J. Lipid Research, July 1963
Volume 4, Number 3

The effect of dietary essential fatty acids upon composition of polyunsaturated fatty acids in depot fat and erythrocytes of the rat*

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[Manuscript received December 27, 1962; accepted February 15, 1963.]

SUMMARY

The fatty acid composition of lipids from erythrocytes and depot fat of rats whose diet was supplemented with various levels of linoleate, arachidonate, or linolenate has been investigated. Dietary linoleate and linolenate are incorporated into the depot fat. The ratio of eicosatrienoic acid to eicosatetraenoic acid, derived from the analysis of depot fat, decreases with increasing amounts of all three dietary essential fatty acids (EFA) in the same manner as observed in organ lipids. The composition of the unsaturated fatty acids of erythrocyte lipids is altered readily by increasing amounts of dietary EFA. The concentration of 20:3 is lowered extensively by all three EFA. The synthesis of arachidonate is inhibited by dietary linolenate and 20:5 and 22:5ω3 (double bond between third and fourth carbons from ω end) can be detected only in animals given linolenate.

Recently we have investigated the changes occurring in the fatty acid composition of liver (1), heart (2), and brain (3) of rats fed diets supplemented with graded levels of highly purified ethyl linoleate, arachidonate, and linolenate. In all these tissues, the course of fat deficiency and the alterations caused by variation of dietary essential fatty acids (EFA) could readily be detected.

Depot fat and erythrocytes are tissues readily available from living animals, and assessment of EFA status through analysis of the fatty acids of these tissues would offer some advantages to the researcher who does not wish to sacrifice his subject of study. Both tissues are known to vary in their fatty acid composition (4, 5, 6) as a consequence of changes in dietary fat composition. It was desirable, therefore, to describe the changes in fatty acid composition of adipose tissue and erythrocytes as a function of the content of single essential fatty acids in the diet.

EXPERIMENTAL METHODS

Weanling rats were kept on a fat-free diet which was supplemented orally with different amounts of either ethyl linoleate, linolenate, or arachidonate. The amounts fed in percentage of calories of the whole diet are shown in Table 1. After 100 days of diet supplementation, the animals were sacrificed by ether anesthesia. The blood was drawn from the heart by syringe, and the erythrocytes were centrifuged. Clotting was prevented by adding a few drops of a citrate solution. The erythrocytes were washed twice with 0.9% saline solution by centrifugation and stored in saline solution at −20° until analyzed. The epididymal fat was removed from the carcasses and stored similarly. Erythrocytes, as well as epididymal fat, were pooled according to groups and the lipids extracted with chloroform–methanol 2:1 in a tissue blendor according to the procedure of Folch, Lees, and Sloane Stanley (7). The lipids were refluxed with HCl-methanol to prepare methyl esters of fatty acids.

The methyl esters were analyzed by gas-liquid chromatography (GLC) using a Barber Colman Model 10 apparatus with argon ionization detector. The U-shaped glass columns of 210 cm x 5 mm i.d. were packed with 20% ethylene glycol succinate polyester on Gaschrom P, 80–100 mesh. Flow rate was 60 ml argon/min at 16 psi. The inlet heater was kept at 250°, the detector cell at 270°. Column temperature was 180° for analysis of fatty acids with retention times shorter than that of 18:3, and 200° for the long-chain acids. Data reported are area percentages. Individual fatty acids were identified by internal standards and by carbon number.

* Supported in part by grants from the National Institutes of Health, U.S. Public Health Service (AM 04524); The Life Insurance Medical Research Fund; and the National Dairy Council.

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TABLE 1. FATTY ACID COMPOSITION OF LIPIDS IN DEPOT FAT (% OF TOTAL FATTY ACIDS)

EFA (% of cal)	Animals in Group	14:0	16:0	16:1	18:0	18:1	18:2	18:3
Fat free	24	1.75	29.27	18.52	2.07	47.71	0.54	
Linoleate								
0.009	6	1.99	31.90	18.19	2.19	45.07	0.65	
0.02	6	1.89	31.21	19.56	1.87	44.57	0.90	
0.05	6	1.75	32.22	17.32	1.98	46.23	0.50	
0.10	6	1.75	27.37	19.45	1.83	48.92	0.68	
0.18	6	1.78	29.26	19.03	2.31	46.89	0.72	
0.32	6	1.96	28.45	17.01	2.00	50.01	0.57	
0.61	6	1.81	29.33	17.55	2.18	48.08	1.05	
1.26	2	1.44	33.05	18.43	2.31	43.18	1.58	
1.79	2	2.26	29.75	17.24	2.18	45.70	2.88	
4.87	2	4.05	32.23	16.48	2.16	37.36	7.73	
Arachidonate								
0.007	6	1.69	28.46	18.49	1.96	48.69	0.71	
0.019	6	1.56	30.50	18.42	2.22	46.72	0.58	
0.04	6	1.71	29.23	16.80	2.27	49.37	0.61	
0.08	6	1.67	32.64	17.59	2.22	45.02	0.85	
0.14	6	1.83	33.67	17.72	2.26	43.82	0.69	
0.27	6	1.76	32.96	18.44	1.84	44.37	0.63	
0.52	6	1.97	30.94	18.32	2.29	45.82	0.67	
1.08	2	2.42	36 60	20.76	2.51	36.76	0.95	
1.39	2	2.71	34.35	21.28	2.32	37.72	0.73	
3.75	2	2.73	36.24	22.38	2.73	30.07	1.17	
Linolenate								
0.009	6	1.77	31.32	18.76	1.41	45.90	0.29	
0.02	6	1.88	30.34	20.74	1.18	44.93	0.44	
0.04	6	2.59	27.27	20.39	1.39	47.63	0.31	
0.08	6	2.33	27.29	18.57	1.36	49.77	0.23	0.05
0.18	6	1.85	30.09	18.33	1.24	47.75	0.32	0.05
0.32	6	2.13	29.40	19.57	1.40	46.81	0.08	0.21
0.61	6	2.12	28.19	21.83	1.29	45.54	0.34	0.47
0.77	4	2.56	42.18	16.33	1.91	35.05		0.97
1.42	4	1.99	26.35	19.81	2.12	46.88	0.48	1.38
2.56	4	3.05	39.45	16.46	3.04	34.42	0.34	2.72
3.56	4	2.32	32.58	20.01	1.76	38.49	0.24	4.16
4.14	4	1.80	31.92	17.79	2.18	40.98	0.51	4.39
9.42	2	2.18	31.95	19.72	1.82	32.36	0.82	10.74

The detailed description of the basic fat-free diet, purity of the essential fatty acids fed, and analytical procedure are given in a previous communication (1).

Since the small amounts of eicosatrienoic and eicosatetraenoic acids in depot fat could just be detected in the chromatograms of the total fatty acid mixture, the saturated fatty acids have been removed by precipitation at low temperature (8). Two grams of the methyl esters from depot fat were saponified with 0.5 N NaOH in methanol. A 10% solution of the free fatty acids in acetone was cooled slowly to −40°. The liquid phase was filtered off by suction through a fritted glass tube. The solvent was removed, and the predominantly unsaturated fatty acids were esterified with diazomethane in diethyl ether.

In chromatograms of the unsaturated fraction (mainly monoenes), the peaks for 20:3 and 20:4 could be detected and identified by internal standards. Since the relations of the unsaturated fractions to the total fatty acids were not determined, only the ratios of the 20:3 to 20:4 were determined by triangulation.

RESULTS

Dietary Alterations in Depot Fat. The fatty acid composition of depot fat is given in Table 1. Almost 50% of the total fatty acids of the adipose tissue in fat-

deficient animals consists of oleate. Supplementation of the diet with EFA tends to decrease the oleate content of the depot fat. The concentration of the saturated fatty acids does not show any consistent changes with increasing amounts of dietary EFA, although the concentration of palmitate at high dietary levels of arachidonate is somewhat raised. Dietary linoleate is readily incorporated into the depot fat. At the level of 5% of calorie intake, the linoleate concentration in the depot fat has increased more than tenfold in comparison with EFA-deficient animals. In animals fed diets supplemented with arachidonate, the 18:2 concentration is rather constant. Animals fed diets supplemented with linolenate show linoleate concentrations that are all lower than in rats fed fat-free diets or diets supplemented with arachidonate.

Dietary linolenate is also incorporated into the lipids of depot fat. Whereas no linolenate can be detected in animals fed linoleate- and arachidonate-supplemented diets, up to 10% of the fatty acids of depot fat consist of linolenate at the highest intake level of that fatty acid. No higher unsaturated fatty acids could clearly be identified in the chromatograms of total fatty acids from depot fat. Chromatograms of the liquid fraction of the low-temperature fractionation showed several peaks beyond 18:3. However, only 20:3 and 20:4 could be identified with certainty. The relationship of dietary EFA to the ratio of 20:3 to 20:4 is illustrated in Fig. 1. The triene/tetraene ratio is lowered by all three dietary polyunsaturated fatty acids.

Dietary Alterations in Erythrocyte Lipids. No consistent changes in the concentration of the saturated fatty acids could be detected. Oleate levels are lowered significantly by increasing amounts of dietary EFA (Table 2). Dietary linoleate gives rise to 18:2, 20:4, and 22:5ω6[1] in erythrocyte lipids. Dietary arachidonate leads to an extremely high 20:4 concentration (more than 25% of the total fatty acids) and increases the 22:5ω6 concentration. Variations in dietary linolenate led to a rather confusing picture in linoleate and linolenate concentrations of erythrocytes in our present experiment. Very inconsistent changes were observed.

Concentration of 20:4 is lowered significantly by dietary linolenate. The fatty acids of the linolenate family, 20:5 and 22:5ω3, could be detected only in rats fed linolenate. The concentration of 20:3 is lowered most significantly by all three dietary polyunsaturated fatty acids. The concentrations of 22:6 were not determined in this experiment.

[1] This notation, indicating the first double bond counting from the terminal methyl group, is necessary to distinguish isomers arising from linoleate (ω6) and linolenate (ω3). Identification of 22:5ω6 was made via ozonolysis by J. Rahm in unpublished experiments.

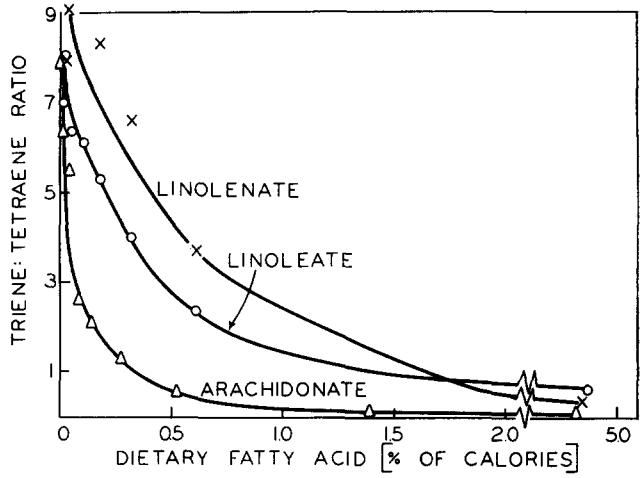

FIG. 1. The effect of varying levels of dietary EFA upon the ratio of 20:3 to 20:4 in depot fat.

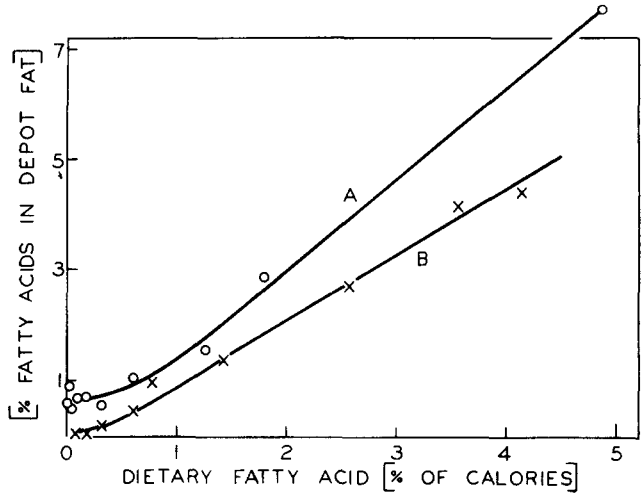

FIG. 2. Deposition of dietary linoleate and linolenate in depot fat of the rat. A = content of linoleate when linoleate is fed, B = content of linolenate when it is fed.

DISCUSSION

The alteration of rat depot fat by different dietary fats has been described in several investigations (4, 5). For example, cod liver oil leads to higher concentrations of unsaturated fatty acids of 20 and 22 carbon chain length and linoleic acid from cottonseed oil is stored in large amounts in depot fat. The present experiment shows that both dietary linoleate and linolenate are deposited in epididymal fat of the rat. The deposition is almost directly proportional to the dietary EFA (Fig. 2) when it comprises up to 5% of total calories. The drop in oleic acid concentration with increasing amounts of

TABLE 2. COMPOSITION OF THE UNSATURATED FATTY ACIDS OF ERYTHROCYTE LIPIDS (% OF TOTAL FATTY ACIDS)

EFA (% of cal)	Animals in Group	18:1	18:2	18:3	20:3	20:4	20:5	22:5ω6	22:5ω3
Fat free	24	20.29	0.93		12.70	5.58		0.34	
Linoleate									
0.009	6	19.03	0.50		15.76	7.90		0.46	
0.02	6	18.43	0.63		12.29	6.90		0.42	
0.05	6	17.98	0.60		14.06	8.55		0.55	
0.10	6	17.66	0.98		11.08	10.73		0.64	
0.18	6	18.34	1.56		8.79	11.51		0.57	
0.32	6	17.30	1.01		7.42	12.62		0.68	
0.61	6	15.67	1.62		4.31	17.27		0.84	
1.26	2	14.06	1.70		1.87	15.12		0.85	
1.79	2	15.78	1.97		0.75	13.88		0.68	
4.87	2	14.25	3.72		0.48	18.74		1.80	
Arachidonate									
0.007	6	19.75	0.30		14.31	7.38		0.54	
0.019	6	19.41	0.38		13.07	7.86		0.70	
0.04	6	18.49	0.37		11.79	8.53		0.60	
0.08	6	20.93	0.68		8.18	15.30		1.18	
0.14	6	18.15	0.29		5.93	15.56		0.96	
0.27	6	17.39	2.02		3.50	14.38		0.86	
0.52	6	15.23	0.26		1.53	22.15		1.16	
1.08	2	15.11	0.32		0.55	22.62		1.97	
1.39	2	15.67	0.87		0.36	25.02		1.53	
3.75	2	10.13	0.59		0.01	25.92		1.98	
Linolenate									
0.61	6	20.22	3.35	2.06	1.64	2.75	2.76	0.66	0.71
0.77	4	18.19	0.47	0.62	1.47	2.64	3.36	0.68	1.81
1.42	4	19.38	0.87	0.99	0.98	1.80	4.71	0.90	1.28
2.56	4	18.30	3.75	3.69	0.22	0.74	3.33	4.65	0.91
3.56	4	12.33	1.02	2.50	0.63	0.56	7.16	2.53	1.28
4.14	4	17.08	0.87	2.85	0.45	0.64	3.11	0.73	1.19
9.42	2	13.15	0.47	3.36	0.67	0.47	6.26	0.96	

dietary EFA has also been observed in lipids of heart (2), liver (1), and brain (3). Mead (9) suggested that the synthesis of the monoenoic fatty acid might be accelerated in fat deficiency to maintain a certain degree of total unsaturation and physical properties of the tissue lipids, which, under normal conditions, are provided by dietary EFA and their metabolites. This might be especially important in the case of the relatively large amounts of depot fat.

The ratio of eicosatrienoic to eicosatetraenoic acids has served as an expression for the metabolic status in regard to EFA supplementation (1) and as an indicator for EFA deficiency (10, 11). The use of the triene/tetraene ratio was based on the analyses of lipids from liver and heart (tissues known as sites of fatty acid synthesis) and from erythrocytes and serum.

Although the amounts of 20:3 and 20:4 are very small in depot fat (less than 0.5% of the total fatty acids in most cases), their ratios follow, in very smooth curves, the pattern found in heart and liver tissue. Arachidonate proves to be the most efficient dietary EFA in lowering the triene/tetraene ratio; linoleate is next best. The same sequence of efficiency in curing fat-deficiency symptoms has been described (1). Even the curve relating dietary linolenate to the ratio of 20:3 to 20:4 follows the same pattern, although the 20:4 concentration does not increase in this case. These findings show that the triene/tetraene ratio is a very sensitive indicator for the EFA status of the rat, not only when measured in the lipids of organs, but also in depot fat.

The changes in the concentration of erythrocyte fatty acids show a larger variation than observed in any other tissue we have studied. Oleate concentration is lowered from approximately 20% in fat deficiency to 14, 10, and 13% for the highest level of dietary linoleate, arachidonate, and linolenate, respectively. Concentrations of 20:3 vary from 15% in fat deficiency to

0.5% of total fatty acids in animals fed diets high in linoleate. Arachidonate levels show more than threefold increases within the ranges of linoleate and arachidonate fed to our animals. Dietary linolenate lowers the concentration of arachidonate by inhibiting its synthesis from linoleate (12). This effect is quite significant. Less than 0.5% arachidonate is present in animals fed diets high in linolenate, whereas more than 5% 20:4 can be found in animals on the completely fat-free diet.

Most of the changes in polyunsaturated fatty acid metabolism described from analysis of heart and liver tissue have been detected in adipose tissue and erythrocytes. The familiar patterns of change of triene/tetraene ratio as a consequence of variations in content of dietary EFA take place in adipose tissue and erythrocytes. Therefore, these readily accessible tissues can be used to assess the EFA status of individuals.

The authors want to thank Herbert Hayes, Joseph Seufert, and Dennis Sukkestad for technical assistance.

REFERENCES

1. Mohrhauer, H., and R. T. Holman. *J. Lipid Res.* **4**: 151, 1963.
2. Mohrhauer, H., and R. T. Holman. *International Conference on Biochemical Problems of Lipids*, Birmingham, 1962.
3. Mohrhauer, H., and R. T. Holman. *J. Neurochem.* (in press).
4. Hilditch, T. P. *The Chemical Constitution of Natural Fats*, 3rd Edition, New York, John Wiley & Sons, Inc., 1956, pp. 85–89.
5. Deuel, H. J., Jr. *The Lipids, Their Chemistry and Biochemistry*, Vol. II, New York, Interscience Publishers, 1955, Chapters V and VI.
6. Witting, L. A., C. C. Harvey, B. Century, and M. K. Horwitt. *J. Lipid Res.* **2**: 412, 1961.
7. Folch, J., M. Lees, and G. H. Sloane Stanley. *J. Biol. Chem.* **226**: 497, 1957.
8. Shinowara, G. Y., and J. B. Brown. *J. Biol. Chem.* **134**: 331, 1940.
9. Mead, J. F. *J. Biol. Chem.* **227**: 1025, 1957.
10. Holman, R. T. *J. Nutr.* **70**: 405, 1960.
11. Hill, E. G., E. L. Warmanen, C. L. Silbernick, and R. T. Holman. *J. Nutr.* **74**: 335, 1961.
12. Mohrhauer, H., and R. T. Holman. *J. Nutr.* (in press)