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Alterations in inflammatory markers following cardiac rehabilitation in patients undergoing coronary angioplasty due to myocardial infarction

Zmiany w stężeniu markerów stanu zapalnego po rehabilitacji kardiologicznej pacjentów leczonych angioplastyką wieńcową z powodu zawału serca

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Abstract

Introduction and Objective. Percutaneous coronary angioplasty, which restores blood flow and increases oxygen delivery after myocardial infarction, can generate oxidative stress and apoptosis, leading to reperfusion injury. The interactions between blood and endothelial cells can be influenced by uric acid, pro-inflammatory cytokines, and polyunsaturated fatty acids. The aim of the study was to assess the concentrations of uric acid (UA), pro-inflammatory cytokine (TNF-α), advanced protein oxidation products (AOPP), and the fatty acids α-linolenic acid (ALA) and arachidonic acid (ARA) in erythrocyte membranes, before and after 8 weeks of cardiac rehabilitation.

Materials and Method. The study group (MI) consisted of 26 patients after myocardial infarction, aged 57.4 ± 5.2 years. The control group (CTRL) consisted of 24 men aged 55.8 ± 7.0 years, non-smokers, and without a history of myocardial infarction. TNF-α was measured by ELISA, AOPP was measured spectrophotometrically using the Witko-Sarsat method; fatty acids ALA and ARA were determined by gas chromatography.

Results. After cardiac rehabilitation, the MI group had significantly higher plasma concentrations of UA, AOPP, and TNF-α than in the control group (UA: 0.29 mmol/L vs. 0.35 mmol/L; AOPP: 52.44 µmol/L vs. 55.21 µmol/L; TNF-α: 9.45 pg/mL vs. 11.36 pg/mL). In the MI group, the percentage of ALA in erythrocyte membranes decreased (from 4.20% to 1.67%), while ARA increased (from 3.67% to 6.71%).

Conclusions. Moderate cardiac exercise reduced markers of inflammation in the control group, but not in the MI group. The results may exert beneficial effects in controlling inflammatory processes and reperfusion injury of the myocardium. To manage these processes, enrichment the daily diet with omega-3 fatty acids and antioxidants should be considered.

Key words

cardiological rehabilitation, α-linolenic acid, inflammatory markers

Streszczenie

Wprowadzenie i cel pracy. Przezskórna angioplastyka wieńcowa, przywracająca przepływ krwi i zwiększająca dopływ tlenu po zawale, może generować stres oksydacyjny i apoptozę, objawiające się uszkodzeniem reperfuzyjnym mięśnia sercowego. Na reakcje między krwią a komórkami śródbłonka mogą wpływać: kwas moczowy i cytokiny prozapalne oraz wielonienasycone kwasy tłuszczowe. Celem pracy była ocena stężeń kwasu moczowego (UA), cytokiny prozapalnej (TNF-α), stopnia utlenienia białek (AOPP) oraz kwasów tłuszczowych: α-linolenowego (ALA) i arachidonowego (ARA) w błonach erytrocytów przed i po 8 tygodniach rehabilitacji kardiologicznej.

Materiał i metody. Grupa badana (MI) składała się z 26 pacjentów po zawale mięśnia sercowego, w wieku 57,4 ± 5,2 lat. Grupę kontrolną (CTRL) stanowiło 24 mężczyzn w wieku 55,8 ± 7,0 lat, niepalących, w przypadku których wywiad nie ujawnił zawału mięśnia sercowego. TNF-α oznaczono testem ELISA, AOPP mierzono spektrofotometrycznie metodą Witko-Sarsat, a kwasy tłuszczowe ALA i ARA oznaczono metodą chromatografii gazowej.

Wyniki. Po rehabilitacji kardiologicznej w grupie MI stwierdzono istotnie wyższe stężenie UA, AOPP oraz TNF-α niż w osoczu kontrolnym (UA: 0,29 mmol/L vs 0,35 mmol/L; AOPP: 52,44 µmol/L vs 55,21 µmol/L; TNF-α: 9,45 pg/mL vs 11,36 pg/mL). Zaobserwowano również, że w grupie MI procentowa zawartość kwasu ALA w błonach erytrocytów zmniejszyła się (z 4,20% do 1,67%), a ARA zwiększyła (z 3,67% do 6,71%).

Wnioski. Umiarkowane ćwiczenia kardiologiczne zmniejszały

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poziom markerów procesów zapalnych w grupie kontrolnej, natomiast efektu takiego nie odnotowano w grupie MI. Uzyskane wyniki mogą mieć korzystne znaczenie dla ograniczania procesów zapalnych oraz uszkodzeń reperfuzyjnych mięśnia sercowego. W celu lepszego kontrolowania tych procesów warto rozważyć wzbogacenie codziennej diety pacjentów leczonych angioplastyką wieńcową z powodu zawału serca o kwasy tłuszczowe omega-3 i przeciwutleniacze.

Słowa kluczowe
rehabilitacja kardiologiczna, markery zapalenia, kwas α-linolenowy

INTRODUCTION

Myocardial infarction is the leading cause of mortality worldwide, and oxidative stress often cited as one of the pathophysiological mechanisms. Percutaneous coronary angioplasty (PCI), a standard therapeutic procedure, restores blood flow and increases oxygen supply to cardiomyocytes after infarction. It can generate oxidative stress and apoptosis, manifesting as myocardial reperfusion injury (MIRI) [1]. Additionally, PCI induces complex inflammatory interactions between blood and endothelial cells. These reactions are regulated in plasma by uric acid (UA) and in erythrocyte membranes by polyunsaturated fatty acids.

The introduction of the stent into the vessel and the inflation of the balloon cause pressure on the atherosclerotic plaque, leading to its microembolization and direct endothelial damage. On the other hand, an increase in the ROS level, especially hydrogen peroxide, and the release of pro-inflammatory cytokines, chemokines and adhesion molecules, have been reported. Activation of platelets and the influx of leukocytes contribute to microvascular obstruction, as well as the release of cholesterol crystals and neutrophil extracellular traps (NETs). High uric acid concentration directly promotes the activation of the NOD-like receptor protein 3 (NLRP3) inflammasome and activates the NF-κB/p65 pathway, leading to the synthesis of IL-1b or TNF-α [2]. Indirectly, UA enhances the suppression of mitochondrial uncoupling protein 2 (UCP 2) expression [3], promotes apoptosis, and may be an independent risk factor for myocardial injury [4].

Irreversible myocardial damage due to reperfusion is believed to result from peroxidation of polyunsaturated fatty acid (PUFA) phospholipids in myocardial, endothelial, and erythrocyte cells. Oxidized PUFAs enriched in phosphatidylethanolamines play a key role, with arachidonic acid and arachidonic acid 15-lipoxygenase-1 as the primary mediators of MIRI [4].

Alpha-linolenic acid (ALA) is incorporated into cell membranes and may inhibit the oxidation and production of phosphatidylcholine species. ALA protects rat cardiomyocytes from apoptosis by reducing DNA fragmentation and caspase activation [5]. It has also been reported to increase myocardial resistance and significantly reduce the TNF-α level in mice with type 2 diabetes induced by streptozocin [5,6]. ALA inhibits IL-6 production after coronary stent implantation [1,7].

Various metabolites of arachidonic acid are developed mainly in the cyclooxygenase (COX) pathway, the lipoxygenase (LOX) pathway, and the cytochrome P450 monooxygenase (CYP) pathway. They are precursors of potent pro-inflammatory mediators, including prostaglandins and leukotrienes. ARA has prothrombotic, pronociceptive and angiogenic properties [4,8].

In turn, cardiac rehabilitation in patients undergoing PCI procedures may prevent cardiovascular complications, re-hospitalization rates, and mortality, likely by reducing oxidative stress and the inflammatory response [9]. Regular exercise may decrease inflammation by modulating CRP, TNF-α and interleukins IL-4, IL-1β, IL-6, and IL-10. Physical activity has also been reported to reduce lipoprotein-associated phospholipase A2, a well-known factor in atherosclerotic plaque formation. Growing evidence suggests that physical activity reduces the activation of nuclear factor NF-κB and the expression of adhesion molecules ICAM-1 and VCAM-1 [9].

OBJECTIVE

MIRI reduces the effectiveness of percutaneous coronary angioplasty and affects the patient's prognosis. Therefore, the prevention and treatment of MIRI are critical, and the study aims to assess whether eight weeks of outpatient cardiological rehabilitation impacts inflammatory markers (uric acid and tumour necrosis factor alpha), oxidative stress markers (advanced oxidation protein products), as well as alpha-linolenic and arachidonic acids in erythrocytes membrane.

MATERIALS AND METHOD

Patients were included in the study after obtaining their written consent for participation. The study group (MI Group) consisted of 18 men aged 40–65 years (mean 57.44 ± 5.24) after coronary angioplasty treatment due to acute myocardial infarction, participating in eight weeks of physical training of the third phase of rehabilitation (outpatient). The control group (CTRL Group) consisted of healthy volunteers and metabolically stable cardiac patients, 18 age-matched men (mean 51.83 ± 7.02), non-smokers, without a history of myocardial infarction.

The men underwent 24 sessions of aerobic endurance training on bicycle ergometers, of medium intensity (40–60% of heart rate reserve), with the effort intensity not exceeding 13 units on the Borg scale. Central haemodynamics were monitored, and maximal workload assessed in metabolic equivalents (METs).

Exclusion criteria from the study included symptoms of acute infection, diseases with chronic inflammation, diabetes mellitus, nicotine addiction, or regular vitamin/antioxidant supplementation.

The study was conducted from 2008 – 2013 in accordance with the Declaration of Helsinki (1975) and approved by the Bioethics and Medical Committee of the Jagiellonian University Medical College in Kraków (Consent Nos. 32/KBL/OIL/2008, KBET/48/B/2008 and KBET/12/B/2005).

Cardiological rehabilitation. Patients from the study group underwent 24 sessions of aerobic endurance training on

bicycle ergometers, of medium intensity (40–60% of heart rate reserve), supplemented with exercises on rowing machines and a stepper, with the effort load not exceeding 13 units on the Borg scale (6–20 units). The training was preceded by a 10-minute warm-up in the form of general exercises performed in various starting positions, and ended with a cool-down period which included low-intensity aerobic exercises, breathing exercises and relaxation (5 min.). In subsequent training sessions, The time of the effort gradually extended from 30–45 minutes.

During the training period, all patients used pharmacotherapy in accordance with the standard treatment after a heart attack, and no exacerbations of coronary artery disease occurred. All patients from the study group completed the planned training sessions.

During rehabilitation period, the men did not use vitamin (antioxidant) supplementation, and undertook average daily physical activity consistent with their lifestyle.

A standard submaximal (85%) exercise test on a bicycle ergometer (load increase of 50 W every 3 min.) was also performed to assess the level of efficiency (MET) and in an echocardiographic examination, measurement of the ejection fraction (EF) before and after training in patients.

Sample collection. Venous blood samples were drawn from an arm vein into K_3EDTA tubes during medical examinations, after 12-hour (overnight) starvation. Blood samples were centrifuged within four hours after obtaining from patient. Plasma samples were collected and stored at -20°C until analysis of uric acid (UA), advanced oxidation end products of protein (AOPPs) and TNF-α.

The following biochemical parameters were measured in the blood of the subjects: blood count (RBCs, WBCs, Ht, Hb), uric acid, lipid profile (total cholesterol and triglycerides, lipoprotein A), and risk markers of cardiovascular disease (fibrinogen, FB).

Advanced Protein Oxidation Products (AOPPs) test. Measured spectrophotometrically according to modified Witko-Sarsat method. The absorbance value was read at a wavelength of 340 nm [10]. Plasma TNF-α levels were analyzed using ELISA kit (R&D Systems, Minneapolis, MN, USA) according to manufacturer's specifications.

All reactions were run in 96-well microplates and the absorbances read on a PolarStar Omega plate reader.

Fatty acid (FAs) analysis in erythrocyte membranes. For FAs analysis, red blood cells (RBC) were separated from plasma by centrifugation (1,500×g; 10 min) and washed with phosphate-buffered saline. Membranes were isolated by centrifugation (10,000×g; 15 min).

Total lipid extraction from RBC membranes was carried out with a solution of chloroform/methanol (2:1). The synthesis of FAs methyl esters of total lipids was carried out with 14% BF_3 in methanol. The FAs methyl esters were analyzed using gas chromatography (Agilent 6890N) and identified according to standards (Sigma-Aldrich, Supelco, USA). The data were analyzed using ChemStation and Excel software. RBC FAs content was expressed in a relative percentage of the total.

Statistics. The difference in variability between the groups was assessed using one-way analysis of variance (ANOVA). In this analysis, Tukey's test was used to search for differences with unequal frequencies (HSD). Additionally, differences between the two patient groups were checked by Kolmogorov-Smirnov test. The difference was significant at the $\alpha = 0.05$ level. The relationship between the variables were estimated using multivariate data analysis, including cluster and principal component analysis. In cluster analysis, data were grouped into clusters based on Euclidean distances and Ward's method of amalgamation. Principal component analysis was performed to detect significant relationships between biochemical data, antioxidants, and FAs. Calculations were made using the TIBCO Statistica version 13.3 statistical analysis software package.

RESULTS

The characteristics of the patient after myocardial infarction and the control group are presented in Tables 1 and 2.

Table 1. Biochemical parameters in groups enrolled in the study: control (CTRL Group; n=26) and after myocardial infarction and PCI procedure (MI Group; n=24), at the beginning of cardiac rehabilitation

	CTRL Group	MI Group	Significance level p
Age (years)	55.8 ±7.0	57.8 ±5.2	ns*
WBC (10^3/ml)	6.6 ±1.7	7.1 ±1.8	ns
RBC (10^6/ml)	4.8 ±0.3	4.9 ±0.4	ns
Hb (g/dl)	14.8 ±1.2	15.3 ±1.3	ns
Hct (%)	44.4 ±2.8	44.7 ±4.2	ns
PLTs (10^3/ml)	212.8±90,6	236.6±64.7	ns
CHOL Tot. (mmol/)	4.8 ±1.1	3.8 ±0.8	<0.01
HDL (mmol/l)	1.4 ±0.3	0.9 ±0.2	<0.01
LDL (mmol/l)	2.7±0.9	2.4 ±0.9	ns
TG (mmol/l)	1.5 ±1.1	1.6 ±0.8	ns
Glucose (mmol/l)	5.4 ±0.8	5.2 ±0.7	ns
Lp(a) (µmol/l)	1.8 ±0.4	1.6 ±0.2	<0.01
Fb (g/l)	3.9 ±1.2	3.7 ±0.7	ns
UA (mmol/l)	0.40 ±0.3	0.30 ±0.1	<0.001
AOPP (µmol/l)	49.4 ±5.4	52.4 ±1.0	<0.01
TNF-α (pg/ml)	4.9 ±2.6	9.5 ±2.4	<0.03

ns* – insignificant difference according to Kolmogorov-Smirnov test

The patients had a lower total cholesterol (TC) concentration ($p<0.001$), a lower level of LDL fraction ($p<0.05$), a higher level of glucose ($p<0.05$) (Tab.1) and a significantly lower ejection fraction level ($p<0.001$) than control individuals. Additionally, the level of metabolic efficiency (MET) in the MI Group increased from 5.64 ±0.94 to 7.01 ±1.31, and was markedly higher than in CTRL Group after cardiac rehabilitation (Tab. 2).

The plasma uric acid concentration in all men in the study ranged from 0.219–0.579 mmol/L, and was significantly higher in the CTRL Group than in the MI Group before the exercise and did not change after rehabilitation. In the group of men after myocardial infarction and PCI procedure, the UA concentration increased significantly (0.297 vs. 0.353 mmol/L) (Fig. 1).

Changes in the concentration of advanced protein oxidation end products are presented in Figure 2. The AOPP level in the reference group was lower than in patients after myocardial

Table 2. Comparison of respiratory and metabolic capacity parameters in the control group (CTRL Group) and the group of post-infarction patients (MI Group), before and after 8 weeks of cardiac rehabilitation

	Before Cardiac Rehabilitation			After Cardiac Rehabilitation		
	CTRL Group	MI Group	Significance level p	CTRL Group	MI Group	Significance level p
EF (%)	72.11 ±6.2	52.8 ±8.34	< 0.001	72.11 ±6.2	62.96 ±7.40	< 0.01
MET (3.5mL O_2/kg/min)	6.07 ±1.26	5.64 ±0.94	ns*	6.07 ±1.26	7.01 ±1.31	< 0.01

ns* – insignificant difference according to Kolmogorov-Smirnov test

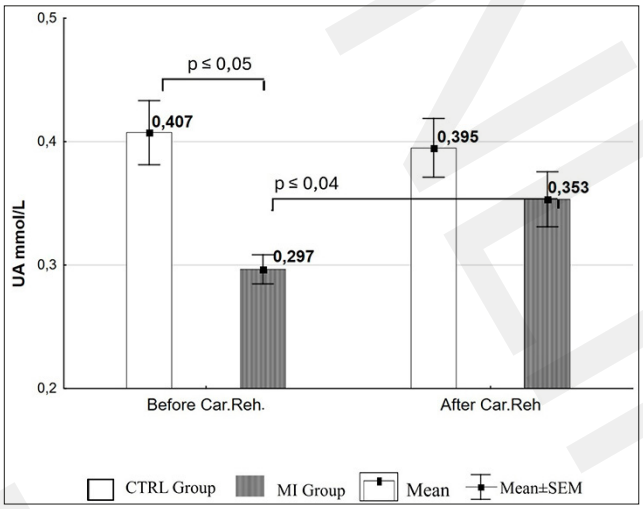

Figure 1. Uric acid levels in control group and patients after myocardial infarction and PCI procedure (MI Group). Points represent mean values and whiskers represent standard error.
MI – Myocardial Infarction; CTRL – Control; Car.Reh – Cardiac Rehabilitation

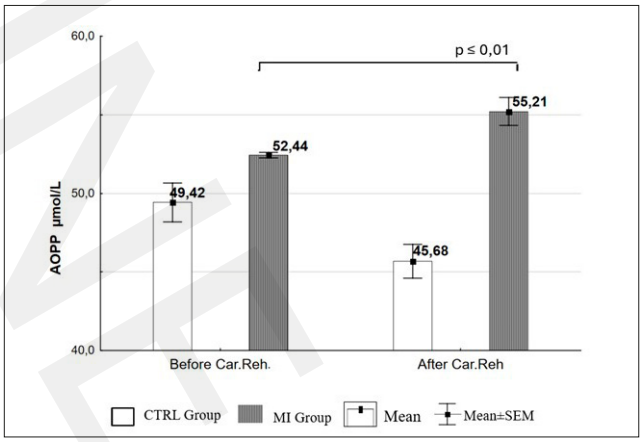

Figure 2. Advanced protein oxidation products (AOPP) level in control and patients after myocardial infarction and PCI procedure (MI Group). Points represent mean values and whiskers represent standard error.
MI – Myocardial Infarction; CTRL – Control; Car.Reh – Cardiac Rehabilitation

infarction, and decreased from 49.4 to 45.7 µmol/L after exercise. In the MI group, a statistically significant increase in AOPP concentration of was observed (Fig. 2).

The TNF-α level was significantly lower in the control than in the plasma of patients after myocardial infarction, and it decreased from 4.99 to 3.56 pg/mL after rehabilitation. In the MI group, moderate exercise increased TNF-α from 9.45 to 11.36 pg/mL (p≤0.01) (Fig. 3).

In patients after myocardial infarction, significantly lower concentrations of ALA and ARA acids were observed in the red blood cell membrane than in the control group.

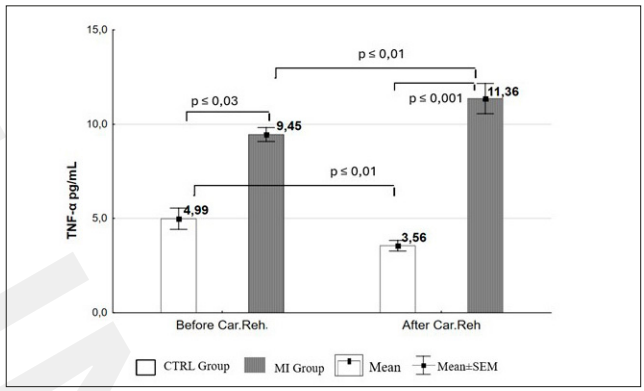

Figure 3. TNF-α level in groups of control group and post-infarction patients and PCI procedure (MI Group). Points represent mean values, whiskers represent standard error.
MI – Myocardial Infarction; CTRL – Control; Car.Reh – Cardiac Rehabilitation

After cardiac rehabilitation, the concentration of ALA in MI patients decreased from 4.2% to 1.7%, while in the CTRL Group it remained unchanged. It was found that the concentration of arachidonic acid in the erythrocyte membrane significantly decreased after cardiac rehabilitation in the CTRL group (24.8% vs. 10.5%). In comparison, in the MI group it increased (3.7% vs. 6.7%) (Fig. 4).

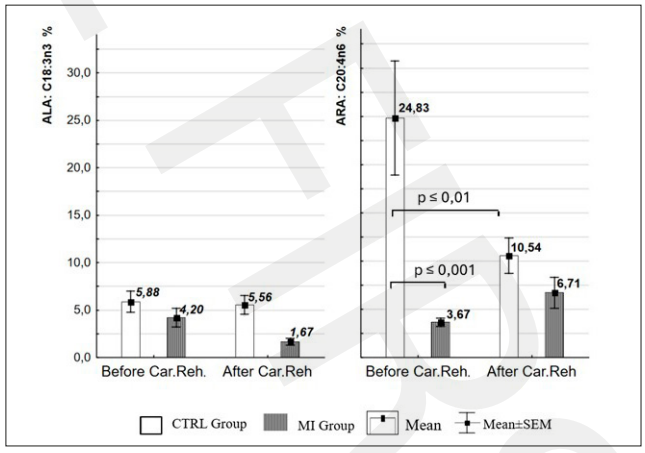

Figure 4. Percentage of fatty acids ALA and ARA in red blood cells membranes in control group patients (CTRL Group), and post-infarction patients (MI Group), at the beginning and after cardiac rehabilitation

Principal component analysis and multivariate cluster analysis were used to find relationships between multiple factors. Figure 5A shows the PCA score plot of all measured variables in the CTRL Group and the MI Group. The points on the plot represent variables and the vectors represent possible relationships between variables. If variables are correlated, their eigenvectors lie on a straight line or at right angles to each other. According to the PCA score plot, in the plane defined by the first component (Factor1:22.7%),

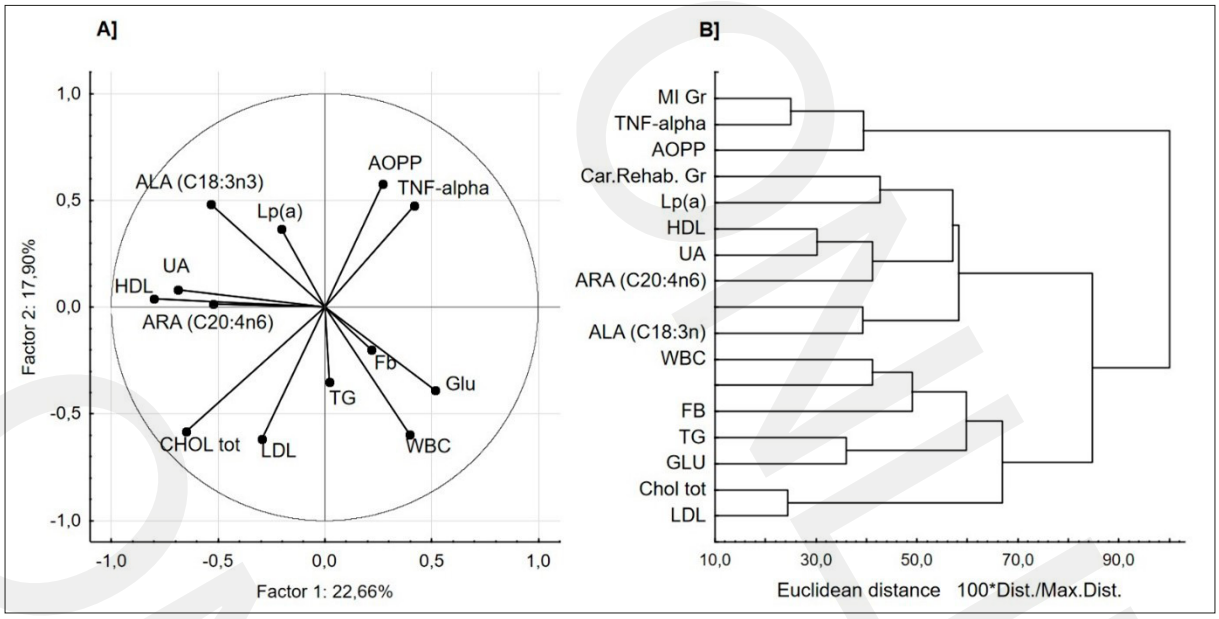

Figure 5. Results of multivariate analysis. On the A] side principal component analysis score plot is presented on the B] tree diagram of cluster analysis (?), where data are standardized to Euclidean distance, and grouped according to Wards method

there were α-linolenic acid, uric acid, lipid markers, and arachidonic acid. It was also shown that ALA may be linked with the white blood cell count and glucose concentration. The highest standardized factor-variable coefficients obtained in principal component analysis were for cholesterol total, cholesterol HDL, uric acid, advanced oxidation protein products as well as tumour necrosis factor-alpha (Tab. 3). In contrast, in the plane defined by Factor 2 (17.9%): ALA and ARA, advanced oxidation protein products (AOPP) and TNF-α proteins were found (Fig. 5A).

In multivariate cluster analysis, a close association was observed between AOPP and TNF-α as well as the myocardial infarction incidence. Uric acid and HDL cholesterol levels were associated with cardiac rehabilitation, and indirectly with arachidonic acid and α-linolenic acid (Fig. 5B).

DISCUSSION

Percutaneous coronary angioplasty (PCI), which restores blood flow and increases oxygen supply to cardiomyocytes after infarction, can generate oxidative stress and apoptosis, manifested by myocardial reperfusion injury (MIRI). Additionally, PCI triggers complex inflammatory interactions between blood and endothelial cells. These reactions can be influenced by plasma uric acid (UA) through activation of the NLRP3 inflammasome and generation of inflammatory cytokines, and in erythrocyte membranes by polyunsaturated fatty acids [2].

For many years, moderate physical exercise in the form of systematic training has been used in cardiac patients as part of their rehabilitation, contributing to the improvement of circulatory system function, the prevention of cardiovascular diseases, and reducing the risk of PCI complications For many years moderate physical exercise in the form of systematic training has been used in cardiac patients as part of rehabilitation, contributing to the improvement of circulatory system function, the prevention of cardiovascular diseases and reducing the risk of PCI complications [13].

Moderate exercise decreases systemic inflammation by modulating diverse inflammatory molecules including CRP, TNF-α, interleukins IL-4, IL-6, and IL-10.

In the current study, the concentrations of uric acid, the pro-inflammatory cytokine TNF-α, the advanced protein oxidation products (AOPP), as well as fatty acids: alpha-linolenic (ALA) and arachidonic (ARA) in erythrocyte membranes, were measured before and after eight weeks of outpatient cardiac rehabilitation.

The study group (MI group) consisted of 26 patients who had undergone myocardial infarction and PCI procedure, aged 40–65 years (mean 57.4 ±5.2). The control group consisted of 24 men age-matched (mean 51.8 ±7.0), without a history of myocardial infarction. Myocardial patients had a lower total cholesterol concentration ($p<0.001$), a lower level of LDL fraction ($p<0.05$), and a higher level of glucose ($p<0.05$) (Tab.1). A significant difference in cholesterol fractions and triglyceride levels may have resulted from statin treatment in this group of patients.

In the presented study, patients who underwent moderate-intensity training (approx. 50 watts) experienced a significant increase in ejection fraction (by 20%) and a 27% improvement in physical capacity per MET, achieving functional improvement. It should be emphasized that the post-training performance level of patients (mean 7.37 ±1.26 METs) was significantly higher ($p<0.005$) compared to healthy individuals (mean 6.1 ±1.26). (Tab. 2). A similar improvement in functional capacity after training in patients with myocardial infarction treated surgically with coronary artery bypass grafting (CABG) was observed by Spiroski et al. [11].

In the current study, in the patient group, in parallel improvement in the effectiveness of cardiac rehabilitation, a significant increase in the uric acid concentration was observed after training in men post-MI and PCI procedure, whereas in the CTRL Group, UA did not change after rehabilitation (Fig.1; Tab.1). Hyperuricaemia concomitant with higher levels of total cholesterol and triglycerides in the control may be associated with the occurrence of hypertension or atherosclerosis. Close correlations were

found between UA and cholesterol HDL (Fig. 5A and 5B).

Some authors have indicated a possible correlation between UA and the risk of MIRI. When UA was above 0.257 mmol/L, each 100 unit increase in UA increased the risk of MIRI by 15%. In other research, uric acid levels in plasma samples obtained from healthy subjects increased slowly during short-term intense exercise by an average of 20%, compared to baseline.

Elevated serum uric acid levels have been identified as an independent risk factor for cardiovascular disease. Individuals with higher levels, have been associated with lower ejection fractions, and longer disease durations [12,13]. Furthermore, UA levels are associated with a more pronounced inflammatory response. Following reperfusion in STEMI patients treated with primary PCI, an increase in C-reactive protein (CRP) and neutrophil counts was observed when the UA level was above 0.404 mmol/L. Moreover, elevated serum uric acid levels above 0.416 mmol/L were reported to be associated with a higher incidence of ventricular arrhythmia due to reperfusion [13]. In the presented study, correlations between myocardial infarction, uric acid, inflammatory cytokine and oxidation of protein were observed (Fig 5A and 5B; Tab.3).

Table 3. Values of standardized factor-variable coefficients obtained in principal component analysis

Parameter	Standardized factor-variable coefficients	
	Factor 1	Factor 2
WBC (103/ml)	-0.23	0.57
CHOL tot (mmol/l)	0.72	0.41
HDL (mmlo/l)	0.73	-0.51
LDL (mmol/l)	0.37	0.44
TG (mmol/l)	0.12	0.74
Glucose (mmol/l)	0.31	0.61
Fb (g/l)	-0.23	0.20
UA (mmol/l)	0.69	-0.22
Lp(a) (µmol/l)	0.25	-0.35
AOPP (µmol/l)	-0.58	-0.51
TNF-α (pg/ml)	-0.56	0.04
ARA (C20:4n-6) (%)	0.42	-0.32
ALA (C18:3n-3) (%)	0.32	-0.25

Uric acid is a low-molecular-weight plasma antioxidant produced by purine metabolism via xanthine oxidase. Under physiological conditions, it exists in the body in dissociated urate form. As UA concentration increases, uric acid crystals precipitate due to environmental saturation and a shift in the dissociation reaction: these crystals mechanically damage cardiomyocytes and vascular endothelium. High UA concentrations also promote the formation of allopurinol radicals, increase dihydrogen dioxide concentration, and increase the expression of ICAM-1, chemoattractant protein-1, VCAM-1, and NOD-like receptor protein 3 (NLRP3) inflammasome. Uric acid exacerbates cardiomyocyte injury by activating the NLRP3 inflammatory cascade, promoting ROS production and activation of the transient receptor potential melastatin 2/Ca^{2+} (TRPM2 channel/Ca^{2+}) pathway [3,14]. On the other hand, UA enhances the expression of NLRP3 by elevating Toll-like receptor 6 (TLR6) levels and activating the NF-κB/p65 pathway [3].

Currently, many markers are used to assess oxidative stress levels. The most commonly used indicators are products of lipid peroxidation, such as isoprostanes F2 and malondialdehyde (MDA). Oxidation of main plasma proteins (albumin, lipoproteins, fibrinogen) results in the formation of carbonyl derivatives or dityrosine, referred to as advanced oxidation protein products (AOPP). The level of advanced protein oxidation products in the body increases with age, and in atherosclerosis, or cardiovascular diseases, reaching pathological concentrations. A significantly increased level of AOPPs in the body was reported in patients with a history of myocardial infarction with ST-segment elevation. AOPP was proposed to be used as a marker of oxidative stress and as a prognostic factor for severe forms of cardiovascular disease [13].

In the current study, the level of AOPP in samples of subjects with MI was higher than in the CTRL group. Additionally, moderate physical exercise, accompanied by cardiac rehabilitation, increased the concentration of AOPPs in the MI Group (Fig. 2; Tab. 1). Similar differences in AOPP between patients with MI and controls were noted in the study of Żurawska-Płaksej et al. [15]. Additionally, the study indicated that the highest level of oxidized proteins in patients with lower cardiac ejection fractions (EF<50%) [15], whereas in the current study, EF<50% was observed in six patients with MI, and only in one before and after cardiac rehabilitation. AOPP level in the mentioned group was also the highest. These results confirm the oxidative stress pathways in MIRI both before and after cardiac rehabilitation.

Affecting the nuclear transcription factors in endothelial cells, AOPP induces pro-inflammatory cytokines (e.g., TNF-α) [16]. As mentioned earlier, AOPP has a close link with TNF- α (Fig.5A and 5B).

In the current study, significantly lower TNF- α levels were found in the control compared to the MI Group ($p \leq 0.01$) (Fig. 3). This finding aligns with the results of Miao and Du [16], who observed similar values. The authors found the highest levels of TNF- α in the group of patients after acute myocardial infarction and 48 hours after PCI. In the third month, the level of this cytokine decreased significantly compared that before the PCI procedure. According to the authors, PCI may induce an inflammatory response, and the high level of TNF-α in plasma may have an important prognostic value for the incidence of major adverse cardiac events. Therefore, higher TNF-α concentration after rehabilitation in our MI group (Tab.1, Fig. 3) may be related to the PCI procedure.

In the present study, cardiac rehabilitation decreased TNF-α level by about 28% in the CTRL Group, whereas in the MI Group, it increased about 20%. In the meta-analysis on the effect of resistance training on inflammatory markers in middle-aged and older adults, reduced CRP and IL6 were reported [17]. Although the resistance exercise analysed in the current study had a similar intensity to the rehabilitation cycle, TNF-α levels did not change significantly, and there were no significant anti-inflammatory effects [17].

In another meta-analysis by Malandish, a significant reduction was observed in TNF-α level after moderate-intensity exercise for middle-aged patients with heart failure and reduced ejection fraction, compared to a control group [18]. The opposite effect of rehabilitation on inflammatory cytokines in CTRL and MI groups may have been be due to different conditions of hypoxia and normoxia. Cardioprotective effect or its lack may also be related to

TNF-α concentration. At lower concentrations (2–5 ng/ml), TNF-α has been reported to activate the Keap1/Nrf2 signals in HL-1 cardiomyocytes and TNF receptor1/2 (TNFR1/2) double knockout mice. This cytokine at a concentration above 10–50 ng/ml markedly suppresses the Keap1/Nrf2 response, increases in the cleavage of caspase-3 and PARP, and suppresses the expression of antioxidant proteins [19].

In a study conducted in adult male Wistar rats, it was shown that under conditions of constant normobaric hypoxia, tumour necrosis factor-alpha may play a role in limiting infarct size by increasing the expression of the TNF-α type 2 receptor. These effects were not observed in the normoxic training group [20].

A critical factor in ischemia/reperfusion injury may be mitochondrial damage through their excessive fission. This fact leads to impaired energy metabolism in cardiomyocytes, damage to the endoplasmic reticulum, increased calcium ion concentration, and inhibition of signal transduction. These changes stimulate apoptosis or necroptosis in the reperfused myocardium. Mitophagy allows autophagosomes to selectively degrade damaged mitochondria, thereby maintaining the efficiency of the mitochondrial network. It has been found that cardioprotection in hypoxia through mitophagy and reduced mitochondrial fragmentation may be activated via the TNF2 receptor and hypoxia-induced nuclear factor-1a (HIF-1a) [21].

The spectrum of signalling in hypoxia after myocardial infarction may be affected by the composition of fatty acids in plasma, cardiomyocytes, and erythrocytes membranes. Different cells have different fatty acid compositions that influence membrane fluidity and flexibility, and the function of the membrane. It was reported that polyunsaturated fatty acids may regulate the antioxidant signaling pathway and modulate inflammatory processes.

Omega-3 FA, α-linolenic acid, was proposed to incorporate into the membrane phospholipids hydrocarbon core region, where it partially displaces arachidonic acid. Phospholipase A2 cleaves ALA from the membrane, and ALA binds to specific transmembrane proteins or voltage-gated channels. Further in the cells it migrates to the nucleus, where it binds transcription factors and promotes or inhibits gene expression. ALA may be converted to anti-inflammatory and antihypertensive lipid mediators by the COX and CYP450 [22]. In the current study, the percentage of ALA contained in RBC membranes, was significantly lower in MI Group of patients than in control, and decreased markedly after eight weeks of cardiac rehabilitation (Fig. 4). In the study by Lazaro I. et al. [23], circulating omega-3 FAs were measured in serum of ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention. The patients who developed major adverse cardiovascular events had markedly lower omega 3 FA levels than those who did not develop any such adverse events. When the level of omega-3 was low, phospholipids in cell membranes (cardiomyocytes or RBC) contained a higher proportion of arachidonic acid. During cardiac ischemia in myocardial infarction, fatty acids cleavage from the membranes of cardiomyocytes or RBC was reported [23]. Therefore, a diet rich in omega-3 fatty acids plays a significant role in the primary and secondary prevention of cardiovascular disease and PCI complications.

It is also imperative to provide α-linolenic acid (ALA) in the diet. ALA is an essential fatty acid that is a precursor to the long-chain n-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). ALA is commonly found in seeds (chia, flaxseed, and hemp), nuts (notably walnuts), and many common vegetable oils. Growing evidence has demonstrated that ALA has a similar cardioprotective effect to that of DHA and EPA. The most significant amounts of DHA and EPA are found in marine fish, such as herring, trout, mackerel and tuna, as well as in fish oils and seafood [23, 24].

Dietary EPA is readily incorporated into the phospholipids of cardiomyocyte membranes. Enriching heart cell membranes with EPA contributes to more efficient oxygen consumption by the heart muscle and, in the case of a myocardial infarction, it may limit the degree of tissue damage [23]. Enrichment the daily diet with omega-3 FAs displaces arachidonic acid in membranes. Released ARA can be converted by cyclooxygenase, lipoxygenase, and cytochrome P450 to oxylipins. Released ARA generates pro-inflammatory eicosanoids that amplify ischemic myocardial damage. ALA converted to anti-inflammatory eicosanoids limits myocardial damage [24].

A four-week ALA supplementation had cardio-protective effects on ischemia-reperfusion myocardium injury by improving left ventricular function, decreasing myocardial enzyme levels CK and LDH, and reducing infarct size. The protective effects of ALA may be due to the blocking of ATP-dependent P2X7R channels in the membrane, inhibition of ischemia-induced Ca^{2+} signals, NF-κB phosphorylation, mRNA release, and reduction of TNF-α. [24].

During exercise, free FAs are released to the circulation, providing a fuel for working muscles. Exercise is associated with increased vasopressor and shear stress, as well as RBC-endothelial interactions. Tissue hypoxia results in the release of oxygen from RBC and may impact the relative content of omega-3 and omega-6 PUFAs. The most consistently observed effect has been an increase in the relative amount of unsaturated, mainly monounsaturated, non-esterified FAs in plasma after moderate intensity for 0.5–2 h running, cycling, or swimming.

In the current study, it was found that the concentration of arachidonic acid in the erythrocyte membrane significantly decreased after cardiac rehabilitation in the control group. In contrast, in the MI group it increased (Fig. 4). Furthermore, the percentage of ARA in RBC was directly linked to uric acid levels and high-density lipoprotein in plasma. On the other hand, ARA content may reflect the post-infarction status of patients (Fig. 5A and 5B).

Some studies have shown that supplementation of omega-3 FAs can potentiate some post-training effects. It was reported that physical exercises alone enhanced defence mechanisms by reducing inflammation through TNF-α modulation and incorporating omega-3 PUFAs supplementation into moderate physical activity, further improved immuno-inflammatory reactions in Wistar rats [25].

CONCLUSIONS

Moderate cardiological exercise reduces markers of inflammatory processes only in the control group. Future research is warranted to examine these relations in larger samples and across various populations. An increase in TNF-α levels after cardiac rehabilitation may be a result of the body's adaptation to ischemia. The results may exert beneficial effects in controlling inflammatory processes and reperfusion injury of the myocardium. To manage these

processes, it is recommended to enrich the daily diet with omega-3 fatty acids and antioxidants.

Strengths and limitations of the study. The strengths of the study included the use of objective (RBC membrane) methods of measuring fatty acids, and that the cardiac rehabilitation conducted for two months justified the search for a relationship between moderate exercise and the ALA and ARA in RBC, the oxidant indicator AOPP, or the level of TNF-α.

This study has at least two significant limitations. First, the patients enrolled had various etiologies of heart failure. The type of percutaneous angioplasty therapy was not known – whether it was balloon dilation or, for example, a stent implantation. The type of PCI therapy may affect the probable impact on complications that may develop in patients, even three months after therapy.

The relatively low numbers of patients in the different subgroups of the control did not allow for reference subgroups to be analyzed.

The final limitation is that the study was observational, and therefore could demonstrate only a probable cause-and-effect relationship between parameters.

Conflict of interest. The authors declare no conflict of interest.

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