

# Composition of phospholipid fatty acids in erythrocyte membranes of children with chronic juvenile arthritis: clinical and biochemical correlations

Anna Górska<sup>1</sup>, Artur Nawrocki<sup>2</sup>, Mirosława Urban<sup>1</sup>, Bożena Florys<sup>1</sup>

<sup>1</sup> 2nd Department of Paediatric Diseases, Medical University in Białystok, Poland

<sup>2</sup> Institute of Physiology, Medical University in Białystok, Poland

**key words:** saturated fatty acids, unsaturated fatty acids, erythrocyte membranes phospholipids, juvenile chronic arthritis

## SUMMARY

*Systemic diseases of connective tissue, including chronic juvenile arthritis are associated with a number of metabolic disorders such as e.g. lipid disturbances. The purpose of the present work was to analyse the composition of fatty acids in erythrocyte phospholipids of children with juvenile chronic arthritis and to determine a correlation between the composition of these acids and patients' clinical status. The study was conducted on 47 children with juvenile chronic arthritis (jca) and 29 healthy subjects. The following fractions of phospholipids were obtained with the help of thin-layer chromatography: phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidyloinositol, cardiolipin, sphingolipin. Fatty acids were analysed with the use of gas chromatograph (Hewlett-Packard 5890). Saturated fatty acids as well as mono- and polyunsaturated (n-3 and n-6) fatty acids were identified. The decrease in the percentage of linolenic acid, PUFA n-6 and PUFA n-3 was found in all the phospholipid fractions in children with jca when compared with control group. There was a concurrent increase in saturated fatty acids, mainly stearic and palmitic acids. The differences in the distribution of fatty acids in erythrocyte phospholipids were observed at early stage of the disease and they became more conspicuous as inflammatory process proceeded.*

### List of abbreviations:

*ra* – rheumatoid arthritis, *jca* – juvenile chronic arthritis, *PUFA* – polyunsaturated fatty acids, *MUFA* – monounsaturated fatty acids, *SFA* – saturated fatty acids, *PG* – prostaglandin, *IL* – interleukin, *LK* – leukotriene, *AA* – arachidic acid, *GLA* – gamma linolenic acid, *DGLA* – dihomogammalinolenic acid, *LA* – linolenic acid, *ALA* – alpha linolenic acid, *EPA* – eicosapentaenoic acid, *DHA* – docosahexaenoic acid

## INTRODUCTION

Changes in the distribution of fatty acids have been documented to take place in many chronic diseases such as rheumatoid arthritis, psoriatic arthritis or colitis ulcerosa [1,2,3]. On the basis of the data from the literature on the subject, it may be claimed presently that first of all, out of C-20 fatty acids, large amounts of eicosanoids (lipid inflam-

matory mediators) are synthesised. The precursor of potent pro-inflammatory human eicosanoids is arachidonic acid (C20:4n-6). The release of this acid from membranous phospholipids is triggered by the stimulation of the receptors found in cell membrane (the inductive factors include: antibodies, growth factors, hormones, peptides or toxins). Then, arachidonic acid undergoes biotransformation stimulated by cyclooxygenases or lipooxyge-

Received: 1999.03.08

Correspondence address: Prof. Mirosława Urban MD PhD, 2nd Department of Paediatric Diseases, Medical University,

Accepted: 1999.10.19

ul. Waszyngtona 17, 15-274 Białystok, Poland

nases and it is metabolised into a series of metabolites forming an arachidonic acid cascade [4]. The metabolites of that cascade are formed rapidly at the sites of an on-going inflammatory process, including synovial membrane of the joints.

Secondly, chronic inflammation may lead to the changes in the metabolism of fatty acids, mainly in the so-called essential unsaturated fatty acids (EUFAs) i.e. linolenic acid (C18:2n-6) and alpha linolenic acid (C18:3n-3). In the circumstances of low EUFA derivative levels - (DGLA/C18:3n-6/, EPA/C20:5n-3) in patients with rheumatoid arthritis, the proliferation of T cells or cytokines may be inhibited, which contributes to the development of chronic inflammatory status [5,6].

The studies conducted so far have dealt primarily with adult patients. As far as children are concerned, the analysis of fatty acids in plasma lipids, erythrocytes or other cells was conducted mainly in patients with developmental disorders or other non-inflammatory diseases [8,9]. The present study was an attempt to answer the following questions: what is the composition of saturated and unsaturated fatty acids in analysed fractions of erythrocyte phospholipids in children with juvenile chronic arthritis?, and is there any correlation between the composition of fatty acids and clinical status of these patients?

## MATERIAL AND METHODS

The study was conducted on 49 children with juvenile chronic arthritis, diagnosed in accordance with the guidelines published by EULAR Paediatric Section in 1982 [10]. Control group was made up of 29 healthy children (12 boys and 17 girls) who reported to our Department for check-up examination having recovered from streptococcal infection. Mean age of healthy children was  $11.9 \pm 2.7$  years, mean age of children with juvenile chronic arthritis was  $11.3 \pm 3.9$  years.

Control group corresponded to the analysed group with respect to socio-economic criteria; the majority of children came from urban areas. It was established on the basis of children's medical history that healthy children were fed in a traditional way, and their food was appropriate for their age in terms of its quality and quantity. Both study groups did not differ statistically significantly as to their height and body mass. Body mass index (BMI) among healthy children was  $18.8 \pm 2.1$  while it was  $17.7 \pm 3.0$  in the group of patients with jca. Children remained

on a light diet, with a lot of fruit and vegetables. Girls prevailed among children with jca (30), while boys constituted 38, 8% analysed group (19 patients). The subjects who have been suffering from the disease for 1–3 years (26) and for more than 3 years (14) represented 81.6% of the whole group, while there were 18.4% those in whom the disease started less than one year earlier (9). The disease involved few joints in more than half of the analysed group (26), while it affected many joints in 23 children (46.9%). The patients with systemic form of the disease were excluded from the study because most of them were treated with high doses of steroids. At the time of the study, none of the patients displayed any clinical symptoms of pancreatitis, diabetes, thyroid dysfunction or liver damage.

Ig M rheumatoid factor (RF) was found in 10 children (20.4%). Disease activity was evaluated on the basis of Mallya and Mace criteria [11], taking into account the duration of morning stiffness (1° up to 10 min, 2° 10–30 min, 3° 31–120 min, 4° over 120 min), Ritchie's articular index (1° without articular pain, 2° 1–7 joints, 3° 8–17 joints, 4° over 18 joints), Hb concentration g/dl (1° above 14; 2° 12–14; 3° 10–12; 4° below 10), ESR mm/h (1° 0–20; 2° 21–45; 3° 46–80; 4° over 81) [11]. These parameters were used to classify patients into four groups: 1° children with slight activity of the inflammatory process – 11 (22.4%), 2° 21 children (42.9%), 3° 10 children (20.4%) with moderate inflammation activity and 4° 7 children (14.3%) with considerable activity of the inflammatory process. Most children were treated with sulfasalazine or methotrexate (38 children – 77.5%, including 25 who received these together with non-steroid anti-inflammatory drugs (NSAID) and 13 – with low doses of prednisone – 5 mg every day or every two days). The remaining 11 children were on periodic or permanent therapy with NSAIDs.

## Determination of fatty acids

Three millilitres of blood were sampled and plasma was removed by rinsing erythrocytes 3 times with double amount of cold 0.9% NaCl. After each rinsing, the sample was centrifuged for 10 min (3000 rpm). Lipids were extracted in accordance with modified Folch method [12]. 2.5 ml cold methanol and 2.5 ml chloroform were added to 0.5 ml erythrocytes, shaking the mixture vigorously in the meantime. Then, 1.25 ml water was added and after centrifugation, chloroform phase was collect-

**Table 1.** Proportion of analysed fractions of erythrocyte phospholipids in children suffering from jca of various activity, and in control group.

Fraction phospholipids	Erythrocytes phospholipids							
	Disease activity				Control group (n=29)		Jca (n=49)	
	1° & 2° (n=32)		3° & 4° (n=17)		Mean	SD	Mean	SD
cardiolipin	47.23	2.30	46.56*	1.67	47.95	1.63	46.90	1.84
phosphatidylinositol	11.69*	1.41	12.25*	1.05	10.92	1.47	11.82*	1.36
sphingomyelin	30.64	1.88	29.47**	1.88	31.35	1.61	30.35*	1.93
phosphatidylethanolamine	8.48** $\pi$	0.82	9.18***	0.82	7.82	0.92	8.65**	0.87
phosphatidylserine	1.18	0.42	1.29	0.39	1.30	0.79	1.21	0.40
phosphatidylcholin	0.65	0.32	0.64	0.34	0.75	0.44	0.65	0.49

\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$  when compared with control group,  $\pi$   $p < 0.05$  when compared with 3 & 4 degree of disease activity

ed. Phospholipids were divided into fractions with the help of thin-layer chromatography (Silica Gel 60) using developing solution composed of: chloroform/methanol/acetic acid/water (100:75:7:4 v/v/v/v). The following fractions were obtained: sphingomyelin (SM), phosphatidylcholine (FCH), phosphatidylserine (FS), phosphatidylinositol (FI), phosphatidylethanolamine (FE), cardiolipin (KA). The plates were sprinkled with 0.2% 2'7'-dichlorofluorescein and they were placed in ammonia vapour. The spots containing particular fractions were identified under UV lamp. The gel with these fractions was placed in test tubes, after which internal standard was added (methylpentadecanoid acid). It was then methylated according to Morrison and Smith method [13]. For the methylation, 14%  $\text{BF}_3$  (14% methanol solution of boron trifluoride) was used (Sigma) at 100°C. Methylated samples were analysed with gas chromatograph (Hewlett-Packard 5890).

The following acids were identified: myristic (C 14:0), palmitic (C 16:0), palmitoleic (C 16:1), stearic (C 18:0), oleic (C 18:1), linolenic (C 18:2n-6), linolenic (C 18:3n-3), arachidonic (C 20:4n-6), eicosapentaenoic (C 20:5n-3). The identification was based upon retention times.

### Statistical analysis

The results of the study were analysed statistically with the use of arithmetical means. The level of investigated parameters in the subgroups was compared with t-Student test or U Mann-Whitney test (depending on the distribution of the parameters). Pearson linear correlation ratio was used in order to evaluate the correlation between the parameters. Alternative hypotheses were considered true when  $p < 0.05$ . Statistica 5.0 manufactured by Stat Soft was the application used for statistical analysis.

The study obtained the approval of the Ethics Supervisory Committee for human and animal studies, at the Medical University in Białystok.

### RESULTS

The proportion of analysed phospholipid fractions of erythrocyte membranes is presented in Table 1. It was found that the highest percentage was observed for FCH and FE in children with jca and in healthy children:  $46.90 \pm 1.84\%$ ;  $47.95 \pm 1.63\%$  and  $30.35 \pm 1.93\%$ ;  $31.35 \pm 1.61\%$ , respectively. Table 2 presents the percentage values of fatty acids in FCH of erythrocytes of children with jca and control subjects. It should be emphasised that higher percentage of palmitic acid C 16:0 ( $p < 0.01$ ) was observed in children with jca when compared with control group –  $36.25 \pm 1.91\%$  and

**Table 2.** Proportion of fatty acids in erythrocyte phosphatidylcholine in children with jca and in control group.

fatty acids	Phosphatidylcholine			
	Jca (n=49)		Control group (n=29)	
	Mean	SD	Mean	SD
C 14 : 0	0.42	0.14	0.40	0.10
C 16 : 0	36.25**	1.91	35.20	1.17
C 16 : 1	1.07**	0.36	0.88	0.11
C 18 : 0	9.56	1.22	9.84	0.64
C 18 : 1	21.03*	1.48	20.25	0.99
C 18 : 2 n - 6	26.51*	2.79	27.92	2.26
C 18 : 3 n - 3	0.39	0.07	0.40	0.08
C 20 : 4 n - 6	4.23	0.80	4.43	0.80
C 20 : 5 n - 3	0.49	0.15	0.66	0.23
SFA	46.26*	1.66	45.45	1.23
MUFA	22.10**	1.69	21.14	1.02
PUFA	31.63***	2.68	33.41	1.53
18:2n-6/20:4n-6 l.b.	6.53	1.66	6.53	1.61

\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$  in relation to control group

**Table 3.** Proportion of fatty acids in erythrocyte phosphatidylethanolamine in children with jca and in control group.

fatty acids	Phosphatidylethanolamine			
	Jca (n=49)		Control group (n=29)	
	Mean	SD	Mean	SD
C 14 : 0	1.49	0.82	1.71	0.94
C 16 : 0	25.65	2.92	25.02	2.30
C 16 : 1	2.94	1.70	2.90	1.70
C 18 : 0	7.11*	0.67	6.42	0.64
C 18 : 1	26.77	3.00	26.29	2.70
C 18 : 2 n - 6	8.87*	1.53	9.71	1.73
C 18 : 3 n - 3	0.54	0.10	0.57	0.16
C 20 : 4 n - 6	24.04	0.18	24.30	0.16
C 20 : 5 n - 3	1.65	0.73	1.75	0.73
SFA	32.76	3.34	33.15	2.05
MUFA	29.51	2.20	29.30	1.66
PUFA	35.10*	3.24	36.33	3.24
18:2n-6/20:4n-6 l.b.	0.38	0.08	0.40	0.08

\* p&lt;0.01; in relation to control group

35.20±1.17% – and there was a significant decrease in linolenic acid C18:2n-6 (p<0.05) in children with jca when compared with control group – 26.51±2.79% and 27.92±2.26%.

Table 3 demonstrates the proportion of fatty acids in FE of erythrocytes of children with jca and control subjects. It was found that significantly higher proportion of stearic acid C18:0 (p<0.05) was typical of patients with jca: 7.11±0.67% in relation to control group: 6.42±0.64%, and there were no significant differences as to the content of other saturated acids. Additionally, this fraction was characterised by a significant decrease in the percentage of linolenic acid (p<0.05) (8.87±1.53%; 9.71±1.73%, respectively) as well as of general content of PUFA (p<0.05) in children with jca (35.10±3.24%; 36.33±3.25%) when compared with control group.

In FS of erythrocytes in children with jca and control subjects, significant differences in the proportion of fatty acids between patients with juvenile chronic arthritis and healthy children were observed in the higher percentage of: stearic acid C18:0 (p<0.01) amounting to: 53.24±3.30% and 51.32±3.11%, respectively, total content of saturated acids (p<0.01): 56.81±2.12% and 54.91±2.97%, and lower total content of PUFA (p<0.05): 30.39±2.38% and 32.09±2.76%, respectively (Table 4).

SM of erythrocyte membranes is characterised by extremely high percentage of saturated acids, which turned out to be significantly higher in chil-

**Table 4.** Proportion of fatty acids in erythrocyte phosphatidylserine in children with jca and in control group.

fatty acids	Phosphatidylserin			
	Jca (n=49)		Control group (n=29)	
	Mean	SD	Mean	SD
C 14 : 0	0.28	0.08	0.25	0.08
C 16 : 0	3.32	0.24	3.22	0.25
C 16 : 1	0.55	0.21	0.58	0.26
C 18 : 0	53.24**	3.30	51.31	3.11
C 18 : 1	11.55	1.59	12.40	2.03
C 18 : 2 n - 6	2.51	0.84	3.31	1.50
C 18 : 3 n - 3	0.07	0.02	0.07	0.03
C 20 : 4 n - 6	27.03	2.52	27.87	3.05
C 20 : 5 n - 3	0.79	0.20	0.85	0.17
SFA	56.81**	2.12	54.91	2.97
MUFA	12.10*	1.73	13.00	2.10
PUFA	30.39*	2.38	32.09	2.76
18:2n-6/20:4n-6 l.b.	0.10	0.03	0.12	0.03

\* p&lt;0.05; \*\* p&lt;0.01; in relation to control group

**Table 5.** Proportion of fatty acids in erythrocyte sphingomyelin in children with jca and in control group.

fatty acids	Sphingomyelin			
	Jca (n=49)		Control group (n=29)	
	Mean	SD	Mean	SD
C 14 : 0	1.42	0.43	1.54	0.34
C 16 : 0	82.40	2.28	82.31	1.63
C 16 : 1	0.74	0.20	0.81	0.21
C 18 : 0	12.66*	1.89	11.45	1.63
C 18 : 1	2.39	0.70	2.19	0.54
C 18 : 2 n - 6	0.65	0.21	0.64	0.15
C 18 : 3 n - 3	0.06	0.03	0.07	0.03
C 20 : 4 n - 6	0.10	0.03	0.11	0.04
C 20 : 5 n - 3	-	-	-	-
SFA	96.48*	2.38	95.29	1.63
MUFA	3.13	0.80	2.99	0.74
PUFA	0.81	0.20	0.82	0.19

\* p&lt;0.05; in relation to control group

dren with jca (p<0, 05) when compared with control subjects, amounting to: 96.48±2.38% and 95.29±1.63%. PUFA content accounted for less than 1%, while the values of EPA acid (C 20:5n-3) could not be measured (Table 5).

It turned out that in both groups, FI of erythrocyte membranes was a phospholipid fraction characterised by the highest percentage of EPA (C 20:5n-3). It was significantly lower (p<0.05) in the group of children with JCA, amounting to 1.47±0.37% when compared with control group – 2.55±0.81% (Table 6).

Increased content of saturated fatty acids was also observed in cardiolipin (constituting less than 1% of all membranous phospholipids):  $54.35 \pm 5.07\%$  and  $49.69 \pm 4.63\%$  ( $p < 0.01$ ) and there was a decrease in PUFA percentage ( $p < 0.05$ ):  $19.43 \pm 3.77\%$  and  $21.67 \pm 3.81\%$  in children with jca when compared with control group (Table 7).

There was a significant decrease in the content of linolenic acid (C18:2n-6) and total PUFA as disease activity increased ( $p < 0.05$ ) in FCH and in FE (Tables 8, 9). Additionally, we also observed posi-

tive correlation between the proportion of linolenic acid as well as total PUFA content, and Hb in FCH, FS, FE and there was a negative correlation with ESR value and the number of platelets (Table 10). In the case of FCH, FE and FI, it is important to emphasise a significant positive correlation between alpha linolenic acid (C18:n-3) and Hb: ( $r = 0.32$ ;  $p < 0.001$ ); ( $r = 0.27$ ;  $p < 0.05$ ); ( $r = 0.28$ ;  $p < 0.05$ ), respectively, and a negative correlation between this acid and ESR value in FE ( $r = -0.30$ ;  $p < 0.05$ ) and the number of platelets in FI ( $r = -0.29$ ;  $p < 0.05$ ).

**Table 6.** Proportion of fatty acids in erythrocyte phosphatidylinositol in children with jca and in control group.

fatty acids	Phosphatidylinositol			
	Jca (n=49)		Control group (n=29)	
	Mean	SD	Mean	SD
C 14 : 0	2.04	0.82	2.32	1.00
C 16 : 0	18.25	3.43	16.81	3.72
C 16 : 1	4.22	2.20	4.47	1.87
C 18 : 0	28.52*	4.34	25.38	4.13
C 18 : 1	15.09	3.60	16.58	4.35
C 18 : 2 n - 6	8.75	2.85	9.47	2.73
C 18 : 3 n - 3	0.38	0.16	0.48	0.20
C 20 : 4 n - 6	21.07	3.37	21.67	3.63
C 20 : 5 n - 3	1.47*	0.37	2.55	0.81
SFA	48.81**	3.67	44.51	3.84
MUFA	19.32	3.47	21.06	3.85
PUFA	31.67*	3.10	34.49	3.60

\*  $p < 0.05$ ; \*\*  $p < 0.01$  in relation to control group

**Table 7.** Proportion of fatty acids in erythrocyte cardiolipin in children with jca and in control group.

fatty acids	Cardiolipin			
	Jca (n=49)		Control group (n=29)	
	Mean	SD	Mean	SD
C 14 : 0	5.53	1.81	5.50	1.72
C 16 : 0	35.32**	4.80	31.36	4.36
C 16 : 1	8.98	3.32	10.78	3.70
C 18 : 0	13.47	1.76	12.83	1.56
C 18 : 1	17.27*	3.34	19.93	3.18
C 18 : 2 n - 6	10.40	2.68	11.61	2.64
C 18 : 3 n - 3	0.67	0.13	0.67	0.16
C 20 : 4 n - 6	8.36	1.64	9.39	1.44
C 20 : 5 n - 3	-	-	-	-
SFA	54.35**	5.07	49.69	4.63
MUFA	26.26***	3.78	30.72	3.19
PUFA	19.43*	3.77	21.67	3.81

\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$  in relation to control group

**Table 8.** Proportion of fatty acids in erythrocyte phosphatidylcholine in children suffering from jca of various activity, and in control group.

fatty acids	Phosphatidylcholine					
	Disease activity				Control group (n=29)	
	1° & 2° (n=32)		3° & 4° (n=17)		Mean	SD
	Mean	SD	Mean	SD		
C 14 : 0	0.42	0.14	0.42	0.13	0.40	0.10
C 16 : 0	36.25*	1.87	37.50***	1.75	35.20	1.16
C 16 : 1	1.05*	0.36	1.17***	0.34	0.88	0.11
C 18 : 0	9.81	1.02	8.78**	1.49	9.84	0.64
C 18 : 1	20.85	1.48	21.56**	1.35	20.26	0.99
C 18 : 2 n - 6	26.16*	2.90	25.20**	2.26	27.92	2.26
C 18 : 3 n - 3	0.39	0.07	0.41	0.05	0.40	0.08
C 20 : 4 n - 6	4.28	0.84	4.13	0.63	4.43	0.80
C 20 : 5 n - 3	0.48	0.29	0.52	0.24	0.66	0.36
SFA	46.48*	1.02	46.69*	1.79	45.45	1.02
MUFA	21.91*	1.67	22.73**	1.63	21.13	1.02
PUFA	31.31*	2.56	30.28***	1.82	33.41	1.53
18:2n-6/20:4n-6 l.b.	6.32	1.66	6.17	1.57	6.57	1.61

\*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$  when compared with control group,  $\square$   $p < 0.05$  when compared with 3 & 4 degree of disease activity

**Table 9.** Proportion of fatty acids in erythrocyte phosphatidylethanolamine in children suffering from jca of various activity, and in control group.

fatty acids	Phosphatidylethanolamine					
	Disease activity				Control group (n=29)	
	1° & 2° (n=32)		3° & 4° (n=17)		Mean	SD
	Mean	SD	Mean	SD	Mean	SD
C 14 : 0	1.56	0.82	1.33	0.83	1.71	0.94
C 16 : 0	25.73	3.00	25.48	2.66	25.02	2.30
C 16 : 1	2.91	1.72	2.90	1.63	2.90	1.70
C 18 : 0	7.99**#	2.57	8.57***	0.46	6.42	0.64
C 18 : 1	26.73	2.57	26.92	3.10	26.29	2.70
C 18 : 2 n - 6	8.63 $\pi$	1.52	7.97*	1.18	9.71	1.73
C 18 : 3 n - 3	0.53	0.18	0.45	0.15	0.57	0.16
C 20 : 4 n - 6	23.27	2.81	23.44	2.21	24.30	0.16
C 20 : 5 n - 3	1.60	0.54	1.66	0.86	1.75	0.73
SFA	35.27**	3.30	35.38**	3.02	33.15	2.05
MUFA	29.69	1.61	29.90	1.84	29.30	1.66
PUFA	34.04** $\pi$	2.13	33.73**	2.11	36.33	2.67
18:2n-6/20:4n-6 l.b.	0.38	0.08	0.36	0.06	0.40	0.08

\* p<0.05; \*\* p<0.01; \*\*\* p<0.001 when compared with control group,  $\pi$  p<0.01; #p>0.01 when compared with 3 & 4 degree of disease activity

**Table 10.** Significant correlation between Hb, SR, platelets and fatty acids in phosphatidylcholine (FCH), phosphatidylserine (FS), phosphatidylethanolamine (FE).

fatty acid fractions	Hb		OB		OB	
	r	p<	r	p<	r	p<
FCH C 16:0	-0.36	0.005	0.37	0.005	0.38	0.005
FCH C 18:2n-6	0.36	0.005	-0.35	0.01	-0.34	0.01
FCH PUFA	0.40	0.002	-0.37	0.005	-0.35	0.01
FS C 18:2n-6	0.28	0.05	-0.12	NS	0.14	NS
FS C 18:3n-3	0.32	0.01	-0.16	NS	-0.02	NS
FE C 18:2n-6	0.28	0.05	-0.28	0.05	-0.21	NS
FE C 18:3n-3	0.28	0.05	-0.30	0.02	-0.25	NS
FE C 18:2n-6/C 20:4n-6	0.28	0.05	-0.30	0.02	-0.25	0.05

NS-statistically insignificant

No significant changes were observed in the content of fatty acids during aggressive treatment (Mtx).

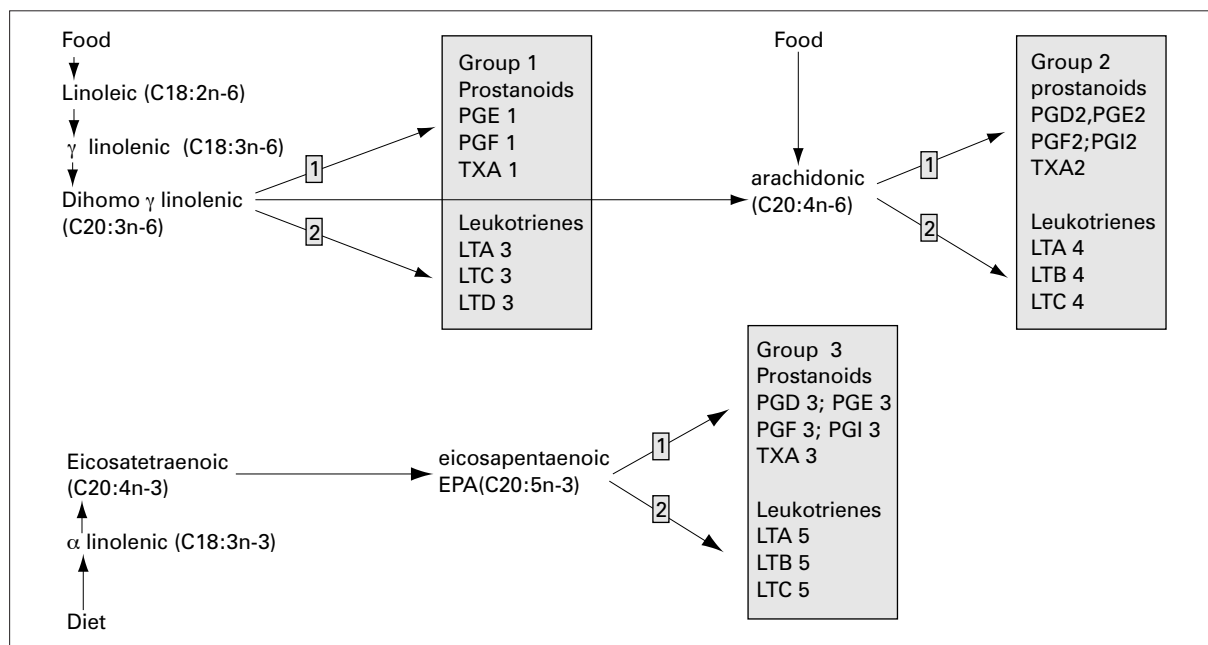
## DISCUSSION

Systemic diseases of connective tissue, including juvenile chronic arthritis are associated with considerable metabolic disorders [14,15]. Special attention has been attracted to the reports describing the effect of chronic inflammatory process on the changes in the constellation of plasma lipids and phospholipids of cell membranes [16,17].

On the other hand, many authors stress the importance of changes in the content of fatty acids of

membranous phospholipids of various cells in the initiation and maintenance of inflammatory process, both through influencing eicosanoid synthesis and the production of pro-inflammatory cytokines [18–20].

In theoretical terms, three polyunsaturated fatty acids may be direct precursors of eicosanoids: arachidonic acid (C 20:4n-6), linolenic acid (C 18:3n-6) and alpha linolenic acid (C 18:3n-3). The presence of these acids in food determines the synthesis of other polyunsaturated fatty acids. For example, gamma linolenic acid (GLA) (C 18:3n-6) and arachidonic acid (AA) are formed of linolenic acid (LA); eicosapentaenoic acid (EPA) (C 20:5n-3), docosapentaenoic acid (DPA) (C22:5n-3) and docosahexaenoic acid (DHA) (C22:6n-3) are formed of alpha linolenic acid (ALA) [4]. It should be pointed out that those three fatty acids compete about the same enzymatic systems – cyclooxygenases and lipoxygenases – in the production of prostanoids and leukotrienes (Figure 1). It was also found that the change in the proportion of unsaturated acids in the diet may influence the type of eicosanoids synthesised [4,8]. Two groups may be distinguished within natural polyunsaturated fatty acids: n-3 and n-6, also marked with the Greek letter  $\omega$ -3 and  $\omega$ -6 or its phonetic form (omega-3, omega-6). Physical properties of phospholipids are largely determined by chain length and the degree of fatty acid saturation. Ample data indicate that the integrity of cell membranes, their physical and chemical properties (permeability, receptor affinity) depend on the



**Figure 1.** Three groups of eicosanoids and their biosynthetic origin from n-6 and n-3 polyunsaturated fatty acids (1-cyclooxygenase pathway, 2-lipoxygenase pathway) [after 146].

degree of saturation, i.e. on the relationship between the amount of unsaturated and saturated acids in membranous phospholipids [21–23].

The studies on the composition of fatty acids in erythrocytes are usually concerned with their content in total phospholipids as well as in phosphatidylcholine and phosphatidylethanolamine. This is related to the fact that FCH, FE and FS are dominant membranous phospholipids. In our study, FCH accounted for nearly 50%, FE – for approx. 30% and FS – for approx. 11% total membranous phospholipids. Sphingomyelin (the main component of phospholipids of nervous cell membranes) was found in 8%. Phosphatidylinositol (playing an important role in membranous transmission) and cardiolipin (found mainly in mitochondrial membranes) constituted approx. 1% each [4,21].

The authors of the present work investigated the content of fatty acids of erythrocyte phospholipids in children with jca under the assumption that the composition of fatty acids in erythrocyte phospholipids was similar to the composition of fatty acids in membranous phospholipids of other cells and that it is less influenced by the daily diet when compared with the composition of the fatty acids of plasma phospholipids.

The comparison of the content of fatty acids in particular erythrocyte fractions among children with

jca and control subjects revealed a significant decrease in the content of linolenic acid and statistically insignificant decline in the content of arachidonic acid. Consequently, this led to a significant decrease in the content of these acids in FCH, FE and FS. As far as the remaining fractions are concerned, no significant differences were observed between analysed groups as to the content of n-6 fatty acids. The content of PUFA n-3 in erythrocyte phospholipids of both alpha linolenic acid (C 18:3n-3) and EPA (C20:5n-3) was lower among children suffering from jca in the majority of fractions (FCH, FE, FS, FI). In sphingomyelin and cardiolipin, the amount of eicosapentaenoic acid (C 20:5n-3) could not be measured in either of the groups. The percentage of saturated acids in particular phospholipid fractions was significantly higher when compared with control group: this applied to palmitic acid (C16:0) in FCH, FS and SM and stearic acid (C18:0) in FCH, FS and FI.

Thus, a definite regularity has been observed: the decrease in the content of unsaturated fatty acids, particularly PUFA n-6, in children with jca, resulted in compensatory increase of saturated fatty acids. These findings correspond in part with the results published by other researchers. Johansson et al. investigated the content of fatty acids in children with jca and they found the increase in the content of saturated fatty acids and decrease in the content of linolenic acid in total phospholipids and phos-

phatidylcholine of erythrocytes [25]. On the other hand, Azzini et al. investigated adult patients with psoriatic arthritis and found significant elevation in the content of palmitic acid (C 16:0) and total saturated fatty acids, with significant decline in the content of linolenic acid (C 18:2n-6) and arachidonic acid (C20:4n-6) in total erythrocyte phospholipids [26]. However, Jacobsson et al. who analysed the cases of adults suffering from rheumatoid arthritis observed lower values of linolenic acid (C 20:4n-6) and alpha linolenic acid (C 18:3n-3) together with the increase in the proportion of saturated fatty acids, both in plasma phosphatidylcholine and in phospholipids of fatty tissue [27].

Considering the data quoted above, and taking into account the findings of the present study, a question may be asked about the mechanism of the disturbances in the content of fatty acids in chronic rheumatoid arthritis. One of the hypothesis trying to account for these results is based on the higher PUFA consumption. It is a fact that an ongoing chronic inflammatory process is associated with the activation of membranous phospholipase A 2 and the release of arachidonic acid from the pool of membranous phospholipids. Consequently, free arachidonic acid is metabolised by means of cyclooxygenase and lipoxygenase into pro-inflammatory eicosanoids, mainly PGE 2 and LTB 4 [17,18]. In the case of simultaneous decrease in the amount of PUFA n-3, there is a competitive elevation in the incorporation of arachidonic acid to membranous phospholipids and its subsequent consumption in cascade lipid peroxidation [28]. On the other hand, better access to enzymes, both desaturases and elongases (also associated with low PUFA n-3 concentrations) results in faster competitive transformation of linolenic acid (C 18:2n-6) into arachidonic acid (C 20:4n-6), and thus, in the decrease in the content of linolenic acid, too [19,24,29]. However, if only such interpretation was accepted, we might expect much lower levels of prostaglandin precursors (including arachidonic acid) than the levels which were actually found in the present study and those that were reported by other investigators [23,25].

The data from literature on the subject indicate the existence of a correlation between changes in the content of fatty acids of membranous phospholipids and the activity of inflammatory process in rheumatoid arthritis [26,29]. The group of children with higher inflammatory activity (3 and 4 degree according to Mallya and Mace) displayed significantly higher content of saturated fatty acids and

lower content of linolenic acid (C 18:3n-6) and arachidonic acid (C 20:4n-6) in FCH, FE and FS when compared with control group and subjects with lower inflammatory activity. Statistically significant correlation observed between the content of saturated acids and PUFA n-6 in analysed phospholipid fractions and Hb concentration, ESR and number of platelets confirms that inflammatory activity influences the content of fatty acids of membranous phospholipids. It should be pointed out that there was a positive correlation between the content of palmitic acid (16:0), ESR and number of platelets, and a negative correlation between these parameters and the content of linolenic acid in FCH.

Having investigated adult patients with rheumatoid arthritis, Jakobssen observed that the changes in the composition of fatty acids in phosphatidylcholine and total plasma phospholipids increased as the disease proceeded in time [27]. These findings were not confirmed during our study. Children suffering from jca for more than 3 years displayed significantly lower increase in the content of saturated fatty acids and significantly lower decline in the percentage of polyunsaturated fatty acids in erythrocyte phospholipids in relation to the content of these acids in children with shorter history of the disease (up to 3 years). It should be remembered, however, that mean disease duration in patients with rheumatoid arthritis in Jakobssen's study was much longer (around 15 years) while in the case of our patients, the longest disease duration was approx. 8 years.

Numerous in vitro studies show changes in the content of fatty acids of various cells obtained from patients with rheumatoid arthritis, including the cells of synovial membrane, polynuclear leukocytes or monocytes [6,17,23]. These investigations also proved that changes in the content of fatty acids influenced the function of these cells [5,30]. For instance, the inhibition of synovial cell proliferation by DHA acid (C 22:6n-3) was proved, and it was probably associated with increased PGE 3 production from DHA and the elevation in the level of cAMP [6,31]. It was also found that the content of fatty acids of lymphocyte phospholipids has an effect on the proliferation of lymphocytes, the permeability of their membranes and the production of cytokines [32].

Therefore, we believe that it is necessary to conduct further studies which would evaluate the content of fatty acids as well as the effect of PUFA on

the function of cells, immune and inflammatory response, both at molecular, cellular and clinical levels. The knowledge of these mechanisms may contribute to the development of a better, comprehensive pharmacological and dietary treatment of patients with rheumatoid arthritis.

## CONCLUSIONS

1. It was found that children with jca manifested significant decrease in the content of polyunsaturated fatty acids with a subsequent elevation in saturated fatty acids in total erythrocyte phospholipids and in analysed fractions, which seems to be associated with the presence of chronic inflammatory process.
2. The differences in the distribution of fatty acids in erythrocyte phospholipids were observed at an early stage of the disease and they became more conspicuous as the activity of inflammatory process increased.

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