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Rat Brain Fatty Acid Composition: Effect of Dietary Fat and Age[1]

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The polyunsaturated fatty acid composition of human brain changes, as well as progressively decreases, with age. To determine if whole brain rat lipid shows similar changes with age, rats born to mothers fed semi-synthetic diets were raised on the same diet as their mothers for varying periods prior to sacrifice. Whole brain lipid composition was determined for female offspring, fed diets containing 20%w (by weight) of either lard or safflower oil, from age 6 days to 730 days. The percentage of 20:4ω6 and 22:5ω6 decreased with age; as in man these changes were compensated for largely by increases in 18:1ω9. In contrast, 22:6ω3 rose gradually from 6 to 730 days of age. Varying the degree of unsaturation and/or the amount of dietary fat, with the exception of lard, did not influence the fatty acid composition of whole brain lipid or of the two major phospholipids, phosphatidyl ethanolamine (PE) and phosphatidyl choline (PC). Lard was found to contain trace amounts of 22:6ω3; this acid was avidly retained in the brain accompanied by corresponding decreases in 22:5ω6. The functional significance of the observed age-related brain lipid changes is unknown; it is likely that the lipid changes are in some way related to the deterioration of the central nervous system with time.

STUDIES on the fatty acid composition of human brain have shown that the over-all spectrum of polyunsaturated fatty acid changes, as well as progressively decreases with age (Rouser, Kirtchevsky, Siakotos, & yamamoto, 1970; Rouser & Yamamoto, 1968). The reason(s) for these changes, and their possible clinical significance, is not known. The purpose of the present study was to determine if polyunsaturated fatty acids in rat brains likewise change and/or decrease with age, and if this change and/or decline is related to the type of dietary fat. To maximize the potential effects of dietary fat on brain lipid fatty acid composition, rats born of mothers on semi-synthetic diets containing lard, safflower-olive (oleinate, a mutant of safflower oil in which oleic acid has replaced linoleic acid) or safflower oil, as a sole source of lipid, were kept on the same diet as their mothers throughout their life-span.

METHODS AND RESULTS

Sprague-Dawley rats were obtained from Charles River Breeding Laboratories at weaning and caged 4/cage (stainless steel, 11½ x 18¼ x 6½ in.). The rats were maintained in an air-conditioned room at 75 - 78 F at a humidity of 50-60%. Cages were changed 2-3 times per week; the bedding was sterilized shredded corn cobs (San-i-Cel, Paxton Processing Co., Paxton, IL). At age 1 mo. the females were placed on semi-synthetic diets (Harman, 1971) containing 5 or 20%w (% by weight) of lard, oleinate, safflower oil, and safflower oil plus 20 mg of α-tocopherol acetate per 100 gm of finished diet; the lipids were of edible grade. The fatty acid composition of these three dietary lipids is shown in Table 1. When the rats were 3 mo. old, 1 male, same age as the females and kept prior to mating on a

Table 1. Dietary Lipids: Fatty Acid Analysis.

Carbon Number	Safflower (%)	Lard[d] (%)	Safflower-Olive (%)
10			0.1[a]±0.1[c]
12			0.2 ±0.1
14	0.5[a]±0.3[c]	1.7[b]±0.1[c]	0.4 ±0.1
16	6.7 ±0.6	26.5	±0.6
16:1ω7		2.1 ±0.4	0.1 ±0.05
18	1.6 ±0.3	12.1 ±0.7	1.2 ±0.1
18:1ω9	11.1 ±0.05	49.8 ±1.7	75.5 ±0.0
18:2ω6	79.7 ±0.1	10.3 ±0.2	15.8
20 or 22 acids	0.1 ±0.05	2.4 ±1.4	

[a]The average of two samples taken at a 6-mo. interval from the dietary oil.
[b]The average of three samples taken at intervals within a year.
[c]Standard error of the mean.
[d]The lard contained a trace amount of 22:6ω3 as the free fatty acid.

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commercial pelleted diet (Rockland, Teklad, Monmouth, IL), was placed in a cage with 4 females for a period of 7 days and then removed. The offspring at 23 days of age were weaned, sexed, caged 4 per cage, and maintained on the same diet as their mothers. Rats were decapitated at 6, 23, 100, 176, and 730 days of age. At sacrifice, whole brains (0.5-2.0 gm, depending on the age of the rat) were taken along with about 2 gm of liver; in a few cases the gastrocnemus muscle (1.5-2.0 gm) was dissected from one leg. These tissues were immediately frozen on dry ice, wrapped in aluminum foil, and stored in nitrogen-filled vials at -70 C. until the time lipid analyses could be carried out.

Rouser et al. (1970) and Folch, Lees, & Sloane-Stanley (1957) methods were used for brain and liver lipid extractions, respectively. The Folch method was used at a later date when extracting skeletal muscle. Phosphatidyl ethanolamine (PE) and phosphatidyl choline (PC), the two major phospholipids in membranes, were also separated by TLC[3] using 65/25/4, chloroform/methanol/water as the eluting solvent (Tami, Matsukawa, & Satake, 1971) and fatty acids analyzed. Cholesterol esters were not separated and the fatty acids analyzed, as only a small fraction of the cholesterol in the central nervous system (CNS) is esterified (McIlwain, 1966).

Neutral lipids were separated into free fatty acid, mono-, di-, and triglycerides by TLC using 90/10/1, petroleum either (B.P. 30-60 °) /ethyl ether/glacial acetic acid as an eluting solvent.

Lipids were hydrolyzed and fatty acids methylated using 14%w/v BF_3 in anhydrous methanol (Rouser et al., 1970) and methyl esters run on an F and M model 810 gas

[3]TLC (thin-layer chromatography) plates were made by spreading a Silica Gel G (Applied Sci. Lab.) slurry (made with water) to 200μ's thickness. Plates were activated for 1 hour and stored in a desiccator until use.

Table 2. Sprague-Dawley Female Rats: Effect of Dietary Fat (20%w) on Fatty Acid Composition of Total Brain Lipid at Age 6, 23, 100, 176, and 730 Days.

A. Lard and Safflower-Olive Diets

Carbon No.	Lard 20% Age (Days) 6[a] (4)[b]	23 (4)	100 (4)	176 (3)	730 (1)	Safflower-Olive 20% Age (Days) 6[c] (4)	23[c] (4)	100 (0)	176 (0)	730 (1)
16:0	26.1 ±0.9[d]	20.8 ±1.2	16.4 ±0.4	17.1 ±0.2	20.2	32.1	21.1	—	—	18.0
18:0	13.6 ±0.8	18.4 ±0.7	20.7 ±0.4	19.2 ±0.2	18.4	14.0	19.0	—	—	20.0
18:1ω9	12.2 ±0.2	14.8 ±0.6	18.1 ±1.1	22.7 ±0.4	28.0	13.5	15.3	—	—	27.8
18:2ω6	0.6 ±0.1	0.5 ±0.1	0.4 ±0.1	0.0 ±0.0	0.5	0.8	0.6	—	—	0.2
20:4ω6	15.6 ±0.9	16.1 ±0.4	12.3 ±0.3	12.8 ±0.2	11.0	17.3	16.0	—	—	12.8
22:4ω6	4.2 ±0.5	5.6 ±0.1	5.2 ±0.2	4.8 ±0.1	3.0	3.5	5.8	—	—	2.7
22:5ω6	10.5 ±0.5	10.4 ±0.2	7.7 ±0.8	7.7 ±0.4	T	9.6	13.9	—	—	5.8
22:6ω3	4.6 ±0.9	9.1 ±0.6	10.0 ±0.5	11.0 ±0.8	12.2	5.1	4.8	—	—	6.4

[a] Rats at age 6 days were not sexed.
[b] Number of rats.
[c] The brains of four rats were pooled.
[d] Standard error.

B. Safflower and Safflower Plus Vitamin E Diets

Carbon No.	Safflower 20% Age (Days) 6[a] (5)[e]	23 (4)	100 (4)[b]	176 (3)	730 (1)	Safflower 20 % plus 20 mg Vit. E Age (Days) 6[a] (4)	23 (4)[c]	100 (0)	176 (0)	730 (1)
16:0	28.9	19.3	17.9 ±0.7	17.0 ±0.3	20.0	29.0	19.5	—	—	20.0
18:0	13.1	18.6	20.7 ±0.6	19.7 ±0.4	19.4	15.6	19.6	—	—	20.0
18:1ω9	9.5	14.9	14.2 ±1.1	21.8 ±0.8	24.0	11.1	13.5	—	—	26.0
18:2ω6	1.9	1.4	1.3 ±0.5	0.4 ±0.1	2.4	1.1	1.7	—	—	0.8
20:4ω6	16.4	16.9	9.7 ±1.1	12.5 ±0.2	11.4	16.3	14.3	—	—	11.0
22:4ω6	5.3	6.5	6.1 ±0.2	5.0 ±0.1	3.4	5.8	6.4	—	—	3.0
22:5ω6	12.5	15.7	13.0 ±1.5	12.8 ±1.0	8.0	13.3	13.6	—	—	6.3
22:6ω3	2.4	3.8	1.7 ±0.2	4.7 ±1.0	4.3	2.3	3.3	—	—	7.8

[a] Number of rats.
[b] The brains of the safflower group rats at 100 and 176 days of age were analyzed individually and standard error computed for each component, while brains of the other groups were pooled for analysis.
[c] Analysis of male brains, as female data are not available; inspection of Table 3 indicates that brain fatty acid composition of male and female rats are almost identical.
[d] Rats at 6 days were not sexed.

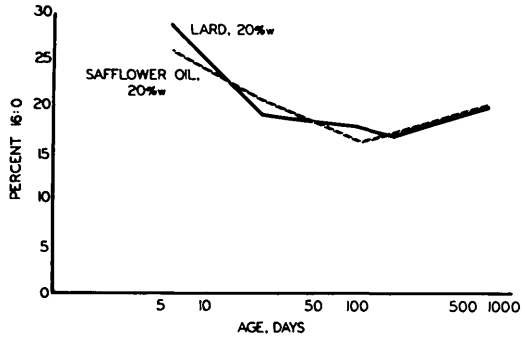

Fig. 1A. Sprague-Dawley female rats: effect of dietary lard (20%w) and safflower oil (20%w) on the percentage of 16.0 in the fatty acids of whole brain lipid.

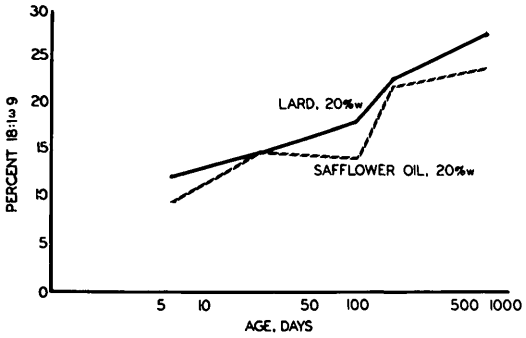

Fig. 1B. Sprague-Dawley female rats: effect of dietary lard (20%w) and safflower oil (20%w) on the percentage of 18:1ω9 in the fatty acids of whole brain lipid.

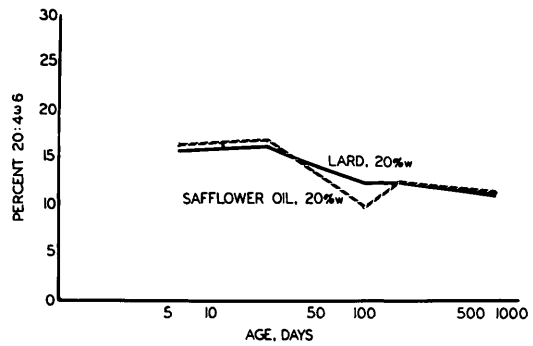

Fig. 1C. Sprague-Dawley female rats: effect of dietary lard (20%w) and safflower oil (20%) on the percentage of 20:4ω6 in the fatty acids of whole brain lipis.

chromatograph[4] with flame ionization detector.

The linearity of response of the flame ionization detector to the various components was

[4]*Column* — 6 ft, ¼ in stainless steel packed with 10% diethylene glycol succinate on 80-100 mesh Diaport s. *Operating Conditions* — Oven temp. 190 C; Det. temp. 273 C; Inj. port 270 C; Air -600 ml/min; Hydrogen -55 ml/min; Nitrogen -70 ml/min.

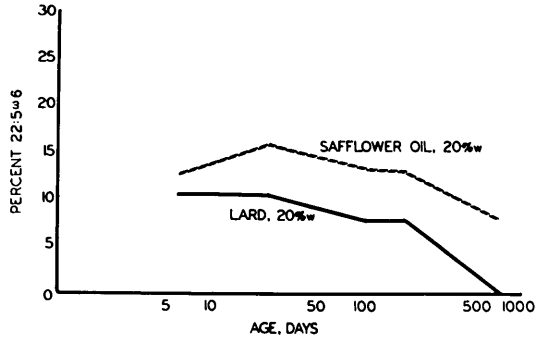

Fig. 1D. Sprague-Dawley female rats: effect of dietary lard (20%w) and safflower oil (20%w) on the percentage of 22:5ω6 in the fatty acids of whole brain lipid.

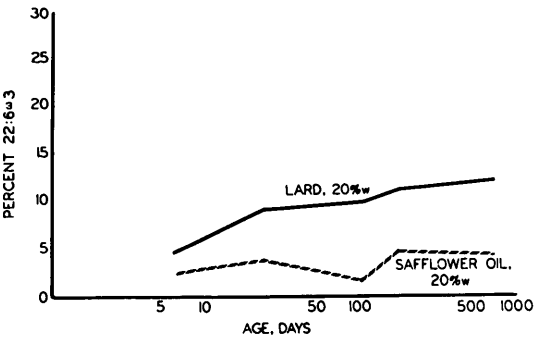

Fig. 1E. Sprague-Dawley female rats: effect of dietary lard (20%w) and safflower oil (20%w) on the percentage of 22:6ω3 in the fatty acids of whole brain lipid.

evaluated using three different standard mixtures of fatty acids (Hormel Institute, Austin, MN). The gas chromatograph was found to be linear within the range C_{14}-C_{22} for both increasing carbon number and unsaturation. Fatty acids higher than C_{22} were not determined; in total, fatty acids higher than C_{22} represent less than 0.50% of the fatty acids present in whole brain (Baker, 1961). The relative retention time for each component was calculated using oleic acid as an internal standard. During the period of analysis individual components were checked frequently, qualitatively and quantitatively, with individual standards (Hormel Institute). The area under each peak was determined and the percentage composition calculated from these values (Bartlet & Smith, 1960). Less than 0.1 μg of fatty acid methyl ester could be detected by the chromatograph in the C_{12}-C_{22} range and <0.5 μg of those C_{22}-methyl esters with multiple double bonds.

The whole brain fatty acid compositions at age 6, 23, 100, 176, and 730 days of age of

Table 3. Sprague-Dawley Rats. Effect of Dietary Fat on Fatty Acid Composition of Total Brain Lipid.

A. At 6 Days of Age.

Carbon Number	Saff. 5%	Saff. 20%	Saff.-Olive 5%	Saff.-Olive 20%	Saff. 5% + 20 mg Vit. E	Saff. 20% + 20 mg Vit. E	Lard 5% + 2.5 mg Vit. E	Lard 20% + 2.5 mg Vit. E ±S.E.[b]
14	2.9%	1.6%	2.7%	1.9%	1.5%	1.5%	2.0%	4.4% ±1.9
15[a]	0.2	2.0	0.1	0.7	0.1	1.2	1.3	0.3 ±0.1
16	28.6	28.9	26.1	32.1	26.4	29.0	26.4	26.1 ±0.9
16:1	1.5	1.0	1.3	1.4	1.7	Trace	2.1	1.7 ±0.3
17:1[a]	0.2	0.2	0.2	Trace	0.2	1.2	0.2	0.3 ±0.1
18	14.1	13.1	13.9	14.0	15.0	15.8	13.6	13.6 ±0.8
18:1ω9	9.4	9.5	11.9	13.5	11.6	11.1	11.2	12.2 ±0.2
18:2ω6	1.3	1.9	0.7	0.8	1.0	1.1	1.6	0.6 ±0.1
18:3 and 20:1ω9	1.8	1.0	1.5	0.2	0.1	0.5	0.3	1.6 ±0.7
20:1ω7[a]	1.5	1.1	0.6	0.4	0.3	1.0	0.3	0.9 ±0.3
20:4ω6	16.0	16.4	15.9	17.3	18.2	16.3	17.2	15.6 ±0.9
22:4ω6	6.2	5.3	3.9	3.5	4.5	5.8	4.5	4.2 ±0.0
22:5ω6	15.2	12.5	12.2	9.6	16.2	13.3	11.9	10.5 ±0.5
22:6ω3	1.4	2.4	5.1	5.1	2.2	2.3	5.2	4.6 ±0.9

B. At 23 Days of Age.

Carbon Number	Sex	Saff. 5%	Saff. 20%	Saff.-Olive 5%	Saff.-Olive 20%	Saff. 5% + 20 mg Vit. E	Saff. 20% + 20 mg Vit. E	Lard 5% + 2.5 mg Vit. E	Lard 20% + 2.5 mg Vit. E S.E.[b]
14	Male	0.4%	0.6%	0.7%		0.1%	0.8	1.1%	0.7% ± 0.3
	Female	0.4	0.4	0.5	0.4	0.6		1.0	0.2 ± 0.1
15[a]	Male	0.2	0.3	0.2		0.6	1.5	0.2	0.8 ± 0.2
	Female	0.9	0.2	0.2	0.2	1.3		0.2	1.1 ± 0.3
16	Male	23.4	19.8	21.2		19.6	19.5	24.1	18.9 ± 1.8
	Female	21.0	19.3	21.6	21.0	20.2		19.1	20.8 ± 1.2
17:1[a]	Male	0.3	0.4	0.3		1.3	1.2	0.3	1.3 ± 0.3
	Female	1.4	0.3	1.1	0.5	0.7		0.2	0.8 ± 0.4
18	Male	19.2	19.3	16.6		19.9	19.6	20.4	17.7 ± 0.3
	Female	19.6	18.6	16.2	19.0	16.9		18.3	18.4 ± 0.3
18:1ω9	Male	13.7	13.2	14.9		13.2	13.5	14.2	14.7 ± 0.4
	Female	12.8	14.9	14.5	15.3	12.2		14.7	14.8 ± 0.6
18:2ω6	Male	1.3	1.7	1.1		1.4	1.7	1.2	0.8 ± 0.1
	Female	1.3	1.4	1.0	0.6	1.5		1.0	0.5 ± 0.1
18:3 and 20:1ω9	Male	0.7	2.4	0.6		4.3	5.0	0.5	1.4 ± 0.3
	Female	3.2	0.6	0.5	1.3	2.1		0.6	2.0 ± 0.3
20:1ω7[a]	Male	0.3	1.3	0.3		1.3	1.5	0.3	0.7 ± 0.2
	Female	1.5	0.4	0.4	0.6	1.3		0.2	1.0 ± 0.1
20:4ω6	Male	16.3	16.0	18.4		15.4	14.3	16.0	17.3 ± 1.1
	Female	14.7	16.9	18.2	16.0	16.1		17.7	16.1 ± 0.4
22:4ω6	Male	6.6	6.6	6.3		6.4	6.4	5.3	6.0 ± 0.2
	Female	6.9	6.5	6.4	5.8	7.5		6.0	5.6 ± 0.1
22:5ω6	Male	14.9	14.6	14.4		15.2	13.6	13.0	11.7 ± 0.5
	Female	14.4	15.7	14.5	13.9	16.9		15.1	10.4 ± 0.2
22:6ω3	Male	1.4	3.4	4.4		2.1	3.3	2.9	8.4 ± 0.2
	Female	1.5	3.8	4.3	4.8	2.3		5.2	9.1 ± 0.6

[a]Tentative identification.

[b]The brain of 4 rats were pooled in all groups except for the group fed the 20% lard diet. These brains were analyzed individually and standard error computed for each component.

female rats receiving the 20% lard and safflower oil diets are given in Table 2 along with safflower-olive and safflower oil + 20 mg Vitamin E data for ages 6, 23 and 730 days; the percentage of the five major fatty acid components — 16.0, 18:1ω9, 20:4ω6, 22:5ω6, and 22:6ω3 in the brains of the lard and safflower oil groups, are plotted as a function of age from 6 to 730 days in Fig. 1A-E.

The percentages of 16:0, 18:1ω9 and 20:4ω6 were about the same for the four diets at all ages. Palmitic acid declined from a value of about 28% at 6 days to 17% at 100 days and then increased to 20% at 730 days. In contrast, 18:1 rose from around 12% at 6 days to approximately 26% at 730 days of age. Arachiodonic acid declined from a level of about 16% at age 6-23 days to around 11% at age 730 days.

The level of 22:5ω6 in the brains of rats fed the lard diet decreased from 10% at 23 days to about 0% at 730 days, while corresponding values for rats receiving the other three diets were 14 and 6%. In contrast, 22:6ω3 in the brain of rats fed the lard diet rose from 5% at 6 days to 12% at 730 days while the corresponding figures for the other three groups were: safflower-olive — 5 and 6%; safflower — 2.5 and 4%; safflower plus 20 mg Vitamin E — 2.3 and 7.8%.

Statistical anaylsis of the data in Table 2 were limited to those cases where the brains were not pooled and more than one brain was analyzed. The changes in the percentages of 18:1ω9, 22:5ω6 and 22:6ω3 in the brains of rats on the lard diet between ages 6 and 176 days, and of 18:1ω9 and 22:6ω3 in the safflower oil group between 100 and 176 days of age, are significant at a level of $p<0.001$ (Mann-Whitney U test). Inspection of Table 2 shows that the changes between age 6 and 730 days in the fatty acid composition of whole brain lipid of rats in the four 20%w dietary groups were similar.

Rats in the 5%w lard and 5%w safflower oil groups and the 5 and 20%w oleinate and safflower oil plus vitamin E groups, with the exception of one female in each of the 20%w oleinate and safflower oil plus Vitamin E groups, were not available for this study beyond 23 days of age. As can be seen in Tables 3A and 3B, at 6 and 23 days of age, there was no effect of any of the 8 diets on brain total lipid fatty acid components except for the elevated levels of 22:6ω3 and lower 22:5ω6 levels in the brain of rats receiving the 5 and 20%w lard diets.

In contrast to the brain lipids, liver fatty acid components at 6 and 23 days of age reflected that of the dietary fat; the lard groups showed the same 22:6ω3 and 22:5ω6 changes as in brain but to a lesser extent; the data for age 23 days are presented in Table 4. Although the fatty acid components of gastrocnemus muscle

Table 4. Sprague-Dawley Rats. Effect of Dietary Fat on Fatty Acid Composition of Total Liver Lipids at Age 23 Days.

Carbon No.	Sex	Saff. 5%	Saff. 20%	Saff.-Olive 5%	Saff.-Olive 20%	Saff. 5% + 20 mg Vit. E	Saff. 20% + 20 mg Vit. E	Lard 5% + 2.5 mg Vit. E	Lard 20% + 2.5 mg Vit. E S.E.[b]
14	Male	0.5%	0.8%	0.8%	%	0.4%	1.0%	0.8%	0.6% ± 0.0%
	Female	0.6	0.8	0.8	1.1	1.0	0.9	0.9	0.6 ± 0.1
15[a]	Male	0.0	0.1	0.1		0.0	0.1	Trace	0.0 ± 0.0
	Female	0.0	0.1	0.0	0.0	0.0	0.1	0.0	0.0 ± 0.0
16	Male	15.5	13.2	15.6		15.6	14.1	14.4	17.2 ± 0.3
	Female	14.1	13.6	15.8	16.3	16.3	12.1	13.7	19.9 ± 1.0
18	Male	16.3	8.3	8.9		17.7	11.7	14.4	14.1 ± 0.5
	Female	15.2	9.8	15.7	12.8	13.7	10.9	12.7	11.2 ± 1.1
18:1ω9	Male	4.5	39.3	19.0		5.2	6.6	5.9	17.8 ± 0.5
	Female	5.8	36.4	13.8	17.2	8.2	7.8	8.0	28.2 ± 3.6
18:2ω6	Male	22.0	17.4	26.0		21.4	36.5	22.7	18.6 ± 0.9
	Female	25.1	16.5	16.3	18.2	26.1	37.5	26.2	12.3 ± 2.5
18.3 and	Male	1.2	0.4	1.6		0.1	0.1	0.9	1.0 ± 0.3
20:1ω9	Female	0.1	0.5	0.2	0.3	0.1	0.1	0.1	0.5 ± 0.3
20:2[a]	Male	3.1	0.2	3.0		0.6	1.7	2.3	1.6 ± 0.1
	Female	0.8	0.1	0.3	0.9	1.1	1.7	1.3	0.4 ± 0.1
20:4ω6	Male	23.6	14.0	12.8		26.1	19.9	24.2	18.0 ± 1.1
	Female	25.6	15.6	25.2	22.6	20.7	19.8	24.9	16.7 ± 1.4
22:4ω6	Male	5.0	1.2	3.0		3.0	2.9	4.3	3.0 ± 0.3
	Female	3.2	1.2	2.0	2.4	3.7	3.3	2.9	1.9 ± 0.3
22:5ω6	Male	7.8	3.9	3.7		9.4	5.0	8.6	4.2 ± 0.3
	Female	8.9	4.3	7.6	6.9	8.3	5.4	7.7	3.9 ± 0.2
22:6ω3	Male	0.2	0.6	0.7		0.3	0.2	1.1	1.6 ± 0.2
	Female	0.3	0.8	1.9	1.1	0.4	0.3	1.2	1.7 ± 0.1

[a]Tentative identification.

[b]The livers of 4 rats were pooled in all groups except for the group fed the 20% lard diet. These livers were analyzed individually and standard error computed for each component.

Table 5. Sprague-Dawley Female Rats: Effect of Dietary Fat (20%w) on the Fatty Acid Composition of Total Muscle (Gastrocnemius) Lipid at Age 8 Mo. - Major Components.

Carbon No.	Safflower (%)	Safflower + 20 mg Vit. E (%)	Lard (%)
16	14:2[a] ± 0.2[c]	13.5[a] ± 0.9[c]	20.7[a] ± 0.4[c]
18	8.1 ± 0.5	8.2 ± 1.1	9.5 ± 0.4
18:1ω9	11.4 ± 0.4	10.1 ± 0.7	34.0 ± 1.9
18:2ω6	45.1 ± 1.8	39.7 ± 2.6	11.3 ± 0.5
20:4ω6	8.9 ± 0.5	10.0 ± 1.4	9.2 ± 1.0
22:5ω6	5.4 ± 0.5	8.4 ± 3.8	1.4 ± 0.3
22:6ω3	0.9 ± 0.2	0.7 ± 0.4	4.6 ± 0.5

[a] Each figure represents an average of five values determined from 5 animals.

[b] Each figure represents an average of three values determined from 3 animals.

[c] Standard error of the mean.

those receiving the other three diets, prompted reanalysis of the dietary lipids. They were separated into free fatty acids, and mono-, di-, and triglyceride fractions by TLC using 90/10/1, petroleum ether/ethyl acetate/glacial acetic acid as a eluting solvent. In safflower oil and in oleinate no components were found beyond a $C_{20:1}$ fatty acid (sometimes mistaken for 18:3ω3, the precurser of 22:6ω3). No long chain components were noted in the lard glyceride fraction but the free fatty acid fraction contained several totaling about 1-3% of the lard fatty acids; one component in this group was identified as 22:6ω3. The small amount of 22:6ω3 in the free fatty acid fraction of lard was evidently the source of the elevated 22:6ω3 in the brain of rats fed the lard diet.

Table 6. Sprague-Dawley Female Rats: Effect of Dietary Fat (20%w) on the Percentage of 22:5ω6 and 22:6ω3 in the Fatty Acids Present in Whole Tissue and the Phosphatidyl Ethanolamine (PE) and Phosphatidyl Choline (PC) Fractions of Liver (Age 23 Day) and Brain (Age 730 Day).

	Safflower (%)						Lard (%)					
Carbon No.	Liver[a] WT[c]	PE	PC	Brain[b] WT	PE	PC	Liver[a] WT	PE	PC	Brain[b] WT	PE	PC
22:5ω6	4.3	14.4	6.9	0.8	11.7	4.2	3.9	14.0	6.0	0.7	1.2	1.0
22:6ω3	0.8	3.5	1.5	4.5	6.4	2.1	1.7	6.9	2.5	12.1	18.7	8.7

[a] 23 days old.

[b] 730 days old.

[c] WT—whole tissue.

Table 7. Sprague-Dawley Female Rats: Effect of Adding 22:6ω3 to the Diet (5%w Safflower Oil + Vitamin E Group) for 30 Days, Starting at Age 26 Days, on the Percentage of 22:6ω3 on Whole Brain Lipids.

	Age (Days)				
	26	36	41	46	57
Controls	1.5 ± 0.1[a]	—	1.3 ± 0.1	—	1.5 ±0.1
Treated	"	3.4 ± 0.1	—	4.6 ± 0.1	7.8 ± 0.2

[a] Standard error.

from 8-mo. old female rats (Table 5) likewise reflected the dietary fat composition, the differences between 22:5ω6 and 22:6ω3 were more pronounced than in liver but less so than in brain.

The alterations in 22:5ω6 and 22:6ω3 observed in whole brain, liver, and skeletal muscle are accentuated in the phosphatidyl ethanolamine fraction and to a lesser extent in The data in Table 7 show that the percentage for liver (age 23 days) and brain (730 days) in Table 6.

The inverse relationship between 22:6ω3 and 22:5ω6 and the higher level of 22:6ω3 in the brains of rats fed the lard diet as compared to

Support of this possibility was obtained by feeding rats for 30 days after weaning, a 5% safflower oil diet plus 50 mg 22:6ω3 (free acid, Hormel Institute) with 20 mg of α-tocopherol per 100 grams of finished diet. The added 22:6ω3 made up 1% of the dietary fatty acids. The data in Table 7 show that the percentage composition of 22:6ω3 of the brains of control and supplemented rats; 22:6ω3 increased steadily in the brain lipids of the supplemented rats over the 30-day period from 1.5% at 26 days to 7.8% at 57 days of age.

Although 22:6ω3 was rapidly incorporated into brain lipids of young rats, this was not the case for older animals. At age 6 mo., female rats receiving the 20%w lard diet were placed on a 20%w safflower oil diet while the same number of females being fed the safflower diet were switched to the lard diet. The rats were fed their new diets for 2 mo. and then killed. Switching the diets for 2 mo. did not alter whole brain fatty acid composition (Table 8).

Rats fed diets containing little or no 22:6ω3 or its precursor, i.e., oleinate and safflower oil diets, derive them from their mothers during nursing (Table 9 — analysis of stomach contents of 6 day old rats) and probably, transplacentally before birth.

Table 8. Sprague-Dawley Female Rats: Effect of Switching Lard (20%w) and Safflower Oil (20%w) Diets for 2 Mo. on Total Brain Lipid Fatty Acid Composition.

Age	Lard 20% 176 Day	Safflower 20%[a] 240 Day	Safflower 20% 176 Day	Lard 20%[b] 240 Day
No. of Animals (Female)	3	4	3	3
Carbon No.	% Composition			
12		0.1 ±0.0		
12:1[c]		0.1 ±0.0		
14	0.2		0.2 ±0.1	0.2 ±0.0
15[c]	0.7 ±0.1[d]	1.9 ±0.0	1.0 ±0.2	0.5 ±0.5
16	17.1 ±0.2	16.9 ±0.2	17.0 ±0.3	17.2 ±0.4
16:1		0.5 ±0.0		0.5 ±0.0
17:1[c]	0.9 ±0.1	2.4 ±0.1	1.3 ±0.3	
18	19.2 ±0.2	20.3 ±0.1	19.7 ±0.4	20.6 ±0.2
18:1ω9	22.7 ±0.4	21.8 ±0.3	21.8 ±0.8	20.9 ±0.5
18:2ω6	0.3 ±0.1	1.2 ±0.1	0.4 ±0.1	1.1 ±0.2
20		0.1 ±0.1		
18:3 and 20:1ω9	3.0 ±0.3[d]	2.9 ±0.3	3.3 ±0.2	3.0 ±0.1
20:1ω7			0.4 ±0.1	
20:2		0.3 ±0.0		
22		0.3 ±0.0		
20:4ω6	12.8 ±0.2	11.4 ±0.1	12.5 ±0.2	10.4 ±0.3
22:1[c]		0.2 ±0.0		
22:4ω6	4.8 ±0.1	3.6 ±0.1	5.0 ±0.1	4.0 ±0.2
22:5ω6	7.6 ±0.4	5.9 ±0.2	12.8 ±1.7	11.4 ±0.1
22:6ω3	11.0 ±0.8	9.8 ±0.1	4.7 ±0.9	3.2 ±0.1

[a]Rat maintained on Safflower 20% for 2 mo. — originally maintained on Lard 20%.
[b]Rat maintained on Lard 20% for 2 mo. — originally maintained on Safflower 20%.
[c]Tentative identification.
[d]Standard error.

Table 9. Sprague-Dawley Rats: Fatty Acid Analysis of Stomach Contents from 6-Day-Old Rats (not sexed).

Fatty Acid Carbon No.	Diet: Lard 20% % Composition	Safflower 20% % Composition
14	5.4 ± 0.6[a]	6.4 ± 1.2
16	29.9 ± 1.4	14.2 ± 0.5
16:1	0.7 ± 0.4	
18	2.0 ± 0.6	0.3 ± 0.2
18:1ω9	51.2 ± 1.6	17.2 ± 2.3
18:2ω6	5.7 ± 0.9	54.4 ± 3.6
20:4ω6	1.3 ± 0.1	4.0 ± 0.4
22:4ω6	0.2 ± 0.0	1.0 ± 0.1
22:5ω6	0.2 ± 0.0	0.9 ± 0.1
22:5ω3	0.1 ± 0.0	
22:6ω3	0.1 ± 0.0	0.1 ± 0.0

[a] Standard error determined from three individual samples in each group.

DISCUSSION

In agreement with human data the percentages of the polyunsaturated fatty acids, 20:4ω6 and 22:5ω6, in whole rat brain lipid, decreases with age; as in human brain these changes are compensated for largely by increases in the level of 18:1ω9. In contrast, whole brain 22:6ω3 rose gradually throughout the period of study, from 6 to 730 days of life. Varying the degree of unsaturation and/or the amount of dietary fat, with the exception of lard, did not influence the fatty acid composition of whole brain lipid or of the two major phospholipids, phosphatidyl ethanolamine (PE) and phosphatidyl choline (PC), while the corresponding data for liver and muscle reflected the dietary fat composition. Addition of α-tocopherol acetate to safflower oil diets had no effect on the lipid composition of any of the three tissues studied — brain, liver, muscle — with the possible exception of increasing the content of 22:6ω3 in the brain at 730 days of age.

In the case of the lard diets the 22:6ω3, present as the free acid in trace amounts — about 2% of the free fatty acid fraction which in turn was about 2% of the lard fatty acids, produced significant elevations of 22:6ω3 in brain lipids, and to a lesser extent in those of liver and muscle, with accompanying decreases in the level of 22:5ω6. The effect of dietary 22:6ω3 was greater in the phosphatidyl fraction (PE and PC) than in whole brains. Previous studies (Manzoli, Barbieri, & Carinci, 1969; Sinclair & Crawford, 1972) have shown 22:6ω3 to be present in high concentrations in the PE and PC fraction of mitochondria/endoplasmic reticulum fractions of the brain gray matter. The present work indicates that 22:6ω3 is passed on from a mother to her nursing offspring and selectively taken up in the brain. Brain 22:6ω3, like retinal 22:6ω3 (Anderson & Maude, 1971), was found to be avidly retained; possibly due, at least in part (by analogy to the resistance of the 22:6ω3 — glycerol ester bond to hydrolysis by pancreatic lipase [Botting, Vandenburg, & Reiser, 1967]) to a relatively low rate of enzymatic hydrolysis.

The mechanism(s) by which the fatty acid composition of whole brain is altered by age remains to be determined. Likewise, the functional significance of age-related brain lipid changes is unknown; it is likely that the lipid changs are in some way related to the deterioration of the central nervous system with time.

SUMMARY

In whole rat brain lipid, the percentages of the fatty acids 20:4ω6 and 22:5ω6 were found to decrease with age; as in human brain, increases in 18:1ω9 largely compensated for these changes. Trace amounts of 22:6ω3 in lard were avidly retained in the brain, accompanied by a corresponding decrease in 22:5ω6. With the ex-

ception of the 22:6ω3 in lard, alterations in the amount and/or degree of unsaturation of dietary fat had no influence on brain fatty acid composition

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