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The effect of fatty acids in red blood cell membranes on the dynamics of inflammatory markers following the coronary stent implantation

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ABSTRACT

The effect of 20 fatty acids in erythrocyte cell membranes on the extent of inflammatory response and cell oxidative stress was evaluated using multidimensional statistical data analysis in 54 patients suffering from ischemic heart disease undergoing percutaneous coronary intervention with coronary stent implantation using multidimensional statistical data analysis. A systemic inflammatory response was indicated by an increase of C-reactive protein (CRP), serum amyloid A (SAA) and ceruloplasmin 48 h after stent implantation and by an increase of interleukin-6 (IL-6) 24 h after intervention. The increase of malondialdehyde (MDA) after 48 h was used as a marker of cell damage by oxidative stress. Multiple linear regression revealed statistically significant relationships between concentration of some fatty acids and the magnitude of inflammatory response, or oxidative stress, after stent implantation. The most significant relationship with an increase of plasma CRP was found for myristic acid and, to a lesser extent, for oleic acid. Trans octadecenoic acid, and to a lesser extent palmitooleic and nervonic fatty acids were found in inverse correlation with the CRP increase. The increase of IL-6 showed a statistically significant correlation with myristic acid, to a lesser extent with cis-9-eicosenoic acid and to the least extent with docosahexaenoic acid, inversely with pentadecanoic, γ-linolenic and stearic acids. An increase of oxidative stress (MDA) significantly correlated only with γ-linolenic acid. Other studied markers of inflammatory response to coronary stenting were SAA and ceruloplasmin (Cp). Statistical evaluation revealed that SAA and Cp are not suitable markers for assessment relationships between inflammation and erythrocyte membrane fatty acids.

1. Introduction

Cardiovascular diseases belong to the most frequent causes of death, especially the most serious type of coronary heart disease (CHD) [1]. The current way of treating CHD lies in percutaneous coronary intervention (PCI) with the implantation of a coronary stent to the narrowed coronary arteries. Different types of coronary stents have been developed. Bare metal stents are still used, along with drug eluting stents and/or biologically degradable stents. Implantation of the coronary stent causes local and systemic inflammatory responses. Their intensity and magnitude promote thrombotic pathways, negatively affect clinical outcome and increase the risk of stent thrombosis and restenosis [2]. Inflammatory and thrombotic pathways share common signalling, contribute to neo-intimal proliferation and are implicated in the pathogenesis of clinical complications [3]. To diminish inflammatory response, anti-inflammatory drug-eluting stents have been developed. Thus, the beneficial effect of these drug-eluting stents might be partly related to a weaker local or systemic inflammatory response after coronary stenting. Numerous studies has been carried out to assess the beneficial effect of drug-eluting stents, unfortunately with rather contradictory results [4–6]. Reliable marker for evaluation of post-stenting inflammatory status was shown hsCRP, concentration of which exhibits maximum 48 h after PCI [7,8]. Basal CRP levels, as well as increase of CRP concentration after coronary stenting, are highly individual and their up-regulation depends on patient reactivity. This was confirmed by the finding no association between the increase of CRP concentration after coronary stenting and laboratory, clinical and demographic or angiographic variables. Some patients exhibit higher sensitivity in inflammatory progression after coronary stenting, as indicate finding of association between basal CRP levels and the magnitude of CRP increase after PCI.

In an Italian study [9], post-procedural increase in CRP concentration correlated with the risk of clinical complications after coronary stenting while no effect of the type of stent was observed.

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Table 1a
Group characteristics. Data are expressed as median (interquantile variance).

Patients with PCI, (n = 54)	
Age	64.0 (14)
Male/Female	40/14
BMI (kg/m2)	29.4 (6.89)
Total Cholesterol (mmol/l)	3.6 (1.5)
HDL-ch (mmol/l)	1.04 (0.47)
LDL-ch (mmol/l)	2.1 (0.89)
Triglycerides (mmol/l)	1.05 (0.69)
Diabetes mellitus (%)	27.8
Smoking cig. (%)	16.7
Statins use (%)	72.2

This finding supports the importance of individual inflammatory patientś reactivity in occurrence of clinical complications.

Vascular inflammation after PCI involves complex interactions between multiple cell types [2]. The spectrum of signaling molecules and cytokines produced by cells at the site of the vascular injury and the activation of signaling pathways can also be affected, to a certain extent, by the composition of available fatty acids in cell membranes.

In order to find novel biomarkers for the prediction of late clinical complications of PCI, such as in-stent restenosis, the aim of this study was to evaluate the role of erythrocyte membrane fatty acids in the oxidative stress and inflammatory response to coronary stenting in patients with significant coronary heart disease and to check whether different profiles of fatty acids in erythrocyte cell membranes influence the intensity of inflammation, which has an impact on the occurrence of complications and the prognosis of patients.

2. Experimental

2.1. Study subjects

Fifty-four patients undergoing PCI with coronary stent implantation for significant coronary stenosis were included in this cross-sectional study as described in [10,11]. Patients with initial levels of hsCRP>10 mg/l and/or patients with serious health complications were excluded. For group characteristics see Table 1a. The appropriate institutional approval of the review board was obtained and the principles outlined in the Declaration of Helsinki for human experimental investigations have been followed. Participants received a description of the study and signed an informed participation consent that included permission to conduct analyses on the blood samples collected. All procedures were performed with a standard technique. 32 patients received drug-eluting stent (DES, Everolimus), 16 patients bioresorbable stent (BVS). Two stents (DES + bare-metal stent) were used in 5 patients and (DES + BVS) in 1 case. Before PCI, patients received weight-adjusted intravenous heparin with a target activated clotting time of 250–350 seconds.

2.2. Blood samples

Venous blood samples were collected in tubes with EDTA before, at 24 and 48 h after PCI. Blood was immediately centrifuged at 1500 *g* for 20 min. and aliquoted into cryotubes as plasma, buffy coat, and erythrocytes. One ml of erythrocytes was taken from the centre of the erythrocyte column and immediately stored at −80 °C.

2.3. Determination of biochemical markers

Hs CRP, HDL-cholesterol, LDL-cholesterol and triglycerides were determined by standard procedures in the Regional Hospital of Pardubice, Czech Republic. Hs CRP was measured with the analytical system VISTA®, IL-6 by the Immulite® immunochemistry analyzer and SAA by the BN ProSpec® laser nephelometer (all provided by Siemens Healthcare Diagnostics Inc., USA). Malondialdehyde (MDA), a marker of lipid peroxidation, was assessed by HPLC as previously described [11]. Plasma MDA was quantified as a malondialdehyde-thiobarbituric acid complex which was made using an isocratic elution on a LiChroCart 250 × 4 mm, Purospher Star RP-18e, 5 μm, analytical column fitted with a LiChroCart 4 × 4 mm, Purospher Star RP-18e, 5 μm, guard column (Merck, Darmstadt, Germany).

2.4. Determination of fatty acids in erythrocyte cell membranes

The patient sample 200 μl of erythrocytes was mixed with distilled water and placed for 15 min in a freezer (−20 °C). After thawing and centrifugation (1 700 × *g*, room temperature, 10 min) membranes of erythrocytes were separated and three times washed. After the last wash, 1 ml of deproteinization solution (2-propanol, n-heptane, ortho-phosphoric acid, 40:20:1, v/v/v) was added to the resulting sediment. The mixture was mixed and incubated (room temperature, 10 min), and 400 μl of internal standard (heneicosanoic acid, C21:0) at a concentration of 9 μg / ml, and 300 μl of distilled water were added. Trans-esterification and the gas chromatographic separation of the fatty acid methyl esters have been described in [10,12]. Quantification of fatty acid methyl esters was carried out based on their peak areas compared to the internal standard peak area by using internal response factors. Fatty acids were labelled x1 through x20 (Table 1b). In order to avoid the potential error given by the different number of erythrocytes in analysed samples the fatty acid concentrations were expressed per g of hemoglobin determined in the same samples of separated erythrocytes (Table 1c).

2.5. Statistical data analysis

The visualization of multi-dimensional data sets can help deal with the flood of information. Visual data analysis techniques have proven to be of high value in exploratory data analysis (EDA). In addition to standard 2D/3D techniques such as scatterplots, scatterplots matrix, bar charts, line graphs and the iconic display or glyphs there are number of more sophisticated classes of visualization techniques of multivariate data matrix, p. 161 in [13]: The glyph *Stars* is composed of equally spaced radii, as many as the number of attributes in the table data, stemming from the centre. The length of the rightmost spike is proportional to the value of the first attribute for a given row and the remaining attributes are assigned to their spikes counter clockwise in this manner. The *Box-and-whisker Plot* shows the variability of data matrix variables. *Correlation Matrix Analysis* (CA) examines the existing interrelationship among variables and tests the basic assumption for the principal component analysis and factor analysis. *Principal Component Analysis* (PCA) performs a dual objective: the transformation of the original variables into new, uncorrelated latent variables called principal component, and dimensionality reduction using only a principal components reflecting the data structure, p. 183 in [13]. *Factor Analysis* (FA) can first identify the separate dimensions (factors) of the structure and then determine the extent to which each variable is explained by each dimension. In the multivariate linear model, the variables are defined as linear functions of the factors, p. 206 in [13]. *Cluster Analysis* (CLU) classifies a set of objects into two or more classes or clusters based on the similarity of the objects for a set of specified variables. It provides an abstraction from individual data objects to the clusters in which those data objects reside, p. 328 in [13]. *Multivariate Linear Regression* (LS) solves the problems in three components of a regression triplet, *i.e.* criticism of the data,

Table 1b
Source matrix of the variables of fatty acids in red blood erythrocyte cell membranes, *Median, n = 54, fatty acids in erythrocyte membranes [μmol/g Hb], SD is the standard deviation [μmol/g Hb]; CV is the variation coefficient [%].

ID	Units	Fatty acid, marker	Molecular formula	Mean	Median	SD	CV [%]
x1	[μmol/ g Hb]	Myristic	C14:0	0.0177	0.0167	0.0012	8.90
x2	[μmol/ g Hb]	Pentadecanoic	C15:0	0.0074	0.0072	0.0003	9.90
x3	[μmol/ g Hb]	Palmitic	C16:0	1.3178	1.3439	0.0767	7.45
x4	[μmol/ g Hb]	Palmitoleic	*cis*-C16:1 N7	0.0159	0.0151	0.0011	6.34
x5	[μmol/ g Hb]	Heptadecanoic	C17:0	0.0249	0.0243	0.0007	7.29
x6	[μmol/ g Hb]	Stearic	C18:0	1.0765	1.1141	0.0520	7.61
x7	[μmol/ g Hb]	Trans octadecenoic	*trans*-C18:1 N9	0.0074	0.0072	0.0019	8.42
x8	[μmol/ g Hb]	Oleic	*cis*-C18:1 N9	0.7243	0.7200	0.0390	7.34
x9	[μmol/ g Hb]	Linoleic	*all cis*-18:2 N6	0.4720	0.4965	0.0303	7.97
x10	[μmol/ g Hb]	γ-linolenic	*all cis*-18:3 N6	0.0024	0.0021	0.0007	8.54
x11	[μmol/ g Hb]	Arachidic	C20:0	0.0160	0.0156	0.0006	8.77
x12	[μmol/ g Hb]	α-linolenic	*all cis*-C18:3 N3	0.0062	0.0060	0.0009	6.86
x13	[μmol/ g Hb]	Cis-9-eicosenoic	*cis*-20:1 N9	0.0123	0.0125	0.0018	9.83
x14	[μmol/ g Hb]	Eicosadienoic	*all cis*-C20:2 N6	0.0116	0.0125	0.0004	4.08
x15	[μmol/ g Hb]	Eicosatrienoic	*all cis*-C20:3 N6	0.0908	0.0954	0.0088	7.36
x16	[μmol/ g Hb]	Behenic	C22:0	0.0568	0.0555	0.0016	7.81
x17	[μmol/ g Hb]	Arachidonic	*all cis*-C20:4 N6	1.0592	1.1871	0.0698	9.49
x18	[μmol/ g Hb]	Eicosapentaenoic	*all cis*-C20:5 N3	0.0400	0.0388	0.0011	4.51
x19	[μmol/ g Hb]	Nervonic	*cis*-C24:1 N9	0.2088	0.2165	0.0183	7.31
x20	[μmol/ g Hb]	Docosahexaenoic	*all cis*-C22:6 N3	0.3041	0.3103	0.0172	6.32
ΔCRP48	[mg/l]*	C-reactive protein			0.8776		
ΔIL6-24	[ng/l]*	Interleukin 6			0.8152		
ΔSAA48	[mg/l]*	Serum amyloid A			0.8223		
ΔMDA48	[μmol/l]*	Malondialdehyd			0.2721		
ΔCp48	[g/l]*	Ceruloplasmin			0.1667		

Table 1c
Time progress of oxidative stress and inflammatory markers during 48 h after PCI with coronary stent implantation, * Median, n = 54.

Marker	0 h	24 h	48 h	Δ24 h	Δ48 h	Maximal relative increase
hsCRP [mg/l]*	2.7050	3.8950	4.1700	1.0800	1.6050	0.8776
IL-6 [ng/l]*	1.9000	4.5900	3.2100	2.0900	0.6700	0.8152
SAA [mg/l]*	4.2500	5.4400	6.1800	1.4450	2.7100	0.8223
MDA [μmol/l]*	1.1201	1.4021	1.4956	0.2033	0.2671	0.2721
Cp [g/l]*	0.2000	0.2200	0.2300	0.0200	0.0300	0.1667

regression model and mathematical method. The classical least-squares method provides accurate parameter estimates only when all assumptions about data and about a regression model are fulfilled. When some assumptions are not fulfilled, the least-squares method is inappropriate. Regression diagnostics represent the procedures for identifying the data quality for a proposed model, the model quality for a given data set and for the fulfilment of all least-squares assumptions, p. 519 in [13] or [14,15]. All statistical analyses were carried out using STATISTICA software (version 12.0, StatSoft, USA) and QCEXPERT software (version 3.5, TriloByte statistical Software, Pardubice, Czech Republic).

3. Results

Inflammatory response to PCI with coronary stent implantation was identified by determining hsCRP, IL-6, SAA and Cp before PCI and in 24 and 48 h, respectively, after the procedure (Table 1). A maximum increase of the studied parameters was found after 48 h in the case of hsCRP, SAA, MDA and Cp, while in the case of IL-6 it was 24 h after the PCI. Relative concentration changes, expressed as ΔCRP48, ΔSAA48, ΔIL6-24, ΔCp48, ΔMDA48 were obtained by expressing the concentration differences per the initial concentration.

3.1. Exploratory data analysis (EDA)

The aim of this work was to investigate whether the fatty acids in the erythrocyte cell membranes affect the change of selected inflammatory markers (CRP, IL-6, SAA and Cp) and the MDA oxidative stress marker in patients undergoing percutaneous coronary intervention followed by stent implantation. Before examining the proposed model, it was necessary to verify the assumptions made on the source data matrix. To examine the relationship of fatty acids the default source matrix contained 54 rows (referred to as P1 to P54) and 20 independent variables (fatty acids) in columns marked x1 to x20, (Table 1b).

The box-and-whisker plot (Fig. 1a) expresses the variability of the individual variables of the source matrix, here the fatty acids. The box-and-whisker plot shows that fatty acids such as palmitic x3, stearic x6, oleic x8, linoleic x9, arachidonic x17 and docosahexaenoic x20 are among the most variable variables. Small variability shows dihomo-γ-linolenic acid x15 and nervonic acid x19. For these acids, a statistically significant effect on the inflammation indicated by a change in marker concentrations can be considered. Acids with minimal or no concentration variability include myristic acid x1, pentadecanoic x2, palmitoleic x4, heptadecanoic x5, trans octadecenoic x7, γ-linolenic x10, arachidic x11, α-linolenic x12, cis-9-eicosenoic x13, eicosadienoic x14, behenic x16 and eicosapentaenoic acid x18. The largest proportions in the erythrocyte cell membranes are palmitic, stearic, oleic, linoleic, and archidonic acids.

From the box-and-whisker plot of the inflammation and oxidative stress markers in Fig. 1a, it is clear that the increase of the inflammatory process, oxidative stress, is relatively small but there are patients who have had an abnormal increase in CRP in 48 h, SAA in 48 h, or IL-6 within 24 h after stent implantation. This was a P14 patient who had a 90-fold increase in SAA in 48 h and more than a 20-fold increase in CRP concentration in 48 h and IL-6 in 24 h after implantation of the stent. In this patient, stent implantation was complicated by ventricular fibrillation, and a transiently elevated troponin I concentration was found to indicate myocardial damage. Patients who experienced a more than 10-fold increase in SAA

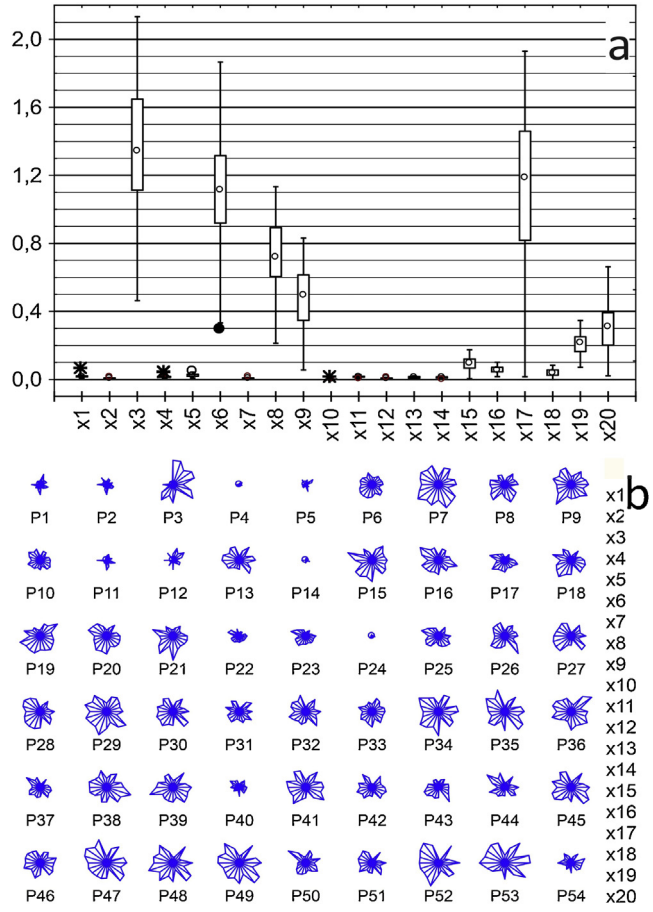

Fig. 1. Exploratory data analysis EDA of the fatty acids profile in red blood cell membranes: (a) The box-and-whisker plot for examination of all fatty acids concentration variability represents the graphical measure of individual x1 to x20 fatty acids, (b) A star glyph showing the composition of 20 fatty acids and 4 markers in red blood cells in P1 through to P54 patients.

include P12, P22 and P24 patients. Patient P12 suffered from respiratory insufficiency for chronic obstructive pulmonary and asthma. Patient P24 suffered from congestive heart failure of 2nd and 3rd degree dysfunction of both ventricles, a thrombus was found in the left ventricle and dilation of all parts of the heart was performed. In addition, the patient suffered from pulmonary hypertension and was a smoker. No specific source of increase in serum concentration of SAA was found in patient P22; however, he was a type 2 diabetic patient suffering from dyslipidemia and hyperuricemia.

The variability and similarity of 20 variables in 54 patients can also be examined using iconic graphs and glyphs (page 168 in Ref. [13]) representing the presentation of a 20-dimensional space projected into the plane. Each patient represents a point in the multidimensional space, and two patients are similar when the Mahalanobis distance between them is minimal (page 154 in Ref. [13]). This fact can be applied to the mutual similarity of patient iconic charts [13].

It is clear from the iconic *Stars graph* in Fig. 1b that the concentration of the individual fatty acids varies considerably between patients. Based on star shape similarities and lengths of their rays, patients with similar concentrations of fatty acids in red blood cell membranes were found. Very small stars are characterized by a group of patients with very low fatty acid concentrations. This group includes patients P1, P2, P4, P5, P11, P12, P14, P22, P24 and P40. The largest stars polygons were found in P7, P9, P29, P34, P35, P36, P38, P39, P41, P45, P47, P48, P49, P52 and P53 patients. This group can be referred to as patients with high concentrations of fatty acids in the erythrocyte cell membranes. Some similarity can be found between patients P47, P48 and P49, or between patient groups P4, P14 and P24. A special status among others is the patient referred to as P3, as the only one exhibiting higher concentrations of saturated and monounsaturated fatty acids compared to polyunsaturated fatty acid concentrations. Upon admission, patient P3 suffered from stable angina pectoris (CSS II, NYHA Class I) previously undergoing myocardial infarction and drug stent implantation with a history of pulmonary embolism. In addition, this patient was treated for type 2 diabetes mellitus and hypertension.

3.2. Correlation analysis (CA)

From the correlation matrix (Table 2 and Fig. 2), there are series of statistically significant correlations between fatty acid concentrations (independent variables). For example, the most positive correlations ($R > 0.8$) were found in stearic acid, which correlates positively with oleic, linoleic, eicosadienoic, behenic, arachidonic, nervonic and docosahexaenoic acid. Similarly, palmitic acid correlates ($R > 0.8$) with oleic, linolenic, behenic, nervonic and docosahexaenoic acid concentrations, and linoleic acid, in addition to the above mentioned correlations ($R > 0.8$) with eicosadienoic, dihomo-γ-linolenic, arachidonic, nervonic and docosahexaenoic. An important prerequisite for a matrix of independent variables for subsequent linear regression, however, is the requirement for independence, originality and uniqueness of independently used variables, here fatty acids x1 to x20. If there is a statistically significant correlation in the matrix of characters, it means that the characters are bound by a hidden internal relationship expressed by the correlation. There is a risk that the least squares method will evaluate the unknown parameters as biased, which means a systematic error of the inappropriate mathematical method. Data showing multicollinearity should be evaluated using the rational rank method or the robust M-estimation method (Welsch), which quantifies unbiased parameter estimates.

With markers, the relative change in CRP at 48 h after coronary stent implantation (ΔCRP48), IL-6 in 24 h after PCI (ΔIL6-24) and SAA in 48 h (ΔSAA48) exhibited mutually positive correlation and they also found numerous statistically significant correlations with fatty acids concentrations in erythrocyte cell membranes. The change in ceruloplasmin concentration at 48 h after stent implantation (ΔCp48) did not correlate with any of the observed variables. This finding supports the idea that the role of ceruloplasmin in the development of cardiovascular disease and inflammation is not entirely clear. Based on the above, we have decided that ceruloplasmin is not suitable for assessment relationships between inflammation and erythrocyte membrane fatty acids. Malondialdehyde, the lipoperoxidation indicator, of all statistically rated signs, significantly correlated only with γ-linolenic acid, suggesting that the inflammatory process and oxidative stress are two different processes largely independent of each other.

3.3. Principal components analysis (PCA)

The objective of the principal component analysis PCA is the representation of the objects and a group of mutually dependent or correlated variables to the *structural matrix* and to the *noise matrix*. This means the transformation of the original variables x_i, j=1, . . ., m, into a smaller number of latent variables y_j, called *principal components*. The principal components represent almost the entire variability of the original data and are uncorrelated.

The graphic tools of PCA is the *Cattel Scree plot of eigenvalues* (Fig. 3a), which serves to identify useful and significant principal components. The *Principal component loadings plot* (PCAW) (Fig. 3b) informs about the relationship between original variables and

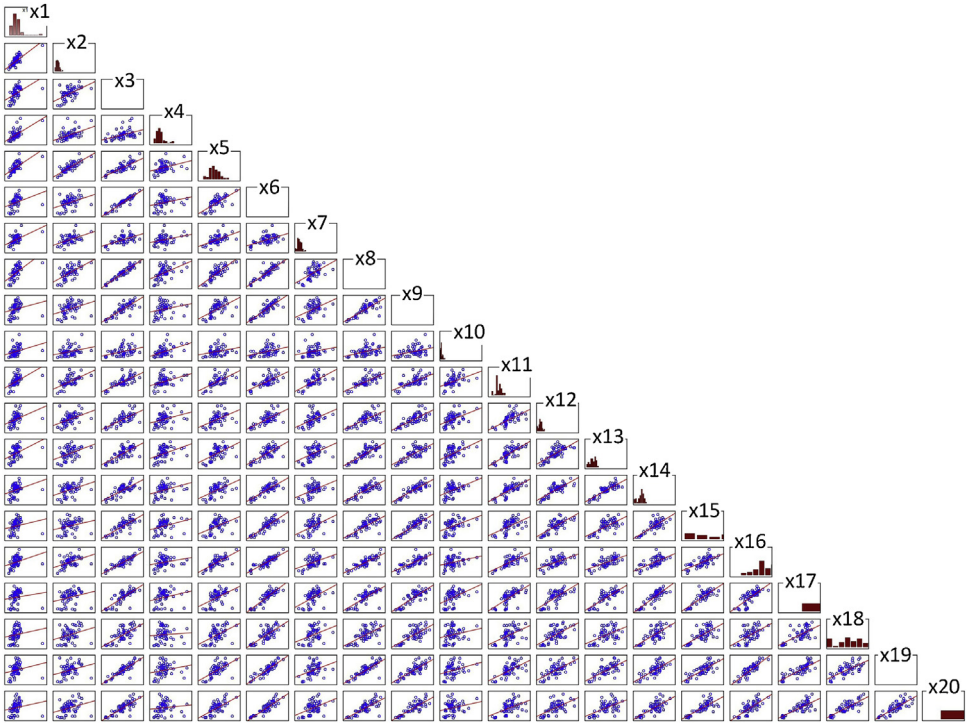

Fig. 2. Scatterplot of correlation matrix indicates which variables (fatty acids and markers) correlate and which do not.

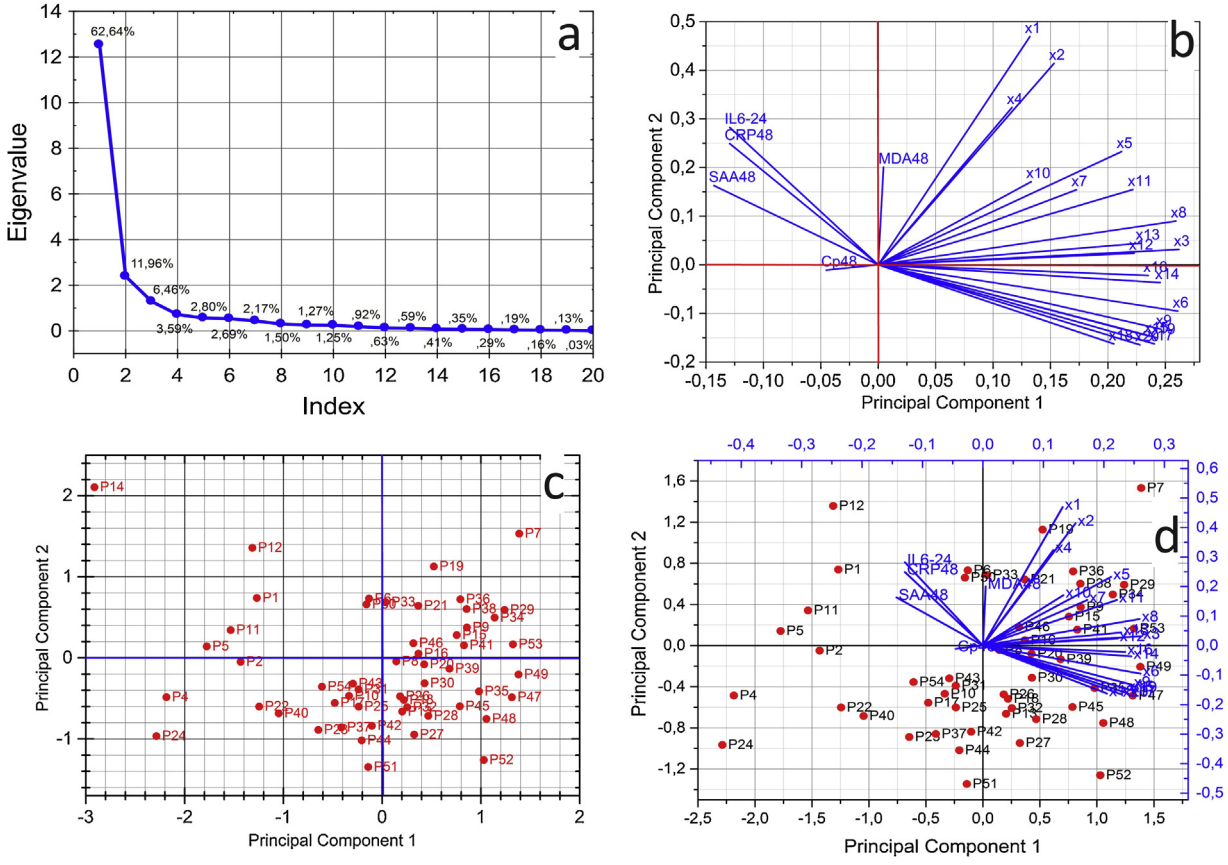

Fig. 3. Principal components analysis PCA of fatty acids profile in red blood cell membranes: (a) The Cattel scree plot of an eigenvalue against the index shows how many significant components to retain, (b) the PCAW1-2 principal component loadings plot of the first two components demonstrates correlation among variables, (c) the PCAS1-2 scatterplot of principal component scores of the first two components exhibits cluster classification of patients.

Table 2
Correlation matrix of fatty acids in erythrocyte cell membranes and inflammatory markers. * Red indicates statistically significant Pearson's pair correlation coefficients, α = 0.05. Statistically significant Pearson's correlation coefficients are written in red. (For interpretation of the references to colour in this table, the reader is referred to the web version of this article.)

Table 3	x1	x2	x3	x4	x5	x6	x7	x8	x9	x10	x11	x12	x13
x1	1,0000	0,7870	0,5157	0,6154	0,6820	0,3330	0,5294	0,6069	0,2186	0,3108	0,5360	0,3857	0,4092
x2	0,7870	1,0000	0,5493	0,4757	0,8203	0,3959	0,5052	0,6024	0,3530	0,2794	0,5949	0,4373	0,4137
x3	0,5157	0,5493	1,0000	0,4274	0,8022	0,9514	0,5695	0,9507	0,8274	0,3765	0,7416	0,6891	0,7212
x4	0,6154	0,4757	0,4274	1,0000	0,3576	0,2575	0,3125	0,4883	0,2290	0,4783	0,3844	0,3253	0,3984
x5	0,6820	0,8203	0,8022	0,3576	1,0000	0,7184	0,5571	0,8160	0,5783	0,2538	0,6398	0,5224	0,5608
x6	0,3330	0,3959	0,9514	0,2575	0,7184	1,0000	0,5289	0,8990	0,8739	0,3681	0,7124	0,6931	0,7332
x7	0,5294	0,5052	0,5695	0,3125	0,5571	0,5289	1,0000	0,5797	0,4704	0,2427	0,6403	0,4903	0,4705
x8	0,6069	0,6024	0,9507	0,4883	0,8160	0,8990	0,5797	1,0000	0,7994	0,4133	0,7412	0,6867	0,7813
x9	0,2186	0,3530	0,8274	0,2290	0,5783	0,8739	0,4704	0,7994	1,0000	0,3643	0,6601	0,7532	0,7224
x10	0,3108	0,2794	0,3765	0,4783	0,2538	0,3681	0,2427	0,4133	0,3643	1,0000	0,4743	0,4796	0,5210
x11	0,5360	0,5949	0,7416	0,3844	0,6398	0,7124	0,6403	0,7412	0,6601	0,4743	1,0000	0,6514	0,7359
x12	0,3857	0,4373	0,6891	0,3253	0,5224	0,6931	0,4903	0,6867	0,7532	0,4796	0,6514	1,0000	0,7459
x13	0,4092	0,4137	0,7212	0,3984	0,5608	0,7332	0,4705	0,7813	0,7224	0,5210	0,7359	0,7459	1,0000
x14	0,3219	0,4139	0,7736	0,3458	0,5668	0,8234	0,4766	0,7902	0,8667	0,5170	0,7321	0,7962	0,8566
x15	0,1953	0,2477	0,7882	0,3040	0,4751	0,8575	0,4970	0,7644	0,8640	0,4234	0,5612	0,7412	0,6890
x16	0,3416	0,4474	0,9004	0,2427	0,7471	0,8802	0,4589	0,8365	0,7725	0,3030	0,6949	0,5983	0,6150
x17	0,1238	0,2450	0,7850	0,2364	0,4938	0,8880	0,5157	0,7508	0,8287	0,4577	0,6935	0,7271	0,7649
x18	0,1627	0,2948	0,6814	0,0689	0,5119	0,7536	0,3720	0,6363	0,6999	0,2304	0,4971	0,6264	0,5241
x19	0,2050	0,2977	0,8863	0,2164	0,6293	0,9040	0,3874	0,8366	0,8224	0,3101	0,6093	0,6828	0,7055
x20	0,1802	0,3142	0,8203	0,1186	0,6167	0,8909	0,4304	0,7575	0,8185	0,2498	0,4850	0,6001	0,5684
ΔCRP4	-0,0952	-0,1128	-0,3868	-0,1895	-0,2385	-0,3946	-0,3252	-0,3800	-0,3736	-0,0705	-0,3159	-0,2806	-0,3091
ΔIL6-24	-0,0098	-0,0950	-0,3637	-0,1437	-0,2045	-0,3968	-0,2048	-0,3396	-0,4344	-0,1876	-0,2345	-0,3424	-0,3089
ΔSAA4	-0,2036	-0,2233	-0,4145	-0,2832	-0,3109	-0,3893	-0,2920	-0,4250	-0,4111	-0,2318	-0,3743	-0,3579	-0,3830
ΔMDA4	0,1074	0,2372	-0,0493	0,1189	0,0410	-0,1221	-0,0757	-0,0493	-0,0313	0,3978	0,0326	0,1761	0,0271
ΔCp48	-0,0410	-0,0266	-0,1334	-0,1878	-0,0985	-0,1416	-0,1690	-0,1130	-0,1682	-0,1244	-0,0510	-0,0914	-0,2345

ID	x14	x15	x16	x17	x18	x19	x20	ΔCRP48	ΔIL6-24	ΔSAA48	ΔMDA4	ΔCp48
x1	0,3219	0,1953	0,3416	0,1238	0,1627	0,2050	0,1802	-0,0098	0,0098	-0,2036	0,1074	-0,0410
x2	0,4139	0,2477	0,4474	0,2450	0,2948	0,2977	0,3142	-0,0950	0,0950	-0,2233	0,2372	-0,0266
x3	0,7736	0,7882	0,9004	0,7850	0,6814	0,8863	0,8203	-0,3637	0,3637	-0,4145	-0,0493	-0,1334
x4	0,3458	0,3040	0,2427	0,2364	0,0689	0,2164	0,1186	-0,1437	0,1437	-0,2832	0,1189	-0,1878
x5	0,5668	0,4751	0,7471	0,4938	0,5119	0,6293	0,6167	-0,2045	0,2045	-0,3109	0,0410	-0,0985
x6	0,8234	0,8575	0,8802	0,8880	0,7536	0,9040	0,8909	-0,3968	0,3968	-0,3893	-0,1221	-0,1416
x7	0,4766	0,4970	0,4589	0,5157	0,3720	0,3874	0,4304	-0,2048	0,2048	-0,2920	-0,0757	-0,1690
x8	0,7902	0,7644	0,8365	0,7508	0,6363	0,8366	0,7575	-0,3396	-0,3396	-0,4250	-0,0493	-0,1130
x9	0,8667	0,8640	0,7725	0,8287	0,6999	0,8224	0,8185	-0,4344	-0,4344	-0,4111	-0,0313	-0,1682
x10	0,5170	0,4234	0,3030	0,4577	0,2304	0,3101	0,2498	-0,1876	-0,1876	-0,2318	0,3978	-0,1244
x11	0,7321	0,5612	0,6949	0,6935	0,4971	0,6093	0,4850	-0,2345	-0,2345	-0,3743	0,0326	-0,0510
x12	0,7962	0,7412	0,5983	0,7271	0,6264	0,6828	0,6001	-0,3424	-0,3424	-0,3579	0,1761	-0,0914
x13	0,8566	0,6890	0,6150	0,7649	0,5241	0,7055	0,5684	-0,3089	-0,3089	-0,3830	0,0271	-0,2345
x14	1,0000	0,7808	0,6704	0,8557	0,6845	0,7343	0,7230	-0,4165	-0,4165	-0,4207	0,0921	-0,1348
x15	0,7808	1,0000	0,7089	0,8459	0,6982	0,7913	0,7835	-0,4101	-0,4101	-0,3721	-0,0393	-0,2087
x16	0,6704	0,7089	1,0000	0,7372	0,5607	0,8683	0,7473	-0,2965	-0,2965	-0,3641	-0,0311	-0,0369
x17	0,8557	0,8459	0,7372	1,0000	0,6916	0,8089	0,7717	-0,4052	-0,4052	-0,3629	-0,1236	-0,1982
x18	0,6845	0,6982	0,5607	0,6916	1,0000	0,7239	0,8224	-0,3621	-0,3621	-0,3125	0,0107	-0,1527
x19	0,7343	0,7913	0,8683	0,8089	0,7239	1,0000	0,8095	-0,3877	-0,3877	-0,4218	-0,0537	-0,0837
x20	0,7230	0,7835	0,7473	0,7717	0,8224	0,8095	1,0000	-0,3777	-0,3777	-0,3284	-0,0506	-0,1403
ΔCRP48	-0,3376	-0,3743	-0,3234	-0,3826	-0,3177	-0,4177	-0,3046	0,7981	0,7981	0,9040	0,1064	-0,0337
ΔIL6-24	-0,4165	-0,4101	-0,2965	-0,4052	-0,3621	-0,3877	-0,3777	1,0000	1,0000	0,8109	-0,0051	0,0821
ΔSAA48	-0,4207	-0,3721	-0,3641	-0,3629	-0,3125	-0,4218	-0,3284	0,8109	0,8109	1,0000	-0,1033	-0,0253
ΔMDA4	0,0921	-0,0393	-0,0311	-0,1236	0,0107	-0,0537	-0,0506	-0,0051	-0,0051	-0,1033	1,0000	0,0156
ΔCp48	-0,1348	-0,2087	-0,0369	-0,1982	-0,1527	-0,0837	-0,1403	0,0821	0,0821	-0,0253	0,0156	1,0000

newly created latent variables called principal components. Each of the matrix points is represented by the variables, which the position and length of the guide axes being judged. The short distance between two variables means a strong correlation. Determining the importance will also determine the variability of the variable. Variables with an angle between 0° guides are strongly correlated, 90° means that characters are totally uncorrelated, while 180° means that characters correlate negatively. The *Scatterplot of prin-*

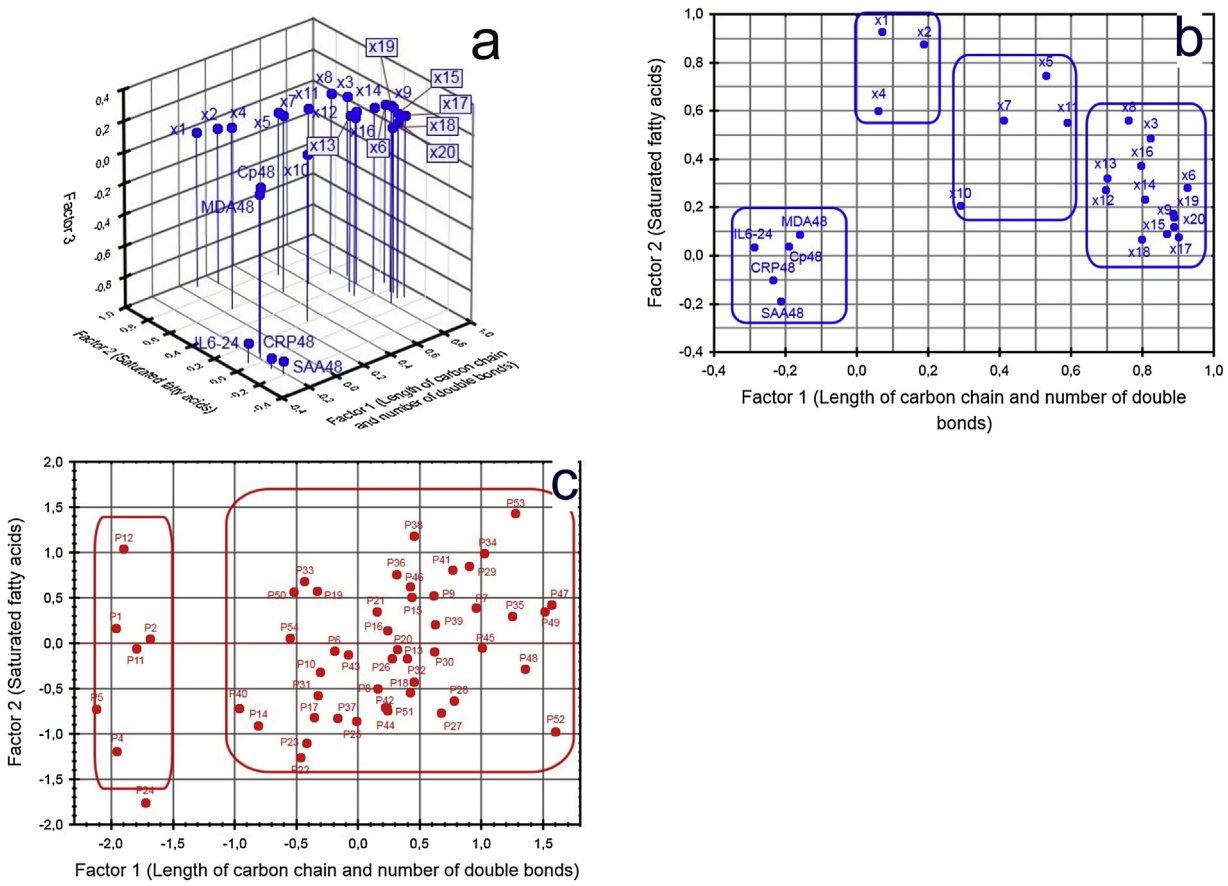

Fig. 4. Factor analysis FA of fatty acids in red blood cell membranes: (a) the 3D plot factor loadings FAW1-2-3 of the first three factors after varimax rotation exhibits classification of variables, (b) the 2D plot factor loadings FAW1-2 of the first two factors after varimax rotation exhibits four clusters classification of variables examined, (c) the 2D scatterplot of factor scores FAS1-2 of the first two factors after varimax rotation presents two clusters of patients.

cipal component scores (PCAS) (Fig. 3c) shows the coordinates of each object on the principal component axes and are used to assess cases in the patients (objects). Patients located close to each other are similar, while far from each other they are dissimilar [13].

The PCA results are shown in Fig. 3. The Cattel graph in our case expresses the most information (62.64% on PC1 + 11.96%) on PC2 is 74.6% of the original data variability. From this graph it is clear that there are quite significant correlations between fatty acids. In order to reduce the number of variables in Fig. 3b, it would be sufficient to leave only myristic acid x1, pentadecanoic x2, palmitic x3, heptadecanoic x5, oleic x8, arachidic x11 and arachidonic acid x17 in the source matrix and release the palmitoleic acid x4, trans octadecenoic x7, linoleic acid x9, γ-linolenic x10, α-linolenic x12, cis-9-eicosenoic x13, eicosadienoic x14, dihomo-γ-linolenic x15, behenic x16, eicosapentaenoic x 18, nervonic x19 and docosahexaenoic x20. Two clusters of patients are visible in the PCAS1-2 scatterplot of principal component scores of the first two components in Fig. 3c. The cluster of patients P1, P2, P4, P5, P11, P12, P14, P22, P24 and P40 corresponds to the group of patients already identified in the Iconic polygons Stars in Fig. 1b in which low fatty acid concentrations were found. We believe that the similarity of these patients could be due to the fact that these patients have significant metabolic disorders such as hyperlipoproteinemia treated with statins and fibrates that affect lipid metabolism, type 2 diabetes mellitus treated with oral antidiabetics or hyperuricemia treated with allopurinol. The *Biplot* is a graph that represents both variables and objects together in two dimension (Fig. 3d) and information about the variables is provided by the variable projections or axes (page 192 in Ref. [13]). Some variables are highly correlated with the index of actual patients.

3.4. Factor analysis (FA)

Factor Analysis (FA) is used to examine internal contexts and to uncover the structures of a data matrix by introducing several latent variables denoted *factors* to which a content meaning is assigned, through which each original variable is explicitly explained in terms of content (Fig. 4).

The basic graphical aids of factor analysis include the *Factor analysis loadings* FAW (Fig. 4a and b), the individual factors which are on the axes. After the axes are rotated, the clusters of variables (fatty acids and markers) usually appear in the graph. Variables located at the end of the coordinate axis have a high factor load on the assigned axis and can be referred to as *factorially-clean variables*. The characters near the beginning (0, 0) have small loads of both factors. The last group concerns variables that are not close to either of the coordinates, which are referred to as *factorially-impure variables*. Using factors with a high factorial load near 1, factors can be assigned to their content meaning. Another aid is the *Scatterplot of factor scores* FAS (Fig. 4c), which is used to find clusters of similar objects but also exceptional object values or outlying objects and, above all, to reveal outlying observations [13,15,16].

Factor analysis of the rotation factors by the Varimax method in the Factor loading plot in Fig. 4b, exhibits a high factor loading for Factor 1 with stearic acid x6, linoleic x9, α-linolenic x12, eicosadienoic x14, dihomo-γ-linolenic x15, behenic x16, arachidonic x17, eicosapentaenoic x18, nervonic x19 and docosahexaenoic x20. All of these acids, except for behenic and stearic acids, are unsaturated fatty acids, and there is a visible trend towards increasing the factor load with increasing carbon chain length and the number of double bonds. Therefore, we decided to name this factor Ľength

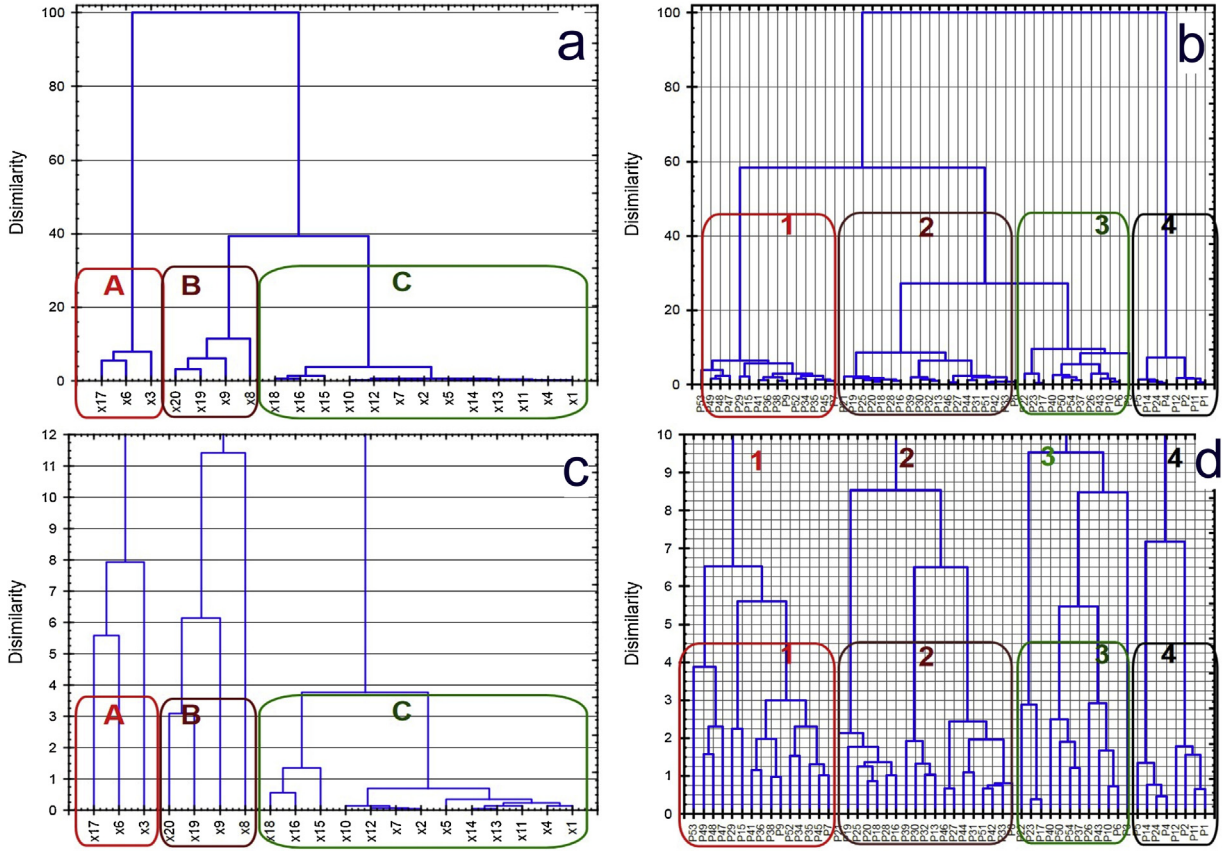

Fig. 5. Cluster analysis CLU of all fatty acids in red blood cell membranes when analyzing the set of 54 patients: (a) three clusters of 20 fatty acids in the vertical Ward dendrogram of variables, (b) four clusters of patients in the vertical Ward dendrogram of objects, (c) the zoom projection of the dendrogram on Fig. 5a, (d) the zoom projection of the dendrogram on Fig. 5b.

of carbon chain of fatty acids and number of double bonds. The highest factorial loading for factor 2 was found in myristic acid x1 and pentadecanoic x2. These acids are among saturated fatty acids, so we named factor 2 Šaturated fatty acids. Factor-impure acids include palmitoleic acid x4, heptadecanoic x5, trans octadecenoic x7, arachidic x11, oleic x8, cis-9-eicosenoic x13 and palmitic x3. These acids are monounsaturated fatty acids or long chain carbonated saturated fatty acids, which is in line with the factors 1 and 2 named. The greatest factor loading for factor 3 shows γ-linolenic acid x10, which fails to name the factor.

Named factors were then transferred to the scatterplot of factor scores in Fig. 4c, which are the culmination of all the exploratory data analysis. It was a success to find consistency between iconic charts, analysis of principal components and factor analysis. The FAS1-2 scatterplot of factor scores confirmed that patient P3 differs significantly from the others as well as the cluster of patients designated P1, P2, P4, P5, P11, P12, P14, 24, when they were included in the icon chart and component score chart, and even patients designated as P22 and P40. At the same time it was confirmed that the naming of the factors is correct since it is clear from the graphs that the patient P3 is unique due to the high concentration of saturated fatty acids and very low concentration of unsaturated fatty acids, which also applies to the cluster of patients that is in the factor score graph near the beginning (0, 0). This is consistent with the fact that these patients are represented in the iconic graph by very small stars, which clearly indicate the low concentration of all fatty acids in the erythrocyte cell membranes.

3.5. Cluster analysis (CLU)

Cluster analysis (CLU) is one of the methods that investigates the similarity of multidimensional objects and classifies them into clusters. The dendrogram of the similarity of the variables, fatty acids here, and the dendrogram of the similarity of the objects, *i.e.* the patients, revealed their similarity and hence the strong correlation. The variables, or objects close to each other are very similar and can therefore be said to be mutually substitutable. There are several clustering procedures, in which case the Ward's method was preferred [13,15,16].

The final step of the exploratory data analysis was to compare the obtained results with fatty acid dendrograms and dendrograms with patients in cluster analysis. The dendrogram of fatty acid clusters in Fig. 5a and in its zoom form in Fig. 5c reveals 3 clusters, marked with the letters A, B, C, and the colours red A, brown B and green C. Red group A includes arachidonic acid x17, stearic x6 and palmitic x3. High concentrations of these acids are very often associated with the promotion of inflammatory processes and the progression of cardiovascular disease. In brown group B there are docosahexaenoic acid x20, nervonic x19, linoleic x9 and oleic x8, which can be said to be predominantly anti-inflammatory and cardioprotective. The fatty acid green cluster C does not exhibit any specific functional similarities between acids. The same results were subsequently marked with fatty acids in the graph of factor loadings in variables (FAW1-2). When comparing character positions in the FAW1-2 plot and the fatty acid dendrogram, it was

found that Group A acids have a high factor load for Factor 1. Fatty acids in Group B correspond to FAW1-2, *i.e.* the axis name. In the created dendrogram of the patients in Fig. 5b and in its zoom form Fig. 5d, clusters marked 1 to 4 were identified and again the same results were subsequently marked by patients in the scatterplot of factor score. A significant match was found when comparing the scatterplot of factor score and the dendrogram of patients. As with factor analysis, patients with P1, P2, P4, P5, P11, P12, P14 and P24 have been clogged in black. As mentioned above, these patients differ significantly from others by very low concentrations of all the fatty acids determined. In addition, a cluster of P7, P35, P34, P36, P41, P15, P29, P47, P48, P49, P47, P48, P49 and P53 patients were found to have long-chain polyunsaturated fatty acids in the erythrocyte cell membranes, with an icon chart, and this cluster is marked in red. Additionally, clusters 2 and 3 were created in the dendrogram of patients, marked with green or brown. These patients are significantly intertwined in the scatterplot of factor score. Therefore, it was not possible to precisely determine what the main cause of patient placement and clustering in the dendrogram of objects was.

3.6. Multiple linear regression

The influence of the concentration of fatty acids determined in the erythrocyte cell membranes on selected markers was studied by means of multiple linear regression [14]. Evaluated inflammatory markers were the relative change of the CRP concentration (labeled ΔCRP48) in serum 48 h after coronary stent implantation and the relative change in serum IL-6 at 24 h post-treatment (labeled ΔIL6-24) and serum amyloid A (labeled ΔSAA48). The influence of fatty acids on the dynamics of cellular oxidative stress was assessed by relative MDA change after 48 h. The proposed regression models were evaluated using a regression triplet technique (data critique, model critique, and regression method critique) [13–15] of the mathematical algorithm used. This technique is sufficiently described in [13,14]. Biological markers are stated here as dependent variables and the relative change of fatty acids concentration as independent variables.

The intensity of the influence of each fatty acid on the selected marker or the chosen dependent variable is defined by the estimate of its parameter β_i, $i = 1, \ldots, 20$. The positive value of the β fatty acid slope when compared to other estimates of β fatty acids parameters means that the fatty acid acts more strongly on the selected marker. Therefore, it can be said that the fatty acid is more strongly involved in the increase in plasma concentration of the given marker in patients, and therefore has a stronger relationship to the process indicated by the marker, *i.e.* the intensity of the inflammatory reaction or the rate of oxidative damage to the cells.

Initially, the effect of all twenty selected fatty acids on the selected dependent variables in the proposed regression model was evaluated. In the data critique, in the regression analysis of the points of influence, there was first the mandatory indication of the outlying points, here patients who had to be excluded from further regression analysis. The Williams graph and other graphical diagnostics [13–16] led to the removal of patients P3, P5, P6, P14, P11, P12, P14, P26, P28, P32, P42, P44 in the regression model ΔCRP48. In the regression model, the ΔSAA48 of the graphical regression diagnostics removed the patients P3, P4, P7, P11, P12, P14, P22, P24, P25, P30, P34, P42, P43 and P46 and the patients P2, P3, P4, P9 and ΔIL6-24 regression model, P11, P14, and P46. The regression model for ΔMDA48 used all 54 original patients.

Estimates of fatty acid slopes including the absolute term b_0 of the tested hypotheses by proposed regression models together with arbitrary model criteria such as the correlation coefficient *R*, the mean quadratic error of prediction *MEP*, the Akaike information criterion *AIC* or the extensive variable, *i.e.* the residual sum of squares *RSS* and the standard deviation of residues $s(e)$ are given in the results of all hypotheses examined [14]. The tables also contain, in addition to parameter estimates b_i, $i = 1, \ldots, 20$, the standard deviation of the parameter estimate $s(b_i)$ and the calculated significance level *p*. It is true that the statistically significant estimate of b_i is for $p < 0.05$. In addition, the Hotteling's lower limit L_l and the Hotteling's upper limit L_u of the interval estimate of the parameter β, representing the precision of the estimates, are also given.

In all proposed regression models, to examine the effect of a concentration of all twenty selected fatty acids on the concentration change in the marker of inflammation, or the oxidation stress, respectively, we primarily used *the classical least squares method LS* (Table 4) and subsequently also *Welsch's method with robust M-estimates* (Table 5) due to the proven correlation between independent variables (here fatty acids) in the regression triplet analysis. On the basis of regression model criticism, a more appropriate method of the classical least squares (LS) was accepted in all cases of proposed models. Regression models constructed in this way include only statistically significant fatty acids and were calculated only by the classical LS method.

In a regression model investigating the effect of twenty given fatty acids on increasing CRP at 48 h after stent implantation (Table 3), it was found that only 7 fatty acids had a statistically significant effect (Table 6). A positive estimate of the β slope was found for myristic acid x1, heptadecanoic x5 and oleic acid x8. A negative value of a statistically significant β slope was found for pentadecanoic acid x2, palmitooleic x4, trans octadecenoic x7 and nervonic acid x19 in Table 6. Table 4 shows the results of the traditional LS method and the results of the robust M-estimates method in Table 5.

In the next step a regression model involving only statistically significant fatty acids was to be developed (Table 7). The regression model was relieved of noise in the form of statistically insignificant fatty acids and can therefore be said to be more correct with respect to the previous model. In this regression model, it has been shown that the effect of pentadecanoic acid x2 and heptadecanoic x5 on the change in CRP concentration is in fact very weak, as estimates of the β slope were already statistically insignificant.

In the last step the regression analysis was repeated only from the remaining statistically significant fatty acids, *i.e.* myristic x1, palmitoleic x4, trans-octadecenoic x7, oleic x8 and nervonic x19 acids. In this model (Table 6 and 7), myristic x1 acid has been shown to be highly positively associated with a change in CRP concentration. The positive value of the β slope was also found for oleic x8 acid but about 15 times smaller than myristic x1 acid. Negative correlations, as well as negative estimates of CRPs, have been demonstrated for palmitoleic x4, trans-octadecenoic x7, and nervonic x19 acids. The results of this mathematical method are shown in Table 8.

In another SAA regression model, which tested the effect of the concentration change of twenty selected fatty acids on increasing the SAA concentration 48 h after stent implantation (Table 1S), it was found that only three fatty acids gained statistically significant values. Pentadecanoic acid x2 has a negative estimate of the β slope and heptadecanoic x5 acid with eicosatrienoic x15 has a positive estimate of the parameter β. The results of the two mathematical methods used are shown in Table 2S (LS) and Table 3S (Welsch). This model was to be cleaned in the next step from statistically insignificant variables. In the development of a regression model involving only pentadecanoic acid x2, heptadecanoic x5 acid and eicosatrienoic x15 acid (Table 4S), it was found that no more fatty acid had a statistically significant effect on the increase in SAA concentration after coronary stent implantation. Since this regression model is a refinement of the overlying model, it can be concluded that the concentration of the fatty acids we evaluated

Table 3
Regression model of ΔCRP48 dependence on the concentration of 20 fatty acids in erythrocyte cell membranes, $n = 39$; $R = 0.9237$; $MEP = 1.2073$; $AIC = -20.2956$; $RSC = 7.8951$; $s(e) = 0.66228$; $\alpha = 0.05$; LS; $y_{\Delta CRP48} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots\ldots + \beta_{20} x_{20}$. (LS) Statistically significant fatty acids are written in red-bold. (For interpretation of the references to colour in this table, the reader is referred to the web version of this article.)

Fatty acid	ID	Molecular formula	Estimate *b*	*s*(*b*)	Significant	*P*	L_L	L_U
Intercept	**β_0**	**---**	**3.7188**	**0.7772**	**Yes**	**0.0001**	**2.3712**	**5.0665**
Myristic	**x1**	**C14:0**	**323.7004**	**69.1930**	**Yes**	**0.0002**	**203.7154**	**443.6855**
Pentadecanoic	**x2**	**C15:0**	**-617.7678**	**155.7994**	**Yes**	**0.0009**	**-887.9338**	**-347.6019**
Palmitic	x3	C16:0	1.0470	2.3003	No	0.6545	-2.9419	5.0359
Palmitoleic	**x4**	***cis*-C16:1 N7**	**-100.9050**	**37.7506**	**Yes**	**0.0155**	**-166.3669**	**-35.4431**
Heptadecanoic	**x5**	**C17:0**	**207.7070**	**62.3266**	**Yes**	**0.0037**	**99.6287**	**315.7853**
Stearic	x6	C18:0	-4.5912	3.2886	No	0.1797	-10.2939	1.1114
Trans	**x7**	***trans*-C18:1 N9**	**-496.8200**	**79.9585**	**Yes**	**0.0000**	**-635.4730**	**-358.1669**
Oleic	**x8**	***cis*-C18:1 N9**	**9.6656**	**2.8309**	**Yes**	**0.0031**	**4.7567**	**14.5746**
Linoleic	x9	*all cis*-18:2 N6	2.6616	2.1099	No	0.2232	-0.9971	6.3203
γ-linolenic	x10	*all cis*-18:3 N6	-71.7873	116.4053	No	0.5452	-273.6415	130.0669
Arachidic	x11	C20:0	-27.9238	69.7474	No	0.6936	-148.8702	93.0226
α-linolenic	x12	*all cis*-C18:3 N3	87.3253	97.5874	No	0.3827	-81.8975	256.5480
Cis-9-eicosenoic	x13	*cis*-20:1 N9	-4.1035	61.3835	No	0.9474	-110.5464	102.3395
Eicosadienoic	x14	*all cis*-C20:2 N6	118.0691	86.9372	No	0.1912	-32.6855	268.8238
Eicosatrienoic	x15	*all cis*-C20:3 N6	-10.7363	9.9807	No	0.2963	-28.0434	6.5708
Behenic	x16	C22:0	-31.0080	24.5266	No	0.2223	-73.5386	11.5227
Arachidonic	x17	*all cis*-C20:4 N6	1.7513	0.8950	No	0.0661	0.1992	3.3034
Eicosapentaenoic	x18	*all cis*-C20:5 N3	-12.8135	10.8515	No	0.2531	-31.6308	6.0038
Nervonic	**x19**	***cis*-C24:1 N9**	**-37.0282**	**9.0414**	**Yes**	**0.0007**	**-52.7066**	**-21.3498**
Docosahexaenoic	x20	*all cis*-C22:6 N3	-0.8824	2.6383	No	0.7419	-5.4574	3.6926

Table 4
In the regression triplet test of the proposed regression model ΔCRP48 on the concentration of 20 fatty acids in erythrocyte cell membranes the efficiency of two minimization methods (LS and Welsch) used is tested, $n = 39$; $R = 0.9237$; $MEP = 1.2073$; $AIC = -20.2956$; $RSC = 7.8951$; $s(e) = 0.66228$; $\alpha = 0.05$; (LS); $y_{\Delta CRP48} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots\ldots + \beta_{20} x_{20}$.

Fisher-Snedecor test of significant regression model		Wald test of autocorrelation	
Experimental criterion *F*:	5.2348	Experimental quantile *WA*:	0.8306
Critical quantile $F_{(1\text{-alfa. m-1. n-m})}$:	1.8777	Critical quantile $Chi^2_{(1\text{-alfa.1})}$:	2.7055
Probability *p*:	0.0005	Probability *p*:	0.8228
Conclusion:	Proven significant model.	Conclusion:	Proven no autocorrelation.
Scott test of multicollinearity			
Experimental criterion *SC*:	−0.1880		
Conclusion:	Proven correct model.		
Cook-Weisberg test of heteroscedasticity in residuals			
Experimental criterion *CW*:	0.0501		
Critical quantile $Chi^2_{(1\text{-alfa.1})}$:	2.7055		
Probability *p*:	0.8228		
Conclusion:	Proven homoscedasticity.		
Jarque-Bera test of normality of residuals			
Experimental criterion JB :	1.7710		
Critical quantile $Chi^2_{(1\text{-alfa.2})}$:	4.6052		
Probability *p*:	0.4125		
Conclusion:	Proven normal distribution.		

in the erythrocyte cell membranes has no effect on the increase in SAA concentration.

In the regression model, which tested the effect of the concentration change of twenty selected fatty acids on increasing IL-6 concentration within 24 h after stent implantation (Table 5S), it was found that 7 fatty acids had a statistically significant effect. In particular, a positive estimate of the β slope of myristic acid x1, heptadecanoic x5, cis-9-eicosenoic x13 and docosahexaenoic acid x20 was calculated, but the influence of docosahexaenoic acid is almost negligible compared to the others. For pentadecanoic acid x2 and α-linolenic x12 acid, a negative estimate of the β slope was calculated. Stearic acid x6 was also calculated as a negative estimate of the β slope, but it is significantly lower compared to the previous ones. The results of two regression methods are shown in Table 6S (LS) and Table 7S (Welsch). In addition, a regression model was constructed (Table 8S), in which the fatty acids were selected independently from the statistical significance in the previous regression model. In this model, it has been shown that the concentrations of 7 selected fatty acids have an effect on the increase in IL-6 concentration. In this model, however, it was found that the intercept term in regression equation is statistically insignificant. Therefore, the regression model was repeated without an intercept term, and the result is shown in Table 9S. It was revealed that there was a strongly positive effect on the increase in IL-6 concentration 24 h after stent implantation, and myristic x1 acid and cis-9-eicosenoic x13 acid, and to a lesser extent docosahexaenoic x20 acid; a significant inverse dependence was found between the increase in IL-6 and pentadecanoic x2 acid and α-linolenic x12 acid and to a much lesser extent in stearic x6 acid. The results of the regression method (LS) used in this model are shown in Table 9S and 10S.

Table 5
In the regression triplet test of the proposed regression model ΔCRP48 on the concentration of 20 fatty acids in erythrocyte cell membranes the efficiency of two minimization methods used is tested, n = 39; R = 0.9812; MEP = 21.5457; AIC = -73.7107; RSC = 2.0070; $s(e)$ = 0.3339; α = 0.05; Welsch robust M-estimates; $y_{\Delta CRP48} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots\ldots + \beta_{20} x_{20}$.

Fisher-Snedecor test of significant regression model		Wald test of autocorrelation	
Experimental criterion F:	23.2334	Experimental quantile WA:	18.8026
Critical quantile $F_{(1\text{-alfa. m-1. n-m})}$:	1.8777	Critical quantile $Chi^2_{(1\text{-alfa.1})}$:	2.7055
Probability p:	0.0000	Probability p:	0.0000
Conclusion:	Proven significant model.	Conclusion:	Proven autocorrelation.
Scott test of multicollinearity			
Experimental criterion SC:	−0.3139		
Conclusion:	Proven correct model.		
Cook-Weisberg test of heteroscedasticity in residuals			
Experimental criterion CW:	7705.4903		
Critical quantile $Chi^2_{(1\text{-alfa.1})}$:	2.7055		
Probability p:	0.0000		
Conclusion:	Proven no homoscedasticity.		
Jarque-Bera test of normality of residuals			
Experimental criterion JB :	438.4081		
Critical quantile $Chi^2_{(1\text{-alfa.2})}$:	4.6052		
Probability p:	0.0000		
Conclusion:	Proven no normal distribution.		

Table 6
Regression model of ΔCRP48 dependence on the concentration of 20 fatty acids in erythrocyte membranes which have been selected according to statistical significance in Table 5a-(ΔCRP48), n = 39; R = 0.7528; MEP = 0.9590; AIC = -4.0554; RSC = 23.3202; $s(e)$ = 0.8673; α = 0.05; (LS); Statistically significant fatty acids are written in red-bold. (For interpretation of the references to colour in this table, the reader is referred to the web version of this article.)

Fatty acid	ID	Molecular formula	Estimate b	$s(b)$	Significicant	p	L_L	L_U
Intercept	**β_0**	**---**	**3.2562**	**0.7185**	**Yes**	**0.0001**	**1.7909**	**4.7215**
Myristic	**x1**	**C14:0**	**178.0368**	**68.4381**	**Yes**	**0.0141**	**38.4564**	**317.6172**
Pentadecanoic	x2	C15:0	-243.3731	141.3501	No	0.0951	-531.6585	44.9124
Palmitoleic	**x4**	***cis*-C16:1 N7**	**-71.8101**	**32.7456**	**Yes**	**0.0359**	**-138.5952**	**-5.0251**
Heptadecanoic	x5	C17:0	69.6567	46.7993	No	0.1467	-25.7910	165.1045
Trans octadecenoic	**x7**	***trans*-C18:1 N9**	**-366.1981**	**76.1462**	**Yes**	**0.0000**	**-521.4994**	**-210.8968**
Oleic	**x8**	***cis*-C18:1 N9**	**8.9871**	**2.5183**	**Yes**	**0.0012**	**3.8509**	**14.1233**
Nervonic	**x19**	***cis*-C24:1 N9**	**-37.2857**	**7.2864**	**Yes**	**0.0000**	**-52.1464**	**-22.4251**

Table 7
Regression model of ΔCRP48 dependence on the concentration of 20 fatty acids in erythrocyte membranes which have been selected according to statistical significance in Table 5d-(ΔCRP48), n = 39; R = 0.723; MEP = 0.9021; AIC = -4.2871; RSC = 25,686; $s(e)$ = 0.8822; α = 0.05; (LS). Statistically significant fatty acids are written in red-bold. (For interpretation of the references to colour in this table, the reader is referred to the web version of this article.)

Fatty acid	ID	Molecular formula	Estimate b	$s(b)$	Significicant	p	L_L	L_U
Intercept	**β_0**	**---**	**2.9155**	**0.6798**	**Yes**	**0.0001**	**1.5325**	**4.2985**
Myristic	**x1**	**C14:0**	**141.8874**	**56.9153**	**Yes**	**0.0179**	**26.0923**	**257.6825**
Palmitoleic	**x4**	***cis*-C16:1 N7**	**-78.4072**	**30.1533**	**Yes**	**0.0138**	**-139.7546**	**-17.0598**
Trans octadecenoic	**x7**	***trans*-C18:1 N9**	**-347.1262**	**75.7928**	**Yes**	**0.0001**	**-501.3279**	**-192.9245**
Oleic	**x8**	***cis*-C18:1 N9**	**8.7618**	**2.3542**	**Yes**	**0.0007**	**3.9720**	**13.5515**
Nervonic	**x19**	***cis*-C24:1 N9**	**-32.4214**	**6.7138**	**Yes**	**0.0000**	**-46.0807**	**-18.7620**

Conclusion: Numerical form of the constructed regression model $y_{\Delta CRP48} = \beta_0(s) + \beta_1(s)\,x_1 + \beta_4(s)\,x_4 + \beta_7(s)\,x_7 + \beta_8(s)\,x_8 + \beta_{19}(s)x_{19})$.
$\boldsymbol{y_{\Delta CRP48}}$ = 2.92 (0.68) + 141.89 (56.92) x_1 + -78.41 (30.15) x_4 + -347.13 (75.79) x_7 + 8.76 (2.35) x_8 + -32.42 (6.71) x_{19}.

Table 11S shows the last regression model of the tested dependent variable which was the change in the MDA the concentration 48 h after stent implantation. Already in the exploratory analysis, oxidative stress has been shown to be largely different and independent of the inflammatory process. In the correlation matrix, it was revealed that only a γ-linolenic x10 acid correlates with a change in the concentration of MDA. In the regression model of all 20 selected fatty acids (Table 11S) it was revealed that the statistically significant effect on the increase of MDA (ΔMDA48) showed only γ-linolenic x10 and eicosadienoic x14 acids. For both of these acids, a very positive estimate of the slope estimate b was found. The results of the classical least squares method (LS) are presented in Table 12S, and the M-Estimates (Welsh) in Table 13S. Furthermore, again, the elimination of statistically insignificant fatty acids and the repetition of the regression model building were again undertaken. The resulting built-up regression model is shown in Table 14S. It has been shown that the effect of eicosadienoic acid on the increase of malondialdehyde concentration is weak, as the effect of eicosadienoic x14 acid has not been statistically significant in this model. In the last step, the regression model of ΔMDA48 dependence on γ-linolenic x10 acid was calculated (Table 14S). The results of the regression methods used are shown in Table 15S and 16S.

Subsequently, the fatty acid relationship selected on the basis of the box-and-whisker plot of Fig. 1a and the principal component analysis of Fig. 3 was evaluated. A regression model involving only fatty acids whose blood concentrations are most scattered, as well as the fatty acid-only model based on the character of the guides in the chart of principal component weights proved to be inappropriate because the fatty acid with proven statistics had a significant

Table 8
In the regression triplet of the proposed regression model ΔCRP48 on the concentration of 20 fatty acids in erythrocyte cell membranes according to Table 5e-(ΔCRP48) has been tested, $n = 39$; $R = 0.723$; $MEP = 0.9021$; $AIC = -4.2871$; $RSC = 25686$; $s(e) = 0.8822$; $\alpha = 0.05$; (LS); $y_{\Delta CRP48} = \beta_0 + \beta_1 x_1 + \beta_4 x_4 + \beta_7 x_7 + \beta_8 x_8 + \beta_{19} x_{19.}$

Fisher-Snedecor test of significant regression model		Wald test of autocorrelation	
Experimental criterion *F*:	7.2281	Experimental quantile *WA*:	0.2697
Critical quantile $F_{(1\text{-alfa. m-1. n-m})}$:	2.5026	Critical quantile $Chi^2_{(1\text{-alfa.1})}$:	3.8415
Probability *p*:	0.0001	Probability *p*:	0.3949
Conclusion:	Proven significant model	Conclusion:	Proven no autocorrelation.
Scott test of multicollinearity			
Experimental criterion *SC*:	−0.3473		
Conclusion:	Proven correct model.		
Cook-Weisberg test of heteroscedasticity in residuals			
Experimental criterion *CW*:	0.7238		
Critical quantile $Chi^2_{(1\text{-alfa.1})}$:	3.8415		
Probability *p*:	0.3949		
Conclusion:	Proven homoscedasticity.		
Jarque-Bera test of normality of residuals			
Experimental criterion JB :	0.8589		
Critical quantile $Chi^2_{(1\text{-alfa.2})}$:	5.9915		
Probability *p*:	0.6509		
Conclusion:	Proven normal distribution.		

impact on the specified dependent variables and there has been a significant deterioration in regression criteria such as MEP or AIC.

Given that each patient is unique and whose blood components can be affected by a number of concealed factors or associated illnesses, it is not possible to expect that all patients will uniquely respond to the regression model found. Of the proposed models, where ΔCRP48, ΔSAA48 and ΔIL6-24 were treated as dependent variables, patients P3, P11 and P14 with different levels were similarly excluded. According to medical records, it was found that the patient P3 previously had a myocardial infarction treated with coronary stent implantation with a history of pulmonary embolism, type 2 diabetes mellitus and hypertension. Patient P11 suffered from gout, treated with allopurinol. The P14 patient showed abnormal increases in the concentration of inflammatory markers. In this patient, stent implantation was complicated by ventricular fibrillation, and a transiently elevated troponin I concentration was found to indicate myocardial damage. In addition, the day before intervention, the patient was subfebrile, although the source of inflammation was not found. Additionally, patients P7, P12, P22, P24 and P42 were eliminated in the regression model ΔCRP48 and ΔSAA48. Patient P7 suffered from statin-treated dyslipidemia, hypertension, gout, and aortic coronary bypass in the past. Patient P12 suffered from respiratory insufficiency for chronic obstructive pulmonary disease and asthma Patient P24 suffered from congestive heart failure 2nd degree congestive heart failure with dysfunction of both ventricles, a thrombus found in the left ventricle tip, dilation of all parts of the heart was performed. This patient also suffered from pulmonary hypertension and he was a smoker. In Patient 22, higher SAA concentrations were found without an obvious cause. However, this patient was type 2 diabetics suffering from dyslipidemia, hyperuricemia and nephrolithiasis. In the regression model ΔSAA48 and ΔIL6-24, patients P4 and P46 were also removed. These patients were consistently treated for type 2 diabetes mellitus, hypertension and dyslipidemia.

In the regression model where the CRP concentration change was chosen as the dependent variable in 48 h, patients P5, P6, P16, P26, P28, P32 and P44 were further excluded. In patient P5, CRP was almost nine times elevated without apparent cause, although, this patient was obese and suffered from hypertension, dyslipidemia and gout. Patient P6 was hypertonic, suffering from dyslipidemia and ischemic disease of the lower limbs. The probable cause of abnormal increase in CRP is that the patient suffered from stenosis of all coronary arteries and 2 stens were simultaneously implanted to him. Patient P26 suffered from significant dysfunction of left ventricle (20–25% ejection fraction), hypertension and obstructive pulmonary disease. Patient P8 was hypertonic and dyslipidemic, the probable cause of CRP variation is the simultaneous implantation of several stents. Patient P44 was treated for dyslipidemia and suffered from chronic obstructive pulmonary disease. In patient P32, the cause of difference and subsequent exclusion is unclear.

In the regression model where a change in SAA concentration 48 h after stent implantation was chosen as the dependent variable, patients P25, P30, P34 and P43 were further removed. All of these patients were equally hypertonic and dyslipidemic. In addition, patient P25 was previously diagnosed with papillary cystadenolymphoma. Patient P30 suffered from liver steatosis, and patient P43 underwent thyroidectomy for thyreotoxicosis. In addition, patients P9, P21, and P2 were excluded from the regression model in which IL-6 was changed at 24 h as a dependent variable. Patient P2 suffered from psoriasis in addition to dyslipidemia, hypertension and diabetes mellitus. Patient P9 was also a diabetic with dyslipidaemia, and was also treated for dysplasia and ischemic disease of the lower limbs. A different patient P21 was of unclear origin. Neither type nor number of stents used had significant effect on the relative increase of inflammatory and/or oxidative stress markers. Given the patients disablement in particular, the proposed regression models are largely inconsistent, and it is clear that the metabolism of these inflammation indicators differs to a great extent.

4. Discussion

The aim of our study was to find out whether there is a relationship between the concentration of individual fatty acids in erythrocyte cell membranes and the increase of the levels of inflammatory markers (CRP, SAA, IL-6 and ceruloplasmin) and the extent of oxidative stress (MDA) after the coronary stent implantation.

Concentrations of 20 fatty acids were determined in the erythrocyte membranes of 54 patients before percutaneous coronary intervention. We chose evaluation of fatty acids in erythrocyte membranes, because the presence of fatty acids in plasma or plasma phospholipids is relatively influenced by dietary fat, while the fatty acid profile of erythrocyte membranes is relatively stable and is considered as indicator of long-term fatty acid intake [17,18]. In addition, erythrocyte cell membranes have been shown to reflect relatively well the composition of fatty acids in cell membranes of a number tissues, such as cardiomyocytes [18].

In monitoring the relationship of fatty acids concentration and dynamics of the inflammatory response, it has been demonstrated that there are a number of statistically significant relationships between fatty acids concentration and inflammatory markers. All evaluated inflammatory markers (CRP, SAA, Cp, IL-6) are consid-

ered as risk factors for the development of coronary artery disease [19,20]. In the correlation matrix, ΔCRP48, ΔSAA48 and ΔIL6-24 have been shown a strong positive correlation with each other. When evaluating the correlation matrix, no statistically significant relationship was found between the change in ceruloplasmin concentration and none of the fatty acid concentration estimated and/or with other inflammatory markers. This finding supports the idea that the role of ceruloplasmin in the development of cardiovascular disease and inflammation is not entirely clear. Cp can act both as an antioxidant, and also as a pro-oxidant, as it is reported that ceruloplasmin bound to copper is released during myocardial infarction and free copper has the ability to cause oxidative damage to cells. Based on our results, ceruloplasmin is not suitable for assessment relationships between inflammation and erythrocyte membrane fatty acids.

In evaluating the effect of fatty acids concentration on the dynamics of oxidative stress, measured as a relative change of MDA in 48 h, a statistically significant correlation was found only with γ-linolenic acid and none of the aforementioned inflammatory markers. These results suggest that although the formation of reactive oxygen species is associated with the development of CHD [21], the inflammatory process and oxidative stress are to a large extent two distinct independent processes.

When evaluating the variation of fatty acids concentrations in erythrocyte cell membranes by the box-and-whisker plot, it was found that the greatest variation in concentrations exhibited palmitic, stearic, oleic, linoleic, arachidonic and docosahexaenoic acid. At the same time, palmitic, stearic, arachidonic, linoleic and γ-linolenic acids are the major fatty acids in red blood cell membranes, with fatty acids occurring in a variety of commonly available foods [22].

The proposed relationship between fatty acids in erythrocyte cell membranes and markers selected on the basis of exploratory data analysis was subsequently examined by multiple linear regression. Fatty acids appeared here as independent variables, and the markers ΔCRP48, ΔSAA48, ΔIL6-24 and ΔMDA48 as dependent variables. In the data critique, outliers were detected in the analysis of the points of influence, here patients who had to be removed from further regression analysis. The generated regression model, in which *y* was ΔCRP48 had 39 patients; the regression model in which *y* was ΔSAA48 included 40 patients; and the regression model in which *y* was ΔIL6-24 consisted of 46 patients. The regression model in which y was ΔMDA48 did not require the removal of any patient.

A group of Icelandic scientists dealt with similar issues [18]. They monitored the dynamics of the inflammatory response indicated by the change in plasma concentration of TNF-α, TNF-β, IL-1β, IL-6, IL-8, IL-10, IL-12, IL-18, IFN- γ, macrophage inflammatory protein α and TGF-β depending on the polyunsaturated fatty acids profile in serum phospholipids, or membranes of erythrocytes in patients undergoing heart surgery. In this study, statistically significant relationships between n-3 and n-6 PUFA concentrations and the change of concentration of TNF-β, IL-1β, INF-γ or TGF-β 72 h post-operative were found. According to this study, the role of eicosapentaenoic acid and arachidonic acid in the inflammatory process is very complex, as their concentrations correlate with pro-inflammatory and anti-inflammatory mediators.

First, a proposed regression model was calculated for each inflammatory marker comprising 20 selected fatty acids. These models were subjected according to regression triplet [13–16] to model criticism and mathematical methods criticism. On the basis of the test criteria, it was decided that in all cases it is preferable to use the least square method (LS). In the next steps, the computation of the regression models was repeated, and only with the fatty acids selected on the basis of statistical significance in the previous models. The conclusion of multiple linear regression was found ($p < 0.05$):

1 Five fatty acids from the original twenty used affect the change in CRP concentration within 48 h after coronary stent implantation. Very strong positive association with increased CRP was detected in myristic acid and approximately 15 times lower positive association of oleic acid. Inverse correlations (this means negative estimates of the β slope) with increased CRP have been demonstrated for trans octadecenoic, palmitoleic and nervonic acid, with trans octadecenoic acid having approximately 5 times more potent inverse association with a dependent variable compared to palmitoleic acid and, in the case of nervonic acid, even more than 10 times higher estimate the b slope. Found regression equations (generally)

$$y = (\beta 0) + \beta 1(s)x1 + \ldots + \beta 20(s)x20) :$$

$$y_{\Delta CRP48} = 2,92(0,68) + 141,89(56,92)x_1 + \text{-}78,41(30,15)x_4 + \text{-}347,13(75,79)x_7 + 8,76(2,35)x_8 + \text{-}32,42(6,71)x_{19}$$

2 In the case of SA, it was found that the fatty acids evaluated did not have a statistically significant effect on its increase.

3 The change in the IL6 concentration within 24 h after stent implantation is affected by 6 fatty acids. A positive effect on the increase in IL-6 concentration was demonstrated in myristic x1, cis-9-eicosenoic x13 and in significantly lesser extent in docosahexaenoic acid x20. The highest estimate of the straight line slope was found for cis-9-eicosenoic acid. Significant inverse dependence was found between an increase in IL-6 and pentadecanoic x2 and α-linolenic x12 acids and, to a lesser extent, stearic acid. Found regression equations:

$$y_{\Delta IL6\text{-}24} = 215,01(34,47)x_1 + \text{-}133,86(63,05)x_2 + \text{-}3,60(0,69)x_6 + \text{-}345,88(59,67)x_{12} + 249,40(39,87)x_{13} + 4,01(1,35)x_{20}$$

4 Only γ-linolenic acid has an effect on the increase of MDA concentration at 48 h after stent implantation. It has been proven that this acid is positively associated with an increase in oxidative cell damage. Found regression equations:

$$y_{\Delta MDA48} = 152,60(48,81)x_{10}$$

Myristic acid has a statistically significantly positive association with an increase in the CRP concentration at 48 h and IL-6 at 24 h after stent implantation. These results are consistent with the Canadian study [23], which found that the concentration of myristic acid correlates positively with CRP in young men and women under 29 years of age. It has been previously reported that SFA with even number of carbon atom C22-C18 may activate Toll-like receptors 2 and 4 [24] and can contribute to the regulation of lipid metabolism by the activation of transcription factors SREBP and LXR [22]. These transcription factors are besides other highly expressed in macrophages, and SREBP activation has been shown to be associated with the pro-inflammatory phenotype M1 macrophages, which is known to produce inflammatory cytokines such as interferon γ, IL-1β, IL-6 or TNF-α. Furthermore, it was shown that excess SFA may lead to mitochondrial dysfunction in macrophages, which again promotes their differentiation in the

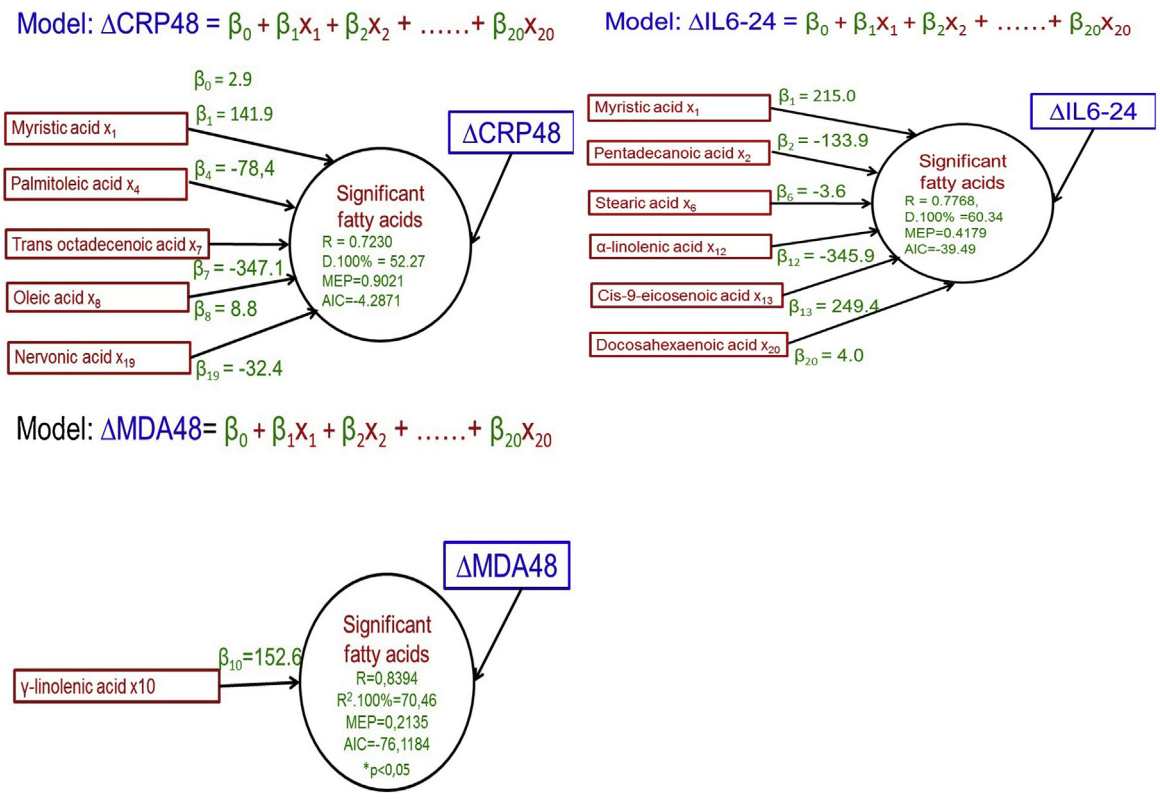

Fig. 6. Found three multiple linear regression models contain the fatty acids selected on the basis of statistical significance in all proposed models tested and respecting a regression triplet.

M1 phenotype, partly due to the functionality of the LXR transcription factor as it regulates the transport of lipids from macrophages. On the other hand, the increased LXR control is associated with down-regulation of NF-κB [25].

The authors of the Canadian study [23] state that the reason for the pro-inflammatory effect of myristic (and palmitic) acid is their ability to up-regulate transcription factor NF-κB through activation Toll-like receptors 2 and 4. An inverse relationship to IL-6 increase in 24 h after intervention, was found in the concentration of pentadecanoic acid. Pentadecanoic acid is a representative of minor odd fatty acids in human diet. It is considered to be an indicator of milk fat intake. It is reported in the literature that pentadecanoic acid is associated with a lower risk of coronary heart disease and type 2 diabetes mellitus [26]. A study of Zheng et al. [27] assessing the relationship of saturated fatty acids to various metabolic indicators has shown that pentadecanoic acid is inversely associated with the concentration of CRP, total cholesterol, TAG, apolipoprotein A1 and B, and hepatic enzymes such as alanine aminotransferase, aspartate aminotransferase. The last saturated fatty acid, which has been shown to have a statistically significant effect on the inflammatory process, is stearic acid. This acid has relatively weak inverted association with an increase in IL-6 concentration, its effect is about 100 times lower than that of α-linolenic acid. Different sources differ in whether stearic acid acts more pro-inflammatory or anti-inflammatory. Stearic acid is reported to be associated with a lower risk of coronary heart disease than myristic or palmitic acids [22]. The results were achieved in the Canadian study [23] or [28], where stearic acid has been shown to correlate inversely with CRP. Also Stryjecki et al. [29] found that plasma stearic acid concentration is inversely associated with plasma CRP concentration and also with stearoyl-CoA desaturase-1 (SCD-1) enzyme activity. The results of their work show that in case reduced activity of SCD-1, which, for example, catalyses the formation of oleic acid from stearic acid, it was suppressed inflammatory status. This could be the reason of our finding that the plasma concentration of oleic acid positively correlated with the concentration of CRP. On the other hand, stearic acid is, as well as palmitic or myristic, capable of up-regulating NF-κB through Toll-like receptors, as well as participating in the already mentioned SREBP and LXR regulation, thereby engaging in inflammation [25].

As concerned MUFA, it has been shown that palmitoleic acid and nervonic acid are inversely associated with increasing the concentration of CRP, while oleic acid is positively associated. Palmitoleic acid has been shown to have a positive effect on human health in [32], where significant decrease of CRP, TAG and LDL levels and an increase of HDL cholesterol have been observed in patients after supplementation with palmitoleic acid for 30 days (220.5 mg/day) [30]. Possible mechanism of this effect could be *via* up-regulation PPAR-α, which is able to inhibit transcription factor NFκB [31]. However, the authors of the above-mentioned publications [28] and [23], contrarily state that the concentration of palmitoleic acid positively correlates with the plasma concentration of CRP. Since the authors [28] found a positive correlation not only between the palmitoleic acid concentration but also between the SCD-1 index (palmitoleic/palmitic acid ratio) and CRP concentration, it was suspected that the relationships found are due to the high intake of SFA diet and SCD-1 activity that could in turn promote inflammation or metabolic disorder that would lead to increased SCD-1 activity.

Positive relationship to the increase in IL-6 concentration has been found in our study for cis-9-eicosenoic acid. The association of cis-9-eicosenoic acid with an inflammatory process was confirmed by a study by German researchers published last year [32], which demonstrated in patients with cardiovascular disease and in healthy ones that the concentration of cis-9-eicosenoic acid inversely correlated with the plasma concentration of LDL and HDL cholesterol and TAG and positively correlated with CRP concen-

tration and endothelial activation. It has been shown that higher concentrations of cis-9-eicosenoic acid pose a higher risk of death from all causes, especially increase the risk of death from cardiovascular causes in women. Cis-9-eicosenoic acid can be ranked as a minority fatty acid and no information is currently available on the mechanism of involvement of cis-9-eicosenoic acid in the inflammatory process and the development of cardiovascular diseases.

Nervonic acid is a significant component of myelin in the form of sphingomyelin, and it is shown that in conditions such as severe depressive disorders its plasma concentration is significantly increased compared to healthy subjects. The authors of the recently published pilot study [33] suggested the concentration of nervonic acid as a biochemical marker of depressive disorder. In the comparison of serum lipids in patients with metabolic syndrome and healthy individuals in the Japanese study [34], it was found that nervonic acid concentrations were demonstrably higher in healthy subjects and positively correlated with HDL-c and inversely with TAG and small dense LDL-c. Finding a positive association of oleic acid concentration with increasing CRP concentration is consistent with the results of the above-mentioned publication [32].

The literature has so far held the predominant view that oleic acid is beneficial to health since several of protective effects have been demonstrated in a number of interventional studies involving olive oil preparations, but the real benefit is uncertain as olive oil contains other ingredients such as squalene, antioxidant hydrophilic phenolic alcohols, polyphenols or vitamin E, which could be responsible for obvious health benefits. The authors of the study revealed that the beneficial effect of oleic acid on human health does not have a linear dependence. The authors state that the dependence of death from all causes on the percentage of oleic acid in the erythrocyte cell membranes is non-linear dependence, but it is U shaped, with 14% being optimal, the values below 13 and above 15% being associated with higher mortality rates. In our group of patients, it was shown that in most cases, the proportion of patients in this group is under 13%. Simultaneously in accordance with our results, the authors demonstrated a positive association of the percentage of oleic acid in erythrocyte membranes with CRP concentration. The significant inverse relationship of trans octadecenoic acid, the only *trans*-MUFA agent assessed, with inflammatory process is quite surprising.

The role of PUFA in the development of inflammatory response is not entirely unambiguous. As mentioned above, n-6 PUFAs are generally referred to as pro-inflammatory and n-3 PUFA as anti-inflammatory [22,35], but this is partly misleading, because some representatives of both PUFAs are capable of forming both pro-inflammatory and anti-inflammatory mediators. Although PUFAs are more of a discussion of fatty acids involved in the inflammatory process, our results concerning PUFAs were considerably limited. A very strong inverse association of α-linolenic acid concentration and a negligible, almost negligible, positive association of docosahexaenoic acid with a change in IL-6 concentration have been demonstrated. The observed important role of α-linolenic acid is in accordance with Zhao et al. [21], who found that increased intake of dietary α-linolenic acid decreases vascular inflammation and endothelial dysfunction due to inhibition of proinflammatory cytokine production, namely IL-6, IL-1β, and TNF-α production in cultured peripheral blood mononuclear cells. In addition, α-linolenic acid is a representative of n-3 PUFA and is a precursor of significant EPA, for which a number of health benefits have been demonstrated. From n-6 PUFA, it has been shown in our study that only γ-linolenic acid has an effect on increasing the concentration of malondialdehyde, which serves as a markerof oxidative stress. It is generally assumed that lipoperoxidation may be subject to fatty acids having a double bond in their structure [21].

In line with some trials [32], we did not observe any relation between an inflammation and arachidonic acid, a representative of n-6 PUFA and/or eicosapentaenoic acid, n-3 PUFAs, although they are widely discussed in the literature for their involvement in the inflammatory process. They have shown no effect on the change in the concentration of the selected inflammatory markers, or marker of the oxidative stress likely due to their relatively low levels.

Despite the careful design of this study, there were several significant limitations. Since this was an observational cross-sectional study, no causality can be drawn. In addition, it should be stressed that the results found are to a large extent limited to elderly patients of Czech nationality, therefore it may not be representative for another population or age category. Results are further limited to patients with ischemic heart disease. Another limitation may be the selection of the markers of inflammatory reaction or oxidative damage since the inflammatory process after stent implantation is very complex and involves a variety of cell types and mediators and that systemic inflammatory reactions may not fully reflect the local inflammatory response at the site of the implanted stent [7].

5. Conclusion

Multiple linear regression has made it possible to quantitatively assess which fatty acids in the erythrocyte cell membranes are involved in increasing or decreasing the plasma concentrations of the selected indicators and to what degree they are involved (Fig. 6). The increase of CRP concentration at 48 h after implantation of the coronary stent is highly correlated with myristic acid and about 15 times less effective with oleic acid. The strongest negative correlation was detected for trans octadecenoic acid, 4 times less for palmitoleic acid and approximately 10 times less for nervonic acid. The increase of IL-6 concentration within 24 h after stent implantation strongly positively correlated with cis-9-eicosenoic and myristic acids, and more than 50 times less with docosahexaenoic acid. A very strong inverse association with an increase of IL-6 concentration in 24 h showed α-linolenic acid, approximately one-third inverse correlation of pentadecanoic acid. The same dependence was found in stearic acid, however, 300 times less than that of α-linolenic acid. As concerned MDA, marker of lipoperoxidation, only γ-linolenic acid was positively associated with oxidative damage 48 h after stent implantation.

Our results may be of practical relevance for improving the clinical outcomes of patients undergoing PCI and stent implantation since the fatty acid profile can be influenced by dietary intake or various supplements.

Authors' contributions

The Working Group (V.M., M.M., A.J. and A.Č.) contributed to the article critically for important intellectual content and approved the final version of the manuscript: V.M. was responsible for study design as chief coordinator, medically interpreted statistical outputs, contributed to draft preparation, review and editing, A.J. was responsible for the chromatographic measurement of fatty acids. M.M. and A.J. performed the multivariate statistical data analysis and drafted the manuscript.

Ethics approval

The study was approved by the Ethical Committee on Human Research of the Regional Hospital of Pardubice, Czech Republic.

Conflict of interest

Authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jpba.2019.01.002.

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