The Effect of Fatty Acids in Red Blood Cell Membranes on the Dynamics of Inflammatory Markers Following Implantation of the Coronary Stent

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A statement that the paper is appropriate

Relevance: This manuscript has not been previously published in any language anywhere and it is not under consideration by another journal.

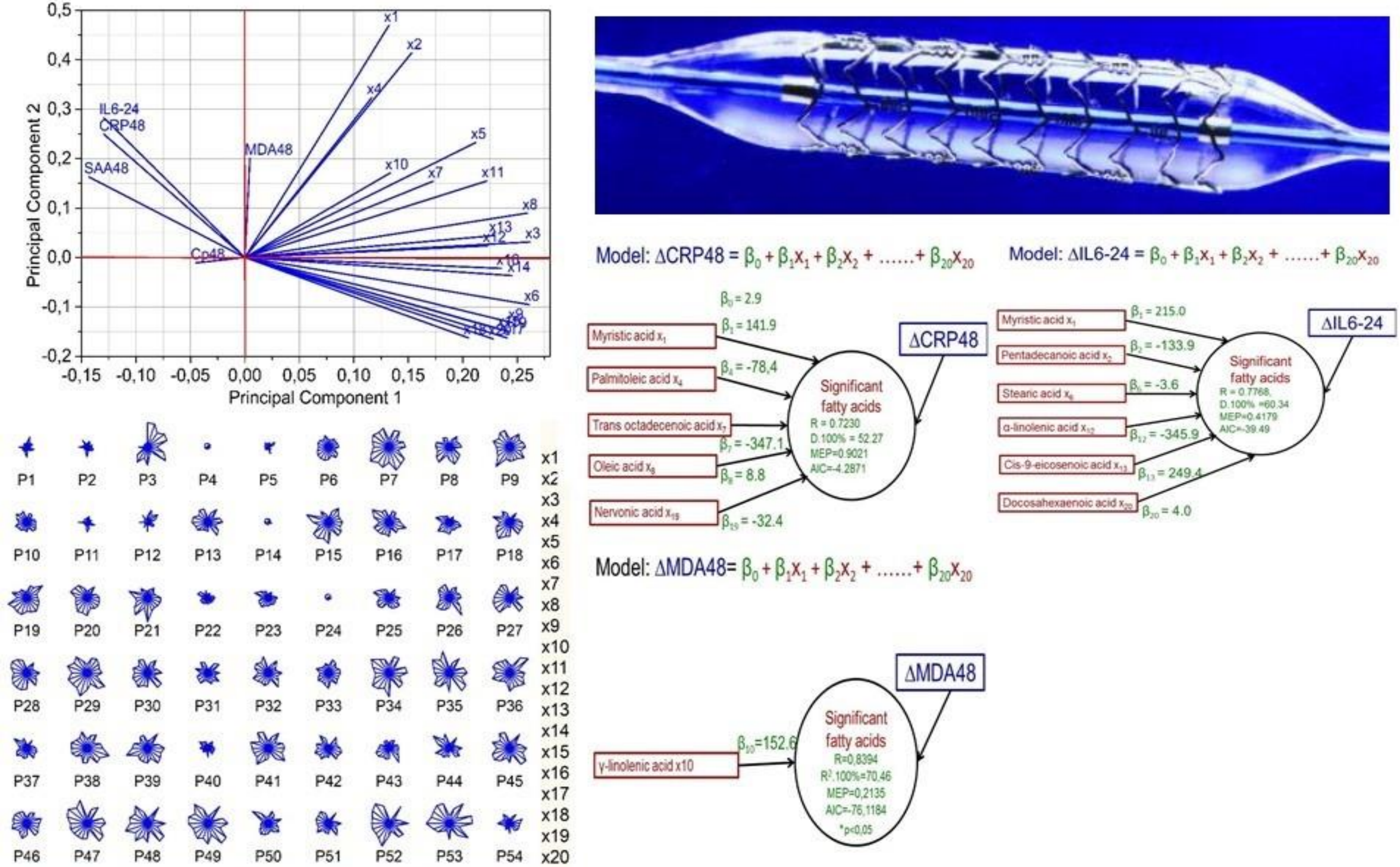
Scientific motivation: The contribution of this work is the finding that fatty acid profile of erythrocyte cell membranes before stenting significantly affects the increase of inflammatory response after percutaneous coronary intervention with drug-eluting stent implantation.

Novelty: The manuscript is aimed to description of the dynamics of inflammatory response and evaluation of the relationship between concentrations of fatty acids in erythrocyte cell membranes and inflammation after percutaneous transluminal coronary angioplasty. 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.

Significance: These findings suggest that the fatty acid analysis represents a promising tool for prediction of patient pre-disposition to inflammatory reaction after coronary angioplasty. We would like to submit this paper for publication in respectful *Journal of Pharmaceutical and Biomedical Analysis.*

GRAPHICAL ABSTRACT

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



SUMMARY

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 hours after stent implantation and by an increase of interleukin-6 (IL-6) 24 hours after intervention. The increase of malondialdehyde (MDA) after 48 hours 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.

METHODS:

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. 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.

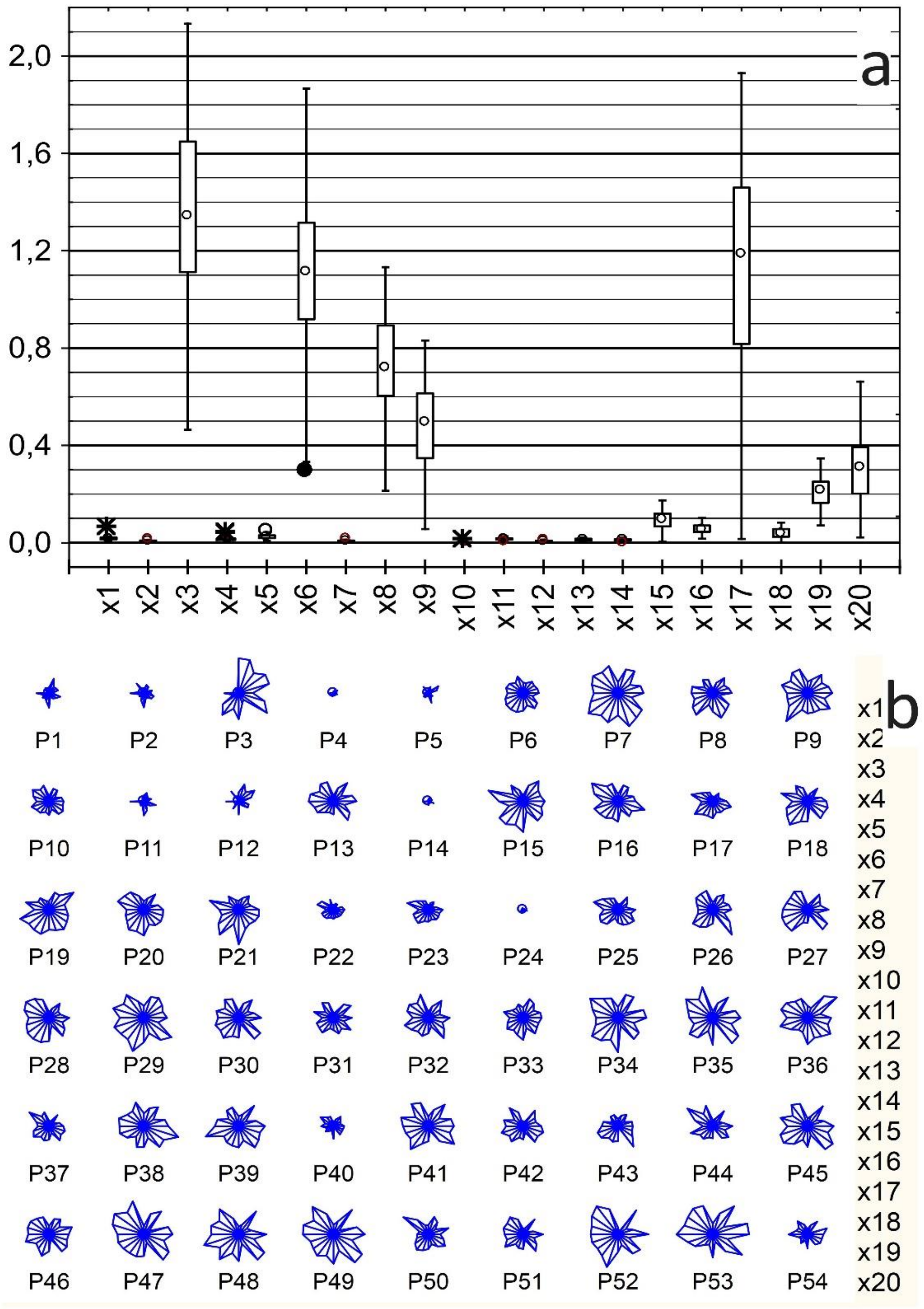


Fig. 1 Exploratory data analysis EDA of the fatty acids profile in red blood cell membranes: (a) The box-and-whisker plot for examination of all fatty acids concentration variability represents the graphical measure of individual x1 to x20 fatty acids, (b) A star glyph showing the composition of 20 fatty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

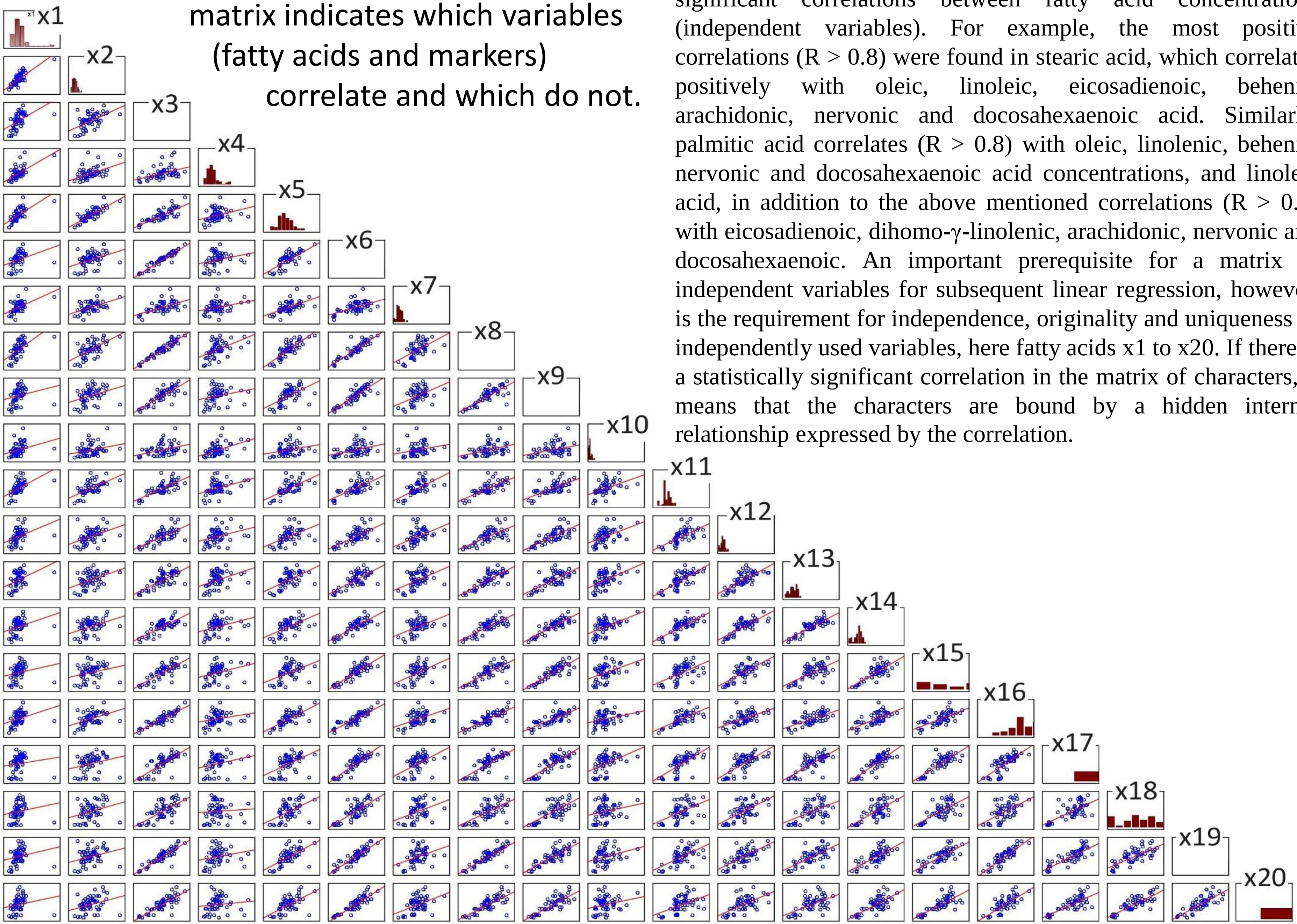
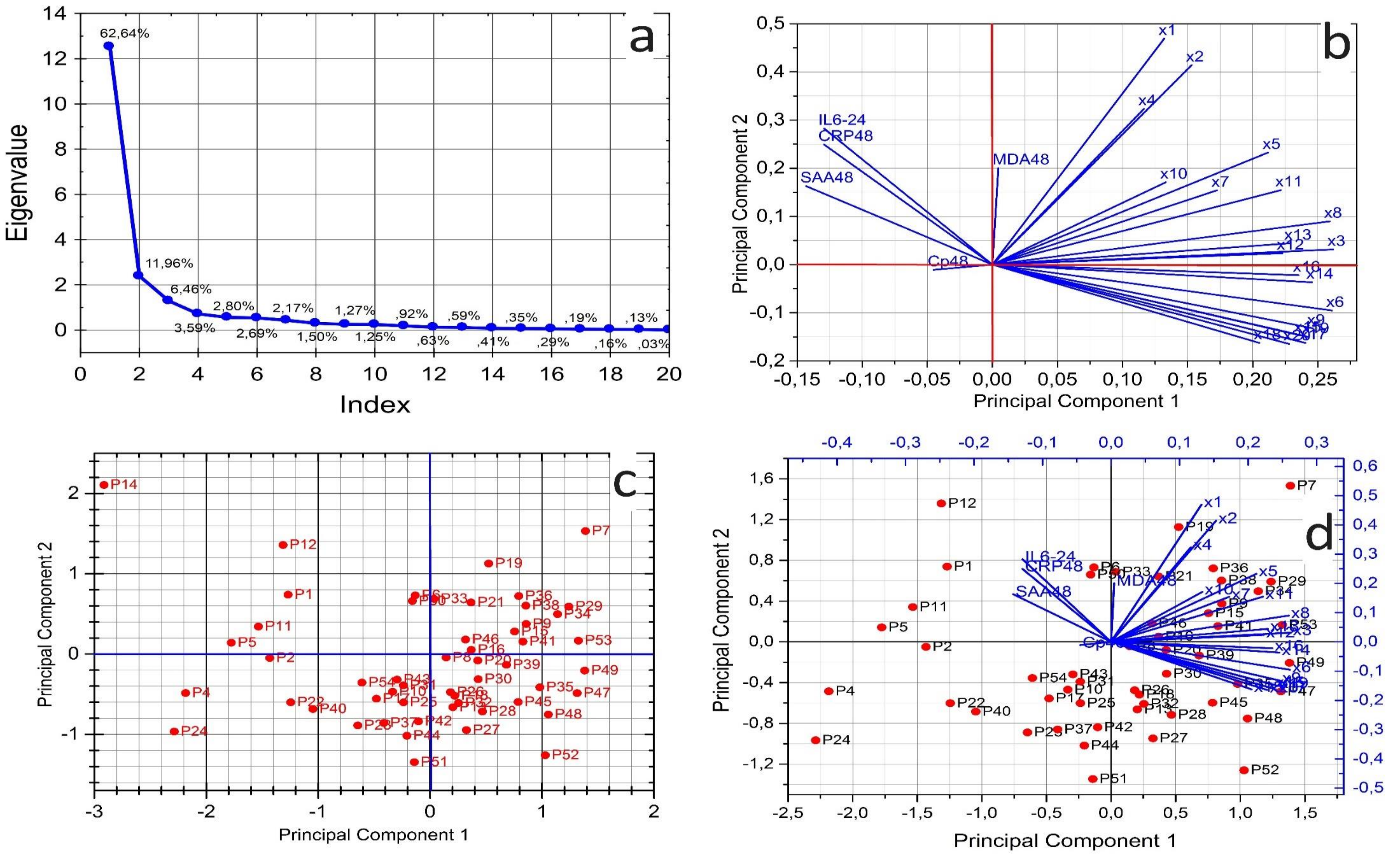
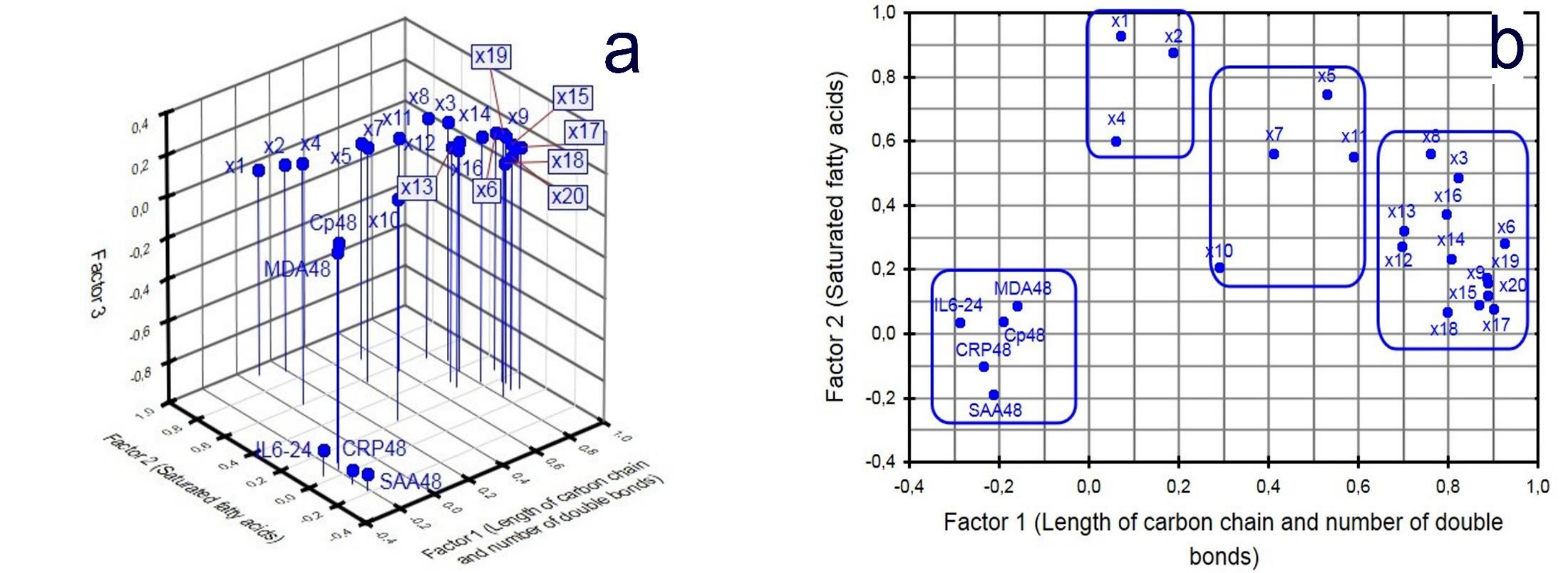


Fig. 2 Scatterplot of correlation matrix indicates which variables (fatty acids and markers) correlate and which do not.

From the correlation matrix, 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.

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. (d) Biplot is the graph b + c together.





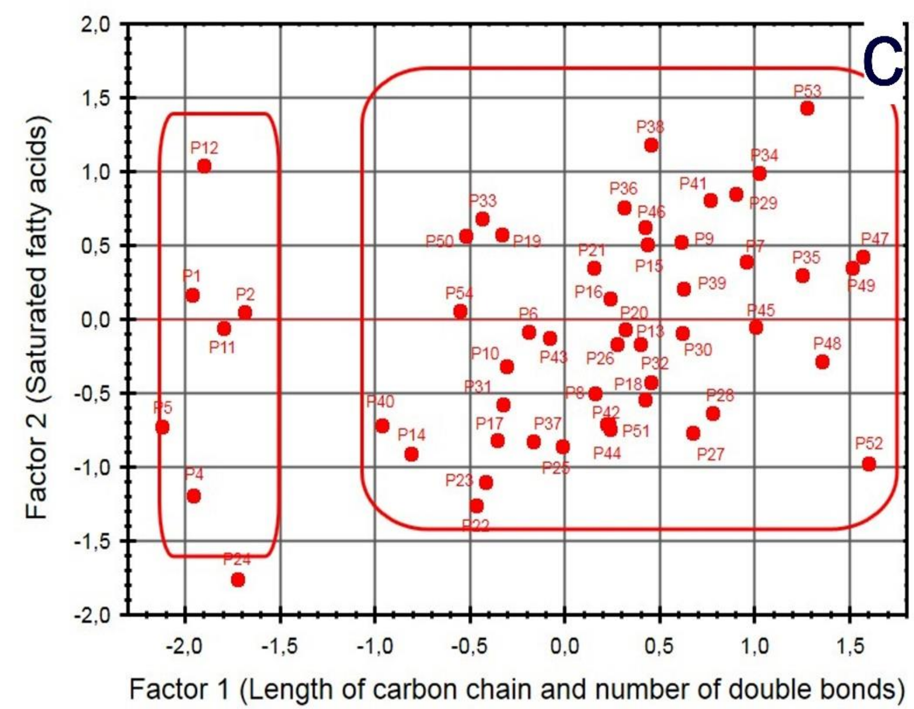
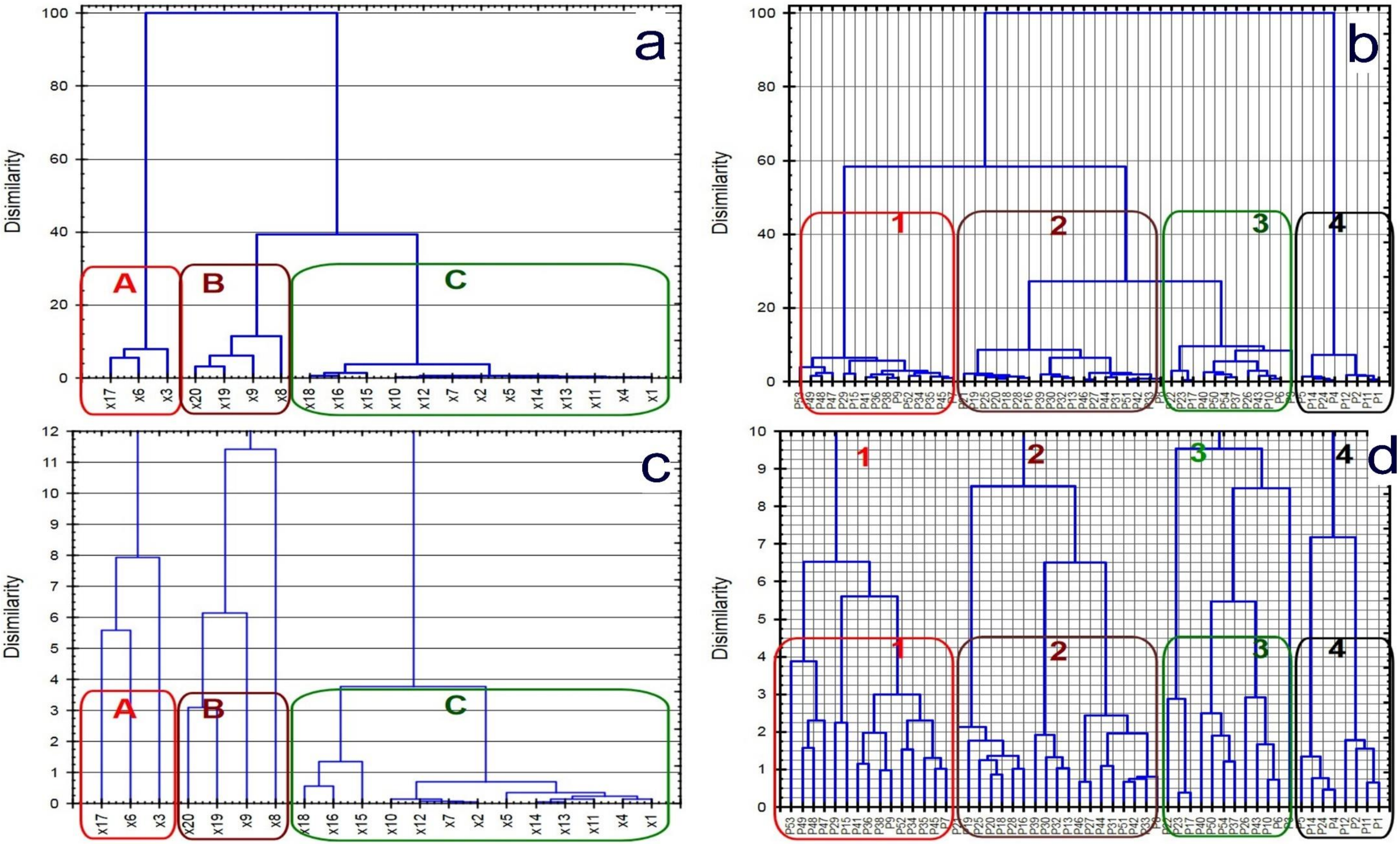
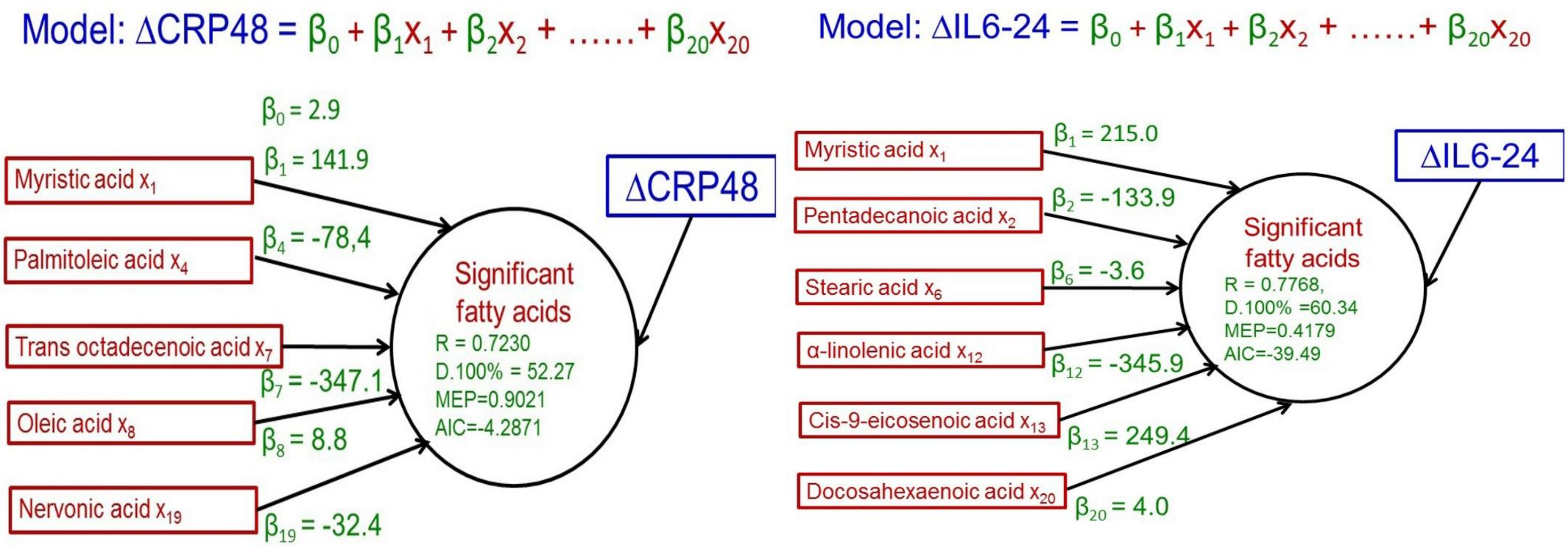


Fig. 4 Factor analysis FA of fatty acids in red blood cell membranes: (a) the 3D plot factor loadings FAW1-2-3 of the first three factors after varimax rotation exhibits classification of variables, (b) the 2D plot factor loadings FAW1-2 of the first two factors after varimax rotation exhibits four clusters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

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.





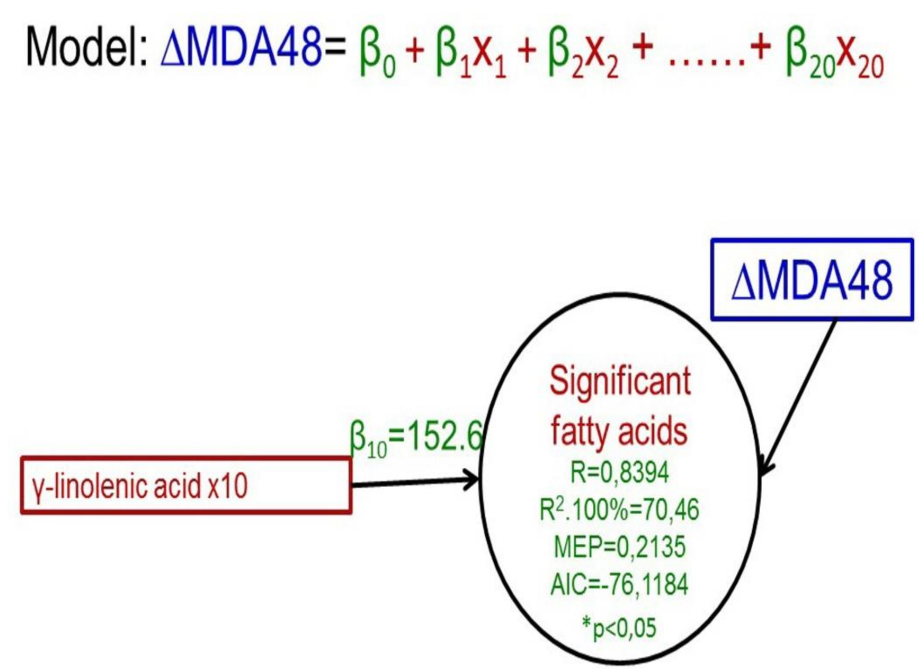


Fig. 6 Found three multiple linear regression models contain the fatty acids selected on the basis of statistical significance in all proposed models tested and respecting a regression triplet.

The increase of CRP concentration at 48 hours 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 hours after stent implementation 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 hours showed α-linolenic acid, approximately one-third inverse correlation of pentadecanoic acid.

TABLES:

Table 1 (a) 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

Table 1 (b) 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 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.

	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
ΔCRP48	-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
ΔSAA48	-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
ΔMDA48	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

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; α = 0.05; LS; $y_{\Delta CRP48} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + ...+ \beta_{20} x_{20}$. (LS) Statistically significant fatty acids are written in red-bold.

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 octadecenoic	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 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.		

In a regression model investigating the effect of twenty given fatty acids on increasing CRP at 48 hours after stent implementation (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.

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. The increase of CRP concentration at 48 hours 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 hours after stent implementation 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 hours 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 hours 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.