Cardiac rehabilitation improves the blood plasma properties of cardiac patients

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Abstract

Cardiac rehabilitation (CR) improves exercise tolerance and general function. However, its effects on blood plasma in cardiac patients remain uncertain. Our aim was to examine the effect of comprehensive CR on the oxidative stress parameters and antioxidant plasma status in patients with coronary artery disease (CAD) after cardiac interventions. Exercise-based rehabilitation was established as ergometer training, adjusted for individual patients' physical efficiency. Training was repeated three times a week for two months. The standard biochemical (total cholesterol, HDL, LDL, triglycerides and erythrocyte sedimentation rate) and metabolic parameters (peak oxygen uptake [VO₂] and peak workload) were determined. We assessed plasma viscosity, lipid peroxidation, carbonyl compounds levels, glutathione (GSH) and ascorbate (ASC) levels and the non-enzymatic antioxidant capacity of plasma in 12 patients with CAD before and after CR. Parameters were examined before exercise, immediately after exercise, and 1 h later. We also compared morphological and biochemical parameters of blood, as well as other parameters such as heart rate and blood pressure (resting and exercise), VO₂max and peak workload (W) before and after CR. Before CR, a significant decrease in GSH concentration was observed 1 h after exercise. Conversely, after CR, GSH, and ASC levels remained unchanged immediately after exercise. However, ASC increased after CR after exercise and 1 h later in comparison to before CR. There was a significant increase in ferric reduction ability of plasma immediately after exercise after CR, when compared with before CR. CR improved several blood biochemical parameters, peak VO₂, induced an increase in systolic blood pressure peak, and patients' peak workload. After CR, improvements were detected in oxidative stress parameters, except in the level of carbonyls. These changes may contribute to the increased functional heart capacity and better tolerance to exercise and functional capacity of the patients. These improvements could indicate better prognosis of future cardiac events and hospitalization and better quality of life.

Keywords: Cardiac rehabilitation, cardiac intervention, exercise training, blood plasma, oxidative stress

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Introduction

Acute cardiac events, as a consequence of different cardiovascular diseases (CVDs), are among the most frequent causes of mortality. Development of CVD is associated with the presence of oxidative stress.¹ Atherosclerosis and ischemic heart disease (IHD) are caused and followed by increased production of reactive oxygen species (ROS).² It has been also shown that surgical interventions, e.g. coronary artery bypass grafting (CABG), are associated with the induction of oxidative stress due to the activation of neutrophils, the presence of hemolyzed blood containing free oxidized hemoglobin, the induction of inflammatory

reactions within the pericardial fluid and postsurgical complications.³ Cardiac patients, particularly after acute cardiac events, have markedly reduced tolerance to physical activity.⁴

Exercise evokes various effects in healthy and diseased subjects dependently on its intensity and duration. Single acute exercise induces significant decrease of GSH in blood plasma of healthy rats indicating enhanced production of ROS.⁵ Exhaustive exercise has been recently found to influence only slightly the oxidative status in young healthy men.⁶ However, in comparison with healthy controls, acute exercise can aggravate oxidative stress and symptoms of preexisting disease in unhealthy subjects.⁷ Patients with

chronic heart failure (CHF) have pronounced oxidative response to exercise in their plasma, such as elevated lipid peroxidation in comparison to control patients. Increased malondialdehyde (MDA) levels during single exercise did not depend on the etiology of CHF.⁸

On the other hand, it is well known that non-strenuous, repetitive physical activity, and mild oxidative stress can positively affect patients with cardiac diseases.

Physical training induces many hematological, hormonal, and metabolic changes,⁹ which are dependent on several factors, mainly the current state of health and intensity and duration of exercise. All of these alterations are adaptations to training which lead to improved efficiency and body fitness. Repetitive training induces physical and biochemical modifications such as increased oxygenation, improved blood flow, and increased metabolism. Chronic exercise induces mild oxidative stress, which in most cases is not detrimental to cardiac patients. Contrary to the acute state, moderate oxidative stress increases the capacity of endogenous anti-oxidative systems, which may attenuate preexisting disease-dependent oxidative stress.¹⁰ Finally, repetitive exercise might have a positive outcome in delaying the progression of disease.⁷ Hence, physical training has been postulated and employed as an important method of rehabilitation in patients suffering from CVDs.¹¹ However, comprehensive rehabilitation of CVD patients is multifactorial and includes nutritional education followed by changes in dietary habits and supplementation,^{12,13} risk factor education, psychological input, and drug therapies to lower low-density lipoprotein (LDL) cholesterol.¹⁴ Exercise training (cardiac rehabilitation [CR]) has been utilized in patients with IHD after myocardial infarction (MI) and other cardiac events, such as percutaneous coronary interventions (PCIs), CABG¹⁵ as well as in the elderly.¹⁶ CR aims to delay disease progression and to increase physical recovery with significant decrease of the risk of recurrent cardiovascular events enabling to return to daily activity. MI patients rehabilitated by exercise training displayed a lower risk of future infarction, lower cardiac mortality, and reduced mortality due to other causes.¹⁷ Another goal of CR is to minimize the number of hospital admissions and their costs.¹⁸ It has been shown that involvement in CR program decreases the risk of recurrent hospital admission and mortality after MI.¹⁹ Patients undergoing CABG with subsequent exercise training during CR had reduced mortality rate and decreased number and duration of hospital readmissions during 10 years after CABG when compared to patients without CR.²⁰

Nevertheless, CR frequently is not properly utilized due to patient- and system-related barriers²¹ and existing programs could be extended and modified to increase accessibility to more patients, e.g. in the form of home-based CR.²² Thus, further research is required to increase physicians' awareness and to encourage them to provide comprehensive CR programs to all eligible patients.

Most of the studies on the physical activity influences in cardiac patients have been focused on the risk factors associated with physical fitness, fibrinolytic system, or lipid profile, whereas investigation of the changes induced in blood remains scarce.²³ The state of patients before

involvement in physical training is particularly underestimated, and may influence the outcomes of exercise-based CR programs.

The aim of this study was to examine the effect of CR and exercise training programs on plasma viscosity, lipid peroxidation (the level of peroxides and thiobarbituric acid-reactive substances [TBARS]), the level of carbonyl compounds, glutathione (GSH) and ascorbate (ASC) concentration and non-enzymatic antioxidant capacity (NEAC) in the plasma of 12 patients with coronary artery disease (CAD) after cardiac interventions. The standard biochemical parameters (total cholesterol, HDL, LDL, triglycerides and erythrocyte sedimentation rate [ESR]) as well as metabolic and hemodynamic parameters (resting and max heart rate, maximal oxygen uptake (VO₂ max) and peak workload) were also determined.

Materials and methods

Chemicals

Xylenol orange, 1,1-diphenyl-2-picrylhydrazyl (DPPH radical) and 4-amino-2,2,6,6-tetramethylpiperidine-1-oxyl (Tempamine), quinaldic acid (QA), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), and ascorbic acid were obtained from Sigma-Aldrich (St. Louis, MO). All other chemicals were analytical grade products from POCh S.A. (Gliwice, Poland).

Subjects and experimental procedures

Patients with CAD after cardiac interventions (PCI and bypass surgery, 12 male volunteers): age (54.5 ± 6 years); weight (86.7 ± 14.9 kg), BMI (29.4 ± 4.5), were examined before and after comprehensive CR. As a part of CR, patients were educated about lifestyle changes including physical activity, healthy diet, stress coping techniques, and effective methods to eliminate nicotine and alcohol and other unhealthy habits. Physical training, healthy lifestyle education, and psychological support were offered for two months in outpatients clinic. Comprehensive CR program was designed as a part of CVD secondary prevention. Each patient underwent 24 interval training sessions on a cycle ergometer three times a week for two months. Workload during physical training was planned individually using formula: $HR_{peak} = \text{resting HR} + (60\text{--}80\%) \text{ of the heart rate reserve (HRR)}$. Duration of a single training session was designed for 35 min cycling on Ergoline Reha System. Interval training was characterized by changing workload: increased gradually from 30 W to maximum of 75 W and further lowered back to 30 W. Each step was performed for 4 min with the limits of pulse rate determined for physical efficiency, and a few min of rest with pulse return to the initial rate. This scheme was modified according to the health state of each patient.

Following exercise, the subjects remained seated at rest for 1 h, and were allowed to drink only water. This specific exercise protocol was used as a model of an stressor inducing oxidative stress.

During CR, all patients were also asked to perform 10–15 min of general conditioning exercises twice daily to

increase by no more than 30% of the resting HR. The interval training sessions were performed three times a week and were monitored by online ECG and BP measurements before and after every session, at the end of each workload peak.

During CR all the patients received unchanged drug therapy, which included beta-blockers, angiotensin-converting enzyme inhibitors, statins, and acetylsalicylic acid.

Venous blood samples were taken from an antecubital vein before the exercise test, immediately after exercise and 1 h after the exercise session.

The experiments have been performed in accordance with the rules of the Declaration of Helsinki and conformed to the ethical principles set by the Belmont Report, Ethical Principles and Guidelines for the Protection of Human Subjects of Research. Approval for investigation was obtained from the Bioethical Committee of Medical University of Lodz. All participants signed an Informed Consent Form prior to enrolment into the study.

For experiments, the blood was centrifuged at 4°C and the plasma was separated. All parameters were measured after completing exercise and were repeated after eight weeks of the rehabilitation program in every patient.

Plasma peroxide concentration

For determination of plasma hydroperoxides, a method with xylenol orange was applied. The reaction was based on the rapid oxidation of Fe(II) ions to Fe(III) in the presence of peroxides.^{24,25} Ammonium iron(II) sulfate of 25 mmol/L in 2.5 mol/L H₂SO₄ and 125 μmol/L xylenol orange with 100 mmol/L sorbitol were mixed (1:100) to prepare a working solution. The samples were then mixed with a working reagent and incubated for 30 min in the dark. Reaction of Fe(III) ions with xylenol orange yielded a violet-colored complex, which was quantified spectrophotometrically at 560 nm. The concentration of peroxides was expressed as μmol/L of plasma.

Plasma viscosity

Plasma viscosity was monitored as described by Morse²⁶ using Tempamine in the electron paramagnetic resonance (EPR) technique. EPR spectra were recorded on the Bruker ESP 300 E spectrometer working at X-band at room temperature (22 ± 2°C), operating at a microwave frequency of 9.73 GHz. The instrumental settings were as follows: the microwave power was 10 mW, the centre field was set at 3480 G, with a sweep range of 80 G, and a 100 kHz modulation frequency, a modulation amplitude of 1.01 G, and a time scan of 128 s. Estimation of the level of Tempamine spin label mobility was possible by calculating the relative rotational correlation time (τ_c) according to equation (1).²⁷

$$\tau_c = kw_0 \left(\sqrt{\frac{h_0}{h_{-1}}} - 1 \right) \quad (1)$$

where

τ_c : time when the spin label undergoes full rotation
 k : constant equal to 6.5×10^{-10} s/G

w_0 : width of the mid-line of spectrum

h_0 : height of the mid-line of spectrum

h_{-1} : height of the high-field line of spectrum.

Plasma viscosity was calculated using data obtained from the EPR spectra according to equation (2).

$$\eta = \frac{\tau_c(\text{PLASMA})}{\tau_c(\text{H}_2\text{O})} \eta_{\text{H}_2\text{O}} \quad (2)$$

where

$\tau_c(\text{PLASMA})$: rotational correlation time for Tempamine in plasma

$\tau_c(\text{H}_2\text{O})$: rotational correlation time for Tempamine in water

$\eta_{\text{H}_2\text{O}}$: water viscosity equal to 1 cP.

Plasma lipid peroxidation

Lipid peroxidation was assayed by determining the level of the production of TBARS using the method described by Rice-Evans *et al.*²⁸ Plasma samples were mixed with working reagent (15% [w/v] 2-thiobarbituric acid (TBA) in 0.25 mol/L HCl containing 2% butylated hydroxytoluene [BHT]) and incubated at 100°C for 10 min. Finally, samples were cooled and centrifuged at $3000 \times g$ for 5 min at 20°C. The absorbance of supernatant was measured at 535 nm against a blank. The TBARS concentration was calculated using the MDA molar extinction coefficient (1.56×10^5 mol/L⁻¹ cm⁻¹) and expressed as μmol/L of plasma.

Concentration of plasma carbonyl compounds

The content of plasma protein carbonyl groups was estimated using 2,4-dinitrophenylhydrazine (DNPH).²⁹ Plasma was mixed with 0.2% DNPH in 2.5 mol/L HCl and incubated at room temperature for 1 h. After the reaction, the proteins were precipitated with 20% trichloroacetic acid (TCA) and centrifuged at $3000 \times g$, for 5 min at 20°C. The precipitate was washed three times with ethyl acetate:ethanol (1:1, v/v), dissolved in 6 mol/L guanidine hydrochloride and the absorbance was measured at 370 nm. The carbonyl content was calculated using molar absorbance coefficient 22×10^3 mol/L⁻¹ cm⁻¹, and expressed in nmol/mg protein.

Plasma thiols concentration

Plasma thiols were measured using Ellman's method.³⁰ Samples were diluted with 0.01 mol/L phosphate buffer, pH 8.0, containing SDS. The 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB) from a 0.01 mol/L stock solution was then added to samples and incubated for 1 h at 37°C. The thiols react with DTNB and form anions, and the strong yellow color was measured at 412 nm. The calibration curve was prepared using different concentrations of reduced glutathione. The concentration of the thiols was calculated and expressed as μmol/mg proteins of plasma.

The level of reduced glutathione

Reduced glutathione concentration was determined using a fluorimetric method with *o*-phthalaldehyde (OPA).³¹

The reaction product of OPA and GSH has a high fluorescence quantum yield. Redox quenching buffer with TCA (RQB-TCA [20 mmol/L HCl, 5 mmol/L diethylenetriaminepentaacetic acid [DTPA], 10 mmol/L ascorbic acid; 5% TCA]), 7.5 mmol/L *N*-ethylmaleimide (NEM) in RQB and 1 mol/L potassium phosphate buffer (KP) (pH 7.0) were added to fresh plasma and incubated at room temperature for 5 min. Finally, 0.1 mol/L KP buffer (pH 6.9) and methanol solution of OPA (5 mg/mL) were added and incubated in the dark at room temperature for 30 min. The OPA-derived fluorescence was measured at 365 nm excitation and 430 nm emission. The content of GSH in plasma was calculated based on the calibration curve for different concentrations of reduced glutathione used as a standard and expressed as nmol/mg protein.

Plasma ascorbic acid concentration

Determination of the concentration of ASC was performed with the method based on its reduction of iron (III). The iron (II) thus formed is complexed with QA and pyridine.³² The plasma samples were mixed with an equal volume of 20% TCA and, after 30 min of incubation, centrifuged (3000 × *g*, 5 min, at room temperature). Solutions of 200 µg/mL Fe (III), 1% QA and pyridine were added to sample supernatant. Finally, water was added to 10 mL and extracted with chloroform. Organic phase was separated and dried with anhydrous sodium sulfate and chloroform to final volume of 10 mL was added. The absorbance was measured at 380 nm. The concentration of ASC was calculated using molar absorption coefficient ($4.229 \times 10^3 \text{ mol/L}^{-1} \text{ cm}^{-1}$) and expressed as nmol/mg protein of plasma.

Plasma protein concentration

The amount of plasma protein was measured by Lowry's method using bovine serum albumin for a standard curve.³³

Non-enzymatic antioxidant capacity

Non-enzymatic plasma antioxidant capacity was determined using two different methods: ferric reduction ability of plasma (FRAP)³⁴ and reduction of DPPH.³⁵

The FRAP assay gives fast, reproducible results with plasma low molecular weight antioxidants. Fresh plasma was added to FRAP freshly prepared reagent (10 mmol/L TPTZ) in acetate buffer (pH 3.6) with 20 mmol/L FeCl₃ and incubated at room temperature for 30 min. Absorbance was measured at 593 nm.

DPPH is a stable colored radical that reacts with low molecular weight antioxidants. Suppression of color intensity is inversely proportional to the antioxidant content in plasma. Fresh plasma was added to 0.1 mmol/L DPPH in methanol, incubated at room temperature for 30 min and absorbance was measured at 517 nm.

A calibration curve was prepared for both methods from different Trolox concentrations in the range of 0–1000 µmol/L. The results were expressed as µmol of Trolox equivalents per 1 L of plasma (µmol/L).

Statistical analysis

All measurement results have been presented as means (± standard deviation (SD)). The normality of data distribution was checked by the Shapiro–Wilk test.

For biochemical and metabolic parameters, statistical differences were estimated using Student's test for paired data. For statistical analysis in the absence of normal distribution of data the Wilcoxon's test was used.

The statistical significance between three different moments of taking of blood during exercise as well as before and after CR was estimated using analysis of variance with repeated measurements. Each difference was estimated using a test for planned comparisons.

The power of the tests used was checked and it was more than 80%. To estimate correlation between measured parameters, the Pearson's correlation coefficient was calculated. All of the statistical analyses were conducted in STATISTICA.PL v.10.

Results

Data were collected using plasma from 12 patients involved in this study. Table 1 shows the characteristics of the participants in comprehensive CR program.

Table 1 Baseline characteristics, clinical, and medications data of CAD patients undergoing cardiac rehabilitation

Parameters	Before cardiac rehabilitation	After cardiac rehabilitation
Weight (kg)	86.7 ± 14.9	86.0 ± 12.2
BMI (kg/m ²)	29.4 ± 4.5	29.2 ± 3.7
Body fat (%)	24.0 ± 4.6	22.9 ± 3.9
WHR	1.00 ± 0.06	0.97 ± 0.05
Drugs (%)		
ASA		100
Statin		100
ACEI		100
β-Blocker		100
Surgical intervention (%)		
PCI		75
CABG		25
EF (%)		61 ± 8
HA (%)		75
Smoking (%)		87.5
Family history (%)		37.5
Anamnesis (%)		66.7
Hypercholesterolemia		16.7
Hyperlipidemia		8.3
Hypertriglyceridemia		8.3
Gastro-esophageal reflux		8.3

ACEI: angiotensin-converting-enzyme inhibitor, ASA: acetylsalicylic acid, BMI: body mass index, CABG: coronary artery bypass graft, EF: ejection fraction, HA: hypertension arterialis, PCI: percutaneous coronary intervention, WHR: waist to hip ratio.

Diagnosis of IHD disease

The basic blood biochemical characteristics of CAD patients before and after CR are shown in Table 2.

No significant differences in the level of total cholesterol, HDL, LDL, and triglycerides were detected after CR compared to before CR. On the other hand, a significant decrease in ESR ($P = 0.0245$) was observed after CR.

At rest, a statistically significant decrease in heart rate ($P < 0.02$) was observed, while an elevation of systolic blood pressure (SBP) peak ($P < 0.005$) was detected after CR (Table 3).

The cardiopulmonary exercise test VO_2 peak before and after CR was also performed. After CR we observed an improvement in maximal workload in Watts and an increase in peak VO_2 expressed in mL/kg/min (Table 3). In the case of both parameters a significant increase was found at the end of rehabilitation in comparison to baseline.

Determination of oxidative stress and the antioxidant potential of plasma was performed using spectrophotometric methods.

The indicators of oxidative stress such as peroxide, carbonyls and TBARS levels as well as the concentration of antioxidants (GSH, ASC and thiols) and FRAP and DPPH were examined in the plasma of patients during exercise

before and after CR. Table 4 shows the details of blood parameters before exercise, post-exercise, and in recovery (1 h) after CR. In general, plasma viscosity did not change after CR; however, no rise in this parameter was observed immediately after exercise when compared to before CR. An increase in the level of -SH groups (approx. 21%) was observed after CR 1 h after exercise, but unfortunately this result was statistically insignificant. On the other hand, a decrease (approx. 10%) in plasma peroxides after CR was noted, but these results were also insignificant. Simultaneously, a slight decline in the level of TBARS was also observed.

Figure 1 presents the results of the concentration of carbonyl groups in the plasma of CAD patients during exercise before and after CR.

We did not find a difference in the level of carbonyl compounds in the plasma before and after CR. On the other hand, a statistically significant rise in carbonyls content immediately after exercise ($P < 0.001$) and 1 h later ($P < 0.01$) in comparison to levels before exercise prior to CR was noted. Similar results were observed after CR before exercise vs. after exercise ($P < 0.05$) and before vs. 1 h recovery ($P < 0.002$).

Figure 2 shows the level of glutathione in the plasma of CAD patients during exercise before and after CR.

Patients with CAD after CR had an insignificantly increased GSH concentration in the plasma. During exercise a tendency for decreases of GSH levels was observed, especially after CR; however, significant results were found for patients before CR 1 h after exercise ($P < 0.05$ before exercise vs. 1 h recovery and $P < 0.02$ after exercise vs. 1 h recovery).

Patients with CAD before CR had a significantly lower ascorbic acid concentration in the plasma immediately after exercise ($P < 0.01$) and 1 h later ($P < 0.02$) in comparison to the concentration before exercise (Figure 3). Significant differences in ASC concentration were also observed between the plasma of patients before CR and after CR just after exercise ($P < 0.002$) and 1 h later ($P < 0.02$). Figure 4 shows increased levels of the ferric reducing ability of plasma after the CR program; however, a significant increase ($P < 0.05$) in FRAP level was observed immediately after exercise, particularly after CR in comparison to before CR (Figure 4).

Similar results were obtained for the plasma of patients examined with DPPH, however, all differences were insignificant (Table 4).

We also estimated correlations between biochemical parameters before and after CR. Significant correlations between DPPH and TBARS, DPPH and -SH groups as well as FRAP and TBARS for all investigated time points are shown in Table 5. An increase of NEAC (DPPH, FRAP) was correlated with decreased TBARS level. In addition, positive correlation between elevated NEAC (DPPH) and an increased level of -SH groups was also found.

Discussion

A sedentary lifestyle, smoking, and obesity are well-known as CAD risk factors, which also include increased level of arterial pressure, left ventricular hypertrophy, the development of type 2 diabetes mellitus, increased LDL,

Table 2 Basic blood biochemical parameters of patients before and after the end of cardiac rehabilitation

Parameters	Before cardiac rehabilitation	After cardiac rehabilitation	P value
T Ch (mg/dL)	122.8 ± 18.3	107.1 ± 15.2	Not significant
HDL (mg/dL)	36.3 ± 4.4	40.6 ± 5.1	Not significant
LDL (mg/dL)	75.7 ± 17.4	66.7 ± 10.6	Not significant
ESR (mm/h)	21.3 ± 11.1	12.0 ± 7.3	0.02450
TG (mg/dL)	90.0 ± 25.2	80.8 ± 26.8	Not significant

ESR: erythrocyte sedimentation rate.

Table 3 Exercise-related metabolic and hemodynamic parameters

Parameters	Before cardiac rehabilitation	After cardiac rehabilitation	P value
Resting HR (bpm)	66.2 ± 7.1	59.4 ± 4.0	0.01105
Exercise peak HR (bpm)	114 ± 10.1	119 ± 7.2	Not significant
SBP peak (mmHg)	149 ± 18.4	162 ± 13.9	0.00362
SBP resting (mmHg)	111 ± 17.5	106 ± 9.5	Not significant
DBP peak (mmHg)	84 ± 8.9	82 ± 9.6	Not significant
DBP resting (mmHg)	74 ± 10.8	70 ± 3.1	Not significant
VO_2 (mL/kg/min)	20.9 ± 0.73	25.6 ± 1.85	0.00506
Peak workload (W)	108 ± 15.5	137 ± 12.2	0.00060

HR: heart rate ([bpm] — number of contractions of the heart per minute), SBP: systolic blood pressure, DBP: diastolic blood pressure.

Table 4 Plasma viscosity and oxidative stress parameters in CAD patients

Parameter	Before cardiac rehabilitation			After cardiac rehabilitation			P
	Pre-exercise	Post-exercise	Recovery (1 h)	Pre-exercise	Post-exercise	Recovery (1 h)	
Viscosity, η (cP)	1.83 $\pm$ 0.09	1.87 $\pm$ 0.08	1.83 $\pm$ 0.11	1.83 $\pm$ 0.06	1.83 $\pm$ 0.06	1.81 $\pm$ 0.12	Not significant
-SH groups (μmol/mg protein)	1.96 $\pm$ 0.22	2.12 $\pm$ 0.3	2.12 $\pm$ 0.47	2.23 $\pm$ 0.56	2.25 $\pm$ 0.40	2.37 $\pm$ 0.39	
Peroxides (μmol/L)	50.9 $\pm$ 10.1	50.2 $\pm$ 11.6	49.1 $\pm$ 9.3	45.9 $\pm$ 8.5	48.7 $\pm$ 11.3	48.7 $\pm$ 10.4	
TBARS (μmol/L)	0.87 $\pm$ 0.14	0.88 $\pm$ 0.14	0.89 $\pm$ 0.14	0.82 $\pm$ 0.13	0.84 $\pm$ 0.13	0.82 $\pm$ 0.11	
NEAC _(DPPH) (μmol/L)	694 $\pm$ 119	687 $\pm$ 106	700 $\pm$ 127	731 $\pm$ 130	709 $\pm$ 125	741 $\pm$ 132

Figure 1 Changes in the content of carbonyl groups in the plasma of CAD patients before exercise, just after exercise, and after 1 h recovery

Figure 3 Changes in plasma ascorbic acid concentration of cardiac patients measured before and after cardiac rehabilitation

Figure 2 Changes in glutathione concentration in the plasma of CAD patients before and after cardiac rehabilitation

Figure 4 Changes in ferric reducing ability of plasma of cardiac patients

triglycerides and total cholesterol, and decreased levels of HDL.^{36–38} All of these factors stimulate production of ROS in the vascular wall.^{39,40} Elevation of ROS, such as superoxide and hydrogen peroxide (H₂O₂), has been detected in patients with CVD and CHF.^{39,41,42}

Physical activity is a major factor in the CR of patients with CAD. CR causes physical recovery of patients after cardiac events and surgery and reduces the risk of recurrent cardiovascular events. CR improves also quality of life (mental health, energy, pain, everyday functioning

Table 5 Pearson correlation coefficient for correlations between the studied parameters

Compared parameters	Pearson correlation	P
NEAC _(DPPH) vs. -SH	0.826	<0.05
NEAC _(DPPH) vs. TBARS	-0.921	<0.05
NEAC _(FRAP) vs. TBARS	-0.815	<0.05

and other), and reduces behavioral characteristics such as depression, anxiety, somatization, and hostility.⁴³

During exercise, the increase of oxygen supply and consumption can lead to mild and well-tolerated oxidative stress, which can induce an increase of antioxidant systems capacity. This idea was employed in CR and aims to improve exercise capacity and physical functioning. Generally, exercise induces many positive metabolic and functional changes in the body. It has been shown that it improves blood and erythrocyte rheology.⁴⁴ However, the details of the mechanisms of CR-related effects on the oxidative stress and cardiovascular system function in patients after MI and bypass surgery still remain unclear. Thus, the aim of the present study was to examine the plasma properties of CAD patients before and after CR.

Analysis of blood lipid parameters in our study confirmed other investigations. A significant decrease in total cholesterol and triglycerides was also reported by other authors.⁴⁵ It has been found that low-intensity and moderate exercise induces an increase in HDL and decreases triglycerides in healthy subjects. However, reduction of total cholesterol and LDL is not always detectable.⁴⁶ In IHD patients, only TGs were significantly decreased after 12 and 24 weeks of CR. Interestingly, the study indicated that long-term CR was not able to reduce the LDL level,⁴⁷ which we did not observe in our study as well. In our study, total cholesterol, LDL, and TG were decreased and HDL was slightly increased. Although these changes were statistically insignificant, observed alterations can be explained as a result of healthy diet in patients during CR. Furthermore, we showed a significant improvement of ESR after CR. This is a very important result, which indicates atherosclerosis development, including advanced stages of this process and predicts coronary heart diseases-related mortality both in short and long term.⁴⁸ Therefore, ESR can be considered as an atherosclerosis marker in healthy middle-aged men and CHD patients. An elevated ESR has been linked to cardiovascular mortality in patients without preceding MI. High value of ESR has been suggested as an indicator of CAD development at threatening level. Individuals with an ESR equal to or higher than 15 mm/h were in a group of high risk of cardiovascular mortality.⁴⁸ In our cohort ESR mean value was 21 mm/h before CR. Relationship between ESR and CHD results in patients with an ESR higher than 21.⁴⁹ On the other hand, the literature suggests that healthy men aged between 50 and 60 years should have ESR below 14.⁵⁰

Our study shows that CR is able to effectively lower ESR to values comparable to those characteristic and recommended for healthy men in patients subjected to surgical

interventions, as the mean value of this parameter was significantly lowered to 12 mm/h. ESR is a marker of inflammation and has been also proposed as a predictive indicator of a heart failure, which can occur after prolonged inflammatory process.⁵¹ A low ESR in patients treated with ACEI suggests a low level of pro-inflammatory reactions.⁵² In our study a low ESR could be viewed as a result of decreased inflammation and oxidative stress parameters after CR.

It has been reported⁵³ that exercise decreases fibrinogen, which is one of the main factors influencing the ESR. However, no significant changes in plasma viscosity were detected in our study, which may indicate lack of plasma fibrinogen changes in cardiac patients before and after CR.

CR also significantly improved hemodynamic and metabolic parameters, such as SBP peak, and ventilatory parameters, e.g. VO₂max and the overall workload of cardiac patients, along with a decrease in resting SBP. The other study showed that ameliorated hemodynamic properties and enhanced functional capacity after CR are also possible in patients who underwent CABG surgery. It was found that CR improved the resting and maximum systolic and diastolic blood pressure, and both resting and maximum heart rate.¹⁵ We detected an improvement of the heart function after CR reflected by positive alterations in blood pressure as SBP peak significantly increased and SBP resting, DBP resting and DBP peak decreased, although the three latter changes were statistically insignificant. Thus, our study provides additional support to the statement that CR should also be prescribed to high-risk patients such as those subjected to various cardiac interventions.

Increased blood and plasma viscosity has been associated with an increased risk of CAD; however, the effects of CR and exercise training on blood rheology in patients with CAD remain uncertain. It has been shown that exercise evokes rapid transient plasma hyperviscosity. On the other hand, repeated exercise, such as during CR, improves blood rheology.⁹ Although no statistical significance in the changes of plasma viscosity was observed, its increase after exercise was detectable before CR, without any effect after CR. Thus, plasma viscosity was improved as a result of CR only in the term that a single bout of exercise did not induce an increase immediately after the completion of training, in comparison to before CR, which could slightly contribute to the amelioration of blood flow. Since a response to exercise was mitigated after CR, this could also reflect an improvement in general exercise capacity. Simultaneously, it is possible that the alterations of blood rheology relate mainly to the changes of blood cells deformability. It has been shown that one of the long-term exercise effects is erythrocytes rheology enhancement. This effect is related to an increased red blood cells deformability and reduced aggregation.⁵⁴

Generally, after CR we observed a slight decrease in oxidative stress parameters such as peroxide and TBARS level, which reflects a positive effect of CR on the improved anti-oxidative activity of plasma. An increase in the level of thiol groups would probably be higher if GSH levels were not decreased as a short-term result of the exercise test. It has been previously shown that exercise reduces plasma GSH levels, and that moderate training induces only

small decrease.⁵ On the other hand, CR influenced slightly antioxidant plasma systems by increasing concentration of GSH seen before exercise and at 1 h after exercise in comparison to before CR. Simultaneously, CR induced another longer-term effect, i.e. FRAP significant augmentation just after exercise test. The other study showed that CR decreased oxidative stress and increased serum antioxidant capacity. These changes are maintained for at least six months after CR completion and contribute to an effective improvement of the exercise results in cardiovascular patients.¹⁰ Moreover, it has been shown that physical training induced moderate oxidative stress, which upregulates expression of the antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase, and catalase in skeletal muscles of CHF patients.^{55,56} An increase of plasma SOD activity and improved endothelial function of brachial artery were also found in patients with CAD after 12 weeks of exercise training.⁵⁷ This antioxidant enzyme defense system enhancement could indirectly decrease lipid peroxidation products, increase FRAP and the level of -SH groups and GSH after CR (before exercise test and 1 hour after recovery) by local oxidative damage and inflammation attenuation. Some of these parameters were well correlated. We observed negative correlation between antioxidant capacity measured by FRAP and DPPH versus TBARS and positive correlation between DPPH and total -SH groups. These results suggest that the increase in NEAC leads to decrease in lipid peroxidation in part by increase of total thiols and ASC.

An increase in ASC concentration in plasma post CR just after exercise and 1 h later in comparison to before CR may contribute to the observed decrease of oxidative stress as a result of CR. Detected ASC level rise may also be affected by dietary changes and nutritional counseling provided during CR, which have been shown to increase plasma vitamin level.⁵⁸ Statistically significant recovery data obtained after CR, i.e. increase of the ASC concentration 1 h after exercise in comparison to before CR and after exercise indicates better tolerance of exercise in trained patients.

Nevertheless, in these experiments both elevated plasma antioxidant capacity and CR-induced oxidative stress decrease were not sufficient to change carbonyl groups level. However, longer training (6 months) in HF patients was shown to decrease proteins oxidative damage.⁵⁹

Observed improvement of functional heart capacity by CR is reflected by an increase of SBP peak, ventilatory parameters $VO_{2,max}$ and workload; this may also result from oxidative stress decrease in cardiomyocytes. Campos *et al.*⁶⁰ showed that exercise induced a reduction of the mitochondrial release of hydrogen peroxide in the cardiomyocytes of trained rats with MI and heart failure.

These results suggest that CR increased exercise capacity with a reduction of oxidative stress and an increase in antioxidant capacity.

The limitations of this study relate to the participants number. Therefore, more investigations with larger number of participants are needed to fully confirm our results and formulate definitive conclusions as well as to investigate molecular mechanisms responsible for observed changes. Additionally, patients were instructed to try to

cease smoking, however none of them reported the change of this habit, which could contribute to less pronounced changes induced by CR.

Conclusions

Our findings show that comprehensive CR is associated with the benefits of an improvement of basic biochemical parameters of blood, without lowering plasma antioxidants such as GSH and ASC. CR also led to the increase of non-enzymatic plasma antioxidant capacity.

The physiological parameters influenced by CR included exercise tolerance improvement, ESR, and blood pressure levels. However, the main physiological benefit of CR is referred to the increased SBP peak, decreased resting SBP, DBP peak and resting as well as increased ventilatory parameters $VO_{2,max}$ and workload. Oxidative stress related factors also potentially contributed to inflammatory processes reduction. Together with other parameter changes the improvement of functional heart capacity and better tolerance of exercise were observed. This may decrease future cardiac events and hospitalization as well as increase life quality.

Author contributions: All authors participated in the design and interpretation of the studies, analysis of the data, and approved the final manuscript; AJ, LP contributed to the assessment of the health of the patients, their enrollment into the study and the collection of blood samples, KG, AP, JC, AJ conducted the experiments, KG, AP, JC wrote the manuscript, and JB contributed in data analyses and reviewed the manuscript.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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