Korean J Nutrition 31(5) : 897~905, 1998

Effects of Dietary Fatty Acids on Fatty Acid Pattern in Developing Rat Brain Phospholipids*

- Effects on P/M/S and ω3/ω6 Fatty Acid Ratios -

Um, Young Sook · Chung, Eun Jung · Lee-Kim, Yang Cha**

Research Institute of Food & Nutrition Sciences, College of Human Ecology, Yonsei University, Seoul 120-749, Korea

*General Education,** Kangnam University, Kyunggido, 449-702 Korea*

ABSTRACT

Docosahexaenoic acid(DHA), a ω3 series fatty acid and arachidonic acid(AA). a ω6 series fatty acid were found in relatively high concentrations in the phospholipids(PLs) of cell membranes of nerve tissues, and they can be affected by various factors. The present study examined the effects of dietary ω6 and ω3 fatty acid composition on P/M/S and on ω3/ω6 fatty acid ratios in brain PLs of 2nd generation rats. The expeimental diets consisted of 10% fat(by wt), which were computer- searched mixed oil('M') with P/M/S ratio, 1 : 1.4 : 1 and ω6/ω3 ratio, 6 : 1 and safflower oil('S') poor in ω3 fatty acids. The experimental diets were started 3 - 4 wks prior to conception. During the lactation period, the feeding mothers were switched 1 wk after birth and provided the pups for 2 wks with milk which had compositions different from that of their natural mother. The same diet as their mothers was provided from weaning to 9 wks of age. The 'M' and 'S' rats were again subdivided into MM, MS, SS, SM rats according to diet which their lactating mothers were fed from the begining of the experiment. The relative percentage of P/M/S fatty acids in brain PLs in all experimental groups converged to a very similar value at 9 wks of age, indicating the existence of a control mechanism for the degree of fatty acid unsaturation. The ω3/ω6 fatty acid ratios of brain PLs converged to about 1.0 in MM & SM groups and to 0.7 in SS & MS groups, suggesting also the existence of some balance between ω3 and ω6 fatty acids in developing rat brain. The concentrations of ω3 fatty acids, especially DHA, in the SM group were increased and became similar to those in MM group at 9 wks of age. The increase in DHA of brain PLs was counterbalanced by a decrease in 22 : 5ω6. Therefore, the ratios of 22 : 6ω6/22 : 5ω6 were higher in both MM & SM groups than those of SS & MS groups at 9 wks of age. Although dietary ω3 and ω6 fatty acids affected 22 : 6ω3 and 22 : 5ω6 contained in rat brain PLs reciprocally, the relative percentage of AA did not appear to be significantly influenced by the diet in all groups at 9 wks of age, suggesting that a mechanism for the maintenance of a certain level of AA in brain PLs exists.

In conclusion, the ω3/ω6 fatty acid and 22 : 6ω3/22 : 5ω6 ratios, but not P/M/S ratio, of rat brain PLs were affected by the postnatal dietary changes. Futher studies are required to clarify the mechanism(s) of ensuring a certain level of DHA and of maintaining a similar level of AA in

Accepted : June 5, 1998

*This research was supported by grants from Korea Science & Engineering Foundation.

rat brain PLs after weaning(9 wks) regardless of prenatal and postnatal dietary changes. (*Korean J Nutrition* 31(5) : 897~905, 1998)

KEY WORDS : brain-phospholipids · P/M/S ratio · docosahexaenoic acid(DHA) arachidonic acid(AA) · ω3/ω6 fatty acids ratio · lactation.

Introduction

Docosahexaenoic acid(DHA, 22 : 6ω3) and arachidonic acid(AA, 20 : 4ω6) are the predominant ω3 and ω6 series long chain polyunsaturated fatty acids(LCPUFA) in the mammalian central nervous system[1]. Both fatty acids are found in membrane phospholipids(PLs) and are known to be essential for development and function of the brain[2-10]. DHA and AA are derived from the essential fatty acids, α-linolenic aicd (α-LnA, 18 : 3ω3) and linoleic acid(LA, 18 : 2ω6), respectively. The capacity to elongate and desaturate essential fatty acid precursors has been a concern for the premature infant born with low fat stores and subsequently minimal stores of LCPUFA[11].

Dietary deficiencies of ω3 fatty acids during development are associated with reductions of levels of DHA in the brain, with a reciprocal increase in levels of 22 : 5ω6[4][11][12]. These changes are accompanied by effects on various measures of visual function in rodents[7][8][13].

Infant formula as a substitute for human breast milk has become an acceptable alternative for feeding preterm infants. Changes to the composition of infant formula designed to optimize brain development by adding of DHA and AA have been studied[14][15]. Dietary ω6/ω3 fatty acid ratio for infants has been recommended to be within the range of 4/1 - 10/1, which is similar to that found in human milk[16].

This study focused on the effects of diets either with desirable ratios of both ω6/ω3 and P/M/S (mixed oil-fed group) or with deficient in ω3 series fatty acids(safflower oil-fed group) on the P/M/S and ω6/ω3 fatty acid ratios in brain PLs of the 2nd generation rat.

Materials and Methods

1. Animals and diets

Female Sprague-Dawley rats weighing 170 - 230g were divided into two groups and fed the experimental diets for 3 - 4 weeks prior to their conception. Maternal experimental diets consisted of 10%(w/w) fat, which were either mixed oil('M') with a P/M/S ratio, 1/1.4/1 and a ω6/ω3 ratio, 6.1/1 or safflower oil(S) poor in ω3 fatty acid. This mixed oil with the desirable ratios of P/M/S and ω6/ω3 was chosen from various combinations of oils generated by a self-developed computer program. The mixed oil consisted of menhaden oil, soybean oil, corn oil, canola oil, palm oil(5, 5, 20, 25 and 45 weight%). The purified menhaden oil containing 1g of tocopherol acetate per kg oil was donated by the Zapata Haynie Corp.(U.S.A.). The purified corn oil & canola oil were donated by the Sam Yang Co., Ltd.(Seoul, Korea) ; the purified soybean oil by the Dong Bang Co., Ltd.(Seoul, Korea) ; the purified palm oil by the Nhong Shim Co., Ltd.(Seoul, Korea).

Three days after delivery, the litters were adjusted to 8 - 10 animals for each mother. During the lactation period, at 1 week after birth, the feeding mothers were switched to provide the new-born pups for 2 weeks with the maternal milks from the same or different mothers until 3 weeks of age. Thus 'M' and 'S' rats were subdivided further into MM, MS, SS and SM groups according to diet which their lactating mothers were fed from the begining of the experiment. After weaning, rats were fed continuously with the same diet as their lactating mothers' up to 9 weeks of age. The DHA contents and ω6/ω3 fatty acid ratios of the 8th day milk were 0.55% and 7.14 for M group, and 0.13% and 50.0 for S group, respectively. The fatty acid compositions of experimental diet and the 8th day milk are shown in Table 1. Animals were sacrificed at 0, 1, 3 and 9 weeks of age to measure fatty acid composition of brain PLs.

2. Lipid and fatty acid analysis

The lipids of brain were extracted according to the method of Folch, et al.[17] Total PLs from brain were

Table 1. Fatty acid compositions of experimental diets and the rat milk at lactation 8th day

Fatty acids	Experimental diet		Rat milk	
	Mixed oil ('M') [1]	Safflower oil ('S')	'M' [2]	'S' [3]
	- wt % of total fatty acids -			
Saturates	28.0	11.4	43.8	44.0
Monounsaturates	40.0	9.1	35.7	14.9
Polyunsaturates	28.1	78.0	18.3	38.0
18 : 2ω6	24.1	77.7	13.8	32.9
20 : 3ω6	-	-	0.40	0.89
20 : 4ω6	-	0.2	1.14	2.05
22 : 4ω6	-	-	0.23	0.69
22 : 5ω6	-	-	0.32	0.40
Totalω6	24.2	78.0	16.1	37.5
18 : 3ω3	2.4	-	0.85	0.33
20 : 5ω3	0.5	-	0.47	0.05
22 : 5ω3	-	-	0.34	0.08
22 : 6ω3	0.5	-	0.55	0.13
Totalω3	3.9	-	2.20	0.56
ω6/ω3	6.1	-	7.14	50.0
P/M/S4)	1.0/1.4/1.0	6.9/0.8/1.0	0.44/0.85/1.0	0.88/0.34/1.0

1) Mixed oil(10% by wt) consisted of 0.5% menhaden oil, 0.5% soybean oil, 2.0% corn oil, 2.5% canola oil, and 4.5% palm oil(This mixture was selected from the computer-searched combinations of various fats and oils for this study).
2) M : Milk of mother group fed mixed oil diet
3) S : Milk of mother group fed with ω3 fatty acid deficient diet
4) P/M/S : Polyunsaturated / monounsaturated / saturated fatty acids.

separated by thin layer chromatography(TLC). The PLs' areas on the silica gel were scraped off immediately after the TLC procedure and methylated by the procedure of Lepage & Roy[18]. The compositions of fatty acid methyl esters were then measured by gas liquid chromatography(GLC, Hewlett-Packard 5890A). For the gas chromatographic separation, a bonded fused-silica capillary column(OMEGAWAX 320, Supelco, USA ; 30m×0.32mm ID×0.25um) was used. The oven temperature of GLC was 200℃. The temperature of injection and detector ports was 260℃. Helium was used as the carrier gas for the column and the flow rate was 1 ml/min with a split ratio of 30 : 1. Methyl esters of various fatty acids were identified by comparison with fatty acid methyl ester standards purchased from the Supelco(Catalog No. 1081) & Nu Chek Prep, Inc., USA(GLC-87A), and were then quantified on the basis of the amount of heptadecanoic acid internal standard obtained from Nu Chek Prep, Inc.(N-17-A).

3. Statistics

Statistical analysis was done using SAS procedure ; the results of fatty acid analysis were presented as mean±SEM and analyzed by one-way analysis of variance(ANOVA) ; the results of Duncan's Multiple Test were presented.

Results

1. P/M/S ratios of fatty acids in brain phospholipids

Fig. 1 illustrates the distribution of the mean values of the polyunsaturated fatty acids, monounsaturated fatty acids and saturated fatty acids in brain PLs at birth and during the experimental period. The levels of PUFA were increased during the 1st week after birth and decreased slowly frow that point. A general trend of an increase in MUFA at the expense of SFA was clearly discernible from the 1st week after birth. The relative percentage of P/M/S fatty acids showed similar values in four dietary groups except for the SS group($p<0.05$ with SM & MM) at 3 wks of age. It was noticeable that the P/M/S fatty acid ratios converged to a very similar

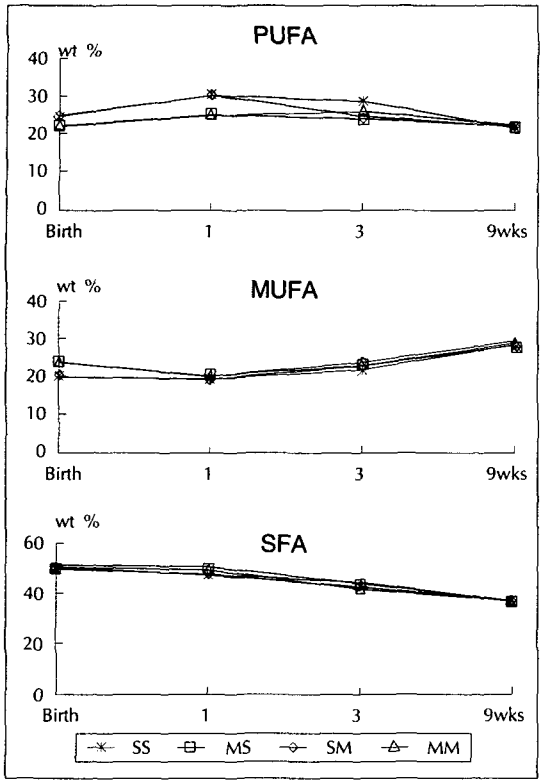

Fig. 1. Changes of polyunsaturated(PUFA), monounsaturated(MUFA) and saturated(SFA) fatty acids in rat brain phospholipids by feeding different types of fats from fetal life to 9 weeks old. Means±SEM, n =10/group.

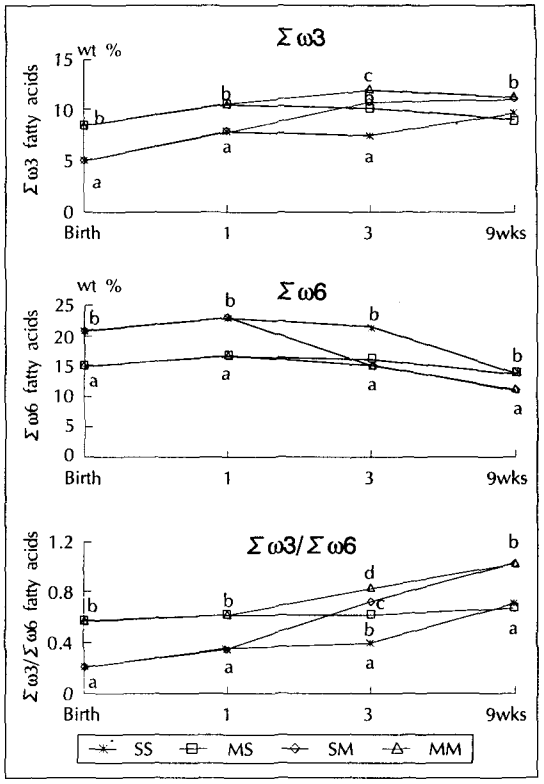

Fig. 2. Changes of Σω3 & Σω6 fatty acids Σω3/Σω6 ratio in rat brain phospholipids by feeding different types of fats from fetal life to 9 weeks old. Values with the different letters are siginificantly different within the same fatty acid at each week($p<0.05$). Means±SEM, n=10/group.

value in all dietary groups at 9 weeks of age.

2. ω6/ω3 fatty acid ratios of brain phospholipids

The compositions of total ω3 & ω6 fatty acids in brain PLs are shown in Fig. 2. The percentages of total ω3 fatty acids, including DHA, were higher in both MM and SM groups which were fed the M-diet either from the very beginning of the experiment(MM) or from day 8 of lactation(SM) to 9 weeks of age, compared to those of SS and MS groups which were fed the S-diet either from the very beginning of the experiment(SS) or from day 8 of lactation(MS) to 9 weeks of age, in brain PLs at 3 and 9 weeks of age. In particular, the concentration of total ω3 fatty acids SM group was increased and became similar to that of MM group at 9 wks of age, although they were significantly lower than those of MS group at 0 and 1 wk of age. In contrast, the concentrations of total ω6 fatty acids were higher in SS and MS groups than in SM and MM groups at 3 & 9 weeks of age. The relative amount of total ω3 and ω6 fatty acids of brain PLs at 9 weeks of age converged to a very similar value in MM and SM groups, and in SS and MS groups, respectively. Therefore, the order of ω3/ω6 fatty acid ratios appeared as SS <MS<SM<MM(0.4<0.6<0.7<0.9) in brain PLs at 3 weeks of age, and ω3/ω6 fatty acid ratios converged to about one in SM & MM groups and to 0.7 in SS & MS groups at 9 weeks of age.

3. 22 : 6ω3/22 : 5ω6 ratios in brain phospholipids

The compositions of DHA and docosapentaenoic acid(22 : 5ω6) of brain PLs are shown in Fig. 3. DHA is the only ω3 fatty acid present in significant amounts in brain PLs. The percentage of DHA was

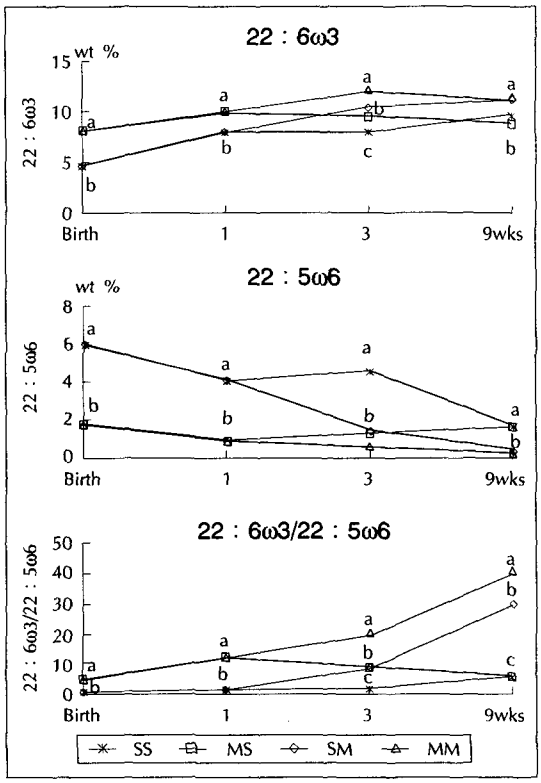

Fig. 3. Changes of 22 : 6ω3 & 22 : 5ω6 and 22 : 6ω3/22 : 5ω6 ratio in rat brain phospholipids by feeding different types of fats from fetal life to 9 weeks old. Values with the different letters are siginificantly different within the same fatty acid at each week($p<0.05$). Means±SEM, n=10/group.

increased by switching the lactating mothers from 'S' to 'M'('S'→SM), and this increase was counterbalanced by a decrease in 22 : 5ω6. The concentration of DHA in MS group, however, was not increased after 1 week of age by changing from 'M' to MS and the value was maintained until 9 weeks of age. It is remarkable to observe that, a considerable amount of DHA was found in the brain PLs of S rats which were born to mothers fed ω3 fatty acid-deficient diet from 3-4 weeks before conception.

Because of reciprocal replacement of ω6 with ω3 fatty acids, for MM and SM groups reared by 'M' diet-fed mothers, 22 : 6ω3/22 : 5ω6 ratios in brain PLs were significantly higher at 9 weeks of age than those in brain PLs of SS and MS groups. The differences in these ratios of brain PLs were more pronouunced at 9 weeks as compared to 3 weeks of age.

Although ω3 fatty acid-deficient diets were shown to cause reciprocal replacement of ω3 with ω6 fatty acids in brain PLs of young rats, the percentages of AA, the most abundant ω6 fatty acids in brain PLs, were not altered significantly affected by either experimental diet 'M' or 'S' except in SS group at 3 and 9 weeks of age, as shown in Table 2. The ω6 precursor fatty acid, linoleic acid, appeared as a minor fatty acid in nervous tissue.

Table 2. Changes of major 3ω3 & ω6 fatty acids of the brain phospholipids by feeding different types of fats from fetal life to 9 weeks old

Period(wk)	ω6 Series				ω3 Series		
& Group	18 : 2ω6	20 : 4ω6	22 : 4ω6	22 : 5ω6	20 : 5ω3	22 : 5ω3	22 : 6ω3
	- g/100g of total fatty acids -						
0 week							
M	0.98±0.12^{a}	9.15±0.24^{a}	2.07±0.15^{a}	1.72±0.10^{a}	0.05±0.01	0.22±0.02^{b}	7.97±0.61^{b}
S	1.51±0.08^{b}	10.6±0.27^{b}	2.99±0.16^{b}	6.05±0.21^{b}	0.05±0.01	0.11±0.02^{a}	4.52±0.30^{a}
1st week							
M	1.19±0.03	11.7±0.38^{a}	2.50±0.08^{a}	0.80±0.03^{a}	0.07±0.02	0.33±0.02^{b}	9.89±0.46^{b}
S	1.54±0.17	13.3±0.26^{b}	3.50±0.08^{b}	4.08±0.11^{b}	0.06±0.01	0.19±0.01^{a}	8.01±0.29^{a}
3rd week							
MM	0.94±0.02^{a}	9.78±0.07^{a}	2.87±0.08^{ab}	0.59±0.03^{a}	0.14±0.03^{b}	0.26±0.03	12.3±0.11^{b}
SM	1.06±0.03^{b}	9.41±0.38^{a}	2.62±0.16^{a}	1.25±0.10^{b}	0.09±0.00^{ab}	0.20±0.03	10.3±0.63^{b}
MS	1.17±0.04^{b}	10.1±0.26^{a}	3.01±0.18^{b}	1.14±0.09^{b}	0.07±0.03^{a}	0.23±0.03	9.63±0.57^{b}
SS	1.24±0.02^{c}	11.6±0.10^{b}	3.67±0.05^{c}	4.50±0.15^{c}	0.07±0.01^{a}	0.19±0.02	8.08±0.31^{a}
9th week							
MM	0.61±0.02^{a}	7.56±0.07	2.52±0.11^{a}	0.29±0.02^{a}	0.11±0.02	0.24±0.02^{b}	11.7±0.69^{b}
SM	0.62±0.03^{ab}	7.32±0.08	2.52±0.04^{a}	0.39±0.02^{a}	0.10±0.01	0.21±0.01^{b}	11.3±0.22^{b}
MS	0.84±0.03^{c}	7.77±0.13	3.19±0.19^{b}	1.55±0.19^{b}	0.08±0.01	0.14±0.03^{a}	9.01±0.46^{a}
SS	0.72±0.06^{b}	7.70±0.12	3.01±0.09^{b}	1.59±0.07^{b}	0.12±0.01	0.13±0.02^{a}	9.45±0.31^{a}

Superscripts a, b, c indicate that values with the different letters are significantly different within the same fatty acid at each week ($p<0.05$). Values are Means±SEM, n=10/group.

Discussion

Patterns of fatty acids in brain PLs can be affected by various factors, *ie.*, age, brain region and brain cell type[15]. The present study examined the effects of dietary fats given 3-4 weeks before conception to 9 weeks old on fatty acid patterns of developing rat brain PLs. Breast-fed pups were switched to different dietary fat and different lactating mothers as stated in experimental design.

As shown in Fig. 1, the relative percentage of PUFA, MUFA & SFA values converged to very similar values in all dietary groups at 9 weeks of age, despite the fact that diets with extremely different fatty acids patterns were given even before conception up to 9 weeks of neonatal age. As suggested from previous works[7][19], this result indicated a possible regulatory mechanism on the degree of fatty acid unsaturation in brain. Thomas, et al.[20] also showed that 22-carbon LCPUFA shares a similar mechanism of incorporation with PL. The percentage of ω3 fatty acids and ω3/ω6 fatty acid ratios in total brain PLs of SM became significantly higher than those of MS at 3 weeks of age, although they were significantly lower at 0 and 1 week of age. Pups in SM group were born to the mothers which were fed the ω3 fatty acid-deficient S diet from 3 weeks before conception up to the 1st week of lactation, totaling 8 weeks and then fed M diet up to 9 weeks of age. At 9 weeks of age, ω3/ω6 ratios of SM group became similar to those of MM group fed 'M' diet with P/M/S ratio, 1/1.4/1 and ω6/ω3 ratio, 6.1/1 throughout experimental period. The results showed that the fatty acid compositions of the newborn rat brain reflect postnatal diet including maternal milk. According to Wainwright, et al.[21], total amount of DHA in the group which received the ω3 supplementation only during the 12 day postnatal period was as high as in the group supplemented during gestation and lactation. This suggests that the ω3/ω6 ratio of the LCPUFA in the brain of the developing offspring can be altered within days through manipulation of the ω3 content of the maternal diet during lactation when the growth of rat brain is at its maximum rate. According to Youyou, et al.[22], the recovery of DHA content in the brain of rat pups started rapidly after changing the maternal diet, *ie.*, from the very first day of changing the diet from sunflower oil to soya oil, but it took more than 2 months for DHA to reach the control value, suggesting the recovery of DHA content by brain cells was slow when the diet was given after lactating period.

It was also remarkable to observe in the SS group that the level of ω3 fatty acids in brain PLs was maintained at $\geq$5% of total fatty acids and the ratios of ω3/ω6 fatty acids even increased at 9 weeks of age, despite receiving a ω3 fatty acid-deficient diet from 7 weeks of prenatal age(Fig. 2). Galli, et al.[23] have shown that rats supplemented with ω3 fatty acids during development maintain certain levels of DHA, even after two months on a ω3 deficient diet. In agreement with previous studies[6][24-26], it seems that dietary ω3 fatty acid deficit during the gestation period can be offset partially by selective retention of DHA by dam. Neuringer, et al.[3] reported that plasma DHA level, although reduced in the neonates which were born to the mothers fed the ω3 deficient diet, was higher than that in their mothers. These suggest that the developing fetus might be preferentially supplied with DHA via placenta and thereby protected from the effects of maternal dietary ω3 fatty acid deprivation. Bazan, et al.[25] suggested that the liver is the organ which actively synthesizes DHA. The maternal adipose tissue was also suggested as a reservoir and provider of DHA[27]. On the other hand, the ω3/ω6 ratios of brain PLs reached a very similar value in MM & SM and SS & MS groups, indicating the effect of postnatal diet overrode that of prenatal diet. This effect may be partly due to the fact that rat brain grows most rapidly during postnatal lactation period. Dietary fatty acid composition and the time of administration of diets can also be regarded as important contributing factors for the fatty acid pattern in brain PLs. Neuringer, et al.[3] suggested that the content of DHA in cerebral cortex approximately doubled in monkeys which were fed ample 18 : 3ω3 throughout pregnancy and received a similar diet from birth, but not in the group fed the diet very low in 18 : 3ω3. The provision of ω3 LCPUFA in brain from α-linolenic acid is an area of further possible study.

As suggested from the previous works[7)10)19)28)29)], the decrease in DHA in rat pups brain caused by changing from MM to MS was compensated for the increase in 22 : 5ω6. Because of compensatory replacement of ω3 with ω6 fatty acids, 22 : 6ω3/22 : 5ω6 ratios in SS and MS rats which were reared by mothers fed ωS' diet were significantly lower than those in MM and SM rats reared by 'M' diet-fed mothers. Levels of 22 : 5ω6 are known to increase in ω3 fatty acid deficiency, thus 22 : 6ω3/22 : 5ω6 ratio in tissue lipids has been suggested to be a more sensitive indicator of the dietary ω3 fatty acid adequacy than percentage of 22 : 6ω3 or 22 : 5ω6 alone[28)]. Despite their similar physical properties, 22 : 5ω6 is not a functional substitute for DHA in neurological functions[3)5)10)30)]. This fact, in addition to the securing of DHA levels in brain PLs in ω3 fatty acid-deficient diet-fed rats, suggests a specific role of DHA beyond contributing to the maintenance of structural stability of biological membrane. Many behavioral studies have shown that congnitive development can be altered in a dietary ω3 fatty acid defeciency in rat models[7)8)13)]. One of the specific roles of DHA could be linked to its presence in brain plasmalogen which is abundant in synaptic membranes[31)]. DHA could have an influence on mechanisms of neurotransmission through plasmalogen mediation[29)].

Even though the compositions of 22 : 6ω3 and 22 : 5ω6 in rat brain PLs can be altered reciprocally by ω3 or ω6 fatty acid diet, the relative percentage of AA did not appear to be significantly affected by the diet in all groups. This suggests that a mechanism for the maintenance of a certain level of AA in brain PLs exists. The suggested effects of AA on neural cells include modulation of neuronal transmembrane signaling, regulation of neurotransmitter release and glucose uptake[32-34)]. Innis, et al.[35)] has also shown that feeding freshwater fish oil which contains higher DHA and lower 20 : 5ω3 than usual marine fish oil did not result in lowering brain 20 : 4ω6 as compared to feeding soybean oil. The ability to maintain brain AA could be partly explained by the presence of specific, high affinity transport systems for AA into brain[36)]. Retroconversion of 22- to 20-carbon chain LCPUFA might also be involved in maintaining AA level[37)].

In conclusion, the ω3/ω6 fatty acid and 22 : 6ω3/22 : 5ω6 ratios, but not P/M/S ratio, of rat brain PLs were affected by the postnatal dietary changes. Further studies are required to clarify the mechanism of ensuring a certain level of DHA in rat brain PLs. An additional study is also needed to identify the mechanism(s) of maintaining a similar level of AA in 9 weeks old rat brain phospholipids regardless of prenatal and postnatal dietary changes.

■ **Acknowledgements**

The authors wish to thank Zapata Haynie Corp. (USA) for providing Menhaden oil, Sam Yang Co., Ltd., for corn and canola oils, Dong Bang Co., Ltd., for soybean oil and Nhong Shim Co., Ltd., for palm oil.

Literature cited

1) Sastry PS. Lipids of nervous tissue : Composition and metabolism. *Prog Lipid Res* 24 : 69-176, 1985
2) Martinez M. Tissue levels of polyunsaturated fatty acids during early human development. *Pediat* 120 : S129-S138, 1992
3) Neuringer M, connor WE, Lin DS, Barstad L, Luck S. Biochemical and functional effects of prenatal and postnatal ω3 fatty acid deficiency on retina and brain in rhesus mokeys. *Proc Natl* 83 : 4021-4025, 1986
4) Bourre JM, Durand G, Pascal G, Youyou G. Brain cell and tissue recovery in rats made deficiency in ω3 fatty acids by alteration of dietary fat. *J Nutr* 119 : 15-22, 1989
5) Lin DS, William EC, Gregory JA, Neuringer M. Effects of dietary ω3 fatty acids on the phospholipid molecular species of monkey brain. *J Neurochem* 55 : 1200-1207, 1990
6) Kim MK, Chee KM, Lee-Kim YC. Effect of maternal dietary ω3 and ω6 polyunsaturated fatty acids on the fatty acid composition of the second generation rat brain. *Kor J Nutr* 26(6) : 661-671, 1993
7) Um YS, Chung EJ, Lee-Kim YC. Effects of dietary ω3 and ω6 fatty acids on the fatty acid composition of RBC and brain synaptosomal, microsomal and mitochondrial phospholipids and on behavioral development of rats. *Kor J Nutr* 29(8) : 849-860, 1996
8) Chung EJ, Um YS, Kim KH, Kim JS, Lee-Kim YC. Effects of ω3/ω6 fatty acids on behavioral developments of rats : Relation with neurotransmitters. *J Korean Neuurology* 15(5) : 952-963, 1997
9) Simopoulos AP, Summary of the NATO advanced Research workshop on dietary ω3 and ω6 Fatty acids : Biologi-

cal effects and Nutritional essentiality. *J Nutr* 119 : 521-528, 1989

10) Bourre JM, Francois M, YouYou A, Dumont O, Piciotti M, Durand G. The effects of dietary α-linolenic acid on the composition of nerve membranes, enzymatic activity, amplitude of electrophysiological parameters, resistance to poisons and performance of learning tasks in rats. *J Nutr* 119 : 1880-1892, 1989
11) Wainwright PE, Huang YS, Bulman-Fleming B, Levesque S, McCutcheon D. The effect of dietary fatty acid composition combined with environment enrichment on brain and behavior in the mice. *Behav Brain Res* 60 : 125-136, 1994
12) Wainwright PE, Huang YS, Coscina DV, Levesque S, McCutchen D. Brain and behavioral effects of dietary(n-3) deficiency in mice : A three generation study. *Dev Psychobiol* 27 : 467-487, 1994
13) Yamamoto N, Saitoh M, Moriuchi A, Noxura M. Effect of dietary linolenic acid/linoleic acid balance on brain lipid compositions and learning ability of rats. *J lipid Res* 28 : 144 -151, 1987
14) Clandinin MT, Chappell JE, Van Aerde JEE. Requirements of newborn infants for long-chain polyunsaturated fatty acids. *Acta Pediatr Scand* 351 : 63-71, 1989
15) Jumpsen J, Lien EL, Goh YK, Clandinin MT. Small changes of dietary(n-6) and(n-3) fatty acid content ratio alter phosphatidylethanolamine and phosphatidylcholine fatty acid composition during development of neural and glial cells in rats. *J Nutr* 127 : 724-731, 1997
16) Clandinin MT, Chappell JE, Heim T. Do low birth weight infant require nutrition with chain elongation-desaturation product of essential fatty acids? *Prog Lipid Res* 20 : 901-904, 1982
17) Folch J, Lees M, Stanley S. A simple method for isolation and purification of total lipids from animal tissues. *J Biol Chem* 226 : 497-509, 1957
18) Lepage G, Roy CC. Direct transesterification of all classes of lipids in a one-step reaction. *J Lipid Res* 27 : 114-120, 1986
19) Horboticky N, McKinnon MJ, Innis SM. Effect of a vegetable oil formula rich in linoeic acid on tissue fatty acid accretion in the brain, liver, plasma and erythrocytes of infants piglets. *Am J Clin Nutr* 51 : 173-182, 1990
20) Thomas SE, Byers DM, Cook HW. Plasmalogen esterification with polyunsaturated fatty acids is more dependent on chain length than on selectivity between(n-3) and(n-6) families. *INFORM* 1 : 315(abs. X4), 1990
21) Wainwright PE, Huang YS, Bulman-Fleming B, Mills DE, Pedden P, McCutchen D. The role of n-3 essential fatty acid in brain and behavioral development : A cross-fostering study in the mouse. *Lipids* 26 : 37-44, 1991
22) Youyou A, Durand G, Pascal G, Piciotti M, Dumont O, Bourre JM. Recovery of altered fatty acid composition induced by a diet devoid of n-3 fatty acid in myelin, synaptosome, mitochondria and microsome of developing rat brain. *J Neurochem* 46 : 224-228, 1986
23) Galli D, Przegalinski E. *Pharmacol Res Comm* 5 : 239-248, 1973
24) Hwang HJ, Chung EJ, Lee JH, Chee KM, Lee-Kim. Effects of ω3 and ω6 polyunsaturated fatty acid diets on fatty acid composition and vitamin E levels in milk and serum of the rat. *Kor J Nutr* 27(2) : 141-152, 1991
25) Bazan NG, Elena B, Rodrigguez T, William C, Gordon C. Supply, uptake and retention of docosahexaenoic acid by the developing and mature retina and brain. In : Lipids, learning and brain fats in infant formulas. Report of the 103rd Ross Conference on Ped Res, pp27-49, 1993
26) Al DM, Vanhouwelingen AC, Kester AD, Hasaart T, Jong A, Hornstra G. Maternal essential fatty acid patterns during normal pregnancy and their relationship to the neonatal essential fatty acid status. *Br J Nutr* 74 : 55-68, 1995
27) Chung HY, Choi YJ, Lee JH, Lee-Kim YC. Effect of n-3/n-6 polyunsaturated fatty acid diets on fatty acid composition of mesenteric and subcutaneous fat of lactating rat. Presented at 6th Asian Congress of Nutrition, Malaysia, Sept. 16-19, 1991
28) Arbuckle LD, McKinnon MJ, Innis SM. Formula 18 : 2(n-6) and 18 : 3(n-3) content and ratio influence long-chain polyunsaturated fatty acids in the developing piglet liver and central nervous system. *J Nutr* 124 : 289-298, 1994
29) Favrelere S, Barrier L, Durand G, Chalon S, Tallineau C. Chronic dietary n-3 polyunsaturated fatty acids deficiency affects the fatty acid composition of plasmenylethanolamine and phosphatidylethanolamine differently in rat frontal cortex, striatum, and cerebellum. *Lipids* 33 : 401-407, 1998
30) Innis SM, Nelson CM, Rioux FM, King DJ. Development of visual acuity in relation to plasma and erythrocyte ω6 and ω3 fatty acids in healthy term gestation infants. *Am J Clin Nutr* 60 : 347-352, 1994
31) Breckenridge W, Gombos G, Morgan IG. The lipid composition of adult rat brain synaptosomal plasma membranes. *Biochim Biophys Acta* 266 : 695-707, 1972
32) Schweitzer P, Madamba S, Champagnat J, Siggins GR. Somatostatin inhibition of hippocampal CA 1 pyramidal neurons : Meditation by arachidonic acid and it metabolites. *J Neurosci* 13 : 2033-2049, 1993
33) Fraser DD, Hoehn K, Weiss S, MacVicar BA. Arachidonic acid inhibits sodium currents and synaptic transmis-

sion in cultured striatal neurons. *Neuron* 11 : 633-644, 1993

34) Yu N, Martin J-L, Stella N, Magistretti PJ. Arachidonic acid stimulates glucose uptake in cerebral cortical astrocytes. *Pro Natl Acad Sci* 90 : 4042-4046, 1993

35) Innis SM, Rioux FM, Auesstad N, Ackman RG. Marine and freshwater fish oils varying in arachidonic, eicosapentaenoic and docosahexaenoic acids differ in their effects on organ lipids and fatty acids in growing rats. *J Nutr* 125 : 2286-2293, 1995

36) Juro J, Trojam J, moro R, Pineiro A. Intracellular uptake of α-fetoprotein : A maker of neural differentiation. *Ann NY Acad Sci* 417 : 321-329, 1983

37) Singh H, Usher S, Aoluos A. Mitochondrial and synaptosomal beta-oxidation of stearic acid and lignoceric acid by rat brain. *J Neurochem* 53 : 1711-1718, 1989