The changes of oxidative stress and endothelial function biomarkers after 6 weeks of aerobic physical training in patients with stable ischemic coronary disease

Promena biomarkera oksidativnog stresa i funkcije endotela posle šestonedeljnog aerobnog fizičkog treninga kod bolesnika sa stabilnom ishemijskom bolesti srca

**Boris J. Djindjić*, Zoran L. Radovanović†, Tomislav Kostić‡,
Nataša M. Djindjić§, Marko V. Lazović‡**

**University of Niš, Faculty of Medicine, *Institute of Pathophysiology, §Institute of
Biomedical Research, Niš, Serbia; Clinical Center Niš, †Institute of Radiology, ‡Clinic of
Cardiology, Niš, Serbia**

Abstract

Background/Aim. Exercise-based cardiac rehabilitation improves endothelial function, reduces cardiac mortality and anginal symptoms in patients with established cardiovascular disease. We evaluated the changes of oxidative stress and endothelial function biomarkers after 6 weeks of aerobic physical training in patients with stable ischemic coronary disease (IHD) participating in a cardiovascular rehabilitation exercise program. **Methods**. Thirty-five patients with stable IHD engaged in cardiovascular rehabilitation program with the regular aerobic physical activity during 6 weeks were consecutively included together with 37 control, age and sex-matched, IHD patients with a sedentary lifestyle. Clinical data about anthropometric and cardiovascular parameters and laboratory data: serum cell adhesion molecules intracellular (sICAM-1) and vascular (sVCAM-1), reactive carbonyl derivatives (RCD), lipid peroxidation products malondialdehyde (MDA) and nitric oxide (NO) concentration were determined at the beginning and after 6 weeks of aerobic training (45 minutes of continuous exercise up to 80% of maximal heart rate, 3 times a week). **Results**. The baseline characteristics of examined groups were similar according to age, gender, and cardiovascular risk profiles. The regular aerobic physical activity induced significant reduction of body mass index, blood pressure, heart rate,

triglycerides, RCD (1.27 ± 0.48 μmol/g proteins vs. 1.04 ± 0.22 μmol/g proteins), sVCAM-1 [100.4, interquartile range (IQR)(78.4–118.3) ng/mL vs. 80.0 IQR(68.5–97.2 ng/mL)] and increasing of NO (64.72 ± 16.06 nmol/mg proteins vs. 74.38 ± 18.57 nmol/mg proteins) and HDL cholesterol ($p < 0.05$), which was not seen in sedentary control RCD (1.16 ± 0.25 interquartile range vs. 1.12 ± 0.14 interquartile range), sVCAM-1 [92.2 IQR (73.6–106.8 ng/mL) vs. 91.3 IQR (73.0–105.3 ng/mL) and NO (68.5 ± 17.9 nmol/mg vs. 65.7 ± 19.6). The values of sICAM-1 were lower in exercise training group baseline without significant changes during observation [80.74 IQR (54.92–97.3) vs. 80.36 IQR (68.1–95.3)] compared to the control [86.35 IQR(57.32–95.8) vs. 84.65 IQR(55.67–93.8). In the exercise training group, values of sVCAM-1 and RCD were significantly lower and NO higher at the end of the study compared to those in the sedentary control. **Conclusion**. Regular physical activity induced reduction of oxidatively modified proteins and vascular cells adhesion molecules alongside with increased NO bioavailability and favorable changes in HDL cholesterol and triglycerides.

Apstrakt

Uvod/Cilj. Redovna fizička aktivnost u sklopu kardiovaskularne rehabiltacije popravlja endotelnu funkciju, redukuje kardiovaskularni mortalitet i anginozne tegobe bolesnika sa kardiovaskularnim oboljenjem. U radu su analizirane promene parametara oksidativnog stresa i endotelne funkcije nakon sprovedenog redovnog aerobnog fizičkog treninga tokom šest nedelja kod bolesnika sa stabilnom formom koronarne bolesti

(KB) uključenih u program kardiovaskularne rehabilitacije. **Metode**. Analizirano je 35 uzastopnih bolesnika sa stabilnom formom KB, uključenih u program kardiovaskularne rehabilitacije sa redovnom aerobnom fizičkom aktivnošću tokom šest nedelja, zajedno sa 37 kontrolnih bolesnika sa KB, uparenih prema polu i starosti, sa sedentarnim načinom života. Anamnestički podaci o komorbiditetima, faktorima rizika od kardiovaskularnih bolesti, primenjenoj terapiji, klinički podaci o antropometrijskim i kardiovaskularnim parametrima kao i laboratorijske

Correspondence to: Boris J. Djindjić, University of Niš, Faculty of Medicine, Institute of Pathophysiology, 18 000 Niš, Serbia.
E-mail: boris_dj@medfak.ni.ac.rs

analize: adhezioni molekuli, derivati oksidativne modifikacije proteina (RCD), derivati lipidne peroksidacije malondialdehid (MDA) i azot monoksid (NO) određivani su na početku i nakon šest nedelja aerobnog treninga. Trening se sastojao od 45 minuta kontinuirane fizičke aktivnosti (do 80% maksimalne srčane frekvencije), tri puta nedeljno. **Rezultati**. Karakteristike grupa na početku studije bile su slične u pogledu polne i starosne strukture, kao i prisustva faktora rizika od kardiovaskularnih bolesti. Redovna aerobna fizička aktivnost dovodila je do značajne redukcije indeksa telesne mase, krvnog pritiska, srčane frekvencije, koncentracije triglicerida, RCD (1,27 ± 0,48 μmol/g proteins naspram 1,04 ± 0,22 μmol/g proteins), sVCAM-1 [100.4 (IQR) (78,4–118,3) ng/mL naspram 80,0 IQR (68,5–97,2 μmol/g proteins)] i povećanja NO (64,72 ± 16,06 μmol/g proteins prema 74,38 ± 18,57) i HDL holesterola ($p < 0,05$), što nije registrovano u sedentarnoj kontroli: RCD (1,16 ± 0,25 naspram 1,12 ± 0,14), sVCAM-1 [92,2 IQR (73,6–106,8) naspram 91,3 IQR(73,0–105,3)] i NO (68,5 ± 17,9 naspram 65,7 ± 19,6). Serumske vrednosti intracelularnog adhezionog molekula sICAM-1 su startno bile niže u grupi sa primenjenim treningom bez značajne promene tokom praćenja [80,74 IQR (54,92–97,3) *vs* 80,36 IQR (68,1–95,3)] u odnosu na kontrolnu grupu [86,35 IQR (57,32–95,8)] *vs* 84,65 IQR (55,67–93,8). U grupi sa fizičkim treningom vrednosti sVCAM-1 i RCD su bile značajno niže a NO više na kraju studije u poređenju sa kontrolnom grupom. **Zaključak**. Redovan fizički trening indukuje smanjenje intenziteta oksidativnog stresa i ćelijskih adhezionih molekula uz porast bioraspoloživog NO i povoljne promene HDL holesterola i triglicerida.

Introduction

Stable ischemic heart disease (IHD) is characterized by systemic endothelial dysfunction. Besides from pharmacological interventions, exercise training improves endothelium-dependent vasodilatation in coronary blood flow and shows positive effects on regression of coronary atherosclerosis and prevention of restenosis. These effects were mediated through stabilization of atherosclerotic lesions and favorable changes in serum lipoproteins in patients with IHD [1]. Exercise has positive effects on an arterial endothelial function by an increased endothelial nitric oxide synthase (eNOS) protein expression and thus increased concentrations of bioavailable nitric oxide (NO) [2].

Exercise training is the major component of cardiac rehabilitation. It has positive effects on the psychosocial well-being and quality of life. Regular exercise improves cardiovascular risk factors' profile and a long-term prognosis in patients with IHD [3]. Results from other studies show that low maximal aerobic capacity is associated with an increased rate of cardiac events [4]. Despite all these benefits, a great proportion of adults in developed countries is physically inactive (about 70% of all Americans), which represents a great pool of individuals at a risk to develop cardiovascular diseases [5].

Increased oxidative stress in endothelium is also a fundamental pathophysiological mechanism of IHD development. The redox signaling pathways have an important role in regulating the cardiac function and vascular tone by reducing NO bioavailability and increased quenching of NO by superoxide [6]. Exercise training plays a positive role in virtually all redox aspects of cardiac and vascular pathophysiology. Regular physical activity of moderate intensity has antioxidant properties and improves endothelial function. It has evolved as an accepted therapy to improve endothelial function. However, the molecular mechanisms by which exercise training improves redox homeostasis in cardiovascular diseases remain unknown and need further investigation [7].

The aim of this study was to evaluate the changes of oxidative stress and endothelial function biomarkers after 6 weeks of aerobic physical training in patients with stable IHD participating in a cardiovascular rehabilitation exercise program.

Methods

The study included 72 patients with stable IHD divided into 2 groups. Exercise training group consisted of 35 patients who spent 21 days under supervising cardiovascular rehabilitation in the medical centre and 21 days of planned physical training at home. They were recruited for the study in a consecutive manner from a cohort of IHD patients included in a cardiovascular rehabilitation program. The control group consisted of 37 age and sex-matched patients with stable IHD who had usual everyday physical activity at home without any other physical activity or physical training 6 weeks before the examination. The control participants were chosen from a cohort of dyslipidemic and hypertensive patients with stable IHD treated in the outpatient Department of Lipid Metabolism and Hypertension, according to the age range obtained from the exercise training group.

The diagnosis of IHD was confirmed by medical documentation of significant coronary stenosis, stenting or bypass procedures or myocardial infarction. The patients were included if they had no anginal symptoms or had stable angina (functional classification of angina: Canadian Society of Cardiology class II / III) diagnosed with the onset of anginal chest pain provoked by physical or mental stress, which disappears after rest or sublingual nitro-glycerine application [8].

The study excluded patients with acute and chronic inflammatory diseases, liver diseases, recent trauma, mild to severe anemia, patients with anti-inflammatory medication (except acetyl salicylic acid), vitamins and antioxidants. Exclusion also comprised those with the acute coronary syndrome within last 3 months, poorly controlled congestive heart failure, signs of myocardial ischemia on electrocardiogram (ECG) at stage 1 of the exercise tolerance test (Bruce protocol), or presence of ECG abnormalities such as left bundle branch block, presence of pacemaker and poorly controlled diabetes or thyroid disease. Patients with non provoked angina's pain in rest were excluded.

The clinical characteristics of patients involved: the presence of concomitant diseases, cardiovascular risk factors, used therapy, anthropometric (data waist and hip circumference, body mass index and waist/hip ratio). The blood pressure was determined according to American Heart Association procedure (the average value from the 3 measurements after 5 minutes resting was adopted) [9].

All patients signed the informed consent to participate in the study and the local Ethical Review Board approved the study.

All the patients had a therapy for secondary prevention of IHD according to the European Society of Cardiology (ESC) guidelines and all cardiac-related medication doses were kept constant during the study. During the study, all patients were required to apply the recommended hypolipemic diet and to avoid any change in their usual housework.

Exercise training protocol

During 6 weeks all patients in the exercise group had 45 minutes of continuous aerobic physical activity using the treadmill, ergo bicycle or walking 3 times a week. The intensity of the physical activity was limited up to 80% of maximal heart rate which was obtained by pre-study ergo test for every patient.

Blood sampling and laboratory measurements

Blood samples were obtained from all patients in the exercise training and the control group at baseline and after 6 weeks. Venous blood samples were taken from the cubical vein in the morning, after 12 h of fasting, before the regular therapy was taken. The following parameters were laboratory determined in obtained samples: total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high- density lipoprotein cholesterol (HDL-C) and triglycerides (TG). In the exercise training group, blood samples were collected at least 24 h after the last physical exercise in order to avoid the immediate effects of exercise. After centrifugation, aliquots were frozen at -80°C until assayed.

Endothelial function

Nitric oxide (NO) release was determined spectrophotometrically by measuring the accumulation of its stable degradation products nitrite and nitrate. Total nitrites were then determined spectrophotometrically by using the Navarro-Gonzalvez et al. [10] (1998), reaction based on Griess reaction. Total nitrate and nitrite concentrations were given in nmol/mg protein.

Cell adhesion molecules, intracellular (CAM-1) and vascular (VCAM-1) were detected by enzyme-linked immunosorbentassay (ELISA), using a commercial test (Beckman Coulter Inc.) on a Bio Systems – ELISA reader. Results were expressed in ng/ml [serum VCAM-1 (sVCAM-1) measuring range: 0-250 ng/mL, sensitivity: 7.4 ng/mL), and sICAM-1 (measuring range: 0-160 ng/mL, sensitivity: 1 ng/mL).

Oxidative stress

Lipid peroxidation intensity was monitored by measuring serum malondialdehyde (MDA), as one of the end products of lipid peroxidation (method by Andreeva et al. [11]). MDA on high temperature in low pH environment, with the addition of Fe^{2+} turns to thiobarbiturate acid and colors the

suspension pink. Chromogen absorption was detected at 523 nm. Levels of serum MDA were presented in µmol/L.

Determination of oxidatively modified proteins was done by spectrophotometric measurement of carbonyl group content in amino acids residues. The carbonyl content was determined by colorimetric reaction with 2,4-dinitrophenylhydrazine (DNPH) and expressed as µmol/g plasma proteins [12]. Protein concentration was determined by the Lowry et al. [13] method.

Statistical analysis

Continuous data with normal distribution were expressed as the mean ± standard deviation (SD) or median (interquartile range, IQR) for skewed data. Categorical data were presented as numbers and percentages. Differences between two groups were compared by Student's t-test and Mann-Whitney U test for continuous variables and χ2 and Fisher test for categorical variables. Statistical analyses were done by software package SPSS 16.0.

Results

The baseline characteristics of examined groups were similar according to age, gender and cardiovascular risk profiles (proportions of smokers, the prevalence of hypertension, dyslipidemia, diabetes mellitus, obesity, family history for coronary artery diseases (CAD). The therapy for secondary prevention of IHD was similar in both groups and was kept constant during 6 weeks (Table 1).

Data are presented as the mean ± SD or n (%); MI – myocardial infarction; PTCA – percutaneous transluminal coronary angioplasty; CAB – coronary artery bypass; CAD – coronary artery disease; ACEI – angiotensin-converting-enzyme inhibitors; ARB – angiotensin receptor blockers; NS – nonsignificant.

The effects of 6 weeks cardiovascular rehabilitation program were visible in a significant reduction of body mass index (BMI), systolic and diastolic blood pressure, heart rate and triglycerides as well as increasing of HDL cholesterol ($p < 0.05$) which was not seen in the group with a sedentary lifestyle (Table 2).

Regular, aerobic, moderate exercise training during 6 weeks induces favorable increase of NO, reduction of RCD and sVCAM-1 level compared with its initial level which is not seen in the control group. The values of sICAM-1 were lower in the exercise training group at the start and at the end of the study without significant changes during the period of observation. In the exercise training group, values of sVCAM-1 and RCD were significantly lower and NO significantly higher at the end of the study compared to the sedentary control group (Table 3).

Discussion

There is a consistent evidence that any type of regular rhythmic physical exercise reduces blood pressure and decrease heart rate independently of weight loss, dietary habits

Table 1
Baseline characteristics of patients

Parameters	Exercise training group	Control group	p
Male/female, n	15/20	18/19	NS
Age (years), x̄ ± SD	57.4 ± 5.7	59.3 ± 6.8	NS
MI, n (%)	23 (65.7)	26 (70.2)	NS
CAB grafting, n (%)	5 (14.2)	4 (10.8)	NS
PTCA, n (%)	7 (20.0)	7 (19)	NS
Duration of CAD (years), x̄ ± SD	4.9 ± 3.8	6.8 ± 5.2	< 0.05
Risk factors for CAD, n (%)			
smokers	14 (40.0)	16 (43.2)	NS
elevated blood pressure	30 (85.7)	30 (81.1)	NS
lipid disorders	15 (42.8)	15 (40.5)	NS
diabetes mellitus	12 (34.2)	13 (35.1)	NS
obesity	11 (31.4)	10 (27.0)	NS
family history for CAD	15 (42.8)	15 (40.5)	NS
Evidence based therapy, n (%)			
beta blockers	30 (85.7)	28 (75.7)	NS
ACEI / ARB	31 (88.5)	33 (89.2)	NS
calcium channel blockers	25 (71.4)	27 (72.9)	NS
statins	31 (88.5)	32 (86.4)	NS
acetylsalicylic acid	33 (94.2)	35 (94.6)	NS
nitrates	20 (57.1)	20 (54.1)	NS

MI – myocardial infarction; PTCA – percutaneous transluminal coronary angioplasty; CAB – coronary artery bypass; CAD – coronary artery disease; ACEI – angiotensin-converting-enzyme inhibitors; ARB – angiotensin receptor blockers; NS – nonsignificant.
x̄ – arithmetic mean; SD – standard deviation;

Table 2
Clinical and biochemical data of patients included in the study

Parameters	Exercise training group		Control group	
	Δ change	% change	Δ change	% change
BMI (kg/m²)	-1.5*	-5.05	-1.5	-5.70[&]
WC (cm)	-1.2	-1.26	+0.6	+0.68
WHR	-0.01	-1.14	-0.01	-1.12
sBP (mmHg)	-7.7[&]	-5.37	-4.7	-3.39
dBP start (mmHg)	-7.4[&]	-8.20	-2.6	-2.98
HR (/min)	-6.3[&]	-8.02	+1.3	+1.71
TC (mmol/L)	-0.2	-3.70	-0.2	-3.77
LDL-C (mmol/L)	-0.3	-9.09	-0.2	-5.56
HDL-C (mmol/L)	+0.23[&]	+23.47	-0.01	-1.00
TG (mmol/L)	-0.2[&]	-11.24	+0.1	+5.88

Data are presented as Δ change from baseline or % change from baseline; BMI – body mass index; WC – waist circumference, WHR – waist/hip ratio; sBP – systolic blood pressure; dBP – diastolic blood pressure; HR – heart rate; TC – total cholesterol; LDL-C – low density lipoprotein cholesterol; HDL-C – high density lipoprotein cholesterol; TG – triglycerides;
***p < 0.05 vs initial values.**

Table 3
Carbonyl contents, lipid peroxydation and markers of endothelial disfunction

Parameters	Exercise training group		Control group	
	before	after	before	after
RCD (μmol/g plasma proteins), x̄ ± SD	1.27 ± 0.48	1.04 ± 0.22[†]	1.16 ± 0.25	1.12 ± 0.14
MDA (μmol/L), x̄ ± SD	14.09 ± 6.65	12.84 ± 3.25	15.12 ± 6.15	13.79 ± 5.23
sVCAM (ng/mL), median (IQR)	100.49 (78.42–118.3)	80.08 (68.5–97.2)†*	92.26 (73.64–106.8)	91.33 (73.01–105.3)
sICAM-1 (ng/mL), median (IQR)	80.74 (54.92–97.3)*	80.36 (68.1–95.3)*	86.35 (57.32–95.8)	84.65 (55.67–93.8)
NO (nmol/mg prot.), x̄ ± SD	64.72 ± 16.06	74.38 ± 18.57[†]*	68.5 ± 17.9	65.7 ± 19.6

x̄ – arithmetic mean; SD – standard deviation; IQR – interquartile rang; MDA – malondyaldechyde; RCD – reactive carbonyl derivatives; NO – nitric oxide; sVCAM-1 – soluble vascular cell adhesion molecule-1, sICAM-1 soluble intercellular adhesion molecule-1; *p < 0.05 vs control; †p < 0.05 vs initial values.

or smoking compared to sedentary ones [14–16]. Aerobic exercise (30–40 minutes at 65% of VO_2 max) three times a week showed effects on vascular function too. It significantly reduces augmentation index (an index of arterial stiffness which measures the reflected wave at the aorta), improves carotid artery compliance, and can restore vascular endothelial function in adults [17, 18].

Considering the similar age, gender distribution, cardiovascular risk factors profile and therapy in both groups of patients at the start of the study, the significant reduction of BMI, systolic and diastolic blood pressure, heart rate and triglycerides as well as increasing of HDL cholesterol could be of pathogenetic importance in the oxidative stress reduction and endothelial function improvement after 6 weeks of regular aerobic exercise training implementation.

Significant reduction of triglyceride concentration and raising of HDL cholesterol level in the group with physical training are in the concordance with data obtained by other authors in similar settings [19]. Volaklis et al. [20] showed that exercise training program during 16 weeks significantly reduced a total cholesterol and triglycerides without altering LDL cholesterol level in patients with CAD.

The cardiovascular risk factor management is widely recognized as a priority in the secondary prevention programs [21, 22]. The regular physical activity and its favorable effects on some cardiovascular risk factors such as blood pressure, dyslipidemia and obesity are important especially in stable asymptomatic patients since it improves the prognosis of this severe disease in which absence of symptoms implies no benignity, as it was presented in this study.

Reactions such as cell injury, adhesion, inflammation, and oxidative stress occur not only at the early stage of risk but persist throughout the process of atherosclerosis. The increased oxidant stress is common to these processes, characterized by the excessive generation of reactive oxygen and nitrogen species (ROS and RNS, respectively) and reduced antioxidant capacity. There is an accumulating evidence from prospective studies for a predictive role of elevated circulating levels of sICAM-1 in initially healthy people, and of sVCAM-1 in patients at high risk or with overt CAD. In those reports that quantify atherosclerosis, sVCAM-1 seems to be more specific for atherosclerosis than other markers. The serum level of sVCAM-1 appears to correlate with the extent of atherosclerosis and might allow for the detection of early stages of atherosclerosis [23]. Thus, adhesion molecules especially VCAM-1 might be useful for clinical risk prediction in IHD patients and serve as therapeutic targets during a cardiovascular rehabilitation program, which is supported by the finding of its significant reduction after 6 weeks of exercise training protocol in conducted study. Similar significant decreasing of VCAM-1 was presented in the study by Lim et al. [24] in elderly obese women after 12 weeks of a healthy life exercise program.

Early therapeutic intervention based on an aerobic exercise program is able to prevent progression and manifestation of the clinical sequelae of atherosclerosis which is also shown by other authors [24, 25]. The continuous or intermittent aerobic exercise training leads to a significant decrease of serum VCAM-1 and ICAM-1 in patients with New York (NYHA) class II–III chronic heart failure and with a left ventricular ejection fraction of 35–55% after 10 weeks [26]. This further spreads the spectrum of patients who benefit from a physical training.

This observation could be applied not only in atherosclerotic cardiovascular disease patients but also in healthy individuals with professionally increased cardiovascular risk such as night shift workers. The intermittent exercise at 10-min bouts (30 min per day), 3 days a week during 10 weeks induces significant decreasing of VCAM-1 and plasma concentrations of some serum's elastases in night shift workers [27]. The common observation in all studies which registered decreasing of CAMs was a reduction of BMI and percent of body fat [19, 24, 26, 27] as seen in conducted study.

The large systematic review about the influence of physical activity on key biomarkers in atherosclerosis suggests that effects on atherosclerotic process may depend on the type, duration, and intensity of physical activity. The cell adhesion molecules seemed to be affected by aerobic exercise while resistance training showed no effect [28]. According to literature data, the effects of aerobic physical training are visible after 4 weeks of aerobic training and persist during the training period of 1 year [29, 30].

Decreasing of reactive carbonyl derivatives but not MDA indicates a specific antioxidative pattern of physical training beyond effects on lipids and endothelial function. The effects of physical exercise training program on blood pressure reduction independently of used antihypertensive treatment could be distinctive antihypertensive mechanism mediated by reactivation of different signaling protein molecules and improving the mitochondrial dysfunction with additional effects on subsequent coronary event prevention [6]. This is in line with novel observations that distinct from the NO pathway different vasoregulative factors improve vasomotion [31]. Further studies confirmed the beneficial effects of exercise on vascular vasomotor function independently of markers of inflammation and oxidative stress and were interrelated with improved exercise capacity [32].

The other important effect of the regular physical training program is increasing concentration of NO. Besides its effects on vasodilatation, it improves the efficiency of myocardial O_2 consumption and produces cardioprotection. These responses are mediated, at least in part, by protein S-nitrosylation, a redox-dependent reversible protein posttranslational modification that involves attachment of a NO moiety to a protein sulphydryl group, further protecting these thiol groups from irreversible oxidative/nitrosative modifications [33, 34] which are also shown in this study.

Conclusion

The exercise training is important as a non-pharmacological tool in treating hypertension, lipid disorders and endothelial dysfunction in selected motivated patients with stable coronary artery disease. Regular physical activity induces the reduction of oxidatively modified proteins and vascular cells adhesion molecules alongside with increased NO bioavailability and favorable changes in HDL cholesterol and triglycerides.

Acknowledgements

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