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Fatty Acids of Plasma and Red Blood Cell Lipids in a Normal Population

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A detailed study of the fatty acid composition of each of the lipids present in plasma and red blood cells of 62 healthy subjects of our area (Barcelona and surrounding counties), by coupling thin-layer and gas chromatography techniques has been made. The results are presented as normative data for comparison with those found in pathological situations. No significant sex differences were found. With increasing age, there was a tendency for the proportion of linoleic acid to decrease. Correlation analyses between the fatty acid composition of different lipids suggested that the interchange of fatty acids between plasma and cells mainly affects the phosphatidylcholine of the latter.

Key words: Fatty acids, Plasma, Red blood cells, Thin-layer chromatography, Gas chromatography.

Polyunsaturated fatty acids (FA) are important constituents of the plasma and membrane lipids. Alterations in their levels have been directly related to several pathologic conditions (6, 8) and suggested as risk factors in cardiovascular disease (3, 11, 13). In spite of this, there are only a few studies dealing with the FA composition of plasma and cell lipids in large groups of normal individuals on a free diet (2, 9, 14).

As the first part of a project to investigate the FA abnormalities in different diseases, we have worked out an appropriate methodology by using thin-layer and gas chromatography techniques, and analyzed in detail each of the plasma and red blood cell (RBC) lipids of 62 healthy subjects from our area, including the city of Barcelona and surrounding counties.

Materials and Methods

Thirty male and 32 female subjects were included in the study. The mean age of the group was 38.2 years, ranging from 17 to

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59 years. They were all in good health, followed a regular free-diet including vegetable oils, had a low or no intake of alcohol, and denied taking any drug. In all cases, the plasma lipid profile (cholesterol, HDL-cholesterol and triglycerides) was in the normal range.

Venous blood was collected from an antecubital vein, in the morning, after an overnight fast. Blood was anticoagulated with EDTA, and centrifuged at 1500 *g* for 30 min, within an interval of 2 hours. The plasma was separated off and the RBC washed three times with a double volume of isotonic saline solution. Lipids were extracted with 2-propanol (1:5 v/v) (7). One ml of the sample was added slowly to 4 ml of the solvent, then shaken during 30 min and centrifuged at 1500 *g* for 15 min. This procedure gave a similar yield of extraction when compared with the more popular chloroform-methanol method (5) in the same samples. The extracted solutions were kept in glass vials, after blowing N_2, at -20° C.

The lipid fractions were separated by thin-layer chromatography on silica gel Woelm plates (22). Extracts of plasma (0.5 ml) and of RBC (1 ml) were applied in a narrow line 1 cm from the bottom of the plate, and developed three times on the same dimension. Plasma samples were developed twice with a solvent mixture of n-hexane / 1,2-dichloroethane / methanol/ formic acid (16:14:8:1 v/v/v/v) up to 12 cm from the origin, and a third time with 1,2-dichloroethane up to 18 cm. For RBC samples, the second system was a mixture of ethyl acetate/2-propanol/water (30:21:9 v/v/v), up to 14 cm. The separated lipid classes were made visible by spraying with dichlorofluorescein and viewed under UV light at 366 nm. Their identification was made by comparison with pure lipids (Sigma) separated in parallel on the same plate.

The distinct bands were scraped off, the lipids converted to methyl esters of FA by boiling in 2 ml of 14 % boron trifluoride/ methanol (15), and then extracted with n-pentane (5 ml) and an isotonic ClNa solution (2 ml) by vigorous shaking. The upper phase was evaporated under a N_2 stream and redissolved in CS_2 (50 µl). All the solvents used were of HPLC grade. The resulting FA methyl esters were separated and measured using a Hewlett-Packard 5830A gas chromatograph with a 2 mm × 3 m glass column packed with 15 % Silar C on Chromosorb P-AW 100-120 mesh. The carrier gas was N_2 at a flow of 20 ml/min. Oven temperature was programmed to rise from 180° to 200°C at 0.5°C/min and then maintained isothermal for 50 min. The flame ionization detector was supplied with H_2 at a flow of 42 ml/min and synthetic air at 300 ml/min. Injector and detector temperature was 250°C. Retention times and peak areas were computed by a Hewlett-Packard 18850CA terminal. The different FA methyl esters were identified by comparison with standard mixtures (Applied Science Co., Nu-Chek Prep Inc.). The profile of one of these mixtures is shown in figure 1. The analysis grade, obtained from comparison between the known composition of a standard mixture and the percentage composition found, was 98.2 %. Statistical analysis included analysis of variance and Pearson correlation tests.

Results

The FA composition of eight lipid fractions of the plasma and seven of the RBC are shown in tables I and II, respectively.

There were no significant differences between male and female subjects, so that both sexes are grouped together. On the other hand, some changes were found in relation to age; the most consistent was a significant tendency for the proportion of linoleic acid in the phosphatidylcholine, triglycerides and cholesteryl esters of the plasma, and in the phosphaditylcholine, phosphatidylserine and phosphatidyl-

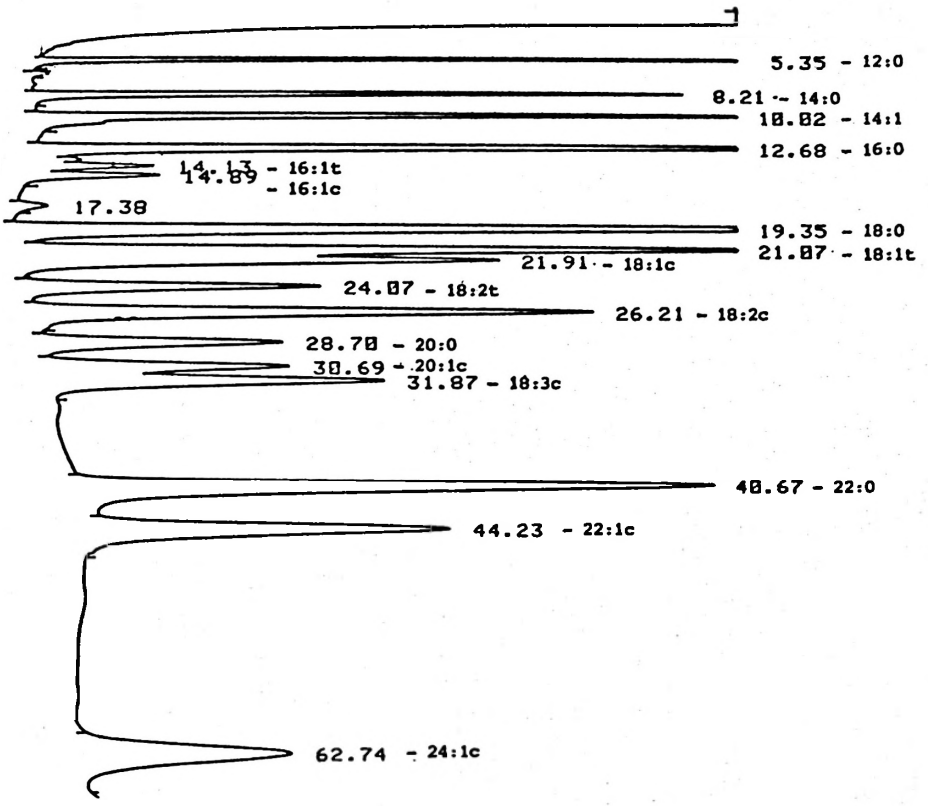

Fig. 1. *Gas chromatographic analysis of a mixture of pure fatty acid methyl esters.*

ethanolamine of the RBC to decrease with increasing age.

The correlation analyses between the FA composition of the plasma and of the RBC lipids are shown briefly in Table III. The main significant trends related the oleic and linoleic acids of phosphatidylcholine, triglycerides and cholesteryl esters of the plasma with the same FA of the phosphatidylcholine of the RBC. Few significant correlations were observed between the FA of the plasma lipids and those of the other RBC lipids.

Discussion

The major purpose of this study was to establish control values for our population against which to compare FA profiles in different pathological circumstances. The combination of thin-layer and gas chromatographic techniques of high resolution has allowed us to perform a detailed analysis of the FA composition of each of the lipid fractions present in plasma and RBC from a healthy population in our area. The original development systems described allow a clear separation by unidimensional thin-layer chromatography of all the lipid classes present in biological samples, and in sufficient amounts for preparative analysis (22). Frequently the FA composition is studied in a total, unfractionated sample or only from the major lipid classes, which does not give information on some minor components, particularly some phospholipids, that may play an impor-

Table I. *Fatty acid composition of the plasma lipids.*
Levels expressed as percentage of the total amount of fatty acids present. Values are means ± s.d.

Fatty acid	Lysophosphatidylcholine	Phosphatidylcholine	Sphingomyelin	Phosphatidylserine	Phosphatidylethanolamine	Non-esterified fatty acids	Triglycerides	Cholesteryl esters
14:0	4.28±2.12	0.40±0.26	1.51±0,84	5.25±1.98	5.80±2.10	2.65±1.68	1.52±0.71	1.44±0.69
14:1	2.26±1.72	0.22±0.14	0.58±0.36	3.05±1.54	3.48±1.98	1.20±0.75	0.43±0.37	0.48±0.31
16:0	45.10±8.88	31.07±4.52	27.60±5.63	28.24±5.61	29.22±6.87	28.52±5.78	25.19±4.43	7.75±2.29
16:1	4.42±2.97	0.69±0.62	1.27±0.75	6.35±2.38	7.13±2.87	3.21±1.17	3.66±1.08	2.92±1.22
18:0	16.87±4.98	17.64±3.01	13.62±3.79	19.03±6.01	17.17±7.31	15.21±4.86	3.99±1.70	1.69±0.96
18:1	13.75±5.47	14.99±2.66	4.82±2.93	21.17±7.14	25.15±7.41	36.87±7.60	47.33±6.91	24.94±5.39
18:2	10.55±5.22	21.86±4.89	3.05±1.55	7.11±2.81	6.18±2.59	11.22±4.08	16.88±6.31	52.72±7.88
20:0			3.87±1.30					
20:3		3.02±1.20						
20:4	1.93±2.07	9.61±2.83	3.70±1.76	9.18±5.08	5.47±6.48		0.53±0.82	6.59±2.24
22:0			12.42±3.56					
23:0			4.70±2.11					
24:0			8.13±3.05					
24:1			13.78±4.68					

Table II. *Fatty acid composition of the RBC lipids.*
Levels expressed as percentage of the total amount of fatty acids present. Values are means ± s.d.

Fatty acid	Lysophosphatidylcholine	Phosphatidylcholine	Sphingomyelin	Phosphatidylserine	Phosphatidylinositol	Phosphatidyl ethanolamine	Phosphatidic acid
14:0	3.42±1.42	0.24±0.10	0.40±0.23	1.33±0.83	1.27±0.68	0.22±0.22	4.05±1.54
14:1	1.23±0.85	0.15±0.06	0.12±0.11	0.56±0.43	0.52±0.34	0.13±0.15	1.34±0.90
16:0	42.57±4.81	37.05±2.46	22.23±6.06	14.09±4.42	17.27±3.55	16.88±4.29	30.97±2.79
16:1	3.76±1.51	0.49±0.33	0.50±0.36	2.37±1.79	2.70±1.73	0.47±0.35	4.63±2.06
18:0	31.09±4.20	13.87±2.33	6.44±1.39	15.86±5.25	41.07±5.98	31.40±7.12	22.06±4.96
18:1	13.41±3.47	21.82±2.46	1.44±0.76	20.95±5.35	16.63±3.57	25.62±3.61	21.37±3.12
18:2	4.42±1.68	20.12±3.04	0.50±0.41	4.19±1.59	4.95±2.37	4.51±1.97	7.66±1.87
20:0			1.76±0.37				
20:3		1.50±0.52		1.12±1.05		1.04±0.39	
20:4	0.23±0.17	4.23±1.68	0.79±0.25	33.04±7.16	15.04±4.92	17.12±2.78	7.58±2.90
22:0			8.35±1.49				
23:0			1.17±0.34				
24:0			21.68±4.01				
24:1			32.39±5.45				
22:4		0.48±0.23	1.98±1.30	5.62±2.65		21.7±0.84	

Table III. *Relationship between the fatty acids of the phosphatidylcholine of the RBC and the fatty acids of the plasma lipids.*
All the correlation coefficients shown are statistically significant at a $p<0.005$ level.

Plasma	RBC Phosphatidylcholine 18:1	18:2	20:3
Phosphatidylcholine			
18:1	0.61	- 0.41	
18:2	- 0.40	0.56	
20:3			0.56
Triglycerides			
16:1		- 0.34	
18:1	0.66	- 0.43	
18:2	- 0.52	0.59	
Cholesteryl esters			
16:1	0.35	- 0.43	0.34
18:1	0.65	- 0.49	
18:2	- 0.53	- 0.49	

tant physiopathological role in certain diseases.

The percentage FA composition of most lipids analyzed is in good agreement with results from other investigators for the plasma (9, 14, 17, 18) and the RBC (4, 10, 16). Differences are substantial for some of the phospholipids present in low amounts in the samples. This is probably due to methodological differences. Incomplete separation by thin-layer chromatography, and an especial interest in the analyses and identification of some particular FA, as those of long chain, may contribute to the disagreements. In most instances, however, the differences between studies may be attributed to the characteristics of the population. Sex is not an important source of variation (9, 14) except during childhood (20, 21). In adults, the lipid FA composition changes slightly in relation to age; this is mainly shown by a progressive decrease of linoleic acid and an increase of oleic and arachidonic acids with increasing age (9). However, the most important factor contributing to variation is the composition of the diet (2, 17). The type of FA in the diet influences the FA profile of the plasma lipids, particularly of the triglycerides (1, 23) and, over a longer term, the cell membrane lipids (12, 16). The relatively high consumption of polyunsaturated lipids from vegetable oils and low intake from fish sources in our country has to be taken into account.

The FA status has been analyzed much more frequently in plasma or serum, than in cell or tissue samples. The RBC constitute a convenient element because they are easily available and relatively stable due to their low metabolic activity (16). The RBC lipids are modified mainly by interchange with the plasma lipoproteins. Changes are substantial for phosphatidylcholine and low for sphingomyelin and acidic phospholipids (19), as shown indirectly by the correlations of FA in plasma and RBC (Table III). The usefulness of cell membrane analysis is that variations in the phospholipids and in their FA proportions affect membrane function and fluidity (19), factors related to the risk of thrombosis and vascular disease.

The results reported are useful for comparison with data from patients with different diseases from our environment. However, because of the changes in composition of the diet, the study of a control group is always recommended for a reliable comparison in other geographical areas.

Resumen

Se estudia, mediante técnicas de cromatografía en capa fina y en fase gaseosa, la composición en ácidos grasos de cada uno de los lípidos presentes en el plasma y en los hematíes de 62 sujetos sanos de nuestra área (Barcelona y comarcas colindantes). Los resultados aportan los valores correspondientes a una población normal y permiten la comparación con los datos obtenidos en diversas situaciones patológicas. En los análisis efectuados no se observaron diferencias significativas entre sexos. Con el aumento de edad se registra una tendencia a disminuir la proporción de ácido linoleico. Las correlaciones entre la

composición en ácidos grasos de los diversos lípidos sugieren que el intercambio entre plasma y células afecta principalmente a la fosfatidilcolina de estas últimas.

Palabras clave: Acidos grasos, Plasma, Eritrocitos, Cromatografía en capa fina, Cromatografía en fase gaseosa.

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