Fatty acid profile in erythrocyte membranes and plasma phospholipids affects significantly the extent of inflammatory response to coronary stent implantation

Short title: Fatty acid profile and inflammation after coronary stenting

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Summary

In coronary heart disease, the treatment of significant stenosis by percutaneous coronary intervention (PCI) with stent implantation elicits local and systemic inflammatory responses. This study was aimed at evaluation of the dynamics of inflammatory response and elucidation of the relationship between the fatty acid profile of red blood cell (RBC) membranes or plasma phospholipids and inflammation after PCI. High-sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6), serum amyloid A (SAA), malondialdehyde (MDA) and the fatty acid profiles were determined in patients with advanced coronary artery disease undergoing PCI before, 24 and 48 hours after drug-eluting stent implantation (n=36). Patients after PCI exhibited a significant increase in studied markers (hsCRP, IL-6, SAA, MDA). Many significant associations were found between the increase of IL-6, resp. SAA and the amounts of n-6 polyunsaturated fatty acids (namely linoleic, dihomo-γ-linolenic, docosatetraenoic and docosapentaenoic acid), resp. saturated fatty acids (pentadecanoic, stearic, nonadecanoic) in erythrocyte membranes. The magnitude of the inflammatory response to PCI is related to erythrocyte membrane fatty acid profile, which seems to be a better potential predictor of elevation of inflammatory markers after PCI than plasma phospholipids.

Keywords: Lipids • Coronary artery disease • Inflammation • Fatty acids • Coronary restenosis

Introduction

Atherosclerosis and diseases of the cardiovascular system belong to the most common causes of death in Central Europe. Percutaneous coronary intervention (PCI) is an invasive procedure resulting in the recovery of vascular patency in patients with coronary atherosclerosis. However, PCI induces an inflammatory reaction that plays a crucial role in the pathogenesis of neointimal proliferation, which is the main cause of stent restenosis (Kornowski *et al.* 1998, Liuzzo *et al.* 1998, Buffon *et al.* 1999). Interleukin 6 (IL-6) concentration increases after PCI, and the magnitude of its elevation correlates with the occurrence of late restenosis (Ikeda *et al.* 2001). Similarly, the associations between high sensitive C-reactive protein (hsCRP) or serum amyloid A (SAA) and restenosis were found (Liuzzo *et al.* 1994, Schillinger *et al.* 2002).

Recently, the association of inflammation with the fatty acid contents was reported (Fernandez-Real *et al.* 2003, Calder 2012, Rangel-Huerta *et al.* 2012). The n-3 and n-6 polyunsaturated fatty acids (PUFA) are precursors of eicosanoids that significantly affect the inflammatory reaction. The n-3 PUFA, particularly eicosapentaenoic and docosahexaenoic acid, compete with n-6 PUFA for enzymes in the biosynthetic pathway of eicosanoids. Ratio of n-3 and n-6 PUFA influences hemodynamics, vascular tone and inflammation (Hirafuji *et al.* 2003). Some epidemiological studies have found that levels of n-3 PUFA in plasma, as well as in erythrocyte membranes, negatively correlate with plasma proinflammatory markers, including CRP and IL-6 in patients with stable coronary artery disease (Ferrucci *et al.* 2006, Farzaneh *et al.* 2009). It was suggested that the successful replacement of n-6 PUFA with n-3 PUFA in cell membranes can result in a decrease of cellular responses to mitogenic and inflammatory stimuli (Bagga *et al.* 2003).

Monounsaturated fatty acids (MUFA) with the cis-configuration are mostly anti-atherogenic, anti-thrombogenic and resistant to lipoperoxidation. MUFA decrease serum low

density lipoproteins (LDL) and positively influence formation of high density lipoproteins (HDL). LDL, rich in monounsaturated fatty acids, are less taken up by macrophages and the risk of lipid peroxidation is therefore reduced (Moreno *et al.* 2008).

Several studies described the effect of n-3 PUFA supplementation on inflammation and restenosis after PCI, but the influence of a whole spectrum of fatty acids should be taken into account. Relations in all groups of fatty acids need to be studied in more details to understand the mechanism of their association with inflammation after PCI. We have focused on evaluation of the role of individual fatty acids in erythrocyte membranes and plasma phospholipids (PL) in the inflammatory response to coronary stent implantation in patients with acute coronary syndrome. Inflammatory response is highly individual and this reaction could be affected by plasma fatty acid composition and composition of fatty acids in cell membranes, what is reflected by the erythrocyte membrane fatty acid profile before stenting. However, analyses of the fatty acid composition of these lipids are usually not available for routine laboratories. The aim of this study is to evaluate the relationship between not only PUFA, but the whole spectrum of fatty acids, and inflammatory response as well as oxidative stress progression after PCI.

Materials and methods

Subjects

This cross-sectional study involved 36 patients diagnosed with significant coronary atherosclerosis undergoing PCI. The study did not include patients with serious health complications, ST Segment Elevation Myocardial Infarction (STEMI), heart failure according to New York Heart Association (NYHA) II-IV, renal failure, thyroid dysfunction, hepatic or oncology disease or patients that regularly consume alcohol (based on the questionnaire). Group characteristics are given in Table 1. The appropriate institutional approval of the

review board has been obtained as well as the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations have been followed. Written informed consent was obtained from all the participants. The study was approved by the Ethical Committee on Human Research of the Regional Hospital of Pardubice (No 434/13.12.2013). All interventions were performed with a standard technique, and all patients received drug-eluting stents (Everolimus). Before intervention, all patients received weight-adjusted intravenous heparin with a target activated clotting time of 250-350 seconds.

Whole blood sample collection

Venous blood samples (9 ml) were collected in tubes with EDTA (The Vacuette Detection Tube, No. 455036, Greiner Bio-One GmbH, Kremsmünster, Austria) before, 24 and 48 h after PCI. Blood was immediately centrifuged at 1500 *x* g for 20 min and aliquots of plasma and buffy coat were separated into cryotubes. One ml of erythrocytes was taken from the center of the erythrocyte column and immediately stored at -80 °C.

Determination of inflammatory markers

HsCRP, IL-6 and SAA were measured in the Regional Hospital of Pardubice. HsCRP was determined by the standard procedure using the analytical system VISTA®; IL-6 with an Immulite® immunochemistry analyzer and SAA with a BN ProSpec® laser nephelometer (all provided by Siemens Healthcare Diagnostics Inc., USA).

Determination of malondialdehyde (MDA)

The analysis of MDA was performed as previously described (Kanďár *et al.* 2002). Briefly, plasma MDA was quantified after its conversion to the malondialdehyde-thiobarbituric acid (TBA) complex which was accomplished using an isocratic elution on a

LiChroCart 250 x 4 mm, Purospher Star RP-18e, 5 μm, analytical column fitted with a LiChroCart 4 x 4 mm, Purospher Star RP-18e, 5 μm, guard column (Merck, Darmstadt, Germany). Mixture of 35 % methanol in 8.3 mM phosphate buffer, pH 7.2, was used as the mobile phase for determining the $MDA(TBA)_2$ derivative. The flow rate was kept constant at 0.7 ml/min. The separation ran at 37 °C. Effluent was monitored with UV/Vis detector at 532 nm.

Determination of erythrocyte membrane fatty acid profile

Defrosted erythrocytes (200 μl) were mixed thoroughly (by vortex) with 1800 μl of distilled water. After thawing and mixing, the samples were centrifuged for 10 minutes at 12050 *x* g. The sediment was washed three times with 1600 μl of distilled water with subsequent centrifugation. 1700 μl of the supernatant was collected after the last washing. One ml of isopropanol/n-heptane/ortho-phosphoric acid (2 M) (40:20:1, v/v/v) was added to the resulting sediment of erythrocyte membranes. The mixture was mixed by vortex and incubated for 10 min at room temperature. The internal standard (*cis*-13,16,19-docosatrienoic acid, 10 μg/ml, Cayman Chemical, Ann Arbor, MI, USA) in a methanol/toluene (1:4, v/v) (400 μl) and 300 μl distilled water were mixed by vortex and centrifuged for 5 minutes at 2910 *x* g. The upper organic layer was transferred to a Pyrex test tube and evaporated to dryness under nitrogen (Linde Gas a. s., Prague, Czech Republic). Two ml methanol/toluene mixture (1:4, v/v) were added to the sample in the Pyrex test tube. 200 μl of acetyl chloride was added to the sample in a thermal block. The mixture was heated for 1 h at 100 °C in a closed Pyrex test tube, cooled and neutralized with 5 ml of 6 % K_2CO_3. Finally, the solution was mixed for 2 min and then centrifuged for 10 min. The organic phase was transferred to a

new test tube and evaporated to a volume of 100 µl under a nitrogen atmosphere (Peter *et al.* 2009).

The samples were analyzed by gas chromatography. An Agilent 7890 gas chromatograph was used for determination of the fatty acid profile. The injector temperature was 250 °C and the FID detector temperature 280 °C. The temperature program was set as follows: 130 °C for 1 min; 2° C/min up to 176 °C, maintained for 2 min; 1 °C/min to 186 °C, maintained for 1 min; 0.2 °C/min to 190 °C, maintained for 1 min; 1 °C/min to 220 °C, maintained for 4 min. The results were obtained after final processing of the peak areas and are reported as a percentage of the total fatty acids. *Cis*-13,16,19-docosatrienoic acid was used as an internal standard. The example of chromatogram with 38 measured fatty acids including internal standard is shown in Figure 1.

Statistical analysis

Descriptive statistics (median, first and third quartile, mean, standard deviation) and all statistical analyses were computed using STATISTICA 12 (StatSoft CR s.r.o., Czech Republic) and Sigmastat version 3.5 (Systat Software Inc., Point Richmond, CA, USA). Analysis of variance (ANOVA) was performed to investigate dynamic changes in levels of inflammatory markers and MDA as a function of sex, age and diabetes. The Wilcoxon test was used to compare the values of inflammatory markers and MDA before, 24 h and 48 h after PCI. Relationships between the content of erythrocytes and plasma fatty acids, inflammatory and oxidative stress markers were expressed as nonparametric Spearman's correlations. $P \leq 0.05$ was considered statistically significant.

Results

The group of patients consisted of 26 men and 10 women undergoing PCI with subsequent stent implantation for stable (16) or unstable (20) angina pectoris. Initial levels of inflammatory markers and MDA (marker of lipoperoxidation), BMI and cholesterol concentrations are included in group characteristic in Table 1.

Using ANOVA, no effect of sex, age, occurrence of diabetes on inflammatory markers and MDA content was found (see Table 2).

Patients after PCI exhibited a significant increase in inflammatory markers. The earliest responses, with maxima after 24 h, were observed in case of IL-6 and hs-CRP, followed by the elevation of SAA, with maximum after 48 h (Figure 2). Also elevation of oxidative stress (estimated via MDA, stable product of lipoperoxidation) reached its maximum after 48 h.

The initial levels of fatty acid groups, namely of saturated fatty acids (SFA), trans-MUFA, cis-MUFA, n-3 PUFA and n-6 PUFA as well as individual fatty acids were correlated with the magnitude of the increase in CRP, IL-6, SAA and MDA concentrations after PCI. Statistically significant correlations are shown in Tables 3-4. Our data show the close relationship of inflammatory markers with fatty acids in erythrocyte membranes, especially in case of IL-6 and SAA. Spearman positive correlations were found between the magnitude of the increase in IL-6 and total n-6 PUFA. Highly significant correlations were found with each n-6 fatty acids, namely with docosatetraenoic acid, docosapentaenoic acid, linoleic acid and dihomo-γ-linolenic acid. Positive correlation was found between IL-6 and n6/n3 ratio. Further we have found close relationship between SAA and erythrocyte membrane SFA, specifically with pentadecanoic acid, stearic acid, nonadecanoic acid and cerotic acid. Contradictory role was found in case of palmitoleic acid in erythrocyte membranes, which significantly contributed to the increase in SAA after PCI, being negatively associated with IL-6.

Discussion

The dynamics of inflammatory markers IL-6, CRP and SAA were studied during 48 hours after the PCI with subsequent stent implantation. The maximum inflammatory reaction was reached 24 and 48 hours after PCI, which is in agreement with previous studies (Inoue *et al*. 2005, Lasave *et al*. 2007, Silva *et al*. 2012). Inoue *et al*. (2005) indicated that increased levels of CRP after PCI predict an increased risk of in-stent restenosis. The increase in proinflammatory cytokine IL-6 with its maximum 24 hours after the procedure is in agreement with the results of Kochiadakis *et al*. (2007). Our results suggest that the progression of the inflammatory response is closely associated with the fatty acid profile in red blood cell membranes and plasma phospholipids before PCI. We have found many significant correlations of fatty acids with IL-6. The obtained results proved a positive correlation between IL-6 and linoleic acid, a precursor of arachidonic acid producing proinflammatory eicosanoids of group 2. It can be assumed that the amount of arachidonic acid in inflammatory cells may also be influenced by the dietary intake of its precursor, linoleic acid (Yaqoob *et al*. 2000). A strong positive correlation was reported between the amount of arachidonic acid in inflammatory cells and the ability of those cells to produce eicosanoids (Peterson *et al*. 1998). Arachidonic acid is usually the major substrate for eicosanoid synthesis but we did not find any statistically significant correlation between this fatty acid and the increase in inflammation. There is a possibility that arachidonic acid was quickly metabolized to eicosanoids, immediately after its formation from linoleic acid.

An increased intake of γ-linolenic acid or dihomo-γ-linolenic acid is supposed to have anti-thrombogenic and anti-inflammatory effects (Kernoff *et al*. 1977, Johnson MM *et al*. 1997). In our study, however, content of these n-6 PUFA, especially of DGLA in erythrocyte

membranes before PCI significantly positively correlated with increased IL-6 after intervention.

Some authors have described a beneficial effect of n-3 PUFA, especially of eicosapentaenoic and docosahexaenoic acid, in terms of a reduction in overall mortality, mortality due to myocardial infarction and sudden death in patients with coronary heart disease (Bucher *et al*. 2002, Albert *et al.* 2002, Hu *et al.* 2002). Other studies reported that n-3 PUFA have impact on the consequences of PCI, namely that diet supplementation with n-3 PUFA results in reduction of the rate of early restenosis after coronary angioplasty (Dehmer *et al*. 1988). Other authors, however, did not observe any effect of n-3 PUFA on treatment and occurrence of restenosis after PCI (Johansen *et al*. 1999). We also did not find any beneficial anti-inflammatory effect of n-3 PUFA, except stearidonic acid in erythrocyte membranes, which negatively correlated with IL-6. This could have been caused by the fact that participants of our study did not use dietary supplements and our findings are based on physiological concentrations, which were extremely low.

The MDA levels correlated positively with the total amount of PUFA in erythrocyte membranes and phospholipids, which is in accordance with the fact that PUFA are the most prone to lipoperoxidation. Plasma concentrations of MDA negatively correlated with the total amount of monounsaturated fatty acids in erythrocyte membranes, which reflects the ability of MUFA to reduce oxidative stress (Perez-Martinez *et al*. 2010).

We have found a positive correlation between cerotic acid and an increase in SAA after 24 hours in case of erythrocyte membranes and phospholipids. Lignoceric acid in phospholipids correlated with increase of IL-6 in 24 hours. These fatty acids belong to the group of VLCSFA. Although VLCSFA are minor components of fatty acids in erythrocyte membranes, they play an important role in atherosclerotic disease (Matsumori *et al*. 2013). Plasma concentration of VLCSFA is influenced both by dietary intake and by activities

of ELOV1, ELOV2, as well as by peroxisomal oxidation. Peroxisomal dysfunction could lead to higher plasma concentration of VLCSFA too (Schutgens *et al*. 1993).

VLCSFA are associated with a higher production of nitric oxide and proinflammatory cytokines. It was also found that higher levels of lignoceric acid in patients with acute coronary syndrome may contribute to the initiation and progression of atherosclerosis by increasing reactive oxygen species production. It has been shown that a higher level of lignoceric acid significantly correlated with higher levels of CRP and LDL particles, which participate in the atherosclerotic process. The accumulation of VLCSFA in macrophages contributed to the progress of inflammatory response in *in vitro* study on macrophages derived from X-linked adrenoleukodystrophy protein deficient mice after stimulation by lipopolysaccharide (Yanagisawa *et al*. 2008, Matsumori *et al*. 2013).

In animal experiments, palmitoleic acid reduced the expression of adipokines and inflammatory markers (Cao *et al*. 2008, Guo *et al*. 2012). Despite this result, association of palmitoleic acid with higher levels of inflammatory markers and with coronary heart disease risk was reported in humans (Perreault *et al*. 2014, Petersson *et al*. 2009, Djoussé *et al*. 2012). The relationship between palmitoleic acid and inflammation in human probably reflects high saturated fat intake. This could be caused by the fact that MUFA arise by desaturation SFA, which stimulate inflammation by activation of TLR-4. In this case cytotoxic and proinflammatory palmitic acid is desaturated to palmitoleic acid. Moreover, the increased content of SFA is associated with high SCD-1 activity, which may promote inflammation (Peterson et al. 2008). We found that palmitoleic acid is positively associated with higher level of SAA, but negatively associated with IL-6 in erythrocyte membranes after PCI, which indicates that the physiological effect of palmitoleic acid is not entirely clear.

Further, total SFA, and especially pentadecanoic, stearic and nonadecanoic acids have been found to be associated with increase of SAA. It corresponds to the fact that there is

direct association between SFA, TNF-α and SAA. SFA increase the expression and secretion of TNF-α (Bradley *et al.* 2008), which subsequently increases SAA levels in serum (Popa *et al.* 2007). Other relationships are shown in Table 3.

Fatty acids were directly measured in erythrocyte membranes and in plasma phospholipids. As no participants were taking dietary supplements, our findings are based on the physiological concentrations. While most authors focused mainly on n-3 and n-6 PUFA, we have studied the whole spectrum of fatty acids - SFA, MUFA, BCFA and VLCSFA in relation to inflammation and oxidative stress after PCI.

The main contribution of our present study is based on the measurement of a wide range of fatty acids (38) of red blood cell membranes and plasma phospholipids. This complex approach allows elucidate in a complex way the associations between fatty acid profile and inflammation after PCI. Further, study includes evaluation of the effect of potential confounding factors, such as gender, age and diabetes.

However, a few limitations should be considered in the interpretation of our results. First, our pilot study contains only 36 participants, predominantly males. Second, since this is an observational cross-sectional study, no causality can be drawn.

In conclusion, erythrocyte membrane fatty acid profile affects significantly the magnitude of the inflammatory response to PCI and seems to be a better potential predictor of elevation of inflammatory markers after PCI than plasma phospholipids.

In erythrocyte membranes, individual n-6 PUFA, namely dihomo-γ-linolenic, docosatetraenoic, docosapentaenoic and linoleic acids, have been associated with elevation of IL-6. VLCSFA, namely cerotic acid, total SFA, and especially pentadecanoic, stearic and nonadecanoic acids, have been associated with increase of SAA. Palmitoleic acid in erythrocyte membranes significantly contributes to the increase of SAA after PCI but it is negatively associated with IL-6.

These findings might be of practical importance for improving clinical outcomes. Fatty acid analysis represents a promising tool for predicting patient´s pre-disposition to inflammatory reaction after PCI. Moreover plasma levels of specific fatty acids can be modified by a food intake or dietary supplementation. Our data indicate possibility to suppress production of proinflammatory compounds as prevention of increased inflammatory reaction and consequent restenosis after PCI.

Conflict of Interest

There is no conflict of interest.

Acknowledgments

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Table 1. Characteristics of the study sample of patients (n=36)

Women (n)	10
Men (n)	26
Age (years)*	67 (14)
Hypertension (n)	25
Diabetes mellitus (n)	12
BMI (kg/m^2)*	28.1 (6.5)
hsCRP (mg/l)*	3.18 (2.11)
IL-6 (ng/l)*	1.61 (3.87)
SAA (mg/l)*	6.01 (4.72)

MDA (μmol/l)*	1.25 (0.41)
HDL-C (mmol/l)*	1 (0.39)
LDL-C (mmol/l)*	2.13 (1.19)
Total-C (mmol/l)*	3.60 (1.57)
P_TG (mmol/l)*	1.17 (0.63)
Albumin (g/l)*	35.50 (3)

BMI – Body Mass Index, hsCRP – high sensitive C-reactive protein, IL-6 – Interleukin 6, SAA – Serum Amyloid A, MDA – Malondialdehyde, HDL-C – HDL Cholesterol, LDL-C – LDL Cholesterol, Total-C – Total Cholesterol, P_TG – plasma triglyceride.

*median (IQR – Interquartile Range)

Table 2. Dynamics of inflammation markers and MDA, marker of lipoperoxidation, as a function of gender, age and diabetes.

	Factor	**F**	**p**	**Power (1-β)**
Δ hsCRP	Sex	0.074	0.788	0.050
24 hours	Age	0.081	0.923	0.050
	Diabetes	1.741	0.197	0.124
Δ hsCRP	Sex	1.164	0.289	0.064
48 hours	Age	2.314	0.116	0.252
	Diabetes	0.016	0.901	0.050
Δ IL-6	Sex	0.374	0.545	0.050
24 hours	Age	1.026	0.371	0.052
	Diabetes	0.383	0.541	0.050

Δ IL-6	Sex	1.104	0.302	0.058
48hours	Age	1.502	0.240	0.120
	Diabetes	2.418	0.131	0.197
Δ SAA	Sex	0.106	0.747	0.050
24 hours	Age	0.152	0.860	0.050
	Diabetes	0.846	0.365	0.050
Δ SAA	Sex	2.133	0.155	0.166
48 hours	Age	2.998	0.066	0.367
	Diabetes	0.187	0.668	0.050
Δ MDA	Sex	0.202	0.656	0.050
24 hours	Age	0.167	0.847	0.050
	Diabetes	0.453	0.506	0.050
Δ MDA	Sex	0.722	0.402	0.050
48 hours	Age	0.251	0.780	0.050
	Diabetes	0.479	0.494	0.050

The F Test Statistic is provided for comparisons within each factor. The p value is the probability of being wrong in concluding that there is a true difference between the groups. There are significant differences if $p < 0.05$. The Power or sensitivity is the probability that the test will detect the observed difference among the groups if there really is a difference. The closer the power is to 1, the more sensitive the test. Traditionally, the power of the performed test should be > 0.8. Less than desired power indicates you are less likely to detect a difference when one actually exists. HsCRP – high sensitive C-Reactive Protein; IL-6 – Interleukin 6; MDA – Malondialdehyde; SAA – Serum Amyloid A.

Table 3. Statistically significant correlations of erythrocyte fatty acids with inflammatory markers and malondialdehyde.

Fatty acids	Δ CRP 24 h (mg/l)		Δ CRP 48 h (mg/l)	
	r	p	r	p
Pentadecanoic	0.39	**0.019**	0.20	0.232
Nonadecanoic	0.33	**0.048**	0.17	0.332

	Δ IL-6 24 h (ng/l)		Δ IL-6 48 h (ng/l)	
	r	p	r	p
Myristic	-0.35	**0.038**	-0.08	0.636
13-Methylmyristic	-0.34	**0.048**	-0.02	0.911
cis-Palmitoleic	-0.37	**0.030**	-0.11	0.534
14-Methylhexanoic	-0.36	**0.032**	-0.19	0.288
trans-Vaccenic	-0.40	**0.018**	-0.20	0.260
Linoleic	0.42	**0.012**	0.08	0.673
Stearidonic	-0.40	**0.018**	0.00	0.978
Dihomo-γ-linolenic	0.46	**0.005**	0.09	0.611
Docosatetraenoic (n6)	0.38	**0.024**	0.03	0.872
Docosapentaenoic (n6)	0.34	**0.048**	-0.04	0.806
Docosapentaenoic (n3)	0.41	**0.013**	0.04	0.827
Total n-6 PUFA	0.34	**0.049**	0.04	0.814
n6/n3	0.41	**0.014**	0.08	0.670
BCFA	-0.37	**0.029**	-0.20	0.250

	Δ SAA 24 h (mg/l)	Δ SAA 48 h (mg/l)

	r	p	r	p
12-Methyltridecanoic	0.46	**0.005**	0.27	0.123
Pentadecanoic	0.40	**0.015**	0.21	0.223
Sapienic	0.34	**0.046**	0.05	0.766
cis-Palmitoleic	0.43	**0.009**	0.15	0.382
Stearic	0.34	**0.045**	0.08	0.648
Nonadecanoic	0.34	**0.041**	0.16	0.371
Eicosapentaenoic (n3)	0.06	0.717	0.37	**0.032**
Cerotic	0.38	**0.023**	0.14	0.445
Total SFA	0.40	**0.017**	0.07	0.686

	Δ MDA 24 h (μmol/l)		Δ MDA 48 h (μmol/l)	
	r	p	r	p
12-Methyltetradecanoic	-0.37	**0.025**	-0.15	0.394
cis-Palmitoleic	-0.22	0.188	-0.36	**0.029**
14-Methylhexanoic	-0.34	**0.044**	-0.33	0.050
Stearic	-0.27	0.116	-0.38	**0.024**
Dihomo-γ-linolenic	0.41	**0.014**	0.25	0.138
Docosapentaenoic (n3)	0.36	**0.030**	0.27	0.108
Total SFA	-0.32	0.054	-0.35	**0.036**

Relationships between the content of erythrocytes fatty acids, inflammatory markers and oxidative stress were expressed as nonparametric Spearman's correlations. The r value means Spearman's correlation coefficient, p value is the probability of being wrong in concluding that there is a true difference between the groups. There are significant differences if $p \leq 0.05$. HsCRP – high sensitive C-Reactive Protein; IL-6 – Interleukin 6; MDA – Malondialdehyde;

SAA – Serum Amyloid A; PUFA – Polyunsaturated Fatty Acids; SFA – Saturated Fatty Acids; BCFA – Branched Chain Fatty Acids.

Table 4. Statistically significant correlations of phospholipid fatty acids (%) with inflammatory markers and malondialdehyde.

	Δ CRP 24 h (mg/l)		Δ CRP 48 h (mg/l)	
	r	p	r	p
Myristic	0.37	**0.028**	0.37	**0.025**
Palmitic	-0.26	0.125	-0.38	**0.022**
Sapienic	0.21	0.222	0.35	**0.034**
Docosatetraenoic (n6)	0.37	**0.028**	0.28	0.096

	Δ IL-6 24 h (ng/l)		Δ IL-6 48 h (ng/l)	
	r	p	r	p
12-Methyltridecanoic	0.13	0.455	-0.34	**0.049**
12-Methyltetradecanoic	0.34	**0.047**	0.24	0.179
Sapienic	0.38	**0.023**	0.14	0.445
Arachidic	0.34	**0.043**	0.21	0.222
α-Linolenic	0.36	**0.031**	0.31	0.070
Lignoceric	0.35	**0.040**	0.11	0.532

	Δ SAA 24 h (mg/l)		Δ SAA 48 h (mg/l)	
	r	p	r	p
Sapienic	0.27	0.116	0.37	**0.033**

	Δ MDA 24 h (μmol/l)		Δ MDA 48 h (μmol/l)	
	r	p	r	p
Vaccenic	-0.34	**0.044**	0.06	0.727
α-Linolenic	0.25	0.144	0.33	**0.048**
n-3 PUFA	0.27	0.118	0.40	**0.016**
n6/n3	-0.20	0.250	-0.37	**0.028**

Relationships between the content of plasma fatty acids, inflammatory markers and oxidative stress were expressed as nonparametric Spearman's correlations. The r value means Spearman's correlation coefficient, p value is the probability of being wrong in concluding that there is a true difference between the groups. There are significant differences if $p \leq 0.05$. HsCRP – high sensitive C-Reactive Protein; IL-6 – Interleukin 6; MDA – Malondialdehyde; SAA – Serum Amyloid A; PUFA – Polyunsaturated Fatty Acids.

Table 5. Survey of all measured fatty acids

Fatty acid	Molecular formula
12-methyltridecanoic acid	12-Me C13:0
myristic acid	C14:0
13-methyltetradecanoic acid	13-Me C14:0
12-methyltetradecanoic acid	12-Me C14:0
pentadecanoic acid	C15:0
palmitic acid	C16:0
sapienic acid	C16:1 N10
cis-palmitoleic acid	C16:1 N7-cis
14-methylhexadecanoic acid	14-Me C16:0

heptadecanoic acid	C17:0
isostearic acid	16-Me C17:0
stearic acid	C18:0
trans-vaccenic acid	C18:1 N11-trans
oleic acid	C18:1 N9
cis-vaccenic acid	C18:1 N7
nonadecanoic acid	C19:0
linoleic acid	C18:2 N6
γ-linolenic acid	C18:3 N6
arachidic acid	C20:0
α-linolenic acid	C18:3 N3
stearidonic acid	C18:4 N3
dihomo-γ-linolenic acid	C20:3 N6
behenic acid	C22:0
arachidonic acid	C20:4 N6
eicosatetraenoic acid (n-3)	C20:4 N3
eicosapentaenoic acid (n-3)	C20:5 N3
lignoceric acid	C24:0
cis-13,16,19-docosatrienoic acid	C22:3 N3
docosatetraenoic acid (n-6)	C22:4 N6
nervonic acid	C24:1 N9
docosapentaenoic acid (n-6)	C22:5 N6
docosapentaenoic acid (n-3)	C22:5 N3
docosahexaenoic acid (n-3)	C22:6 N3
cerotic acid	C26:0

tetracosatetraenoic acid (n-6)	C24:4 N6
tetracosapentaenioc acid (n-6)	C24:5 N6
tetracosapentaenioc acid (n-3)	C24:5 N3
tetracosahexaenoic acid	C24:6 N3

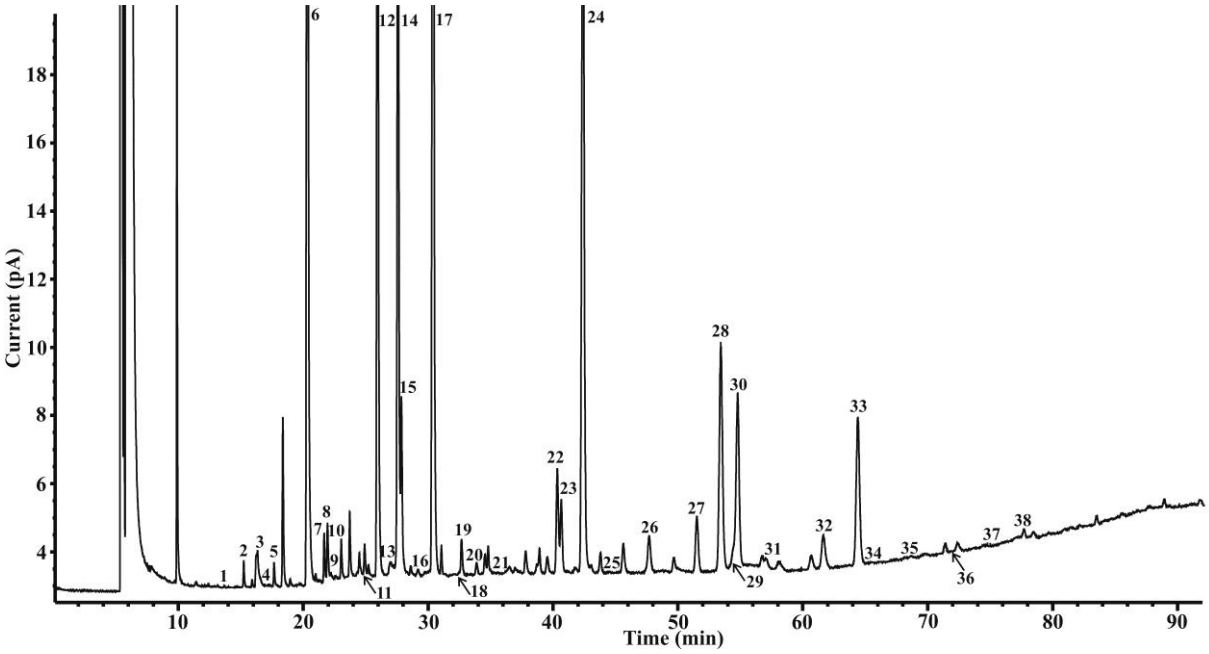

Figure 1. Representative chromatogram of fatty acid profile of plasma sample. Peaks: ***1.*** *12-methyltridecanoic acid* ***2.*** *myristic acid* ***3.*** *13-methyltetradecanoic acid* ***4.*** *12-methyltetradecanoic acid* ***5.*** *pentadecanoic acid* ***6.*** *palmitic acid* ***7.*** *sapienic acid* ***8.*** *cis-palmitoleic acid* ***9.*** *14-methylhexadecanoic acid* ***10.*** *heptadecanoic acid* ***11.*** *16-methylheptadecanoic acid* ***12.*** *stearic acid* ***13.*** *trans-vaccenic acid* ***14.*** *oleic acid* ***15.*** *cis-vaccenic acid* ***16.*** *nonadecanoic acid* ***17.*** *linoleic acid* ***18.*** *γ-linolenic acid* ***19.*** *arachidic acid* ***20.*** *α-linolenic acid* ***21.*** *stearidonic acid* ***22.*** *dihomo-γ-linolenic acid* ***23.*** *behenic acid* ***24.*** *arachidonic acid* ***25.*** *eicosatetraenoic acid (n-3)****26.*** *eicosapentaenoic acid (n-3)****27.*** *lignoceric acid* ***28.*** *cis-13,16,19-docosatrienoic acid INTERNAL STANDARD* ***29.*** *docosatetraenoic acid (n-6)* ***30.*** *nervonic acid* ***31.*** *docosapentaenoic acid (n-6)* ***32.*** *docosapentaenoic acid (n-3)* ***33.***

docosahexaenoic acid (n-3) ***34.*** *cerotic acid* ***35.*** *tetracosatetraenoic acid (n-6)* ***36.*** *tetracosapentaenoic acid (n-6)* ***37.*** *tetracosapentaenoic acid (n-3)* ***38.*** *tetracosahexaenoic acid (n-3).* GC conditions: Temperature of injector and FID was 250°C and 280°C; inject volume was 1µl (split ratio 10:1); the flow rate of helium was 3 ml/min; stationary phase was the capillary column HP-88 (100 m in length, 250 µm i.d, 0.25 µm in film thickness). The initial temperature was 130 °C for 1 min. The temperature gradient was as follows: 2° C/min up to 176 °C, maintained for 2 min; 1 °C/min to 186 °C, maintained for 1 min; 0.2 °C/min to 190 °C, maintained for 1 min; 1 °C/min to 220 °C, maintained for 4 min.

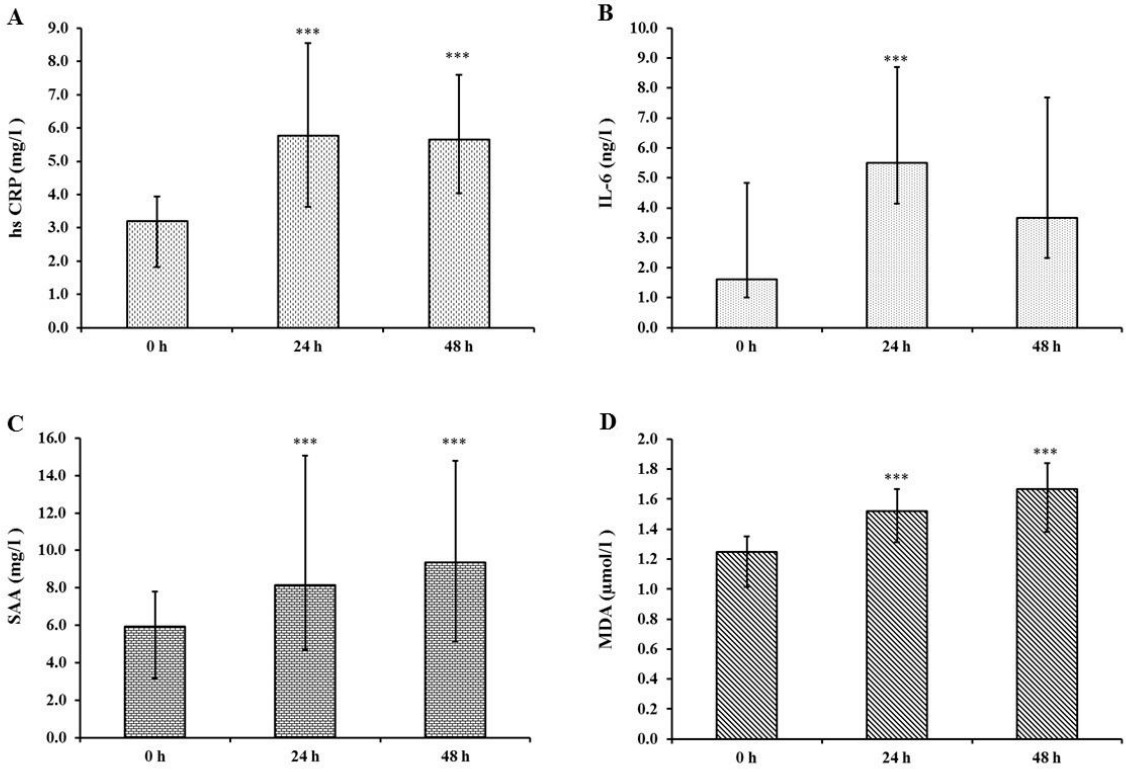

Figure 2. Dynamics of the inflammatory response and oxidative stress after PCI. (A) Levels of IL-6 during 48 hours after PCI; (B) - Levels of hsCRP during 48 hours after PCI; (C) SAA levels during 48 hours after PCI; (D) MDA levels during 48 hours after PCI. IL-6: interleukin-6; PCI: percutaneous transluminal coronary angioplasty; hsCRP: high-sensitivity C-reactive protein; SAA: serum amyloid A; MDA: malondialdehyde. The results are expressed as median and IQR (Interquartile range). *p<0.05; **p<0.01; ***p<0.001.