—Review—

n-6/*n*-3 Ratio of Dietary Fatty Acids Rather Than Hypercholesterolemia As the Major Risk Factor for Atherosclerosis and Coronary Heart Disease

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Classic lipid nutrition for the prevention of chronic, elderly-onset diseases was apparently established before 1960, assuming that hypercholesterolemia is the major risk factor and that raising the polyunsaturated/saturated (P/S) ratio of dietary fatty acids is hypocholesterolemic. However, the hypocholesterolemic effect of linoleic acid (LA) was found to be transient. Furthermore, hypercholesterolemia itself is unlikely to be a serious risk factor for diseases in the elderly because serum cholesterol level is positively correlated with longevity. Instead, a high *n*-6/*n*-3 ratio of dietary fatty acids was found to increase thrombotic tendency, decrease peripheral blood flow and lead to persistent inflammation, which was proposed to be the major risk factor for atherosclerosis and related diseases. Based on animal experiments and epidemiological studies, we recommend a reduction in the intake of LA from a current value of >6 en% to half, and a reduced *n*-6/*n*-3 ratio from the current value of >4 to 2. Simply decreasing LA intake would produce the recommended *n*-6 and *n*-3 fatty acid balance in Japan due to the typical Japanese diet, but both decreasing the intake of LA and increasing that of *n*-3 fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), is necessary in Western industrialized countries for the effective prevention of atherosclerosis and related diseases, as well as of apoplexy, allergic hyper-reactivity and cancers typical in Western populations.

Key words —— atherosclerosis, inflammation, *n*-3 fatty acid, CHD, hypercholesterolemia

INTRODUCTION

Hypercholesterolemia had long been recognized as the major risk factor for atherosclerosis and related thrombotic diseases such as coronary heart disease and cerebral infarction. Dietary saturated fatty acids (S) were observed to be hypercholesterolemic compared with vegetable oils enriched with polyunsaturated fatty acid (P), essentially linoleic acid (LA). Based on these observations, classic recommendations for the prevention of these diseases were apparently established before 1960: the intake of cholesterol should be reduced and the P/S ratio of dietary fatty acids should be increased.[1,2] Basically, a decreased intake of animal fats was advised, whereas increased intake of margarine and high-linoleic acid (LA) vegetable oils was recommended. During the past 40 years, the average intake of animal fats in Japan increased 3-fold but the current level (~60 g/d) is still considerably below the levels of Western countries (~90 g/d). The average intake of LA has also increased to a 3-fold higher level in Japan and is comparable to those of Western countries. Accordingly, the percentage of LA among the total ingested fatty acids is significantly higher in Japan than in Western countries. Along with these changes in lipid nutrition, disease patterns have also been changing to those of Western countries.[3]

In this review, we summarize the evidence that suggests that increased dietary LA associated with elevated *n*-6/*n*-3 ratios of polyunsaturat-

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ed fatty acids is the major risk factor for cardiovascular and cerebrovascular diseases, as well as for Western type cancers and allergic hyperreactivity.[4] Understanding the metabolism of *n*-6 and *n*-3 fatty acids in our body is essential for interpreting a large number of experiments performed so far regarding the dietary factors in atherosclerosis and coronary heart disease (CHD).

1. Three Series of Fatty Acid Metabolism in Mammals and Variability of Tissue *n*-6 and *n*-3 Fatty Acid Compositions

Major dietary fatty acids can be classified mainly into three series, depending on their metabolism in mammals: the saturated and monounsaturated fatty acid series, the LA (18 : 2*n*-6) series and the αLNA (18 : 3*n*-3) series (Fig. 1).

Saturated fatty acids, as well as monounsaturated fatty acids, are synthesized in mammals from excess energy (carbohydrates and proteins) and are relatively enriched in animal foods (meats, dairy products) and in some vegetable oils. Oleic acid is desaturated and elongated to eicosatrienoic acid (20 : 3*n*-9) only when the supply of *n*-6 and *n*-3 fatty acids is restricted. LA is not synthesized in mammals but is synthesized in plants and is generally stored in large amounts in grains. When ingested, LA is desaturated and elongated to form γ-linolenic (18 : 3*n*-6, γLNA), dihomo-γ-linolenic (20 : 3*n*-6), arachidonic (20 : 4*n*-6, AA) and adrenic (22 : 4*n*-6) acid. Docosapentaenoic acid (22 : 5*n*-6) is synthesized from adrenic acid in significant amounts only under conditions of prolonged *n*-3 fatty acid deficiency. αLNA is also synthesized in plants and is relatively enriched in leaves (photosynthetic cells) but is not synthesized by mammals. When ingested, αLNA is desaturated, elongated and chain-shortened to form eicosapentaenoic (20 : 5*n*-3, EPA) and docosahexaenoic (22 : 6*n*-3, DHA) acid.[5-7] Although the retro-conversion of fatty acids, *e.g.* from DHA to EPA, occurs in mammals,[8-11] no inter-conversion among the three different series shown in Fig. 1 occurs in mammals. Nutritionally, it is important to note that different foods contain different proportions of the three series of fatty acids; vegetable oils are classified into four types based on their major component fatty acid (Fig. 1). Therefore, the proportions of the three series, particularly those of the *n*-6 and *n*-3 fatty acids in tissue lipids, change significantly, depending on the choice of foods. For example, the *n*-6/*n*-3 ratios of plasma lipids were 3.3 and 0.36 for Danes and Greenland Innuits, respectively.[1,2] LA level in breast milk easily changes from 9% to over 20%, depending

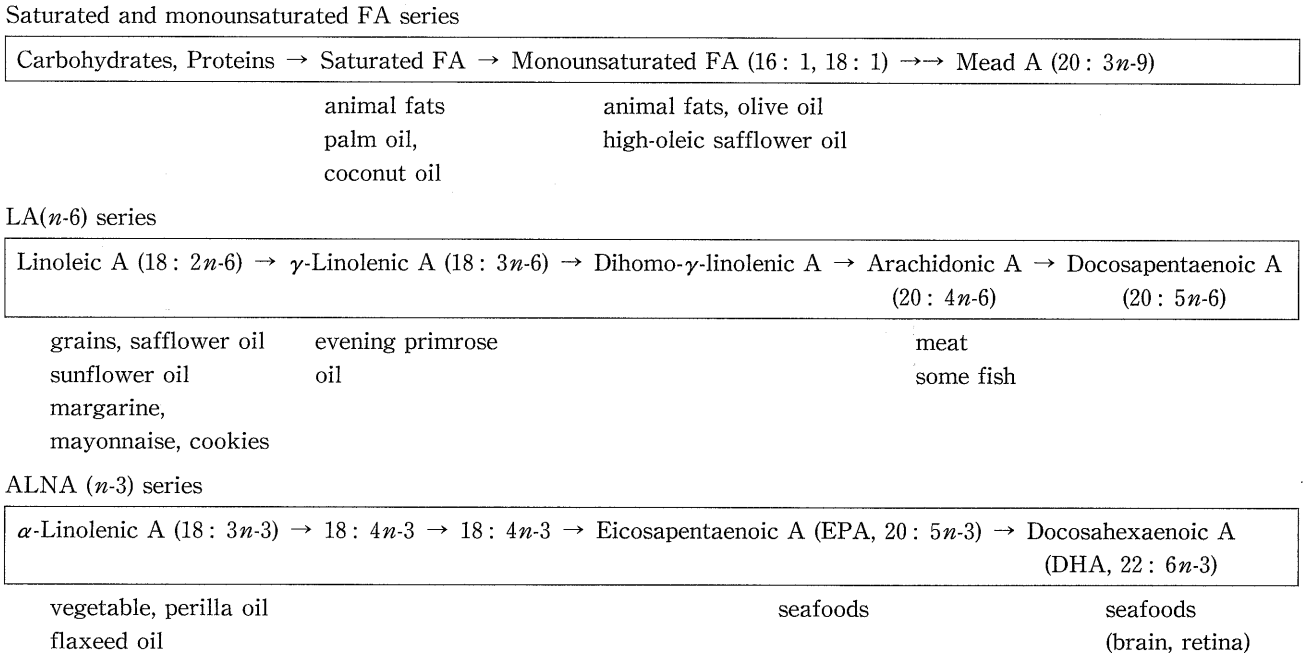

Fig. 1. The Three Series of Fatty Acid Metabolism in Mammals and Associated Food Chains

FA and A stand for fatty acid and acid, respectively. Fatty acids are designated by the carbon chain : the number of double bonds with the first double bond numbered from the methyl terminus as *n*-6 or *n*-3.

on mothers' choice of fats and oils.[13]

In the desaturation-elongation-chain shortening enzyme systems, *n*-3 fatty acids are the preferred substrates, followed by *n*-6 fatty acids and then monounsaturated fatty acids.[14,15] In the incorporation of these fatty acids into membrane phospholipids, *n*-6 and *n*-3 fatty acids are effective competitive inhibitors of each other in phospholipid acyltransferase systems.[16–19] The *n*-6 and *n*-3 fatty acids also compete in cyclooxygenase and lipoxygenase systems to form eicosanoids.[22–24] Saturated and monounsaturated fatty acids are relatively poor competitive inhibitors in the metabolism of LA to form eicosanoids through AA, although they probably compete effectively in β-oxidation steps. Because of the competitive nature of *n*-6 and *n*-3 fatty acids, not only the absolute amounts of *n*-6 and *n*-3 fatty acids, but also the *n*-6/*n*-3 ratio, are the important factors regulating the AA content in phospholipids and eicosanoid tone in tissues, which will be discussed in relation to diseases in Section 6.

2. LA Is Not Hypocholesterolemic Compared with Saturated and Monounsaturated Fatty Acids after Long-Term Feeding

Nutritional recommendations for the prevention of CHD were apparently established in Japan when empirical equations for the types of dietary fatty acids and plasma cholesterol levels were published 30 years ago,[1,2] indicating that dietary polyunsaturated fatty acids (in effect LA) lower and saturated fatty acids (S) raise plasma cholesterol levels. In fact, a 2-fold difference in plasma cholesterol levels was observed in college students by feeding them high-LA safflower oil and butter weekly.[25] Hence, "raising the P/S ratio of foods" became the major focus in Japan as part of the recommendations designed to prevent atherosclerosis and hypercholesterolemia-related elderly diseases. However, there was a major problem with interpreting such short-term clinical data in the context of long-term nutritional recommendations for the prevention of chronic, elderly diseases. Although a 2-fold difference was noted when safflower oil and butter were fed alternately for weeks, "raising the P/S ratio" by increasing the intake of LA-rich vegetable oil and decreasing the intake of animal fats over a period of years does not lower plasma cholesterol significantly.[26–28] Now we understand that dietary LA induces transient cholesterol accumulation in liver and arteries; however, subsequent metabolic responses appear to occur in the liver that result in the secretion of accumulated cholesterol as lipoproteins, so that no 2-fold difference in plasma cholesterol occurs over the longer term by raising the P/S ratio of foods.[29,30]

The results of a large-scale multiple risk factor intervention trial performed in the U.S.A. (MRFIT) on more than 12 000 males are worth careful examination.[31] In this trial, the major risk factors for atherosclerosis and CHD were assumed to be hypertension, hypercholesterolemia and smoking. Besides using hypotensive drugs and counselling to quit smoking, a reduction of the intake of saturated fatty acids and cholesterol was advised, as well as an increase in LA from *ca.* 7 en% on average to 10 en%. After 7 years, the diastolic blood pressure and plasma cholesterol decreased only by ca 4% and 2%, respectively, while the smoking rate decreased by almost 30%. However, the mortality from coronary heart disease, as well as all causes of mortality, was essentially unchanged. Among the hypertensive subjects with abnormal electrocardiograms, CHD mortality increased significantly by the intervention: 29.2 *vs.* 17.7/thousand in the control group, and mortality from all causes also increased, from 39.7 to 60.0/thousand. These results are consistent with our recent interpretation that raising the P/S ratio does not lower plasma cholesterol after long-term intervention, but could be risky for CHD.

Later, the MRFIT study was analyzed by Dolecek and Grandits[32] from the point of view of *n*-6 and *n*-3 fatty acids in the ingested foods. The results can be summarized as follows:

(a) intake of αLNA was negatively correlated with mortality from cerebrovascular disease (CVD) and all causes,

(b) intake of fish oil (EPA, 22 : 5*n*-3 and DHA) was negatively correlated with mortality from CHD, CVD and all causes,

(c) a high αLNA/LA ratio was negatively correlated with cancer mortality,

(d) total *n*-3/total *n*-6 ratio was negatively correlated with mortality from cancer, CVD and all causes,

(e) no significant association with LA intake on any mortality group was observed.

The above results (a)—(d), but not (e), are

consistent with our interpretation that a high n-6/n-3 ratio of dietary fatty acids is the major risk factor for atherosclerosis and CHD. Supporting the (a), αLNA in serum lipids was found to be negatively correlated with stroke.[33] The apparent failure to reveal a positive correlation between the intake of LA and CHD mortality in this trial (e) is interpreted by the tissue phospholipids being saturated with n-6 eicosanoid precursors, even in the control group ingesting average amounts of LA (n-6), but with very small amounts of competitive n-3 fatty acids in the U. S.A. subjects, which will be discussed more in detail in Section 6.

Muldoon *et al.*[26] summarized the results of several clinical trials involving nutritional intervention and/or hypocholesterolemic drugs. On average, a 10% decrease in plasma cholesterol and ~15% lower incidence of cardiac death in the treated group was noted, but these differences were not statistically significant. Instead, the mortality from cancer and violent death was found to be significantly higher in the treated group, and the overall death rates were not different between the control and treated group. Since these trials usually included other recommendations, such as increasing exercise and restricting caloric intake, the observed hypocholesterolemic effects on the order of ~10 % could well be brought about by such recommendations, other than the increase in P/S ratio.

Despite a failure to demonstrate the effectiveness of classic lipid nutrition, *i.e.*, limiting dietary cholesterol and raising the P/S ratio (high-LA vegetable oil/animal fat ratio), such recommendations have continued until very recently in all the industrialized countries, hoping that a longer-term follow-up trial would prove the effectiveness of such recommendations. However, a 15-years follow-up in Finland[27,28] revealed fatal features of such classic lipid nutrition. When typical, classic nutritional recommendations were made to decrease the intake of animal fats and high-cholesterol foods and to increase LA in the form of soft margarine, plasma cholesterol was not affected after 10 years. The incidence of cardiac and total death were not much different for up to 10 years but the mortality rates from CHD and all causes at 15 years were 2.4- and 1.4-fold higher in the intervention group than in the control group, respectively. Although hypotensive and hypocholesterolemic drugs were used for the initial 5 years, the proportion of subjects using these drugs was similarly low at 10 years, and the differences in mortalities began to increase significantly after 10 years. Therefore, we interpret that it was not the drugs used but the nutritional factors (*i.e.* increased LA) that increased the incidence of CHD and shortened the longevity.

In rodents, the hypocholesterolemic effect of dietary LA has been observed in young animals after relatively short feeding periods, but the difference tends to be smaller after longer feeding periods.[29,30] Dietary LA caused cholesterol to accumulate in the liver, but αLNA, EPA and DHA did not.[34,35] In aged mice and rats, no significant difference in plasma cholesterol levels was seen between the two groups fed high-LA safflower oil versus lard.[35] Thus, the age of the subjects and the feeding period, as well as the fat energy %, appear to be critical determinants of the effects of dietary fatty acids on plasma cholesterol. In monkeys, a shift from a basal diet (2 en% fat) to an atherogenic diet (40 en% fat) for a short period (8 weeks) resulted in ~2-fold increases in plasma cholesterol.[36] In a longer feeding study (2—5 years),[37] a butter diet group had 1.6-fold higher plasma cholesterol, while hepatic cholesterol was significantly lower as compared with a safflower oil fed-group. Medium-chain fatty acids (*e.g.* coconut oil) were hypercholesterolemic compared with LA-rich vegetable oil,[38] but palmitate, a major component of animal fats, was found to be as effective as LA.[39] When saturated fats and unsaturated fats were compared in baboons for 7—8 years (up to young adult), plasma cholesterol was only 1.13-fold higher in the saturated fat group. Importantly, atherosclerotic lesions tended to be fewer (0.49—1.1) in the saturated fat group than in the unsaturated fat group, although the difference was not statistically significant.[40]

Thus, relatively long-term feeding studies in monkeys have not supported the proposal that the major risk factor for atherosclerosis is dietary saturated fats which raise plasma cholesterol levels leading to atherosclerosis, or that high-LA vegetable oils are beneficial for the prevention of atherosclerosis. It is important to note that in aged rodents and humans, LA is not hypocholesterolemic as compared with animal fats after long-term feedings (>1/10 the life span).[28,35] The results of clinical studies on elderly diseases

based on relatively short-term feedings must be carefully evaluated, and the results should not be used as a basis for nutritional recommendations for the prevention of elderly-onset diseases.[25,28]

3. Hypercholesterolemia Is a Marker in Some Populations but May Not Be a Risk Factor for CHD — Those with Higher Cholesterol Values Survive Longer

A method to evaluate the extent of atherosclerosis was developed by Hasegawa and coworkers,[41] and pulse wave velocity was established to be positively correlated with the degree of atherogenesis. Pulse wave velocity increased along with aging, and was shown to decrease by the ethyl ester of EPA, an anti-atherogenic drug registered in Japan.[42] However, the pulse wave velocity was essentially constant when plotted as a function of plasma cholesterol level in more than 100 000 Japanese, indicating that hypercholesterolemia is not a risk factor for atherosclerosis, as judged by the pulse wave velocity.[41]

Recently, the results of a 25-years follow-up study on plasma cholesterol and CHD mortality in seven countries were summarized (Fig. 2).[43] In northern Europe and the U.S.A., the mortality from CHD increased along with increased plasma total cholesterol; the CHD mortality of the highest cholesterol group was ~2.3-fold higher than in the lowest group of the cholesterol quartile. However, such a relationship was not apparent in Japan; only a 1.13-fold difference was noted.

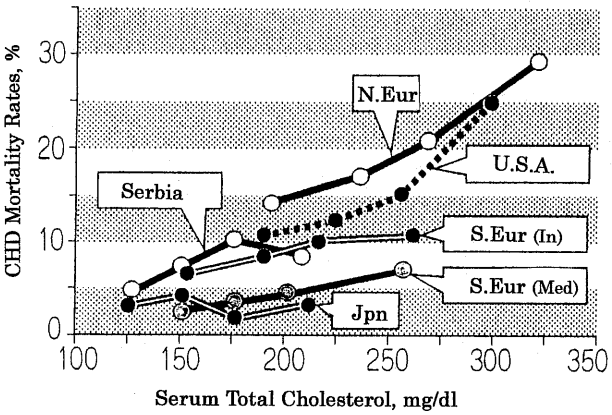

Fig. 2. Twenty-Five-Year Follow-Up of the Seven Countries Study

Redrawn from the data by Verschuren, W.M.M. *et al.*, 1995.[43] The cohort was divided into 6 groups: N.Eur (north Europe, Finland and Zutphen), S.Eur (In) (south Europe Inland, Rome, Crevalcore, Belgrade), S.Eur (Med) (south Europe Mediterranean area, Crete, Corfu, Montegiorgio, Dalmatian) and Jpn (Japan, Ushibuka and Tanusimaru).

Furthermore, CHD mortality at 210 mg/dl (5.45 mmol/liter) varied between 15% in northern Europe and 12% in the U.S.A. but was 4—5% in Japan. A similar difference was seen in comparing data from the Mediterranean area and northern Europe at 250 mg/dl cholesterol. Even the mortality of the lowest cholesterol quartile in northern Europe (193 mg/dl) was >4 fold higher than that of the highest quartile in Japan (212 mg/dl). Similarly, there was an 8-fold difference in CHD mortality between west Scotland and Catalonia in Spain, without a significant difference in blood cholesterol concentrations.[44] These observations clearly indicate that plasma cholesterol is one of the markers (indicators) of CHD in some countries, but that other factors can be more important. The observed positive correlation between plasma cholesterol and CHD mortality in some countries (Fig. 2) could well be the consequence of the increased intake of fats and oils with high n-6/n-3 ratios. It is now obvious that a high n-6/n-3 ratio of foods, but not antioxidants in red wines, is the major risk factor for CHD; Japanese and Mediterranean people are known to ingest relatively large amounts of seafood enriched with n-3 fatty acids, which will be explained in more detail in Section 5.

When the relationship between all causes of mortality and serum cholesterol was examined, we reached a definitely different conclusion from proposals based on classic lipid nutrition. The summary by Muldoon *et al.*[26] indicated that the classic nutritional recommendation, to raise the P/S ratio of foods, does not decrease the mortality from all causes. The 15-years Finland study[28] also indicated that such recommendations actually increase CHD mortality and shorten longevity. Similar observations have been reported for Japanese urban dwellers. For men and women aged 40 years and over in Toda City,[45,46] the 10-year mortality from all causes tended to be even lower in the highest plasma cholesterol group (207—419 mg/dl) than the lowest tertile group (90—176 mg/dl). For men aged 40—69 years old in Yao City, there was also a significant inverse association of serum cholesterol with all causes of mortality and with cancer mortality (Fig. 3), although the association was not statistically significant for women.[47]

Very similar conclusions have been reported in clinical studies performed in Western countries. The 30-year follow-up Framingham study

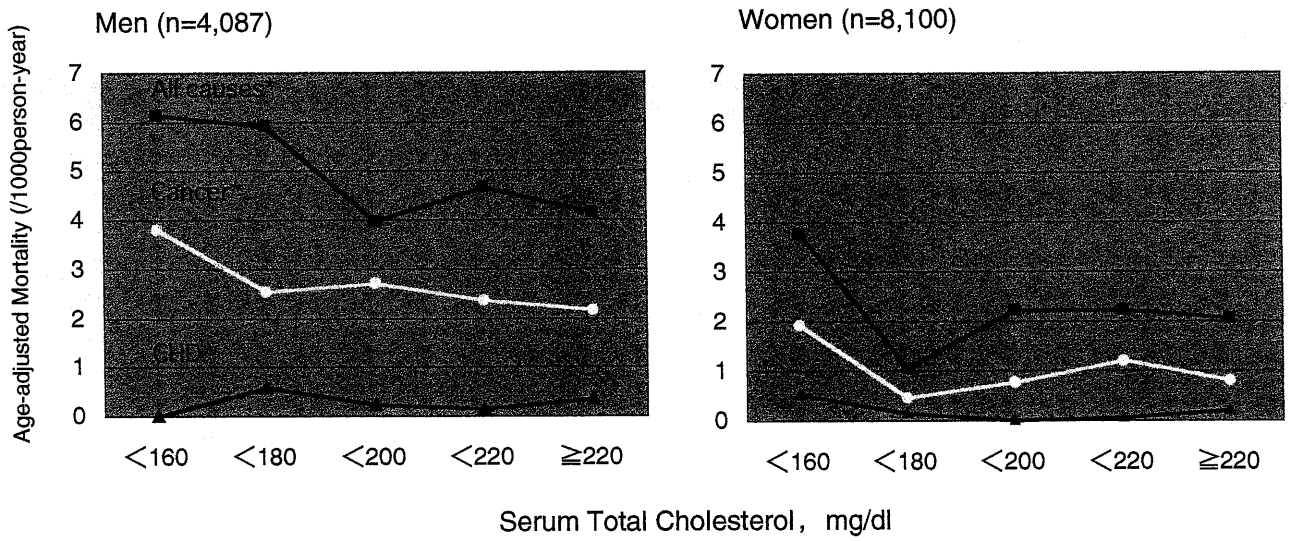

Fig. 3. The Age-Adjusted Mortality According to Serum Total Cholesterol Level

Yao citizens aged at 40—69 years with no history of stroke and coronary heart disease at baseline were followed on an average of 8.9 years (1975—1988). Data taken from Iso H. *et al.*, 1994.[47]

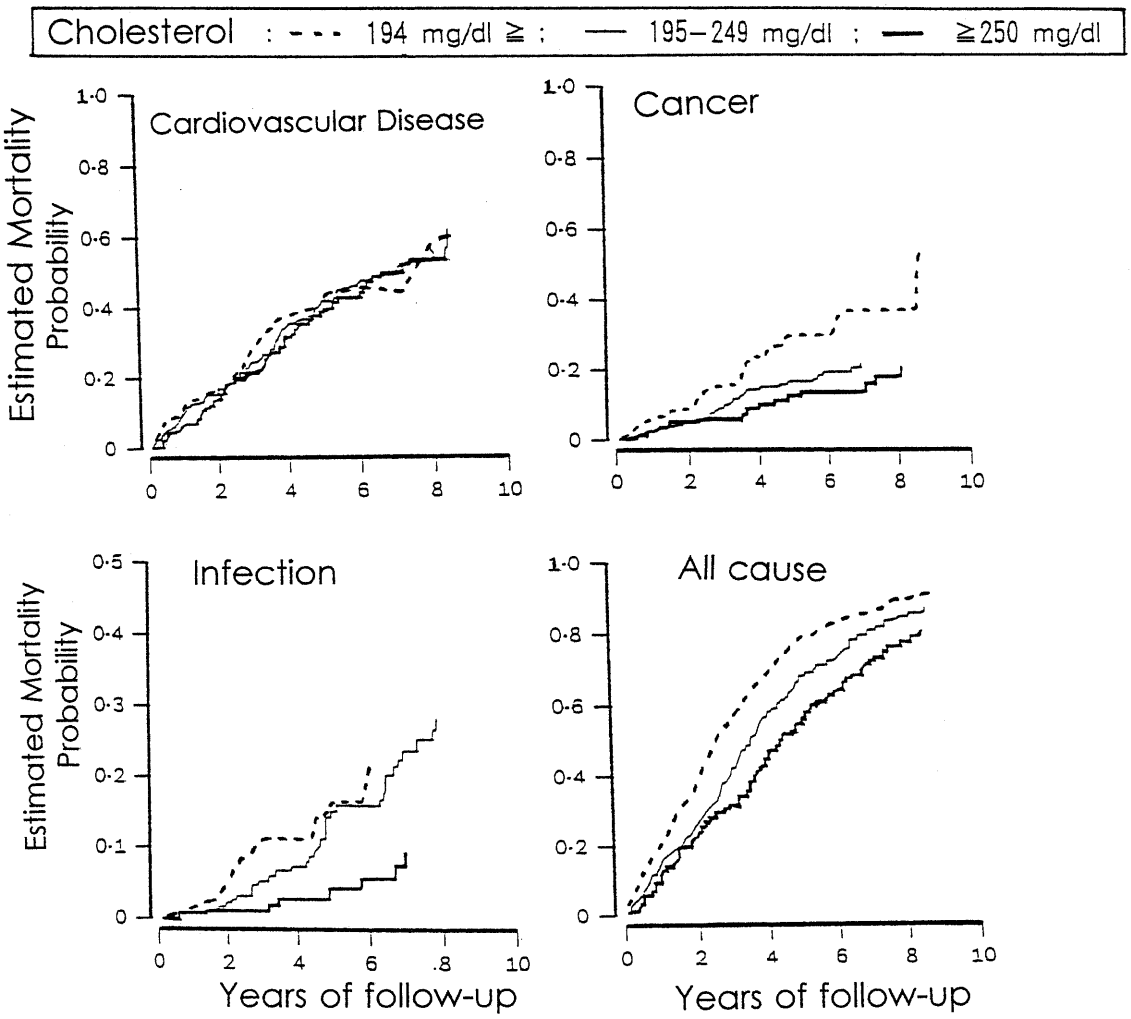

Fig. 4. Cumulative Mortality for Various Causes of Death

The influence of total cholesterol concentrations on specific and all causes of mortality in people aged 85 years and over was assessed ($n=724$). Data taken from Weberling-Rijinsburger A.W.E. *et al.*, 1997.[49]

revealed a very clear negative correlation of serum cholesterol with survival for men aged 30'.[48] However, the correlation was not clear in older groups (~55 years old), and no significant correlation was observed for women throughout the age groups. Thus, hypercholesterolemia was a marker of shorter survival only for young men, possibly for those with genetic factors such as familial hypercholesterolemia (FH). Even in this group with FH, it is not certain whether hypercholesterolemia itself is a major risk factor for shortened survival, because inadequate uptake of plasma low-density lipoprotein (LDL) into cells through LDL receptors is expected to cause many kinds of physiological disturbance other than hypercholesterolemia.

Among the oldest old followed for 10 years in the Netherlands (over 85 years old),[49] no significant correlation was found between serum cholesterol level and CHD mortality (Fig. 4). Instead, both cancer mortality and mortality from infectious diseases were higher when serum cholesterol was lower, and all causes of mortality were negatively correlated with serum cholesterol level. A similar conclusion has been obtained when people older than 70 years were followed for 10 years in the U.S.A.[50]

From these long-term follow-up studies performed in Japan and in Western countries, it is concluded that those with higher plasma cholesterol survive longer, possibly due to decreased cancer mortality, decreased mortality from infectious diseases, and/or decreased apoplexy; low serum cholesterol has been identified as a risk factor for hemorrhagic stroke. The negative correlation between serum cholesterol and cancer mortality observed in these studies could be due to decreased prenyl intermediates in cholesterol biosynthesis. Cholesterol and its oxidation products are negative feedback effectors of 3-hydroxy-3-methylglutaryl-Coenzyme A (HMG-CoA) reductase, the rate-limiting step of cholesterol synthesis. Inhibition of this enzyme results in decreased prenyl intermediates and farnesylated ras protein with cell-proliferative activity, thereby suppressing carcinogenesis (Fig. 5). In fact, HMG-CoA reductase inhibitor has been shown to suppress carcinogenesis.[51]

Thus, the risk of hypercholesterolemia must be reevaluated very carefully in terms of longevity and healthy life span as end points, and the critical plasma cholesterol level, above which hypocholesterolemic drugs are prescribed, should be carefully determined; we cannot find scientific evidence for the current cholesterol level value proposed by a group of the Japan Atherosclerosis Society (>220 mg/dl). It should be noted that the American Physicians' Association published significantly revised guidelines for measuring plasma cholesterol, already in 1996.[52]

4. Comparison of a Hypocholesterolemic Drug

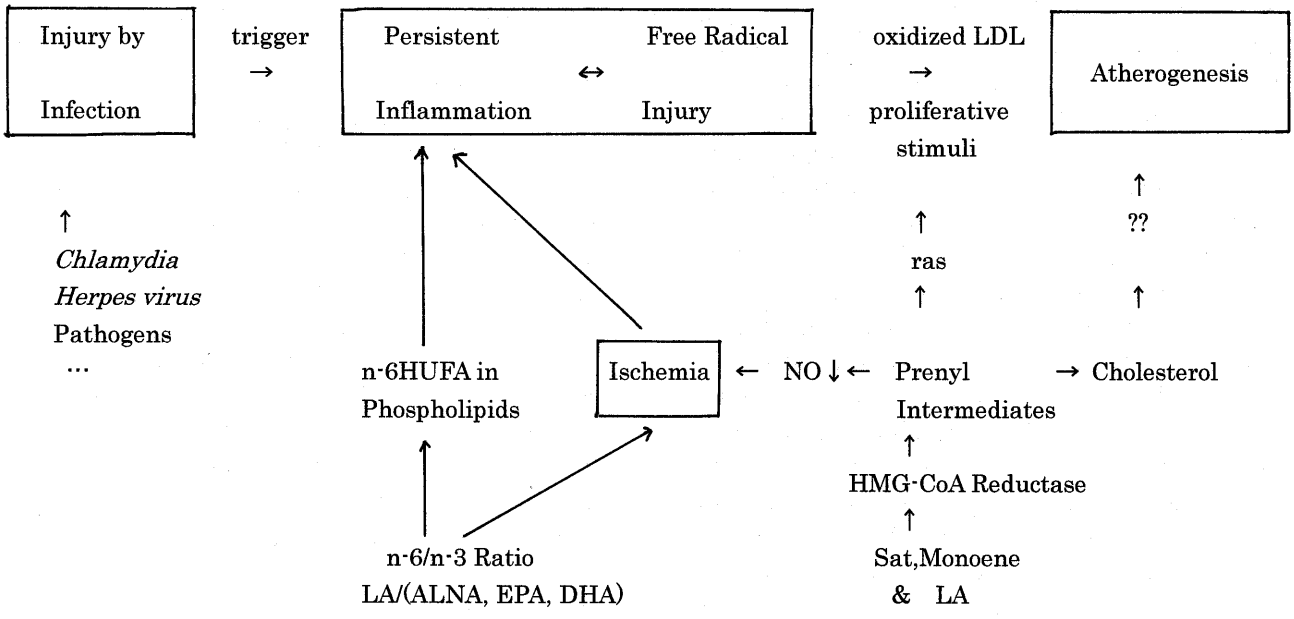

Fig. 5. Infection–Inflammation–Ischemia Theory of Atherogenesis
See text for details.

and Choice of Oils

Recently, a new type of effective hypocholesterolemic drug, HMG-CoA reductase inhibitor, was developed. This type of inhibitor is expected to lower not only plasma cholesterol but also the availability of prenyl intermediates required for the covalent modification of some proteins and for heme, dolichol and ubiquinone syntheses (Fig. 5).

Clinical side effects have so far been reported not to be serious, and a 30% risk reduction was observed in the secondary prevention of CHD.[53,54] However, the hypocholesterolemic activity of these drugs may not be the critical factor for reducing CHD mortality.[55] Inhibition of HMG-CoA reductase results in decreased prenyl intermediates such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate, leading to increased production of vasodilative NO and decreased activity of cell-proliferative ras protein, which would protect against the progression of atherosclerosis and CHD.[56,57] On the other hand, a possible decrease in heme and ubiquinone synthesis from prenyl intermediates may be related to their side effect, rhabdomyolysis.

Interestingly, the Lyon Diet Heart Study group reported in the same year (1994) that the choice of oils and foods was also effective for the secondary prevention of CHD; 70% risk reduction was achieved[58] compared with 30% risk reduction with an HMG-CoA reductase inhibitor.[53] Although, these two studies cannot be strictly compared, the importance of choosing appropriate vegetable oils was clearly established. Olive oil and rapeseed oil recommended in the Lyon Diet Heart Study are characterized by a relatively low LA (*n*-6) content and high αLNA (*n*-3) and oleic acid content, supporting the contention that an excess intake of *n*-6 fatty acid is a major risk factor; oleic acid is neutral and *n*-3 fatty acids suppress thrombotic diseases.[4,59,60] The seven-country study[47] also suggests that the oleic acid-rich Mediterranean diet is beneficial for CHD. However, it should be noted that the safety of olive oil and rapeseed oil in large quantities has not been established, at least in animal experiments (Section 7). These studies do not support the proposal that increasing the intake of large amounts of low-LA and high-oleic vegetable oils (olive oil, rapeseed oil and others) is beneficial for the prevention of thrombotic diseases, but rather, we emphasize the importance of "decreasing LA intake" while taking a significant amount of *n*-3 fatty acids from seafood and vegetables.

The classic lipid hypothesis that "dietary saturated fats increase serum cholesterol, leading to atherosclerosis and thrombosis and thereby increase CHD mortality" was recently reviewed critically by Gurr[61] and us.[4] We conclude that the major risk factor for atherosclerosis and CHD is a high *n*-6/*n*-3 ratio of dietary fatty acids, and that hypercholesterolemia is a marker for CHD in those populations whose diets include relatively small amounts of *n*-3 fatty acids from seafood and large amounts of saturated and monounsaturated fatty acids and LA.

5. Fatty Acid Balance Affecting the Process of Atherogenesis and CHD

Age- and sex-adjusted morbidity from thrombotic diseases in Innuits (native Greenlanders) was less than 1/10th of that seen in Danes, despite the fact that Innuits consumed a large amount of animal fat (saturated and monounsaturated fatty acids in ~30 en%) and the intake of cholesterol was almost twice that of Danes.[12,62,63] Obviously, for Innuits, dietary animal fats and cholesterol were not the major risk factors for thrombotic diseases. Similarly, hypercholesterolemia was not associated with mortality of all causes or CHD mortality, as described above. The mechanisms by which dietary fish oils (EPA and DHA) suppress thrombotic tendencies and improve hemodynamics have been reviewed by many groups,[64–81] and we understand the mechanisms to be as follows.

(a) Increased LA and AA raise the thromboxane (TX) A_2/prostaglandin (PG) I_2 ratio and thrombotic tendency[82–85]; (b) *n*-3 fatty acids competitively inhibit LA metabolism, suppressing the overproduction of AA metabolites (Sections 1 and 6); (c) EPA is a relatively poor substrate for cyclooxygenases (COX 1 and 2), and the thrombotic activity of TXA_3 from EPA is much less than that of TXA_2, while PGI_3 is as effective as PGI_2 in inhibiting platelet aggregation[67–73,82–85]; (d) *n*-3 fatty acids decrease blood viscosity and pulse wave velocity of the aorta, while increasing erythrocyte deformability[86–90]; (e) one *n*-3 fatty acid (22 : 5) stimulates the chemotaxis of endothelial cells while suppressing that of smooth muscle cells, a process presumed to be involved in atherogenesis[91]; (f) *n*-3 fatty

acids suppress the production of inflammatory cytokines and neutrophil adhesiveness[92-96]; (g) *n*-3 fatty acids suppress the production of platelet-derived growth factor (PDGF) while stimulating the production of endothelial cell-derived relaxation factor (EDRF)[97,98]; (h) *n*-3 fatty acids suppress free radical injury involving the production of oxidized LDL and foam cell formation (Section 8); (i) *n*-3 fatty acids inhibit cardiac arrythmia[83,99,100]; (j) epoxygenase products of EPA and DHA inhibit platelet aggregation[101]; and (k) EPA enhances α-agonist-induced production of ATP and adenosine, both which have hypotensive activities.[102]

Fish oils have been shown to suppress restenosis after angioplasticity.[103,104] Many intervention trials have shown the effectiveness of fish oil supplements on CHD,[105-110] indicating that dietary LA and the accompanying overproduction of AA metabolites and its associated persistent inflammation are the major risk factors for CHD. Although negative results have also been reported,[111,112] supplementing several grams of *n*-3 fatty acids while ingesting ~20 grams of LA daily for relatively short periods would not be effective in lowering the tissue AA/EPA ratio, as will be discussed in the next section.

6. Failure to Reveal the Causal Relationship between LA Intake and CHD

Because *n*-6 and *n*-3 fatty acids compete with each other at many steps of their metabolism, not only their absolute amounts but also the *n*-6/*n*-3 ratio is a critical factor influencing inflammatory tone related to arachidonic acid and derived inflammatory mediators. The effectiveness of increasing the intake of *n*-3 fatty acids for suppressing CHD means that an excessive intake of *n*-6 fatty acids, essentially LA in our current food environment, is a risk factor for CHD. In fact, the intake of LA and the *n*-6/*n*-3 ratio of ingested fats were much higher in Danes than in Innuits in a well-known epidemiological study. In industrialized countries, the intake of LA increased to roughly 3-fold higher levels in the past several decades, and the *n*-6/*n*-3 ratio of human foods increased from more or less 1 in the hunting and gathering age to 4—5 in Japan and 7 in the U.S.A., for example (Fig. 7).[3,113] Although the age-adjusted mortality from CHD has tended to decrease these days in Japan, this is mainly due to an improved medicare system; it does not

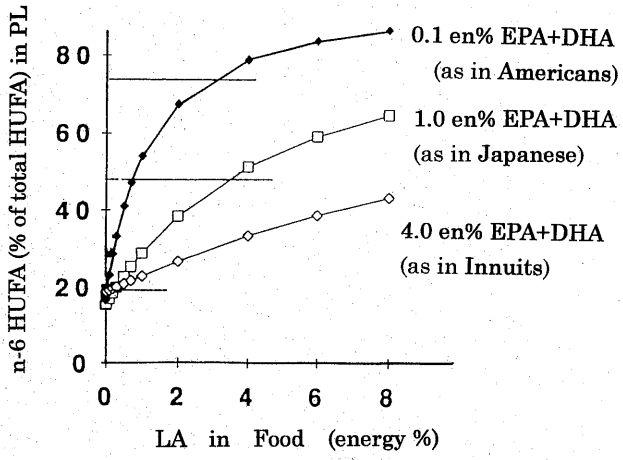

Fig. 6. Competitive Aspect of *n*-6 and *n*-3 Fatty Acids Affecting the Proportion of *n*-6 Eicosanoid Precursors in Phospholipids That Is a Marker of Eicosanoid Tone and Inflammatory Tone

The data from Lands *et al.*[114] are plotted as a function of *n*-6 HUFA (% of total HUFA) in plasma phospholipids (ordinate), and their precursor LA (*n*-6) in the diet (abscissa) and their competitive effector *n*-3 fatty acids (EPA, DHA).

appear that the age-adjusted morbidity is also decreasing in Japan.

In analysis of the MRFIT study, no positive correlation was observed between LA intake and CHD mortality, as described above (Section 2). However, this is not contradictory to our proposal that an excessive intake of LA is a cause of CHD. A more proximate marker for CHD is "*n*-6 eicosanoid precursors in phospholipids" such as AA and dihomo-γ-linolenic acid derived from LA, which are called *n*-6 HUFA (highly unsaturated fatty acids). The *n*-6 HUFA in cellular phospholipids (expressed as % of the total HUFA) is a function of both their precursor, LA and their competitive effector, *n*-3 fatty acids, in the diet, as shown by Lands' empirical equation (Fig. 6).[114,115]

Decreasing the intake of LA alone without competitive amounts of *n*-3 fatty acids in foods, as in the average American diet, is quite ineffective in lowering the levels of *n*-6 eicosanoid precursors in plasma phospholipids, because saturated and monounsaturated fatty acids are relatively poor competitive inhibitors of *n*-6 fatty acid incorporation into phospholipids. In the presence of competitive amounts of *n*-3 fatty acids found in traditional Japanese and Mediterranean foods, decreasing the intake of LA is expected to effectively decrease the amounts of *n*-6 HUFA in phospholipids. These competitive aspects revealed in animal experiments have also

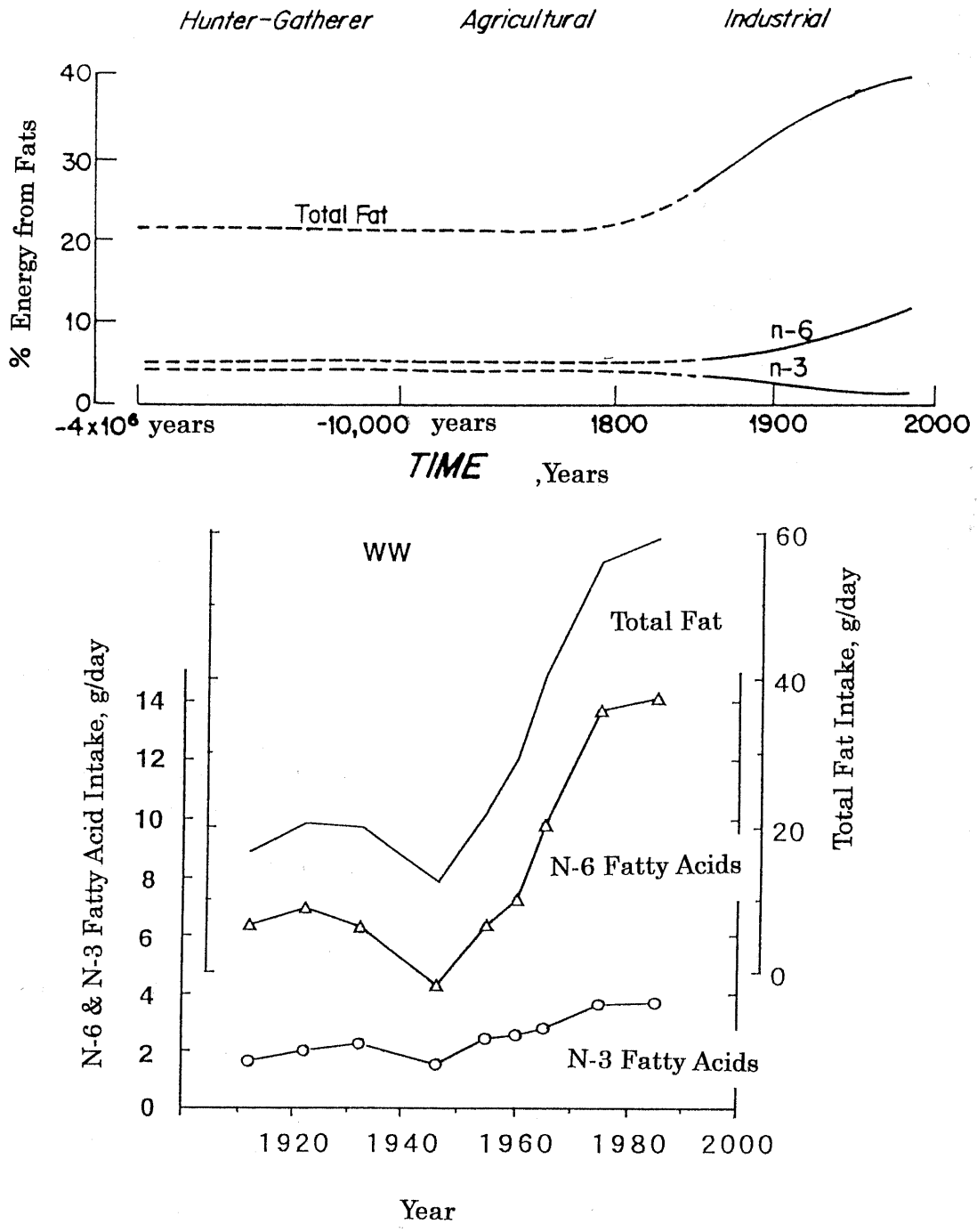

Fig. 7. Trends of *n*-6 and *n*-3 Fatty Acid Intake

The upper panel shows a hypothetical scheme of the relative percentages of fat and different fatty acid families in human nutrition extrapolated from cross-sectional analysis of contemporary hunter-gatherer populations and from longitudinal observations and their putative changes during the preceding 100 years. Modified from the data by Leaf *et al.*, 1987.[3,113] The lower panel shows the trend of fatty acid intake in the past 80 years in Japan.

been demonstrated clinically.[116–118]

The apparent failure to reveal a causal relationship between LA intake and CHD mortality in the MRFIT study (U.S.A.) could be interpreted as their cellular phospholipids being saturated with *n*-6 HUFA, regardless of the high or low intake of LA (4—10 en%), because the amounts of competitive *n*-3 fatty acids were very low in their diets. It is emphasized that both a decrease in the intake of LA and an increase in the intake of *n*-3 fatty acids, particularly EPA and DHA, are necessary in order to decrease the *n*-6 HUFA content in phospholipids and thus decrease CHD mortality as well as other diseases related to the

excessive intake of LA and elevated n-6/n-3 ratios.[4]

7. Apoplexy Is Not Enhanced but Suppressed by n-3 Fatty Acids —Problems of Minor Components in Some Vegetable Oils—

Morbidity due to apoplexy was higher in Innuits than in Danes,[62] which raised the possibility that an excess intake of n-3 fatty acids might increase the tendency to bleed and thereby increase the incidence of apoplexy. However, cerebral bleeding is mainly a problem associated with the strength of blood vessels and high blood pressure, and does not appear to be due to a decreased thrombotic tendency, decreased blood pressure or improved hemodynamics resulting from the intake of n-3 fatty acids. Physicians have encountered difficulties in clinical trials which attempt to demonstrate the expected decreases in platelet aggregability and thrombotic tendency by supplementing diets with fish oil. That is, supplementation with fish oil in clinical trials does not appear to yield levels of n-3 fatty acids which cause adverse effects.[2,119] In fact, the ISSFAL (International Society for Study on Fatty Acids and Lipids) noted that "although n-3 fatty acid supplementation lengthens bleeding times, there is no evidence to suggest that it may cause clinical bleeding episodes."[2,120]

In the MRFIT analysis, cerebrovascular disease mortality was negatively correlated with the intake of αLNA, intake of fish oil (EPA, 22 : 5n-3 and DHA) and total n-3/total n-6 ratio, as described above. Vitamin C is a cofactor for hydroxylases required for the synthesis of collagen and elastin, components important for the integrity of blood vessels. Innuits used to derive vitamin C from uncooked seals and fish. However, Western culture may have affected these eating habits such that Innuits, by cooking their meats and avoiding the vitamin C-rich parts of their foods, decreased their intake of vitamin C. In fact, native Greenlanders are said to want to be called "Innuits" rather than "Eskimos" because the term "Eskimo" means "people who eat raw meat". Thus, the intake of n-3 fatty acids does not increase but rather suppresses apoplexy.

Stroke-prone spontaneously hypertensive (SHRSP) rats derived from SHR develop the highest blood pressure among available rat strains and frequently die of apoplexy, particularly when administered salt solutions.[121–123] The risk factors for apoplexy in this animal model have been found to be high-salt and low-protein diets. The incidence of apoplexy was once very high in certain areas of Japan where grains and salted vegetables were the major foods, but this situation has changed significantly following recommendations to decrease the intake of salt and increase that of protein (meats).

Using the SHRSP animal model, we have shown that the survival time is significantly longer (by *ca.* 10%) in the groups fed DHA-rich fish oil, perilla oil or flaxseed oil as compared the groups fed soybean oil or safflower oil.[124] Thus, in animal experiments, a relatively large amount (10 wt%) of these n-3 rich oils was found to suppress apoplexy,[125] possibly as a consequence of decreased blood pressure (by ~10%) and improved hemodynamics.[126–131] αLNA as well as fish oil has been reported to be hypotensive in humans[132] and in SHR rats.[125] Similarly, in SHRSP rats, feeding cholesterol at 1% of the diet increased the plasma cholesterol level 2-fold, suppressed the onset of apoplexy and prolonged the survival time.[133] Thus, a low n-6/n-3 ratio of dietary fatty acids is a beneficial factor for an animal model of apoplexy, SHRSP rats.

We have encountered another problem in some vegetable oils. Rapeseed oil (Canola type) was considered a good choice of cooking oil among the major vegetable oils produced, because it contains ~55% oleic acid, ~25% LA and ~8% αLNA (n-6/n-3=~3). The old type of rapeseed oil contained erucic acid (22 : 1n-9) and thyrotoxic sulfur compounds derived from glucosinolates, but a selected strain (Canola) has much less erucic acid and sulfur compounds. The safety of the double-low rapeseed oil (Canola) appeared to have been established.[134] However, cardiac lipidosis was observed in SD rats fed this double-low rapeseed oil,[135] although this adverse effect was not seen in pigs. Later, the rapeseed oil-induced lipidosis was ascribed to its unique fatty acid composition; high in oleic and αLNA but low in saturated fatty acids.[136] Because most people consume an abundance of saturated fatty acids from other sources, the observed effect of the double-low rapeseed oil on cardiac lipidosis has been overlooked in human nutrition, although rapeseed meal appears to induce hepatic hemorrhage in hen.[137]

Using the double-low rapeseed oil that was designed for human use, we have noted that it

shortens the survival time of SHRSP rats by > 40% as compared with fish oil, perilla oil, flaxseed oil and soybean oil at 10 wt% of diet.[138] Rapeseed oil diluted 4-fold with soybean oil shortened the survival time significantly. Evening primrose oil, high-oleic safflower oil, high-oleic sunflower oil, partially hydrogenated rapeseed oil, partially hydrogenated soybean oil and olive oil were found to shorten the survival times of SHRSP rats comparably to that found with rapeseed oil, but lard, sesame oil, a microbial oil and high-LA safflower oil, as well as soybean oil, were relatively safe in this animal model. Free fatty acid fractions obtained after the lipase hydrolysis of rapeseed oil and high-oleic safflower oil did not exhibit such activity.[139] Therefore, we presume that some vegetable oils contain a factor other than fatty acids which is toxic to SHRSP rats (stroke-stimulating factor or survival-time shortening factor, SSF).

Recently, phytosterols were proposed to form a part of SSF, but our observations do not support such a proposal. Rapeseed oil and partially hydrogenated soybean oil decreased platelet counts and stimulated proteinuria, which was accompanied by an increased expression of renin, transforming growth factor (TGF) β and fibronectin genes. Lesions in renal blood vessels and glomeruli have also been noted in SHRSP rats.[140]

The concept that some vegetable oils contain physiologically active components other than fatty acids is supported by observations that olive oil stimulated, but high-oleic safflower oil suppressed, colorectal carcinogenesis,[141,142] and that mice fed rapeseed oil showed unusual behavioral patterns compared with those fed other oils, and that these differences cannot be accounted for by differences in LA/αLNA ratios.[143] In piglets, decreased platelet counts and unusual sensitivity to iron injection were noted in the rapeseed oil-fed group compared with the soybean oil-fed group. Increased lung and liver carcinogenesis observed in non-smoking Chinese women may also be related to rapeseed oil.[144,145]

Longevity has not been commonly used as an end point for the nutritional assessment of vegetable oils, but now we have to face the problems of minor components of fats and oils. Because of their hydrophobic nature, these factors might accumulate in the body through the food chain, and influence brain function. In moving into the 21st century, the collaboration of scientists is requested to identify presumed unfavorable minor components in vegetable oils and find a means to improve the safety of these oils.

8. Infection–Inflammation–Ischemia Theory of Atherosclerosis and CHD

Oxidized LDL is detected in the circulation and its uptake by macrophages is recognized as an early event of atherogenesis and the progress of CHD.[146] However, *in vivo* situations demonstrating how oxidized LDL increases in the circulation remain to be defined. A simple explanation has been provided by the so-called free radical theory or lipid peroxide theory of atherosclerosis; free radicals and/or reactive oxygens produced in cells are believed to attack polyunsaturated fatty acids to form lipid peroxides, which then decompose in chain reactions to form more free radicals, and the resulting deluge of free radicals injure cellular components accelerating the processes of atherogenesis. In this theory, free radical injury and lipid peroxide injury are used almost synonymously. This kind of theory is often extended in nutrition circles to imply that the ingestion of large amounts of easily autoxidizable lipids, such as *n*-3 fatty acids, leads to the accumulation of lipid peroxides in tissues. In fact, the original free radical theory of aging by Harman and others came from results comparing dietary fats and oils with different autoxidizability.[147–149] However, many lines of evidence indicate that long-term feeding of *n*-3 fatty acids as compared with *n*-6 fatty acids suppresses so-called free radical diseases including atherogenesis-CHD, aging and carcinogenesis, indicating that lipid peroxidation *in vitro* does not correspond to the *in vivo* setting.[4]

Fish oil supplementation usually results in elevated plasma TBA-RS (thiobarbituric acid-reacting substances) values and decreased vitamin E levels. However, plasma lipid peroxides account for only a couple of molecules among tens of thousands of fatty acids in plasma when measured by a chemiluminescence method.[150] Interestingly, the plasma, liver and brain lipid peroxide levels determined by the chemiluminescence method were not proportional to the autoxidizabilities of fats and oils in the diets given to rats, (*e.g.* the plasma lipid peroxide level was higher in the LA-rich safflower oil group than in the αLNA-rich perilla oil group).[151,152] Lipid

peroxide levels in erythrocytes from fish oil-fed rats tended to be lower than in the safflower oil-fed group when measured by a chemiluminescence method.[153] Furthermore, autoxidizability in an air-atmosphere appears to be quite different from that in water; DHA and EPA are more stable than LA when suspended in aqueous media.[154]

On the other hand, the decrease in plasma vitamin E levels after fish oil supplementation is probably not due solely to the consumption of vitamin E through peroxidation reactions, but also to decreased triacylglycerol levels[155-157]; the plasma α-tocopherol/lipid ratios appear to be maintained at a relatively constant value by a mechanism in liver which selects only the α-isomer of tocopherols to be secreted into plasma as lipoproteins.[158]

In animal experiments, EPA ethyl ester was found to render LDL less susceptible to oxidative modification[159] and to inhibit cholesterol ester accumulation in macrophages.[160] Despite the very high susceptibility to autoxidation in air of EPA and DHA, fish oils containing these fatty acids have been established to be beneficial in suppressing atherosclerosis and thrombotic diseases (Section 5). We interpret that these fatty acids serve as scavengers of free radicals and reactive oxygen species rather than serving as free radical proliferators. Several lines of *in vivo* evidence which support this interpretation are summarized as follows: (a) pre-treatment of cats with fish oil suppressed the ischemia-induced loss of brain function (righting reflex)[161]; (b) free radical injury induced in an ischemia-reperfusion model was suppressed by pre-treatment with EPA[162-164]; (c) administration of EPA ethyl ester lowered plasma lipid peroxide levels and suppressed LDL oxidation and foam cell formation in macrophages[159,150]; (d) free radical (and reactive oxygen) injury (thrombosis) induced in rats by perfusion with rose bengal and laser irradiation was suppressed by pre-treatment with DHA[165]; (e) native Greenlanders had much less atherosclerotic plaque and had a lower incidence of thrombotic diseases than Danes, and many intervention trials have established that fish oil supplementation is beneficial in the prevention of thrombotic diseases (Section 5); (f) a long-term feeding of rats and mice with perilla oil as compared with safflower oil lowered tissue lipid peroxide levels and suppressed thrombotic tendency[150,151,166]; (g) increasing the intake of high-LA margarine and decreasing the intake of animal fats resulted in an increased incidence of thrombotic diseases[28]; (h) decreasing the intake of LA and increasing the intake of αLNA and oleic acid were effective for the secondary prevention of coronary heart diseases[58]; and (i) EPA administration decreased platelet lipid peroxides produced by an SH reagent.[167]

Although not directly related to atherosclerosis, the following observations are also inconsistent with the so-called lipid peroxide theory of free radical diseases: (a) bleomycin-induced pulmonary fibrosis, a presumed free radical injury, was suppressed by fish oil administration[168,169]; (b) diets containing lipid peroxides decreased the levels of hepatic precancerous cells[170]; (c) in CCl4-induced hepatitis, an injury presumed to be caused by the CCl3 radical, the proportion of DHA increased and that of AA decreased[171], indicating that eicosanoid formation from AA is involved, rather than a free radical attack on DHA.[172] In addition, the extent of fatty liver was less in the fish oil group than in the safflower oil group[171]; and (d) oxygen toxicity in the lung was suppressed by supplementation with fish oil or polyunsaturated fatty acids.[173-175]

Free radicals produced in cells easily react with polyunsaturated fatty acids to form lipid radicals (L・), which react with oxygen to form peroxy radicals (LOO・). LOO・ has a very short life and is typically converted to hydroperoxy fatty acid (LOOH) by a reaction with vitamin E present in the membranes. Oxidized vitamin E is reduced in the presence of vitamin C, the oxidized vitamin C is reduced by consuming glutathione, and the oxidized glutathione is reduced by NADPH, the formation of which is regulated in living cells. There are other coupling systems available to maintain vitamins E and C in their active reduced forms *in vivo*.[176,177] Even *in vitro*, the chain reaction of lipid peroxidation does not proceed until all the vitamins E and C are oxidized.[177] Lipid hydroperoxides (LOOH) are relatively stable and do not easily undergo spontaneous breakdown to form radicals such as LO・, HO・ and L・; the equilibrium favors the formation of LOOH, and such free radicals are only formed in the presence of coupling oxidation reactions, *e.g.*, $Fe^{++} \rightarrow Fe^{+++}$. Glutathione peroxidases are present in various tissues to reduce LOOH to more stable hydroxy fatty acids (LOH),

which are then β-oxidized. In the presence of these coupling systems in living cells, fatty acids which are reactive with free radicals can serve as free radical scavengers. However, *n*-3 fatty acids would serve as free radical proliferators under the conditions of vitamins C and E -deficiency and/or in the presence of impaired coupling systems.

We accept that free radicals can injure cellular components under pathological conditions,[146,164,165] leading to accelerated atherogenesis. However, *in vivo* experiments indicate that long-term feeding of *n*-3 fatty acids as compared with *n*-6 fatty acid suppresses such diseases, despite the fact that *n*-3 fatty acids are more susceptible to autoxidation in an air atmosphere than the corresponding *n*-6 fatty acids.

We hypothesize that free radicals and reactive oxygen species are produced *in vivo* mainly by hypoxia (ischemia) and/or inflammation (Fig. 5). Persistent ischemia and inflammation result in the overproduction of free radicals and reactive oxygen species, which injure cellular components leading to the so-called free radical diseases. Excessive intake of *n*-6 fatty acids, essentially LA in our food supply, and an elevated *n*-6/*n*-3 ratio of tissue lipids accelerate these events through the overproduction of inflammatory mediators (eicosanoids and platelet-activating factor) and unbalanced production of eicosanoids from AA (*e.g.* decreased PGI2/TXA2 ratios). *n*-3 Fatty acids increase erythrocyte deformability, decrease blood viscosity, decrease leukocyte adhesiveness, decrease thrombotic tendency and thereby decrease ischemic tendency and inflammatory tone. *n*-3 Fatty acids would also be able to trap free radicals more effectively than *n*-6 fatty acids in the presence of antioxidant vitamins and their associated coupling systems.

Serum cholesterol levels are elevated by dietary animal fats and high LA vegetable oils as compared with oils enriched with *n*-3 fatty acids. Elevated prenyl intermediates (geranylgeranyl pyrophosphate) by ingesting animal fats and high-LA oils appear to suppress the formation of NO, a vasodilative mediator leading to increased ischemic tone.[56] On the other hand, farnesylated ras protein may serve as a proliferative stimulus enhancing atherogenetic processes.[57] Thus, saturates, monounsaturates and LA would also serve to accelerate atherogenesis, while HMG-CoA reductase inhibitors suppress atherogenesis through these pathways. It is very likely that hypercholesterolemia itself serves to lower prenyl intermediates by a feedback control mechanism and does not stimulate atherogenesis through these pathways.

Recently, Chlamidia, Herpes virus and other pathogens have been considered to eventually injure blood vessels.[178–180] Inflammatory reactions would follow to repair the injury, and inflammatory lipid mediators are necessary for the repair processes. However, we interpret that elevated *n*-6/*n*-3 ratios would tend to lead to persistent inflammation after the blood vessel injury. Inflammatory cells produce active oxygens and free radicals to attack LDL and form oxidized LDL. Thus, we interpret that an elevated *n*-6/*n*-3 fatty acid ratio of cellular lipids, that is an elevated proportion of *n*-6 HUFA in phospholipids (Figs. 5 and 6), leads to ① persistency in inflammatory reactions triggered by infection, and ② elevation of ischemic tendency through altered hemodynamics. Inflammation leads to ischemia and *vice versa*, and inflammatory cells as well as ischemic cells produce reactive oxygen species to increase oxidized LDL and thereby to stimulate atherosclerosis-CHD. Animal fats would serve to accelerate atherogenesis through the elevation of prenyl intermediates, but hypercholesterolemia itself would be suppressive for these processes. Although some revisions would be required for the schemes shown in Fig. 5, the effectiveness of lowering *n*-6/*n*-3 fatty acid ratios in food for the suppression of atherosclerosis and CHD has been well established clinically, as described above (Sections 5 and 6).

9. Recommended *n*-6 and *n*-3 Fatty Acid Balance for the Prevention of Elderly-Onset Diseases

After World War II, grain production was increased by farm mechanization beyond the demand for human use in some industrialized countries, and excess grains were fed to domestic animals after removal of their oils. Along with these industrial changes, the intake of meats and vegetable oils increased. The trend in Japanese fatty acid intake is common to most industrialized countries, although the trend in Japan probably trails by a couple of decades those of Western countries, and the Japanese continue to

consume more *n*-3 fatty acids from seafood.[1)]

Two major concerns have led to the widespread acceptance of an increased intake of LA as being healthful-LA is essential and LA is hypocholesterolemic. However, the nutritional requirement for LA is ~1 en% and the hypocholesterolemic activity of LA is only transient; no significant difference in plasma cholesterol levels is found in aged subjects ingesting animal fats versus high-LA vegetable oils after long-term feedings. Thus, there is no rational empirical basis for increasing LA intake to current levels (>6 en% in Japan and >7 en% in the U.S.A.). Indeed, increased mortality due to Western type cancers can be ascribed to an excess intake of LA which causes an over- and unbalanced-production of *n*-6 eicosanoids leading to enhanced ischemic and inflammatory tendencies.[4)] The usefulness of inhibitors of AA metabolism (steroidal and non-steroidal anti-inflammatory drugs, lipoxygenase inhibitors) and the action of lipid mediators (anti-allergic drugs) in suppressing cancers, atherosclerosis and allergic hyperreactivity supports the concept that an excess intake of LA is a major risk factor for these diseases and for other diseases for which steroidal anti-inflammatory drugs are known to be effective. Although the trend toward an increase in total fatty acid intake in Japan trails that in Western countries, the proportion of LA among the total ingested fatty acids is currently much higher in Japan because the Japanese ingest less animal fat. Fortunately, the average intake of competitive *n*-3 fatty acids is also higher in Japan, although the quantity and quality of fatty acids ingested by younger Japanese are almost comparable to those of average Americans. We are afraid that the excess LA syndrome[4)] will soon become prevalent in Japan.

The competitive aspects of the three series of fatty acids (Figs. 1 and 6) should be taken into account in modifying current lipid nutrition. In Western countries, simply decreasing the intake of animal fats without increasing the intake of *n*-3 fatty acids could be quite risky, because saturated and monounsaturated fatty acids are relatively poor competitive effectors of LA metabolism, and *n*-6 fatty acids in tissue lipids would thus be expected to increase to even higher levels. As predicted by Lands' equation (Fig. 6), decreasing LA intake while consuming competitive amounts of *n*-3 fatty acids is the most effective approach.

Table 1. *n*-6 and *n*-3 Fatty Acid Intakes in Japan and the U.S.A., and Recommended Adequate Intakes (AI) or Dietary Reference Intakes (DRI)

Fatty acid	Current status		Recommended by		
	Japan	U.S.A.	Jpn. Min. 1999	Jpn. Soc. Lipid Nutr., 1997	ISSFAL 1999
n-6, en %	6.4	7.1	4.8—6.0	3.8	2—3
n-3, en %	1.6	0.9	1.2—1.5	1.9	1.3
n-6/*n*-3	4.0	8.3	4.0	2.0	1.5—2.3

Jpn. Min., The 6th revision of "Dietary Reference Intakes" from the Ministry of Health and Welfare, Japan, published in 1999; Jpn. Soc. Lipid Nutr., The President's Summary from the Japan Society for Lipid Nutrition published in 1997; ISSFAL, Adequate Intakes recommended at an International Workshop supported by NIH, the International Society for the Study of Fatty Acids and Lipids, which was held in Washington D.C., U.S.A. in 1999.

The 5th revision of the Dietary Reference Intakes for Japanese was issued in 1994 from the Ministry of Health and Welfare of Japan. A total fat intake of 20—25 en% and a saturated: monounsaturated: *n*-6: *n*-3 ratio of 1: 1.5: 0.8: 0.2 were recommended for healthy adults with a moderate physical activity. This fatty acid balance is based on the current nutrition of Japanese, who are enjoying the longest life span in the world. The 6th revision, published in 1999, was essentially unchanged with respect to lipid nutrition (Table 1). However, age-adjusted mortalities from Western type cancers are increasing rapidly and the current rate of increase of colorectal cancer in Japan is faster than that seen in the past in the U.S.A. More than 1/4 of Japanese infants are being diagnosed as atopic, although the occurrence of atopic dermatitis is reported to be 8% of 3 year-old infants, according to newly instituted standard diagnosis. Considering these trends in disease patterns and fatty acid intake, and also through discussions at the several annual meetings of the Japan Society for Lipid Nutrition, we recommend for the Japanese (and those living in temperate regions) that the intake of *n*-6 fatty acids (essentially LA) should be decreased from the current level of >6 en% to 3.8 en%, which is the level of the average Japanese 35 years ago, and that the intake of *n*-3 fatty acids should be increased to 2—3 en% from the current level (1.6 en%).

The *n*-6/*n*-3 ratios increased from 2.8 in 1955 to a current level of 4—5 in Japan. The ratio of foods consumed during the age of hunting and gathering is estimated to be 1,[1,113)] and the ratios for native Greenlanders and Danes' diets were

Table 2. Adequate Intakes (AI) for Adults Recommended at an International Workshop (ISSFAL 1999)

Fatty Acid	Gram/day (2000 kcal diet)	% Energy	Footnote
LA (*n*-6)	4.44	2.0	1
(upper limit)	(6.67)	(3.0)	
αLNA (*n*-3)	2.22	1.0	
DHA+EPA (*n*-3)	0.65	0.3	2
DHA to be at least	0.22	0.1	
EPA to be at least	0.22	0.1	
trans-FA (upper limit)	(2.00)	(1.0)	3
Saturates (upper limit)	-	(8.0)	4
Monounsaturates	-	-	5

1. Although the recommendation is for AI, the working group felt that there is enough scientific evidence to also state an upper limit for LA of 6.67 g/d based on a 2000 kcal diet, or of 3.0% of energy.
2. For pregnant and lactating women, ensure 300 mg of DHA.
3. Except for dairy products, other foods under natural conditions do not contain *trans*-FA. Therefore, the working group does not recommend *trans*-FA to be in the food supply as a result of hydrogenation of unsaturated fatty acids or high temperature cooking (reused frying oils).
4. Saturated fats should not comprise more than 8% of energy.
5. The working group recommended that the majority of fatty acids be obtained from monounsaturates. The total amount of fat in the diet is determined by the culture and dietary habits of people around the world (total fat ranges from 15—40% of energy) but with special attention to the importance of weight control and reduction of obesity.

0.36 and 3.3, respectively. We proposed a ratio of 2 or below as the first step toward preventing the excess LA syndrome described above.[4,181] For the people living in frigid zones, even lower *n*-6/*n*-3 ratios are recommended. The safety of foods with very low *n*-6/*n*-3 ratios has been assessed in animal experiments. We find no scientific evidence to support the recommended *n*-6/*n*-3 ratios of 5—10 proposed elsewhere.

In early 1999, an international workshop was held in Washington, D.C. to discuss the essentiality of and recommended dietary intake of *n*-6 and *n*-3 fatty acids.[182] More than 30 specialists from 10 countries agreed to recommend adequate intakes for adults, as shown in Table 2. Three major changes from the classic lipid nutrition advice to raise the P/S ratio for the prevention of elderly-onset diseases were, (1) decreasing the intake of LA from current levels of >6 en% to 2% was recommended, setting an upper limit of 3 en%, (2) adequate amounts of αLAN, EPA and DHA were set at 1, >0.1, and >0.1 en%, respectively, recognizing the importance of *n*-3 fatty acids, and (3) unfavorable effects of partially hydrogenated vegetable oils were recognized, setting an upper limit at 0.1 en%. Saturated fatty acids with 16 and 18 carbons are easily converted in our body to monounsaturated fatty acids, but no eicosanoids are formed from monounsaturates such as oleic, palmitoleic and *cis*-vaccenic acid. Therefore, monounsaturated fatty acids are considered relatively safe in a range that does not lead to obesity.

The hypothesis of cholesterol posing the major risk factor for atherosclerosis and related diseases has lost its scientific basis, and even raising the P/S ratio has been proved to be risky; Israel has one of the highest P/S ratios in the world, but is suffering from these diseases--hence, The Israeli Paradox.[183] Instead, an increased intake of LA associated with elevated *n*-6/*n*-3 ratios has been proved to be the major factor for these diseases. Comparing current lipid ingestion with the recommended adequate intakes (Table 2), the average Japanese are advised to decrease the intake of LA to below half the current level, whereas both decreasing the intake of LA and increasing the intake of *n*-3 fatty acids, particularly EPA and DHA, are advised for those in Western industrialized countries.

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