Minimum Requirements of n-3 and n-6 Essential Fatty Acids for the Function of the Central Nervous System and for the Prevention of Chronic Disease (43412)

HARUMI OKUYAMA[1]
Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabedori, Mizuhoku, Nagoya 467, Japan

Abstract. **General behavioral patterns of rats or mice fed 5 wt% safflower oil (75% linoleate [n-6] and <0.1% α-linolenate [n-3]) for two generations were significantly different from those of animals fed 5 wt% perilla oil (15% n-6 and 55% n-3). Also, brightness-discrimination learning ability and retinal function were higher in the perilla group than in the group fed 5 wt% soybean oil (53% n-6 and 4.7% n-3) or safflower oil, indicating that the requirement of n-3 for the maximum responses of the nervous system is above 0.6 en% when there is 6.8 en% linoleate n-6. Perilla oil has been found to be beneficial for the suppression of carcinogenesis, allergic hyperreactivity, thrombotic tendency, apoplexy, hypertension, and aging in animals, as compared with soybean oil and safflower oil. These results are against a lipid peroxide theory of aging, carcinogenesis, and chronic diseases. Animal experiments and epidemiological studies lead to a recommendation that the intake of n-6 should be decreased to as low as 2~4 en% and that of n-3 be increased to levels higher than linoleate n-6 for the prevention of chronic diseases prevailing in the industrialized countries.** [P.S.E.B.M. 1992, Vol 200]

The desaturation-elongation activities to form 20 and 22 carbon highly unsaturated fatty acids from 18 carbon polyunsaturated fatty acids were considered by some researchers to be very low in humans as compared with experimental animals. However, several lines of evidence now indicate that the lower activities in humans may be due to the negative feedback control of the enzyme system by large amounts of highly unsaturated fatty acids in their diets. Nevertheless, the activities are enough to provide 20 carbon highly unsaturated fatty acids in human tissues. As a result, the dietary linoleic acid (LA) and α-linolenic acid (α-LnA) must be evaluated as precursors of arachidonate (20:4n-6) and eicosapentaenoate (20:5n-3), which are eicosanoid precursors and competitive effectors of each other.

The essentiality of LA for the maintenance of growth, reproductive physiology, and skin conditions in mammals was established 50 years ago. However, the essentiality of α-LnA for the maintenance of brain and retinal functions at high levels was established relatively recently. Thus, LA and α-LnA are essential fatty acids with different physiological activities in mammals.

Besides being essential, these fatty acids have been implicated in many kinds of chronic diseases in animals. After evaluating the consequences of relatively long-term feedings of vegetable oils with different proportions of LA and α-LnA, together with the results of epidemiological studies, we have reached a new recommendation in lipid nutrition for the prevention of chronic diseases, as described below.

Animals and Diets

For the estimation of the effects of dietary LA and α-LnA on nervous system functions, semi-purified diets supplemented with different vegetable oils (5 wt%) were fed to rats or mice for two generations (1). For the estimation of the effects of dietary oils on animal models of chronic diseases, a conventional diet (Nihon Clea Co., Ltd.; CE2) was treated with hexane, then vegetable oils (10%) and a vitamin mixture (2%) were

---

[1] To whom requests for reprints should be addressed at Faculty of Pharmaceutical Sciences, Nagoya City University, 3-1 Tanabedori, Mizuhoku, Nagoya 467, Japan.

0037-9727/92/2002-0174$3.00/0

supplemented and the mixtures were pelleted (2). Diets with peroxide values below 20 mEq/kg were served.

Effects of Dietary LA and α-LnA on Behavior of Rats and Mice

Brightness-discrimination learning ability has been tested in strains of spontaneously hypertensive rat (SHR), Wistar/Kyoto (normotensive control of SHR), aged and young Donryu (a conventional strain), and Sprague-Dawley rats (1, 3, 4). During the initial 30 sessions (days), diet pellets were given for the lever-pressing responses under a bright light, while no pellet was given for the responses under a dark light. In all the strains examined, the learning ability was higher in the perilla oil group than in the safflower oil group. After the 30 sessions, conditions for the following 30 sessions were changed. No pellet was given for the responses under both the bright and dark lights, or conditions for the bright and dark lights were reversed. In these experiments, the ability to discriminate the new conditions was significantly higher in the perilla group than in the safflower group (Table I). These results indicate that α-LnA is essential in mammals for the maintenance of discrimination-learning ability at a high level (1).

A series of general behavioral tests were performed with mice (ICR, male) fed different diets for two generations. The anxiety caused in the elevated plus maze task was more in the perilla group than in the safflower group (Table I). The learning ability in Morris' water maze test was higher in the perilla group, but no significant difference was observed in the correct response ratios in Y-shaped maze brightness discrimination or in elevated plus maze task. The safflower group was more sensitive to pentobarbital in inducing sleeping and was less sensitive to scopolamine in inducing hyperlocomotion as compared with the perilla group.

The mechanisms by which dietary vegetable oils affect general behavioral patterns remain to be elucidated. However, these results clearly indicate that the choice of foods affects behavior of mammals through the essential fatty acid balance.

Table I. Effect of Dietary Oils on Animal Behavior

Behavioral patterns	Safflower animals	Perilla animals	Reference
Habituation	Worse	Better	—[a]
Anxiety caused	Less	More	—[a]
Brightness-discrimination learning test			
Shaping	Faster	Slower	1, 3, 4
Learning ability	Lower	Higher	1, 3, 4
Memory	Worse	Better	3
Water maze learning	Lower	Higher	—[a]
Retinal function	Lower	Higher	5
Sensitivity to			
Pentobarbital	Higher	Lower	—[a]
Scopolamine	Lower	Higher	—[a]

[a] Y. Nakashima, S. Yuasa, Y. Hukamizu, H. Okuyama, T. Ohhara, T. Kameyama, T. Nabeshima, manuscript in preparation.

The brightness-discrimination learning ability and electroretinographic responses decreased in the order of the perilla group followed by the soybean group and then the safflower group (5). The fatty acid compositions of brain and retinal phospholipids were very similar between the perilla and soybean groups (1, 4, 5), but the physiological responses were significantly different. Therefore, the amount of α-LnA in the soybean diet (0.6 en%) was not sufficient to reach maximum responses in nervous system functions.

Effects of Dietary LA and α-LnA on Animal Models of Chronic Diseases

Effects of dietary oils on carcinogenesis were examined in dimethylbenzanthracene- and dimethylhydrazine-induced carcinogenesis models of rats (6) and in a spontaneous mammary tumorigenesis model in mice (2). Tumor metastasis was examined by measuring the number of pulmonary metastatic foci using Yoshida ascites tumor cells in rats (7). The effect on tumor necrosis factor production was measured in mice (8). Thrombotic tendency was estimated by measuring platelet aggregability and serotonin release in rats (9). The effects on apoplexy, hypertension, and mean survival time were estimated using stroke prone SHR, SHR rats, and Donryu rats (10). Allergic hyperreactivity was estimated by measuring leukotriene and platelet-activating factor production in rats (11, 12). For the estimation of aging, the mean survival time and brightness-discrimination learning ability in aged rats were used as the criteria (3).

In all these models of chronic diseases, perilla oil was beneficial as compared with the soybean and safflower oils (Table II).

Contrarily, the severities of anti-glomerular base-

Table II. Effects of Dietary Oils on Animal Physiology[a]

Perilla oil is better than soybean and safflower oils in:
  Learning ability (1, 3, 4), retinal function (5), suppression of carcinogenesis (6), spontaneous tumorigenesis (2), metastasis (7), thrombosis (9), hypertension (10), apoplexy (10), allergy[b] (11, 12), and aging (survival time and discrimination learning) (3, 10)
Safflower oil is better than perilla oil in:
  Suppression of anti-glomerular basement membrane nephritis (13) and lipopolysaccharide-galactosamine-induced hepatitis (14)
Dietary α-LnA/LA balance does not affect:
  Growth, reproductive physiology (♀) (17), erythrocyte deformability (18), tissue lipid peroxide levels (3, 19), radiation damage,[c] chronic nephritis,[b] lipopolysaccharide-induced nephritis (14), and teratogenicity (17)

[a] References are shown in parentheses.
[b] Soybean oil group was not included.
[c] Unpublished observations.

ment membrane-induced nephritis (13) and endotoxin plus galactosamine-induced hepatitis (14) were lower in the safflower group than in the perilla group (Table II). However, the severities of chronic nephritis seen in an autoimmune model of mice (MRL) and endotoxin (alone)-induced hepatitis were not affected by these oils. The interpretation of the effects of dietary α-LnA/LA balance on nephritis and hepatitis is not simple, but obviously there are pathological conditions under which eicosanoids derived from arachidonate play protective roles. These acute models differ from the chronic models in that the nutritional guidelines described below do not apply, and eicosanoid derivatives may be used as medicines.

Nutritional Recommendation for the Prevention of Chronic Diseases

Lipid nutrition for the prevention of chronic diseases must be considered in terms of both the n-3/n-6 balance and the total amounts ingested daily. Perilla oil with an n-3 to n-6 ratio of as high as 3~4 suppressed the chronic diseases listed in Table II, but soybean oil with an n-3 to n-6 ratio of 0.1~0.2 and safflower oil with a ratio below 0.01 stimulated those chronic diseases. The n-3 to n-6 ratios of Eskimos and Danes' diets were 2.8 and 0.3, respectively (15), whereas those of average Japanese adults, Japanese children, and American adults were 0.26, 0.15, and 0.12, respectively (16). Based on a combination of the animal experiments and epidemiological studies, the following guidelines in lipid nutrition are recommended for the prevention of chronic diseases prevailing in the industrialized countries.

1. The intake of LA (n-6) should be decreased to as low as 2~4 en%. The required amount is less than 1 en%.

2. The intake of α-LnA (n-3) should be increased to levels higher than linoleate n-6. The amount of n-3 as high as 15 en% was the best in animal experiments.

3. The n-3 to n-6 ratio should be raised to more than 1 from the current values ~0.2. The ratio of 3~4 was the best in animal experiments.

4. Total fat intake should preferably be below 20 en%, because fat-related diseases began to increase when it exceeded 15 en% in 1965 in Japan.

---

1. Yamamoto N, Saitoh M, Moriuchi A, Nomura N, Okuyama H. Effect of dietary α-linolenate/linoleate balance on brain lipid composition and learning ability of rats. J Lipid Res **28**:144–151, 1987.
2. Kamano K, Okuyama H, Konishi H, Nagasawa H. Effects of a high-linoleate and a high-α-linolenate diet on spontaneous mammary tumorigenesis in mice. Anticancer Res **9**:1903–1908, 1989.
3. Yamamoto N, Okaniwa Y, Mori S, Nomura M, Okuyama H. Effects of a high-linoleate and a high-α-linolenate diet on the learning ability of aged rats. Evidence against an autoxidation-related lipid peroxide theory of aging. J Gerontol **46:**B17–22, 1991.
4. Yamamoto N, Hashimoto A, Takemoto A, Okuyama H, Nomura M, Kitajima R, Togashi T, Tamai Y. Effect of dietary α-linolenate/linoleate balance on lipid compositions and learning ability of rats. II: Discrimination process, extinction process and glycolipid compositions. J Lipid Res **29:**1013–1021, 1988.
5. Watanabe I, Kato M, Aonuma H, Hashimoto A, Naito Y, Moriuchi A, Okuyama H. Effect of dietary alpha-linolenate/linoleate balance on the lipid composition and electroretinographic responses in rats. Adv Biosci **62**:563–570, 1987.
6. Hirose M, Masuda A, Ito N, Kamano K, Okuyama H. Effect of dietary perilla oil, soybean oil and safflower oil on DMBA and DMH-induced mammary gland and colon carcinogenesis in female SD rats. Carcinogenesis **11**:731–735, 1990.
7. Hori T, Moriuchi A, Okuyama H, Sobajima T, Tamiya-Koizumi K, Kojima K. Effect of dietary essential fatty acid balance on pulmonary metastasis of ascites tumor cells in rats. Chem Pharm Bull **35:**3925–3927, 1987.
8. Watanabe S, Hayashi H, Onozaki K, Okuyama H. Effect of dietary α-linolenate/linoleate balance on LPS-induced tumor necrosis factor production in mouse macrophages. Life Sci **48:**2013–2020, 1991.
9. Watanabe S, Suzuki E, Kojima N, Kojima R, Suzuki Y, Okuyama H. Effect of dietary α-linolenate/linoleate balance on collagen-induced platelet aggregation and serotonin release in rats. Chem Pharm Bull **37**:1572–1575, 1989.
10. Shimokawa T, Moriuchi A, Hori T, Saitoh M, Naito Y, Kabasawa A, Nagae Y, Matsubara M, Okuyama H. Effect of dietary alpha-linolenate/linoleate balance on mean survival time, incidence of stroke and blood pressure of spontaneously hypertensive rats. Life Sci **43:**2067–2075, 1988.
11. Hashimoto A, Katagiri M, Torii S, Dainaka J, Ichikawa A, Okuyama H. Effect of the dietary α-linolenate/linoleate balance on leukotriene production and histamine release in rats. Prostaglandins **37:**3–16, 1988.
12. Horii T, Satouchi K, Kobayashi Y, Saito K, Watanabe S, Yoshida Y, Okuyama H. Effect of dietary α-linolenate on platelet-activating factor production in rat peritoneal polymorphonuclear leukocytes. J Immunol **147:**1607–1613, 1991.
13. Watanabe S, Suzuki E, Kojima R, Suzuki Y, Okuyama H. Effect of dietary α-linolenate/linoleate balance on crescent type anti-glomerular basement membrane nephritis in rats. Lipids **25:**267–272, 1990.
14. Watanabe S, Okuyama H. Effect of dietary α-linolenate/linoleate balance on endotoxin-induced hepatitis in mice. Lipids **26:**467–471, 1991.
15. Dyerberg J. Linolenate-derived polyunsaturated fatty acids and prevention of atherosclerosis. Nutr Rev **44:**125–134, 1986.
16. Lands WEM, Hamazaki T, Yamazaki K, Okuyama H, Sakai K, Goto Y, Hubbard VS. Changing dietary patterns. Am J Clin Nutr **51:**991–993, 1990.
17. Naito Y, Ishizaki K, Yamamoto H, Okuyama H. Studies on the safety of perilla oil. Effects on reproduction and skeletal teratogenicity. J Food Hyg Soc Jpn **31:**251–254, 1990.
18. Sakai K, Okuyama H, Kon K, Sekiya M, Shiga T, Reitz RC. Effects of high α-linolenate and linoleate diets on erythrocyte deformabilty and hematological indices in rats. Lipids **25:**793–797, 1990.
19. Sakai K, Okuyama H. Effects of a high α-linolenate and high linoleate diet on hemolysis and lipid peroxidation on rat erythrocytes. Chem Pharm Bull **39:**1504–1506, 1991.