JOURNAL OF THE AMERICAN GERIATRICS SOCIETY
Vol. XXV, No. 5

Printed in U.S.A.

Free Radical Theory of Aging: Effect of Age, Sex and Dietary Precursors on Rat-Brain Docosahexanoic Acid*

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ABSTRACT: Increasing the peroxidizability of dietary fat has an adverse effect on the function of the central nervous system (CNS) in the rat. This effect may be influenced by the level of docosahexanoic acid, a highly unsaturated fatty acid, selectively concentrated in the phospholipids of brain membranes. This study was aimed at determining the influence of age, sex, and the nature of a dietary lipid supplement – linolenic acid (18:3*w*3), docosahexanoic acid (22:6*w*3), or the same amount of 22:6*w*3 in the form of menhaden oil triglycerides – on the rate of increase in the percentage of 22:6*w*3 in the whole-brain fatty acids of rats between the ages of 1 and 12 months. The dietary lipid supplements were reflected in linear increases in the brain 22:6*w*3 of female rats throughout the study. Between 6 and 12 months of age, the rate of incorporation of dietary 22:6*w*3 and its precursors into the brain 22:6*w*3 of male rats dropped. At 12 months it was about half that for females in the case of 22:6*w*3 and menhaden oil, and about zero for 18:3*w*3. It is suggested that dietary 22:6*w*3 and its precursors may modify CNS function by altering membrane function and peroxidizability through changes in the concentration of 22:6*w*3 in membrane phospholipids.

Rat performance in maze and discrimination learning situations is impaired as the peroxidizability of dietary fat is increased (1). This effect of dietary fat may be produced, at least in part, by enhancing the level of more-or-less random free radical reactions throughout the brain. The enhancement is brought about through increases in the peroxidizability of the dietary fat or through the provision of easily oxidized lipids or their precursors that are concentrated in brain tissues, thereby enhancing the susceptibility of the tissues to free radical reactions.

The major contributor to the possible latter mode of brain dysfunction may be the ω3 family of fatty acids, the group of polyunsaturated fatty acids whose first double bond, starting from the CH_3 end, is between carbons 3 and 4. Both linolenic acid (carbon number 18:3ω3) and docosahexanoic acid (22:6ω3) are avidly taken up from the diet (2–4) and reflected in increased brain levels of 22:6ω3. Brain 22:6ω3 is tenaciously retained, resulting in increased levels with increasing age, largely at the expense of 22:5ω6 (2).

Brain 22:6ω3 is concentrated in the phospholipids of neurons, both in the perikaryon and the synaptic area (5–8); the percentages of 22:6ω3 in the acyl chains of the phosphatidylethanolamine, phosphatidylserine plus phosphatidylinositol, and phosphatidylcholine fraction are about 20–30, 10–15 and 1–3 respectively. Retinal rods are also rich in 22:6ω3 (9). Presumably the presence of this highly unsaturated, readily peroxidized, fatty acid in phospholipids has a beneficial effect on membrane permeability to water and organic compounds (10) and on the activities of membrane-bound enzymes (11).

The minimal dietary requirements, if any, for 22:6ω3 or its precursors (mainly 18:3ω3) for satisfactory central nervous system (CNS) function, has not been determined because of difficulties in

* The authors gratefully acknowledge the financial assistance of the late Mrs. Adah Millard, Omaha, Nebraska and of the U.S. Department of Agriculture.
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eliminating 18:3ω3 from the diet (3). It is also not known if there is a value or a range of values of brain 22:6ω3 associated with optimal brain function.

The purpose of the present study was to determine the influence of age, sex, and the nature of the dietary lipid supplement – 18:3ω3, 22:6ω3 or distilled triglycerides of menhaden oil – on the rate of increase in the percentage of 22:6ω3 in the fatty acids of whole-brain lipid.

METHODS AND RESULTS

The 130 rats used in this study were the seventh generation offspring born in this laboratory of Sprague-Dawley rats obtained (Charles River Breeding Laboratories, Wilmington, MA) at weaning in October 1972. The rats were maintained in an air-conditioned room at 75–78°F at a humidity of 50–60 percent. They were kept, 4 to a cage (stainless steel, 11.5 × 18.5 × 6.5 inches). Cages were changed two or three times per week; the bedding was sterilized shredded corn cobs (Sani-i-Cel, Paxton Processing Co., Paxton, IL). Food and water were provided ad libitum. The 23 dams were given a semisynthetic diet (12) containing 5%w (by weight) of edible grade safflower-olive (oleinate, a mutant of safflower oil in which oleic acid replaces linoleic acid) (Pacific Vegetable Oil Corp., Richmond, CA) and 20 mg of α-tocopherol acetate per 100 grams of finished diet; at 115 days of age, and thereafter, unesterified α- tocopherol was used instead of the acetate. When the rats were 117 days old, 1 male, same age as the females and given prior to mating the same diet, was placed in a cage with 4 females for a period of 7 days and then removed. The offspring (seventh generation) were culled to 8 per litter at 6 days of age. At 23 days of age the 130 rats (67 females and 63 males) were weaned, sexed and caged 4 per cage.

At age 26 days the offspring were divided into three approximately equal groups as shown in Table 1.

The rats of *Group I* were divided into 4 dietary subgroups: 1) control diet; 2) control plus 50 mg of 18:3ω3 (Nu Chek Prep, Inc., Elysian, MN); 3) control plus 50 mg of 22:6ω3 (90 percent grade, Nu Chek Prep, Inc.); or 4) control plus 377 mg of distilled triglycerides of menhaden oil (kindly supplied by Dr. Neva L. Karrick of the Northwest Fisheries Center, U. S. Dept. of Commerce, Seattle, WA), per 100 grams of finished diet. The fatty acid analyses of the four finished diets are listed in Table 2. The base diet was prepared every ten to fourteen days and the supplemented diets every six to seven days; the diets were kept in a deep-freeze before use. The rats were fed fresh food daily. Two male and 2 female members of Group I were killed by decapitation on days 0, 15 and 30, and like numbers of rats from each of the supplemented groups were killed after days 10, 20 and 30 of the study.

Group II rats were given the control diet until age 147 days, and *Group III* rats until age 391 days, before being divided into the 4 dietary subgroups. The decapitation schedule for the supplemented subgroups of Group II and III was the same as for Group I; the numbers of rats for analysis were also the same except that only 1 male was available in the 18:3ω3 subgroup of Group III. In the control subgroup of Group II, 2 male and 2 female rats were killed at the beginning and at the end of the 30-day supplemented period whereas only 1 male and 2 females were available for the control subgroup of Group III; these were killed at the start of the 30-day supplemented period.

After decapitation, whole brains (1.0–1.9 grams, depending upon the age of the rats) were taken immediately, frozen on dry ice, wrapped in aluminum foil and stored in nitrogen-filled vials at −70°C, until the lipid analyses could be carried out. The procedures for brain lipid extraction, conversion of the fatty acid moieties to methyl esters, and analysis of fatty acid composition by gas chromatography have been described previously (2).

The mean ± the standard error of the mean for the major brain fatty acids for the 4 dietary subgroups for the three supplemented periods are shown in Table 3. The male and female data for Groups I and II, diets supplemented for 30 days starting at age 27 and 147 days respectively, are combined in the table, as they were essentially the same. Individual male and female rat brain data are shown for Group III – diets supplemented for 30 days starting at age 391 days, along with the averages of the combined data. The data of Table 3 for total brain 22:5ω6 and 22:6ω3, are presented graphically in Figure 1, A and B. The changes in the percentage of 22:4ω6, 22:5ω6 and 22:6ω3 in total brain lipids of rats on the base diet are shown in Figure 2 as a function of age from 26 to 391 days.

The differences between the percentages of a given fatty acid at the end of the 30-day supplemented dietary fat period and at the start for each of the given dietary subgroups, for each of

TABLE 1

Effect of Age, Sex, and Dietary Fat on Fatty Acid Composition of Total-Brain Lipids in Sprague-Dawley Rats. (Supplemental Lipid Feeding Periods and Autopsy Schedule)

Group	Age (days)	Dietary Fat Supplement* None	18:3ω3	22:6ω3	Menhaden Oil
		Autopsy Schedule			
1–48 rats	26	2M†,2F‡			
	36		2M,2F	2M,2F	2M,2F
	41	2M,2F			
	46		2M,2F	2M,2F	2M,2F
	56	2M,2F	2M,2F	2M,2F	2M,2F
2–44 rats	147	2M,2F			
	157		2M,2F	2M,2F	2M,2F
	167		2M,2F	2M,2F	2M,2F
	177	2M,2F	2M,2F	2M,2F	2M,2F
3–38 rats	391	1M,2F			
	401		1M,2F	2M,2F	2M,2F
	411		2M,2F	2M,2F	2M,2F
	421		2M,2F	2M,2F	2M,2F

* At 26, 147 and 391 days of age, groups of rats that had been receiving base diet were subdivided into 4 dietary groups by adding either nothing (control), 50 mg of 18:3ω3, 50 mg of 22:6ω3, or 377 mg of distilled triglycerides of menhaden oil to the diet.

† Male.

‡ Female.

the three supplemented periods, are shown in Table 4. Changes in 22:6ω3 are shown in Figure 3.

DISCUSSION

The percentage of 22:6ω3 in the total-brain lipid of rats receiving the base diet rose steadily, almost linearly for females and somewhat less so for males, throughout the course of this study – from age 26 days to age 391 days. The increases in brain 22:6ω3 with age are due to the small amount (about 0.1 percent) of 18:3ω3 present in the safflower-olive (oleinate) in the base diet. The increases in brain 22:6ω3 were roughly paralleled by the decreases in 22:5ω6.

Increases in dietary 18:3ω3, 22:6ω3 or distilled triglycerides of menhaden oil were reflected in virtually linear increases in the percentage of brain 22:6ω3 during the three age periods studied, viz, 26–56, 147–177 and 391–421 days. The increases for the age period 26–56 days were nearly the same when the diet was supplemented with either 50 mg of 22:6ω3 per 100 grams of diet or 377 mg of distilled triglycerides of menhaden oil (containing 50 mg of 22:6ω3 in ester form) – 8.1 per cent and 10.6 per cent respectively. The increase produced by adding 50 mg of 18:3ω3 to 100 grams of the base diet was about half, i.e., 3.9 per cent. The increase in brain 22:6ω3 resulting from administration of the three lipid supplements during the second age period, 147–177 days, was roughly half that observed for the first age period. During the third age period, 391–421 days, the percentage increase of 22:6ω3 in brain total lipid was the same as for the second age period for all three lipid supplements for females, but about half for males receiving 22:6ω3 or menhaden oil and practically zero for males receiving 18:3ω3.

Previous work (2) indicated that the level of 22:6ω3 in total brain lipid would increase little after 6 months of age; therefore the present experiment was programmed for one year. This impression was in error. Instead, at 12 months of age, the female rat continues to convert dietary 18:3ω3 and 22:6ω3 (the latter either as the free acid or in glyceride form) into brain 22:6ω3 at the same steady linear rate as observed at 147–177 days of age. This ability is significantly impaired in males, particularly for 18:3ω3.

The maximum percentage of 22:6ω3 in the total brain lipid of male and female rats that can be achieved by dietary manipulation, is not known. In this study the highest brain 22:6ω3 level, 13.1

TABLE 2

Fatty Acid Composition of the Diets

Carbon Number	Diet Base*	18:3ω3**	22:6ω3†	Menhaden Oil‡
10	0.10	0.14	0.21	–
12	–	–	–	0.22
14	0.20	0.23	0.23	1.16
15§	0.01	0.02	0.02	0.08
16	5.79	5.43	5.36	8.20
16:1ω7	–	–	–	0.57
17:1§	–	–	–	0.13
18	0.67	0.44	0.75	0.67
18:1ω9	71.43	70.78	69.38	61.66
18:2ω6	19.74	19.29	19.70	20.61
20	–	–	0.12	0.23
18:3ω3	0.10	2.47	0.13	0.08
20:1ω7	0.18	0.10	0.17	0.86
20:2§	1.56	1.04	0.24	0.44
20:4ω6	–	–	–	0.13
20:5ω3	–	–	–	2.31
22:1	–	–	–	0.43
22:6ω3	–	–	3.65	1.68

* Semisynthetic diet containing, as sole source of lipid, 5%w of oleinate and 20 mg of α-tocopherol per 100 grams of finished diet.

** 50 mg of 18:3ω3 added per 100 grams of base diet.

† 50 mg of 22:6ω3 (90% grade) added per 100 grams of base diet.

‡ 377 mg of distilled triglycerides of menhaden oil (equivalent to 50 mg of 22:6ω3) added per 100 grams of base diet.

§ Tentative identification.

TABLE 3

Effect of Age, Sex, and Dietary Fat on Fatty Acid Composition of Total-Brain Lipids in Sprague-Dawley Rats

A. *Group I: Control diet supplemented with 18:3ω3, 22:6ω3 or menhaden oil, from 27 to 46 days of age*

Carbon Number	Control Diet (CD)			CD + 18:3ω3		
	Age, Days			Age, Days		
	26	41	56	36	46	56
16	20.6 ± 0.4	18.5 ± 0.2	18.3 ± 0.1	19.7 ± 0.2	18.7 ± 0.3	18.2 ± 0.1
18	18.9 ± 0.2	19.7 ± 0.4	19.8 ± 0.1	20.0 ± 0.2	20.4 ± 0.2	20.7 ± 0.3
18:1ω9	20.0 ± 0.6	21.7 ± 0.5	22.6 ± 0.4	21.4 ± 0.2	22.2 ± 0.4	21.9 ± 0.3
20:4ω6	15.0 ± 0.1	13.2 ± 0.1	12.2 ± 0.7	13.4 ± 0.3	12.7 ± 0.1	12.2 ± 0.1
22:4ω6	4.5 ± 0.1	4.2 ± 0.1	4.3 ± 0.1	4.1 ± 0.1	3.8 ± 0.2	3.9 ± 0.1
22:5ω6	14.6 ± 0.2	14.4 ± 0.4	14.0 ± 0.3	13.7 ± 0.2	11.8 ± 0.1	10.8 ± 0.4
22:6ω3	2.1 ± 0.2	2.0 ± 0.1	2.3 ± 0.0	3.2 ± 0.1	4.5 ± 0.1	6.0 ± 0.2

Carbon Number	CD + 22:6 3			CD + Menhaden Oil		
	Age, Days			Age, Days		
	36	46	56	36	46	56
16	19.3 ± 0.1	18.4 ± 0.2	17.9 ± 0.1	18.9 ± 0.2	18.7 ± 0.2	17.9 ± 0.1
18	20.0 ± 0.3	20.6 ± 0.2	20.2 ± 0.1	19.7 ± 0.2	20.2 ± 0.1	20.7 ± 0.2
18:1ω9	20.3 ± 0.1	22.0 ± 0.4	22.5 ± 0.3	21.4 ± 0.4	22.3 ± 0.3	23.0 ± 0.4
20:4ω6	13.2 ± 0.1	12.0 ± 0.2	11.8 ± 0.1	13.0 ± 0.2	12.1 ± 0.2	11.4 ± 0.2
22:4ω6	4.1 ± 0.2	3.6 ± 0.1	3.3 ± 0.2	3.7 ± 0.1	3.5 ± 0.1	3.6 ± 0.4
22:5ω6	13.1 ± 0.1	10.1 ± 0.4	8.4 ± 0.2	11.1 ± 0.2	7.4 ± 0.1	5.4 ± 0.1
22:6ω3	4.8 ± 0.2	7.6 ± 0.3	10.1 ± 0.3	6.7 ± 0.3	10.8 ± 0.4	12.7 ± 0.1

B. *Group II: Control diet supplemented with 18:3ω3, 22:6ω3 or distilled triglycerides of menhaden oil, from 147 to 177 days of age*

Carbon Number	Control Diet (CD)			CD + 18:3ω3		
	Age, Days			Age, Days		
	147	167	177	157	167	177
16	18.2 ± 0.3		17.0 ± 0.2	17.4 ± 0.2	17.1 ± 0.3	17.4 ± 0.2
18	18.8 ± 0.1		19.5 ± 0.6	19.8 ± 0.5	19.8 ± 0.7	18.8 ± 0.3
18:1ω9	26.2 ± 0.3		27.9 ± 0.3	27.0 ± 0.4	27.0 ± 0.1	28.0 ± 0.2
20:4ω6	12.8 ± 0.3		12.1 ± 0.1	11.8 ± 0.0	12.0 ± 0.1	12.1 ± 0.2
22:4ω6	3.7 ± 0.1		3.6 ± 0.1	3.4 ± 0.1	3.5 ± 0.1	3.4 ± 0.2
22:5ω6	13.3 ± 0.1		10.8 ± 0.5	10.7 ± 0.3	10.3 ± 0.3	10.1 ± 0.3
22:6ω3	3.9 ± 0.4		4.4 ± 0.5	3.8 ± 0.3	5.2 ± 0.4	6.2 ± 0.3

TABLE 3—*Continued*

B. *Group II: Control diet supplemented with 18:3ω3, 22:6ω3 or distilled triglycerides of menhaden oil, from 147 to 177 days of age (Continued)*

Carbon Number	CD + 22:6ω3			CD + Menhaden Oil		
	Age, Days			Age, Days		
	157	167	177	157	167	177
16	17.3 ± 0.2	17.5 ± 0.1	17.1 ± 0.3	17.3 ± 0.2	17.3 ± 0.2	17.1 ± 0.2
18	20.1 ± 0.9	18.7 ± 0.3	18.8 ± 0.2	19.1 ± 0.2	20.7 ± 0.4	19.5 ± 0.2
18:1ω9	26.6 ± 0.7	27.6 ± 0.2	27.7 ± 0.4	27.2 ± 0.2	26.5 ± 0.3	27.7 ± 0.1
20:4ω6	11.8 ± 0.2	12.2 ± 0.2	11.7 ± 0.2	12.3 ± 0.1	11.6 ± 0.2	11.6 ± 0.3
22:4ω6	3.3 ± 0.2	3.3 ± 0.2	3.2 ± 0.1	3.8 ± 0.2	3.8 ± 0.2	3.2 ± 0.0
22:5ω6	11.1 ± 0.6	9.8 ± 0.3	8.8 ± 0.2	10.3 ± 0.2	8.1 ± 0.1	6.8 ± 0.4
22:6ω3	4.5 ± 0.3	6.5 ± 0.4	8.1 ± 0.4	5.2 ± 0.4	6.8 ± 0.4	8.8 ± 0.4

C. *Group III: Control diet supplemented with 18:3ω3, 22:6ω3 or distilled triglycerides of menhaden oil, from 391 to 421 days of age*

Carbon Number	Control Diet (CD)				CD + 18:3ω3			
	391 Days of Age				401 Days of Age			
	Female		Male	Avg. ± S.E.M.	Female		Male	Avg. ± S.E.M.
16	17.3	17.2	16.8	17.1 ± 0.2	16.6	16.5	17.4	16.9 ± 0.3
18	19.8	19.4	19.9	19.7 ± 0.1	19.6	20.4	19.1	19.7 ± 0.4
18:1ω9	27.8	27.9	28.5	28.1 ± 0.2	28.2	27.9	28.8	28.3 ± 0.3
20:4ω6	11.3	11.7	11.9	11.6 ± 0.2	11.6	10.8	11.8	11.4 ± 0.3
22:4ω6	3.0	3.2	3.5	3.2 ± 0.1	3.2	3.0	3.7	3.3 ± 0.2
22:5ω6	9.6	8.2	9.2	9.0 ± 0.4	7.4	9.5	9.5	8.8 ± 0.7
22:6ω3	7.0	8.1	6.1	7.0 ± 0.6	8.6	7.8	6.4	7.6 ± 0.7

Carbon Number	CD + 18:3ω3					CD + 18:3ω3				
	411 Days of Age					421 Days of Age				
	Female		Male		Avg. ± S.E.M.	Female		Male		Ave. ± S.E.M.
16	16.5	16.1	16.7	16.7	16.5 ± 0.2	17.2	15.4	16.4	17.3	16.6 ± 0.5
18	19.4	20.1	19.4	19.1	19.5 ± 0.2	19.4	19.5	19.0	19.3	19.3 ± 0.1
18:1ω9	29.2	28.8	29.6	29.3	29.2 ± 0.2	28.7	28.8	29.5	29.1	29.0 ± 0.2
20:4ω6	11.3	11.1	11.7	11.8	11.5 ± 0.2	10.9	11.3	11.9	11.8	11.5 ± 0.3
22:4ω6	3.2	2.8	3.5	3.5	3.3 ± 0.2	2.9	3.2	3.4	3.2	3.2 ± 0.1
22:5ω6	7.1	8.3	8.5	8.5	8.1 ± 0.3	7.8	6.6	9.6	8.8	8.2 ± 0.7
22:6ω3	9.8	9.1	6.6	6.2	7.9 ± 0.9	9.2	10.4	5.8	6.3	7.9 ± 1.1

TABLE 3—*Continued*

C. *Group III: Control diet supplemented with 18:3ω3, 22:6ω3 or distilled triglycerides of menhaden oil, from 391 to 421 days of age*

CD + 22:6ω3

Carbon Number	401 Days of Age					411 Days of Age					421 Days of Age				
	Female		Male		Avg. ± S.E.M.	Female		Male		Avg. ± S.E.M.	Female		Male		Avg. ± S.E.M.
16	16.6	16.4	17.1	16.7	16.7 ± 0.2	16.7	17.0	16.8	16.9	16.8 ± 0.1	17.1	15.8	16.9	16.7	16.6 ± 0.3
18	19.0	19.4	19.4	19.9	19.4 ± 0.2	19.6	19.1	20.0	20.2	19.7 ± 0.3	19.4	19.5	19.8	19.6	19.6 ± 0.1
18:1ω9	28.6	28.9	28.8	29.3	28.9 ± 0.1	28.2	28.3	28.8	30.0	28.8 ± 0.4	28.1	28.6	29.4	29.2	28.8 ± 0.3
20:4ω6	11.4	11.3	11.9	11.7	11.6 ± 0.1	11.5	11.6	11.3	11.0	11.4 ± 0.1	11.1	11.2	11.5	11.2	11.3 ± 0.1
22:4ω6	3.3	3.0	3.6	3.5	3.4 ± 0.1	3.2	3.3	3.1	3.2	3.2 ± 0.1	3.3	3.3	3.0	3.4	3.3 ± 0.1
22:5ω6	8.9	8.3	8.7	9.1	8.8 ± 0.2	6.8	7.5	8.0	7.6	7.5 ± 0.3	5.7	6.6	7.7	7.0	6.7 ± 0.4
22:6ω3	8.5	9.3	6.5	5.8	7.5 ± 0.8	10.3	9.3	7.5	7.7	8.7 ± 0.7	11.7	11.2	8.0	8.5	9.8 ± 0.9

CD + Menhaden Oil

Carbon Number	401 Days of Age					411 Days of Age					421 Days of Age				
	Female		Male		Avg. ± S.E.M.	Female		Male		Avg. ± S.E.M.	Female		Male		Avg. ± S.E.M.
16	16.1	16.9	17.0	16.5	16.6 ± 0.2	16.6	16.7	16.6	16.6	16.6 ± 0.0	16.1	17.2	17.1	16.8	16.8 ± 0.3
18	19.4	20.0	18.4	20.0	19.4 ± 0.4	18.9	18.3	19.9	20.2	19.3 ± 0.4	19.2	19.9	18.7	19.0	19.2 ± 0.3
18:1ω9	30.7	28.3	29.5	28.8	29.3 ± 0.5	28.4	28.8	29.1	30.0	29.1 ± 0.4	28.2	26.3	29.7	28.2	28.1 ± 0.7
20:4ω6	10.8	11.3	12.2	11.9	11.6 ± 0.3	11.5	11.6	11.7	11.0	11.5 ± 0.2	11.3	12.4	11.4	11.4	11.6 ± 0.3
22:4ω6	3.0	3.1	3.4	3.3	3.2 ± 0.1	3.1	3.2	3.7	3.2	3.3 ± 0.1	3.3	3.2	3.0	3.1	3.1 ± 0.1
22:5ω6	7.6	8.5	8.6	8.8	8.4 ± 0.3	6.6	6.4	7.3	6.9	6.8 ± 0.2	5.1	7.3	7.9	6.9	6.8 ± 0.6
22:6ω3	9.2	8.6	6.5	6.7	7.7 ± 0.7	10.7	11.4	7.6	7.6	9.3 ± 1.0	13.1	11.5	8.0	9.7	10.6 ± 1.1

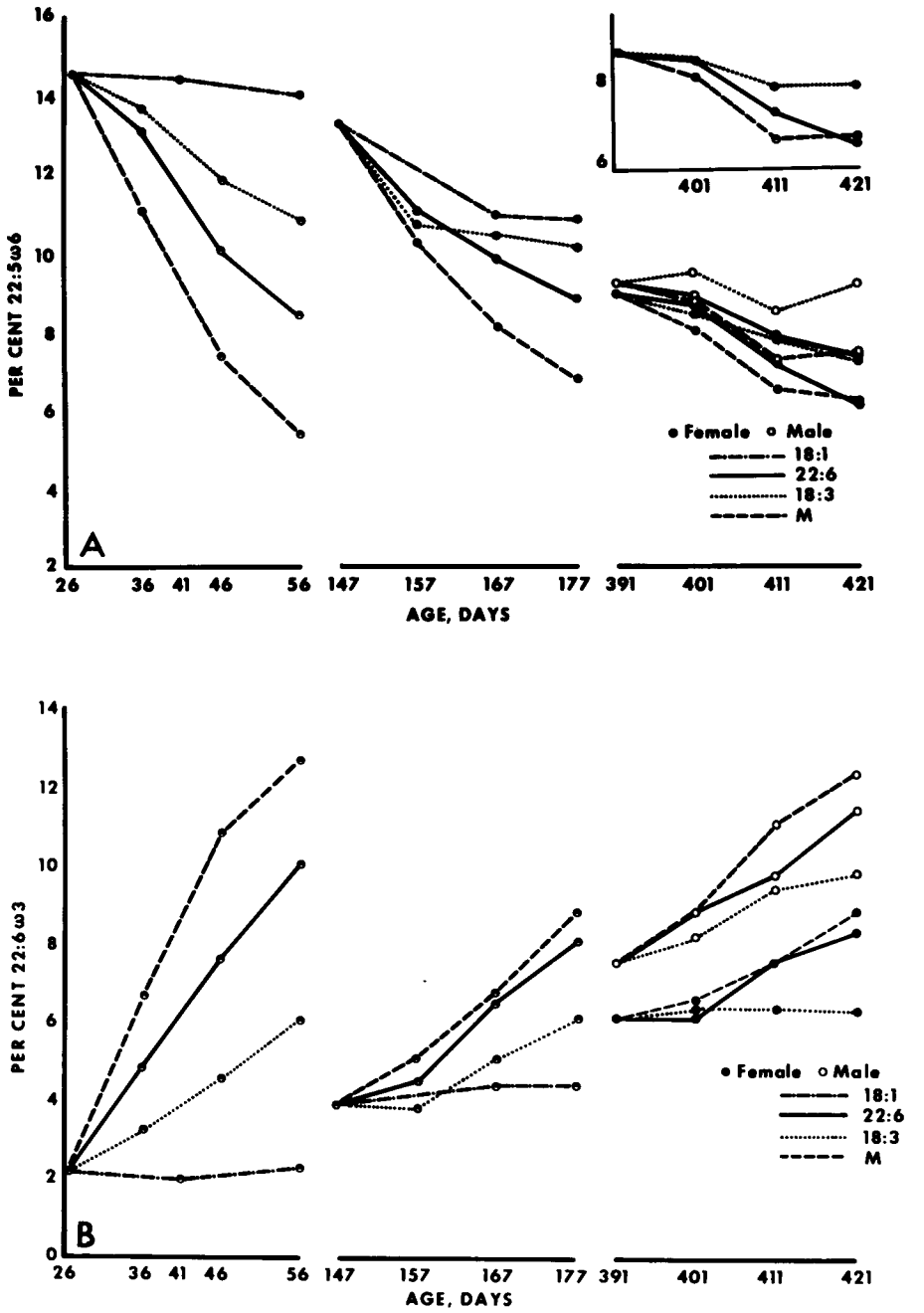

Fig. 1. Effect of age, sex and dietary fat on: A. The percentage of 22:5ω6 in the fatty acids of whole-brain lipid. B. The percentage of 22:6ω3 in the fatty acids of whole-brain lipid. (Sprague-Dawley rats.)

per cent of total fatty acids, was observed for a female rat given menhaden oil supplement for 30 days starting at age 391 days; the corresponding value for males from the same menhaden oil group was 9.7. It is also not known if neuronal populations differ in fatty acid composition and ability to concentrate 22:6ω3, nor if the composition of the components of the synaptic areas mirror corresponding elements of the perikaryon.

The fact that 22:6ω3 is selectively concentrated in brain phospholipids suggests that it has a beneficial effect on membrane function, probably on permeability and membrane-bound enzymes. Hence the findings of the present study imply that variations in CNS function between individuals may be partially a reflection of the variables influencing brain 22:6ω3 levels, viz, age, sex and dietary precursors. This suggestion is supported by the recent observation (13) that the performance of rats in a discrimination learning situa-

tion was correlated positively with the level of 22:6ω3 in brain phosphatidylethanolamine and phophatidylserine-phosphatidylinositol. Brain 22:6ω3 levels were altered by feeding semisynthetic diets containing safflower oil, corn oil or soybean oil as the sole source of lipid.

It would be anticipated that as the percentage of 22:6ω3 in total brain lipid increased, lipid peroxidation would increase (even though dietary vitamin E was more than sufficient to prevent overt vitamin E deficiency) and at some point might result in clinically significant behavioral changes. These changes would not necessarily be accompanied by decrements in other body tissues because of the lesser tendency of 22:6ω3 to concentrate in them. Thus, enhanced lipid peroxidation in the synaptic areas, which are rich in polyunsaturated fatty acids, might contribute to deleterious alterations in behavior in a manner similar to that caused by β-hydroxydopamine (14) or by the anesthetic, halothane (15, 16). Support for this possibility comes from studies of neuritic plaques (senile plaques). The plaques, present in the cortex and basal ganglia of normal

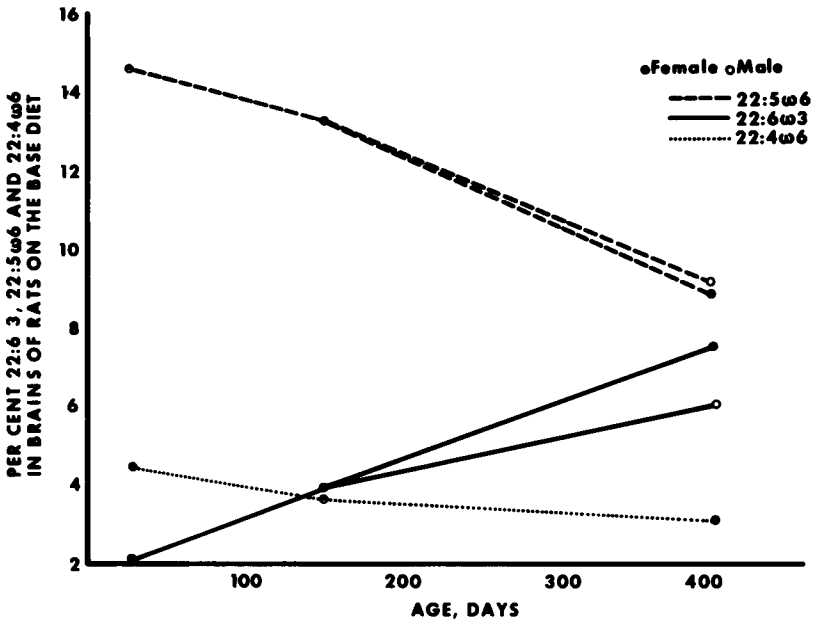

Fig. 2. Effect of age on the percentage of 22:4ω6, 22:5ω6, and 22:6ω3 in the fatty acids of whole-brain lipid of rats receiving the base diet.

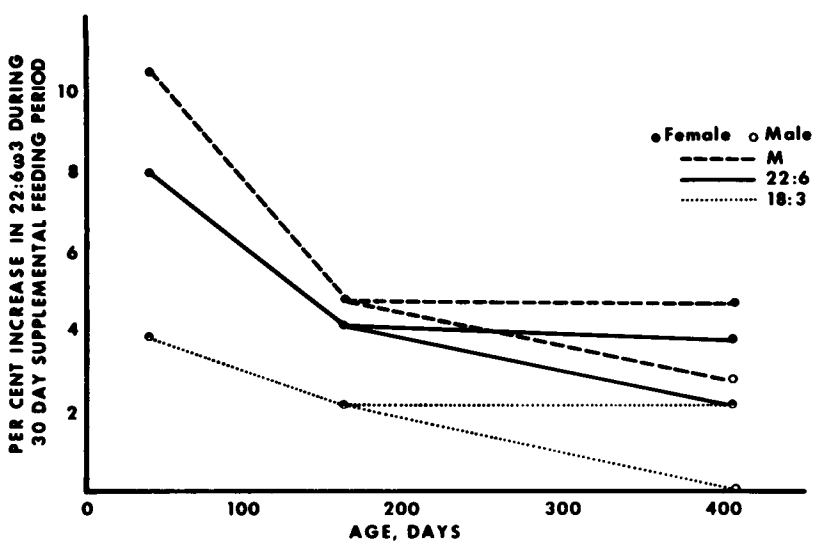

Fig. 3. Effect of age, sex and dietary fat on the percentage increase in 22:6ω3 in the fatty acids of whole-brain lipid during each of the three 30-day supplemented feeding periods.

TABLE 4

Effect of Age and Sex on Changes in Whole-Brain Fatty Acid Composition Produced by Supplementing the Diet for 30 Days with Either 18:3, 22:6 or Menhaden Oil (Sprague-Dawley)

Age (days)	Diet	Percent Change in Fatty Acid Composition													
		Carbon Number													
		16:0		18:0		18:1ω9		20:4ω6		22:4ω6		22:5ω6		22:6ω3	
27-56	Base	−2.35		0.80		2.61		−2.87		−0.24		−0.60		0.19	
	" + 18:3*	−2.39		1.68		1.92		−2.78		−0.62		−3.75		3.93	
	" + 22:6	−2.71		1.23		2.51		−3.13		−1.19		−6.20		8.06	
	" + Menh.	−2.77		1.70		2.96		−3.53		−0.94		−9.18		10.60	
147-177	Base	−1.21		0.71		1.77		−0.68		−0.11		−2.50		0.52	
	" + 18:3	−0.76		−0.03		1.80		−0.61		−0.33		−3.21		2.25	
	" + 22:6	−1.08		0.01		1.51		−1.05		−0.27		−4.55		4.15	
	+ Menh.	−1.05		0.65		0.52		−1.12		−0.54		−6.55		4.85	
		M†	F‡	M	F	M	F	M	F	M	F	M	F	M	F
391-421	Base**	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	" + 18:3	0.13	−0.98	−0.70	−0.12	0.75	0.87	−0.16	−0.41	−0.30	−0.11	−0.01	−1.73	−0.06	2.24
	" + 22:6	0.04	−1.04	−0.17	−0.14	0.78	0.47	−0.54	−0.31	−0.27	0.20	−1.89	−2.73	2.07	3.81
	" + Menh.	0.22	−0.61	−1.02	−0.02	0.41	−0.60	−0.50	−0.39	−0.41	0.11	−1.79	−2.69	2.77	4.78

* The base diet was supplemented with either 50 mg of linolenic acid (18:3ω6), docosahexanoic acid (22:6ω3) or 377 mg of distilled triglycerides of menhaden oil, containing 50 mg of 22:6ω3 as the triglyceride.

** Data available only for rats aged 391 days.

† Male.

‡ Female.

old people (17), are increased in senile persons (18). The first changes seen in the development of the plaques are alterations in the mitochondria of the axon terminals (19). These mitochondrial changes may be due to peroxidation, for the mitochondria have both a high degree of lipid unsaturation and a high rate of oxygen utilization. The foregoing may be the basis for the observation that rats receiving a semisynthetic diet containing distilled triglycerides of menhaden oil performed more poorly in a Hebb-Williams maze than had been anticipated from mortality data (1).

The present study also suggests that variability in the period of senility prior to death in humans may be due, at least in part, to the differences in brain 22:6ω3 attributable to sex and variation in the amount and kind of brain 22:6ω3 dietary precursors consumed throughout life. Further, it would be anticipated that variable degrees of dysfunction might occur throughout the CNS with time because of local differences in factors, other than lipid, which influence lipid peroxidation. Among these factors are the rate of oxygen utilization, the concentration of α-tocopherol, and the concentration of peroxidation catalysts such as copper and iron (20).

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