EFFECT OF DIETARY COLUMBINIC ACID ON THE FATTY ACID COMPOSITION AND PHYSICAL MEMBRANE PROPERTIES OF DIFFERENT TISSUES OF EFA-DEFICIENT RATS

Elisabet C. Mandon[1], *Irma N.T. de Gómez Dumm*[1]
and Rodolfo R. Brenner[2]

Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP),
CONICET-UNLP
La Plata, Argentina

SUMMARY

The effect of columbinic acid (5 trans, 9 cis, 12 cis, octadeca-trienoic acid) supplemented to a fat-free diet on the fatty acid composition and its correlation to the physical properties of several tissues of rats, was studied. The absence of lipids in the diet produced the typical changes in the fatty acid composition characteristic of essential fatty acid (EFA) deficiency, namely a significant increase in the relative percentage of monoenoic fatty acids with a concomitant decrease in linoleic and arachidonic acids and a rise in eicosa-5,8,11-trienoic acid in liver, kidney, lung and spleen homogenates.

Columbinic acid supplemented to a fat-free diet for 24 or 48 hr was incorporated into the different tissues and was partially elongated to 7 trans, 11 cis, 14 cis eicosatrienoic acid, but it was not desaturated. It modified the fatty acid spectrum of the lipids in the different tissues returning it to a similar composition of non-EFA deficient animals, except for a decrease of linoleic acid. The absence of lipids in the diet produced an increase in the 1-6 diphenyl-1,3,5-hexatriene (DPH) steady-state fluorescence anisotropy (r_s) in liver microsomes, that was corrected by the administration of columbinic acid for 24 hr. It is concluded that columbinic acid produced a change in the pattern of total fatty acid composition of the different tissues studied which induced a favorable effect on the physical properties of the liver microsomal membranes (r_s), leading to an improvement on the fatty acid deficiency in those mem-

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1 Members of the Carrera del Investigador Científico, Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina, Instituto de Investigaciones Bioquímicas de La Plata (INIBIOLP), La Plata, Argentina.

2 Director of the Instituto de Investigaciones Bioquímicas de La Plata, (INIBIOLP), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) Facultad de Ciencias Médicas, Universidad Nacional de La Plata (UNLP), Calles 16 y 120 (1900), La Plata, Argentina.

branes. Besides, columbinic acid would also exert a favorable effect in the short term, but not in the long-term eicosanoids production.

INTRODUCTION

Linoleic acid (18:2ω6) is the dietary precursor of a family of polyunsaturated acids having the same terminal ω6 structure where cis double bonds are alternated with $-CH_2-$ groups. The acids of this series which includes arachidonic acid (20:4ω6) are considered the principal essential polyunsaturated fatty acid in mammalian tissues, since animals are unable to synthesize linoleic acid *de novo*. The absence of polyunsaturated fatty acids of this series in the diet leads to an essential fatty acid (EFA) deficiency in young rats, characterized by macroscopical and microscopical symptoms, and biochemical alterations (1-3). Supplementation of the diet with acids of linoleic family return the afore-mentioned parameters towards normality (1,3,4). This effect undoubtedly depends on the specific structure of the fatty acid molecule since other polyunsaturated acids with similar unsaturation but different double bond disposition or trans structure, do not exert the same effect. It is well-known that in animals these acids are important components of phospholipids and cholesterol esters, and then are indispensable in the structure of membranes and lipoproteins. Besides, it has been demonstrated that important physiological functions of polyunsaturated fatty acids of ω6 series are determined by their conversion to eicosanoids: prostaglandins, tromboxanes and leukotrienes.

Linoleic acid is converted by an alternated series of desaturating and elongating reactions to 18:3ω6 (γ-linolenic acid) → 20:3ω6 (dihomo-gamma-linolenic acid) → 20:4ω6 (arachidonic acid) → 22:4ω6 → 22:5ω6. Dihomogammalinolenic and arachidonic acids may be converted by cyclooxygenase and lipooxygenase enzymes to eicosanoids. Therefore, on some occasions it has been proposed that probably all or at least the largest part of EFA deficiency symptoms are a consequence of eicosanoids deficiency.

To elucidate this point and to establish if linoleic acid structure *per se* has specific essential functions in animals, it is therefore important to develop an experimental model to test it.

Houtsmüller (5) introduced columbinic acid (5t, 9c, 12c 18:3) to study various functions of essential fatty acids. The most important characteristic of this acid is the Δ5 trans double bond present in addition to the structure of linoleic acid which blocks its Δ6 desaturation and the further conversion to dihomogammalinolenic acid and arachidonic acid. Therefore, it can not be a substrate for eicosanoids synthesis. However, it can be incorporated without any transformation into complex lipids. On the other hand, hydrogenation of the trans double bond of columbinic acid that would convert the fatty acid into linoleic acid, does not occur in rats (5). Columbinic acid supplemented to the diet of EFA-deficient rats improves some physiological and biochemical aspects of the deficiency: e.g. restoration of skin permeability (6), and reduction of hepatic content of fatty acid synthetase (7) as well as microsomal fatty acid Δ^9 desaturase (8).

It has been largely suggested that the physical dynamic properties of the membrane lipid matrix can regulate several membrane-associated biological functions (9), such as substrate transport (10-12) or enzyme activities (13-15). Phospholipids are necessary constituents of membranes and their structure and physical properties depend on the fatty acid composition. The unsaturated:saturated fatty acid ratio is considered in many cases as a factor that determines transition temperature and "fluidity" changes of phospholipids (16), and it is known that this ratio is modified by many physiological and pathological agents (17-19).

Taking into account the preceeding considerations, this report deals with a study of the fatty acid composition of the homogenate and microsomal fraction of several tissues, and their correlation with the physical properties of microsomal membranes of rats fed a balanced diet, a fat-free diet and a fat-free diet supplemented with columbinic acid.

MATERIAL AND METHODS

Animals and Their Treatment

The experiments were carried out with 24 male weanling rats of the Wistar strain. Animals were fed *ad libitum* for one month on either a balanced diet consisting of: (in cal) 55°/o starch, 20°/o casein and 25°/o sunflower seed oil (6 animals), or on a fat-free diet comprising 73.4°/o starch and 26.6°/o defatted casein (18 animals). Both diets were supplemented with 4g°/o of minerals and 2g°/o of a vitamins mixture (20). Water was also given *ad libitum*. After a month, the rats on the fat-free diet were divided in three groups of six animals each. One group was maintained on the same diet, while in the other groups the diet was supplemented with columbinic acid (1.5g°/o) only for 24 or 48 hr. At the end of this time all the rats were sacrificed. The control groups of rats fed the fat-free diet and those receiving the balanced diet, were also sacrificed at the same time.

Analytical Procedure

The rats were killed by decapitation and exsanguinated. Blood was allowed to clot, serum was removed and the fatty acid composition analyzed. Livers, kidneys, lungs and spleens were excised, immediately placed in ice-cold homogenizing medium, and homogenized (21).

The microsomal fraction was obtained from an aliquot of the homogenate of different tissues by differential ultracentrifugation at 4°C, as already described (22). The pellet was resuspended in the corresponding homogenizing solution (1:2 v/v) and the protein content measured by the Lowry's technique (23). The lipids of the homogenates, microsomes and serum were extracted with chloroform-methanol (2:1 v/v) following the procedure of Folch, Lees and Sloane-Stanley (24). The fatty acids of lipids were converted to methyl esters and analyzed on a Hewlett-Packard, Model 5840-A, gas liquid chromatograph equipped with a flame ionization detector. The column was packed with 10°/o SP 2330 coated on Chromosorb WAW 100-120 mesh, Supelco Inc., Bellefonte, PA, USA. The

oven temperature was programmed from 140 to 220°C at 3°C/min to separate methil esters ranging from 12 to 22:6ω3. Retention time and peak areas were determined electronically using a Hewlett-Packard Reporting Integrator. Identification of methyl esters was made by comparison with known methyl ester standards.

Steady-State Fluorescence Anisotropy Determinations (r_s)

The fluorescence probe 1,6-diphenil-1,3,5-hexatriene (DPH) was purchased from Aldrich Chemical Co. The fluorescence anisotropy measurements (352 nm excitation - 435 nm emission) were done at 37°C, following the procedure of Shinitzky and Barenholz (25) in an Aminco-Bowman spectrofluorometer equipped with two glan prism polarizers. Light scattering was less than 5°/o and fluorescence values were corrected correspondingly. The phospholipid:diphenyl-hexatriene ratio was always maintained at more than 200:1 (mol:mol) in order to minimize probe-probe interactions and perturbations of membrane bilayer.

The steady-state fluorescence anisotropy was calculated using the equation:

$$r_s = \frac{I_{||} - G\, I_{\perp}}{I_{||} + 2\, G\, I_{\perp}}$$

where $G = I_{hv}/I_{hh}$ is a correction factor arising from instrumental factors; I_{hh} and I_{hv} are the fluorescence intensities detected with the excitation polarizer in horizontal position and the analyzer in horizontal or vertical positions, respectively.

Results were calculated as mean ± SEM. Statistical analyses were made by the conventional "t" test.

RESULTS AND DISCUSSION

Effect of EFA Deficiency

The relative percentages of total fatty acids in serum, liver, kidney, lung and spleen of rats fed for a month, either a balanced diet or a fat-free diet are shown in Table 1. In all tissues studied (except serum) the major percentage of non-essential fatty acid was palmitic acid. The higher level of palmitic acid in lung, as compared to other tissues, may reflect the relevance of dipalmitoyl-phosphatidylcholine molecule in this tissue since it is a pulmonary surfactant (26,27) critical for the organ function.

Although it has been described that the contribution of kidney, lung and spleen on the fatty acid synthesis does not exceed 2°/o of the total fatty acid synthesis in the rat (28), the effect of the absence of polyunsaturated fatty acids in the diet is clearly reflected in the fatty acid composition of all of them. It resulted in a significant increase of monounsaturated fatty acids in all the tissues, expressing an increase in $\Delta 9$ desaturation activity already shown in the livers of rats maintained under this kind of diet (29-31). A significant decrease in dipalmitoyl-phosphatidylcholine was also described in lungs of the EFA-deficient rats (32-34).

The relative percentages of linoleic acid as well as its metabolic product, arachidonic acid, decreased as a consequence of the absence of linoleate in the diet. Nevertheless, as the linoleic acid decrease was larger than that of arachidonic acid, the ratio 20:4ω6/18:2ω6 increased. Oleic acid was converted into eicosa-5,8,11-trienoic acid; this non-essential fatty acid increased significantly in all the tissues analyzed rising the triene:tetraene ratio (20:3ω9/20:4ω6) several folds above the control levels.

Effect of Columbinic Acid

The effect of the addition of columbinic acid for 24 and 48 hr, to the fat-free diet on the total fatty acid composition of serum, liver, kidney, lung and spleen is shown in Tables 2 and 3. It is clear that the patterns obtained reflect the dietary fatty acid administered. Columbinic acid was rapidly incorporated in all the tissues investigated, but the relative percentages of this acid in kidney, lung and spleen were lower than in liver and serum. The lower percentage incorporation of columbinic acid in those tissues can be the result of different enzyme activity or specificities in these tissues or of a delayed supply. The presence of 22:3 (9t, 13c, 16c) acid in the tissues (Tables 2 and 3) suggests that some of the columbinic acid was further metabolized via chain elongation. Nevertheless, the presence of the Δ5 trans double bond impedes the introduction of double bonds.

The addition of columbinic acid to the diet evoked reduced ratios of 16:1/16:0 and 18:1/18:0 in liver (Table 2) suggesting that columbinic acid in the diet can inhibit the activity of Δ9 desaturase, effect that was also shown in kidney and lung. These conclusions are in accordance with previous results showing that columbinic acid added to the diet produced a decrease of fatty acid synthetase, acetyl-CoA carboxilase (35) and Δ9 desaturase (8). Similarly, the administration of cis-unsaturated acids of the linoleic acid family also produced a deactivation of Δ9 desaturase previously increased by a fat-free diet (29). Columbinic acid not only evoked the afore-mentioned effect on monoenoic acid, but also produced a reduction in the relative percentages of 20:3ω9 in all the tissues studied in this work, suggesting that columbinic acid is able to interrupt the eicosatrienoic acid synthesis. A decrease in 20:3ω9 level was also shown feeding EFA–deficient rats with linoleate, linolenate or arachidonate (36,37). Therefore, columbinic acid seems to be as efficient as polyunsaturated ω6 fatty acids in depressing the level of 20:3ω9.

Columbinic acid induced a decrease on the relative concentration of linoleic acid in all the tissues studied. Since in spite of the trans Δ5 double bond, columbinic acid has a very similar structure to linoleic acid, this effect could be due to the replacement of linoleic acid by columbinic acid in phospholipid molecules. In consequence, the linoleic acid released can be further desaturated and elongated via arachidonic acid. Arachidonic acid would be then released to serum where the relative concentration of this acid was found to increase significantly (Table 2). This statement is agreement with the increase of 20:4ω6/18:2ω6 found in all tissues studied under columbinic acid treatment. Therefore, we may conclude that columbinic acid would produce a release of linoleic acid from depot

TABLE 1

COMPARATIVE FATTY ACID COMPOSITION (PER CENT OF TOTAL FATTY ACIDS) OF SERUM, LIVER, KIDNEY LUNG AND SPLEEN LIPIDS OF RATS FED A FAT-FREE DIET (FFD) AND A BALANCED DIET (BD) FOR A MONTH

Fatty	Serum		Liver		Kidney		Lung		Spleen	
acids	BD	FFD	BD	FFD	BD	FFD	BD	FFD	BD	FFD
14:0	0.4 ± 0.1[a]	0.9 ± 0.05	0.2 ± 0.05	0.4 ± 0.1	0.7 ± 0.02	1.0 ± 0.1	1.6 ± 0.2	2.6 ± 0.3	0.5 ± 0.03	0.6 ± 0.05
16:0	17.8 ± 0.6	29.8 ± 0.6*	19.1 ± 0.8	21.4 ± 0.5	21.7 ± 0.7	22.0 ± 0.8	29.8 ± 0.8	32.5 ± 0.4	24.2 ± 0.6	23.5 ± 0.8
16:1	1.7 ± 0.1	6.6 ± 0.5*	1.5 ± 0.2	5.7 ± 0.5*	2.6 ± 0.2	7.1 ± 0.5*	4.8 ± 0.6	11.5 ± 0.6*	2.1 ± 0.5	4.6 ± 0.3†
18:0	10.0 ± 0.5	12.4 ± 0.5†	15.0 ± 0.7	13.9 ± 1.0	14.7 ± 0.8	11.0 ± 0.3†	8.2 ± 0.4	6.6 ± 0.3†	12.1 ± 0.6	15.0 ± 0.4†
18:1	18.7 ± 0.3	21.9 ± 1.0	13.6 ± 0.5	21.0 ± 1.1*	15.2 ± 0.6	24.6 ± 0.7*	18.6 ± 0.5	30.0 ± 0.7*	17.4 ± 0.7	27.0 ± 0.8*
18:2	28.1 ± 0.7	7.0 ± 0.3*	15.2 ± 0.6	7.8 ± 0.4*	14.2 ± 0.7	6.9 ± 0.6*	15.9 ± 0.6	2.8 ± 0.4*	15.3 ± 0.2	3.9 ± 0.6*
20:3ω9	0.9 ± 0.1	7.8 ± 0.5*	1.0 ± 0.1	5.8 ± 0.3*	0.8 ± 0.1	2.9 ± 0.4*	0.5 ± 0.08	2.1 ± 0.2*	1.4 ± 0.3	3.8 ± 0.5†
20:3ω6	0.9 ± 0.2	1.3 ± 0.2	1.0 ± 0.3	1.4 ± 0.1	1.1 ± 0.4	1.2 ± 0.1	0.9 ± 0.5	0.8 ± 0.4	1.0 ± 0.4	1:3 ± 0.2
20:4ω6	21.6 ± 0.8	12.3 ± 0.4*	24.7 ± 0.8	14.9 ± 0.9*	23.4 ± 0.9	17.0 ± 0.8†	10.8 ± 0.6	6.8 ± 0.3*	18.3 ± 0.5	11.9 ± 0.6*
22:4ω6	—	—	2.0 ± 0.2	1.2 ± 0.2	2.4 ± 0.3	2.0 ± 0.2	3.3 ± 0.4	1.6 ± 0.5	4.2 ± 0.3	3.6 ± 0.5
22:5ω6	—	—	3.6 ± 0.3	2.9 ± 0.1	2.1 ± 0.5	3.0 ± 0.6	2.8 ± 0.1	2.0 ± 0.6	1.6 ± 0.4	1.8 ± 0.6
22:5ω3	—	—	0.6 ± 0.1	0.6 ± 0.04	0.3 ± 0.1	tr	1.2 ± 0.2	tr	1.0 ± 0.2	1.4 ± 0.4
22:6ω3	—	—	2.8 ± 0.1	3.0 ± 0.2	0.8 ± 0.2	1.3 ± 0.1	1.6 ± 0.3	0.8 ± 0.1	0.9 ± 0.1	1.6 ± 0.3
16:1/16:0	—	—	0.08	0.27	0.11	0.32	0.16	0.35	0.09	0.20
18:1/18:0	—	—	0.91	1.51	1.03	2.23	2.27	4.55	1.44	1.80
20:4ω6/ 18:2	—	—	1.62	1.91	1.61	2.46	0.68	2.43	1.20	3.05
20:3ω9/ 20:4ω6	—	—	0.04	0.39	0.03	0.17	0.05	0.31	0.08	0.32

a Results are the mean of 6 rats ±1 SEM. † $p < 0.01$ compared to the balanced diet. * $p < 0.001$ compared to the balanced diet.

TABLE 2

COMPARATIVE FATTY ACID COMPOSITION (PER CENT OF TOTAL FATTY ACIDS) OF SERUM, LIVER, KIDNEY, LUNG AND SPLEEN LIPIDS OF RATS FED A FAT-FREE DIET (FFD) AND THE SAME DIET SUPPLEMENTED WITH COLUMBINIC ACID FOR 24 AND 48 Hr

Fatty acids	Serum			Liver			Kidney		
	FFD	FFD+Columb. 24 hr	FFD+Columb. 48 hr	FFD	FFD+Columb. 24 hr	FFD+Columb. 48 hr	FFD	FFD+Columb. 24 hr	FFD +Columb. 48 hr
14:0	0.9 ± 0.05[a]	0.8 ± 0.1	0.7 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	1.0 ± 0.2	1.1 ± 0.3	0.7 ± 0.3
16:0	29.8 ± 0.6	22.5 ± 0.6*	22.2 ± 0.5*	21.4 ± 0.5	19.5 ± 0.2†	19.4 ± 0.6	22.0 ± 0.8	22.5 ± 0.6	21.5 ± 0.4
16:1	6.6 ± 0.5	4.8 ± 0.5	4.3 ± 0.4†	5.7 ± 0.5	4.0 ± 0.3	3.7 ± 0.2†	7.1 ± 0.5	5.7 ± 0.3	5.0 ± 0.6
18:0	12.4 ± 0.5	12.4 ± 0.5	13.0 ± 0.2	13.9 ± 1.0	15.1 ± 0.5	15.2 ± 0.9	11.0 ± 0.2	11.0 ± 0.4	11.0 ± 0.3
18:1	21.9 ± 1.0	17.5 ± 0.5†	15.5 ± 0.6*	21.0 ± 1.1	17.4 ± 0.9	16.5 ± 0.9†	24.6 ± 0.7	22.8 ± 0.7	22.0 ± 0.4†
18:2ω6	7.0 ± 0.3	3.0 ± 0.3	3.2 ± 0.3*	7.8 ± 0.4	4.0 ± 0.1	3.5 ± 0.2*	6.9 ± 0.6	5.3 ± 0.4	5.1 ± 0.2
18:3 Columb.	—	13.4 ± 0.3	15.6 ± 0.6	—	9.4 ± 0.4	10.1 ± 0.5	—	2.3 ± 0.2	4.7 ± 0.5
20:3ω9	7.8 ± 0.5	3.0 ± 0.2*	3.5 ± 0.1*	5.8 ± 0.3	3.1 ± 0.2*	2.3 ± 0.2*	2.9 ± 0.4	2.3 ± 0.5	2.0 ± 0.3
20:3ω6+(7t, 11c, 14c) 20:3	1.3 ± 0.2	2.6 ± 0.1*	2.7 ± 0.2*	1.4 ± 0.1	1.4 ± 0.1*	1.2 ± 0.2*	1.2 ± 0.1	1.4 ± 0.3	1.5 ± 0.5
20:4ω6	12.3 ± 0.4	20.0 ± 0.5	19.3 ± 0.4	14.9 ± 0.9	15.8 ± 0.5	15.1 ± 0.6	17.0 ± 0.8	16.5 ± 0.6	16.5 ± 0.4
(9t, 13c, 16c)22:3				—	1.6 ± 0.1	2.2 ± 0.2	—	1.4 ± 0.3	2.3 ± 0.2
22:4ω6				1.2 ± 0.2	1.4 ± 0.1	2.0 ± 0.2	2.0 ± 0.2	2.1 ± 0.4	2.4 ± 0.1
22:5ω6				2.9 ± 0.1	3.0 ± 0.1	4.0 ± 0.4	3.0 ± 0.6	2.5 ± 0.2	2.9 ± 0.3
22:5ω6				0.6 ± 0.04	0.6 ± 0.1	0.9 ± 0.2	tr	1.0 ± 0.6	tr
22:6ω3				3.0 ± 0.2	3.4 ± 0.1	3.6 ± 0.5	1.3 ± 0.1	2.1 ± 0.3	2.4 ± 0.6
16:1/16:0				0.27	0.20	0.19	0.32	0.25	0.23
18:1/18:0				1.51	1.15	1.08	2.13	2.07	2.00
20:4/18:2				1.91	4.02	4.31	2.46	3.11	3.23

a Results are the mean of 6 rats ± 1 SEM. † $p < 0.01$ compared to the fat-free diet. * $p < 0.001$ compared to the fat-free diet.

TABLE 3

COMPARATIVE FATTY ACID COMPOSITION (PER CENT OF TOTAL FATTY ACIDS) OF LUNG AND SPLEEN LIPIDS OF RATS FED A FAT-FREE DIET AND THE SAME DIET SUPPLEMENTED WITH COLUMBINIC ACID FOR 24 hr AND 48 hr

Fatty acids	Lung			Spleen		
	FFD	FFD + Columb. 24 hr	FFD + Columb. 48 hr	FFD	FFD + Columb. 24 hr	FFD + Columb. 48 hr
14:0	2.6 ± 0.3	1.9 ± 0.2	2.2 ± 0.4	0.6 ± 0.05	0.7 ± 0.1	0.7 ± 0.05
16:0	32.5 ± 0.4	33.0 ± 0.4	33.5 ± 0.5	23.5 ± 0.8	23.5 ± 0.5	25.0 ± 0.7
16:1	11.5 ± 0.6	9.5 ± 0.7	9.0 ± 0.1†	4.6 ± 0.3	4.4 ± 0.3	4.5 ± 0.4
18:0	6.6 ± 0.3	8.5 ± 0.3†	9.8 ± 0.2*	15.0 ± 0.4	10.2 ± 0.6*	8.5 ± 0.6*
18:1	30.0 ± 0.7	21.0 ± 0.2*	20.0 ± 0.6*	27.0 ± 0.8	21.0 ± 0.3*	23.5 ± 0.6†
18:2ω6	2.8 ± 0.4	2.3 ± 0.4	3.1 ± 0.4	3.9 ± 0.6	2.9 ± 0.4	2.6 ± 0.5
18:3 Columb.	— —	— —	1.8 ± 0.2	— —	1.2 ± 0.1	1.8 ± 0.3
20:3ω9	2.1 ± 0.2	1.7 ± 0.2	1.4 ± 0.4	3.8 ± 0.5	2.5 ± 0.4	2.4 ± 0.2
20:3ω6 + (7t, 11c, 14c) 20:3	0.8 ± 0.4	1.2 ± 0.3	1.3 ± 0.2	1.3 ± 0.2	1.5 ± 0.2	1.3 ± 0.1
20:4 (9t, 13c, 16c) 22:3	6.8 ± 0.3 — —	10.0 ± 0.2* 1.8 ± 0.4	10.2 ± 0.3* 2.0 ± 0.5	11.9 ± 0.6 — —	18.0 ± 0.6* 2.5 ± 0.2	16.6 ± 0.5* 2.7 ± 0.3
22:4ω6	1.5 ± 0.5	3.8 ± 0.4	2.6 ± 0.3	3.6 ± 0.5	4.4 ± 0.5	2.8 ± 0.4
22:5ω6	2.0 ± 0.6	2.7 ± 0.5	0.6 ± 0.1	1.8 ± 0.6	3.4 ± 0.3	3.7 ± 0.5
22:5ω3	tr	1.0 ± 0.2	1.0 ± 0.3	1.4 ± 0.4	1.5 ± 0.2	1.6 ± 0.2
22:6ω3	0.8 ± 0.1	1.6 ± 0.4	1.5 ± 0.4	1.6 ± 0.3	2.3 ± 0.1	2.3 ± 0.4
16:1/16:0	0.35	0.29	0.27	0.20	0.19	0.24
18:1/18:0	4.54	2.47	2.04	1.92	2.06	2.76
20:4/18:2	2.43	3.33	3.29	3.05	6.21	5.77
20:3ω9/20:4ω6	0.31	0.17	0.14	0.32	0.14	0.16

a. Results are the mean of 6 rats ± 1 SEM. † $p < 0.01$ compared to the fat-free diet. * $p < 0.001$ compared to the fat-free diet.

evoking a new supply of substrate for eicosanoids biosynthesis that would persist until the total consumption of linoleic acid. On the other hand, Elliot *et al.* (38) have recently shown that columbinic acid can be a substrate for cyclooxygenase and lipoxygenase. The products principally formed were hydroxiderivative not cyclized structures. The topical application of the lipoxygenase product to paws of EFA-deficient rats produced a resolution of the scaly dermatitis similar to that induced by columbinic acid itself.

Effect on Microsomal Membrane Dynamics

The effect of the different diets on the fluorescence anisotropy of DPH microsomal membranes is shown in Table 4. The fat-free diet produced an increase in the DPH steady-state fluorescence anisotropy in liver microsomes indicated by a decrease in the rotational mobility of the probe in the membrane lipid phase, as compared to the microsomes obtained from animals maintained on a balanced diet. The previously mentioned effect of the fat-free diet on the membrane dynamics was corrected after the administration of columbinic acid during 24 hr.

The different diets did not produce any modifications in the rotational mobility of the probe in the membrane lipid phase in lung or kidney.

In connection with these results obtained by fluorescence measurements in the rats fed a fat-free diet, it is important to remark that liver

TABLE 4

VARIATION OF FLUORESCENCE ANISOTROPY (r_s) of 1,6-DIPHENYL-HEXATRIENE (DPH) BY DIFFERENT DIETS IN MICROSOMAL MEMBRANES OF KIDNEY, LIVER AND LUNG

Tissue	Diet	r_s
Kidney	BD	0.187 ± 0.001[a]
	FFD	0.184 ± 0.0006
	FFD + Columbinic 24 hr	0.186 ± 0.0005
Liver	BD	0.114 ± 0.0005
		$P < 0.001$
	FFD	0.124 ± 0.0009
		$P < 0.001$
	FFD + Columbinic 24 hr	0.109 ± 0.003
Lung	BD	0.189 ± 0.0005
	FFD	0.182 ± 0.0004
	FFD + Columbinic 24 hr	0.186 ± 0.0003

[a] Results are the mean of 6 rats ± 1 SEM.

microsome composition showed typical changes of EFA deficiency (Table 5), namely an increase in the levels of monoenoic acids and 20:3 ω9, concomitantly with a decrease of linoleic and arachidonic acids. These changes altered the unsaturated:saturated fatty acid ratio from 5.13 to 3.68, value that increased to 4.92 when the animals were fed with columbinic acid.

If the relative proportion of unsaturated to saturated fatty acids could be one of the factors that control the fluidity of lipids (39), the increase

TABLE 5

COMPARATIVE FATTY ACID COMPOSITION OF LIVER MICROSOME LIPIDS OF RATS FED A BALANCED DIET, A FAT-FREE DIET AND A FAT-FREE DIET SUPPLEMENTED WITH COLUMBINIC ACID FOR 24 hr

Fatty acid	Balanced diet %	Fat-free diet %	Fat-free diet supplemented with columbinic acid %
14:0	0.2 ± 0.04[a]	0.5 ± 0.04	0.3 ± 0.03
16:0	18.5 ± 0.5	22.4 ± 0.4	18.2 ± 0.3
16:1	1.2 ± 0.2	5.5 ± 0.5	2.8 ± 0.1*
18:0	16.0 ± 0.5	14.8 ± 0.9	16.8 ± 0.7
18:1	12.2 ± 0.3	21.1 ± 0.9	14.8 ± 0.7*
18:2ω6	13.8 ± 0.8	6.9 ± 0.3	3.7 ± 0.1*
18:3 (5t 9c 12c)	— —	— —	10.9 ± 0.6*
γ - 18:3	0.4 ± 0.1	0.5 ± 0.1	tr
20:3ω9	0.9 ± 0.05	5.0 ± 0.2	3.1 ± 0.2*
20:3ω6	1.3 ± 0.1	1.7 ± 0.4	
20:3 (7t 11a 14c)	— —	— —	1.5 ± 0.2
20:4ω6	26.1 ± 0.5	14.4 ± 0.6	17.0 ± 0.04
22:3 (9t 13c 16c)	— —	— —	1.7 ± 0.2
22:4ω6	1.8 ± 0.2	0.8 ± 0.1	1.0 ± 0.1
22:5ω6	3.9 ± 0.3	2.8 ± 0.1	3.3 ± 0.1
22:5ω3	0.8 ± 0.1	0.6 ± 0.1	0.8 ± 0.1
22:6ω3	2.9 ± 0.3	3.0 ± 0.2	4.1 ± 0.2
20:3ω9/20:4ω6	0.03	0.35	0.18
20:4ω6/18:2ω6	1.89	2.09	4.59
OBI/SFA[b]	5.13	3.68	4.92

a Results are the mean of 6 rats ± 1 SEM.

b $\frac{\text{Double bond index}}{\text{Saturated fatty acid}} = \frac{\Sigma \text{ (number unsaturated mol x number double bonds)}}{\Sigma \text{ number saturated mol}}$

* $p < 0.01$ compared to the fat-free diet.

in the r_S observed in the liver microsomes of rats fed a fat-free diet could be attributed to a fall on the unsaturated:saturated fatty acid ratio. In this respect, it is important to state that the administration of columbinic acid returns the dynamic properties of the liver membranes towards the values obtained in the animals fed a balanced diet, in correspondence with an increase of the unsaturated:saturated fatty acid ratio.

CONCLUSIONS

The results obtained in the present experiment clearly show that, as already known, the absence of lipids in the diet produces typical changes in the fatty acid composition, not only in the liver but also in kidney, lung and spleen, which are characteristics of EFA deficiency. Columbinic acid supplemented to a fat-free diet is rapidly incorporated into the different tissues displacing linoleic acid due to its similar structure, and permitting the conversion of the released linoleic acid to arachidonic acid. Therefore, it produces a partial return of the pattern of total fatty acid composition to normality. This effect, however, may be only effective in arachidonic acid production and eicosanoids biosynthesis until linoleic acid depot becomes exhausted, since columbinic acid is not converted to arachidonic acid.

Notwithstanding columbinic acid would apparently replace linoleic acid in EFA deficiency in membrane structures in a beneficial way, since it not only improves the skin permeability defect and the scaly dermatitis produced by EFA deficiency (4,5,38) but it also leads to a recovery of the dynamic properties (r_S) of membrane lipid bilayers.

It is not easy to deduce if the inhibitory effect produced by columbinic acid on $\Delta 9$ desaturation and oleic, palmitic and eicosa-5,8,11-trienoic acid (20:3ω9) biosynthesis is either beneficial or not. In EFA deficiency when arachidonic acid is not synthesized because of the absence of substrate (linoleic acid), the increase of oleic acid compensates the decrease of unsaturated acid (linoleic acid) in membranes and 20:3ω9 occupies the place of arachidonic acid in phospholipids. Therefore, although 20:3ω9 is not converted to eicosanoids, oleic and 20:3ω9 acids, it would favor membrane fluidity maintenance.

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RESUMEN

EFECTO DEL ACIDO COLUMBINICO ALIMENTARIO EN LA COMPOSICION DE ACIDOS GRASOS, Y PROPIEDADES FISICAS DE MEMBRANAS DE DIFERENTES TEJIDOS DE RATAS DEFICIENTES EN ACIDOS GRASOS ESENCIALES (AGE)

Se estudió el efecto del agregado de ácido columbínico (5 trans, 9 cis, 12 cis octadeca-trienoico) a una dieta libre de grasas sobre la composición de ácidos grasos de distintos tejidos de rata, y estos datos se correlacionaron con las propiedades físicas de dichos tejidos. La ausencia de lípidos en la dieta produjo cambios en la composición de ácidos grasos que son característicos de la deficiencia de ácidos grasos esenciales (AGE). Se observó un incremento significativo del porcentaje relativo de ácidos grasos monoenoicos acompañado de una disminución de los ácidos linoleico y araquidónico y un aumento del ácido eicosa-5,8,11-trienoico en los homogenatos de hígado, riñón, pulmón y bazo.

El ácido columbínico agregado a una dieta libre de grasas durante 24 ó 48 horas se incorporó en los distintos tejidos, elongándose parcialmente al ácido eicosa-7 trans, 11 cis, 14 cis-trienoico, pero sin ser desaturado. El ácido columbínico modificó el perfil de composición de ácidos grasos de los lípidos en los distintos tejidos, de manera tal que su porcentaje de distribución fue similar al observado en los animales no deficientes en AGE, excepto por el descenso del ácido linoleico. La ausencia de lípidos en la dieta produjo un incremento en la anisotropía de fluorescencia determinada con excitación continua (r_s) del 1,6-difenil-1,3,5-hexatrieno (DPH) en microsomas hepáticos, que se corrigió con la administración de ácido columbínico durante 24 hr.

Se concluye que el ácido columbínico produjo un efecto favorable de corto alcance sobre las propiedades físicas de la membrana microsomal hepática (r_s) atribuible a las modificaciones en la composición de ácidos grasos. El ácido columbínico, por lo tanto, induciría también un efecto favorable a corto plazo sobre la producción de eicosanos, pero no así a largo plazo.

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