers of adherence and participation as possible. Based on various psychological theories [such as Theory of Planned Behavior (TPB) by Ajzen, I (34-37), Social Cognitive Theory (SCT) by Bandura, A.(38-40), Self-Determination Theory (SDT)(41), Trans Theoretical Model (TTM), Health Belief Model (HBM) (42)] which all examine the relationship between individuals determination (motivation) to exercise and objectively measured participation and adherence, ACSM 2014(4) concludes that special attention should be paid not only to attitudes, perceived benefits, barriers and relapse prevention but as well to the following determinants: **self-efficacy** (confidence that one is able to accomplish the task and develop **competence** and mastery), **autonomy** (e.g. choice of intensity), intrinsic motivation (**joy, pleasure, fun**), extrinsic motivation (e.g. exercising for one's **attractiveness** to be seen by others), **reinforcement** (acknowledgement, appraisal), **group/exercise leaders** (modeling, support and caring), **social support** (meaningful interactions with others within the frame of friend group exercising together where cohesion is enhanced by the sense of common goals, distinctiveness of group, exhibited by T-shirt uniforms for exercisers, outdoor events or organization of closed type parties for the exercise group) and **avoidance of boredom** (by offering challenge).

According to the studies conducted by Ekkekakis, P (43) special attention should be paid to affective reactions to exercise. Generally, lower and moderate intensity is associated with **positive affect** (44-48) during exercise and especially in beginners, but high intensity exercise or high intensity interval exercise may be associated with positive affect after the training is completed, giving a sense of accomplishment to (experienced) exercisers (49, 50). Authors speculate that high intensity interval aerobic training may give a rise to an overall positive affect, together with spending a lot of energy (51). Namely, high intensity bouts give the participants the feeling of “a job well done”, while low intensity intervals give them opportunity to recover for another high intensity interval.

Moreover, authors who examined the most and the least preferred context of engaging in aerobic physical activity, claim that female university students would rather choose exercising with others (group activity) than for exercising alone(52).

Having in mind these positive determinants, for our intervention we opted for a modern commercial aerobic dance program Body Combat, Les Mills company, New Zealand (53), which is performed in group, along with modern energetic music in order to increase feelings of pleasure and joy. Choreography consists of simple movements originating from martial arts, that may boost the sense of self-efficacy and competence more than choreography with regular non-martial arts movements (54), while the participants are constantly being verbally encouraged by the instructor to think of themselves as “modern strong tribe (group) of warriors who stay with the fight”. Choreography is changed every three months in order to avoid boredom. The instructor and music beat suggest the pace and intensity, but it is finally the matter of participants’ choice, since easier options are constantly shown.

In order to address affect, we aimed to classify intensity of Body Combat, so we implemented equations[1] which relate percentage of maximum heart rate at which exercise is performed (%HR_{max}), average energy expenditure and age of participants to the certain amount of metabolic equivalents (METs) (4). (For Body Combat in particular, the former two were determined in a study performed in 2007 (55)). Result of the equation classifies Body Combat as vigorous or moderate-to-high-intensity aerobic dance class for the average age of our study participants. Although Body Combat cannot be classified as typical high interval intermittent training (HIIT), it is indeed interval training with regular exchange of moderate and high intensity bouts, similarly as for continuous moderate intensity aerobic trainings and HIIT, which could raise the positive affect as well (51). Moreover, it is performed to music, which distracts attention from fatigue and thus reinforces positive affect (49).

[1] MET is the rate of EE while sitting at rest. By convention, 1 MET is equal to an oxygen uptake of 3,5 mL x kg^{-1} x min^{-1}); Equation that relates caloric expenditure to METs of certain activity: **kcal x min^{-1}=[(METs x 3,5 mL x kg^{-1} x min^{-1} x body weight (kg)) ÷1000) x 5] ,** as defined by 4. American College for Sports Medicine (ACSM). ACSM's Guidelines for Exercise Testing and Prescription. 9th ed. Baltimore: Wolters Kluwer Lippincott Williams & Wilkins, 2014.pg. 176

1.2.5. PHYSICAL SELF-CONCEPT AND PHYSICAL SELF-DESCRIPTION QUESTIONNAIRE (PSDQ)

Physical self-concept (PSC) is a multidimensional construct which reflects one's perception of its own self in physical appearance and health, physical components such as strength, endurance and sports competence. PSC association with exercise behavior, physical fitness, promotion of individual well-being is documented cross-culturally in numerous studies (56-60) and consensus exists among researches about the relevance of PSC for the development of an active and healthy lifestyle.

For the measurement of PSC, Marsh HW, Richards GE, Johnson S, Roche L and Tremayne P developed and published in 1994 (61) their Physical Self-Description Questionnaire (PSDQ) which assesses 9 specific scales of: Health, Strength, Endurance, Body Fat, Physical Activity, Sports Competence, Coordination, Appearance and Flexibility, and 2 general dimensions: General Physical Self-Concept and Self-Esteem. They documented its internal robustness and reliability as well as its consistency with the previously invented and widely used instruments (such as physical self-perception profile (PSPP) or its shorter version). PSDQ reliability and both internal and external consistency are validated in numerous studies conducted in different nationalities, age populations and languages (56-58, 60, 62).

In our study we wanted to explore whether the kind of exercise we chose may cause positive changes in previously mentioned determinants of positive exercise behavior (self-efficacy, attitudes etc.) as well as in certain indices of body image of females. We wanted to assess as many determinants as possible so we needed a locally validated and translated psychometric instrument capable of multi-trait measurement both pre and post-intervention. The choice was made for the Serbian version of Marsh's Physical Self-Description Questionnaire (61) recently (2014) validated in Serbian population of adolescents (60).

1.3. ARONIA MELANOCARPA (CHOKEBERRY) JUICE CONTENT AND HEALTH RELATED BENEFITS

1.3.1. CONTENT AND HISTORY OF USE

Although beneficial effects of regular physical activity and balanced diet with plenty of vegetables and fruit are wide-known, individuals still fail to comply with the recommendations. Moreover, medicines for the treatment of cardiometabolic risk factors are often accompanied by side-effects. Thus, science is turning to exploring the potential therapeutic effects of natural plant products.

Chokeberry (Aronia *melanocarpa*) and its juice, berries and lyophilized tablets, are among these plant products with promising health-promoting effects. Research on them has started quite recently, with the first laboratory reports of their potential useful effects being published in the mid 1990s (63, 64). Aronia is a shrub, native to North America, whose fruit is often used in Russian traditional medicine to attenuate atherosclerosis and hypertension. Nowadays, it has become very popular for its potent antioxidant properties and therefore it is often cultivated in countries of East and South-East Europe, like Poland and Serbia. Fruits of aronia are dark blue berries rich in vitamin C, E, K, Mg and fiber but their main health related advantage lies in their high polyphenol, anthocyanin content, which is regarded highest of the other berries (65, 66). The latter study showed that in comparison with blueberries, cranberries and lingonberries, chokeberries also exhibited the greatest ORAC (Oxygen Radical Absorbance Capacity) value. Polyphenols found in aronia are mostly proanthocyanidins and anthocyanins, followed by smaller quantities of flavonoids, quercetin, coumaric, cafeic and chlorgenic acid. Its high polyphenol content renders aronia protective against free radicals and beneficial for diseases caused (or worsened) by oxidative stress while cardiovascular and metabolic disorders are being just one of these (63, 67)

1.3.2. HEALTH BENEFITS OF ARONIA: three A (“AAA”)

Mechanisms by which aronia protects and improves health can be summarized by three A letters, „AAA“, standing for: Antioxidant, Antibacterial (67) and Anti-inflammatory. A review (63) of human clinical studies published up to 2009 indicates the following beneficial effects after acute (2-24 hours) or prolonged use (4-8 weeks or 12 weeks) of aronia products:

- Normalization of oxidized LDL (oLDL) in patients with increased levels
- Improvement in fasting blood glucose, HbA1c, TC, lipids, blood pressure
- Higher counts of CD4 and CD8 T lymphocytes
- Beneficial change in platelet aggregation and all blood lipids except for HDL-C
- Increase in superoxide dismutase (SOD) and gluthathione peroxidase (GP) activity
- Increase in antioxidant capacity and inhibition of lipid peroxidation
- Improvement in endothelial function and lipid metabolism

Summarizing the key cardio protective characteristics of aronia in recent clinical trials and laboratory studies up to 2016, Polish authors (68), also referred to its antioxidant, anti-inflammatory, anti-hyperlipidemic, vasorelaxant/hypotensive, antiplatelet/antithrombotic properties and their regenerative effect on endothelial progenitor cells.

A group of Serbian authors(69) found hypotensive and lipid lowering effects (significantly reduced TG level and reducing effect on TC and LDL-C) after 4-week administration of aronia juice in hypertensive but not pharmacologically treated subjects. The other trial with healthy women consuming aronia juice for 12 weeks (70) found fine modulation of pro-oxidant/ antioxidant balance, as well as a reduction in thiobarbituric acid reactive substances (TBARS) (the latter are habitually used as biomarkers of oxidative stress). The same year, evidence was provided for a significant decrease of systolic blood pressure, BMI and waist circumference, as well as a decrease in ratio between n-6 and n-3 polyunsaturated fatty acids in the membranes of erythrocytes of postmenopausal women with abdominal obesity who consumed glucomann-enriched aronia juice for 4 weeks (71).

Moreover, Boncheva, M and Turnovska, T in 2014 (72) showed that 2 months of aronia juice daily supplementation in overweight subjects with an increased waist circumference significantly changed blood levels of resistin, leptin anad adiponectin compared to their baseline value. Another study (73) showed that aronia may exhibit a thermo regulative effect by increasing plasma noradrenalin concentrations and inducing elevation in body surface temperature as well as attenuated feeling of cold in all four limbs and hips of healthy women with cold constitution.

Reviews on various laboratory ex vivo studies with human cells or experimental studies on rats or mice models report of aronia glucose and insulin regulating effect (67, 74, 75), lipid-lowering, antihyperlipidemic effects (76, 77), obesity and weight gain suppression (78), normalization of obesity-related disorders of neutrophils (79), anxiolytic and antidepressant effect (80-82), anti-inflammatory effect in eye (83), hepatoprotective effect with attenuation of de novo lipogenesis in mice model with non-alcoholic fatty liver disease (NAFLD) (84) as well as possible inhibitory effect on proliferation of liver cancer cells.

It seems that the mechanism by which aronia exhibits its favorable metabolic effects depends on its high content in proanthocyanidins which exert inhibitory effect on digestive enzymes, such as lipase (85), amylase (86), α-glucosidase or enzymes that cleave incretins such as dipeptyl peptidase IV (DPP IV) (75, 87). Some authors report on proanthocyanidins anti-obesity effects (86) and claim their activating effect on sympathetic nervous system innervation of brown adipose tissue followed by activation of uncoupling proteins (UCP1, UCP2, UCP3). Aronia potential for gene expression regulation (genes for PPARγ, GLUT1, GLUT4, FABP, FAS,LPL, IL1β, IL6 and TNFα) and modulating effect in insulin, adipogenic and inflammatory signaling pathways was also documented in rat models (75).

Moreover, according to a recent review of scientific literature (67) evidence is accumulated for the protective role of aronia against the toxic effects of various xenobiotics: pesticides (paraquat), fertilizers (nitrates), food preservatives(nitrosamines), tobacco smoke, heavy metals (for lead and cadmium trials were performed by the authors of the review) and against various medicines side effects, such as those used in chemotherapy (88), schizophrenia, antibiotics, paracetamol etc.

At the same time, aronia seems to confer a beneficial impact in the field of exercise and aesthetics as well. A Serbian group of authors led by Šavikin K, 2014 found improvement in cellulite morphology and reduction of edema in women who consumed aronia juice for 90 days (89). In order to address the augmenting effect of excessive strenuous training on production of reactive oxygen species (ROS), which could possibly exceed organism's antioxidant capacity, Polish authors (90) designed a study which showed that 1 month of 150 ml daily aronia juice supplementation was sufficient to increase antioxidant activity and limit the exercise induced oxidative damage to red blood cell membranes in elite male rowers. Other Polsih study (91) found that ingestion of 150 ml of chokeberry juice daily for 8 weeks caused significantly lower post-exercise levels of inflammatory tumor necrosis factor α (TNFα), increased iron and improved TAC (total antioxidative capacity) in the supplemented group of elite male rowers compared to their non-supplemented counterparts.

Controversially, another Serbian group of authors in 2016 (92) showed that 4 weeks of aronia supplementation in young male and female handball players did not confer a significant increase in polyunsaturated fatty acids as expected by antioxidative protective properties of aronia. A significant decrease was seen in serum TG and lipid peroxidation markers only in male but not in female players who consumed aronia, indicating the possibility of gender-related differences in aronia effect on regularly exercising individuals. Therefore, one of the aims of our study is to further elucidate its effects on recreationally active women, who were previously sedentary.

1.4. AIM AND HYPOTHESIS OF THE STUDY

Elaborating on the evidence mentioned in previous sections we realized following:

- The conducted literature search shows a lack of human clinical studies on aronia effects in healthy non-elite exercising women, especially regarding their body composition and psychological outcomes.
- Young adulthood is characterized by substantial lack of participation and continuation in physical activity, sufficient fruit and vegetable ingestion and time for building on healthy habits being possibly detrimental for CVD risk in older adulthood.
- Possible psychological barriers related to physical activity may prevent participation in it and oxidative stress may occur when increasing exercise intensity.

Therefore we designed a relatively short, not time demanding and pleasant exercise intervention for young healthy women. In order to address the issue of insufficient fruit intake and oxidative stress, we additionally included aronia juice supplementation. We opted for intervention which does not require any change in dietary pattern or culinary skill mastery, but demands 8-week participation in modern aerobic dance group exercise classes for 180 minutes per week and daily ingestion of the juice**.**

Aim of the study: To examine the effects of relatively short intervention consisting of 8 weeks of aerobic dance exercise with or without supplementation with polyphenol-rich aronia juice on body composition, metabolic health and physical self-description outcome in young healthy sedentary women.

Null- Hypothesis (Ho): The observed anthropometric parameters, body composition, biochemical, hematological parameters and score on physical self-description questionnaire will remain unchanged after the intervention.

Alternative Hypothesis (H1): Intervention will significantly change some of the parameters observed:

Primarily: body composition and segmental body composition- represented by positive effects on body fat%, fat-free mass, anthropometric indices such as blood pressure and circumferences, blood lipids such as HDL and triglycerides, as well as general PSDQ score and specific score for the PSDQ Body Fat scale.

Secondary: Aronia juice ingestion will additionally augment the effect of improvements accomplished by the exercise intervention.

2. MATERIALS AND METHODS

2.1. STUDY DESIGN

Study was designed as a controlled randomized clinical trial with two intervention groups and one control group. Graphic representation of the study design is available in Figure 1.

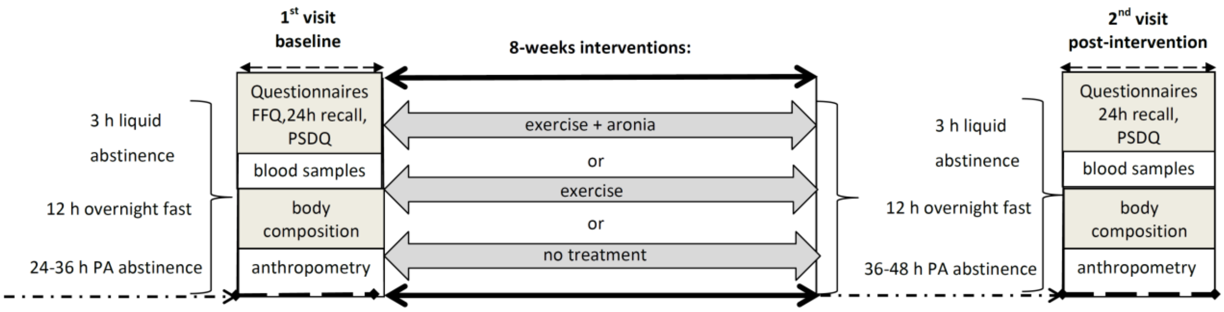

Figure 1- Graphic representation of the study design
FFQ *–Food Frequency Questionnaire;* ***PSDQ****- Physical Self-Description Questionnaire*

Two sampling occasions were separated by 8 weeks of intervention and defined as follows: 1) the initial, 1-3 days before the beginning of the intervention, in order to obtain baseline data and 2) the final, at least 36 hours after the last exercise session and juice ingestion, in order to isolate as many chronic, not acute effects of intervention as possible. On both occasions, participants arrived at the laboratory in the morning between 8-10.30 a.m., after an overnight fast and at least 3 hours of abstinence from water (or other beverage) intake and 36 hours of abstinence from physical activity.

Treatments applied on two intervention groups lasted for 8 weeks and consisted of Body Combat aerobic exercise 3 times a week for 60 minutes together with daily ingestion of 100 ml of aronia juice after the main meal (BCA group) or 8 weeks of Body Combat exercise alone (BC group). Participants in the control group were instructed not to change their physical activity levels, dietary habits and supplementation[2]. Both intervention groups were also instructed not to change their dietary habits and supplementation.

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[2] The supplementation was not forbidden as long as it did not contain any additional doses of strong antioxidants (such as aronia, astaxanthin, other carotenoids etc.) and remained unchanged from the period preceding the intervention until the completion of the protocol.

The study was designed with respect to the Declaration of Helsinki and approved by the Institutional Ethics Committee of the Medical Clinical Center in Zemun (Belgrade, Serbia). All participants were provided with an informational brochure about the study protocol prior to the signing of Informed consent. For the purpose of safe inclusion in exercise intervention the participants were also asked to fill in Physical Activity Readiness Questionnaire (PAR-Q).

2.2. STUDY PARTICIPANTS

Participants were recruited through the advertisements on social networks and through word of mouth and personal contacts of researchers. Most of the participants, however, came through the advertisements on social networks attracted by the opportunity for free recreational exercise. The size of the study sample was determined by G power calculator and by reviewing the existing literature on similar intervention and sample type (young healthy females). Minimum sample size per group was defined as 11 participants. Diagram of participants' flow and attendance per each phase of the study is provided in the Results section, Figure 2.

Fifty five (n=55) young women were screened for the following eligibility criteria: young (19-29 years old) healthy (absence of therapy or diagnosis of CVD or metabolic disease) sedentary (defined as: less than 1 hour and 30 minutes of moderate physical activity or 3 hours of light physical activity per week) women. Exclusion criteria were: regular physical activity, diagnosis of any chronic, metabolic or cardiovascular disease, regular use of medication for any chronic disease including contraceptives, corticosteroid drugs etc., smoking, pregnancy, recent blood donation or recent weight-loss or weight-management or cosmetic treatment intended for weight management, as well as lack of readiness to maintain the same level of nutrition, physical activity and supplementation throughout the study. For the estimation of compliance with the latter three we relied on subjective participants reports.

Forty three (n=43) participants met eligibility criteria and were included in the study. The study population was characterized by average (±SD) age of 25±3 years, weight: 63±13 kg, height: 169±7 cm and BMI: 22±4. The participants were further assigned to control or intervention groups in a randomized way. The initial number of participants in groups was as follows: n (BC) =14 participants, n (BCA) = 15 participants, n (CON) =14.

2.3. SAMPLING METHODS AND DATA ACQUISITION

2.3.1. ANTHROPOMETRIC, MORPHOLOGICAL AND BLOOD PRESSURE MEASUREMENTS

For anthropometry and body composition measurements participants were requested to remain barefoot and lightly dressed (shorts and top). Body height was measured to the nearest 0,1 cm by a standard stadiometer (seca 213, SECA GmbH, Germany). Waist and hip circumferences were measured by measuring non-elastic tape. Waist to hip ratio was calculated for each participant.

Body weight, as well as the other 23 variables related to body composition or metabolic estimation were measured or calculated on semiprofessional 8-electrode biompendance analyzer (BC-1500 Ironman, Tanita, Japan) with the help of its integrated software (Healthy Edge Lite, Tanita, Japan). A 8-electrode biompedance (BIA) analyzer was chosen since evidence exists that provided recommended measuring conditions (93, 94) these analyzers perform better and provide more reliable body fat and muscle mass measurements than standard 4-electrode BIA analyzers, thus having greater consistency and agreement of results with dual X ray absorptiometry (DEXA) method (95-97). Notably, the latter is regarded a gold-standard method for body composition estimations. The following variables were directly measured on the analyzer:

- body weight (kg)
- total body composition (body fat mass (BFM), total body fat percentage (BF%), fat free mass (FFM), muscle mass (MM), bone mass (BM), body water mass (BW), body water percentage (BW%)
- segmental body composition (muscle masses (MM) of right arm (RA-MM), left arm (LA-MM), right leg (RL-MM), left leg (LL-MM), central body (CB-MM), as well as fat percentages (F%) of these segments (right arm fat percentage (RA-F%), left arm fat percentage (LA-F%), right leg fat percentage (RL-F%), left leg fat percentage (LL-F%) and central body fat percentage (CB-F%)

Moreover, the integrated software calculated following metabolic estimations (by relating some of the measured variables to existing databases of population average values for that variable or by calculating the ratio between two measured variables (98-101)):

- basal metabolic rate (BMR) (98) and estimated daily calorie intake (calculated by applying the validated population equations that use age, weight measurement and activity level)
- metabolic age- which refers to a number calculated by comparing the result of BMR to the average BMR of the population chronological age group (101)
- visceral fat rating[3](99) - an estimation of visceral adipose tissue accumulation calculated on the basis of the measured body fat percent compared to known populations representative sample image analysis of magnetic resonance imaging (MRI)
- physique rating (calculated as relative ratio of measured body fat and muscle mass of participant and compared to population average for the age).

Pulse, systolic (SBP) and diastolic blood pressure (DBP) measurements were performed on a digital sphygmomanometer (Digital Blood Pressure Monitor HEM -907, Omron, Germany) by a skilled nurse, prior to the blood draw.

All anthropometry and body composition measurements (except pulse and blood pressure measurements) were performed by the same researcher on both occasions.

[3] According to the manufacturer's technical bulletin (99. Tanita Corporation of America Inc. Tanita Technical Bulletin: Visceral Fat Measurement. Illinois: Tanita Corporation of America Inc,, 2013. [Tanita Corporation of America I) visceral fat rating refers to the calculation of the visceral adipose tissue (VAT) accumulation risk by BIA measurement of body fat percent and its comparison to the image analysis applied to the magnetic resonance imaging (MRI). The VAT accumulation risk is calculated by estimating the VAT area by the BIA method on the basis of MRI image processing and by assigning a value from 1 to 59. A rating of 1-12 indicates a healthy level, while 13-59 indicates an excess of visceral fat.

2.3.2. BLOOD SAMPLING, BIOCHEMICAL AND HEMATOLOGICAL ANALYSES

After anthropometry, body composition and blood pressure measurements were completed, trained nurse collected venous blood sample from over-night fasted participants (13- 16 ml blood per participant). Blood was collected into sample tubes for serum and EDTA evacuated tubes for further analysis.

Biochemical parameters such as lipid status (total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), triglycerides (TG)) as well as glucose (Gluc) , liver aminotransferases (AST;ALT) activities, creatinine, urea, uric acid, lactate dehydrogenase (LDH) were determined in serum (after centrifugation and coagulation) the day of the sample collection, using a clinical chemistry analyzer (Cobas c111,Roche Diagnostics, Switzerland) and Roche Diagnostics colorimetric kits, according to the manufacturer's instructions. Moreover, atherogenic risk (LDL to HDL ratio) was immediately calculated from the obtained data. Full blood count was determined on the same day, from the EDTA evacuated samples (homogenized by gentle manual mixing) using automated hematological analyzer ABX Micros 60 (this device works on the photometry method and uses the manufacturer prescribed reagents for red blood cell lysis, device cleaning and maintenance).

2.3.3. QUESTIONNAIRE ADSMINISTRATION

2.3.3.1. PSDQ QUESTIONNAIRE

The participants filled in the validated Serbian version of PSDQ questionnaire (60) consisting of 70 items and describing 9 specific scales (Health, Strength, Endurance, Body Fat, Physical Activity, Sports Competence, Coordination, Appearance and Flexibility) and 2 general dimensions (General Physical Self-Concept and Self-Esteem).

Participants indicated their level of agreement with each statement of the questionnaire by circling the number on the 6-point Likert scale (from "1"- representing "not correct at all" to "6"- representing "fully correct"). Since each scale or dimension is described by several items and since not all scales are described with equal number of items, the total score for each scale is actually the averaged score computed as a sum of the individual scores of all items which describe the scale, divided by the number of these items. On both occasions, a trained psychologist was at participants' disposal for any clarifications regarding PSDQ.

2.3.3.2. DIETARY QUESTIONNAIRES

Food Frequency Questionnaire (FFQ) was fulfilled by participants only on the baseline visit. Since it estimates the food frequency intake over the last three months and we designed a study of shorter duration (8 weeks = 2 months) we would not be able to isolate the changes yielded by our intervention solely, so we decided to exclude it from the final visit in order to diminish the burden imposed on participants.

However, three 24-hour recall questionnaires were filled out. Two of them related to regular working days (one administered on 1st and other on 2nd visit) and one for the first day of weekend following the baseline visit (to take home and return to the laboratory as soon as filled out). For the questionnaires filled in at our facility a trained medical doctor, specialized in Nutrition, assisted participants by interviewing them and showing them pictures of portions sizes, while reminding them not to forget to note down any beverage or snack ingested.

Questionnaires were analyzed using the Serbian Food Composition Database and dietary assessment software tool DIET ASSESS (102-105). The purpose of the analysis was to determine the dominating type of diet for our participants as well as their levels of habitual antioxidants intake through foodstuffs. The number of filled out 24hrs recall questionnaires (2 at the baseline and 1 post-intervention) was not sufficient to monitor for the dietary changes during the intervention. For the latter purpose we would need to assess at least 3 separate daily intakes at each time point (at the baseline and post-intervention) which would pose additional burden on the already overloaded participants so the extrapolation of the between and within the group dietary differences was not planned. The dietary data collection was intended just for the screening whether the participants intake comply with or deviates from the WHO population goals, since the previous study (8) in young adults from Serbia report the deviation.

2.4. EXERCISE INTERVENTION

Commercial aerobic dance exercise program named Body Combat, Les Mills, New Zealand(53), was performed by participants 3 times a week, for 60 minutes mostly in the evening hours, at three exercising facilities located in Belgrade (Serbia) certified by Les Mills (namely, 2 fitness clubs “Ethnogym” and fitness club “SC Ranković”, which kindly donated free sessions to our participants). Participants were assigned to one or another facility based on their preference and accessibility of the facility (close to their home or workplace).

Body Combat choreographies activate both upper and lower body muscle groups in a Hi-Lo interval fashion. A previous study showed its average intensity to be approximately 73,2±7,3 % of maximum heart rate (HR_{max}) and its average energy cost of 9,7±2,0 kcal x min^{-1} (55). The instructors leading the training are certified by the local branch of Les Mills Company and are performing the same official choreography prescribed by the company, thus having the same music, same beats per minute as well as same distribution of Hi, Lo intervals and breaks. The instructors are advised through their certification training to verbally encourage participants to exercise at highest possible intensity and to lead by example performing at their own maximum intensity. Having taken this into account, we assumed that there were no differences in the training quality and intensity between the facilities.

The participants were allowed to miss up to 2 trainings from the overall 24 planned training sessions. However, when skipping their planned training session, they were allowed to compensate for it during the following days in the same or another facility.

2.5. ARONIA JUICE SUPPLEMENTATION

Aronia juice was a kind donation of the manufacturer *Rheapharm d.o.o., Belgrade, Serbia*. The dietary and polyphenol analysis of the juice was conducted in the previous CENM study (82) which used the same batch of the juice and published its results a month before the beginning of our intervention. Namely, as authors stated the juice contains in g per L: glucose 45, fructose 28, sorbitol 50, citric acid 1,2, K-citrate-monohydrate 6,0 ; in mg per L: vitamin C 29, vitamin B1 0,38, vitamin B2 0,71, vitamin B6 0,44, niacin 0,39, CaCl2 × 2H2O 559, MgCl2 550 and NaCl 760. Regarding phenolic compounds content, 6484 mg/L of total phenols and 240 mg/L of total proanthocyanidins were identified. Other identified compounds were (in mg/L):

phenolic acids (chlorogenic acid: 1389 and neocholorgenic acid: 1057), anthocyanins (cyanidin-3-O-glucoside: 21, cyanidin-3-O-galactoside: 301, cyanidin-3-O-arabinoside:101, cyanidin-3-O-xyloside 13) and flavonols (quercetin-3-O-glucoside (isoquercetin): 53, quercetin-3-O-galactozide (hyperoside): 97, quercetin-3-O-rutinozide (rutin): 194).

The juice was packed in transparent glass bottles of 1 liter. The total quantity of juice provided to the participants was from the same batch. Participants were instructed to keep the beverage in the refrigerator after opening. One bottle of juice was provided to the participants in the BCA group, every 10 days by the researcher who visited the exercise facility and monitored the exercise adherence. Delivery of the exact amount enough for 10 days only was applied in order to prevent ingestion of larger quantity than prescribed (100 ml a day). Moreover, since polyphenols in aronia juice are susceptible to degradation due to increased temperature and prolonged storage (106, 107) we wanted to minimize the quantity of juice possibly exposed to different storage conditions applied by each participant.

2.6. STATISTICAL ANALYSIS

The data obtained from PSDQ was transformed by computing the averaged scores for each of the 11 scales and dimensions. This was needed since various scales were not represented by the equal number of items, and likewise had unequal score opportunity.

Afterwards, all data were tested for the normality of distribution by the Shapiro-Wilk's test and visual evaluation of distribution histograms. Those normally distributed, or obtaining normal distribution after log-transformation, were analyzed with parametric statistical tests. Paired t-test was applied for within the group analysis (analysis of related samples data pre- and post-intervention) while Mixed Model ANOVA (or repeated measures ANOVA) test was applied for "between and within groups" analysis (all groups in 2 time points: before and after intervention).

Those non-normally distributed were analyzed with non-parametric Wilcoxon signed rank test in a quest for "within the group" differences. Since there is no equivalent non-parametric test to the Mixed Model or repeated measures ANOVA, in order to incorporate the sense of change during the intervention into the analysis we performed Kruskal-Wallis test not on the raw post-intervention data, but on the folded change (baseline to post-intervention values) of each variable. In order to check between which groups significant differences occurred, pairwise comparisons and post-hoc Bonferroni test were applied for PSDQ variables and Tukey’s post-hoc test for all the other variables.

Results were considered significant at $p < 0,05$ level. All analyses were performed using the R software package or the SPSS, 21 software package.

3. RESULTS

3.1. ATTENDANCE AND DIETARY DATA

Thirty eight out of 43 participants successfully completed the study protocol and provided complete data (baseline and post-intervention) and thus included in the final per-protocol analysis. The drop-out rate was 12%, with 5 participants leaving the study. Out of those, 3 left due to professional and academic duties, 1 due to disease and 1 for disliking the Body Combat training program. Characteristics of the analyzed study sample were as follows: age 25±3 years, body height: 169±7 cm, body weight: 62±13 kg, BMI:22±4. A detailed flow chart of participants attending or leaving the study phases is shown in Figure 2. Among the exercising groups training attendance rate was 91% with the average attendance of 22 sessions out of 24 (based on participants' claims and occasional monitoring by the facility staff or the researcher).

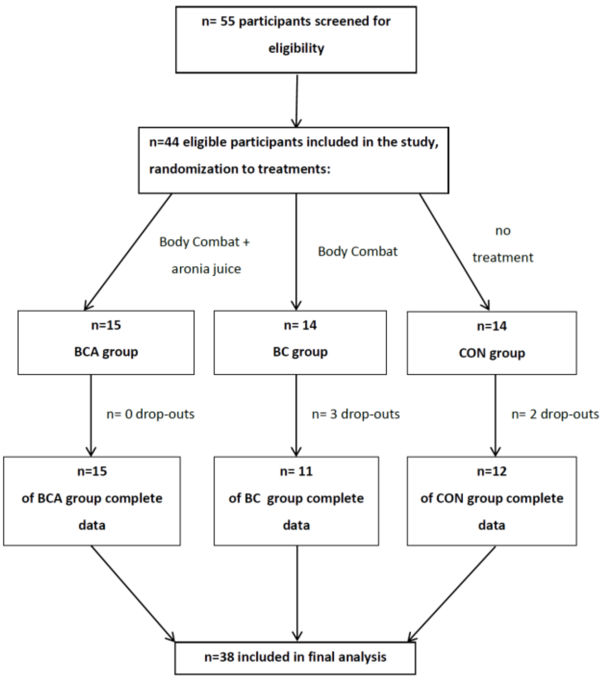

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Figure 2- Chart of participants' flow and attendance at different study phases

As shown in Table 1, the dietary data extracted from the three 24 h recall questionnaires and FFQ analysis show <u>lack of compliance with the recommended population goals (WHO)</u> for macronutrients and polyphenol intake both in the whole sample and in each group. Regarding macronutrients intake, expressed as the percentage of the total energy (TE) intake, participants' diet is characterized as predominantly high-fat, exceeding the population goal for fat intake by at least 10% (45% vs. the recommended upper limit of 35%). The intake of carbohydrates was also lower than recommended (38% vs. at least 50% recommended).

Table 1- Dietary pattern of participants across the groups based on three 24h recall questionnaires

nutrient type	*BCA group*	*BC group*	*CON group*	*population goals (WHO)*
total energy (TE) (kcal)	1861±358	1820±429	1946±569	
proteins (%TE)	15±3	16±4	16±3	10-20 % TE
fats (% TE)	46±8	42±8	44±4	25-35 % TE
carbohydrates (%TE)	35±9	38±9	36±5	50-60 % TE
polyphenols (mg)	152±150	256±169	196±221	600-750 mg/day

Data expressed as mean ±SD. ***TE*** *– total energy intake per day;* ***BCA-*** *group under intervention of Body Combat training and aronia juice consumption,* ***BC****- group under Body Combat training intervention,* ***CON****- control group, under no treatment*

Participants' daily intake of polyphenols (being a major group of dietary antioxidants) was 196,1±180,8 mg thus being much lower than the recommended goal of 600-750 mg/day. Most commonly consumed polyphenol containing foods were apples and apple juice, strawberries, dark chocolate, green tea, oranges, blueberries, raspberries, sweet cherries, onion and potatoes. Only five participants seemed to consume adequate or nearly adequate levels of at least 500-700 mg polyphenols daily mostly originating from apples and strawberries. An analysis of the dietary changes during the treatment or analysis for the between and within the group was not performed since we did not monitor daily intakes in a number of days necessary for such an extrapolation (3 days at baseline and 3 post-intervention).

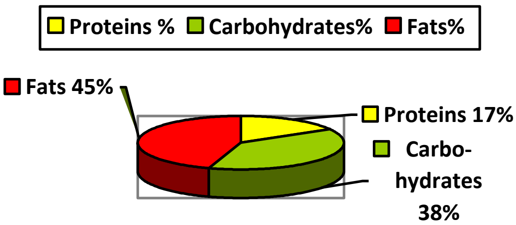

Chart 1- Distribution of macronutrient intake of the whole sample expressed as percentage of total energy intake. Data obtained from analysis of "24 h recall" questionnaires taken on 3 separate days.

3.2. DEMOGRAPHIC, ANTHROPOMETRIC AND BLOOD PRESSURE DATA

Analysis between the groups shows that three groups were homogenous at the baseline in regard to their weight (p value=0,86) and BMI (p=0,57). Similarly, as shown in Table 2, none of the anthropometric or blood pressure data differed significantly with the completion of the study protocol (neither within the group, nor between the three groups).

Table 2- Demographic, anthropometric and blood pressure data of participants pre- (1st visit) and post-intervention (2nd visit)

group	*BCA*	*BCA*	*BC*	*BC*	*CON*	*CON*
visit	*1st*	*2nd*	*1st*	*2nd*	*1st*	*2nd*
age (yrs)	26±3		26±3		25±4	
height (cm)	168±9		170±5		168±6	
weight(kg)	63±12	63±12	60±6	61±5	63±18	63±18
BMI(kg/m²)	22±3	22±3	21±1	21±1,0	22±7	22±7
WC (cm)	80±9	79±9	78±5	78±4	80±14	79±14
HC (cm)	102±9 100(94;110)	99±8 99(94;106)	97(95;102)	96(94;102)	99±14 94(89;104)	99±13 94(89;105)
WC:HC ratio	0,79±0,05 0,79 (0,74;0,81)	0,79±0,05 0,79 (0,76;0,81)	0,79 (0,77; 0,86)	0,80 (0,77; 0,85)	0,81±0,06 0,82 (0,78; 0,83)	0,80±0,07 0,79 (0,76; 0,85)
SBP (mmHg)	119±11 120(111;130)	114±12 115(104;126)	126±10	116±8	116±12	107±8
DBP (mmHg)	66±11	68±9	71±9	66±7	65±7	62±7

Data expressed as average ±SD if following normal distribution, or as median (followed by quartiles Q1, Q3) if variable was not normally distributed in a specific group or where non parametric test was applied for the differences among the three groups. ***BCA-*** *group under intervention of Body Combat training and aronia juice consumption,* ***BC****- group under Body Combat training intervention,* ***CON****- control group, under no treatment* ***WC****-waist circumference,* ***HC****-hip circumference,* ***WC: HC*** *–waist to hips ratio,* ***SBP****- systolic blood pressure,* ***DBP****-diastolic blood pressure. Differences are considered significant if p< 0,05.*

3.3. BODY COMPOSITION DATA

As shown in Table 3 interventions produced similar effects on most of the measured body composition and metabolic estimation variables with only several exceptions. Analysis for pre- and post-intervention changes within each group showed significant decreases in body fat mass (BFM), left leg and right leg fat percentage (LL-F% and RL-F%) with simultaneous increases in FFM, BW%, BWM, MM, right and left leg muscle mass (LL-MM and RL-MM) and BMR in all groups (e.g. in the BCA group p-values for all the mentioned variables $<0{,}001$). However, visceral fat rating improved significantly ($p=0{,}037$) only in the BC group, physique rating only in the BCA group ($p = 0{,}025$), while the central body muscle mass improved in both the exercising groups ($p = 0{,}003$ and 0,008 for the BCA and BC, respectively) but not in the CON group.

"Between and within" the group analysis showed significantly different decrease in visceral fat rating and left leg fat percentage (LL-F%) ($p=0{,}040$ and 0,033, respectively) between the groups. Post-hoc Tukey's test showed that for the former the significant difference exists actually between the BC and CON group ($p=0{,}009$), while for the latter between the BCA and CON group ($p < 0{,}001$).

Table 3- Participants' total and segmental body composition and metabolic estimation data pre- (1st visit) and post-intervention (2nd visit)

group	*BCA*	*BCA*	*BC*	*BC*	*CON*	*CON*
visit	*1st*	*2nd*	*1st*	*2nd*	*1st*	*2nd*
BF %	28±6	26±6	26±5	24±4	28±11 24(20;39)	26±11* 22(17;38)
BFM (kg)	19±8	17±7*	16±4	14±3*	20±14	18±13*
FFM (kg)	44±5	46±5*	44±3	46±3*	44±5	45±5*
BW%	53±4	55±4*	55±3	56±3*	53±8	55±8*
BWM(kg)	33±4	34±4*	33±2	34±3*	32±4	33±4*
MM (kg)	42±5	44±4*	42±3	44±3*	41±5	43±5*
BM (kg)	2,3±0,2 2,3 (2,1;2,4)	2,3±0,2 2,3 (2,1;2,5)	2,2 (2,2;2,4)	2,3* (2,2;2,5)	2,2 (2,1;2,4)	2,3* (2,1;2,4)
LA-F %	29±7 26 (23;34)	27±7 26 (20;33)	25±4 24 (23;28)	24±4 24 (21;26)	25 (19;38)	20 (16;39)
LA-MM (kg)	2,0±0,3 2,0 (1,8;2,2)	2,1±0,3 2,1 (1,8;2,3)	2,0 (1,9;2,2)	2,0 (2,0;2,3)	2,0±0,4 2,0 (1,8;2,1)	2,0±0,4 2,0 (1,8;2,2)
RA-F %	26(22;33)	25(20;32)*	24±4 23(21;28)	22±4 22(19;25)	21 (17;37)	19* (16;35)
RA-MM (kg)	2,0 (1,8;2,2)	2,1 (1,9;2,4)	2,0±0,2 2,0 (2,0;2,2)	2,1±0,2 2,1 (2,0;2,3)	2,0±0,3 2,0 (1,8;2,1)	2,0±0,3 2,1 (1,8;2,2)
LL-F%	32±4	29±4*¥	31±3	29±3*	33±8	31±8*¥
LL-MM(kg)	7,1±0,8	7,4±0,8*	7,0±0,4	7,2±0,4*	6,9±0,9	7,1±1,0*
RL-F%	32±4	29±5*	31±4	29±3*	32±8	30±8*
RL-MM (kg)	7,2±0,8	7,6±0,7*	7,1±0,3	7,4±0,4*	7,1±0,9	7,3±1,0*
CB-F%	26±8 25(19;34)	23±8 24(16;29)	23±6 23(19;29)	21±5 20(17;26)	21(15;39)	18(12;38)*
CB-MM (kg)	24±2	25±2*	24±2	25±2*	23±2	24±3
visceral fat rating º	2,0(1,0;4,0)	1,0(1,0;3,0)	2,0(1,0;2,0)	1,0(1,0;1,0)*¥	1,0(1,0;3,5)	1,0(1,0;4,3)¥
metabolic age º	27±16 26(13;44)	23±14 20(12;32)	22±8 21(12;30)	18±6* 17(12;23)	30±26 14(12;48)	28±24 13(12;52)
physique rating º	5,0(2,0;5,0)	5,0(5,0;8,0)*	5,0(5,0;5,0)	5,0(5,0;8,0)	4,0(3,3;7,3)	6,0(3,3;8,0)
BMR (kcal)	1373±145	1405±141*	1363±83	1403±86*	1358±177	1384±186*

Data expressed as average ±SD if following normal distribution, or as median (followed by quartiles Q1, Q3) if variable was not normally distributed in the specific group or where non parametric test was applied for the

differences among the three groups. ***BCA****- group under intervention of Body Combat training and aronia juice consumption,* ***BC****- group under Body Combat training intervention,* ***CON****- control group, under no treatment.* ***BF*** *- total body fat expressed as percentage* ***(BF %)*** *or as mass (****BFM (kg))*** *- total body fat mass in kg;* ***FFM (kg) -*** *total fat free mass;* ***BW****-total body water expressed as percentage (****BW %****) or as mass (****BWM (kg)****);* ***MM (kg) -*** *total body muscle mass;* ***BM*** *(kg)-total body bone mass;* ***LA-F %****- left arm fat percentage;* ***LA-MM (kg)****- left arm muscle mass;* ***RA-F % -*** *right arm fat percentage;* ***RA-MM (kg)*** *– right arm muscle mass;* ***LL-F%*** *- left leg fat percentage;* ***LL-MM(kg)*** *– left leg muscle mass;* ***RL-F%*** *- right leg fat percentage;* ***RL-MM (kg****) – right leg muscle mass;* ***CB-F%*** *- central body (trunk) fat percentage;* ***CB-MM (kg)*** *–central body muscle mass;* ***BMR*** *– basal metabolic rate;*
Symbol º denotes an estimation calculated by the Healthy Edge Lite™ software incorporated into the Tanita BC-1500 Ironman™ biompedance analyzer based on the ratios between the measured body composition parameters, entered age, activity level and validated population equations or cross-sectional imaging (see p.28, Materials & Methods).- difference from baseline to post- intervention data is statistically significant within the same group; ¥ - symbol denotes significantly different data between the groups sharing it. Differences are considered statistically significant if p<0,05.*

3.4. BIOCHEMICAL DATA

As shown in Table 4, analysis between and within the groups revealed that only LDH significantly (p=0,008) differed due to the treatment. Post-hoc Tukey's test showed that the difference was observed between the BCA and CON group (p=0,015), with the former showing significantly greater increase than the latter. Within a group analyses showed that significantly different values from baseline to post intervention were observed only in the CON group in TC and AST levels (p=0,037).

Table 4- Biochemical blood test results of participants after overnight fast pre- (1st visit) and post- (2nd visit) intervention

group	*BCA*	*BCA*	*BC*	*BC*	*CON*	*CON*	*Ref.*
visit	*1st*	*2nd*	*1st*	*2nd*	*1st*	*2nd*	*value*
Gluc (mmol/L)	4,6(4,3;4,7)	4,3(4,1;4,5)	4,5±0,3 4,6(4,2;4,8)	4,1±0,5 4,1(3,8;4,4)	4,7±0,4 4,6(4,4;4,9)	4,2±0,4 4,1(4,0;4,7)	3,9-5,8
TG (mmol/L)	0,67±0,20 0,63 (0,52;0,77)	0,67±0,16 0,71 (0,51;0,75)	0,66 (0,45;1,1)	0,62 (0,47;1,1)	0,77±0,24 0,76 (0,57;0,88)	0,68±0,31 0,57 (0,51;0,69)	0,46-1,70
TC (mmol/L)	5,1±0,8 4,9(4,5;5,7)	4,7±0,6 4,6(4,3;5,2)	4,7±0,8 4,8(4,0;5,4)	4,2±0,8 3,9(3,6;5,1)	4,3(4,1;5,4)	4,0(3,3;4,6)*	3,9- 5,2
HDL-C (mmol/L)	1,8±0,4 1,7(1,6;1,9)	1,7±0,3 1,7(1,6;1,9)	1,7±0,2 1,7(1,6;1,9)	1,7±0,2 1,6(1,6;1,9)	1,5(1,3;1,9)	1,5(1,3;1,7)	>1,3
LDL-C (mmol/L)	2,4±0,6 2,2(2,0;2,7)	2,5±0,5 2,5(2,1;2,7)	2,1±0,6 2,1(1,6;2,4)	2,0±0,5 2,0(1,6;2,5)	1,9(1,7;2,3)	2,0(1,8;2,5)	< 3.4
Athero$^{\alpha}$ risk	1,4±0,5 1,4(1,0;1,8)	1,5±0,5 1,5(1,3;1,8)	1,2(1,0;1,4)	1,1(0,91;1,5)	1,2(1,1;1,7)	1,3(1,1;2,0)	<3,0
Creatinine (μmol/L)	64±10 61(57;70)	57±8 53(52;62)	68(57;82)	63(56;72)	63±10 63(54;71)	61±7 61(56;68)	53-115
Urea (mmol/L)	3,7±1,0	4,0±1,1	4,0±1,1	3,9±1,5	3,8±1,0	4,1±0,7	2,1-7,1
Uric acid (μmol/L)	233±53	243±54	279±49	267±44	266±65	274±34	155-357
ALT (U/L)	13±4	16±8	14±4	15±5	15±7	15±8	<40
AST (U/L)	18±3	19±3	21±4	21±6	19±4	18±3*	< 40
LDH (U/L)	139±29	163±31¥	141±19	164±18	150±36 143 (127;159)	155±17¥ 157 (140;169)	125-200

Data expressed as average ±SD if following normal distribution, or as median (followed by quartiles Q1, Q3) if variable was not normally distributed in the specific group or where non parametric test was applied for the differences among the three groups. ***BCA-*** *group under intervention of Body Combat training and aronia juice consumption,* ***BC****- group under Body Combat training intervention,* ***CON****- control group, under no treatment.* ***Gluc*** *– fasting glucose;* ***TG****- blood fasting triglycerides;* ***TC****- fasting total cholesterol;* ***HDL-C*** *– high density lipoprotein cholesterol;* ***LDL-C****- low density lipoprotein cholesterol;;* ***ALT****- alanine transferase;* ***AST****- aspartate transferase;* ***LDH****- lactate dehydrogenase* ***U/L*** *stands for unites of enzyme per liter.* $^{\alpha}$*- atherogenic risk estimation is based on ratio between* ***LDL: HDL*** *cholesterol.* ***Ref****. denotes reference range value of variable accepted as normal by our laboratory,* *******- difference from baseline to post- intervention data statistically significant within the group;* **¥** *- symbol denotes the data significantly different between the groups sharing it. Differences are considered statistically significant if p<0,05.*

3.5. HEMATOLOGICAL DATA

"Between and within" group analysis shows significantly different changes among the groups only in erythrocyte blood count (RBC), with p value=0,046 and in several other variables estimated on the basis of RBC, such as HCT, MCHC, and RDW (p=0,047; <0,001; and 0,030, respectively). Post-hoc Tukey's test revealed that the difference in RBC occurred between the BCA (4,6±0,4 x 10^9/L pre- vs. 4,5±0,3 x 10^9/L post-intervention) and CON group (4,5±0,2 x 10^9/L vs. 4,6±0,2 x 10^9/L) (p<0,001).

Within the group analysis showed that RDW increased significantly only in the CON group (13±0% vs. 14±1%, p<0,001). HCT also increased only in the CON group, but not statistically significantly (p=0,256). However, HGB concentration and MCHC decreased significantly from baseline to post-intervention values in all three groups (HGB, in BCA: 119±9 g/L vs. 111±6, p<0,001; BC: 117±8 g/L vs. 109±11 g/L, p=0,002; CON: 115±6 g/L vs. 108±6 g/L, p<0,001), but this decrease was significantly different between and within the groups (BCA compared to CON, p<0,001) only for MCHC (respective pre- to post-intervention values for BCA: 296±5 g/L vs. 284±4 g/L, BC: 294±7 g/L vs. 279±8 g/L, CON: 299±7 g/L vs. 279±5 g/L and p value in each group <0,001). There was also a slight increase in GRA%, but it reached significance level only in the BCA group (61±6% vs. 64±8%, p=0,031), similarly to the decrease in MON% observed in all the groups but reaching the statistical significance only in the CON group (6,3±2,9% vs. 4,1±1,1%, p=0,043).

3.6. PSDQ DATA

As shown in Table 5, analysis for the between and within group differences showed that intervention differently affected scales of Health Condition, Fatness and Endurance (p=0,004; 0,002 and 0,008, respectively), with obviously more prominent improvements in the exercising groups (BCA and BC). The post-hoc Bonferroni test showed that the for the Endurance scale actually the BC improved significantly more than the CON, while for the Health Condition pairwise comparisons showed that significant difference occurred between the BCA and BC (p= 0,003) groups, with the former increasing and the latter decreasing its score. For the Fatness scale CON group decreased its score and thus significantly differed from both exercising groups which actually increased their Fatness scores with the intervention (p= 0,012 and p=0,004 when CON compared with the BC and BCA, respectively).

Analysis for within the group changes from baseline to post-intervention values showed that the CON group significantly increased its score only on 1 out of 10 scales and dimensions, namely on the dimension of General Physical Condition. The BCA group increased significantly the score of all the scales and dimensions of PSDQ. The BC group increased its score in 6 out of 10 scales and dimensions examined (the Physical activity scale was omitted from further analysis due to the logical difference between the exercising and non-exercising groups). Namely, in the BC group, significant improvements were observed in the scales of Fatness, Sports Competence, Appearance, Strength, and Endurance and in the dimension of General Physical Condition. Improvements in all the other scales were also seen in the BC group but they did not reach statistical significance.

Table 5- Averaged scores of participants on the PSDQ scales and dimensions pre- (1st visit) and post- intervention (2nd visit).

group	*BCA*	*BCA*	*BC*	*BC*	*CON*	*CON*
visit	*1st*	*2nd*	*1st*	*2nd*	*1st*	*2nd*
Health Condition	4,6±1,0 4,9(3,8;5,4)	4,9±0,8*¥ 5,1(4,5;5,5)	5,4±0,6 5,5(5,1;5,9)	5,2±0,7¥5,5(4,1;5,8)	4,7±0,8 4,8(4,2;5,3)	4,8±0,5 4,8(4,4;5,3)
Coordination	4,2±0,8	4,6±0,9*	4,9±0,9	5,0±0,6	4,3±1,1	4,2±1,3
Physical Activity	2,2±1,1	4,4±1,3	2,3±1,3	4,9±1,1	2,0±0,8	2,0±0,8
Fatness	4,4±1,5 4,8(3,2;5,8)	5,0±1,0* 5,2(4,2;5,8)	5,0±0,7 5,3(4,5;5,7)	5,6±0,5* 5,7(5,3;6,0)	4,9±1,7 5,7(3,5;6,0)	4,8±1,5‡ 5,3(4,3;5,9)
Sport Competence	3,6±1,2	4,3±0,9*	4,1±1,3	4,5±1,2*	3,2±0,9	3,4±1,2
Appearance	4,6±0,8 4,7(4,0;5,3)	5,1±0,7* 5,0(4,7;5,8)	5,2±0,3 5,2(5,0;5,5)	5,5±0,3* 5,7(5,2;5,8)	4,6±0,9 4,9(4,3;5,2)	4,8±0,9 5,0(4,6;5,2)
Strength	3,6±1,0	4,5±0,6*	4,4±1,0	4,9±0,8*	3,9±1,0	4,0±0,9
Flexibilty	3,9±1,2	4,6±1,0*	4,5±1,0	4,9±0,9	4,3±1,1	4,5±1,1
Endurance	2,5±0,8	3,6±0,7*	3,4±1,1	4,1±0,9*¥	2,6±0,8	2,8±0,9¥
General Physical condition	3,9±0,9	4,7±1,0*	4,5±0,8	5,4±0,5*	4,1±0,9	4,4±1,0*
Self-Appreciation	5,2±0,6 5,3(5,0;5,5)	5,4±0,6* 5,5(5,1;5,8)	5,6±0,4 5,8(5,6;5,9)	5,7±0,3 5,8(5,6;5,9)	5,1±0,6 5,1(4,7;5,6)	5,2±0,5 5,1(5,0;5,7)

Data represent averaged scores of answers given on all the items of 9 scales and 2 general dimensions of PSDQ, Maximum attainable value of an averaged score is 6. Data expressed as average ±SD if following normal distribution, or as median (followed by quartiles Q1, Q3) if a variable was not normally distributed within the specific group or where non parametric test was applied for the differences among the three groups. ***BCA****- group under intervention of Body Combat training and aronia juice consumption,* ***BC****- group under Body Combat training intervention,* ***CON****- control group, under no treatment.* *******- difference from baseline to post- intervention data statistically significant within the group;* **¥** *- symbol denotes the data significantly different between the groups sharing this symbol.* *‡- symbol denotes the data in this group significantly differ from the both of the other groups. Differences are considered statistically significant if p<0,05.*

4. DISCUSSION

The results of our study showed that an 8-week aerobic dance exercise intervention was not sufficient either alone, or in combination with aronia juice supplementation to make any significant changes in most of the cardiovascular risk factors examined when analysis between and within the groups was applied. None of the observed anthropometric variables (weight, BMI, WC, HC, DBP and SBP), total body composition (BF%, BFM, FFM, MM, BM, BW% or BWM) or segmental body composition values (limbs and central body fat percentage and muscle mass) differed significantly between the groups due to the intervention. The only exceptions were significantly differently decreased visceral fat rating and left leg fat percentage between the groups. Although restricted, these significant changes may imply potential effectiveness of aerobic dance exercise and aronia juice supplementation in the improvement of cardiovascular risk factors.

Regarding biochemical results, LDH was the only variable that differed significantly between the groups due to different treatments. This increase is normally expected in the exercising groups and could be regarded an adaptation to the intense training, when the latter is performed above the anaerobic threshold (108) thus causing an increase in lactic acid formation. If still remaining within the normal range, increased LDH is not detrimental and could, on the contrary, be regarded protective for CVD prevention according to recent cross-sectional (109) and cohort retrospective studies (110). They found higher but still normal levels of LDH in physically active individuals than in their sedentary counterparts. Authors of the latter study speculated that this increase could help the body adapt better to increased LDH levels and cope better with ischemic conditions in case of their sudden onset.

Regarding the hematological variables the only significant difference between the groups were observed in the RBC (blood count of erythrocytes, being decreased in both exercising groups and slightly increased in the CON) and the variables related to it, namely, HCT, MCHC and RDW. However, post-hoc tests revealed that the difference actually reached the significance only between the BCA and CON groups. HCT and MCHC decreased within all the groups and RDW increased in the control group. These variables are actually calculated as concentrations, so they could have been affected by different body water content as well or could represent one of the normal adaptations to the aerobic training. Notably, our findings are in disagreement with the findings of the study performed by Polish authors in 2015 (111) who found absence of significant changes in blood count and other hematological indices in young women after 12 weeks of exercise. In this study, intensity of exercise was increased gradually over the weeks, so maybe our findings of changed RBC and associated parameters (HCT, MCHC, RDW) could be regarded, similarly to LDH increase, as specific adaptations to the sudden and steep increase in the training volume and intensity applied to the previously sedentary individuals. These hematological findings thus have only limited importance in providing the evidence that participants of BCA and BC groups indeed attended the training sessions.

Regarding the overall CVD risk factor outcome, the results of previous **intervention studies** in the field (17, 101-115), with prolonged exercise in healthy females, showed inconsistent results.

Three studies found similar results to ours. Grant S, Todd K, Aitchison TC, Kelly P, Stoddart D (112) analyzed between and within the group differences in overweight, older than ours (mean age 63±4 years) and dietary advised sample after applying 12-week aerobic plus resistance exercise intervention or no treatment (control group) and found no statistical significant differences between the treatments. Within the group analysis, however, revealed a significant improvement in body mass, BMI, SBP and the Life Satisfaction Index score in the exercising but not in the control group. As for the results of the Physical Self Perception Profile, contrary to our findings, they failed to show a significant difference between the groups. Similarly, an absence of significant differences in body composition or blood lipid profiles (with the only significant exception of the Apo-B decrease) was found in 2006 by Ring-Dimitriou S, von Duvillard SP, Paulweber B, Stadlmann M, LeMura LM, Peak K, Mueller E (113). Notably, their sample was middle aged and some of their participants were overweight and slightly dyslipidemic (elevated Apo-B levels) at the baseline. No significant differences in body composition (as estimated by BIA and skinfolds method) and biochemical indices emerged within the normoweight group of a Polish study (111), even after longer exercise duration than ours. However, this study lacked a control sedentary group thus the reducing the possibility of equal comparison with ours.

Partial agreement with our results was found in other two studies (114, 115), but unfortunately both of them lacked a control group. Namely, an Italian group of authors (114) concluded that 9 weeks of aquatic moderate to high intensity training were not sufficient to modify the total body mass or the total body composition, but caused small changes in the segmental body composition. They found significant reduction in waist, arm and thigh circumference, skin folds sum and percentage of fat mass when applying the skinfold method, while with the DXA, no significant changes were observed for the total body data and segmental analysis showed a significant improvement in the fat free mass of arms and trunk only. In addition to their different body composition estimation methodology, their sample also differed in terms of pre-intervention physical activity level and their study design included a monitoring for additional physical activities or modified dietary intake. Absence of significant biochemical changes was found as well by Ceylan Hİ, Babayiğit İrez G, Saygın Ö (115) after 12-week of aerobic dance or step dance exercise in a mixed sample of young adults (18-22 years old, with the baseline weight status not clearly indicated). They also found a significant hemoglobin (HGB) decrease, but no other significant changes from the baseline to post-intervention values after the aerobic dance intervention. Opposite to our findings, the step dance intervention yielded a significant decrease in triglycerides and an increase in the red blood cell count (RBC) and hematocrit (HCT).

Finally, the studies which disagree with our in terms of results are numerous but only several of them had a similar sample, were controlled and well designed. A good example of these, is a very well-designed controlled trial by LeMura LM, von Duvillard SP, Andreacci J, Klebez JM, Chelland SA, Ruso J (116) where 16 weeks of aerobic training in young healthy sedentary females produced significant improvements in BF%, TG and HDL compared to their matched counterparts under combined, resistance, or no training condition. The strengths of this study included the doubled duration, body density determination by the underwater weighing, objective exercise intensity monitoring and adjustment (if needed), monitoring of the dietary intake and general physical activity as well as several sampling occasions at different time points. The authors state their inability to test all the participants at the same phase of menstrual cycle as the major limitation of the study, which could possibly have affected the changes in the blood lipids.

Arikawa AY, Thomas W, Gross M, Smith A, Phipps WR, Kurzer MS, Schmitz KH (18) also found a significant decrease in body fat percentage and an increase in lean mass when applying the double duration of the aerobic exercise intervention and DXA based body composition estimation. Their further strengths were monitoring for the oxidative stress, dietary intake, exercise intensity and menstrual cycle phase changes. Their note of competitive dietary restriction observed in the control group as well as their findings of the lowered systemic oxidative stress observed only in the exercising participants with the highest baseline stress levels provide valuable guidelines for the design of our future studies, especially having in mind that we plan to perform additional tests of oxidative status change on cryopreserved samples of our participants' serum/ plasma. They justify our decision not to include another non-exercising aronia consuming control group since according to them significant changes are not to be expected due to the exercise.

A similar improvement in the body composition values was seen in the study conducted by Smith MM, Sommer AJ, Starkoff BE, Devor ST (117), but their results should be discussed with caution since the sample was mixed in terms of gender, BMI and dietary restriction (Paleolithic diet or no restriction) and exposed to a higher frequency (5 days a week) and different type (cross-fit based) of exercise.

By applying longer duration (12-week) of aerobic dance and a different methodology for body composition estimation another group of authors from Serbia and Croatia(118) found a significant reduction in BF% (-10,1%), sum of the lower body (-21%) and overall skinfolds (-17%) as well as the different percentage of muscle mass between the experimental and the control group. Similarly, Trapp E, Chisholm D, Freund J, Boutcher S (119) almost doubled the intervention duration, monitored for the menstrual cycle phase, used DXA and found significant fat mass loss in the high intensity intermittent exercise (HIIE) group compared with the control or with the steady state exercise (SSE) group while a significantly different reduction in total body mass, fat percentage of lower limbs, trunk and central abdominal fat was seen only between the HIIE and the control group. Opposite results to ours, such as a significant decline in triceps skin fold, body fat percentage, fat mass and increase in fat free mass and HDL were also found by a group of Brazilian authors (120). However, they also applied a longer duration intervention (12 weeks), different methodology for body composition estimation, monitored objectively the exercise intensity and chose a different sample in terms of gender (mixed), age (adolescents) and BMI (overweight). Another two studies, one of equal (121) and the other of longer duration (14 weeks) (122), were similar in sample but opposite in results to ours. They claimed a significant decrease in body weight and TG (121, 122), LDL and TC (122), resting heart rate, DBP and SBP (122) and an increase in HDL (121, 122) only in the exercising group but both relied only on the results of the within the group paired t-test without any the "between and within" the groups analysis.

There are several other studies claiming disagreement with ours, but they not only had a different sample or methodology than those previously mentioned, but they also lacked a control group. An example is a previously mentioned Polish study (111) which found significant body composition changes (a reduction in weight, BMI, skinfold thickness and blood lipids and increase in FFM, total BW%) only in the over- and under-weight groups (increase in BMI and weight). A significant BF loss, skinfold and circumference reduction after a longer duration (12 weeks) treadmill training was also observed in a sample similar to ours (123). A greater reduction in circumference and total body mass was achieved in the group that exercised at a lower intensity (approx. 50% VO_{2max}) than in the group exercising at a higher intensity. The latter gained more FFM. Similarly, authors from Chile (124), found a significant reduction in the overall BF% and fat mass of the trunk and arms after longer duration (12-week) and different type (non-dominant leg muscles localized endurance resistance) training in a previously active mixed gender sample.

The other two Serbian studies (125, 126) lacking control group found a significant pre-post intervention reduction in BMI, BFM, BF% and lipid profile parameters (125, 126) such as TC and TG (125), LDL decrease with an increase in HDL (125, 126). However, the first (125) lasted longer, applied dietary regimen control and had mixed gender, hyperlipidemic sample of older adults, while the other chose an overweight sample.

The above presented brief discussion on our results and those presented in the other recent intervention studies, points out the inconsistency of the results, design and methodology applied thus underlining the need to recall the summary of the previously presented (in Introduction section) review and meta-analysis of the up to date literature on exercise benefits in young women. Namely, cross-sectional epidemiological (16) and meta-analysis studies (17), advocates (the former) the inverse correlation between the time spent in light physical activity and triglycerides or lipid accumulation product levels (16) and claims (the latter) efficacy of aerobic exercise interventions of duration equal or longer than 8 weeks in improving blood lipid profile in women of all ages (average reductions of TC, LDL and TG by 2%, 3% and 5%, respectively and the average increase of HDL by 3%) (17). ACSM's (4) and AHA (7) standpoints also claim the beneficial effects of exercise on CVD risk factors in healthy populations of all ages. Thus, we are inclined to revise the methodology we applied and presented in the Methods and Materials section. Namely, there is an intervention study performed by a group of Finish authors (127), who applied prolonged (21-week) exercise intervention in middle aged females and claimed that compared to DXA, BIA and skinfolds methods indeed underestimate the training induced positive changes in the body composition.

In addition to the body composition estimation method, there may be other four possible explanations for the absence of significant changes in the most of the body composition and biochemical variables examined in our study. Firstly, in regard to biochemical markers, our participants' results were within the normal reference range as well prior to the intervention. Secondly, the duration of the intervention may be too short to cause significant changes. Thirdly, seasonality (the change from winter to spring season) with associated change in food choices may have affected the body water content making it increased in all the groups. Indeed, a group of Japanese authors (128) points out the possibility for seasonal variations in exercise-induced weight loss and body composition changes. Since biompedance method is very sensitive to water content alterations this may have affected all the other body composition variables we examined, so significant within the group improvements were seen in all the groups regardless of the treatment applied. Last but not least, different hormonal and appetite response to exercise observed between males and females, with tighter energy compensation (by the means of greater dietary intake following exercise) in the latter (129) may minimized women' possibility for weight loss solely with the exercise.

Summarizing the effects of our exercise intervention, improving our study design by applying longer intervention duration, closer monitoring of the exercise intensity, dietary intake, menstrual cycle phase and seasonality, as well as the simultaneous analysis of samples from both the baseline and post-intervention visits at the same time point may be necessary to better elucidate and document the potential of the Body Combat exercise program for CVD risk factors improvement in young healthy normoweight women.

In order to compare our results to those of up to date literature on the effects of chronic aronia juice consumption on the body composition or weight loss outcomes, we searched for available human intervention studies published in this field and noticed obvious scarcity, thus implying the significance of our study which adds up novel information to the pool of not so much researched scientific topic. A somewhat greater body of evidence exists for aronia consumption effects on blood pressure, lipid status and inflammation markers. However, there is a lack of literature addressing the effects of pure aronia juice consumption (rather than powdered extracts) on CVD risk factors in controlled human trials. Thus, our study is again of great importance, aiming to provide new findings also to the field of dietary intervention studies with polyphenol-rich aronia juice.

Similar findings to ours were observed in several trials conducted on healthy samples. Namely, when it comes to the anthropometry results, a study conducted in Serbia by the members of the CENM and a cooperating laboratory (89) aimed to examine aronia effects on cellulite and found a significant reduction in subcutaneous tissue thickness (-1,9 mm on average), subcutaneous tissue fascicles length (-1,18 mm) and a reduction or total disappearance of tissue edema in the sample of normoweight and overweight apparently healthy women aged 25-48 years after 90 days of aronia juice consumption at the same dose as ours. However, neither hip circumference nor any other anthropometric, body composition and biochemical variable reached a statistical significant improvement thus implying an agreement with our findings.

An absence of significant improvements in anthropometric, biochemical or blood pressure results of already healthy subjects (even though slightly older (35±7,8 years old)), was also seen in the previous studies performed by CENM researchers (70, 130) even after longer (12 weeks) aronia juice consumption at the same dose as ours. In the first study (70), however, they found positive correlation between age, BMI, WC, BF%, blood pressure and the analyzed marker of lipid peroxidation which was influenced by the juice consumption.

A group of authors from Poland included both unhealthy and healthy participants in their studies (131, 132) and showed no significant improvements in the healthy ones, but significant changes in TC, LDL, TG concentrations as well as in SBP, DBP after 2 months of daily aronia ingestion (600 mg a day) only in those participants with diagnosed metabolic syndrome. (Notably, the participants in the former study (131) also followed a restricted low fat diet even up to 3 months prior to the study intervention).

Neither the study (72) which applied supplementation over the same period as ours but with a slightly increased dose (150-200 ml daily) and aimed to examine aronia effect on plasma levels of adipocyte hormones in overweight sample with increased WC found any significant improvements in the lipid metabolism between the supplemented and the non- supplemented groups. However, within the group of supplemented participants, levels of resistine, leptine, adiponectin and glycosylated hemoglobin (% HbA1c) improved significantly and participants self-reported improvement in their mood and feeling of strength, thus implying agreement with the significant improvement in all PSDQ scales we observed in the BCA.

Opposite results to ours, were found in other two trials performed in CENM laboratory (69, 71), on somewhat older (averaged age 47,5±10,4 years (69)) or post-menopausal adults (71), with already increased CVD risk factors (higher normal BP or grade I hypertension according to the European Society of Hypertension (ESH) (69) or abdominal obesity (BMI: 36±4,4 kg/m^2, WC: 105±10 cm) (71)). The former applied a consumption of doubled dose (200 ml) of aronia juice over halved duration (4 weeks) and found significant reductions in blood pressure parameters (average 24-h and awake SBP and DBP, the 24-h pulse pressure and the SD of awake SBP that represents BP variability during the day) and fasting triglycerides (baseline 1,95±1,42 mmol/L vs. 1,57±0,99 mmol/L post-intervention) coming into the normal range after the intervention. The latter (71) shows a significant reductions in BMI, SBP and triglycerides levels even at the same dose (100 ml) of the juice, additionally enriched with glucomann fibers. However, even after the statistically significant reduction, participants were still clinically categorized as abdominally obese while the SBP value came into the normal range (baseline 127,6±16,9 vs. 116,4±16,1 mmHg post-intervention).

Worth of mentioning is significant blood hemoglobin concentration decrease within all groups of our participants which is in an agreement with the previously mentioned exercise intervention study results (115) but in disagreement with the study which applied the same duration of aronia juice supplementation on elite male rowers (79). The latter found higher blood iron levels during the recovery phase in the supplemented than in their non-supplemented counterparts. The difference between our and their results may stem from their elite training level or due to the menstrual losses of iron in our female participants. However, this study and the recently published study (92) which found different fatty acid profiles between male and female handball players after supplementation with aronia juice imply the possibility for the gender differences of aronia effectiveness in healthy exercising populations.

Summarizing, it seems that aronia juice reserves its beneficial effects on CVD risk factors only for the individuals with already increased values, thus possibly providing an additional advantage when being added to their regular diet or treatment. Nevertheless, in healthy individuals, it may be beneficial as an antioxidant supplement intended for the post-exercise oxidative stress reduction and improved recovery (90, 91).

Moreover, the results of our study show significant and numerous improvements overtime in PSDQ scores in both intervention groups, but the BCA group, which consumed aronia juice, improved significantly on more (namely, on all) PSDQ dimensions and scales, while BC group significantly improved on fewer scales, namely, Fatness, Sports Competence, Appearance, Strength, Endurance and on the dimension of General Physical Condition. These results indicate the dominant role of aronia juice consumption in providing even better scores on PSDQ, being in agreement with the studies which document anxiolytic and mood-bolstering effects of aronia juice (72, 81, 82) and studies which suggest an increased feeling of well-being and happiness in individuals who regularly consume more fruit than their counterparts (133), as well as boosted optimism in those with higher serum antioxidant levels (134).

Between and within the group analysis shows that the scores for the scales of Health Condition, Fatness and Endurance changed significantly differently between the three groups due to different treatments. Results on Health Condition scale significantly differed between BCA and BC (with the improvement in the former and deterioration in the latter), thus implying increased perception of health improvement with the consumption of aronia. The BC group decrease on Health Condition scale may reflect their perception of themselves being deprived of aronia health related benefits. Thus, it is seems prudent to include a placebo juice in future studies in order to prevent such speculations. Increase on Endurance scale score differed between the BC and CON group, while Fatness score was significantly lower in the absence of treatment compared to both intervention exercising treatments.

Since both of the exercising groups improved on Body Fatness and Appearance scales it seems that the exercise intervention had a positive effect on the perception of one’s body image. However, exercise alone (without aronia juice consumption) was not regarded sufficient for a significant improvement in the self-perception of Health Condition, Flexibility, Coordination and Self-Appreciation which inclines us to think that individuals may need something additional (in the form of dietary change or supplement) to perceive themselves as better in regard to these scales.

To better elucidate the effects of exercise on PSDQ scales, we would like to turn to the results of the previous intervention studies in this field. Some of them report on improvement or partial improvement (135-138) while others fail to provide sufficient evidence of improvement (139, 140). The inconsistency of their results may stem from the various study populations in terms of gender and age or due to the different measurement instruments (PSDQ, PSPP or short version of PSPP), intervention type (single activity exercise sessions or various exercise activities, with or without the educational discussions and dietary intervention) and duration (6, 8, 10 weeks or several months or years).

We reached partial agreement in results with a group of Turkish authors (138) who chose a similar study sample (university sedentary female students) and applied similar exercise modality (step dance or aerobic sessions) and intensity (approx. 60-80% of the heart rate reserve) for 10 weeks. Exercisers increased their scores more than the control group on 4 out of 11 PSDQ scales, namely, Physical Activity, Coordination, Sports Competence and Flexibility. (We omitted the first from our analysis since our intervention in itself was exactly the increase in physical activity so the improvement on this scale was warranted.) Thus, if turning to the between and within the group differences both our study and the Turkish one found significant differences in 3 out of the 10 observed scales. However, when considering only the results of within the group analysis we found improvement in more than 3 scales in both of our exercising groups thus exceeding the Turkish results. If we observe only the BC group which is comparable in treatment to the Turkish exercising group, we document double the improvement in the number of scales. Since the type of scales improved is also different, this may imply the difference in the beneficial potential due to the different exercise modality (e.g. the Body Combat program is not exactly a dance but rather martial arts based aerobics, and this fact may predispose a greater improvement in Strength and Endurance perception). Both studies found an improvement in the Coordination scale (in our study in the BCA group only), which is expected due to the nature of aerobic dance intervention which requires coordination of body part movements to music.

Similarly, we state partial agreement with the findings of an early work (141) advocating the beneficial improvements in physical self-perception with a similar sample and intervention duration but a different exercise type (aerobic program on a bicycle ergometer). Although the authors used another measurement instrument, (PSPP), they also found significant improvements in three scales: Physical Condition, Strength and Physical Self-Worth, with the latter two being in reasonable correspondence with the PSDQ Strength, Sports Competence and General Physical Self-Concept scales, while the former may reflect the items from the PSDQ Physical Activity (which we omitted from the statistical analysis) and Endurance scales (61). Similarly, the PSPP Body Attractiveness Scale is closely related to the PSDQ Appearance and Body Fatness scales.

So we agree on the improvement in Endurance (PSPP Condition) and Strength scale and disagree on exercise potential to improve perceptions of one's Body Attractiveness.

The authors from the United Kingdom (137) also found improvement in Body Attractiveness, Strength and Condition scales of PSPP after 10 weeks of exercise intervention in their slightly older (31,9±9,37 years) mixed sample who followed individual trainings at a public gym. These results were found only in the subpopulation of adherers and authors state positive correlations with the significantly improved BF% and BMI. We did not perform any correlation analysis. According to the performed "within the group" analysis all of our groups (even the control group) improved their body composition, so if there was a positive correlation the control group would improve in the equal number of the scales as the other two, but that actually did not occur. So, we do not agree that physiological or body composition change is prerequisite for increase in PSC.

There is also agreement with the results of four other studies (136, 142-144); however, their sample and intervention design differed from ours. Taylor, HA and Fox, KR in 2005 (142) found significant improvements during the post-intervention period in two or even three PSPP out of the possible 4 scales (physical self-worth, physical condition and physical health) when observing at 16 weeks and 37 weeks, respectively. Opposite to our study, they found significant positive correlations of PSPP scales with the reduction in skinfolds and circumferences of their much older sample which had at least one coronary heart disease (CHD) risk factor increased. A significant improvement in Body Attractiveness, Physical Self-Worth and Sports Competence as well as in BMI was also found in exercising adolescent girls after a 6-week aerobic dance intervention compared to their counterparts who only participated in the classical physical education classes (143). Possible correlations were not examined. Another adolescent study (136) also claimed small magnitude improvements in Sports Competence, Physical Condition and Physical Self-Worth with absence of any correlation with the body composition or anthropometry change after 6 months of exercise intervention combined with healthy life style discussions. Similarly, study performed in children (144) in addition to the strength exercise intervention also applied dietary restrictions and found improved Strength and Endurance perception in the overweight/obese exercising participants, while in the group only on the dietary restrictions just the former scale improved. In both groups improvements were accompanied by favorable changes in body composition, BMI and physical performance tests, while lean body mass significantly differed between the groups.

On the contrary, we found four studies with the opposing results. An early investigation in this field (145) failed to find different responses between the groups after 10 weeks of aerobic intervention. Only their study number 2, with a mixed male-female sample and a different exercise type (weight training) improved Strength and Physical self-efficacy perception of exercisers compared to the control group enrolled in standard physical education classes. Several years later, the abovementioned Turkish group of authors (146) similarly failed to prove any significant group effects on the 5 scales of PSPP questionnaire between the control and exercise intervention groups of female university students, when the latter attended either aerobics or step exercise sessions for 8 weeks. Neither did the female adolescent study (139) with physical activity of preference performed 5 times a week together with the regular educational sessions on PA benefits show any significant change regarding the PSDQ scores in comparison to the traditionally held physical activity education classes. No difference between the groups emerged either in BF% or BMI. A study conducted in a sample of younger children (140) found changes in physical fitness parameters after 8 weeks of fitness circuit program which were not accompanied by improvement in PSC. Namely, the scores of Physical Appearance, Strength and Self-Esteem even decreased in the control group (which attended standard physical education classes). Authors speculate that the high baseline PSDQ scores (possible ceiling effect) and the absence of the sedentary control group biased the results.

It is clear from the above mentioned that we report improvement in a greater number of PSC scales than any other study discussed, at least in case of the combined Body Combat exercise and aronia juice consumption. Though it may be attractive to conclude that our intervention outbalances all the other intervention protocols discussed above, such a conclusion is not to be made until further controlled trials examine different exercise program effects on the PSC or differentiate between the separate contributions of Body Combat training and aronia juice to the overall PSC improvement. Moreover, two other issues also provide implications for the future studies. Firstly, since there is no ideal placebo for the exercise, the most appropriate study design would be a cross-over with sufficient washout period between no treatment and 2 different exercise treatments (BCA and BC). Such a design would also minimize interindividual variability, since each participant represents a „matched“ control for itself. However, that design is challenging in terms of the participants’ adherence due to a much longer protocol duration and a greater number of blood samplings. Secondly, since our control group who did not exercise or consume aronia juice also showed improvement on the General Physical Condition dimension and several body composition and biochemical parameters, we may speculate that their inclusion in the study was a sufficient trigger for them to improve their life style habits or to develop competiveness against the exercising groups. Thus, they may also have engaged in some kind of leisure physical activity or dietary restriction without reporting it. Considering these two issues, the greatest limitations of our study may be the absence of the double blinded randomization, an appropriate placebo (e.g. a juice equal in sensory properties to aronia juice but depleted of its active components), objective monitoring of exercise intensity, dietary intake, menstrual cycle phase and the additional control sedentary but aronia consuming group as well as the small sample size.

Although our study failed to prove significant improvements in most of the CVD risk factors observed (body composition, blood biochemical parameters or metabolic estimations) in healthy young sedentary females after 8 weeks of Body Combat aerobic exercise with or without aronia juice consumption, to the best of our knowledge this is the first controlled intervention study that combines aronia juice consumption with an aerobic dance/martial arts exercise in young healthy previously sedentary females with a simultaneous examination of both the physiological and psychological effects. Both of our intervention treatments showed strong potential for the PSC improvement and caused small improvements in at least two components of body fat (LL-F% and visceral fat rating) which is a known CVD risk factor. Our findings as well as the findings of several abovementioned studies show that a change in body composition and physiological parameters is not prerequisite for improvement in the PSC. We conclude that even the awareness and ability of doing something good for one's own physical-self (exercising, consuming healthy juice) is beneficial for the development of positive PSC and could possibly slow down the increase in eating disorders and body image distortion prevalence among generations brought up in the modern "era of dieting" and "hunt for leanness"(11). Therefore, we suggest that the creators of national labor and academic policies incorporate free access to exercising facilities, fresh fruit and juices to their young female staff or students in order to protect them from body image and PSC distortions and body weight gain during the challenging period of transition from high school to university or working environment.

To sum up, the fact that our participants were healthy young individuals whose CVD or metabolic disease risk is low compared to the older sedentary adults possibly prevented striking physiological change. Thus, we conclude that beneficial changes in psychological outcome represented by the improved PSC may be of greater clinical importance for the young adults, having in mind that a high score in physical self-perception is associated with a greater body and life satisfaction, increase in current quality of life and higher rate of frequent participation and continuation in physical activity which is a proven method for the prevention of CVD in later adulthood.

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