Nutritional Biochemistry of Omega-3 Fatty Acids

– Their Essentiality and Importance for the Prevention of Chronic Diseases –

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Ladies and Gentlemen, it is a great honor for me to be invited to exchange informations on lipid nutrition at the Scientific Meeting of the Korean Nutrition Society, which has a relatively long history. We founded a new society last year, the Japan Society for Lipid Nutrition, for which I am serving as the President. Many people asked me "why nutrition at the end of the 20th century ? ". However, I am quite certain that lipid nutrition is one of the most important medical fields to be explored toward the 21st century, because good medicines would not be easily available for the treatments of chronic, elderly diseases such as cancer, thrombotic diseases, allergy and aging, but lipid nutrition is deeply involved in such diseases.

Current nutritional recommendations for the prevention of elderly diseases have long been to increase the intake of vegetable oils rich in linoleic acid and to decrease the intake of animal fats and cholesterol. However, the usefulness of such nutritional recommendations had not been proved clinically, and a long-term intervention trial performed in Finland has clearly indicated that such recommendations are even dangerous for cardiovascular diseases.

Before going into the details of the intervention trial performed in Finland, I would like to remind you of the three pathways of fatty acid metabolism in our bodies(Table 1).

As most of you are very familiar, saturated and monounsaturated fatty acids are synthesized in our bodies from carbohydrates and proteins. These are enriched in animal fats, olive oil and high-oleic vegetable oils.

Linoleic acid is synthesized only in plants, but is converted in our bodies to arachidonic acid and other ω6 fatty acids. Linoleic acid, enriched in most of common vegetable oils and oil products, is known to be essential for the maintenance of growth, skin conditions and reproductive physiology. However, the required amount is very small and we are currently ingesting several times more linoleic acid than the amount that is required(ω6 series).

α-Linolenic acid is also synthesized in plants, but is converted to EPA and DHA in our bodies. We and others have recently established that the deficiency of α-linolenic acid results in decreased learning ability and visual acuity. These ω3 fatty acids are relatively enriched in vegetables ; perilla oil and linseed oil are particularly rich in α-linolenic acid. As you know, seafoods are very rich in EPA and DHA(ω3 series).

Nutritionally, it is important to note that no interconversion occurs among these three series. Another important aspect is that various hormone-like substances such as prostaglandins, thromboxanes and leukotrienes are synthesized from these 20 carbon fatty acids, which have been found by many scientists to be deeply involved in chronic, elderly diseases.

Finland study(Strandberg et al(1991) JAMA)

In the Finland Study, more than 12 hundered business executives with risks for elderly diseases were divided into 2 groups ; one group was left free as a control and another group was treated with these drugs for the begining 5 years when necessary, and the mortality was followed for up to 15 years. Nutritional recommendations shown here are probably very familiar to most of you, which advise to decrease the intake of total calorie, animal fats, cholesterol and others and to increase the intake of polyunsaturated fatty acid and others(Table 2). As will be discussed in this paper, I am going to emphasize that increasing the intake of linoleic acid in the form

Table 1. Three series of fatty acid metabolism in mammals

S and M Series :

Saturated FA → Monounsaturated FA
18 : 1ω9, 18 : 1ω7

Animal fats — Olive oil, High-oleic oils
Palm oil, Coconut oil

ω6(n-6) Series :

Linoleic A → γ-Linolenic A → Dihomo-γ-Linolenic A → Arachidonic A → Docosapentaenoic A
(18 : 2ω6) — (20 : 4ω6) — (22 : 5ω6)

Safflower oil, Sunflower oil,
Margarine, Mayonnaise, Meats — (Meats)

ω3(n-3) Series :

α-Linolenic A → 18 : 4 → 20 : 4 → Eicosapentaenoic A → Docosahexaenoic A →
(18 : 3ω3) — EPA(20 : 5ω3) — DHA(22 : 6ω3)

Vegetables,
Perilla oil, Linseed oil — Seafoods — Seafoods, Brain & Retina

Table 2. Long-term mortality after 5-year multifactorial primary prevention of cardiovascular diseases in middle-aged men(Strandberg et al, 1991)

Dietary and daily instructions	
Advised to reduce :	Advised to increase :
Intake of calories	Polyunsaturated fats
Saturated fat, Cholesterol, Alcohol, Sugar	(soft margarine)
	Fish, Chicken, Veal, Vegetables
Smoking	Physical activity
When necessary :	
Hypotensive drugs(β-blocker, diuretics)	
Hypolipidemic drugs(probucol, clofibrate, niacin, neomycin sulfate, cholestyramine)	

of soft margarine was unfavorable for the prevention of elderly diseases.

In the Finland study, the mortality from cardiac deaths was similar for the beginning several years but the difference between the two groups became clearer after 10 years, and the mortality in 15 years was 2.4-fold high in the intervention group than in the control group. The total death was also significantly higher in the intervention group. Then, one must think which was wrong, medicines or nutrition ? (Fig. 1)

Because hypotensive and hypolipidemic drugs were used only for the beginning 5 years, the percentage of subjects receiving medicines was significantly higher in the intervention group at the 5th year(Table 3). The serum cholesterol, blood pressure and body weight decreased significantly in the intervention group. Therefore, the treatments appear to be quite successful. At the 10th year, the number of subjects receiving medicines was similar in both groups, indicating that these drugs were not the cause of the increased mortality from cardiovascular diseases.

It should be noted that no difference was seen in the serum cholesterol levels of the two groups despite 10 years' nutritional recommendations. Animal experiments also supported these observations. The hypocholesterolemic effect of vegetable oils is only transient ; after long-term feedings of vegetable oils and animal fats, very little difference was seen in the plasma cholesterol levels.

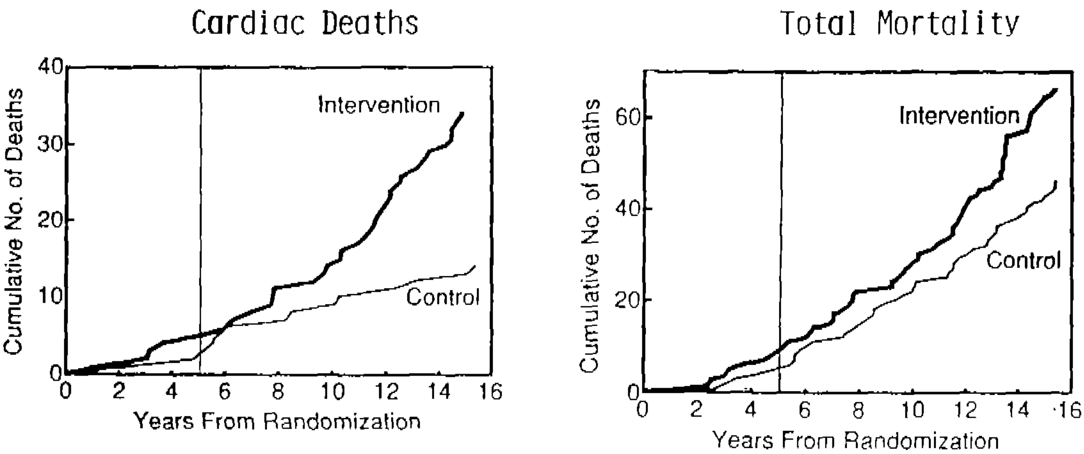

Fig. 1. Cutting cholesterol increases danger of heart attacks(Strandberg et al, 1991).

Table 3. Characteristics of the study groups of a multifactorial primary prevention trial(Strandberg et al, 1991).

	At 5 years		At 10 years	
	Control Group	Intervention Group	Control Group	Intervention Group
Medication %				
Hyportensive	15	32**	22	27
Hypolipidemic	0	37**	2	2
Serum Cholesterol(mmol/l)	7.2	6.7**	7.1	7.1
Blood Pressure(mmHg)	142	135*	142	141
Relative Body Weight(%)	118	114**	117	115**

Therefore, I cannot recommend people to increase the intake of linoleic acid only because of its transient hypocholsterolemic effect.

The well known epidemiological studies on Eskimos and Danes must be reevaluated. Both of these populations took so much animal fats(saturated and monounsaturated fatty acids) in amounts of more or less 30 energy %(Fig. 2). Furthermore, the intake of cholesterol was two-fold greater in Eskimos than in Danes, but the incidence of cardiovascular diseases was much less in the Eskimos' population(1/10th). Therefore, we must interpret these data that both the animal fats and cholesterol in diets are not serious risk factors for thrombotic diseases.

In contast, the intake of linoleic acid was much more but the intake of EPA and DHA from seafoods was much less in Danes than in Eskimos. These differences in the intake of linoleic acid and EPA plus DHA brought about a strong impact on thrombotic tendency, which was proved to be the major factor for thrombotic diseases.

As most of you already know, many scientists have established that over-production of eicosanoids derived from linoleic acid through arachidonic acid(thromboxane A_2) increases thrombotic tendency while EPA competitively inhibit arachidonate metabolism(Fig. 3). Anti-thrombotic PGI_3 is synthesized from EPA but pro-thrombotic thromboxane A_3 is not synthesized in significant amounts. Furthermore, fish oils rich in EPA and DHA have been shown to increase erythrocyte deformability, and to decrease blood viscosity and leukocyte adhesiveness. All these effect of ω3 fatty acids are understood to serve to decrease thrombotic diseases.

Suppression of stroke by dietary high-ω3 vegetable oil

In our experiments, we used vegetalbe oils. Various vegetable oils differ very much in their

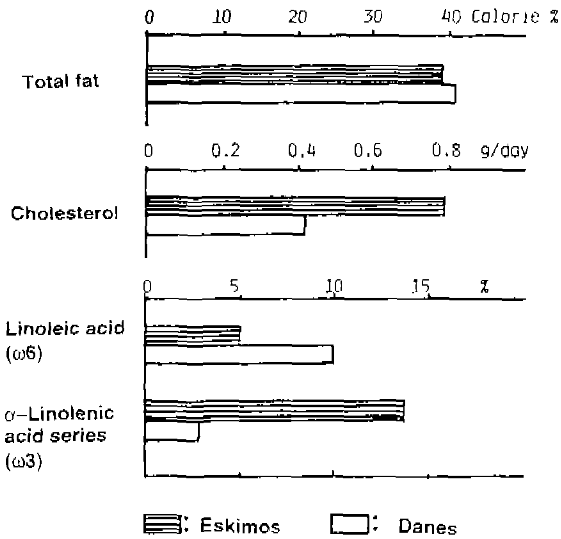

Fig. 2. Diets of Eskimos and Danes(Dyerberg, 1986).

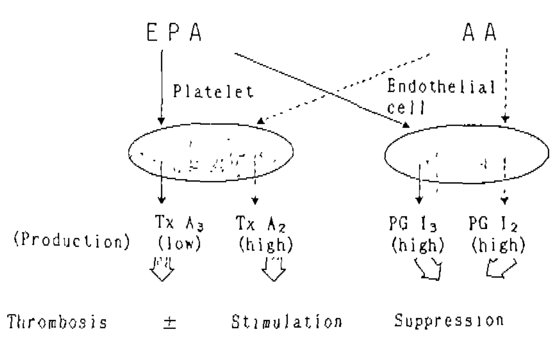

Fig. 3. Eicosapentaenoate(EPA) & arachidonate(AA) in thrombosis.

fatty acid compositions, but most of currently available oils are very rich in linoleic acid(ω6)(Fig. 4). Safflower oil had been considered to be good for health because of its very high content of linoleic acid. Perilla seed oil is very familiar to you. I know it is good for "Bosintan" cooking. It contains a relatively small amount of linoleic acid and a large amount of α-linolenic acid(ω3). We compared mainly safflower oil and perilla oil ; soybean oil was used as a control. Feeding these oils to rats or mice through two generations did not bring about any difference in appearance ; all the groups grew apparently normally.

In this experiment(Table 4), we used stroke-prone spontaneously hypertensive rats(SHR-SP), which have the highest blood pressure among the available strains of rats, and die mostly of cerebral bleeding.

The mean survival time was significantly longer in the perilla oil group than in the safflower oil group or soybean oil group. Donryu is a conventional stain, but again more than 10% difference was observed in longevity. Even if a specific medicine for cancer could be found, the life expectancy of Japanese is calculated to be elongated by only 4%. Therefore, the >10% difference expected to be brought about by the choice of oils would have a strong impact in preventive medicine.

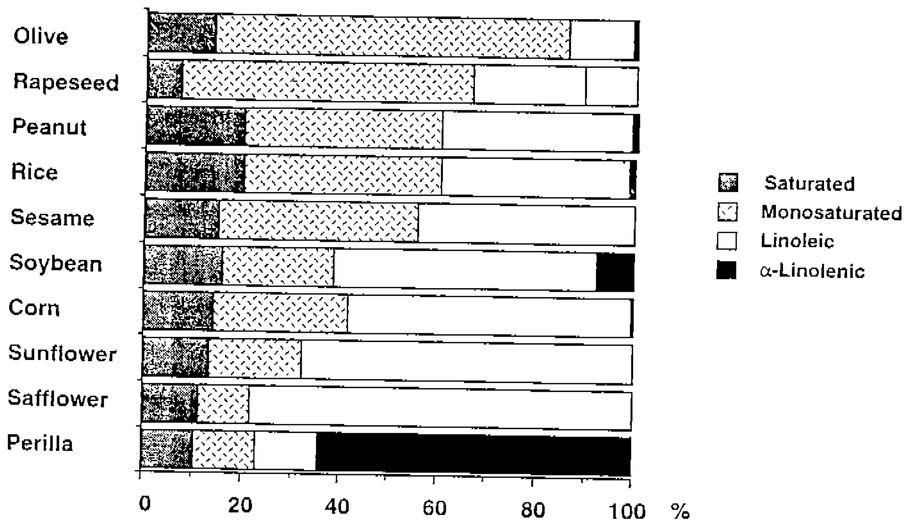

Fig. 4. Fatty acid compositions of vegetable oils.

Table 4. Effects of dietary oils on mean survival times of rats

Rat Strain	Perilla Oil Group	Soybean Oil Group	Safflower Oil Group
SHR-SP♂	100(13.9Mo)	88.6	85.5
SHR-SP♀	100(20.2Mo)	nd	87.0
Donryu♂	100(27.2Mo)	nd	88.1
SHR-SP♂*	100(12.4Mo)	79.6	nd
SHR-SP♂*	100(6.4Mo)	78.0	nd

*, 1% NaCl was loaded as drinking water
nd, not determined
( ), mean survival time in month

Effects of dietary ω3 and ω6 fatty acids on carcinogenesis and metastasis of tumor cells

In the past 30 years in Japan, the mortalities from stomach and uterus cancers tended to decrease, and hopefully would reach the very low levels seen in the United States(Fig. 5). On the other hand, the mortalities from lung, colon, breast and several other cancers have been increasing rapidly and are going to approach the very high levels seen in the western countries. For most of these western type cancers, it has been shown in animal experimets that dietary linoleic acid promotes but ω3 fatty acids suppress carcinogenesis.

One of such experiments by Yonekura and Sato showed that mammary tumourigenesis was significantly higher in the corn oil group than in the perilla oil and fish oil groups(Fig. 6). Similar conclusions have been obtained in our laboratory and by many scientists in the world.

Smoking is known to be a major risk factor for lung cancer, but lung cancer is classified mainly into squamous cell carcinoma type which is highly correlated with smoking habit and adenocarcinoma type which is not correlated. In the growing number of lung cancer in Japan, more than half is adenocarcinoma type. Recently, corn oil enriched with linoleic acid was shown in animal experiments to stimulate lung adenocarcinoma(Imaida et al).

In the past 30 years in Japan, the intake of both animal fats and linoleic acid increased roughly 3-fold. Then, one can ask a question which of the animal fats and linoleic acid is more serious risk factor for cancers. Dr. Yanagi's group has shown that butter increased mammary tumourigenesis slightly, but safflower margarine and safflower oil were much more potent stimulators of carcinogenesis, indicating that it is linoleic acid that is a more serious risk factor for carcinogenesis(Fig. 7). The stimulatory effect of linoleic acid on western type cancers appears to be mediated through

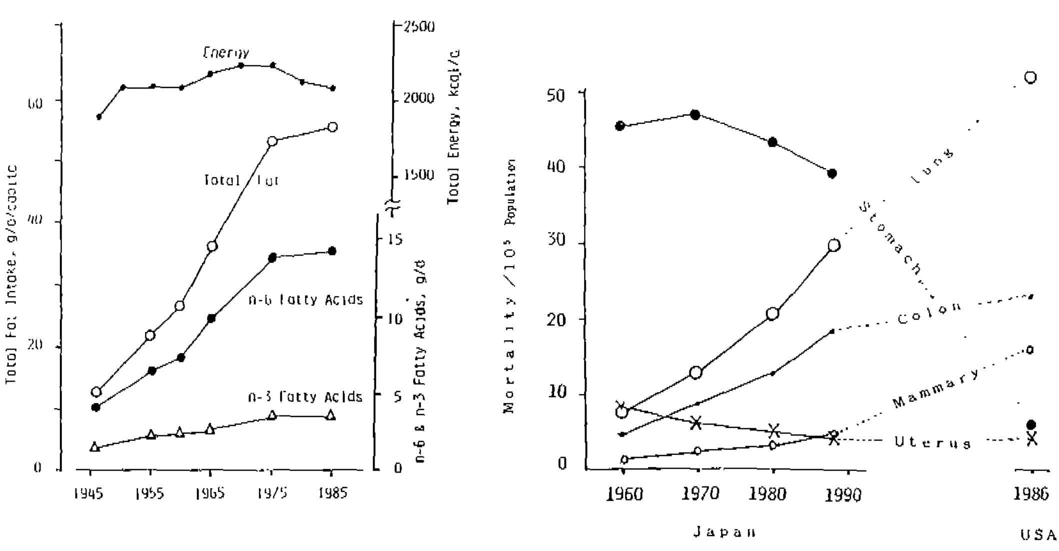

Fig. 5. Trends of fat intake and cancer mortality in Japan.

over-production of prostaglandin(PG) E_2, which mediates the proliferation of such cells as fibroblasts and epithelial cells. PG E_2 suppresses host immune system allowing tumor cells to grow rapidly. It also suppresses the production of tumor necrosis factor(TNF). Linoleic acid epoxide may also play a role as a promoter of carcinogenesis. Stimulation of platelet aggregability is probably associated with stimulated metastasis of tumor cells observed in the high-linoleic acid group as compared with high ω3 groups. I interpret that all these mechanisms work together in the stimulation of carcinogenesis and metastasis by the dietary linoleic acid.

Hirayama and coworkers reported the results of 17 years cohort study for more than 265 thousand Japanese people(Table 5). As in the case of MRFIT study carried out in the USA, the total deaths, mortalities from cerebrovascular and cardiac diseases as well as from these cancers were significantly less in the populations eating seafoods daily as compared with those who do not eat so much. Thus, the conclusions from animal experiments and epidemiological studies fit very well, allowing to conclude that the intake of ω3 should be increased and that of ω6(mainly linoleic acid) should be decreased in order to prevent western type cancers and thrombotic diseases.

Effect of dietary ω3 and ω6 fatty acids on immune system functions

Allergic patients have increased several-folds in the past 30 years in Japan and about 1/3 of Japanese infants are diagnosed as atopy.

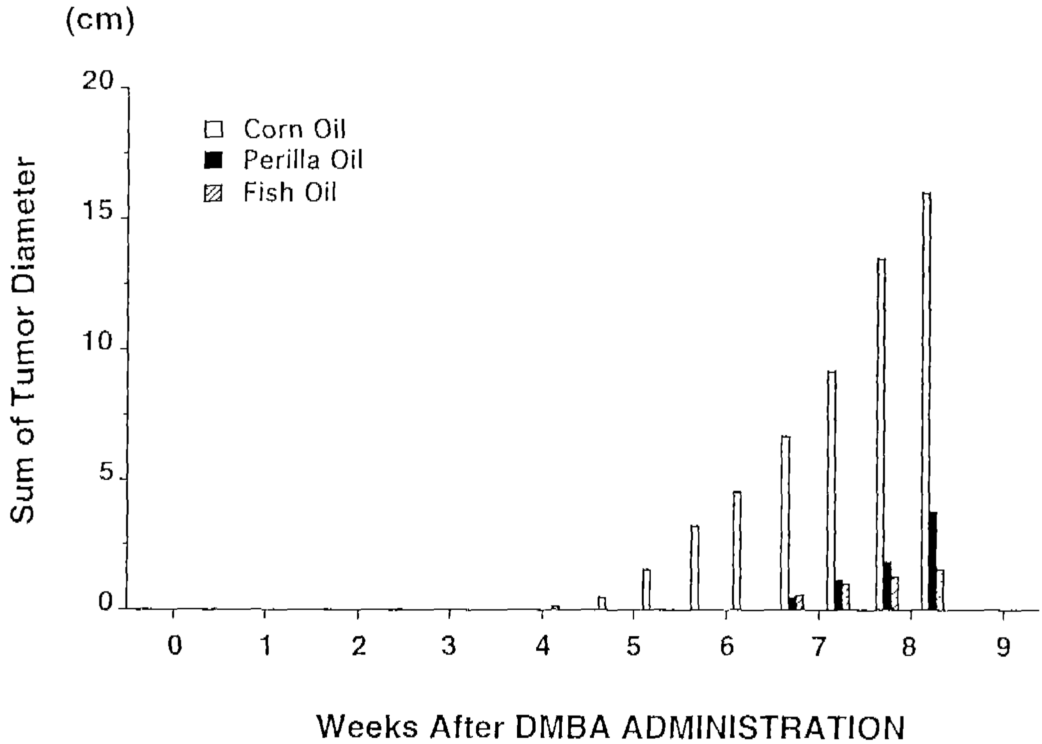

Fig. 6. Effect of dietary oils on DMBA-induced mammary tumorigenesis(Yonekura & Sato, 1991).

As has been established, IgE antibody is formed when allergen enters the body, and IgE attaches to such cells as mast cells and basophiles(Fig. 8). When the allergen enters again, it binds to IgE and activate such cells, resulting in the release of mediators such as histamine, leukotrienes(LT), platelet-activating factor(PAF) and other eicosanoids, which induce allergic and inflammatory reactions. We have shown earlier that perilla oil(high-ω3) suppresses the productions of LT B_4, thromboxane(TX) A_2, slow-reacting substances of anaphylaxis(SRS-A) and PAF as compared with safflower oil. Here, I would like to show you that IgE production in mice is also affected by dietary ω3 and ω6 fatty acids.

In repeated assays, the IgE production was found to be significantly less in the perilla oil group as compared with the safflower oil group(Fig. 9). Under the experimental conditions, the level of IgE was not much elevated from the control level. Therefore, the second booster injection of

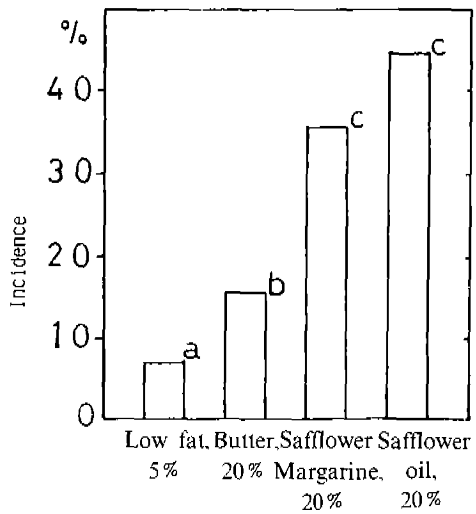

Fig. 7. Effect of fat & oil on spontaneous mammary tumorigenesis in mice(Yanagi, S. et al, 1989).

Table 5. Frequency of seafood intake vs normalized mortality - A Cohort Study in Japan(Y.Hirayama, 1992)

Population : 265, 111 ; Period, 17 years

Cause of Death	Frequency of Seafood Intake				P
	Daily	Occasional	Rare	None	
Total death	1.00	1.07	1.12	1.32	$p<0.0001$
Cerebro-vascular	1.00	1.08	1.10	1.10	$p<0.0001$
Cardiac	1.00	1.09	1.13	1.24	$p<0.0001$
Hypertension	1.00	1.55	1.89	1.79	$p<0.0001$
Cirrhosis	1.00	1.21	1.30	1.74	$p<0.0001$
Stomach Cancer	1.00	1.04	1.04	1.44	$p<0.05$
Hepatic Cancer	1.00	1.03	1.16	2.62	$p<0.05$
Cervical Cancer	1.00	1.28	1.71	2.37	$p<0.0001$

(Relative Risks)

Ovalbumin(OVA) was made. Again, the level of IgE tended to be lower in the perilla oil group as compared with the safflower oil group. Under the conditions, however, a significant proportion of mice died of anaphylaxy-like shock. The mortality by second challenge of OVA or DNP-OVA as antigens was significantly lower in the perilla oil group than in the safflower oil group(Fig. 10).

From these and other data, we conclude that dietary ω3 fatty acids suppress allergic hyper-reactivity of body by decreasing the productions of IgE and allergic, inflammatory madiators such as LTB_4, SRS-A, TXA_2 and PAF but linoleic acid(ω6) stimulates the hyper-reactivity.

The above conclusion would be acceptable if you consider the action sites of various anti-allergic drugs(Fig. 11). Steroidal anti-inflammatory drugs are known to suppress the expression of phospholipases A_2 and cycloxygenase II, while most of the anti-allergic drugs used in Japan are known to suppress the liberation of LT, TX A_2 and PAF.

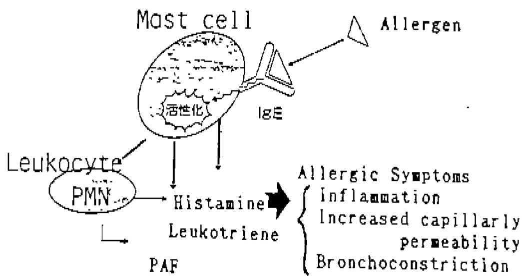

Fig. 8. Mediators of allergy.

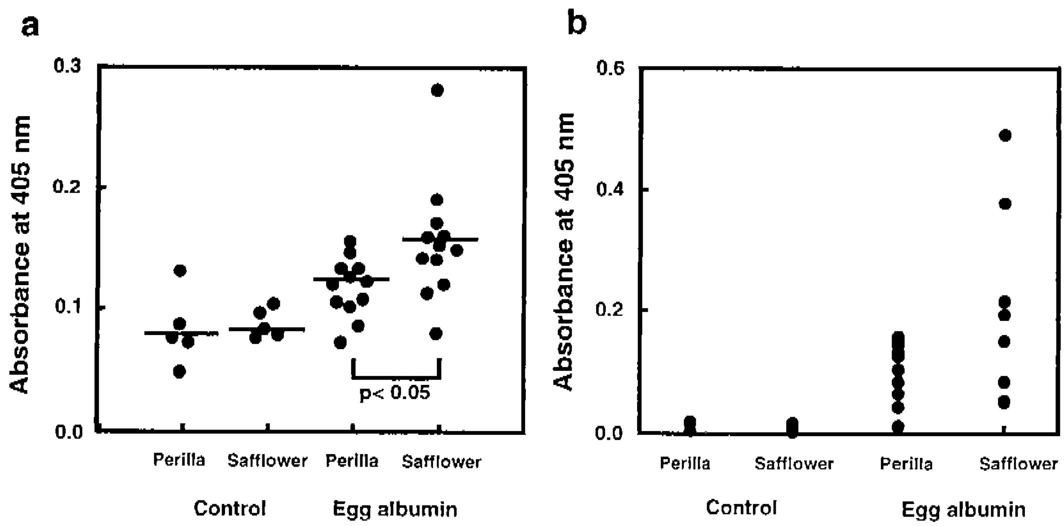

Fig. 9. Effect of a high α-linolenate(perilla) and a high linoleate(safflower) diet on the production of anti-egg albumin IgE antibodies in mice.

Brain functions are affected by dietary essential fatty acid balance

In the brightness-discrimination learning testes, rats were fasted·by limiting the diet and then trained so as to press the lever to obtain diet pellets(shaping process). Within several days, all the animals learnt how to press the lever to obtain diet pellets. Then in the brightness-discrimination test, either a bright light or a dim light was presented on this screen randomly but equally. Diet pellets were given to the lever-pressing responses under the bright light while no pellet was given to the responses under the dim light, and the number of lever-pressing responses was counted through 30 sessions(days).

The open circle(Fig. 12) represents the responses under the bright light that is proportional

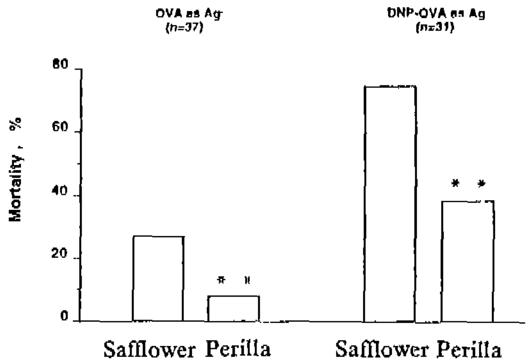

Fig. 10. Effect of dietary oils on the mortality induced by second challenge with OVA or DNP-OVA in ICR mice.

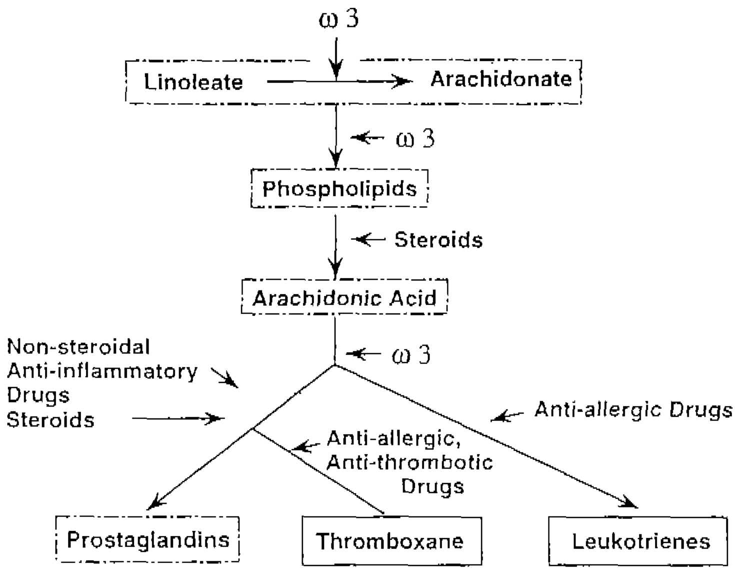

Fig. 11. Inhibitors of linoleate metabolism.

to the number of pellets he got, and the closed circle represents the responses under the dim light. The difference between the open circle and closed circle, that is, the area of the shaded portion represents the ability to discriminate the brightness. Then, we can say that the learning ability was higher in the perilla oil group than in the safflowr oil group. You will see the major difference of the two groups in the negative responses shown in the closed circles.

Safflower oil contained very little α-linolenic acid. Feeding safflower oil from mothers to offspring resulted in a decrease in the DHA content of brain, which was associated with inferior learning performance as compared with the perilla oil group. We have found that this link is very reproducible in 5 different stains of rats. DHA rich in fish oil was as effective as perilla oil or ethyl ester of α-linolenic acid. When α-linolenic acid was supplemented after the weaning, the decreased leaning ability was restored(reversibility). Chronic administration of indomethacin did not affect the learaning behavior, indicating that over-production of eicosanoids is not the cause of decreased learning ability seen in the safflowewr oil group. This link was seen both in young and aged animals, which forms one of the bases for our claim that perilla oil is useful for the suppression of aging proceess.

Biochemical bases for the dietary ω3/ω6 balance affecting the behavior

The question many researchers are asking these days is what is the biochemical mechanisms for the dietary essential fatty acid balance affecting the learning behavior of mammals. Na, K-ATPase activity has been reported from a few laboratories to be reduced in ω3-deficiency. In one experiment, we observed a slightly lower Na, K-ATP ase activity at a suboptimal concentration of the substrate(ATP)(Table 6). However, this was not reproducible in anothe enzyme preparation, synaptosomal plasma membranes. This phospholipase C was found in our laboratory to be specific

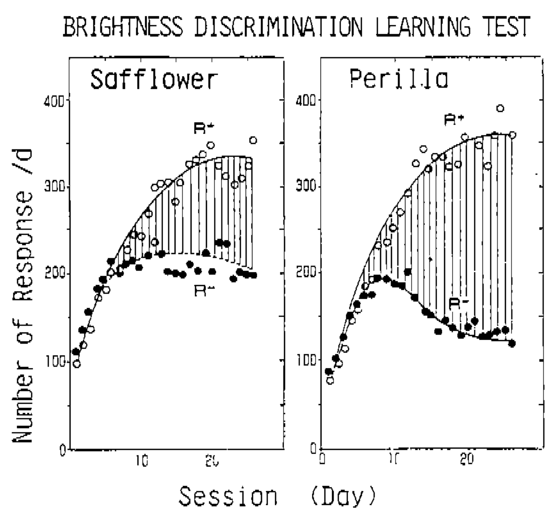

Fig. 12. Brightness discrimination learning test.

for lysophosphatidylinositol and to be localized at synaptosomal plasma membranes. The activity ratio was similar to that of Na, K-ATPase in the two dietary groups. So far, we found no direct evidence to show lower activity of this enzyme in the ω3-deficient animals. Activities of serveral other enzymes in brain were compared to find no significant differences in the safflowr and perilla oil groups.

However, in the safflower oil group, the anesthesia onset time induced by pentobarbital was shorter and anesthesia duration was longer than in the perilla group(Fig. 13). Barbiturate is known to act on chloride channel through GABA receptor. Therefore, these processes may be affected by ω3-deficiency, although we have no direct evidence so far.

One of our coworkers measured the density of synaptic vesicles by using an image-analyzer to find a significant difference between the safflower group and perilla group(Fig. 14). Average synaptic vesicle density was greater in the perilla oil group than in the safflowr oil group. There are two possibilities for the reduced synaptic vesicle density in the safflowr group ; the utilization of synaptic vesicles may be accelerated or the formation of synaptic vesicles may be suppressed. We do not know which is the case. As shown here and in the previous figures, we found some clues for the elucidation of biochemical mechanisms, but at present we are far from explaining the mechanisms of food-learning behavior link in biochemical terms. All of you are welcome to this very interesting field of research, that is, neurobiology of essential fatty acids.

Table 6. Effect of dietary oils on synaptic plasma membrane enzymes

Dietary Group	Synaptosomes Na$^+$, K$^+$-ATPase at		Synaptic Plasma Membranes Na$^+$, K$^+$-ATPase at		
	3mM ATP	50μM ATP	3mM ATP	50μM ATP	lysoPl-PLC
Perilla	986± 105	63± 8	1073± 164	48± 13	8.7± 2.0
Safflower	928± 104	49± 8**	1152± 77	55± 7	9.7± 1.8

(n=6, nmol/min/mg protein)

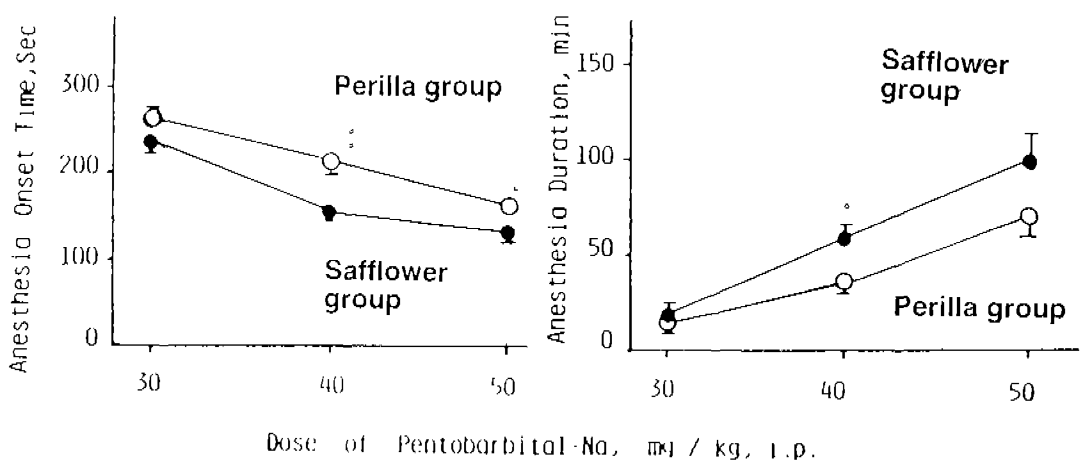

Fig. 13. Effect of dietary oils on pentobarbital-induced anesthesia.

New nutritional recommendations for the prevention of chronic, elderly diseases described above

Finally, I would like to come back to the new nutritional recommendations for the prevention of chronic, elderly diseases mentioned so far. Various foods can be classified mainly into three groups depending on the major consituent fatty acids(Fig. 15).

Saturated and monounsaturated fatty acids present abundantly in animal fats, olive oil and high-oleic vegetable oils are interpreted not to be so risky. They do not elevate plasma cholesterol after long-term feedings and they do not promote carcinogenesis so much. Therefore, these are condidered to be relatively safe energy sources.

Linoleic acid abundant in many types of vegetable oils and their products is an essential fatty acid. However, the required amount is very small and average people are ingesting several times more linoleic acid than the amount required. Excess intake of linoleic acid does not lower plasma cholesterol but results in over-production of arachidonate metabolites which is the major cause of western type cancers, thrombotic diseases, allergy and aging. Therefore, the intake of foods enriched with linoleic acid should be decreased. The amount of arachidonic acid in our food environment is relatively small(0.2~0.3g/d/capita).

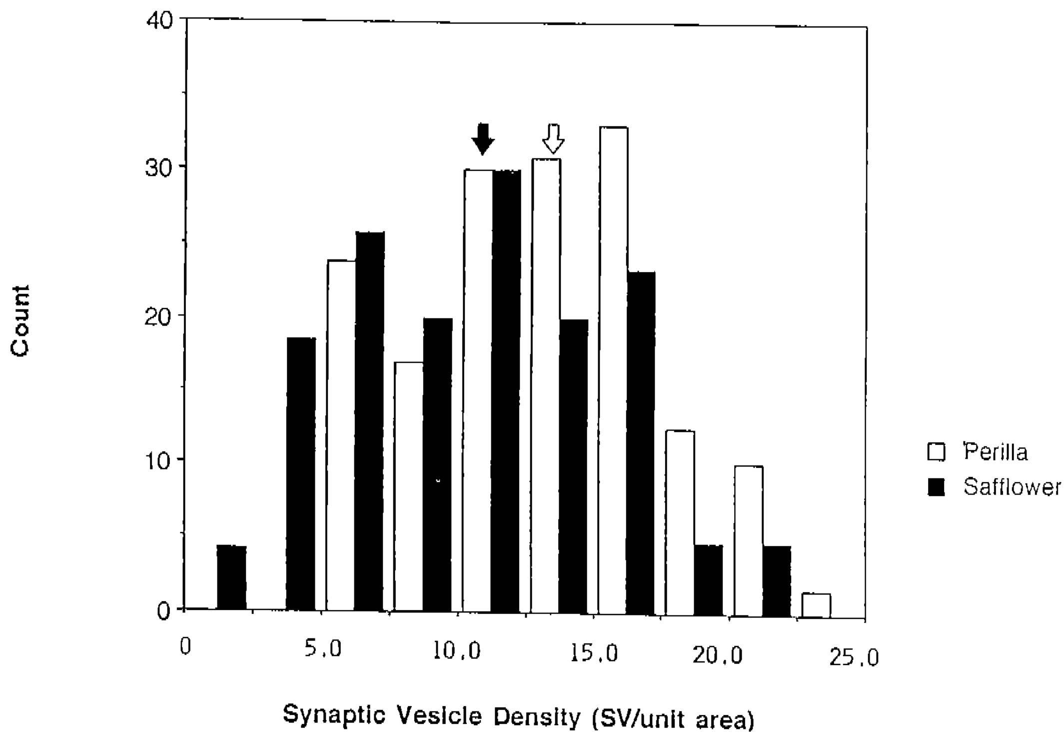

Fig. 14. Synaptic vesicle density in rat hippocampus CA1.

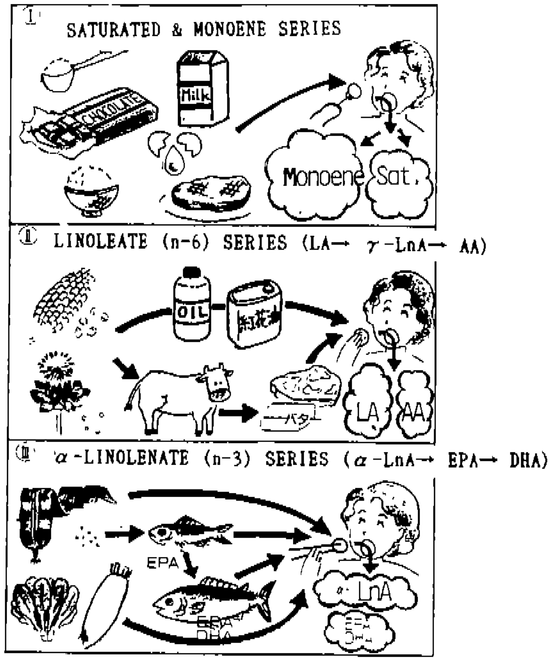

Fig. 15. Food chain of three fatty acid series.

α-Linolenic acid is also an essential fatty acid with a different physiological potency. EPA and DHA derived from α-linolenic acid can competitively suppress the side effects caused by excess linoleic acid. Therefore, the intake of vegetables and seafoods containing ω3 fatty acids should be increased in order to prevent chronic, elderly diseases noted so far.

The nutritional recommendations I presented today are much different from the current ones, but I believe that the available evidence is almost enough to ask nutritionists to change the current nutritional recommendations. Thank you very much for your attention(May 1, 1993 in Seoul).