韓國營養會誌 : 第 18 卷 • 第 4 號 • 1985
Korean J. Nutr. Vol. 18, No. 4, 1985

Effect of Dietary Lipid on Fatty acid Pattern in Developing Brain Mitochondria in Rats

Eui-Ju Yeo and Haymie Choi

Department of Food and Nutrition, Seoul National University, Seoul 151, Korea

식이 중 지방 함량이 성장기 쥐의 뇌 미토콘드리아 지방산 조성에 미치는 영향

여 의 주 • 최 혜 미

서울대학교 식품영양학과

□ 국 문 초 록 □

식이 중 지방함량이 성장기 뇌의 미토콘드리아 지방산 조성에 미치는 영향을 살펴보기 위해 옥수수기름을 각각 10, 2, 0.5% 포함한 3종류의 식이를 임신한 Sprague Dawley종의 흰쥐에게 임신말기부터 제공했다. 수유기간 중의 영향을 살펴보기 위해 대조군(10% 옥수수기름 섭취) 의 일부에 분만 후 0.5% 결핍식이를 섭취시켰고, 또 이유후부터 0.5% 결핍식이군의 일부에 10% 정상식이를 주어 회복효과를 관찰하고자 하였다. 전 실험기간 동안 체중과 뇌 무게, 단백질 함량을 측정하였고 뇌에서의 미토콘드리아를 분리하여 지방산 조성을 관찰하였다.

출생후 수유기간 동안에는 대조군에 비해 지방결핍식이군에서 체중과 뇌의 무게가 다소 높았으나 이유 후 부터 점점 감소하여 유의적인 차이를 볼 수 없었고 특히 0.5% 식이군의 체중은 생후 6주와 7주에 유의적인 감소를 보였다. 그러나 체중에 대한 뇌무게와 뇌의 단백질 함량에 있어에는 대조군과 결핍군 사이에 유의적인 차이를 볼 수 없었다. 따라서 지방결핍이 뇌 성장에 미치는 영향은 다른 영양소에 비해 크지 않음을 알 수 있었다. 뇌 미토콘드리아의 지방산 조성은 나이와 식이에 따라 변하였다. 나이를 먹음에 따라 단쇄 포화 지방산은 감소하고 다 불포화 지방산의 함량은 증가하였다. 또한 결핍식이군과 대조군 사이에 뇌 미토콘드리아 지방산조성을 살펴 본 결과 지방 결핍의 좋은 지표가 되고 불포화도, triene 과 tetraene 의 비, 그리고 $w-6$ 계열과 $w-9$ 계열의 비에 있어서 큰 차이를 볼 수 있었다. 이들로 부터 성장초기에 지방함량을 조절한 식이에 의해 뇌 미토콘드리아 지질 조성이 크게 변하고 이에따라 미토콘드리아의 기능도 유의적인 영향을 받고 있음을 알 수 있었다.

접수일자 : 1985 년 12월 1일

INTRODUCTION

Current model of the biomembrane is that peripheral and integral membrane proteins arranged in lipid bilayer of the membrane. Therefore, membrane bound enzyme functions, lipid requiring transport systems or protein-lipid interactions with the membrane may be dependent on the physical properties of the phospholipids, and the content of cholesterol in lipid bilayer of the membrane[1)2)].

Fatty acid composition is a major factor influencing the physical state of membranes[2)]. Alteration of fatty acid composition of membrane lipid results in a shift in transition temperature[4)5)6)], implying that fluidity of the membrane can be modulated by altering the fatty acyl chain components of membrane phospholipids. The motional state of the lipid phase may physically determine the ability of the protein to undergo conformational changes necessary for optimal catalytic activity of the enzyme - substrate complex[7)8)]. Changes in membrane lipid composition have been obtained experimentally by alteration of environmental temperature in poikilotherms and bacteria, or in homeotherms by feeding diffecent dietary fats[9)10)11)]. But previous studies on the effect of diet fat on different membrane lipids have suggested that brain membranes are the most resistant to change[12)13)]. Furthermore, since the brain completes most of its growth during the per iod and early in life dietary factors, particularly in the early stages of development, can influence the chemical composition of the brain[14)]. Therefore the present study was carried out to investigate the effect of dietary lipid on mitochndrial fatty acid pattern in developing rat brain. Since the corn oil is rich in linoleic (54.2%) and oleic (29.2%) acids, it can be used as a good source of essential fatty acids. In this study, we select three kinds of experimental diet with different amounts of corn oil to investigate the effect of dietary lipid on mitochondrial fatty acid pattern.

MATERIALS AND METHODS

Materials

Bovine albumin (Cohn Fraction V, 96-99% albumin and remainder mostly globulins) and BF-methanol (14% boron trifluoride in methanol) were obtained from Sigma Chemical Company. GP 10% SP 2330 (a cyano silicone) on 100/120 Chromosorb W, AW was obtained from Supelco, Inc.

Animals and Diets

Virgin female Sprague Dawley rats weighing 200-250gm, supplied by Animal Bleeding Laboratory of Seoul National University, were used in this experiment. After pregnancy, female rats were individually housed in a plexglas cage and fed experimental diets (Table 1) according to the feeding design of Fig. 1. After parturition, litter size was adjusted to 8 pups. The temperature and humidity were kept 20±1℃ and 55±10%, respectively. Light was also controlled and food and water provided ad libitum.

Control group rats were fed a balanced control

Table 1. Compositions of experimental diets

Ingredient \ Group	C	D-0.5	D-2
Corn starch	58.7	69.4	67.6
Casein	20	20	20
Cellulose[2]	6	5.37	5.46
Corn oil	10	0.50	2.1
Vitamin mixture[4]	1	0.89	0.91
Salt mixture[4]	4	3.58	3.64
Methionine[5]	0.3	0.27	0.27

[1)] C, control diet (10% corn oil) ; D-0.5, deficient diet with 0.5% corn oil; deficient diet with 2% corn oil; [2)] α-cellulose from sigma chemical company[3)]; vitamin mixture from ICN pharmaceuticals, Ind. Life seience group cleveland, ohio, U.S.A.; [4)] salt mixture from ICN Nutritional biochemicals, cleveland, ohio, U.S.A.; [5)] DL-methionine from BDH chemicals Ltd. poole, England.

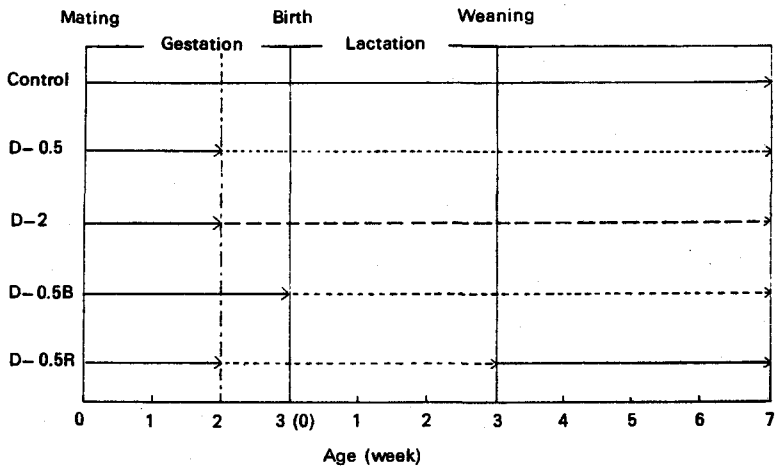

Fig. 1. Scheme of experimental design of feeding. D−0.5, deficient diet with 0.5g corn oil; D−2, deficient diet with 28 corn oil: D−0.5B, deficient diet from birth with 0.5% corn oil; D−0.5R, deficient diet with 0.5% corniol and rehabilitation at weaning. 10% corn iol diet, ——→ 0.5% corn oil diet, ·····→ ; 2% corn oil diet, — — — →

diet (10% corn oil diet) during the experimental period. The deficient rats were fed 0.5% corn oil diet (D − 0.5) and 2% corn oil diet (D − 2) from 15th day of gestation. One half of control group rats were fed 0.5% corn oil diet at birth (D − 0.5 B). After weaning at 21 days, one half of D − 0.5 group rats were rehabilitated with the control diet (D −0.5 R). Body weights of all offspring rats were weighed weekly. At the age of 0, 1, 2, 3, 5 and 7 weeks, offsprings were randomly chosen and decapitated during late afternoon hours. The brains were immediately frozen and stored at −60℃ until use.

Determination of total protein

After the brain was washed once and weighed, the 10% brain homogenate (W/V) was prepared in 0.32 M ice − cold sucrose solution by a glass homogenizer with 45 strokes. The protein of whole brain homogenate was determined colorimetrically by the method of Lowry et al.[15] with bovine serum albumin as standard. Because the protein in the range of 0.06 − 0.14mg can be determined by this method, 10% homogenate was diluted to 50 volumes and the absorbance at 500nm was measured.

Lipid extraction from mitochondrial fraction

The mitochondrial fraction of rat brain was isolated by the method of Wittaker and Baker[16] as described previously[17]. Lipids in brain mitochondrial were extraced by the modified method of Folch et al.[18]

Fatty acid composition of the brain mitochondria

Mitochondrial lipid extract was transmethylated by heating with BF_3−methanol[19]. One milliliter of mitochondrial lipid extract was evaporated to dryness under nitrogen in a centrifuge tube provided with a teflon −lined screw cap. One milliliter of BF_3− methanol reagent was added under nitrogen, and the tube was closed tightly with the screw cap. The tube was then heated in a boiling water bath for 30minute. After cooled, the esters were extracted by adding 2ml of heptane, then 1ml of water, shaking briefly, and centrifuging until both layers were cleared. The resulting methyl esters were determined by gas − liquid chromatography (Yanaco model G 80 with a flame ionization detector). A 3

Table 2. Effect of dietary lipid on body weight of offsprings (g)

Group / Week	C	D-0.5	D-2	D-0.5B	D-0.5R
0	6.05±0.02[a] (48)[b]	6.13±0.14 (55)	6.14±0.03 (119)	6.05±0.02 (48)	6.13±0.04 (55)
1	15.07±0.17 (72)	15.14±0.14 (128)	16.52±0.11** (104)	15.80±0.27* (56)	15.14±0.14 (128)
2	32.04±0.54 (54)	32.85±0.25 (95)	36.35±0.30** (72)	34.09±0.46** (42)	32.85±0.25 (95)
3	54.55±1.19 (36)	55.31±0.58 (64)	59.05±0.64** (48)	54.85±0.97 (28)	54.79±1.91 (22)
4	82.88±2.11 (22)	80.43±1.61 (30)	90.89±1.27** (30)	82.28±0.78 (16)	92.58±2.00** (14)
5	127.40±3.06 (11)	121.12±2.11 (30)	136.11±2.09* (30)	127.91±0.85 (18)	136.54±2.84 (14)
6	172.49±3.99 (11)	156.41±4.32* (15)	180.90±2.61 (15)	168.29±4.43 (9)	178.53±4.26 (14)
7	209.48±4.96 (11)	189.00±5.95* (15)	215.17±3.37 (15)	210.37±5.43 (9)	213.10±5.31 (14)

[a] : Mean±S.E.
[b] : Number of animals used for calculation.
* : $P < 0.05$, significantly different from control.
** : $P < 0.01$, significantly different from control.

mm×3m glass column packed with GP 10% SP 2330 on 100/120 Chromosorb W, AW (maximum temperature; 275℃) was used with a nitrogen flow of 22.5 ml/min. Column temperature was programmed from 160℃ to 230℃ with increase of 4℃/min and injection temperature was operated at 200℃. Identification of the esters was performed by comparison of retention times with those of the standard esters chromatographed under the identical conditions. Peak areas of each fatty acid ester were obtained by an integrator and expressed as % of total area.

Experimental data from control and deficient groups were analyzed by the student's t-test.

RESULTS AND DISCUSSION

Effect of Dietary Lipid on Body and Brain Development

In this experiment 0.5% and 2% corn oil diet provided suboptimal level of essential fatty acids. No signs of deficiency symptoms were noticed in deficient dams and pups. Throughout the experimental period, body and brain weights and total protein content of brain were observed and shown in Table 2, 3, 4 respectively

Before weaning, body weight of D-0.5 group rats were a little higher than that of the control group, but there was no significant difference between these two groups After weaning, a decrease in the body weight of D-0.5 group rats were observed. At 6 and 7 weeks of age D-0.5 group rats were significantly ($P < 0.05$) lower in body weight than the control group.

Body weight of D-2 and D-0.5B group rats were a little higher than that of the control group throughout the postnatal period. But its increasing tendency slowed down and the significant difference disappeared. And the brain weight of deficient groups were higher than that of the control group. However, there was no significant difference

Table 3. Effect of dietary lipid on brain weight of offsprings (g)

Week \ Group	C	D − 0.5	D − 2	D − 0.5B	D − 0.5R
0	0.242±0.004[a] (34)[b]	0.232±0.003* (58)	0.234±0.003 (28)	0.242±0.004 (34)	0.232±0.003* (58)
1	0.688±0.014 (16)	0.697±0.013 (32)	0.731±0.007** (31)	0.717±0.018 (14)	0.697±0.013 (32)
2	1.224±0.014 (18)	1.284±0.012** (31)	1.321±0.009** (24)	1.294±0.018** (14)	1.284±0.012** (31)
3	1.503±0.014 (14)	1.548±0.014* (15)	1.582±0.017** (15)	1.560±0.019* (10)	1.548±0.014* (15)
5	1.718±0.020 (11)	1.785±0.018* (15)	1.786±0.015** (15)	1.806±0.023* (9)	−
7	1.860±0.018 (11)	1.910±0.023 (15	1.945±0.021** (15)	1.932±0.037 (9)	1.903±0.017 (14)

[a]: Mean±S.E.
[b]: Number of animals used for calculation.
*: P < 0.05. significantly different from control.
**: P < 0.01. significantly different from control.

Table 4. Effect of dietary lipid on brain protein of offsprings (mg/g tissue)

Week \ Group	C	D−0.5	D−2	D−0.5B	D−0.5R
0	82.47±2.98[a]	83.05±1.49	74.80±0.63*	82.47±2.98	83.05±1.49
1	68.83±2.26	69.38±1.86	74.91±0.42	71.95±0.75	69.38±1 86
2	95.45±1.35	95.53±1.38	95.50±1/4	97.95±1.62	95.53±1.38
3	109.78±3.64	113.33±1.21	110.61±2.25	112.62±1.55	113.33±1.21
5	123.25±1.21	125.44±3.41	121.48±2.30	123.67±0.91	−
7	128.60±0.68	128.95±3.36	130.35±2.62	127.32±3.33	129.59±1.57

[a]: Mean±S.E.
*: P < 0.05 significantly different from control.

in the percentage of the brain to body weight among the groups. Brain protein of deficient groups were not different from the control group.

It is expected that a little bit higher body and brain weights in deficient groups could be stemmed from the increased food intake. Since deficient diets had only 90% caloric density of the control dlet, the amount of food consumed by the deficient groups was more than that of the control group. Thus the intake of the protein and other nutrients in deficient groups may be more than that of the control group and resulted in good development of the deficient group rats. But the present data demonstrated that the difference between the control and the deficient groups decreased with age. Therefore, lipid deficiency of the present study seems to have an effect on physical development of the pups, but not as severe as in other nutrients like energy, protein, or vitamins investigated previously by other workers [20)21)].

Table 5. Effect of dietary lipid on fatty acid patterns in brain mitochondria of offsprings

(percentages of total area)

Week \ fatty acids below / Group	$C_{16:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{16:2}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:0}$	$C_{18:3}$	$C_{20:1}$	$C_{20:3}$	$C_{20:4}$	$C_{22:3}$	$C_{22:4}$	$C_{22:5}$	$C_{22:6}$	$C_{24:4}$	$C_{24:5}$
0 C	3.21	27.60	3.74	1.11	–	16.56	13.82	1.04	–	0.54	0.43	0.52	9.51	–	2.67	3.72	5.24	5.95	4.64
D–0.5	3.64	26.81	4.05	1.21	–	16.61	14.76	0.95	–	0.27	0.45	0.51	9.18	0.22	2.31	6.19	4.34	5.04	4.34
D–2	3.86	26.75	3.99	1.14	–	16.73	13.78	0.60	–	0.39	0.78	0.53	9.62	0.21	2.48	5.67	3.93	4.64	4.51
1 C	5.44	26.21	2.33	1.01	–	18.56	10.29	1.16	0.15	0.86	0.41	0.36	11.21	0.12	2.69	3.87	6.28	4.71	4.16
D–0.5	5.12	26.19	3.09	1.41	–	17.62	11.94	0.96	0.28	0.57	0.33	0.40	10.61	0.15	2.48	3.78	5.83	5.06	4.30
D–2	6.88	25.17	2.81	1.25	–	15.95	11.51	0.92	0.20	0.39	0.38	0.47	11.60	0.31	2.57	4.03	6.28	4.55	4.49
D–0.5B	5.58	26.04	2.93	1.45	–	17.22	11.11	0.78	0.20	0.63	0.36	0.41	10.75	0.10	2.46	3.02	7.75	4.20	4.08
2 C	2.53	23.78	1.56	2.01	0.43	16.93	12.52	1.92	0.13	0.40	0.54	0.37	11.65	0.10	3.21	3.81	6.89	5.01	6.17
D–0.5	3.49	23.74	1.62	1.93	0.40	18.77	12.78	1.22	0.13	0.78	0.40	0.47	11.08	0.15	2.68	3.73	6.28	5.06	6.02
D–2	2.97	23.96	1.54	2.00	0.31	18.90	12.20	1.24	0.16	0.42	0.40	0.34	10.82	0.24	2.93	3.80	6.16	5.23	6.33
D–0.5B	2.95	23.44	1.42	2.02	0.43	18.43	12.70	0.95	0.14	0.62	0.32	0.38	11.18	0.21	2.94	3.21	7.69	5.27	6.14
3 C	2.12	19.27	0.58	2.60	0.87	17.74	13.69	1.72	0.25	0.72	0.57	0.54	10.05	0.35	3.61	3.32	7.34	6.45	8.07
D–0.5	2.00	19.95	0.61	2.34	0.70	12.68	13.28	0.41	0.25	0.88	0.41	0.55	9.27	0.49	3.18	3.10	5.28	6.35	8.21
D–2	2.24	19.50	0.64	2.50	0.81	19.49	13.92	0.88	0.26	1.01	0.46	0.63	9.66	0.40	3.45	3.72	6.35	5.65	8.38
D–0.5B	2.41	19.82	0.69	2.64	0.82	18.07	13.79	1.61	0.30	0.94	0.53	0.61	10.10	0.30	3.46	3.16	7.50	5.80	7.41
5 C	2.02	16.05	0.36	3.01	1.58	16.53	14.90	1.41	0.37	1.17	0.56	0.61	8.80	0.47	3.78	4.30	7.72	7.72	9.42
D–0.5	2.04	16.39	0.44	3.05	1.48	16.68	15.13	0.59	0.26	1.24	0.45	0.65	8.95	0.48	3.52	4.47	6.73	7.35	8.85
D–2	2.02	16.97	0.32	3.02	1.35	17.12	15.01	0.84	0.26	1.14	0.46	0.65	8.99	0.46	3.55	4.69	7.19	7.24	8.92
D–0.5B	2.04	16.65	0.45	3.03	1.42	16.78	15.35	0.65	0.24	1.11	0.47	0.66	9.05	0.43	3.44	4.18	7.73	7.32	8.88
7 C	2.00	16.80	0.30	3.38	1.47	16.74	14.60	0.96	0.31	1.19	0.48	0.50	8.79	0.43	3.72	3.93	8.47	7.59	9.21
D–0.5	1.95	17.05	0.45	3.25	1.96	17.02	15.72	0.70	0.27	1.19	0.47	0.58	8.59	0.49	3.40	4.06	7.65	7.39	8.70
D–2	1.90	16.47	0.31	3.28	1.61	17.49	15.20	0.67	0.25	1.34	0.41	0.47	8.44	0.45	3.47	4.82	6.74	7.55	9.11
D–0.5B	2.03	16.21	0.34	3.25	1.88	16.87	15.45	0.19	0.28	1.55	0.35	0.64	8.51	0.52	3.39	4.23	7.46	7.26	9.73
D–0.5R	1.85	16.25	0.30	3.09	1.53	16.86	14.97	1.00	0.28	1.65	0.55	0.53	8.69	0.38	3.64	3.90	7.11	7.96	8.94

Effect of Dietary Lipid on Brain Mitochondrial Fatty Acid Pattern

Lipid extract from the brain mitochondria was analyzed for fatty acid composition. Palmitic ($C_{16:0}$) stearic ($C_{18:0}$) and oleic ($C_{18:1}$) acids were the major fatty acids comprising about 50%, and arachidonic ($C_{20:4}$) and polyunsaturated fatty acids, such as $C_{22:4}$ $C_{22:5}$ and $C_{22:6}$ occupied the rest. And also small amount of saturated and polyunsaturated fatty acids were appeared in the chromatogram. It is summarized in Table 5.

It is interesting to notice that fatty acids of saturated short chain, such as fatty acids below C_{16} palmitic ($C_{16:0}$) and palmitoletic ($C_{16:1}$) acid decreased and fatty acids of $C_{17:0}$ and $C_{16:2}$ and most of polyunsaturated fatty acids increased with age.

As expected, it was found that the lipid deficient diets caused an altered fatty acid composition of brain mitochondria. Throughout the experimental period, saturated and short chain fatty acids, such as fatty acids below C_{16}, $C_{16:0}$, $C_{16:1}$, $C_{17:0}$, $C_{18:0}$ and $C_{18:1}$ were higher in the deficient groups than control group. But polyunsaturated and/or long chain fatty acids were mostly lower in the deficient groups than the control group. Particularly, linoleic ($C_{18:2}$), arachidonic acids ($C_{20:4}$), and polyunsaturated fatty acids longer than $C_{22:6}$ were significantly lower than the degree of unsaturation, a good indicator of membrane fluidity, was significantly lower in deficient groups than the control group, indicating that mitochnodrial membrane fluidity in deficient group was lower than that of the control group.

Some investigators[22)23)] suggested the ratio of trienes (20:3) to tetraenes (20:4, arachidonic acid) as a biochemical index of essential fatty acid deficiency. Therefore, the ratio of $C_{20:3}/C_{20:4}$ in brain mitochondria of offsprings were calculated and are shown in Table 6. The ratio of the deficient groups were a little higher than that of the control groups throughout the experimental period The ratio of $C_{20:3}/C_{20:4}$ in the D-0.5 and D-2 were significantly ($P<0.05$) higher than that of the control group during the lactation periods. However, after weaning, there were no significant differences in the ratio among the groups. It seems likely that the decreasing tendency in the ratio is associated with the adaptation of brain to the nutritional stress.

Other investigators[24)] also suggested that the $w-6/w-3$ ratio may be a good index of essential fatty acid deficiency in brain. But our data were not consistent with their results. In our experiment, the most noticeable changes in the mitochondrial fatty acids were the low percentages of $w-6$ series to $w-9$ series fatty acids in the deficient groups. The $w-6/w-9$ ratios are shown in Table 7. The $w-6$ family of linoleic acid are arachidonic (20:4), do-

Table 6. Effect of dietary lipid on the ratio of fatty acid $C_{20:3}/C_{20:4}$ in brain mitochondria

Week \ Group	C	D-0.5	D-2	D-0.5B	D-0.5R
0	0.055±0.006[a]	0.056±0.001	0.057±0.009	0.055±0.006	0.056±0.001
1	0.032±0.001	0.038±0.002**	0.041±0.001**	0.038±0.001	0.038±0.002**
2	0.032±0.002	0.048±0.002**	0.032±0.002++	0.033±0.001++	0.048±0.002**
3	0.054±0.001	0.060±0.001*	0.065±0.003*	0.059±0.004	0.060±0.001*
5	0.070±0.007	0.073±0.005	0.072±0.003	0.075±0.004	-
7	0.057±0.005	0.064±0.004	0.058±0.004	0.068±0.006	0.062±0.008

[a]: Mean± S.E.
*: $P<0.05$. significantly different from control.
**: $P<0.01$. significantly different from control.
++: $P<0.01$. significantly different from D-O.S.

Table 7. Effect of dietary lipid on the ratio of fatty acid $w-6/w-9$ in brain mitochondria

Week \ Group	C	D−0.5	D−2	D−0.5B	D−0.5R
0	52.94	38.37	37.19	52.94	38.37
1	57.92	49.44	36.10	49.59	49.44
2	67.62	48.05	52.38	50.32	48.05
3	37.33	29.35	30.82	34.66	29.35
5	32.81	29.85	30.84	30.75	-
7	36.77	30.69	37.02	28.63	37.51

cosatetraenoic (22: 4), docosapentaenoic (22:5), C $C_{24:4}$ and $C_{24:5}$ acids. And the $w-9$ family of oleic are eicosatrienoic (20:3) and docosatrienoic (22:3) acid. At 0, 1, 2, 3, 5 and 7 weeks of age, the ratios of $w-6/w-9$ in D−0.5 group showed 27.5, 14.6, 28.9, 21.4, 9.0 and 16.5% reduction, respectively, compared with the control. And at 0, 1, 2, 3 and 5 weeks of age the ratios in the D−2 group showed 37.7, 22.5, 17.4 and 6.0% reduction, respectively, as compared with the control group. Throughout the experimental period, the ratios of the D−0.5 group were lower than those of the D−0.5B and D−2 groups. But the D−0.5R group rehabilitated normally. It seems likely that the $w-6/w-9$ ratio in the mitochondrial fatty acid is a good indicator of lipid deficiency.

Summary

The effect of dietary lipid on brain mitochondrial fatty acid pattern was examined by feeding pregnant Sprague Dawley rats with diets containing 10(C), 2(D−2), 0.5% (D−0.5) of corn oil. At birth one half of the control fed 0.5% diet (D−0.5B) and at weaning, D−0.5% rats were rehabilitated (D−0.5R) with the control diet. Throughout the experimental period, body and brain weights and total protein content of brain were determined, and the brains were analyzed for mitochondrial fatty acid pattern.

Body and brain weights of the deficient groups were a little higher than that of the control group during the lactation period. After weaning, however their increasing tendency slowed down and the significant difference disappeared. Furthermore, at 6 and 7 weeks of age D−0.5 group rats were significantly ($P<0.05$) lower in body weight than the control group. But there was no significant difference in the brain to body weight and brain protein among the groups. Therefore, the effect of lipid deficiency on physical development appears not as severe as in other nutrients. Fatty acid pattern in brain mitochondria changed with age and diet. Fatty acids of saturated short chain decreased and most of polynusaturated fatty acids increased with age. The degree of unsaturation, the ratios of triene (20:3) to tetraene (20:4) and $w-6$ to $w-9$, which may be a good indicator of lipid deficiency, showed significant differences between the control and the deficient groups. These observations, therefore, provide an evidence that the alteration of dietary lipid can affect the fatty acid composition and functions of brain mitochondria.

REFERENCES

1) Boggs, J.M.: *Intermolecular hydrogen bonding between lipids; Influence on organization and function of lipids in membranes. Can. J. Biochem. 58: 755-70, 1980.*

2) Foot, M., Cruz, T F. & Clandinin, M.T.: *Influence of dietary fat on the lipid composition of rat brain synaptosomal and microsomal membranes. Biochem. J. 208: 631-640, 1982.*

3) Innis, S M. & Clandinin, M.T.: *Dynamic modulation of mitochondrial inner-membrane lipids in rat heart by dietary fat. Biochem. J. 193: 155-167, 1981.*

4) Raison, J.K., Lyons, J.M., Mehlhorn, R.T. & Keith, A.D.: *Temperature induced phase changes in mitochondrial membranes detected by spin labeling. J. Biochem. 246: 4036-4040, 1971.*

5) King, M.E., Stavens, B.W. & Spector, A.A.: *Diet-induced changes in plasma membrane fatty acid composition affect physical properties detected with a spin-label probe. Biochemistry. 16: 5280-5285, 1977.*

6) McMurchie, E.J. & Raison, J.K.: *Membrane li-*

pid fluidity and its effect on the activation energy of membrane associated enzymes. Biochim. Biophy. Acta. 554. 364-341, 1979.

7) Sanderman, H.: *Regulation of membrane enzymes by lipids. Biochim. Biophy. Acta 515 : 209 -237, 1978.*

8) Sanderman, H.: *Lipid dependent membrane enzymes. Eur. J. Biochem. 127:123-128, 1982.*

9) Baldassare J J., Rhinehart, K B & Silbert, D.F. : *Modification of membrane lipid;physical properties in relation to fatty acid structure. Biochemistry 15:2986-2994, 1976.*

10) Innis, S.M & Clandinin, M.T.: *Mitochondrial membrane polar-head group composition is influenced by diet fat. Biochem. J. 198:231-234 1981.*

11) Ehrstrom, M., Harms-Ringdahl, M. & Alling, C. : *Osmotic fragility and fluidity of erythrocyte membranes from rats raised on an essential fatty acid deficient diet. -A spin label study. Biochim. Biophys. Acta. 644:175-182, 1981.*

12) Century, B. & Horwitt, M.T.: *Effect of dietary lipid upon some enzymes of significance in biogenic amine metabolism in the rat. J. Nutr. 95:509-516, 1968.*

13) Tahin, Q.S., Blum, M. & Carafoli, E.: *The fatty acid composition of subcellular membranes of rat liver, heart and brain;diet-induced modifications. Eur. J. Biochem. 121, 5-13, 1981.*

14) Matheson, D.F., Oei, R. & Roots, B.I.: *Effect of dietary lipid on the acyl group composition of glycerophospholipids of brain endothelial cells in the developing rat. J. Neurochem 36: 2073-2079, 1981.*

15) Lowry, O.H., Rosebrough, N.J., Farr, A.L & Randall, R.J : *Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275, 1951.*

16) Wittaker, V.P. & Baker, L.A : *In Methods of Ne urochemistry. Fried, R. ed. Marcel, Dekker, N.Y. 1972.*

17) Yeo, E.Z, Choi, H. & Choi. M.U.:*Effect of Dietary Lipid on Monoamine oxidase activity of developing rat brain. Korean Biochem. J. 18: 157-163, 1985.*

18) Folch, J , Lees, M. and Sloane-Stanley, G.H. : *A simple method for isolation and purification for total lipids from animal tissues. J. Biol. Chem. 226: 497-509, 1957.*

19) Morrison, W.R. & Smith, L.M.: *Preparation of fatty acid methyl esters and diemthylacetals from lipids with boron fluoride methanol J. Lipid Res. 5:600-608, 1964.*

20) Kenney, M.A. & Barton. E.B.: *Malnutrition and fetal development in two generations of rats. Nutr. Rep. Int. 11:243-250, 1975.*

21) Driskell, J.A. & Foshee D.P.: *Bahavioral patterns and brain nucleic acid and pyridoxal phosphate contents of male rat progeny from vitamin B_6- repleted dams. J. Nutr. 104:810-818, 1974.*

22) Galli, G. Trzeciak, H.I. & Paoletti, R.: *Effect of EFA deficiency on myelin and various subcelluar structures in rat brain. J. Neurochem. 19: 1863-1867, 1972.*

23) Holman, R. T.: *The ratio of trienoic to tetraenoic acids in tissue lipids as a measure of EFA requirement J. Nutr. 70:405-416, 1960.*

24) Paoletti R. & Galli, G.:*Effects of EFA deficiency on the CNS in the growing rat. In; Lipids, Malnutrition and the Developing Brain, pp. 121-140, Associate Scientific Publishers, Amsterdam, 1972.*