Effects of Dietary Fish Oil during the Fetal and Postnatal Periods on the Learning Ability of Postnatal Rats

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The effects of a diet containing fish oil with a low level of docosahexaenoic acid fed during the fetal to newborn stage on the learning ability of postnatal rats were evaluated.

Learning ability estimated by the swimming test was superior with the fish oil group of 6-wk-old rats to that with the non-fish oil group. These findings suggest that the ingestion of fish oil, and especially of docosahexaenoic acid, during the period from the fetal to newborn stage, but not during the weaning period, and the accumulation of a large amount of dietary docosahexaenoic acid in the brain of fetal and newborn rats may be related to an improved cerebral function of postnatal rats.

Polyunsaturated fatty acids (PUFA), i.e., linoleic acid (18:2n-6), arachidonic acid (20:4n-6), γ-linolenic acid (18:3n-3), and docosahexaenoic acid (22:6n-3), have been identified in breast milk, a major nutritional source for infants, and play an important role in the growth and development of the infant's brain and retina. Lampetly and Walker have reported that a deficiency of dietary n-3 PUFA impaired the learning ability of rats. Yamamoto et al. have studied the effects of a diet containing 5% perilla oil with 64.0% 18:3n-3 on the brain lipid composition and learning ability. We have also studied the effects of a diet containing 10% soybean oil with 6.5% 18:3n-3, the learning ability of the soybean oil-fed rats being superior to that of safflower oil-fed rats. There have been a few studies on the effect on learning ability of weaning rats due to feeding with a diet containing 22:6n-3. However, there have been no studies on the effect of feeding a diet with a low level of 22:6n-3 on a fetus or neonate concerning the learning ability of rats. In this study, therefore, the influence of a diet containing fish oil at a low level, i.e., 3.4% as eicosapentaenoic acid (20:5n-3) and 3.0% as 22:6n-3, which was administered to rats during the period from the fetal to newborn stage, on their learning ability was examined.

Materials and Methods

Materials. Fish oil (sardine oil), lard oil, palm oil, soybean oil, and coconut oil were prepared by Nippon Oil & Fats Co., Ltd. (Tokyo, Japan). Casein was purchased from the New Zealand Dairy Board (New Zealand), l-methionine from Wako Pure Chemicals Ltd. (Osaka, Japan), and cornstarch from Nippon Shokuhin Kakou Ltd. (Tokyo, Japan).

Animals and diets. Nine-wk-old female Wistar rats, weighing about 200 g and obtained from Japan SLC Inc. (Shizuoka, Japan), were housed individually in a room with controlled temperature, humidity and light (23 ± 2°C, 55 ± 2% RH and 06:00 to 18:00h, respectively). The rats were divided into two groups: the FS group was fed on a diet containing 10% of fat comprising basin of oil and fish oil (sardine oil) mixed in the proportion of 70-30, and the control (CN) group was fed on a diet containing 10% of basin of oil only. The basin of oil was composed of palm oil, lard oil, soybean oil, and coconut oil (10, 55, 30, and 5%, respectively). The diets were prepared according to the formula recommended by AIN, the fatty acid composition of each diet being shown in Table 1.

Table 1. Fatty Acid Composition of Dietary Fats

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>CN (%)</th>
<th>FS (%)</th>
<th>Fish oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:0</td>
<td>2.0</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>14:0</td>
<td>1.9</td>
<td>2.9</td>
<td>8.7</td>
</tr>
<tr>
<td>16:0</td>
<td>20.8</td>
<td>18.9</td>
<td>19.5</td>
</tr>
<tr>
<td>16:1n-7</td>
<td>—</td>
<td>2.4</td>
<td>6.1</td>
</tr>
<tr>
<td>18:0</td>
<td>9.2</td>
<td>7.3</td>
<td>2.7</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>37.5</td>
<td>31.2</td>
<td>13.3</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>20.8</td>
<td>15.3</td>
<td>1.6</td>
</tr>
<tr>
<td>20:3n-6</td>
<td>—</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>—</td>
<td>0.2</td>
<td>0.8</td>
</tr>
<tr>
<td>22:5n-6</td>
<td>—</td>
<td>0.1</td>
<td>0.3</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>2.0</td>
<td>1.7</td>
<td>1.2</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>—</td>
<td>3.4</td>
<td>13.2</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>—</td>
<td>0.6</td>
<td>2.1</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>—</td>
<td>3.0</td>
<td>11.1</td>
</tr>
<tr>
<td>n-6n-3</td>
<td>10.4</td>
<td>1.8</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Abbreviations: CN group, the group fed with a diet containing 10% of basin oil; FS group, the group fed with a diet containing 10% of fat comprising basin oil and fish oil; PUFA, polyunsaturated fatty acids; 18:2n-6, linoleic acid; 20:4n-6, arachidonic acid; 18:3n-3, γ-linolenic acid; 22:6n-3, docosahexaenoic acid.
all the rats were able to reach the safety platform within the time allowed. Therefore, all data from the swimming test were analyzed. This experiment was repeated eight times daily for three days (day 1 to day 3) with the safety platform at different positions. After the rats had reached the safety platform, they were allowed to rest on it for 180 s, and were then made to swim again. On the fourth day (day 4), the safety platform was transferred to a position directly opposite its previous position, and the test was again performed six times. The number of times each rat returned to the place where the platform was formerly located (i.e., “visits”) was counted.

Throughout this experiment, all external environmental cues remained constant.

**Statistical methods.** All data are expressed as the mean ± SD. Data for the time required to reach the platform in the swimming test on day 1 to day 3 were analyzed for statistical significance by four-way ANOVA, with the fed diet as factor A (i.e., the difference between the FS and CN groups), individual rat as factor B (i.e., the difference among the rats used in the swimming test), treatment day as factor C (i.e., the difference among the days on which the swimming test was performed), and trial number as factor D (i.e., the difference among the trials). Data from the swimming test for the second to sixth trials on the fourth day were analyzed for statistical significance by three-way ANOVA, with the fed diet, individual rat, and trial number as factors.

**Results**

**Learning ability of young rats**

The 20:4n-6 and 22:6n-3 concentrations of phospholipids in the liver and brain of the rats fed on the experimental (FS) and control (CN) diets during the fetal and postnatal periods were measured.12)

The results of the rats’ swimming test as an index of learning ability are shown in Fig., while the results of four-way ANOVA for the swimming test on day 1 to day 3 are shown in Table II.

There were significant differences in the fed diet, individual rat, treatment day and trial number factors. The time required to reach the safety platform for both groups became shorter as the swimming frequency increased and treatment day advanced. The mean times required to reach the platform for trials 1–8 on day 1 were 19.8 ± 35.5 s (n = 160) for the FS group and 29.0 ± 37.8 s (n = 160) for the CN group, the FS group thus taking a significantly shorter time than that for the CN group. The mean times for trials 1–8 on day 2 were 10.9 ± 15.3 s (n = 160) for the FS group and 13.8 ± 17.1 s (n = 160) for the CN group, and the times on day 3 were 6.6 ± 5.1 s (n = 160) for the FS group and 7.4 ± 6.3 s (n = 160) for the CN group.

When the platform position was transferred to the opposite side on the 4th day (day 4), the time required to reach the safety platform for both groups was longer in the 1st trial on the 4th day compared with that on the previous day. The times in the 1st trial on the 4th day were 66.0 ± 46.5 s (n = 20) for the FS group, and 38.5 ± 36.3 s (n = 20) for the CN group, showing a significantly longer time for the FS group, because the rats more frequently visited the previous platform location, than that for the CN group (the mean numbers of “visits” were 10.2 ± 0.5 (n = 20) for the FS group and 5.7 ± 0.4 (n = 20) for the CN group, which shows significant difference at p < 0.05). The results of three-way ANOVA on the data from trials 2–6 showed significant differences with the trial number as the factor. The time required to reach the safety platform for both groups became shorter as the swimming frequency was increased. The times for the 2nd trial were 22.8 ± 27.1 s (n = 20) for the FS group and 15.5 ± 14.2 s (n = 20) for the CN group (the mean numbers of “visits” were 8.5 ± 0.5 (n = 20) for the FS group and 4.2 ± 0.6 (n = 20) for the CN group, being significantly different at p < 0.05), showing the same trend as that in the 1st trial. However, the mean numbers of “visits” in trials 3–6 were 4.2 ± 0.6 (n = 80) for the FS group and 4.1 ± 0.8 (n = 80) for the CN group, showing no significant difference between the groups. When the time is presented as the mean value for trials 3–6 on the 4th day, the mean time required to reach the safety platform was 8.4 ± 1.5 s (n = 80) for the FS group and 10.4 ± 2.2 s (n = 80) for the CN group, with the FS group again taking a significantly shorter time than that for the CN group.

**Discussion**

Large amounts of 22:6n-3 and 20:4n-6 are contained in the brain and liver, playing an important role in the development of the brain and retina.12) In this study, a large amount of 22:6n-3 in the liver and brain of the FS group was observed during the fetal and neonatal periods, although no difference was observed between the FS and...
CN groups in the 22:6n-3 content of the brain a 7 wk of age, and no influence from 22:6n-3 fortification of the feeds was observed.\textsuperscript{12} Thus, we have suggested that a high level of 22:6n-3 in the liver and brain of fetal and neonatal rats can be achieved by the intake of fish oil by maternal and postnatal rats.\textsuperscript{12}

Clear data could not be obtained from a swimming test on 1- and 4-wk-old rats, because they were not physically strong enough to swim. Therefore, the swimming test on 6-wk-old rats was conducted to evaluate their learning ability.

Differences in the learning ability of the 6-wk-old rats were observed, the learning ability of the FS group being superior to that of the CN group.

In the evaluation of learning ability by the swimming test used in the present study, the difference in learning ability that was initially observed between the two groups diminished gradually, because of the time required to reach the safety platform for both groups becoming shorter with repeated swimming. In order to eliminate this phenomenon, a new environment was created on the 4th day by changing the position of the platform to its opposite location in the tank. As a result, the frequency of visiting the place in which the platform had been previously set was higher for the FS group than for the CN group during the early part of day 4, suggesting a better memory for the former. The mean time required to reach the platform in trials 3–6 was also shorter for the FS group than for the CN group. The influence of a dietary regimen on the learning ability, which may have been reduced as a result of repeated swimming, thus seems to have been re-established by changing the position of the platform.

In light of the report by Cosicina et al.,\textsuperscript{10} and judging from our own data, it was found that the shorter the time required for reaching the safety platform on day 1, the greater the number of "visits" to the old platform position in the first trial on day 4, and the shorter the time required for the rats to reach the new platform position once they had discovered it. These facts may indicate a superior learning ability of the FS group when compared with that of the CN group.

Yamamoto et al.\textsuperscript{5} have reported that the brightness-discrimination learning ability of young rats fed on a diet containing 5% of perilla oil rich in 18:3n-3 was superior to that of young rats fed on a diet containing 5% of safflower oil rich in 18:2n-6, and that a large amount of 22:6n-3 for the perilla oil group and a large amount of 20:4n-6 for the safflower oil group was observed in the brain phospholipids. We have also reported that the presence of large amounts of 22:6n-3 in the tissue phospholipid of soybean oil-fed neonatal rats without any alteration of their 20:4n-6 levels may contribute to an enhanced learning ability in rats.\textsuperscript{6}

Small premature human infants, who had not received the necessary amounts of nutritional elements from the maternal body, even though either breast milk or an artificial formula had been administered, showed an intellectual development at the age of 5 years inferior to that of normal infants.\textsuperscript{13} From these facts, it is assumed that the accumulation of 22:6n-3 in the rat brain is related to the improved learning ability, and it is suggested that nutritional elements may play an important role in the brain development of human infants.

The ratio of n-6/n-3 in the dietary fat was 10.4 for the CN group and 1.8 for the FS group, there thus being a large difference between the two groups. However, the dietary fat for the CN group was composed of 18:2n-6 and 18:3n-3, with a 18:2n-6/18:3n-3 ratio of 10.4. The 18:2n-6 and 18:3n-3 concentrations in the FS group were not significantly different from those in the CN group with a ratio of 9.0. Thus, the lower n-6/n-3 ratio for the FS group must have been due to 20:5n-3 and 22:6n-3 of dietary fish oil origin. The possible contribution of the direct incorporation of 20:5n-3 and 22:6n-3 derived from dietary fish oil to the accumulation of 22:6n-3 in the rat liver and brain is indicated. In practice, 20:5n-3 in the rat liver and brain is almost undetectable (A. Yonekubo et al., unpublished results), although both 20:5n-3 and 22:6n-3 were contained in the diets for the FS group. Therefore, the contribution of dietary 20:5n-3 to the improved learning ability may be small. Furthermore, this study reveals that 22:6n-3 produced from 18:3n-3 during the process of biosynthesis may be contained in the brain of rats of the CN group fed with the diet that did not contain 22:6n-3. It is also apparent that a larger amount of 22:6n-3 was accumulated in the rat brain from the fetus to newborn stage for the FS group than for the CN group, although no significant difference was observed in the concentration of 22:6n-3 in the brain of 7-wk-old rats between the two groups.

Based on these findings, it is suggested that the ingestion of 22:6n-3 during the period from the fetal to newborn stage, but not during the weaning period, and the accumulation of a large amount of dietary 22:6n-3 in the brain of fetal and newborn rats may be related to an improved cerebral function in 6-wk-old rats.

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References