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Effects of ω6 and ω3 Fatty Acid Diets on the Fatty Acid Composition of the Mesenteric and Subcutaneous Fat of Lactating Rats*

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ABSTRACT

Long chain polyunsaturated fatty acids (LCPUFA) are important components of brain phospholipids and play important role (s) in brain function. In rats, the maximum brain growth occurs during the period of lactation even though it happens during the third trimester of gestation in human. Since milk contained docosahexaenoic acid (DHA) even though the maternal diet had no DHA and/or a very small amount of its precursor, α-linolenic acid (α-LnA), an emphasis was given to maternal adipose tissue as a reservoir of this fatty acid. We, therefore, investigated the mesenteric and subcutaneous adipose tissues for their fatty acid composition in dams reared with different fat diets. Diets containing various amounts of ω6 and ω3 fatty acids were given to adult female rats (200 – 250 g) throughout the pregnancy and lactation periods. Diets were composed of 10% (wt/wt) corn oil (CO), soybean oil (SO), perilla seed oil (PO) containing about 60% α-LnA, or fish oil (FO) rich in eicosapentaenoic acid (EPA) and DHA. The fatty acid compositions of mesenteric and subcutaneous fat were measured and evaluated at Day-2 and Day-15 after parturition. In general, major characteristics of dietary fatty acid composition was reflected on the fatty acid composition of adipose tissues. Dietary fatty acid composition was reflected more on mesenteric fat as compared to subcutaneous fat. Mesenteric fat was found to contain less arachidonic acid (AA) and mesenteric fats of CO, SO and PO groups contained less DHA than did the subcutaneous fat. The P/M/S ratios of adipose tissues were similar between experimental groups while dietary P/M/S ratios differed significantly. It was noticeable that a small proportion of DHA was found in the adipose tissues of animals of CO, SO and PO groups (Day-2) and in SO and PO groups (Day-15), the groups which do not contain DHA in their diets. The percentage of DHA in mesenteric fat of CO, SO and PO groups decreased as lactation continues, while the proportion of DHA in FO group increased. Adipose tissues of FO group had higher DHA/EPA ratio as compared to the diet. Considering the fact that the body contains a large amount of adipose tissues, our present finding suggests that the adipose tissue can serve as a reservoir of DHA for pregnant and lactating rats.

KEY WORDS: mesenteric fat, subcutaneous fat, lactating rat, ω6 and ω3 fatty acids, docosahexaenoic acid.

INTRODUCTION

Docosahexaenoic acid (22 : 6ω3, DHA) and arachidonic acid (20 : 4ω6, AA) are the predominant long chain polyunsaturated fatty acids (LCPUFA) of brain phospholipids.[1-3] Although DHA can be synthesized from α-linolenic acid (18 : 3ω3, α-LNA), the capacity of synthesis is restricted in human and animals.[4]

In humans, LCPUFA, required for brain development, accumulates mostly during fetal period and brain phospholipid synthesis reaches its peak at around 1 year after parturition. In rats, however, the maximum brain growth occurs during the period of lactation. A study on prenatal accumulation of fatty acids in rat brain reported that there was a steep increase in the concentration of all the fatty acids during embryonic days 14 and 17. After this period and up to birth, the concentration of the fatty acids plateaued, except that of DHA, which continued to accumulate further.[5] It was shown that the percentage of DHA in brain was negatively correlated to the number of errors made in maze developmental tests.[6-8] It was also reported that, in human and experimental animals, dietary essential fatty acid and LCPUFA intake during developmental stage can affect the plasma and cell membrane fatty acid composition and visual acuity.[9-10] But young animals cannot synthesize essential fatty acids enough for brain development. However, mother's milk can provide both arachidonic acid and DHA.[11] Several studies have indicated that infants should obtain LCPUFA from their mother's milk.[12-14]

Many researchers demonstrated that diet can influence fatty acid composition of milk.[15-18] And some reported the similarity between mother's milk and brain of infants in regard to fatty acid composition.[11] So it is important for

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mothers to consume appropriate quantity and quality of fat especially during gestation and lactation periods. However some researchers reported that the brain of the second generation rat contained DHA even when mothers were fed diets deficient in ω3 LCPUFA.[19,20] During gestation, metabolic adaptation occurs in mother's body to meet the developmental needs of fetus. This includes transfer of AAω6 and DHAω3 to fetus. A ninety percent increase in dam's hepatic EPA content implies that DHA, necessary for fetal brain growth, is synthesized in pregnant rat's hepatic tissue.[21]

It is not fully understood whether fetus obtains DHA from blood supply through placenta or synthesize itself certain amount of DHA. The impact of milk supply on neuronal development of newborn babies has been of interest to many researchers.[22,23]

Adipose tissue is generally considered to be a storage organ for excess energy, but it has other physical and dynamic functions as well like architecture, insulation and storage of fatty acids and fat-soluble vitamins. While it is quite well documented that dietary fat intake can influence fatty acid composition of milk,[15-17] fat reservoir of the body, as a source of milk fatty acids, has been poorly investigated. But fatty acid composition of adipose tissue is the key element which determines the quality of fat reservoir. Sinclair *et al.*[24] found that saturation of adipose tissue fatty acids through dietary modification is more difficult than desaturation of those fatty acids. Lin *et al.*,[25] reported that the degree of accumulation differs by the degree of desaturation of each fatty acids.

Adipose tissues, according to their location, are different in size and metabolic rate. So many researchers have been trying to compare the fatty acid compositions of various adipose tissues in human body. Especially, visceral fat (i.e. mesenteric fat) has drawn much attention since, when compared to subcutaneous fat, it is metabolically more active.

In this study, we investigated the effects of diets containing various composition of ω3 and ω6 fatty acids on fatty acid composition of dam's adipose tissue which can act as a reservoir of DHA during lactation in Sprague-Dawley rats.

MATERIALS AND METHODS

1. Animals

Female Sprague-Dawley rats (n = 40), with body weight of 200 – 250 g, were used as experimental animals. After pregnancy was confirmed, the rats were reared on experimental diets. After 3 weeks of gestation period, dams were randomly divided into two groups. One group is sacrificed 2 days after parturition (Day-2) and the other is sacrificed 15 days (Day-15) postpartum. Mesenteric fat and subcutaneous fat in the lower abdomen area were collected and frozen at −20℃ for further analysis.

2. Experimental diets

Experimental diets are divided into 4 groups. The composition of test diets are shown in Table 1. Corn oil was used as source of ω6 LA in CO group. SO contains soybean oil which has 6% α-LNA (18 : 3ω3) and 55% LA (18 : 2ω6); the ω6/ω3 ratio of SO is about 9.6. In PO group, perilla seed oil (Pulmuone Co., Ltd) was used as source of ω3 α-LNA (56.8%). Fish oil (Pulmuone Co., Ltd), rich in EPA (20 : 5ω3, 22.3%) and DHA (22 : 6ω3, 6.1%), were used in FO group. In FO group, 10% of fat was replaced with soybean oil to prevent LA deficiency. Table 2 shows the fatty acid composition, ω6/ω3 ratios and Polyunsaturated/Monounsaturated/Saturated Fatty Acids (P/M/S) ratios of test diets.

3. Fatty acid analysis of adipose tissues

To analyze fatty acid composition, an accurate amount of adipose tissues (0.1 g for mesenteric fat and 0.4 g for subcutaneous fat) were weighed and mixed with 0.9% NaCl solution to make 20% homogenate solution. An aliquot of 100 μl homogenized solution of mesenteric fat and of 300 μl of subcutaneous fat were directly methylated by the method of Lepage & Roy.[26] An aliquot of samples were dissolved in 2 ml of methanol-benzene 4 : 1 (v/v) solution. While stirring, 200 μl of acethyl chloride was slowly added over a period of 1 min. Tubes were then tightly closed with Teflon-lined caps and subjected to methanolysis at 100℃ for 1 h. After the tubes had been cooled in water, 5 ml of 6% K_2CO_3 solution was slowly added to stop the reaction and to neutralize the mixture. The mixtures were then centrifuged, and an aliquot of the upper benzene phase was collected and injected into the gas liquid chromatography system for fatty acid analysis (Hewlett Packard 5890 A).[27] Supelco wax 10 capillary column (30 m × 0.32 mm ID × 0.25 μm) and FID (Flame Ionization Detector) were used for the analysis. Oven temperature was programmed from 150℃ to 250℃. Injector and detector temperature was 260℃. Nitrogen gas was used as carrier gas and flow rate was 2 ml/min. Injected sample volume was 1 μl for mesenteric fat and 2 μl for subcutaneous fat. The split ratio was 20 : 1. Each peak of fatty acid methyl esters was identified by comparison with fatty acid methyl ester standard, GLC

Table 1. Composition of experimental diets (Unit : Wt %)

Ingredients	Experimental groups			
	CO	SO	PO	FO
Carbohydrate[1]	65	65	65	65
Protein: Casein	18	18	18	18
DL-Met.	0.1	0.1	0.1	0.1
Fat: Corn oil	10			
Soybean oil		10		
Perilla seed oil			10	
Fish oil[2]				10
Salt mixture[3]	4	4	4	4
Vitamin mixture[4]	1	1	1	1
CMC[5]	2	2	2	2

1. Starch : Sucrose = 80 : 20
2. Fish Oil : Soybean Oil = 9 : 1
3. Salt mixture (g per 100g salt mixture): $CaCO_3$ 29.29 ; $CaHPO_4 \cdot 2H_2O$ 0.43 ; KH_2PO_4 34.31 ; NaCl 25.06 ; $MgSO_4 \cdot 7H_2O$ 9.98 ; $Fe(C_6H_5O_7) \cdot 6H_2O$ 0.623 ; $CuSO_4 \cdot 5H_2O$ 0.156 ; $MnSO_4 \cdot H_2O$ 0.121 ; $ZnCl_2$ 0.02 ; KI 0.0005 ; $Na_2SeO_3 \cdot H_2O$ 0.0015 ; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 0.0025.
4. Vitamin mixture (mg per kg diet): Thiamin HCl 5 ; Riboflavin 5 ; Nicotinamide 25 ; Ca-Pantothenate 20 ; Pyridoxine HCl 5 ; Folic acid 0.5 ; Biotin 0.2 ; Vitamin B12 0.03 ; DL-α-tocopherol acetate 100 ; Retinyl palmitate 2.196 ; Cholecalciferol 10 (μg) ; Choline chloride 2000 ; Ascorbic acid 50 ; Menadione 0.5 ; Inositol 100.
5. CMC: Carboxymethyl Cellulose Sodium Salt.
6. CO: Corn Oil Group, SO: Soybean Oil Group, PO: Perilla Oil Group, FO: Fish Oil Group. (No additional Vit. E is supplemented)

68B (Nu Check Prep. Inc., USA).

4. Statistical analysis

All values appearing in the table and figures are expressed as mean ± SEM. The significance between experimental groups were tested by one way ANOVA and Duncan's multiple range test. The significance between experimental periods and adipose tissue site were tested by Student's t-test.[28] The data were analyzed by the SPSS/PC$^+$ package.

RESULTS

1. Fatty acid composition of mesenteric fat

Fatty acid composition of mesenteric fat of Day-2 and Day-15 are shown in Table 3. In case of Day-2 mesenteric fat, LA levels of CO and SO were 36.25% and 35.19%, respectively, and significantly higher than those of PO and FO ($p < 0.05$). α-LnA composition was highest in PO (13.16%). AA, which can be synthesized from LA, showed the lowest level in PO, possibly because of a very high quantity of α-LnAω3 in PO which competitively inhibit the synthesis of AAω6 from LAω6. EPA showed the highest level in FO (1.51%). PO showed the second highest EPA level (0.43%). Along with EPA, DHA showed the significantly high level in FO (4.85%) and DHA level in CO was lowest (0.37%).

Table 2. Relative composition of total fatty acids of dietary oils[1]

Fatty acids	Experimental groups			
	CO	SO	PO	FO
12 : 0	0.23	ND[2]	ND	ND
14 : 0	0.18	ND	ND	8.68
15 : 0	ND	ND	ND	0.1
16 : 0	10.5	11.2	6.81	14.0
16 : 1 ω7	0.05	ND	0.10	13.0
17 : 0	ND	ND	ND	ND
17 : 1	ND	ND	ND	0.15
18 : 0	1.95	3.71	2.34	1.11
18 : 1 ω9	26.1	21.7	20.3	14.8
18 : 2 ω6	58.1	54.7	13.1	3.92
18 : 3 ω3	0.82	5.78	56.8	0.14
18 : 4 ω3	ND	ND	ND	3.61
20 : 1 ω9	0.30	ND	ND	4.14
20 : 2 ω6	0.05	0.03	ND	ND
20 : 4 ω6	0.07	0.67	0.18	0.09
20 : 5 ω3	ND	0.01	ND	22.3
22 : 1 ω9	0.11	ND	ND	3.02
22 : 5 ω3	ND	ND	ND	0.7
22 : 6 ω3	ND	ND	ND	6.07
24 : 1 ω9	0.10	0.09	ND	0.94
SFA[3]	12.8	14.9	9.15	23.9
MUFA[4]	26.6	21.7	20.4	36.1
PUFA[5]	59.1	61.2	70.1	36.9
ω6/ω3	71.01	9.58	0.23	0.12
P/M/S[6]	4.6/2/1	7/4.1/1	5.1/2.2/1	1.5/1.5/1

1) Values are expressed as the relative % of total fatty acids.
2) Not Detected..
3) SFA = C12 : 0 + C14 : 0 + C15 : 0 + C16 : 0 + C17 : 0 + C18 : 0
4) MUFA = C16 : 1 + C17 : 1 + C18 : 1 + C20 : 1 + C22 : 1 + C24 : 1
5) PUFA = C18 : 2 + C18 : 3 + C18 : 4 + C20 : 2 + C20 : 4 + C20 : 5 + C22 : 5 + C22 : 6
6) Polyunsaturated/Monounsaturated/Saturated Fatty Acids.

The fatty acid composition pattern was similar in Day-15 mesenteric fat when compared to that of Day-2. But, in Day-15 mesenteric fat, AA composition was different from that of Day-2. PO and FO showed the lowest AA level and CO showed the highest level of 1.20%. No EPA and DHA were detected in CO group. These results indicate that fatty acid composition of mesenteric fat was influenced by dietary fatty acid composition.

2. Fatty acid composition of subcutaneous fat

Fatty acid composition of subcutaneous fat of Day-2 and Day-15 is featured in Table 4. Analysis of Day-2 subcutaneous fat revealed that CO had the highest level of LA. In case of α-LnA, PO showed the significantly highest level, 10.33% as expected. The level of AA was highest in SO, but the difference was not statistically sig-

Table 3. *Relative composition of total fatty acids of mesenteric fat at Day-2 & Day-15*[1-3]

	Day-2				Day-15			
	CO	SO	PO	FO	CO	SO	PO	FO
14 : 0	*1.39 ± 0.12c†	*1.22 ± 0.07c†	1.98 ± 0.30b	2.78 ± 0.18a	*2.26 ± 0.05b	*2.00 ± 0.24b	2.03 ± 0.12b	3.46 ± 0.65a
14 : 1, ω7	0.05 ±	0.06 ±	0.43 ± 0.18	0.17 ± 0.06	0.14 ±	0.15 ± 0.03	0.17 ± 0.04	0.20 ±
16 : 0	21.36 ± 0.80ab	*18.5 ± 1.24b	21.66 ± 1.46ab	23.51 ± 0.44a	24.45 ± 1.85	*26.9 ± 1.73	24.85 ± 1.08	25.74 ± 1.61
16 : 1, ω7	*1.96 ± 0.18b	*1.76 ± 0.36b	2.97 ± 0.52ab	3.81 ± 0.73a	*3.22 ± 0.28	*4.61 ± 0.75	5.26 ± 1.00	4.66 ± 0.98
18 : 0	4.96 ± 0.60	5.37 ± 0.62†	6.43 ± 1.17	6.23 ± 1.63	5.10 ± 0.61	5.42 ± 0.77	4.87 ± 0.18	5.18 ± 0.76†
18 : 1, ω9	27.44 ± 0.53	26.77 ± 1.09†	24.80 ± 1.14	27.66 ± 2.49	26.85 ± 2.51	26.79 ± 0.67	25.63 ± 0.68	24.56 ± 1.50
18 : 1, ω7	2.17 ± 0.15c	2.95 ± 0.25ab	2.34 ± 0.26bc	3.30 ± 0.05a	2.59 ± 0.07b	2.41 ± 0.12b	2.39 ± 0.05b	2.96 ± 0.24a
18 : 2, ω6	*36.3 ± 0.74a	*35.2 ± 1.27a†	19.42 ± 2.20b	19.89 ± 0.97b†	*26.2 ± 1.29a	*25.1 ± 2.22a	12.39 ± 2.53b	19.24 ± 2.87ab
18 : 3, ω3	0.67 ± 0.10b†	1.46 ± 0.23b	13.16 ± 1.82a	*1.22 ± 0.02b	1.89 ± 0.80b	1.49 ± 0.08b	16.95 ± 1.42a†	*1.10 ± 0.03b
20 : 0	0.17 ± 0.02	0.25 ± 0.08	0.19 ± 0.07	0.13 ±	0.31 ±	0.22 ± 0.03	0.15 ±	0.17 ±
20 : 1, ω9	*0.40 ± 0.02b	0.43 ± 0.02b	0.51 ± 0.13b	1.07 ± 0.00a†	*0.53 ± 0.00b	0.31 ± 0.09b	0.36 ± 0.04b	1.43 ± 0.69a
20 : 2, ω6	0.32 ± 0.07	0.41 ± 0.14	0.17 ± 0.04†	0.19 ± 0.09	1.19 ± 1.00	0.37 ± 0.14	0.16 ± 0.02	0.26 ±
20 : 3, ω6	0.19 ± 0.02	0.25 ± 0.06	0.13 ± 0.04	0.18 ± 0.12	0.35a ±	0.21 ± 0.01ab	0.12 ± 0.03b	0.21ab ±
20 : 4, ω6	0.43 ± 0.04ab	0.49 ± 0.07a†	0.23 ± 0.02b†	0.60 ± 0.16a†	1.20 ± 0.62	0.45 ± 0.03†	0.33 ± 0.12†	0.36 ± 0.00
20 : 5, ω3	ND[4]	0.14 ±	0.43 ± 0.02	1.51 ± 0.35	ND	0.24 ± 0.03	0.82 ± 0.39	3.26 ± 1.69
22 : 0	ND	0.05 ±	ND	ND	ND	0.11 ±	ND	ND
22 : 1, ω9	ND	0.04 ±	ND	ND	ND	ND	ND	ND
22 : 6, ω3	0.37 ± 0.17b	0.67 ± 0.15b†	0.73 ± 0.23b†	4.85 ± 1.51a	ND	0.47b ±	0.60 ± 0.24b†	5.75 ± 1.58a
24 : 0	ND	0.14 ± 0.11	ND	ND	0.52 ±	0.01 ±	ND	ND
24 : 1, ω9	ND	ND	ND	ND	ND	ND	ND	ND
Others	2.11 ± 0.30	4.61 ± 1.35	5.53 ± 1.84	5.81 ± 1.88	4.60 ± 2.87	3.49 ± 0.60	3.56 ± 0.54	3.96 ± 2.04
Σ SFA[5]	27.80 ± 1.35bc	*25.4 ± 0.66c†	30.15 ± 1.60ab	32.55 ± 1.37a	32.08 ± 1.45	*34.5 ± 2.49	31.79 ± 1.06†	34.44 ± 2.36
Σ MUFA[6]	31.98 ± 0.50ab	31.75 ± 0.93ab†	30.68 ± 0.99b	35.44 ± 2.63a	33.07 ± 2.62	34.27 ± 1.27	33.71 ± 1.06	32.22 ± 2.14
Σ PUFA[7]	*38.1 ± 0.71a†	*38.2 ± 0.91a†	33.68 ± 2.43a	26.20 ± 2.60b	*30.3 ± 1.20†	*27.7 ± 2.49	30.94 ± 2.05	29.38 ± 2.00
P/M/S[8]	1.39 / 1.17 / 1	1.51 / 1.25 / 1	* 1.15 / 1.04 / 1	0.81 / 1.10 / 1	0.94 / 1.04 / 1	0.83 / 1.01 / 1	0.98 / 1.06 / 1	0.86 / 0.95 / 1
(EPA + DHA) / AA	0.66 ± 0.31b	1.45 ± 0.23b	4.15 ± 1.16b	8.02 ± 2.21a	Not Calculated	0.69 ± 0.35b	4.30 ± 0.38b†	13.19 ± 3.39a

1) Values are expressed as the relative % of total fatty acids (Mean ± SEM).
2) Values with superscript, *: are siginificantly different between Day-2 and Day-15.
3) Values with superscript, †: are siginificantly different between mesenteric and subcutaneous fat.
4) Not Detected
5) SFA = C14 : 0 + C16 : 0 + C18 : 0 + C20 : 0 + C22 : 0 + C24 : 0
6) MUFA = C14 : 1 + C16 : 1 + C18 : 1ω9 + C18 : 1ω7 + C20 : 1 + C22 : 1 + C24 : 1
7) PUFA = C18 : 2 + C18 : 3 + C20 : 2 + C20 : 3 + C20 : 4 + C20 : 5 + C22 : 6
8) Polyunsaturated / Monounsaturated / Saturated Fatty Acids.

Table 4. Relative composition of total fatty acids of subcutaneous fat at Day-2 & Day-15[1-3]

	Day-2				Day-15			
	CO	SO	PO	FO	CO	SO	PO	FO
14 : 0	1.39 ± 0.06	1.72 ± 0.18†	*1.68 ± 0.11	2.02 ± 0.37	2.78 ± 0.75	1.65 ± 0.15	*2.24 ± 0.17	2.48 ± 0.47
14 : 1, ω7	0.10 ±	1.07 ±	0.35 ± 0.17	1.10 ±	ND	0.65 ± 0.44	0.21 ±	0.13 ± 0.05
16 : 0	22.53 ± 0.81	22.57 ± 1.40	22.66 ± 1.41	23.64 ± 0.47	26.60 ± 1.79	22.47 ± 1.23	25.28 ± 1.50	23.30 ± 2.51
16 : 1, ω7	3.00 ± 0.64	2.97 ± 0.67	4.03 ± 0.71	4.56 ± 1.25	3.67 ± 1.23	4.15 ± 0.75	4.98 ± 1.48	3.48 ± 1.90
18 : 0	6.68 ± 1.14	8.43 ± 1.04†	5.52 ± 0.61	9.04 ± 1.69	8.91 ± 1.85	8.21 ± 2.87	11.15 ± 1.43	9.78 ± 0.89†
18 : 1, ω9	23.24 ± 2.03	21.28 ± 0.78†	23.64 ± 1.10	21.98 ± 1.43	22.51 ± 2.75	23.92 ± 1.89	22.14 ± 2.91	16.59 ± 2.06
18 : 1, ω7	2.74 ± 0.32^{ab}	2.57 ± 0.11^{b}	2.90 ± 0.25^{ab}	3.69 ± 0.39^{a}	2.56 ± 0.44	2.90 ± 0.25	2.51 ± 0.49	3.59 ± 0.52
18 : 2, ω6	28.28 ± 2.15^{a}	25.52 ± 0.59^{a}†	*19.6 ± 1.20^{b}	14.89 ± 1.27^{b}†	20.53 ± 2.26^{ab}	24.42 ± 2.31^{a}	*13.4 ± 2.07^{b}	15.41 ± 2.79^{b}
18 : 3, ω3	1.29 ± 0.32^{b}†	1.54 ± 0.23^{b}	10.33 ± 2.28^{a}	1.51 ± 0.55^{b}	0.43 ± 0.06^{b}	1.52 ± 0.19^{b}	8.48 ± 1.24^{a}†	1.98 ± 0.51^{b}
20 : 0	0.13 ±	ND	ND	ND	ND	ND	ND	ND
20 : 1, ω9	0.25 ±	0.65 ± 0.30	0.25 ± 0.09	0.75 ± 0.02†	0.25 ±	0.34 ± 0.08	ND	0.98 ± 0.22
20 : 2, ω6	0.62 ± 0.36	ND	0.68 ± 0.19†	1.92 ±	0.29 ±	0.26 ± 0.04	ND	ND
20 : 3, ω6	0.21 ±	0.56 ± 0.28	0.20 ±	0.39 ± 0.17	0.13 ±	0.23 ± 0.05	ND	ND
20 : 4, ω6	1.50 ± 0.39	3.71 ± 0.99†	1.29 ± 0.06†	2.55 ± 0.69†	2.25 ± 1.03	1.89 ± 0.33†	1.39 ± 0.26†	1.78 ± 0.44
20 : 5, ω3	0.17^{b} ±	ND	*0.44 ± 0.03^{b}	2.53 ± 0.61^{a}	ND	ND	*1.29 ± 0.13^{b}	3.03 ± 0.32^{a}
22 : 0	ND[4]	ND	ND	ND	ND	0.72 ±	ND	0.29 ±
22 : 1, ω9	ND	ND	ND	ND	ND	0.30 ±	ND	ND
22 : 6, ω3	1.23 ± 0.66^{b}	*2.66 ± 0.33^{ab}†	1.70 ± 0.04^{b}†	4.77 ± 1.50^{a}	1.43 ± 0.81^{b}	*1.10 ± 0.35^{b}	1.54 ± 0.18^{b}†	6.55 ± 1.95^{a}
24 : 0	ND	0.88 ±	ND	1.93 ±	ND	0.23 ±	ND	ND
24 : 1, ω9	ND	ND	ND	ND	ND	ND	ND	ND
Others	5.16 ± 1.96	6.08 ± 1.81	6.76 ± 2.00	7.25 ± 0.73	8.40 ± 2.75	6.33 ± 1.67	7.24 ± 2.09	10.51 ± 2.71
Σ SFA[5]	30.64 ± 4.76	32.93 ± 1.53†	*29.9 ± 1.96	35.35 ± 2.44	38.29 ± 4.38	32.57 ± 2.60	*38.7 ± 2.00†	35.65 ± 2.34
Σ MUFA[6]	29.10 ± 2.50	*26.8 ± 0.55†	30.87 ± 1.73	31.09 ± 4.05	28.82 ± 4.44	*31.8 ± 2.00	28.87 ± 2.65	24.40 ± 3.99
Σ PUFA[7]	32.43 ± 2.67†	33.72 ± 1.71†	32.51 ± 2.02	26.31 ± 2.08	24.16 ± 1.56	29.31 ± 2.49	25.22 ± 3.36	28.10 ± 2.62
P/M/S[8]	1.09 / 0.99 / 1	1.03 / 0.82 / 1	1.11 / 1.05 / 1	0.75 / 0.90 / 1	0.68 / 0.85 / 1	0.93 / 1.00 / 1	0.66 / 0.76 / 1	0.81 / 0.68 / 1
(EPA + DHA) / AA	0.74 ± 0.43	0.86 ± 0.21	1.69 ± 0.03	3.27 ± 1.72	0.34 ± 0.17^{b}	0.62 ± 0.17^{b}	1.91 ± 0.82^{b}†	5.64 ± 0.84^{a}

1) Values are expressed as the relative % of total fatty acids (Mean ± SEM).
2) Values with superscript, *: are siginificantly different between Day-2 and Day-15.
3) Values with superscript, † : are siginificantly different between mesenteric and subcutaneous fat.
4) Not Detected.
5) SFA = C14 : 0 + C16 : 0 + C18 : 0 + C20 : 0 + C22 : 0 + C24 : 0
6) MUFA = C14 : 1 + C16 : 1 + C18 : 1ω9 + C18 : 1ω7 + C20 : 1 + C22 : 1 + C24 : 1
7) PUFA = C18 : 2 + C18 : 3 + C20 : 2 + C20 : 3 + C20 : 4 + C20 : 5 + C22 : 6
8) Polyunsaturated / Monounsaturated / Saturated Fatty Acids.

nificant. EPA proportion of FO was 2.53% and significantly higher than those of other groups. In case of DHA, FO showed the significantly higher level in comparison with CO and PO.

In Day-15 subcutaneous fat, α-LnA composition was highest in PO and lowest in CO group as a consequence of dietary influence. In case of EPA, FO showed the significantly higher level (3.03%) when compared to PO, and no EPA was detected in CO and SO group. DHA composition in FO showed the significantly highest level (6.55%).

It was noticeable that DHA was found in adipose tissues of lactating rats of CO, SO and PO groups (with the exception of Day-15 mesenteric fat of CO) although these rats were not supplied by DHA from their diets.

Dietary P/M/S ratios were different among the experimental groups ranging from 7.7/4.1/1 to 1.5/1.5/1 (Table 2). But in mesenteric and subcutaneous fat, P/M/S ratios seemed to be controlled quite considerably. In mesenteric and subcutaneous fat, the ratio of [DHA + EPA]ω3/AAω6 was shown to be significantly higher in FO in comparison with other groups possibly due to the relatively high level of EPA and DHA and the low level of AA in its diet (Table 3, 4 and Fig. 1). However, the difference is not statistically significant in Day-2 subcutaneous fat.

3. The Comparison of fatty acid compositions of mesenteric and subcutaneous fat

In each experimental groups, the comparison of fatty acid composition of mesenteric fat and subcutaneous fat revealed somewhat consistent differences in levels of oleic acid (18 : 1ω9, OA), LA, AA and DHA (Fig. 2). OA level of mesenteric fat was higher than that of subcutaneous fat in every experimental groups; especially the difference was significant in Day-2 SO ($p < 0.05$). The level of LA in mesenteric fat was higher than that in subcutaneous fat in every experimental groups other than PO; significant differences were found in Day-2 SO ($p < 0.01$) and Day-2 FO ($p < 0.05$). On the contrary, AA, the LCPUFA of LA levels were higher in subcutaneous fat; significant differences were found in all experimental groups other than CO and Day-15 FO. Along with AA, DHA was found to be present in higher proportion in subcutaneous fat than in mesenteric fat of all experimental groups other than Day-2 FO. The differences were significant in Day-2 SO ($p < 0.01$), Day-2 PO ($p < 0.05$) and Day-15 PO ($p < 0.05$).

4. The changes in fatty acid compositions during experimental period

The observation on the changes in compositions of PUFA, MUFA and SFA revealed that, at Day-15, SFA level tends to be higher when compared to Day-2, with the exception of SO subcutaneous fat. On the contrary, PUFA level was lower at Day-15 in every experimental groups other than FO (Table 3, 4 and Fig. 3). In mesenteric fat, SFAs like myristic acid (C14 : 0) and palmitic acid (C16 : 0) composition have increased in proportion and LA has decreased in all groups along with experimental period. In subcutaneous fat, stearic acid (C18 : 0) composition was higher at Day-15 in every groups, except SO. But the difference was not statistically significant. The compositions of LA were lower at Day-15 in every experimental groups other than FO. As a result, P/S ratio of CO, SO and PO decreased along with the experimental period. The differences in CO mesenteric fat ($p < 0.01$), SO mesenteric fat ($p < 0.01$) and PO subcutaneous fat ($p < 0.05$) were statistically significant. But FO group was different with other groups in that its

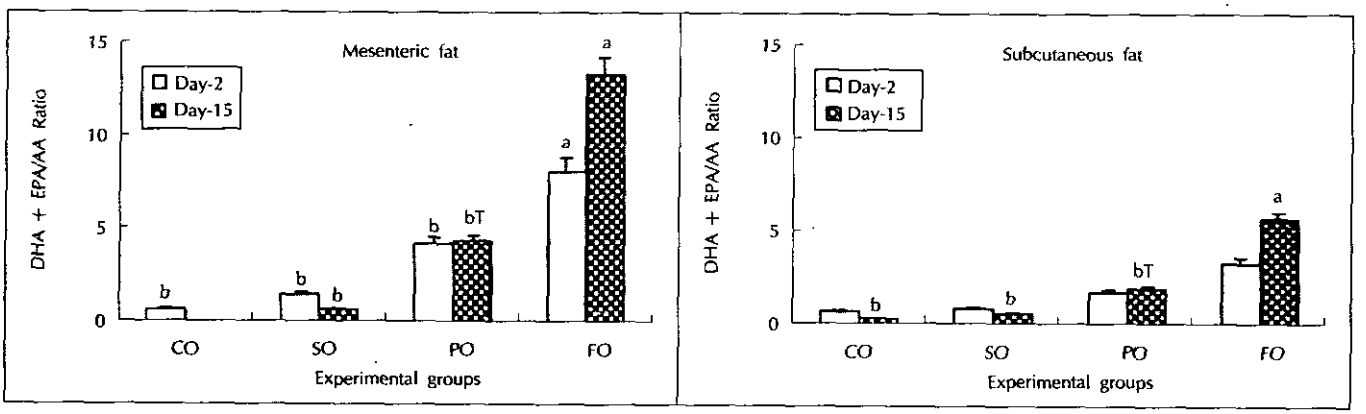

Fig. 1. [DHA+EPA] (ω3)/AA (ω6) Ratios of Mesenteric & Subcutaneous Fat. 1) Values with superscript * are siginificantly different between Day-2 and Day-15. 2) Values with superscript † are siginificantly different between mesenteric and subcutaneous fat.

PUFA proportion had increased (Data not shown).

Observation over the DHA composition change along with the experimental period showed that, in case of mesenteric fat, DHA level has decreased in CO, SO and PO groups, but that of FO has increased (Fig. 4). In subcutaneous fat, DHA level has decreased in SO ($p < 0.05$)

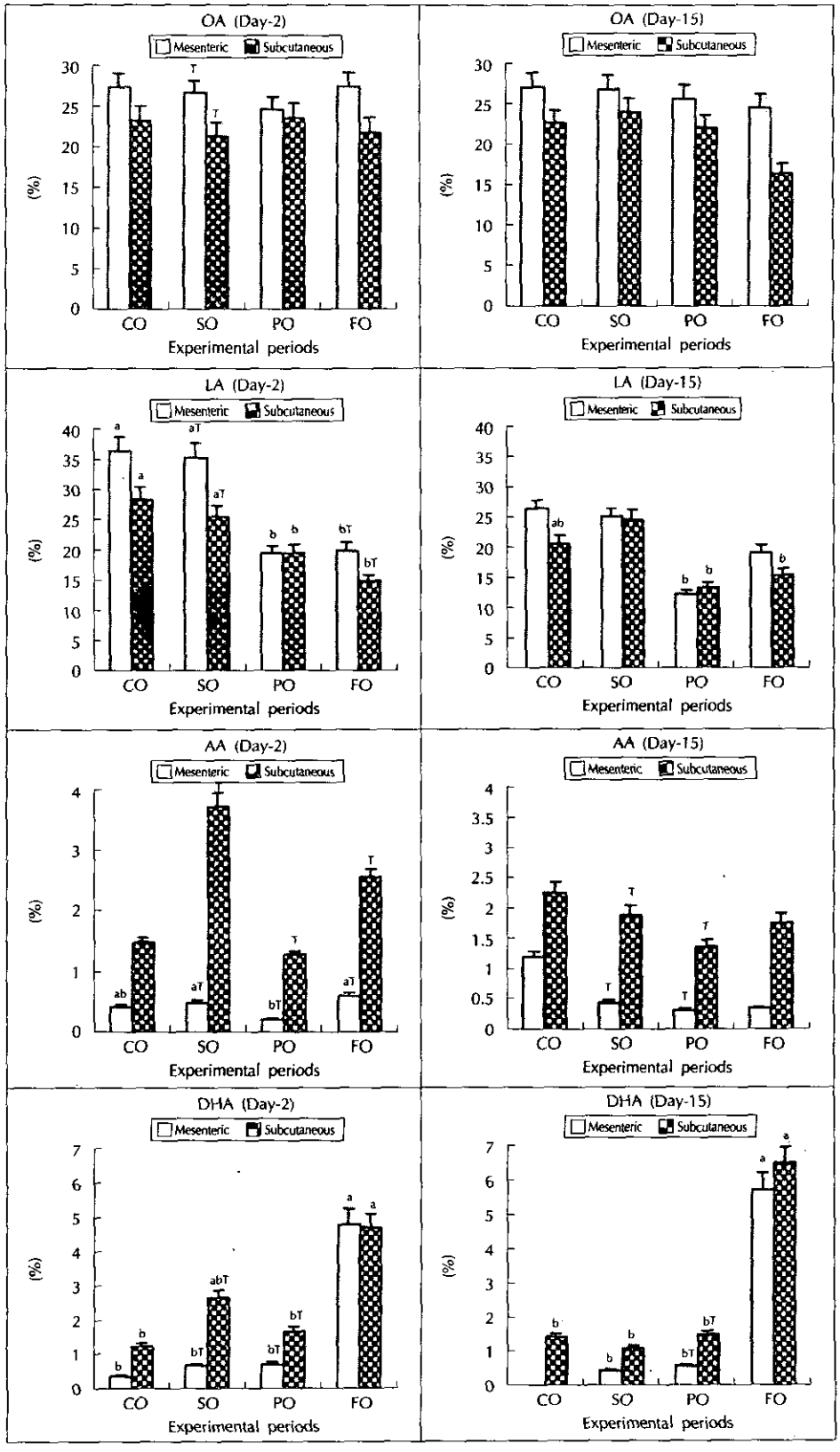

Fig. 2. Comparison of Oleic acid (OA), Linoleic acid (LA), Arachidonic acid (AA) and Docosahexaenoic acid (DHA) composition of Mesenteric & Subcutaneous Fat. 1) Values with superscript * are siginificantly different between Day-2 and Day-15. 2) Values with superscript † are siginificantly different between mesenteric and subcutaneous fat.

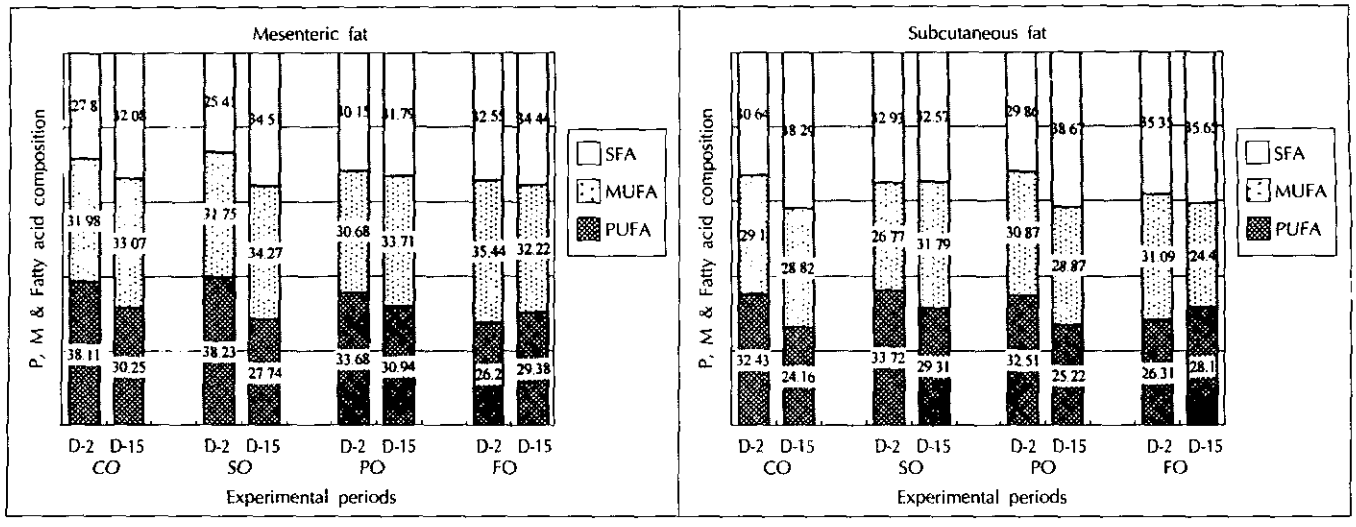

Fig. 3. Change of PUFA, MUFA & SFA Composition of Mesenteric & Subcutaneous Fat During The Lactation Period. 1) Values with superscript * are siginificantly different between Day-2 and Day-15. 2) Values with superscript † are siginificantly different between mesenteric and subcutaneous fat.

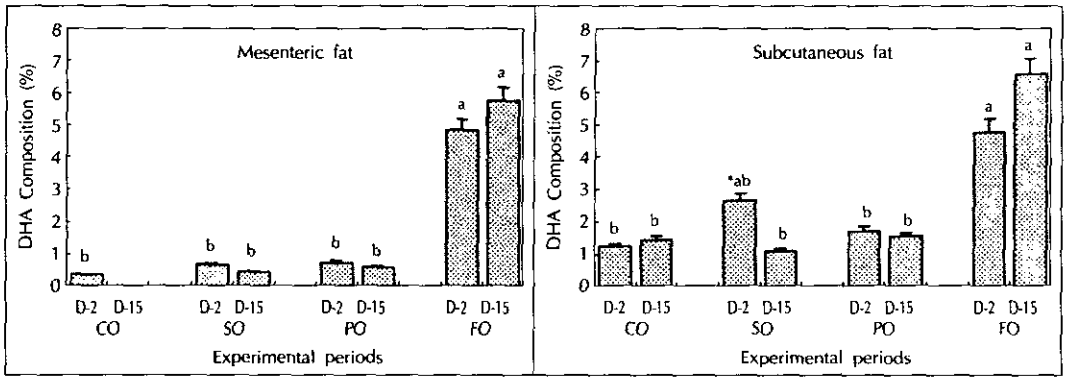

Fig. 4. Change of The DHA Composition of Mesenteric & Subcutaneous Fat During The Lactation Period. 1) Values with superscript * are siginificantly different between Day-2 and Day-15. 2) Values with superscript † are siginificantly different between mesenteric and subcutaneous fat.

and PO groups and has increased in CO and FO groups. In case of AA, no significant differences were found along with the experimental period in mesenteric and subcutaneous fat.

DISCUSSION

In rats, brain develops most vigorously during lactation. The effects of diets containing various amount of ω3 and ω6 fatty acids on fatty acid composition of mesenteric and subcutaneous fat were studied during lactation to test the possibility of adipose tissue to act as a reservoir of DHA.

In this study, the fatty acid compositions of mesenteric and subcutaneous fat were found to be influenced by experimental diets. However mesenteric fat seems to be more influenced by diets than subcutaneous fat. It may be because mesenteric fat, as a channel between absorption-related blood vessels, lymphatics and neuronal systems, participate in the active inter-organ exchange of fatty acids in the postabsorptive phase. The composition of OA was higher in mesenteric fat than in subcutaneous fat in every experimental groups. Also the level of LA was higher in mesenteric fat in every groups other than PO. On the contrary, the composition of AA was higher in subcutaneous fat. The composition of DHA was higher in subcutaneous fat, but Day-2 FO was exceptional in that matter, possibly because of high DHA content in its diet. This is in good agreement with the result of Calder *et al.*[29] who reported the differences in the fatty acid composition of subcutaneous fat vs deep visceral adipose tissue. They studied the fatty acid composition of triglyceride from various adipose depots taken from human subjects and reported that some adipose depots including mesenteric fat have particular site-specific properties. The difference in fatty acid composition between these two adipose tissues can be explained by the differences in fatty acid deposition, mobilization and endogenous synthesis of fatty acids in each adipose tissues.[25,30]

In our previous study the resemblance between fatty acid composition of mesenteric fat and dam's milk was found. On the other hand, fatty acid composition of sub-

cutaneous fat resembled mother's serum fatty acid pattern.[19] Martin *et al.*[31] also reported a positive correlation in LA composition of human milk and white adipose tissue, with the correlation factor of r = 0.52 – 0.64. From these results, it can be assumed that adipose tissue can contribute to fatty acid supply for milk production.

Although P/M/S ratios of experimental diets are different from each other, the ratios of adipose tissues are quite similar (Table 3 and 4). This suggests the homeostatic control of fatty acid composition in the body.

Experimental diets in this study contain more EPA than DHA in FO group. But higher DHA to EPA ratio were found in adipose tissues of lactating rats. This result is in accord with Lin's results[25] which reported that DHA/EPA ratio was higher in triglyceride fraction of rabbit adipose tissue when compared to experimental diet. This suggests preferential incorporation and/or retention of DHA in the adipose tissue. Alternatively, some of the EPA could have been converted to DHA since it is a precursor to DHA. Such a conversion would be a natural phenomenon because DHA is the most common ω3 fatty acid in the membranes of the body. In cheeks, feeding EPA led to increase in DHA composition in the brain and retina.[25] Also there is a possibility of preferential oxidation of EPA. In developed countries the level of EPA in human plasma phospholipids is often only about one-fifth the concentration of DHA. Hodge *et al.*[32] suggested that this is mainly due to increased β-oxidation of EPA relative to DHA.

It is noticeable that DHA is found in adipose tissues of lactating rats in all experimental groups, even though some of these groups did not contain DHA in their diets. DHA may be accumulated in mother rats' adipose tissues even before the start of the study or it may be synthesized from it's precursor by the activity of elongase and desaturase to meet the developmental need. But, in sheep, it is reported that fatty acid synthesis in adipose tissue during lactation has declined.[33] Moreover fatty acid synthesis capacity of adipose tissue is limited as compared to liver. So DHA might be synthesized in liver and then transported to adipose tissues, rather than produced in situ. Increase in hepatic blood flow rate during gestation[34] can contribute to transport of DHA to various parts of the body, including adipose tissues and mammary gland. Chen *et al.*[21] reported 90% increase in EPA proportion and 22% decrease in DHA proportion in female rat liver during late pregnancy. They suggested the possibility of DHA, necessary for fetal growth, being synthesized in pregnant rat's hepatic tissue and transferred to fetus.

Along with the result of previous study from our laboratory about fatty acid composition of pups' brain fed with same experimental diets,[35] our result implies that decrease in PUFA and increase in SFA is due to transport of DHA from dam's adipose tissue to pups. From the fact that DHA level decreased in CO (mesenteric fat only), SO and PO groups as lactation continued, it could be assumed that DHA in mother's adipose tissues were removed and then transported to pups for brain development. But in FO group, DHA composition had increased, possibly because FO diet provided high amount of DHA exceeding the physical needs of rats.

There is a large body of evidence supporting the importance of AA in maintaining normal brain function.[36-38] In our study, no significant change in AA composition were found in every experimental groups. It could be the result of less requirement of AA than DHA during lactation in rats. A study on prenatal accumulation of fatty acids in rat brain reported that there was a steep increase in the concentration of all the fatty acids during embryonic days 14 and 17. After this period and up to birth, the concentration of the fatty acids plateaued, except that of DHA, which continued to accumulate further.[5] But more studies are needed to be done to explain this.

It was reported that during lactation, nutrient demands of the mammary gland usually exceed those of the rest of the body.[39] This increased demand is met primarily by massive increases in food intake, mobilization of body tissues, especially adipose tissue lipid and synthesis in the mammary gland and hepatic tissue. The relative importance of each source depends on species and on the nutritional and physiological states of the animal. Barber *et al.*[40] estimated that adipose tissue lipid can contribute 10 – 20% of milk fat production in rats.

From our results, it may be concluded that adipose tissues of lactating rat can serve as a reservoir of DHA during gestation/lactation, even when dams are fed diets deficient in DHA. To determine the exact mechanism of supply system of LCPUFAs which are essential to brain development, further studies are needed on comparison of dam's liver, placenta, mammary gland and adipose tissue fatty acid metabolism and on relative importance of phospholipid, triglyceride, cholesterol ester and free fatty acid in supplying essential fatty acids.

Literature cited

1) Innis SM. The role of dietary n-6 and n-3 fatty acids in the developing brain. *Dev Neu* 22(5-6): 474-480, 2000

2) Youdim KA, *et al.* Essential fatty acids and the brain: possible health implications. *Int J Dev Neu* 18(4-5): 383-399, 2000
3) Uauy R, *et al.* Essential fatty acids in early life: Structural and functional role. *Pro Nutr Sci* 59(1): 3-15, 2000
4) Hanson JM, Kinsella JE. Fatty acid content and composition of infant formulas and cereals. *J Am Diet Assoc* 78: 250-254, 1981
5) Green P, *et al.* Developmental changes in rat brain membrane lipids adn fatty acids. The preferential prenatal accumulation of DHA. *J Lipid Res* 40(5): 960-966, 1999
6) 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
7) Lim SY, Suzuki H. Effect of dietary DHA and phosphatidylcholine on maze behavior and fatty acid composition of plasma and brain lipids in mice. : Int J Vit Nutr Res 70(5): 251-259, 2000
8) Greiner RS, *et al.* Rats with low levels of brain DHA show impaired performance in olfactory based and spatial learnings tasks. *Lipids* 34 suppl: s239-s243,1999
9) Carlson S, Ford A, Werkman S, Peeples J, Koo W. Visual acuity and fatty acid status of term infants fed human milk and formulas with and without docosahexaenoate and arachidonate from egg yolk lecithin. *Pediat Res* 39: 882-888, 1996
10) Birch EE, *et al.* Visual acuity and the essentiality of DHA and AA in the diet of term infants. *Pediatr Res* 44(2): 201-209, 1998
11) Xiang M, *et al.* Long chain polyunsaturated fatty acids in human milk and brain growth during early infancy. *Acta Pediatr* 89(2): 142-147, 2000
12) Dietary fish oil increase ω3 long chain polyunsaturated fatty acids in human milk. *Nutr Rev* 43: 302-303, 1985
13) Cuthbertson WF. Essential fatty avid requirements in infancy. *Am J Clin Nutr* 29: 559-568, 1976
14) Calson SE, Rhodes RG, Ferguson MG. Docosahexaenoic acid status of preterm infants at birth and following feeding with human milk or formula. *Am J Clin Nutr* 44: 798-804, 1986
15) Francois CA, *et al.* Acute effects of dietary fatty acid on the fatty acids of human milk. *Am J Clin Nutr* 67(2): 301-308, 1998
16) Mansbridge RJ, *et al.* Nutritional factors affecting the fatty acids compositions of bovine milk. *Br J Nutr* 78 suppl: s37-s47, 1997
17) Lien EL, *et al.* Effect of maternal dietary arachidonic or linoleic acid on rat pup fatty acid profiles. *Lipids* 29(1): 53-59, 1994
18) Berger A, *et al.* Anandamide and diet: inclusion of dietary arachidonate and docosahexaenoate leads to increased brain levels of the corresponding N-acylethanolamine in piglets. *Proc Natl Acad Sci USA* 98(11): 6402-6406, 2001
19) Hwang HJ, Chung EJ, Lee JH, Chee KM, Lee YC. 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, 1994
20) Neuringer M, Connor WE, Petten CV, Barstad L. Dietary ω3 fatty acid deficiency and visual loss in infant rhesus monkeys. *J Clin Nutr Inv* 73: 272-276, 1984
21) Chen Z-Y, Yang J, Menard CR, Cunnane SC. Linoleoyl-enrichment triacylglycerol species increase in maternal liver during late pregnancy in the rat. *Lipids* 27: 21-24, 1992
22) Reynolds A. Breastfeeding and brain development. *Pediatr Clin of Nor Am* 48(1): 159-171, 2001
23) Willatts P, *et al.* Effects of long chain polyunsaturated fatty acids in infant formula on problem solving at 10 months of age. *Lancet* 352(9129): 688-691, 1998
24) Sinclair RG. The metabolism of the phospholipids. VI Relative proportion of saturates and unsaturated fatty acids in phospholipid of different degrees of saturation. *J Biol Chem* 111: 261-273, 1935
25) Lin DS, Connor WE. Are the n-3 fatty acids from dietary fish oil deposited in the triglyceride stores of adipose tissue? *Am J Clin Nutr* 51: 535-539, 1990
26) Lepage G, Roy CC. Direct transesterification of all classes of lipids in a one-step reaction. *J of Lipid Res* 27: 114-120, 1986
27) McNair HM, Bonelli EJ. Basic gas chromatography. 5th ed., Varian Instrument Division Offices, 1968
28) Kim BS, Ahn YK, Yoon KJ, Yoon SW. Statistical Analysis using SPSS. Bakyoungsa, 1987
29) Calder PC, *et al.* Site-specific difference in the fatty acid composition of human adipose tissue. *Lipids* 27: 716-720, 1992
30) Rebuffe-Scrive M, Enk L, Crona N. Fat cell metabolism in different regions in women. Effect of menstrual cycle, pregnancy and lactation. *J Clin Inv* 75: 1973-1976, 1985
31) Martin JC, Bougnoux P, Fignon A, Theret V, Lamisse F, Couet C. Dependence of human milk essential fatty acids on adipose stores during lactation. *Am J Clin Nutr* 58: 653-659, 1993
32) Hodge J, Sanders K, Sinclair A. Differential utilization of eicosapentaenoic acid and docosahexaenoic acid in human plasma. *Lipids* 28: 525-531, 1993
33) Vernon RG, Finley E. Roles of insulin and growth hormone in the adaptations of fatty acid synthesis in white adipose tissue during the lactation cycle in sheep. *Bioc J* 256: 873-878, 1988
34) Lomax MA, Baird GD. Blood flow and nutrient exchange across the liver and gut of the dairy cow. *Br J Nutr* 49: 481-496, 1983
35) Kim MK, Chee KM, Lee 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
36) Crawford MA, Costeloe K, Ghebremeskel K, Phylactos A, Skirvin L, Stacey F. Are deficits of arachidonic and docosahexaenoic acids responsible for the neural and vascular complications of preterm babies? *Am J Clin Nutr* 66(4Suppl.): 1032s-1041s, 1997
37) 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 neuronal and glial cells in rats. *J Nutr* 127(5): 724-731, 1997
38) Ward GR. Long chain polyunsaturated fatty acid levels in formulae influence deposition of DHA and arachidonic acid in brain and red blood cell of artificially reared neonatal rats. *J Nutr* 128(12): 2473-2487, 1998
39) Vernon RG, *et al.* (Eds). Physiological strategies in lactation, Academic Press, London, pp.119-145, 1984
40) Barber MC, *et al.* Lipid metabolism in the lactating mammary gland. *Bioc dt Biop Acta* 1347: 101-126, 1997