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Effect of Fish Oils on Brain Fatty Acid Composition and Learning Performance in Rats

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ABSTRACT

The effects of sardine oil(high in eicosapentaenoic acid : EPA) and tuna oil(high in docosahexaenoic acid : DHA, also high in EPA) on fatty acid composition of brain and learning ability were evaluated in male weanling Sprague-Dawley rats and compared with the effects of corn oil and beef tallow. Animals assigned by randomized block design to one of the four experimental diet groups containing dietary lipid at 15%(w/w) level were given ad libitum for 7 weeks. Food intake and body weight gain of the fish oil groups were significantly lower than those of the corn oil and beef tallow groups. However, brain weights of the groups were not significantly different. In the brain fatty acid composition, the corn oil group showed high concentrations of n-6 fatty acids, the fish oil groups of n-3 fatty acids, and the beef tallow group of saturated fatty acids. Brain EPA and DHA contents of the fish oil groups showed significantly higher than the other groups while the brain ratio of saturated/monounsaturated/polyunsaturated fatty acid was controlled in a narrow range. In a maze test, the fish oil groups appeared to arrive at the goal faster than the corn oil and beef tallow groups. It explained that EPA in diets might efficiently convert to DHA resulting in DHA accumulation in brain tissue and might increase the learning performance as DHA did.

KEY WORDS : sardine oil · tuna oil · brain fatty acid composition · maze test · learning ability.

Introduction

The nervous system is the organ with the second greatest concentration of lipids, immediately after adipose tissue. These lipids are practicularly all structural and participate directly in the functioning of cerebral membranes. The phospholipids of excitable membranes of the central nervous system are unusual in that there is a prevalence of the polyunsaturated fatty acyl chain docosahexaenoic acid(DHA)[1]. Membranes from nonneural tissues contain phospholipids with much lower amounts of this fatty acyl chain.

The DHA occurs mainly at the C_2 position of the glycerol backbone of phosphlipids. While saturated or monounsaturated fatty acids, such as palmitic, stearic, and oleic acids, are usually present at the C_1 position, polyunsaturated fatty acids, such as archidonic acid(AA) and DHA usually occupy the C_2 position. Unlike AA, which is widely distributed in relatively large amounts

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in cell membranes of all tissues, DHA is scarce in nonneural tissues[2]. However, in the synaptic membranes of the brain, and in the neural and photoreceptor outer segments of the retina[3)4)], the DHA content of phospholipids is high. High levels of DHA in the cerebrum and the retina suggest that n-3 fatty acids may be indispensable for neural development in newborns. The requirement for DHA is very large during the development and differentiation of the central nervous system. This coincides with synaptogenesis and with the biogenesis of photoreceptor membranes. Depletion of DHA early in life has been associated with loss of visual acuity in infants of nonhuman primates[5)6)] and abnormalities of learning performance in rats[7)8)].

Docosahexaenoic acid is the end product of a series of elongations and desaturations of linolenic acid(LNA)[9]. However, the change from α-LNA to DHA seems to be limited in human body while the change from eicosapentaenoic acid (EPA) to DHA does not[10-12]. Dietary EPA increased the brain concentration of DHA in growing animals[13], which explained that provided fatty acid EPA was preferentially moved to brain and used after changed to DHA. In this case, providing C_{20} EPA is more useful than providing C_{18} LNA. Therefore, it is necessary to find out what differences are between the providing EPA and DHA. This experiment is to discover how two kinds of dietary fish oils, sardine oil containing 34.17% of EPA and tuna oil containing 26.40% of DHA and 26.40% of EPA, effect the brain fatty acid composition and the learning ability of weanling rats. Also, the water maze used was intended to exclude the possibility of sight impairment and alterations in vision-dependent behaviors due to retinal photoreceptors and to test the learning ability due to reduction in the DHA content of the brain.

Materials and Methods

1. Animals and diets

Forty male weanling Sprague-Dawley rats weighing about 50－60g were housed singly in stainless steel cages, exposed to light daily between 23：00h and 11：00h, kept at a room temperature of 20± 2℃ and humidity of about 60%. The animals were assigned by randomized block design to one of the four experimental diet groups(n＝10 animals per group) and were given ad libitum access to food and water for 7 weeks. The amounts of food intake were measured every day and rats were weighed every week 2 hours after removing food cups.

The diets were based on AIN-76 rodent diet and contained by weight(%)：corn starch, 60； casein, 20； lipid, 15； mineral mixture(Bio-Serv. Inc., N.J. USA), 3.5； vitamin mixture(AIN-76 rodent diet), 1； DL-methionine, 0.3； choline chloride, 0.2. The provided lipids were 4 different kinds：beef tallow(Lotte-Samkang Co.) as the source of saturated fatty acid, corn oil(Dong-bang Oil Co.) linoleic acid(n-6), sardine oil(Kohap Bio. Co.) EPA(n-3), and tuna oil(Kohap Bio. Co.) DHA(n-3), respectively. The fatty acid composition of the four test diets is shown in Table 1.

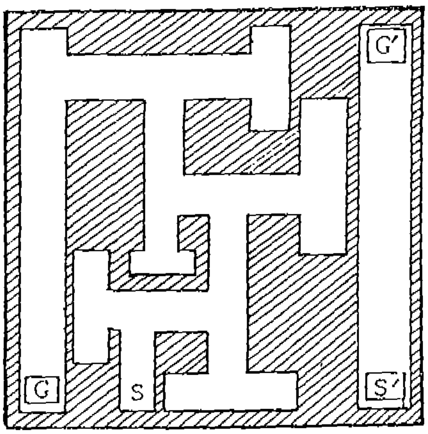
2. Water maze test

The water maze(shown in Fig. 1) used before at our laboratory[14] was filled with water to a platform to make it possible for rats to swim.
Rats were put the S-G section in the water maze and the time when the rats swam from start(S) to goal(G)was measured. Before starting the test, the rats practiced the way how to swim in the S'－G' section. The only distinguishable light was preserved by turning up two infra-red lamps in

Table 1. Fatty acid composition of dietary lipids[1] (%)

Fatty acid	Corn oil	Sardine oil	Beef tallow	Tuna oil
14 : 0	0.13	9.10	6.27	4.65
14 : 1	ND[2]	0.57	0.99	0.22
16 : 0	10.00	15.37	29.45	12.84
16 : 1n7	0.14	11.43	3.50	7.25
18 : 0	2.46	3.89	17.77	3.14
18 : 1n9	26.31	10.86	39.51	13.47
18 : 2n6	59.62	1.69	2.51	1.87
18 : 3n3	0.42	1.20	ND	1.09
20 : 0	0.44	ND	ND	ND
20 : 1n9	0.48	0.41	ND	1.28
20 : 4n6	ND	1.79	ND	2.51
20 : 5n3	ND	34.17	ND	25.28
22 : 6n3	ND	9.52	ND	26.40
SFA	13.03	28.36	53.49	20.63
MUFA	26.93	23.27	44.00	22.22
PUFA	60.04	48.37	2.51	57.15
Total n9[3]	26.79	11.27	39.51	14.75
Total n6[4]	59.62	3.48	2.51	4.38
Total n3[5]	0.42	44.89	ND	52.77
P/S ratio[6]	4.61	1.70	0.05	2.77

1) Values are expressed as the relative % of total fatty acids.
2) Not Detected
3) Total n9=C18 : 1n9+C20 : 1n9+C22 : 1n9
4) Total n6=C18 : 2n6+C20 : 2n6+C20 : 3n6+C20 : 4n6
5) Total n3=C18 : 3n3+C20 : 5n3+C22 : 6n3
6) P/S ratio=PUFA/SFA



water tank 1200×1200×360mm
maze width 110mm
maze height 360mm

Fig. 1. Shape of the water maze.

the laboratory. This test was performed daily at the beginning of dark cycle(11 : 00h) six times a week. The test was done for three weeks from the fifth week. The water maze we used tried to attenuate the effect of visual acuity on learning ability by eliminating task with visual cues has recently been discussed[15].

3. Brain fatty acid composition

The animals were deprived of food overnight and decapitated. Their brains were rapidly removed and frozen. Their fatty acid composition was measured using Fletcher's method[16] and Lapage and Roy's method[17] by gas liquid chromatography(GLC). The conditions of GLC analysis are shown in Table 2.

4. Statistical analysis

Data were presented as the means± SEM. Statistical evaluations were performed by ANOVA, and followed by Duncan's multiple range test for differences between means. Nonparametric Kruskal-Wallis test was used for the verification of the results of the water maze test[18]. For all comparisons, the level of statistical significance was set at $p<0.05$.

Results and Discussion

1. Food intake and body weight gain

The total food intake, body weight gain, and food efficiency ratio(FER) are shown in Table 3. The corn oil group had the highest food intake. Next were the beef tallow, the tuna oil, the sardine oil in that order. Compared to the corn oil group, the sardine oil group ate a significantly small amount. In the body weight gain, the fish oil groups were significantly smaller than the corn oil or beef tallow group. The body weight gain of the corn oil group was the highest. The FER of the corn oil and beef tallow groups was significantly higher than that of the fish oil groups. There was no difference between the two fish oil groups.

In the case of having lots of polyunsaturated fatty acids for a long time, vitamin E deficiency may occur because of the increase of demand and the peroxidation[19)20]. The peroxidation value of the fish oils we used was 1.0meq/kg. Since the fish oil itself had α-tocopherol in it(about 300mg α-tocopherol/100g fish oil) and α-tocopherol was supplemented to the diets(50mg α-tocopherol/kg diet), it seemed that the reason of low food intake of the sardine oil group was not peroxidation of the oil. Also, food intake related to factors such

Table 2. Instrument and operating condition for GLC

Instrument	Hewlett Packard 5890 series II
Detector	Flame Ionization Dector(FID)
Column	30m Fused Silica Capillary Column 0.25mm I.D., 0.25μ Film Thickness
Carrier gas(flow rate)	He(3ml/min)
Injection temperature	260℃
Detection temperature	270℃
Column temperature	Initial 195℃ 30min Programmed temperature from 195℃ to 198℃ at 0.5℃/min Final 198℃ 21min
Sample injected	0.4μl

Table 3. Food intake, body weight gain, and food efficency ratio in rats fed different type of dietary lipids for 7 weeks[1)]

Group	Food intake (g)	Body weight gain (g)	Food efficency ratio
Corn oil	655.2 ± 41.3^{a}	135.2 ± 7.8^{a}	0.21 ± 0.01^{a}
Sardine oil	526.4 ± 16.8^{b}	73.1 ± 8.2^{b}	0.14 ± 0.01^{b}
Beef tallow	627.2 ± 28.7^{ab}	116.6 ± 6.4^{a}	0.20 ± 0.01^{a}
Tuna oil	565.6 ± 42.0^{ab}	77.3 ± 11.5^{b}	0.14 ± 0.01^{b}

1) Values are means± SEM. Values in a column not sharing a same superscript differ significantly($p<0.05$) by Duncan's multiple range test.

as palatability and n-6 fatty acid deficiency could be attributed. However, the 15% single fish oil diet(w/w) showed very low FER and, therefore, lower level of oil in diets or mixing with n-6 and saturated fatty acids could be considered.

2. Brain weight

The brain weights of the experimental groups are shown in Table 4. There was no significient

Table 4. Brain weight in rats fed different type of dietary lipids[1)] (g)

Group	Brain weight
Corn oil	1.65 ± 0.03
Sardine oil	1.59 ± 0.03
Beef tallow	1.62 ± 0.03
Tuna oil	1.57 [illegible]

1) Values are means± SEM and do not differ significantly(p<0.05) by Duncan's multiple range test among groups.

Table 5. Brain fatty acid composition in rats fed different type of dietary lipids[1)] (%)

Fatty acid	Experimental group			
	Corn oil	Sardine oil	Beef tallow	Tuna oil
14 : 0	0.20 ± 0.01^{b}[2)]	0.23 ± 0.01^{ab}	0.23 ± 0.01^{ab}	0.24 ± 0.01^{a}
16 : 0	23.07 ± 0.65	22.02 ± 0.79	23.78 ± 0.63	22.74 ± 0.89
16 : 1n7	0.44 ± 0.02^{c}	0.62 ± 0.01^{a}	0.53 ± 0.02^{b}	0.69 ± 0.04^{a}
18 : 0	24.04 ± 0.35	23.88 ± 0.40	24.86 ± 0.34	23.90 ± 0.28
18 : 1n9	20.56 ± 0.29^{b}	21.65 ± 0.44^{ab}	21.95 ± 0.28^{a}	20.95 ± 0.46^{ab}
18 : 2n6	1.48 ± 0.03^{a}	0.43 ± 0.07^{c}	0.71 ± 0.09^{b}	0.50 ± 0.09^{bc}
18 : 3n3	ND[3)]	ND	0.05 ± 0.03	ND
20 : 0	0.63 ± 0.09	0.59 ± 0.07	0.66 ± 0.05	0.74 ± 0.05
20 : 1n9	1.83 ± 0.08	1.51 ± 0.12	1.77 ± 0.11	1.59 ± 0.08
20 : 2n6	0.29 ± 0.04^{a}	ND^{c}	0.14 ± 0.03^{b}	0.05 ± 0.04^{bc}
20 : 3n6	0.35 ± 0.06	0 27 ± 0.06	0.34 ± 0.04	0.37 ± 0.02
20 : 4n6	12.04 ± 0.22^{a}	9.29 ± 0.18^{c}	11.17 ± 0.19^{b}	9.53 ± 0.19^{c}
20 : 5n3	ND^{b}	0.40 ± 0.08^{a}	0.02 ± 0.02^{b}	0.30 ± 0.08^{a}
22 : 0	0.56 ± 0.08	0.46 ± 0.12	0.37 ± 0.09	0.43 ± 0.15
22 : 1n9	0.02 ± 0.01	0.06 ± 0.04	0.02 ± 0.01	0.06 ± 0.04
22 : 6n3	12.44 ± 0.54^{b}	16.76 ± 0.35^{a}	12.16 ± 0.36^{b}	16.53 ± 0.97^{a}
24 : 0	0.77 ± 0.25	0.76 ± 0.33	0.50 ± 0.20	0.62 ± 0.31
24 : 1n9	1.28 ± 0.43	1.07 ± 0.59	0.74 ± 0.28	0.79 ± 0.33
SFA	49.27 ± 0.67^{ab}	47.94 ± 0.83^{b}	50.39 ± 0.68^{a}	48.68 ± 0.77^{ab}
MUFA	24.12 ± 0.28	24.92 ± 0.44	25.00 ± 0.35	24.08 ± 0.40
PUFA	26.60 ± 0.48^{a}	27.15 ± 0.45^{a}	2[illegible].59 ± 0.49^{b}	27.28 ± 0.98^{a}
Total n3[4)]	12.44 ± 0.54^{b}	17.16 ± 0.36^{a}	12.23 ± 0.37^{b}	16.83 ± 0.95^{a}
Total n6[5)]	13.87 ± 0.23^{a}	9.99 ± 0.25^{c}	12.22 ± 0.25^{b}	10.41 ± 0.29^{c}
Total n9[6)]	23.97 ± 0.29^{ab}	24.29 ± 0.44^{ab}	24.61 ± 0.35^{a}	23.45 ± 0.39^{b}
P/S ratio[7)]	0.54	0.56	0.49	0.56

1) Values are means± SEM and expressed as the relative weight % of total fatty acids.
2) Values in a row not sharing a same superscript differ significantly(p<0.05) by Duncan's multiple range test among groups.
3) Not Detected
4) Total n3=C18 : 3n3+C20 : 5n3+C22 : 6n3
5) Total n6=C18 : 2n6+C20 : 2n6+C20 : 3n6+C20 : 4n6
6) Total n9=C18 : 1n9+C20 : 1n9+C22 : 1n9+C24 : 1n9
7) P/S ratio=PUFA/SFA

difference among the groups.

3. **Brain fatty acid composition**

The fatty acid composition of brain is shown in Table 5. In the content of n-3 fatty acids, LNA (18 : 3) was not detectable in the corn oil, sardine oil, and tuna oil group and also was very small in the beef tallow group. In the experiments used perilla oil as lipid source[21], there was little LNA accumulation in brain tissue, and it is evident that dietary LNA may be changed to DHA in the body and, then DHA may accumulate in the brain.

The EPA and DHA concentrations were high in the sardine oil and tuna oil groups, compared to the corn oil and beef tallow groups. The DHA content of the tuna oil and sardine oil groups(16.53%, 16.76%) was significantly higher than that of the corn oil and beef tallow groups(12.44%, 12.16%). The fact that there was no difference between the tuna oil and sardine oil groups indicates that EPA in sardine oil could be successfully converted to DHA and then accumulated in brain tissue.

The n-6 fatty acids were the highest in corn oil group, and beef tallow group, fish oil groups in that order.

The ratio of saturated fatty acid(SFA)/monounsaturated fatty acid(MUFA)/polyunsaturated fatty acid(PUFA) in brain tissue was 1.00/0.49/0.54 of the corn oil group, 1.00/0.52/0.56 of the sardine group, 1.00/0.50/0.49 of the beef tallow group, and 1.00/0.49/0.56 of the tuna oil. These were different from the fatty acid composition of diets. It suggests that the ratio in brain may be controlled with a narrow range regardless of dietary lipid composition.

4. **Water maze test**

The results of the water maze test are shown in Fig. 2. There was no significant difference among the groups, but the sardine oil and tuna oil groups appeared to swim faster than the corn oil and beef tallow groups to arrive at the goal platform. In the first week, the corn oil group took the longest time in the test, and the tuna oil, the sardine oil, the beef tallow in order. In the last week of the test, however, the fish oil groups performed the maze faster than the corn oil and beef tallow groups. Therefore, it indicated that the fish oil groups had a higher learning ability than the other groups, which agreed with other studies reported that the consumption of n-3 fatty acids had an effect on improving learning ability. Chung[21] reported that fish oil group made less errors in visual discrimination test than corn oil group. In addition, when Lampty & Walker[22] provided safflower oil or soy bean oil as lipid source to rats, safflower oil group showed lower learning ability in performing Y-shaped maze. Many experiments[23-25] about pregnant and lactating animals provided LNA or DHA-deprived diets have been reported that there occurred low brain level of DHA in their babies and the damage of their learning ability. Therefore, consumption of fish oil abundant either in EPA or in DHA seemed to be helpful in performing the maze test.

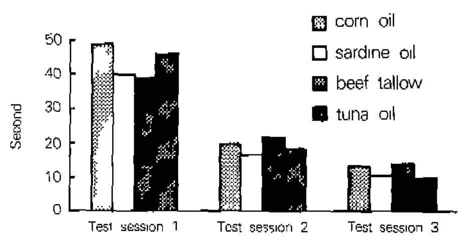


Fig. 2. Time spent in the water maze test in rats fed different type of dietary lipids. Bars are means and do not differ significantly by nonparametric Kruskal-Wallis test among groups.

Conclusion

This experiment investigated the effect of EPA or DHA of fish oils on the brain fatty acid composition and the learning ability in weanling rats.

The food intake and body weight gain of the sardine oil and tuna oil groups were significantly lower than the corn oil and beef tallow groups. The food efficiency ratio of the fish oil groups was lower than the others while there was no difference between the two fish oil groups. However, average brain weights of the groups were not significantly different.

In the brain fatty acid composition, the corn oil group showed a high concentration of n-6 fatty acids, the fish oil groups of n-3 fatty acids, and the beef tallow group of saturated fatty acid. The fish oil groups, sardine and tuna, had almost same EPA and DHA contents but significantly higher than the other groups. However, the brain ratio of SFA/MUFA/PUFA was controlled within a narrow range.

In the maze test, the fish oil groups tended to arrive at the goal faster than the corn oil and beef tallow groups.

This study indicated that dietary EPA efficiently converted to DHA resulting in DHA accumulation in brain tissue and might increase the learning performance as DHA did. Also, the brain fatty acid composition could be changed by providing fish oils even after lactating period, when brain growth is known to be mostly completed.

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= 국문초록 =

어유 섭취가 흰쥐의 뇌조직내 지방산 조성 및 학습행동에 미치는 영향

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본 연구에서는 n-3계 지방산인 EPA 및 DHA를 공급하였을 때 뇌조직의 지방산 조성과 학습행동에 나타나는 영향을 포화지방산과 n-6계 지방산 공급과 비교하여 알아보고자 하였다. 이유한 흰쥐를 체중에 따라 10마리씩 네 군으로 나누어 지방급원을 각각 옥수수유, 정어리유, 쇠기름, 참치유로 하여 식이내 15%(w/w) 첨가하여 7주간 공급한후 희생시켜 뇌조직내 지방산을 분석하였다. 미로검사는 실험 5주부터 3주간 시행하였다. 식이섭취량과 체중증가량이 어유섭취군이 옥수수유군이나 쇠기름군보다 낮았다. 뇌의 무게는 실험군간에 차이가 없었다. 뇌조직내 지방산 조성은 식이내 지방산 조성과 비슷하였는데, 옥수수유군은 n-6계 지방산의 농도가 유의적으로 높았고, 쇠기름군은 포화지방산이, 어유섭취유군은 n-3계 지방산의 농도가 높았다. 그러나 옥수수유, 정어리유, 쇠기름, 참치유의 식이내 포화/단일불포화/고도불포화지방산의 비는 각기 달랐으나 각 실험군의 뇌조직내 지방산 조성은 좁은 범위에서 조정되고 있는 것으로 나타내었다. EPA가 많은 정어리유 섭취군과 DHA가 많은 참치유 섭취군이 다른 두 군에 비하여 뇌조직내에 EPA와 DHA의 수준이 높았으나 두 어유군간에는 차이가 없었다. 즉 식이내의 EPA가 체내에서 효율적으로 DHA로 전환되어 뇌에 축적이 되는 것으로 나타났다. 미로검사에서는 n-3계 지방산함량이 높은 어유섭취군이 옥수수유군이나 쇠기름군보다 빠른 경향을 보여 n-3계 지방산의 섭취가 학습효과 증진에 도움을 주리라고 보여진다.