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Medicines and Vegetable Oils as Hidden Causes of Cardiovascular Disease and Diabetes

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Key Words
Statin · Warfarin · Canola oil · Hydrogenated oil · *Trans*-fat · Cardiovascular disease · Diabetes mellitus · Vitamin K_2 · Osteocalcin · Testosterone

Abstract
Background: Positive associations have been observed between cardiovascular disease (CVD) and type 2 diabetes mellitus (DM), but their causal relationship has not been clarified. Nevertheless, guidelines from relevant medical societies recommend using cholesterol lowering medication (statin) for both types of patients. Medicines with several different action mechanisms have been developed, and the effectiveness of different lifestyle modifications has been studied extensively for the prevention of DM, which was successful in improving clinical marker status in relatively short-term treatments, but none have been shown to be effective in improving long-term outcomes (mortality from CVD and all causes). ***Summary:*** Statin-induced suppression of prenyl intermediates in the cholesterol biosynthetic pathway has been linked to stimulated atherosclerosis and heart failure. On the other hand, certain types of vegetable oil and hydrogenated oil shortened the survival of stroke-prone spontaneously hypertensive rats by decreasing platelet number, increasing hemorrhagic tendency and damaging kidney functions, which could not be accounted for by their fatty acid and phytosterol compositions. These vegetable oils and medicines such as statin and warfarin share, in part, a common mechanism to inhibit vitamin K_2-dependent processes, which was interpreted to lead to increased onset of CVD, DM, chronic kidney disease, bone fracture and even mental disorder. Impaired vitamin K_2-dependent processes by some types of vegetable oils and medicines, but not plasma high low density lipoprotein cholesterol, were proposed as the cause of CVD, DM and other lifestyle-related diseases. High n-6/n-3 fatty acid ratio of ingested foods, but not animal fats, was emphasized to be another risk factor for many of the diseases described above. ***Key Messages:*** To date, no randomized controlled trials (RCTs) have been performed to prove the above interpretation. However, the opposite types of RCT trials have been performed by increasing the intake of high-linoleic vegetable oils and reducing that of animal fats, which resulted in increased CVD and all-cause mortality. The amounts of these vegetable oils to exhibit adverse effects in animal studies are not huge (<6 energy %), which should not be overlooked nor disregarded.

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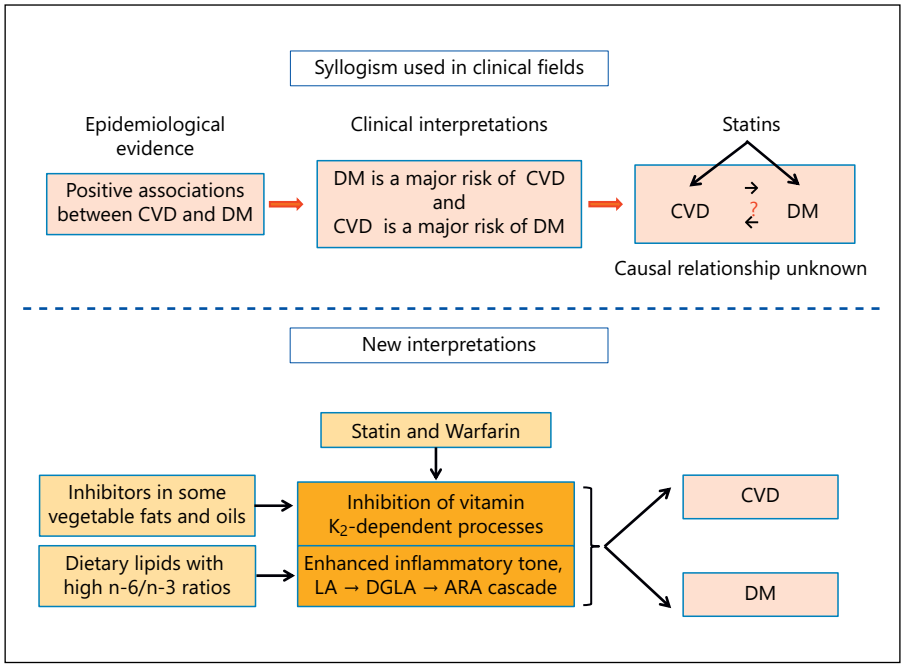

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Fig. 1. Syllogism often used in clinical fields to explain the positive association between CVD and DM and our new interpretations.

Introduction

A positive association between cardiovascular disease (CVD) mortality and prevalence of diabetes mellitus (DM) has been widely recognized, albeit with some exceptions found for the association [1], and CVD is regarded as a major risk factor for DM and vice versa (fig. 1, upper panel). In contemporary medicine, reducing the risks of a disease is generally accepted to be useful for preventing the disease. For example, even though the mechanisms by which high levels of low density lipoprotein cholesterol (LDL-C) cause DM and the mechanisms by which diabetic conditions cause CVD are still unclear, the latest guidelines from the American Diabetes Association (2015) and the American College of Cardiology/American Heart Association (2013) recommend that all diabetics take statins. Similarly, Japanese medical societies set an upper LDL-C level for diabetics, which is 40 mg/dl lower than the value for those without CVD risks.

In a previous review, we highlighted a dramatic change in the reported efficacy of statins for the prevention of CVD after new penal regulations for clinical trials came into effect in the EU in 2004 [2]; most of the large randomized controlled trials (RCTs) published after 2004 have reported no statistically significant beneficial effects of statins on CVD [3]. Moreover, the pharmacological mechanisms underlying statin-induced atherosclerosis and heart failure have since been clarified.

In this review, we present evidence that statins and warfarin, as well as certain types of vegetable oil, cause both CVD and DM in part through a common mechanism involving the inhibition of vitamin K_2-dependent processes. This provides the rationale behind the observed positive association between CVD and DM, that is, these substances cause both CVD and DM, resulting in the apparently positive association between the 2 diseases (fig. 1, lower panel).

I. Statins Induce CVD and DM

1. Statins Inhibit Several Biochemical Steps, Leading to Acceleration of Atherosclerosis

Statins are Mitochondrion-Toxic and Cytotoxic

Statins inhibit HMG-CoA reductase to lower the levels of prenyl intermediates in cholesterol biosynthesis as well as lower blood cholesterol levels. Coenzyme Q_{10} and heme A derived from prenyl intermediates are essential components of the mitochondrial electron transport chain, and thus statins inhibit the generation of adenosine triphosphate (ATP) that serves as an energy source for cellular activities. ATP depletion is the major cause of atherosclerosis progression under ischemic conditions (fig. 2). Under diabetic conditions, glucose is not utilized efficiently as an energy source and hydrophilic ketone bodies, synthesized from fatty acids partly in mitochon-

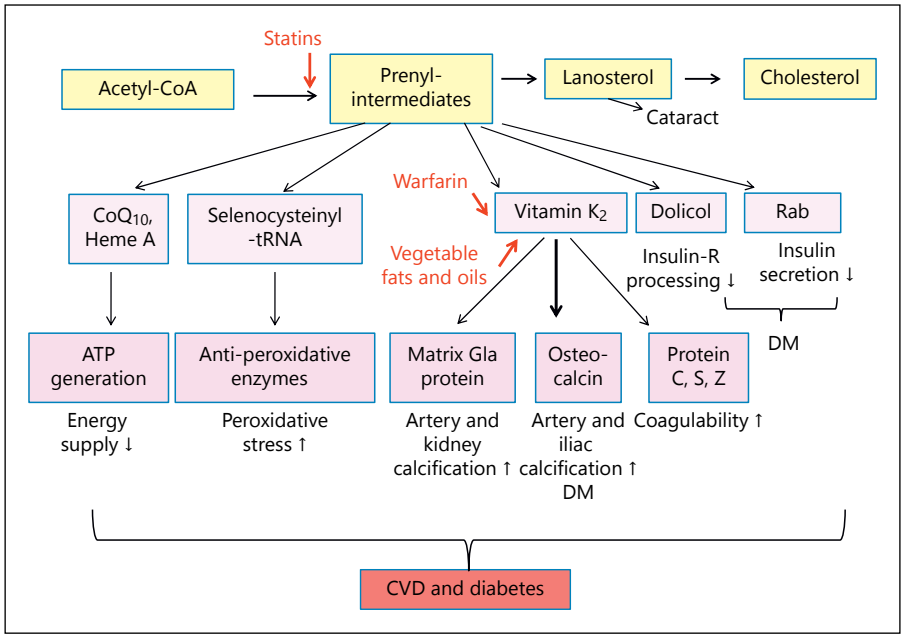

Fig. 2. Mechanisms of medications and vegetable oils serving as possible causes of CVD and DM. Modified from Okuyama et al. [3].

dria, are transported to the tissues and utilized as an energy source. Statins inhibit ketone body synthesis [4].

Mitochondrial membranes are relatively rich in cholesterol, and a decrease in cholesterol content caused by statins is likely to alter mitochondrial integrity. In the case of steroidogenic cells, steroidal hormone synthesis in mitochondria is inhibited, as will be explained later (Section IV-3). Thus, statins are mitochondrion-toxic in all tissues, accelerating atherosclerosis and exacerbating diabetic conditions.

Statins Inhibit the Synthesis of Selenium-Containing Proteins

Selenium (Se) is a trace element that is essential for the synthesis of Se-containing proteins (selenoproteins) such as glutathione (GSH) peroxidase involved in suppressing peroxidative stress, selenoprotein P involved in insulin signal transduction, thioredoxin reductase essential for synthesizing deoxyribonucleotides for DNA synthesis and iodothyronine deiodinase involved in thyroid hormone synthesis. Isopentenyl adenine from a prenyl intermediate is a minor base of transfer RNA that carries selenocysteinyl residue for protein synthesis. Anti-peroxidative enzymes other than GSH peroxidase, such as superoxide dismutase and catalase, are not selenoproteins but their synthesis is also suppressed by statins [5]. Interestingly, dietary canola rapeseed oil also suppresses such peroxidative enzyme activities (see Section III for details).

These statin-induced changes in biochemical parameters correlate with heart failure, for example, congestive heart failure is the major symptom of Keshan disease, an endemic disease known as Se deficiency in China [3].

Statins Inhibit the Conversion of Vitamin K_1 to Vitamin K_2 via Vitamin K_3

Vitamin K_1 is a cofactor of enzymes activating coagulation proteins (named after *Koagulation* in German). It is abundant in vegetable oils, such as canola and soybean oil, and in vegetables in general. Therefore, it is not commonly deficient in people getting sufficient energy from food. When ingested, vitamin K_1 is converted to vitamin K_2 utilizing the prenyl intermediate geranylgeranyl pyrophosphate in various organs including the brain, pancreas, bone, testis and kidney, in which vitamin K_2 is more abundant than vitamin K_1 [6] and is used as a cofactor for enzymes that γ-carboxylate the glutamyl residues of proteins. Statins inhibit this process by restricting the supply of geranylgeranyl pyrophosphate, resulting in vitamin K_2 deficiency in various tissues (fig. 2).

Intercellular matrix Gla protein in its γ-carboxylated form conserves calcium and phosphate, but its undercarboxylated form loses the capability to hold calcium, leading to artery and tissue calcification, a marker of atherosclerosis and kidney injury.

Osteocalcin (Ocn), a hormone produced in osteoblasts, is γ-carboxylated by a vitamin K_2-dependent enzyme to form carboxylated Ocn (c-Ocn), which is stored in the ma-

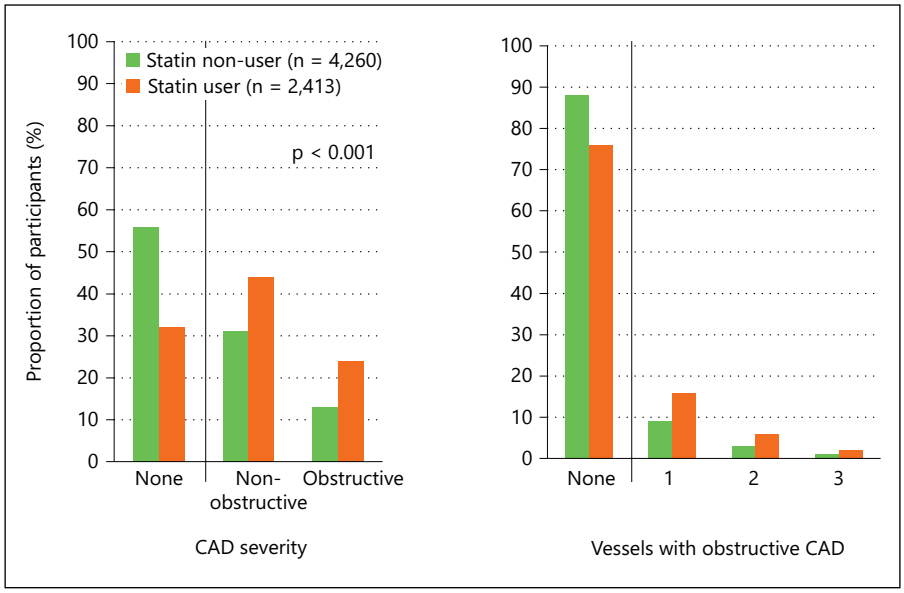

Fig. 3. Evaluation of statin effects by coronary CT angiography. Data taken from Nakazato et al. [15]. International multicenter CONFIRM registry. Participants aged 59 ± 11 years with no known CAD and available statin use status were evaluated by coronary CT angiography.

trix of the bone. When acid conditions are produced by osteoclasts, c-Ocn is decarboxylated to its under-carboxylated Ocn (uc-Ocn) form [7]. Both c-Ocn and uc-Ocn are secreted into the bloodstream and reach their target organs to affect metabolism in the pancreas (insulin secretion), intestine (incretin secretion), testis (testosterone production), bone (homeostasis), and adipocytes (adiponectin secretion). In some organs related to energy metabolism, uc-Ocn produced from c-Ocn has been reported to be the active form. Both circulating c-Ocn and uc-Ocn are taken up by neurons through a putative G-protein coupled receptor and converted to c-Ocn; they are even transported to the fetal brain through the blood–brain barrier, affecting the development and behavior of offspring [8].

Protein C is also γ-carboxylated and serves to inactivate coagulation factors Va and VIIIa. Statin-induced vitamin K_2 deficiency leads to modification of blood coagulability. Protein C is also known to play a role in inflammation, cell death and maintenance of blood vessel walls. Proteins S and Z are cofactors of protein C in the inactivation of coagulation factors. Interestingly, protein C deficiency was associated with neonatal hemorrhagic stroke [9]. In this way, statins affect many organs through pathways involving vitamin K_2-dependent processes as well as vitamin K_2-independent processes (fig. 2).

Statins Inhibit Dolichol Synthesis and Rab Activation

Dolichol is a homologous polyisoprenoid alcohol containing 14-24 isoprene units, and it is required for the biosynthesis of biologically important N-linked glycoproteins related to DM. N-glycosylation of the insulin receptor is required for its intracellular transport to the cell surface [10]. Glycosylation of insulin-like growth factor receptor in placenta is also inhibited by statins [11].

Rab is a small GTP-binding protein belonging to the Ras super family. Rab is activated by prenylation and plays an essential role in membrane trafficking of the insulin receptor and other proteins. Inhibition of dolichol synthesis and Rab prenylation by statins leads to cellular glycosphingolipid remodeling [12]. Other aspects of statins to stimulate the onset of DM have been reviewed by Brault et al. [13].

Early in 1979, a decade before the first statin appeared on the market, Compactin with the same inhibitory step as other statins was introduced into clinical studies, and it was shown in animal experiments that inhibition of dolichol synthesis caused abnormal fetal development [14]. In early clinical trials, the teratogenic activities of statins were confirmed, and they became contraindicated in women of childbearing age. Currently, more women than men are prescribed statins.

Clinical Evaluation of the Effects of Statins on Coronary Artery Disease by Coronary CT Angiography

In the International Multicenter CONFIRM registry, coronary artery status was evaluated by coronary CT angiography [15]. Compared with the group not taking statins, those taking statins had a higher prevalence of

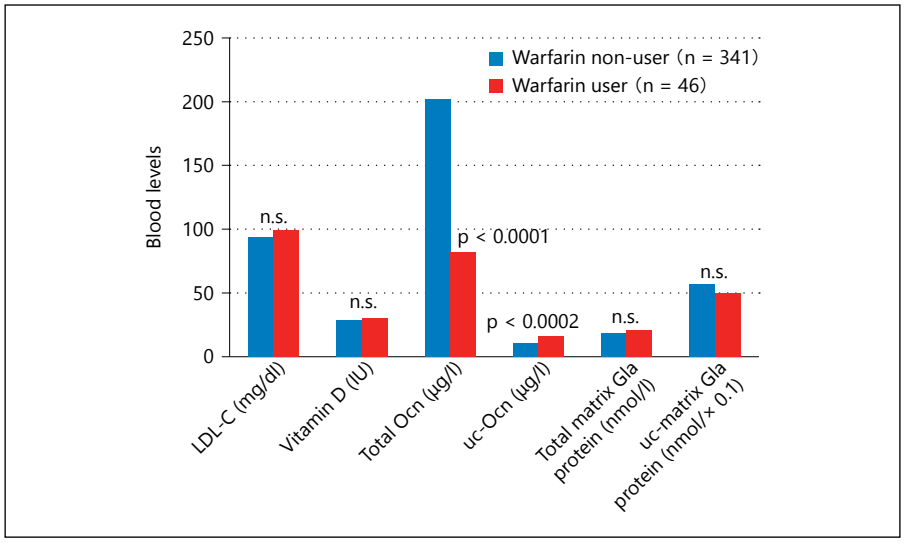

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Fig. 4. Effects of warfarin on blood markers in hemodialysis patients. Data from Fusaro et al. [16]. A multi-center, cross-sectional study with 3-year follow-up. Patients on hemodialysis for >1 year (n = 46) taking warfarin were compared with controls not treated with warfarin (n = 341).

obstructive coronary artery disease (CAD; fig. 3). In multivariable analyses, statin use was associated with increased numbers of coronary segments possessing mixed plaque and calcified plaque (data not shown in fig. 3). Thus, statin use was associated with an increased prevalence and extent of coronary plaques possessing calcium, which can be interpreted as being due to inhibition of the γ-carboxylation of matrix Gla protein and Ocn. These data, together with those described previously [3], clearly indicate that statins accelerate the progression of atherosclerosis, and clinical data consistent with these mechanisms have been reported.

2. Warfarin Inhibits Vitamin K Recycling, Leading to Tissue Vitamin K_2 Deficiency and Kidney Calcification

Warfarin, which is often used by patients with angina pectoris or myocardial infarction (MI) and those undergoing hemodialysis, inhibits the recycling of vitamin K (reduction of oxidized vitamin K) and reduces coagulability. Chronic administration may lead to vitamin K_2 deficiency in tissues and cause the same adverse effects as described above for statins.

In patients undergoing hemodialysis, those using warfarin showed significantly lower levels of total Ocn and significantly higher levels of uc-Ocn compared with warfarin non-users (fig. 4, 5) [16]. Levels of LDL-C, vitamin D and matrix Gla protein were unaffected by the warfarin treatment.

As shown in figure 5, the association between warfarin use and lower levels of Ocn was confirmed in a multiple regression model. Vertebral fracture was more frequent in male warfarin users ($p < 0.04$) but not in female warfarin users ($p = 0.6$). The use of warfarin was associated with a higher OR for aortic and iliac artery calcification. Warfarin users had a higher risk of all-cause mortality. Significantly fewer warfarin users survived after 3 years compared with non-users. When the grade of aortic valve calcification was estimated, calcifications in patients receiving preoperative treatment with oral anticoagulant (marcoumar) were significantly higher (2-fold) than in non-treated patients [17].

Thus, warfarin and a related anticoagulant were shown to induce calcification in the aortic, iliac and valve tissues and to cause irreversible adverse effects in hemodialysis patients through the inhibition of vitamin K_2-dependent pathways.

3. Potential Benefits of Supplementation with Vitamin K_2 for the Prevention of CVD under Current Nutritional Conditions

It is generally accepted that vitamin K deficiency will not occur among people with sufficient dietary intake because vitamin K_1 is abundant in vegetable oils and vegetables. Intestinal microflora is also known to supply vitamin K_2. However, recent epidemiological studies have revealed that a substantial proportion of the general population might be suffering from vitamin K_2 deficiency, possibly because of ingesting inhibitors of vitamin K_2-dependent processes.

In the Rotterdam Study, the participants aged ≥55 years were non-statin users with no history of MI [18], meaning there were likely to be a small proportion of warfarin users in the study, if any. It was found that vitamin

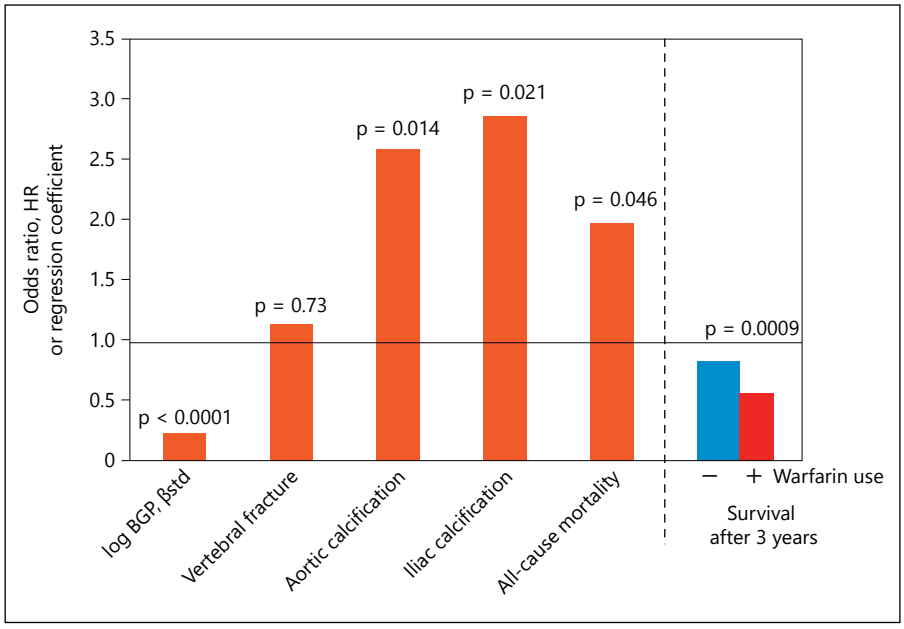

Fig. 5. Effects of warfarin on bone status and mortality in hemodialysis patients. Data from Fusaro et al. [16]. A total of 77 patients died during follow-up (2.7 ± 0.5 years on average).

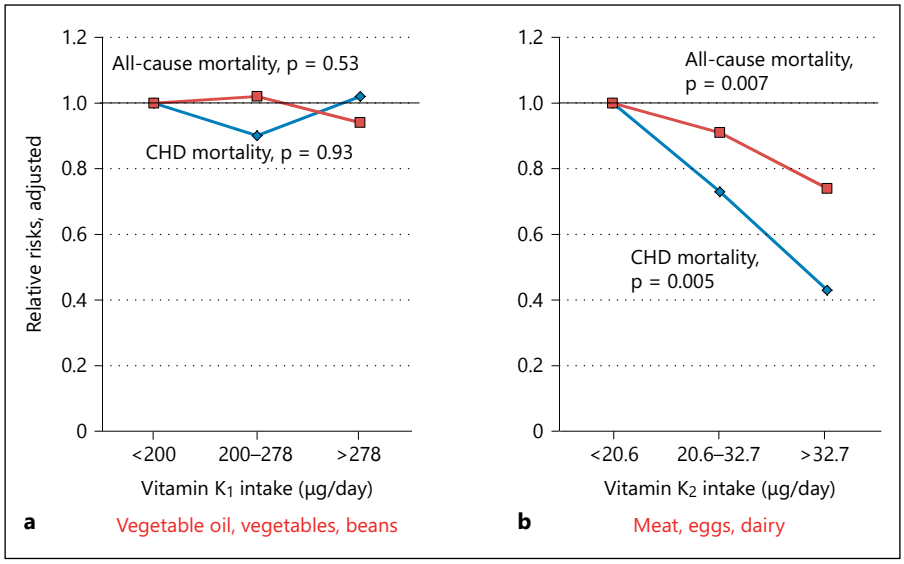

Fig. 6. Dietary intake of vitamins K_1 and K_2 and mortality: the Rotterdam Study. Data from Geleijnse et al. [18]. Residents of Rotterdam (n = 4,807, aged ≥55 years, non-statin users and without a history of MI) were followed for 7.2 years on average.

K_2 intake was inversely associated with coronary heart disease (CHD) mortality (relative risk (RR) 0.4, $p < 0.005$) and all-cause mortality (RR 0.75, $p = 0.007$), while no significant association was seen with respect to vitamin K_1 intake (fig. 6).

Severe aortic calcification was also associated inversely with vitamin K_2 intake (OR 0.48; $p < 0.001$). Thus, the impact of increasing vitamin K_2 intake on aortic calcification and CHD mortality cannot be disregarded in comparison with reducing other known risk factors [18].

II. Worldwide Prevalence of DM Rapidly Increased after 1980

1. DM-Associated Markers, But Not Long-Term DM-Associated Complications, Can Be Controlled by Lifestyle Interventions

DM is a typical lifestyle-related disease, and its major causal factor has been suspected for many years to be over-nutrition or energy imbalance (consumed energy < ingested energy). In fact, 3 types of dietary intervention

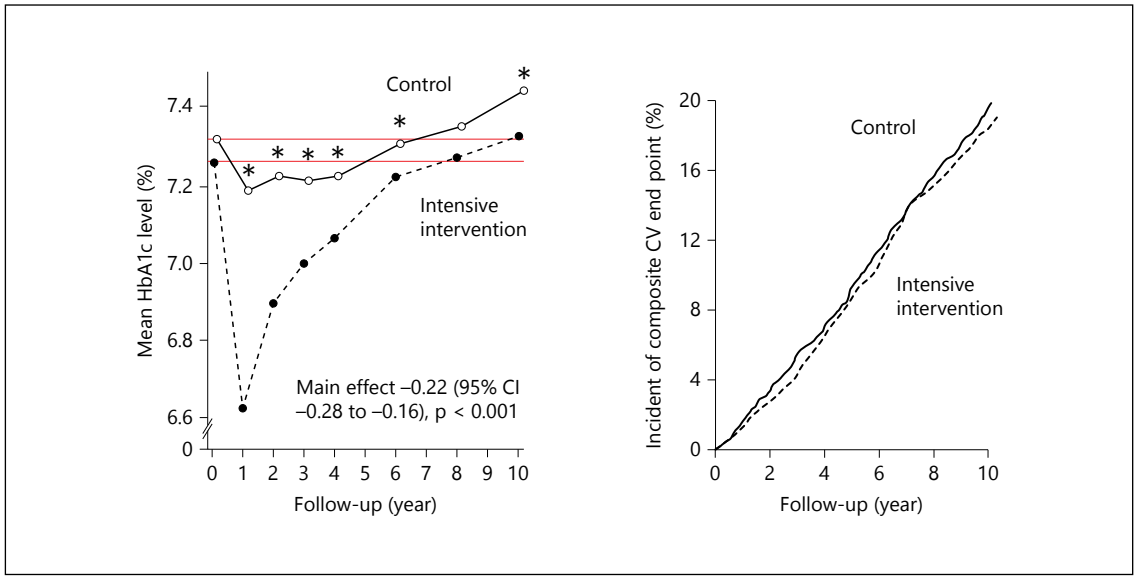

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Fig. 7. Effects in DM patients of standard and intensive lifestyle intervention on HbA1c levels and incidence of composite cardiovascular end points: the Look AHEAD Study. Data were reproduced from Look AHEAD research group [20]. US overweight and obese adults were randomized to an intensive lifestyle intervention group (n = 2,570) and control group (n = 2,575) and followed for 9.6 years. * $p < 0.05$ for the between-group comparison.

performed at an Israeli hospital [19] were shown to improve some markers of DM. Among the low-fat, Mediterranean and low-carbohydrate diets that they examined, the low-carbohydrate diet was more effective in general but adherence rate was lower than for the other diets. In the Look AHEAD Study, intensive lifestyle intervention also resulted in significant improvement in DM markers at 1 year [20] (fig. 7).

Despite successful reduction of the DM-associated parameters by the 1-year lifestyle intervention program [21], rapid rebound occurred, reaching baseline levels again after several years (fig. 7). Moreover, no significant difference was observed in the incidence of the composite cardiovascular end point, which included cardiovascular death, non-fatal MI, non-fatal stroke and hospitalized angina. When the primary and secondary end points included CVD, MI and/or stroke, DM-associated complications were not successfully reduced during the 10 years of follow-up (fig. 8). Based on the results of a futility analysis, the trial was stopped early at a median follow-up of 9.6 years.

2. Is Increased Intake of Carbohydrates the Major Cause of DM?

Differences in the prevalence rates of DM across countries may provide a clue as to whether overnutrition alone accounts for the DM prevalence (fig. 9) [22]. Canada, the US and the UK are similar with respect to nutritional status on average, but the DM prevalence in Canada is >2-fold greater than that in the UK. In Asia, the prevalence decreases in the order of China, Korea and Japan, differences which may not be accounted for by overnutrition alone. We suspect that something other than overnutrition underlies the recent rapid increase in DM prevalence worldwide.

In the US, the American Heart Association recommends replacing fats with carbohydrates for the prevention of prevailing CVD, and the intake of carbohydrates has increased gradually since 1960 (fig. 10). The increase in the number of diabetics appears to have occurred in 2 phases, with a steep increase seen after 1980. As pointed out by Gross et al. [23], the increase in the intake of high-fructose corn syrup (HFCS) preceded the rapid increase in DM patients. Thus, the data imply that the increase in DM is associated with an increase in carbohydrate intake, particularly fructose, which is several-fold more reactive than glucose with hemoglobin [24]. In fact, compared with glucose-sweetened beverages, fructose-sweetened beverages supplemented at 25 energy percent for 10 weeks increased visceral adiposity and lipids and decreased insulin sensitivity in those who were overweight or obese [25]. However, the extent to which increased fructose intake affects DM prevalence needs further careful study. India and Denmark do not

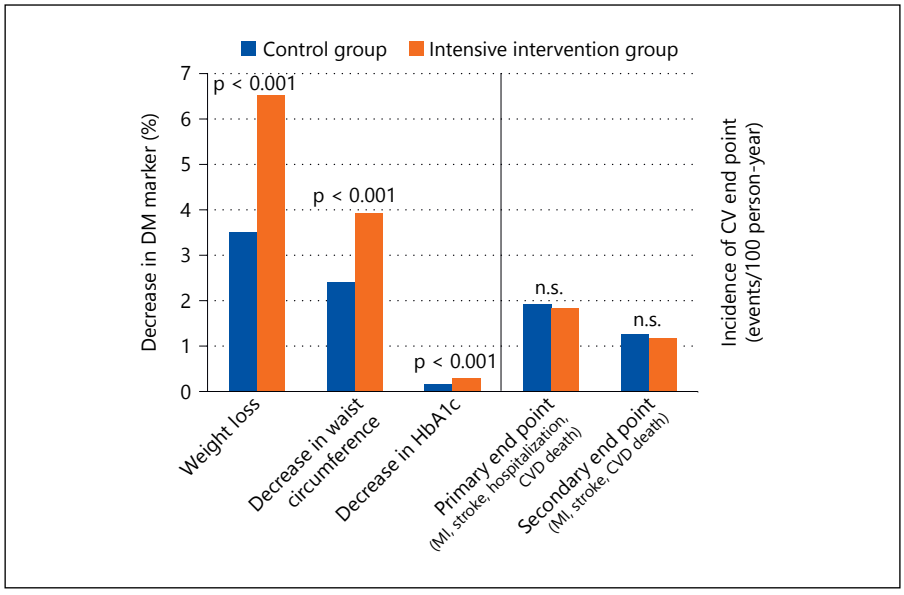

Fig. 8. Effect of long-term lifestyle intervention on cardiorespiratory fitness in adult DM patients. The Look AHEAD research group [20].

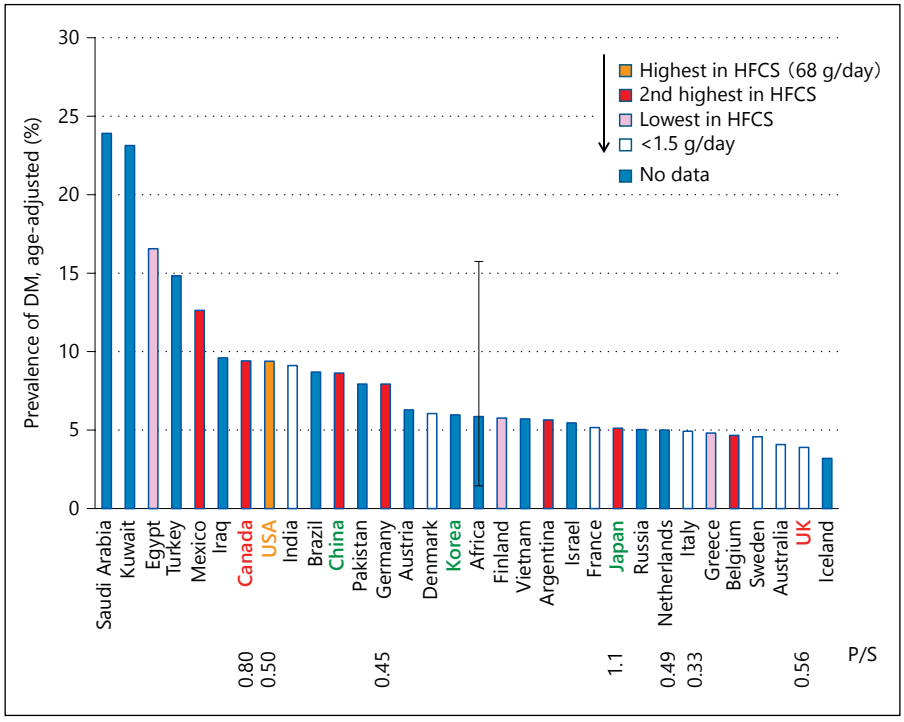

Fig. 9. Prevalence of DM across countries, adjusted for the world standardized age distribution (20–79 years of age). International Diabetes Federation DIABETES ATLAS, 6th Edition, 2014. Because fructose intake is a suspected risk factor for DM, the intake of HFCS is shown in different colors (Goran et al. [22]).

permit the use of HFCS, which is produced by enzymatic isomerization of starch, as food, but the DM prevalence in these countries is also relatively high. Similarly, Japan belongs to the second highest HFCS intake group, whereas France, which has the same DM prevalence as Japan, does not allow HFCS use. The difference in DM prevalence is relatively small between the high HFCS intake group (8%) and low intake group (6.7%) [22].

Thus, the increased intake of carbohydrates and HFCS in the US is associated with increased prevalence of DM, but other factors should also be taken into account when considering the causal relationship between the rapid increase in DM prevalence and trends in nutrient intake.

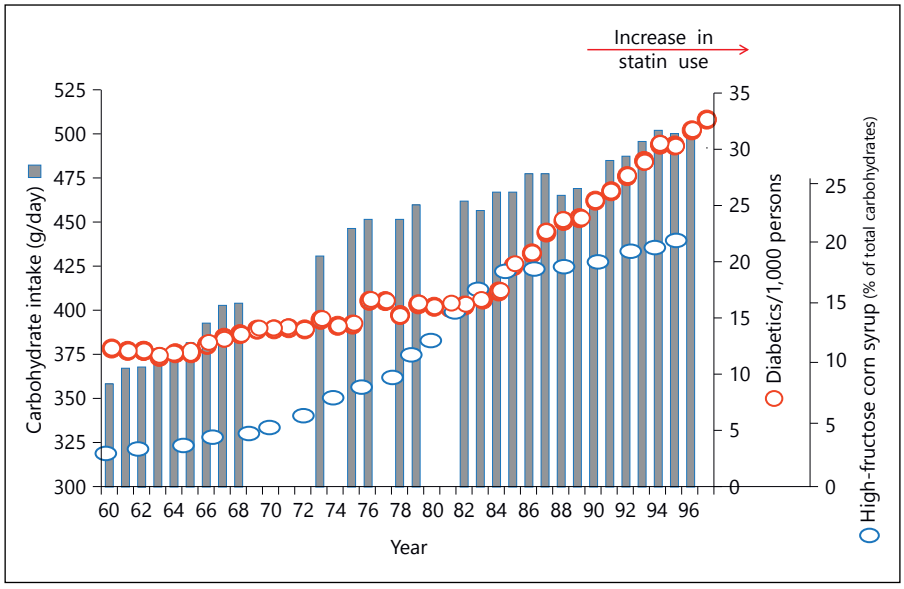

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Fig. 10. Trends in DM prevalence, carbohydrate intake and fructose intake in the USA. Gross et al. [23].

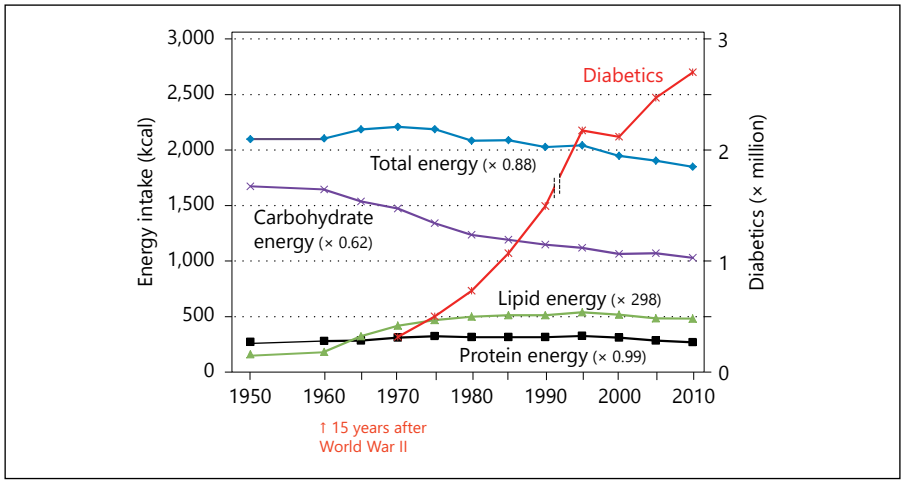

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Fig. 11. Trends in nutrient intake and DM prevalence in Japan. Data from the Ministry of Health, Labour and Welfare, Japan (www.mhlw.go.jp/toukei/itiran/gaiyo/dl/k-eisei-seigo-h23.pdf#search www.nhlw.go.jptoukei_itiran_gaiyo_dl_keiseiseigoh23.pdf). The number of diabetics before 1990 was adopted from Kuzuya et al. [26]. Figures in parentheses denote the ratio of the value for 2010 to the value for 1950.

Although statins are associated with the onset of DM as pointed out above, their use increased rapidly after 1990, and therefore, they cannot be the major contributing factor for the steep increase in DM that occurred in the US after 1980 (fig. 10).

Unlike the situation in the US after 1960 (fig. 10), in Japan, the amount of carbohydrate energy decreased by 40%, total energy intake decreased by 20% and lipid energy amount increased 3-fold after 1960, and DM prevalence increased several-fold (fig. 11) [26].

It seems then that lipid intake, not carbohydrate intake, is the most likely dietary risk factor for DM at least in Japan. The intake of animal fats and vegetable oils are roughly equal in Japan. It should be noted that the intake of vegetable oils increased rapidly from 1965 to 1975 in Japan, the increase of the US's intake preceding by several years. Therefore, vegetable fats and oils need to be considered as one of causative factors for DM in both countries, as will be explained later in Section III.

Dispute Over-Interpretation of DM Data in Japan's Hisayama Study

Hisayama town (population just under 10,000) is located near Fukuoka city in the westernmost area of Japan. The Hisayama Study is recognized worldwide for its diagnostic accuracy of cause of death due to the very high

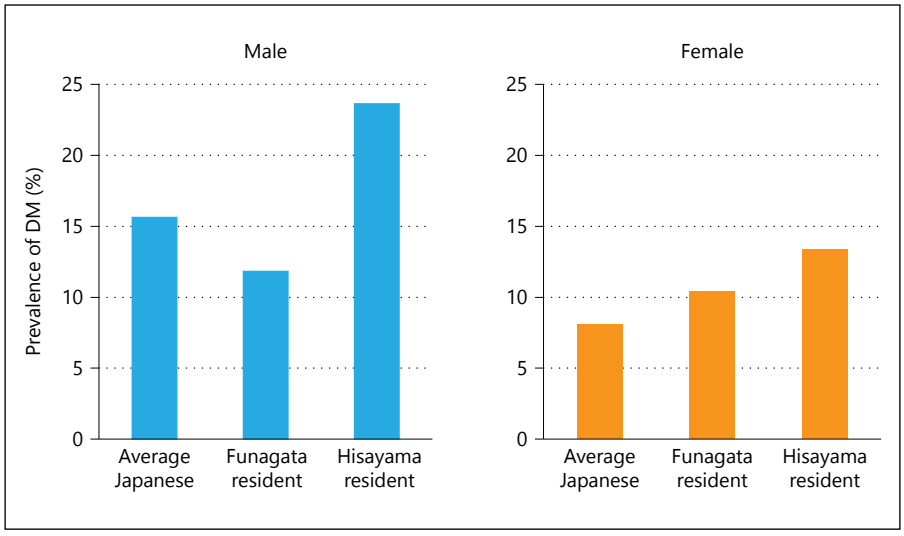

Fig. 12. Comparison of DM prevalence in Hisayama in Fukuoka prefecture, Funagata in Yamagata prefecture and the general Japanese population. Data from Dr. Kouji Ebe's website (in Japanese), February 21, 2012. http://koujiebe.blog95.fc2.com/blog-entry-2836.html., and Sekikawa et al. [28].

proportion of autopsies performed in collaboration with Kyushu University School of Medicine. For health promotion purposes, lifestyle intervention (advice) has also been continued over several decades for the town's residents. The dispute started when Dr. Kouji Ebe pointed out that the prevalence of DM among Hisayama residents was much higher than among the general Japanese population (fig. 12).

Professor Yutaka Kiyohara, the present leader of the Hisayama Study, from Kyushu University School of Medicine, responded that the apparently high DM prevalence in Hisayama town is due to the high proportion of residents receiving medical check-ups (≥70%) as well as the accuracy of diagnosis that includes an oral glucose tolerance test [27]. This explanation seems rational, but the study results from residents of Funagata town in Yamagata prefecture were inconsistent with this explanation (fig. 12). Both the rate of having medical check-ups and the method of diagnosis were comparable to those of the Hisayama Study [28]; the only difference was that lifestyle intervention (advice) was not provided to Funagata residents. Thus, lifestyle advice, and particularly the dietary advice given to Hisayama residents, is a possible causative factor for DM. The people responsible for offering the nutritional advice followed the guidelines of medical societies, and the leader of the Japanese Society of Clinical Nutrition provided direct advice. So, there is a clear possibility that the nutritional guidelines officially adopted in Japan are wrong and are leading to increased DM prevalence. In fact, no significant improvements in metabolic syndrome (MetS)-related parameters were noted after 4 years of the National Lifestyle Modification Project conducted by the Japanese government. Moreover, receiving such guidance was significantly associated with an increase of HbA1c at the 4-year evaluation mark (OR 2.49, 95% CI 1.18–5.24) [29].

Dr. Kouji Ebe analyzed the nutrient intake of Hisayama residents from the data published by those participating in offering the nutritional advice [30], and he found a significant increase in carbohydrate intake (energy %) from 1998 to 2004, which led him to deduce that increased carbohydrate intake was the major cause of the higher than average prevalence of DM among Hisayama residents, just as in the case of the US population shown in figure 10.

However, this interpretation is not consistent with the trends seen in nutrient intake and increased DM prevalence in Japan (fig. 12) – no increase in carbohydrate intake is apparent during this period in Japan. Examining the basis for the apparent increase in carbohydrate energy percent among Hisayama residents noted by Dr. Kouji Ebe, we found a critical problem associated with our government's database, that is, the classification of foods was changed starting in 2001.

Mayonnaise and salad dressing were reclassified from the food group containing fats and oils to the group of 'seasoning and spices'. Mayonnaise and salad dressing constituted roughly one-third of the fats and oils consumed by the average Japanese, hence the intake of fats and oils appeared to have dropped substantially after 2001 (fig. 13). The intake of rice products and whole grains also increased abruptly and unusually after 2001, which does not reflect the actual intake of these foods, namely a gradual decrease in carbohydrate intake (fig. 11).

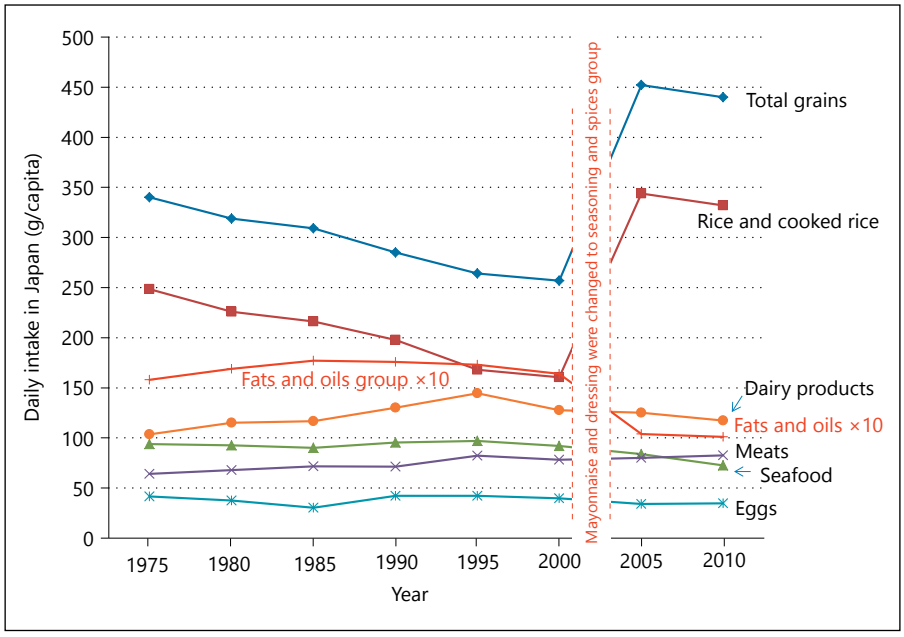

Fig. 13. Annual trends in food group intake among the Japanese after abrupt changes in the classification of food groups in 2001. Data from the Ministry of Health, Labour and Welfare, Japan.

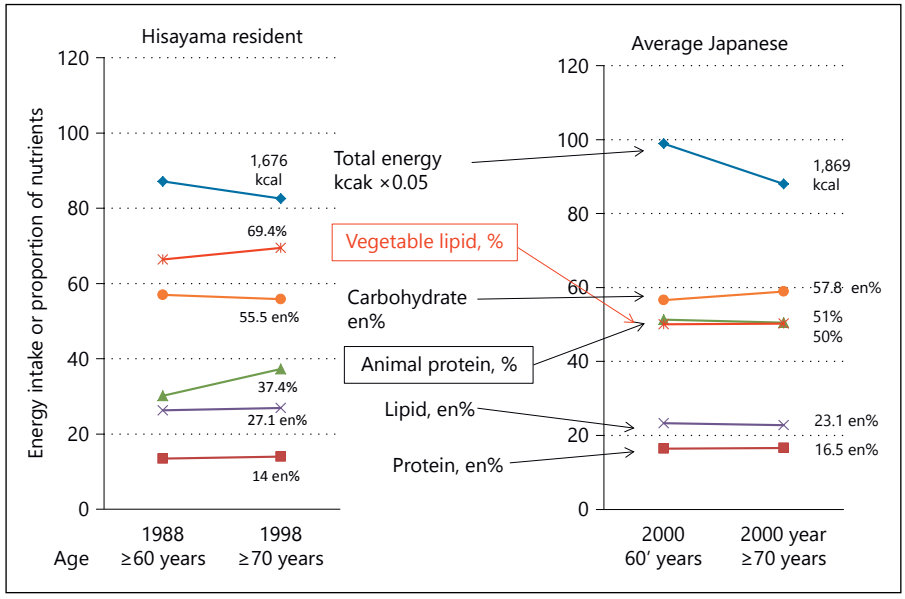

Fig. 14. Nutrient intake of Hisayama residents aged ≥70 years in 1998 compared with that of the general Japanese population 2000. Data for Hisayama residents are from Shirota et al. [31] (in Japanese), and those for the general Japanese population are from the National Health and Nutrition Survey 2000, Ministry of Health, Labour and Welfare, Japan.

We can find no rational explanation for these changes in food group classification, and we cannot find how the government's data (e.g. fig. 11) have been modified and adjusted to account for these changes. However, these changes obviously misled many nutritionists to correlate increased carbohydrate intake with higher DM prevalence among Hisayama residents.

In an effort to guess what type of nutritional advice was given in Hisayama before 2001, we found a report on the intake of nutrients by its residents aged ≥70 years in 1998 [31], which was compared with the data for the general Japanese population aged ≥70 years in 2000 (fig. 14); this difference of 2 years was considered relatively small compared with the changes made to the food group classification in 2001.

Hisayama residents, compared with the general Japanese population, had a slightly higher total lipid intake (27.1 vs. 23.1 energy %, respectively), slightly lower

carbohydrate intake (55.5 vs. 57.5 energy %) and slightly lower total energy intake (1,676 vs. 1,869 kcal). Striking differences were noted in the proportions of animal protein (37.4 vs. 50%) and vegetable oils (lipids, 69.4 vs. 51%). The ratio of vegetable oils to animal fats was 2:1 among Hisayama residents but 1:1 among the general population.

These differences came from the intensive dietary advice to (1) increase the intake of soy products, emphasizing the benefits of soy isoflavones, (2) reduce the intake of cholesterol and animal fats but increase that of vegetable oils and (3) emphasize the safety of *trans*-fatty acids. This dietary advice was exactly the opposite of what the Japan Society for Lipid Nutrition recommended, but it was consistent with the recommendations of lipid nutritionists in Kyushu region as well as that of the major clinical nutrition societies in Japan.

We conclude, therefore, that the higher intake of vegetable oils may be associated with the higher prevalence of DM among Hisayama residents than among the general Japanese population. We will explain in detail the responsible mechanism later in Section III.

3. Intensive vs. Standard Treatment to Lower HbA1c Is Not Beneficial for the Prevention of DM Complications

The complications of DM include CVD, retinopathy, renal failure and arteriosclerosis obliterans. Blood glucose reacts with proteins to form advanced glycation end products (AGEs), and this reaction is assumed to cause DM complications. Therefore, blood glycated hemoglobin (HbA1c) level is used widely as a measure of hyperglycemic status. Several types of medication for DM have been developed, and it is now relatively easy for doctors to maintain patients' blood HbA1c levels as desired.

Several large intervention trials comparing intensive versus standard medical therapy have been reported. In the randomized controlled ACCORD trial, median HbA1c levels of 6.4 and 7.5% were achieved at 1 year in the intensive and standard therapy groups, respectively [32]. However, the trial was discontinued after a mean of 3.5 years because of higher mortality in the intensive therapy group (fig. 15). CVD mortality was also higher although non-fatal MI, a less objective end point, was lower in the intensive therapy group.

The ACCORD trial was discontinued at 3.7 years, but a follow-up of 1.2 years was added (ACCORD-ON) [33]. The new finding was that MI was less frequent in the intensive therapy group than in the standard therapy group during active treatment (hazards ratio, HR 0.80, p = 0.015; table 1). The interpretation of the authors was that raised

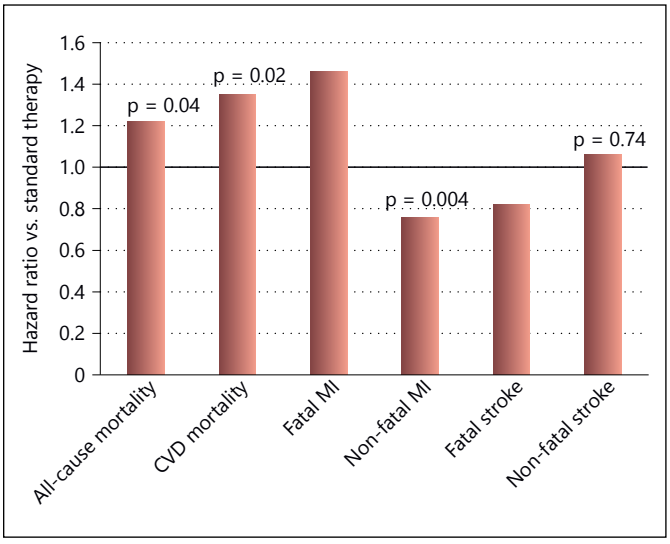

Fig. 15. Effects of intensive glucose lowering in DM: the ACCORD trial. Data from Action to Control Cardiovascular Risk in Diabetes Study Group, 2008 [32]. Patients with a median HbA1c level of 8.1% (n = 10,251) were randomly assigned to receive intensive therapy (targeting an HgA1c level <6.0%) or standard therapy (targeting a level of 7.0–7.9%). The trial was discontinued after a mean of 3.5 years because of higher mortality in the intensive therapy group.

glucose concentration is a modifiable risk factor for ischemic heart disease in middle-aged people with DM and other cardiovascular risk factors [33].

Simply reading the summary of the ACCORD-ON trial, many readers might believe that lowering blood glucose level is effective for reducing CVD in DM patients, because there is no description of the increased mortality rates from CVD and all-cause mortality by the intensive treatment, which were described only in the ACCORD trial (fig. 15).

In VADT, another large-scale trial, the effects of intensive treatment versus standard treatment were compared in the US diabetic veterans [34]. Again, no significant beneficial effects on all-cause mortality, CVD mortality or major CVD events were noted (fig. 16). When the follow-up was extended from 5.6 to 11.8 years (VADT-ON Study), no improvement in all-cause mortality was seen [35].

Similar to the ADVANCE and ADVANCE-ON trials, no significant improvement was seen in the intensive therapy group with respect to all-cause mortality or CVD, MI or stroke events. In contrast, there were more major hypoglycemia events in the intensive therapy group (HR 1.85, p < 0.001).

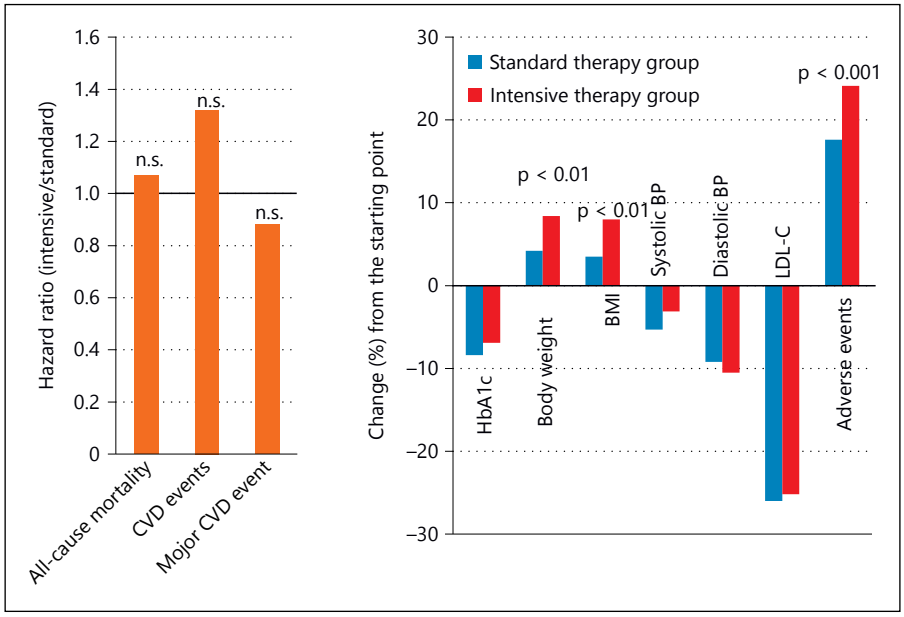

Fig. 16. Effects of blood glucose control on CVD events in US veterans (VADT trial). Data from Duckworth et al. [34]. Diabetic veterans with a mean age of 60.4 years (n = 1,791) were followed for 5.6 years (median), and 264 and 235 cases were noted in the standard therapy group and intensive therapy group, respectively. Sulfonylurea drugs were used for the control of blood glucose. Hypotensive drugs were also used.

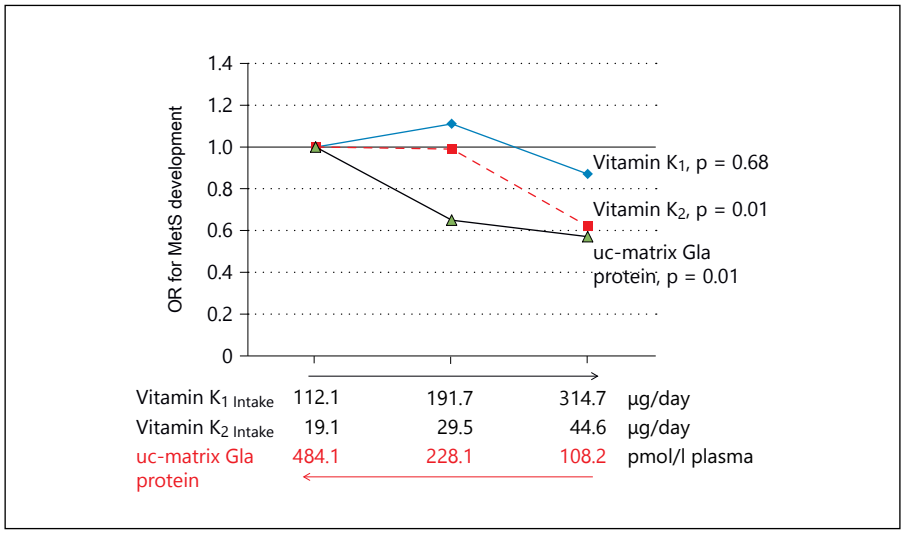

Fig. 17. Relationship between the intake of vitamin K_1 and K_2, plasma uc-matrix Gla protein level and the development of MetS. Data from Dam et al. [36]. Dutch older adults (aged 40–80 years, 400 men, 402 women) were followed for 8.14 years. Development of MetS was diagnosed by waist circumference (men ≥102 cm, women ≥88 cm), blood pressure (SBP ≥130 mm Hg, DBP ≥85 mm Hg, or hypotensive drug), HDL (men <1 mmol/l, women <1.3 mmol/l, or hypolipidemic drug), fasting glucose ≥5.6 mmol/l or drug) and neutral lipid level (≥1.7 mmol/l). Observed number of adults with MetS was 151.

These observations lead us to conclude that while the levels of blood glucose and HbA1c can be successfully controlled by medication, this does not lead to long-term benefits in terms of a reduction in cardiovascular complications or in all-cause mortality. Thus, the formation of AGEs is unlikely to be a major causative factor for CVD in patients with DM.

Given the above observations (fig. 16, 17 and table 1), a committee of the Japan Geriatric Society proposed that all medication used for DM by older people should be classified under Screening Tool of Older Person's potentially inappropriate prescriptions, and we support this proposal (www.jpn-geriat-soc.or.jp/).

4. Involvement of Vitamin K_2-Dependent Pathways in DM

The involvement of vitamin K_2-dependent processes in the development of CVD and DM was partly summarized in figure 2, and the clinical data relevant to CVD were shown in figures 3–6. In a Dutch Study, the development of MetS was inversely associated with the intake of

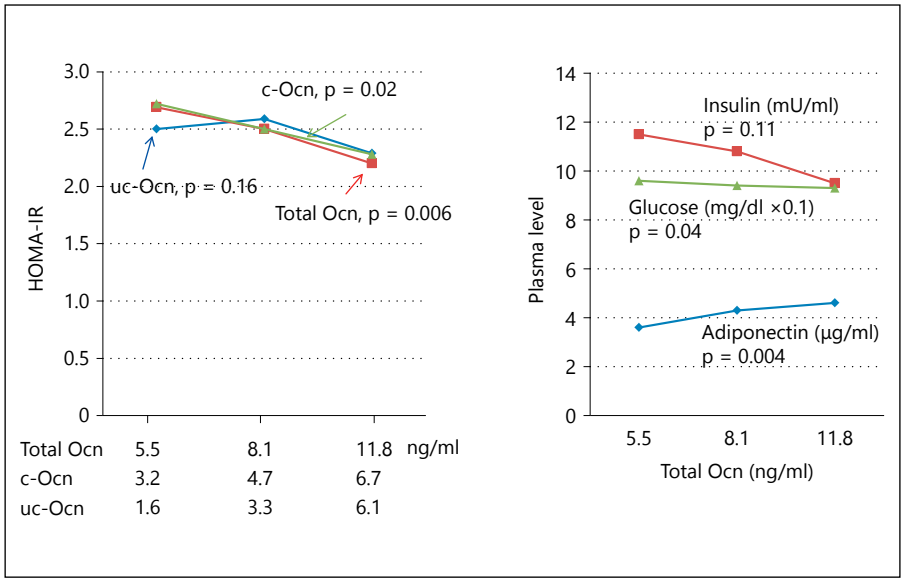

Fig. 18. Relationship between type of plasma Ocn and a marker of insulin resistance. Data from Shea et al. [37]. Cross-sectional associations between serum measures of the type of Ocn were examined in 348 nondiabetic men and women (mean age 68 years; 58% female) by using the HOMA-IR. Associations between each form of Ocn at baseline and 3-year change in HOMA-IR were examined in 162 adults (mean age 69 years; 63% female) who did not receive vitamin K supplementation.

Table 1. Comparison of the ACCORD and ACCORD-ON trials by Gerstein et al. [32]

Reference	Standard treatment	
	Gerstein et al. [32] (ACCORD)	Gerstein et al. [33] (ACCORD-ON)
Number of diabetics, n	10,251	10,251
Age, years	62.2 (average)	40–79
Average HbA1c, %	8.1	8.3
Target HbA1c, %	7.0–7.9, 6.0 (intensive)	7.0–7.9, 6.0 (intensive)
Female, %	38	Not described?
History of CVD, %	35	Those with risks of IHD
Follow-up, years	3.5[1]	3.7+1.2[2]
HR for the major endpoints[3]	0.90, p = 0.16	Data not shown
HR for all-cause death	1.22, p = 0.04	Data not shown
HR for MI	Data not shown	0.80, p = 0.015

[1] The trial stopped when all-cause mortality became significant in the intensive treatment group.
[2] Follow-up period was added.
[3] Non-fatal MI, non-fatal stroke and cardiovascular death.

vitamin K_2 but not with that of vitamin K_1 (fig. 17). Matrix Gla protein is also γ-carboxylated through a vitamin K_2-dependent processes, and the plasma level of uc-matrix Gla protein was positively associated with MetS [36].

In older adults, elevated levels of c-Ocn and total Ocn, but not of uc-Ocn, were associated with lower insulin resistance [37] (fig. 18). These results are consistent with the scheme shown in figure 2 where the inhibition of the vitamin K_2-dependent processes to γ-carboxylate Ocn leads to DM onset.

In relation to these observations, an intervention trial was performed using vitamin K_1 supplement for 3 years (fig. 19). Fasting insulin level and homeostasis model assessment of insulin resistance (HOMA-IR) value were significantly lower in the supplemented group only in men; the effects on these blood markers of DM were not significant in women [38]. This randomized trial did not use vitamin K_2 but vitamin K_1, which proved effective, but which is not consistent with the data shown in figure 17, where only vitamin K_2 was associated with insulin resistance. The amount of vitamin K_1 supplement used in this 3-year intervention study was 5-fold greater than the recommended daily amount, which may have resulted in increased tissue vitamin K_2 levels. Taken together, these

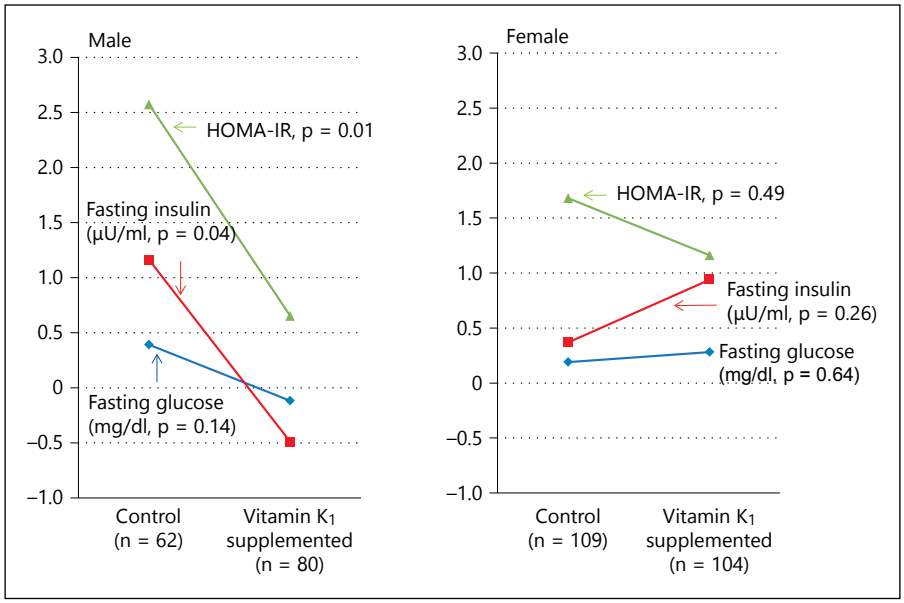

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Fig. 19. Effect of vitamin K_1 supplementation on insulin resistance in elderly people. Data from Yoshida et al. [38]. Elderly people without DM (aged 60–80 years) were randomly divided into 2 groups, with 1 group receiving vitamin K_1 supplementation for 3 years.

clinical data imply that a vitamin K_2-dependent process is involved in suppressing the development of DM and that inhibitors of vitamin K_2 formation result in DM onset.

III. Some Vegetable Oils and Fats Inhibit the Vitamin K_2-Dependent Processes, Leading to Cerebral Bleeding, Kidney Injury, DM and CVD

1. Vitamin K_2-Dependent Biochemical Pathways and Their Inhibitors: An Overview

We saw the diverse roles of prenyl intermediates as precursors of essential cellular components in figure 2. The side chain of vitamin K_2 comes from one of the prenyl intermediates, the geranylgeranyl group, and this is why statins interfere with the formation of vitamin K_2 and why warfarin inhibits the recycling of vitamin K, both leading to tissue vitamin K_2 deficiency (fig. 20).

Proteins C, S and Z, matrix Gla protein and Ocn are activated by vitamin K_2-dependent enzymes. Among them, Ocn produced in the bone has been revealed to act as a hormone and is involved in normal brain function as well as CVD and DM. γ-Carboxylated c-Ocn produced in osteoblasts accumulates in the bone matrix. When osteoclasts work, the acidic conditions produced lead to decarboxylation of c-Ocn to form uc-Ocn [7, 8]. Both c-Ocn and uc-Ocn released from the bone are carried in the bloodstream to their target organs and serve as bone-derived hormones.

In most tissues, there is a higher content of vitamin K_2 than vitamin K_1, and vitamin K_2 has unique functions, as shown in figures 2 and 20. Statins inhibit the supply of the geranylgeranyl group, leading to vitamin K_2 deficiency in tissues.

2. Certain Types of Vegetable Oil Show Inhibitory Activities Toward Vitamin K_2-Dependent Processes

In lipid nutrition studies, the period of intervention is critical. For example, Ancel Keys' equation and related equations were derived from more or less 1 month of intervention, but these equations did not predict the plasma levels of cholesterol after several years of intervention, as reviewed elsewhere [39].

In an effort to evaluate the nutritional value of vegetable oils with different fatty acid compositions, stroke-prone spontaneously hypertensive (SHRSP) rats were fed a diet supplemented with 10% fat or vegetable oil from weaning and their survival was compared (fig. 21). The group given n-3 fatty acid-rich fish oil or perilla (seed) oil had a longer survival time than that of the group given n-6-rich soybean oil. The fatty acid compositions of major fats and oils are shown in figure 22 for reference purposes.

The n-6 to n-3 ratio of the rapeseed oil (canola type) was much lower than that of soybean oil, but the group given rapeseed oil or n-6-rich evening primrose oil had an unusually short survival time [40].

When 1% salt solution was given to SHRSP rats, the mean survival time was shortened but the difference in

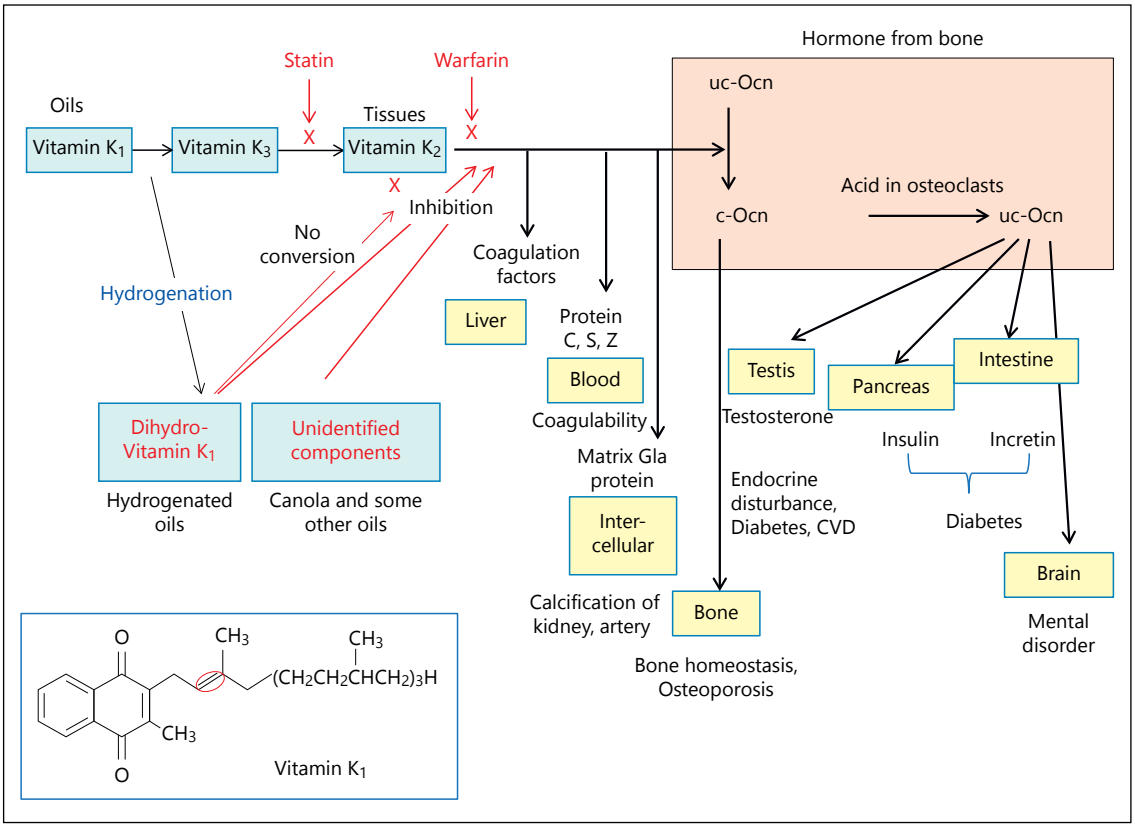

Fig. 20. Diverse roles of vitamin K_2-dependent processes related to CVD and DM. The roles played by uc-Ocn and c-Ocn shown in this scheme may require some updates as we learn more into the future.

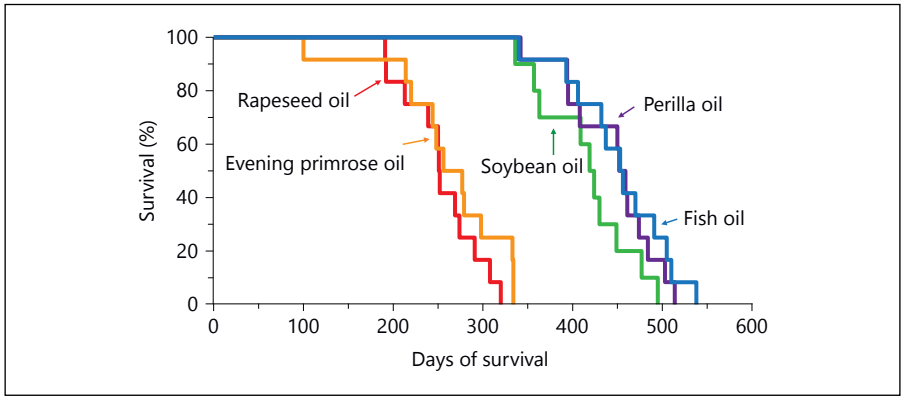

Fig. 21. Effect of different fats and oils on the survival of SHRSP rats. Data from Huang et al. [40]. A conventional laboratory chow supplemented with 10% fat or oil was fed to SHRSP rats from weaning (n = 12 in each group) and survival was noted.

survival time was reproduced in the soybean oil group and in the canola rapeseed oil (fig. 23). High erucic rapeseed oil was even worse, and canola type rapeseed oils produced in different years and by different manufacturers were similar in toxicity, indicating that the activity is not due to contaminants during the processing of oil for human use.

Partially hydrogenated soybean oil shortened survival similar to canola oil, indicating that something toxic to the SHRSP rat was produced during industrial hydrogenation. Ratnayake et al. [41, 42] at Health Canada confirmed the survival-shortening activity of canola rapeseed oil. Moreover, olive oil and corn oil were shown to exhibit similar anti-nutritional activities.

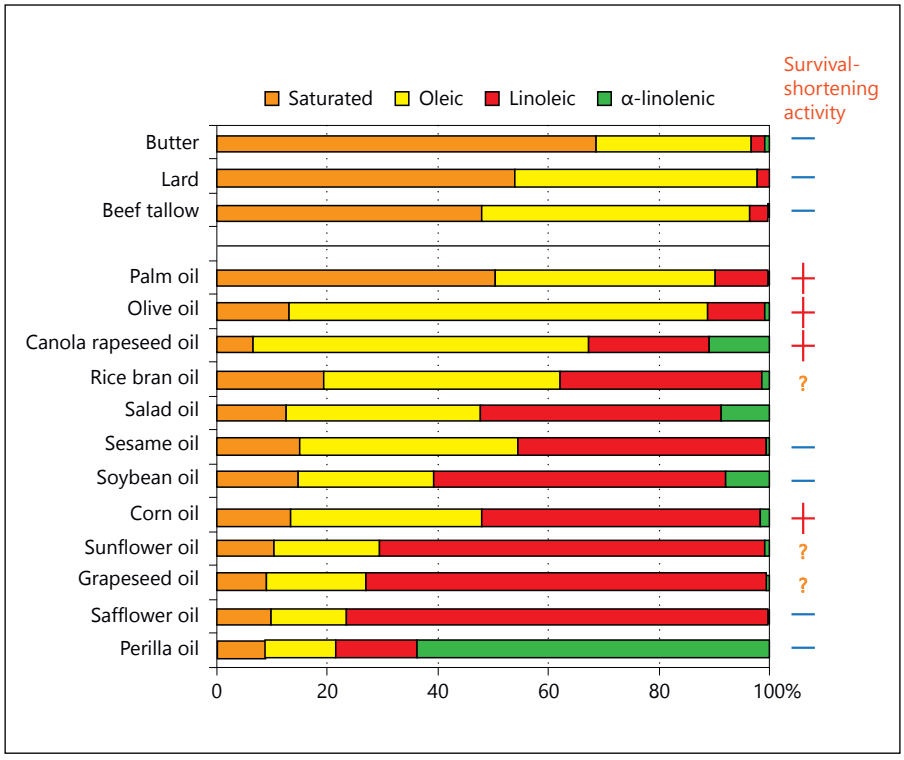

Fig. 22. Fatty acid compositions of common fats and oils. The plus sign (+) indicates those oils with survival-shortening activity in the SHRSP rats in figure 21. Those with question mark (?) have not been examined in this animal model.

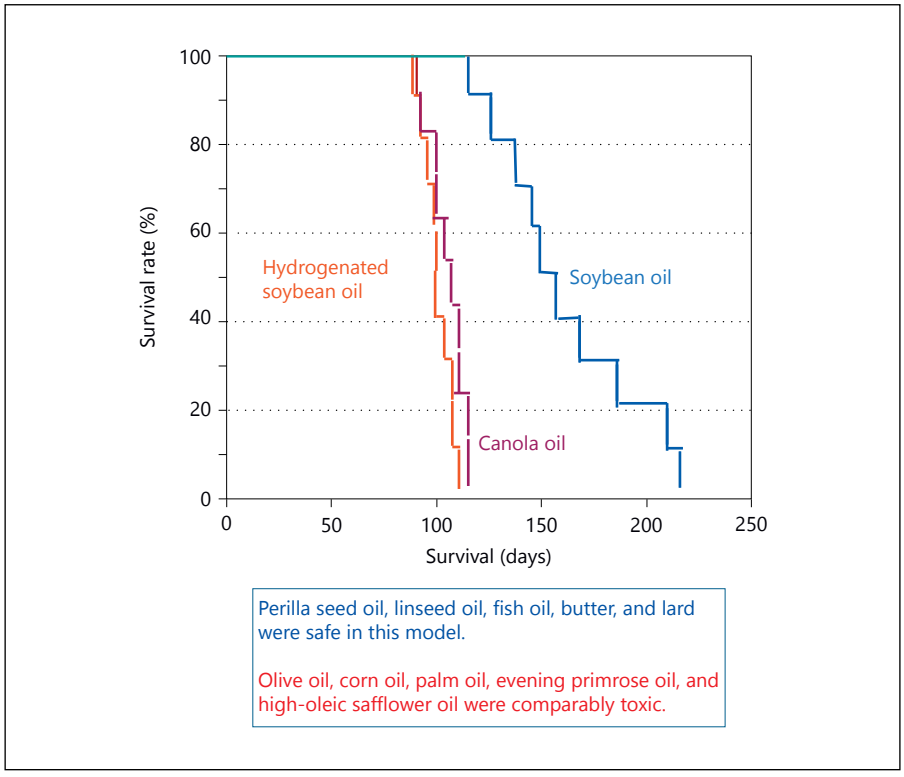

Fig. 23. Effects of dietary fats and oils on the survival of SHRSP rats. Data from Tatematsu et al. [43]. Ten rats in each group were fed a diet supplemented with 10% fat or oil from weaning and 1% NaCl solution as drinking water.

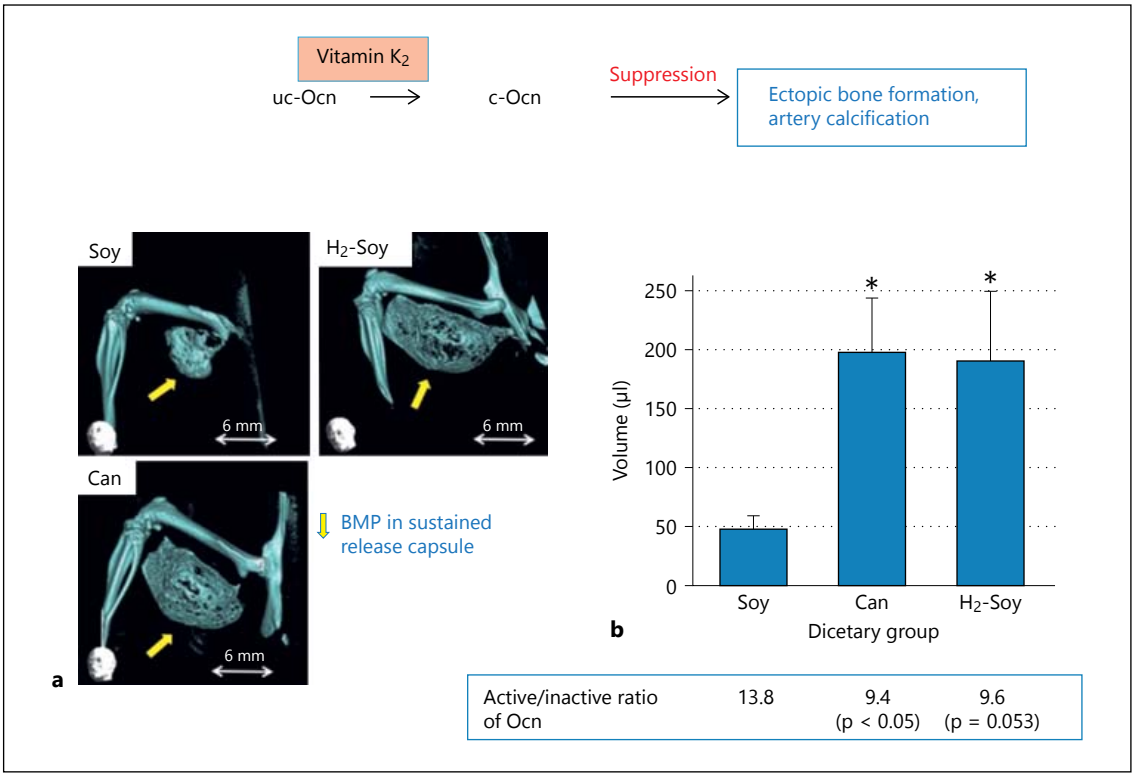

Fig. 24. Effects of dietary fat or oil on ectopic bone formation induced by BMPs. Data from Hashimoto et al. [49]. Mice were fed a diet containing soybean oil (Soy), canola oil (Can) or hydrogenated soybean oil (H_2-Soy) together with an essential amount of linoleic acid. The BMP capsule was then implanted and ectopic bone formation was observed 3 weeks after the implantation. The volume (μl) of newly formed bone was quantified using 3-dimensional X-ray micro-CT. * $p < 0.05$ vs. Soy group.

As to the principle of the toxic effects of canola oil, Ratnayake et al. [42] proposed phytosterols, but the amount of phytosterols in different oils and their survival-shortening activity are not in parallel [43–45], and 5-fold more phytosterols were needed to reproduce the toxic activity of canola rapeseed oil [46]. Furthermore, the free fatty acid fractions obtained by lipase or alkaline hydrolysis retain similar compositions of phytosterols and fatty acids, but the survival-shortening activity was lost (canola oil) or reduced significantly (hydrogenated soybean oil) [47]. Thus, components other than phytosterols and fatty acids must be mainly responsible for the toxicity [43–45]. We proposed dihydro-vitamin K_1 as one of the components responsible for the survival-shortening activity of hydrogenated soybean oil (fig. 20) [48].

Dose Dependency and Affected Physiology

The survival-shortening activity of canola oil in the SHRSP rat was dose-dependent; the amount corresponding to 6% of energy still exhibited significant activity, indicating that the current Japanese intake of these oils is comparable to the toxic dose in the SHRSP rat model. Kidney injury, cerebral bleeding, decreased platelet count, decreased tissue testosterone level, onset of diabetes and altered bone metabolism and brain function have been reported by various laboratories, which we will discuss, in part, below.

3. Link between the Survival-Shortening Activity of Fats and Oils and the Vitamin K_2-Dependent Processes

Bone morphogenetic proteins (BMPs) are a group of growth factors originally discovered for their ability to induce bone and cartilage formation. When a BMP preparation was sealed in a suspended release capsule and implanted into a gap in the fascia of the right femoral muscle of mice, ectopic formation of new bone was observed (fig. 24). The volume of the newly formed bone was approximately 4-fold greater in the canola and hydrogenated soybean oil groups than in the soybean oil group [49].

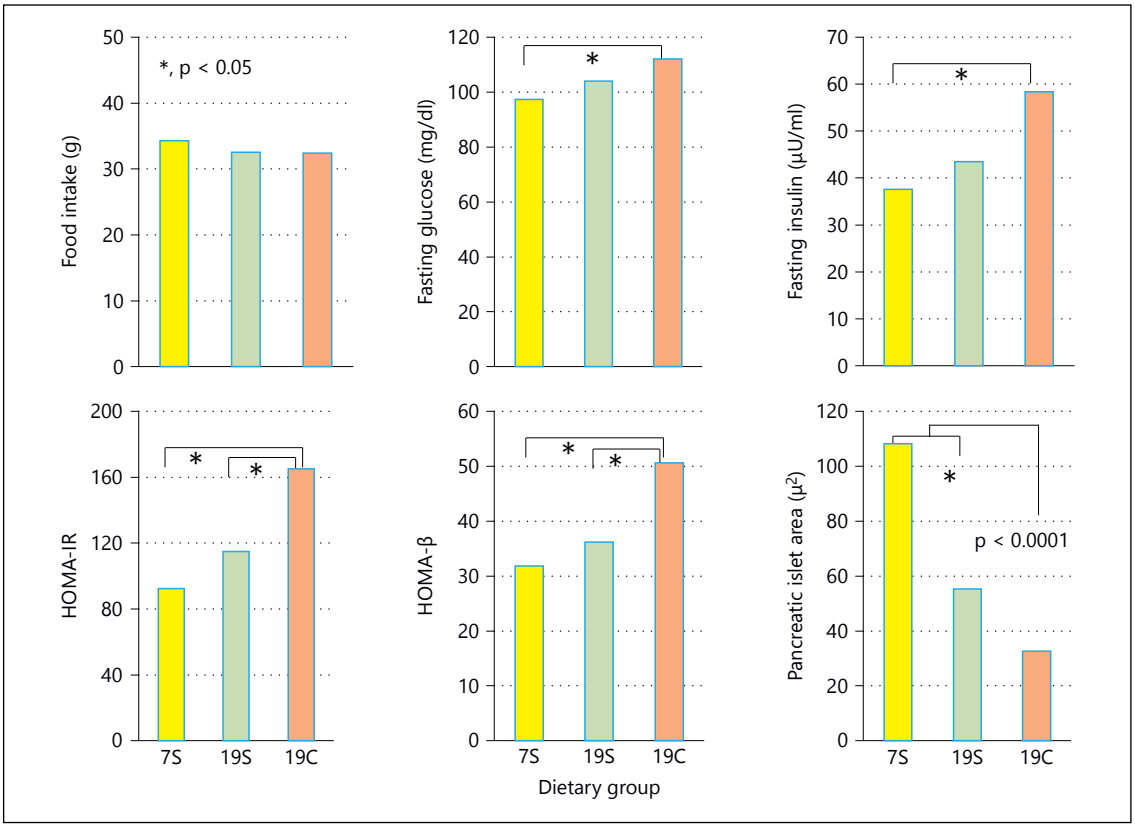

Fig. 25. Effects of dietary canola oil (C) and soybean oil (S) on diabetes-related parameters in young male rats. Data from da Costa et al. [51]. After weaning, rats (n = 12 in each group) were fed with a control diet (7S) or a high-fat diet containing soybean oil (19S) or canola oil (19C) until 60 days. Both 19S and 19C groups showed higher body mass, body length and retroperitoneal fat mass.

Ocn in its γ-carboxylated form (c-Ocn) is known to suppress BMP-induced formation of ectopic bone. Therefore, the results in figure 24 seem to indicate that a vitamin K_2-dependent process is inhibited in the canola and hydrogenated soybean oil groups, both of which exhibited survival-shortening activities in the SHRSP rat (fig. 23). In fact, these groups showed a tendency for a decreased c-Ocn to uc-Ocn ratio compared with the soybean oil group.

Dihydro-vitamin K_1 in the hydrogenated soybean oil is the likely candidate for inhibiting the γ-carboxylation of uc-Ocn because it is not converted to vitamin K_2, it is absorbed to a lesser extent, and it has no measurable biological effects on the measures of bone formation and resorption [50]. Canola oil does not contain dihydro-vitamin K_1, but we presume it has a component with physiological properties similar to those of dihydro-vitamin K_1. Thus, hydrogenated soybean oil and canola oil are linked by a common property that serves to inhibit the vitamin K_2-dependent processes. Other vegetable oils with survival-shortening activities in the SHRSP rat need to be evaluated for their potential inhibitory activities toward this process (fig. 23).

More direct evidence demonstrating that canola oil and soybean oil – 2 major vegetable oils consumed worldwide – differ in their effects on markers of DM in rats is shown in figure 25. In the figure 25, the 19C (19% canola oil) group showed higher insulin resistance (HOMA-IR) and more damage to pancreas β-cells than the 19S (19% soybean oil) and 7S (control) groups. The 19C group presented lower percentage of pancreatic islets area in comparison to 19S (–41%) and 7S group (–70%, $p < 0.0001$) [51]. DM patients in Japan are characterized by exhibiting both insulin resistance and impaired insulin secretion. This animal study is the first to demonstrate that a common vegetable oil, accounting for more than 40% of vegetable fats and oils consumed in Japan, impaired pancreatic function to secrete insulin. Similarly, these 2 vegetable oils differentially affected oxidative stress-related

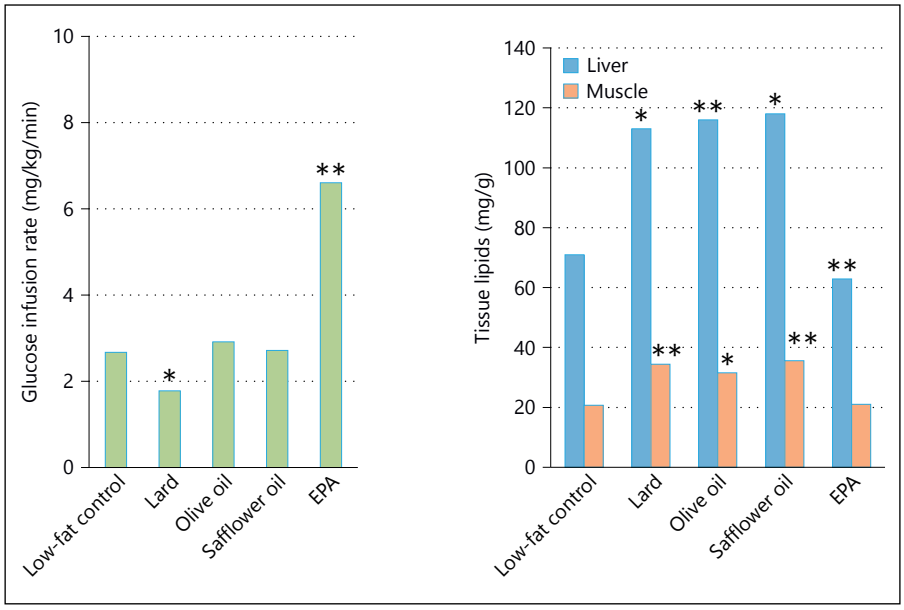

Fig. 26. Effects of dietary oils on the parameters of DM in model rats. Data taken from Mori et al. [53]. Spontaneous non-insulin-dependent DM with obesity (OLETF) rats were fed a diet supplemented with fish oil (EPA-E), lard, olive oil or high-linoleic safflower oil for 17–18 weeks and the glucose-infusion rate was determined in a euglycemic, insulin-glucose clamp test. * $p < 0.05$ and ** $p < 0.01$ vs. low-fat control group.

enzymes in erythrocytes and vascular properties in the SHRSP rat [52].

da Costa et al. [51] ascribed the difference in these parameters observed between the canola and soybean oil groups to the difference in the proportion of monounsaturated oleic acid and polyunsaturated linoleic acid (fig. 25). However, no differences were seen in the effects of an oleic acid-rich olive oil diet or a linoleic acid-rich soybean oil diet on the parameters of DM (fig. 26) [53]. Therefore, the observed differences are likely to be due to the factor inhibiting the vitamin K_2-dependent processes.

The glucose infusion rate (rate of disappearance of glucose from bloodstream) in a euglycemic insulin-glucose clamp test was approximately 3 times greater in the eicosapentaenoic acid ethyl ester (EPA-E)-treated group than in the other dietary groups [53]. Fat accumulation in skeletal muscle and liver was consistently lower in the EPA-E group compared with the lard, olive oil and safflower oil groups. Using the same animal model, we have shown that the glucose infusion rate of the α-linolenic-rich perilla oil group was comparable to that of the fish oil group, but with no significant difference with the lard or soybean oil groups (Yamashita T, Masters' degree thesis, Nagoya City University, 1999). These observations in animal experiments (fig. 25, 26) lead us to think that the difference in the effects of canola oil and soybean oil on glucose tolerance is not due to their fatty acid compositions but rather due to the difference in their inhibitory activities toward the vitamin K_2-dependent processes (fig. 20).

4. Differential Association of Industrial and Ruminant Trans-Fats with CVD and DM

Partial hydrogenation of vegetable oils produces *trans*-fats (industrial) and dihydro-vitamin K_1. Ruminant microorganisms also produce *trans*-fats (ruminant); hence, meat and dairy products contain significant amounts of ruminant *trans*-fats. The positional isomer compositions differ – the position of major *trans* double bonds in the industrial *trans*-fats are at positions 6-14 numbered from the carboxyl terminal, whereas that of ruminant *trans*-fats is mainly at position 11 – although the physicochemical properties of these positional isomers of *trans*-fatty acids may not differ much as shown earlier (fig. 27) [54].

The legal regulation of *trans*-fat in Western countries preceded that in Japan, and the World Health Organization recommends keeping its intake <1% of total energy intake. The American Food and Drug Administration (FDA) Agency excludes ruminant *trans*-fat from the regulation. There is good reason to treat industrial and ruminant *trans*-fats separately; a meta-analysis recently reported that their associations with CVD and DM events and all-cause mortality differ (fig. 28) [55].

Total *trans*-fat intake was shown to be harmful for all-cause mortality and CHD mortality (RR 1.34 and 1.28, respectively), while no positive association was found for ruminant *trans*-fats. Interestingly, total *trans*-fats tended to be harmful for DM, whereas ruminant *trans*-fats were protective (RR 0.58, $p < 0.001$). Industrial *trans*-fats and ruminant *trans*-fats differ in their positional isomer composition (fig. 27), but their effects on plasma LDL-C were

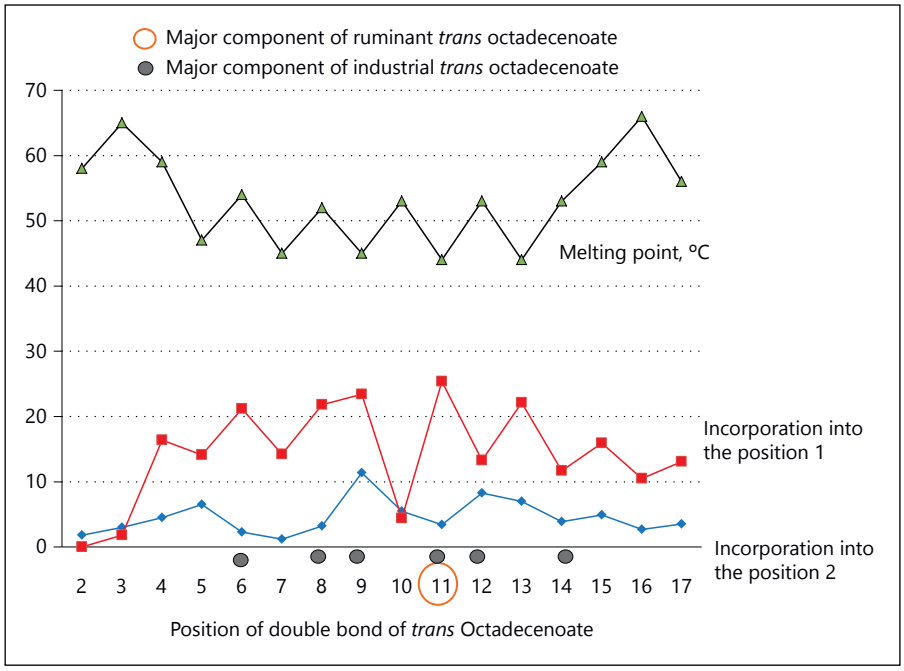

Fig. 27. Melting points of *trans*-octadecenoyl isomers and their acyl transfer rates into positions 1 and 2 of lysophosphatidylcholine in rat liver microsomes. Data from Okuyama et al. [54].

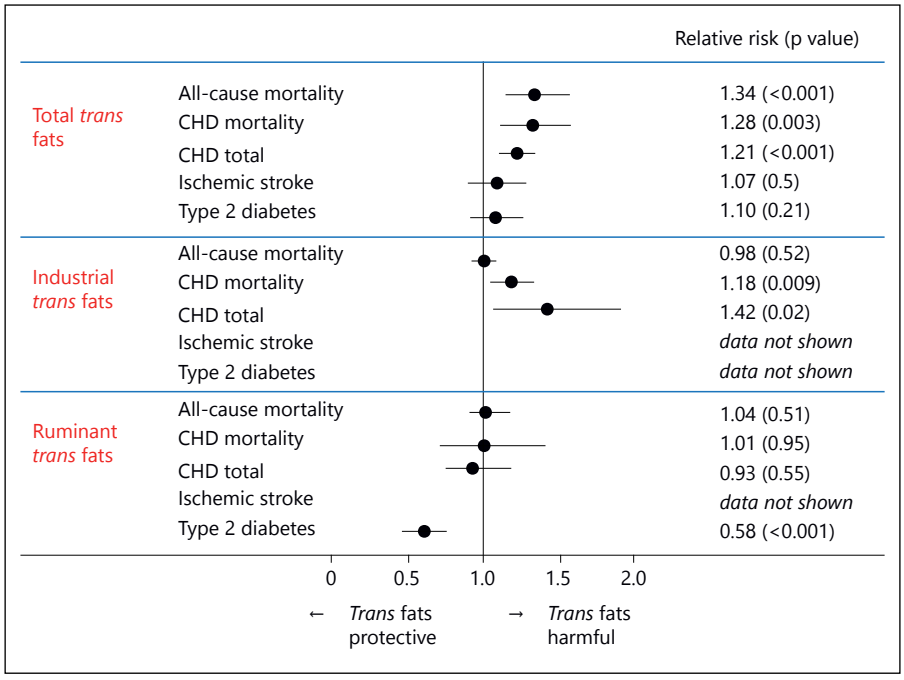

Fig. 28. Intake of *trans*-fats and risk of all-cause mortality, CVD and DM: a meta-analysis of observational studies. Data reproduced in an abbreviated form from de Souza et al. [55].

relatively similar, with ruminant *trans*-fats being slightly but still significantly more hypercholesterolemic than industrial *trans*-fats [56]. Therefore, there is no rational explanation for such a difference between industrial and ruminant *trans*-fats that show opposite correlations with CVD and DM. Instead, we infer that industrial *trans*-fats are a surrogate marker of dihydro-vitamin K_1 that interferes with the vitamin K_2-dependent processes to increase all-cause mortality and CVD mortality (fig. 6). In fact, dihydro-vitamin K_1 has been shown to decrease bone

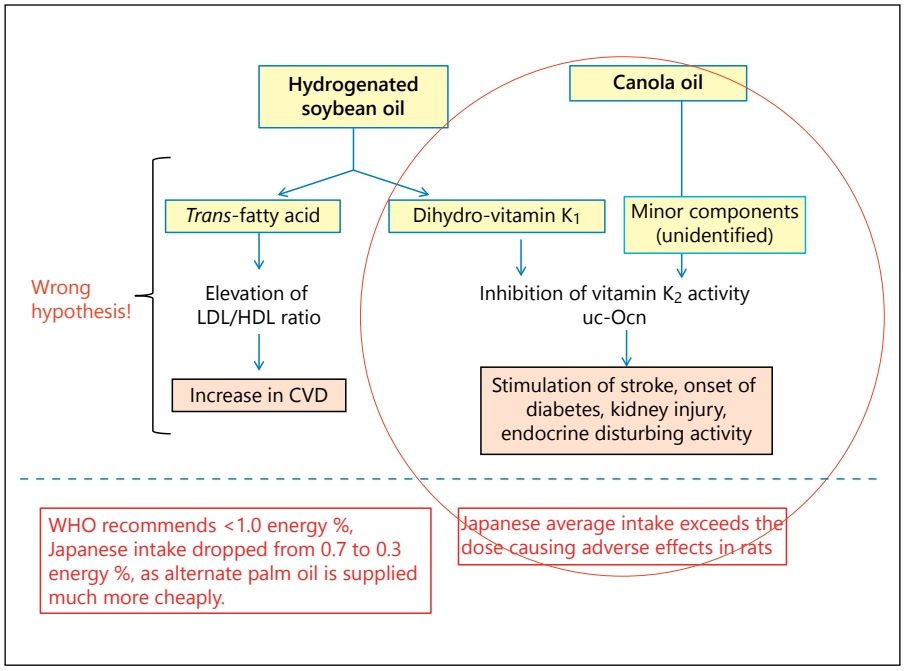

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Fig. 29. *Trans*-fats and dihydro-vitamin K_1 as risk factors for CVD and DM.

mineral density in humans [57]. Similarly, ruminant *trans*-fats are likely to be a surrogate marker of vitamin K_2, both of which are relatively abundant in meat and dairy products. Increased intake of vitamin K_2 is associated with decreased incidence of DM and MetS (fig. 6, 17).

Ocn is a major regulator of bone homeostasis, and positive association of the incidence of hip fracture with *trans*-fat intake was observed in the European countries. Using goldfish scales (tissues very similar to mammalian bone), *trans*-fatty acids (vaccenic and elaidic acids) were shown to decrease alkaline phosphatase activity, a marker of osteoblasts, and mRNA expression for collagen and Ocn, suggesting that *trans*-fats per se could be causatively related to hip fracture [58].

5. Problems Associated with Restricting the Intake of Industrial Trans-Fats and with the Safety of Palm Oil, an Alternative Zero Trans-Fat Vegetable Oil

The reasons for the legal restriction of industrial *trans*-fats were based on the interpretation that (1) increased intake of industrial *trans*-fats elevates the LDL-C to high-density lipoprotein cholesterol (HDL-C) ratio in blood and thereby increases CVD risk, and (2) lowering the LDL-C to HDL-C ratio is effective for reducing CVD. However, there has been serious criticism of both (1) and (2). The effects of dietary industrial *trans*-fats on the LDL-C to HDL-C ratio in blood have been shown by short-term interventions (e.g., 1 month), but such effects are unlikely to be reflected in the ratio after more than several years, as indicated by the results of intervention trials to raise the polyunsaturated to saturated fatty acid ratio for the prevention of CVD as reviewed elsewhere [39, 59]. Moreover, although medications that effectively lower the LDL-C to HDL-C ratio have been developed, such as statin alone or statin + cholesterol ester transport protein (CETP) inhibitor (torcetrapib), no beneficial effects have been observed for the prevention of CVD. In fact, all-cause mortality was increased by lowering the LDL-C to HDL-C ratio using such medications [60]. Among general populations aged ≥50 years, the LDL-C level is inversely associated with all-cause mortality, and high blood cholesterol is a predictor of longevity [39].

The recommendations from WHO and the FDA to restrict the intake of industrial *trans*-fats were based on interpretations (1) and (2) described above (fig. 29). However, we showed above that it is not *trans*-fats per se but the presumed inhibitors of vitamin K_2 activity in vegetable fats and oils which are more likely to be the real risk factors for CVD and DM.

In the food industry worldwide, an alternative zero-*trans* vegetable oil has already been developed and can be supplied much more cheaply than hydrogenated oils; this alternative is palm oil, with physical properties similar to those of margarines. If *trans*-fats are the causative factor

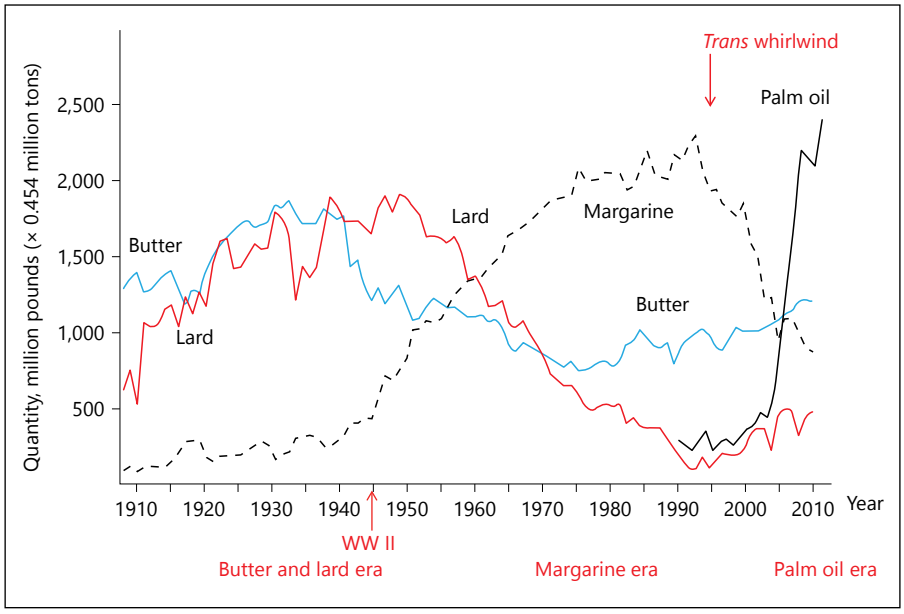

Fig. 30. Trends in US fat consumption, 1909–2010. Data from http://www.cnpp.usda.gov/USFoodSupply-1909-2010. The trend toward palm oil occurred in the USA later than it did in Japan, where butter is often unavailable at local markets.

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for CVD, the recommendations from WHO and the FDA will prompt the food industry to shift to palm oil. However, the real risk factor is likely to be dihydro-vitamin K1 in the hydrogenated oil and its related compound in canola oil, both of which have inhibitory activity toward the vitamin K_2-dependent processes (fig. 29). In addition to palm oil, the safety of some other vegetable oils with survival-shortening activity comparable to that of canola oil in the SHRSP rat (fig. 22) are of serious concern in many countries.

Animal Experiments Show Palm Oil Is Not Safe

Before the end of World War II, butter and lard were the major fats consumed in the US, and these animal fats were subsequently replaced with margarine (partially hydrogenated vegetable oils; fig. 30). The so-called *trans*-fat whirlwind blew around 1995 and palm oil then replaced margarine. Sooner or later, the problems associated with *trans*-fats will be resolved because cheap palm oil is now available. Japan preceded the US in replacing animal fats with palm oil, and this kind of shift is likely going on worldwide.

When butter was replaced with purified palm oil in processed foods, few consumers could tell the difference, although some report actually preferring the taste of foods containing palm oil. Careful Japanese consumers who examine the labeling of processed foods will not find the ingredient 'palm oil' however. Instead, plant (vegetable) fats or processed fats are often stated on the label, and consumers in Japan do not know what specific types of fats are used. Butter is often in short supply at local markets as the import of butter is regulated to protect small-scale cattle breeders in Japan, which has served to accelerate the shift from butter to palm oil.

Despite the palatability of palm oil, its safety has not been established even though many articles from exporting countries emphasize its safety. For example, palm oil unusually promotes mutagen-induced colon carcinogenesis comparable to high-linoleic safflower oil, using perilla oil as a control (fig. 31) [61]. The difference in the rate of carcinogenesis between perilla oil and safflower oil has been explained in terms of the n-6/n-3 balance and the established concept of the arachidonic acid (ARA) cascade promoting carcinogenesis. However, given that the linoleic acid content of palm oil is very low, unidentified minor components must be responsible for the unusual cancer-promoting activity observed. Similarly, olive oil increased mutagen-induced aberrant cryptic foci in rats [62], and the involvement of minor components in olive oil is suspected given that oleic-rich safflower oil with a fatty acid composition similar to that of olive oil does not promote carcinogenesis [63].

In addition to its cancer-promoting activity (fig. 31), palm oil was shown to shorten the survival of mice [64] and induce hyper-insulinemia in rats [65]. We strongly argue, therefore, that increasing the supply of palm oil as an alternative to industrial *trans*-fat is not a safe path to take.

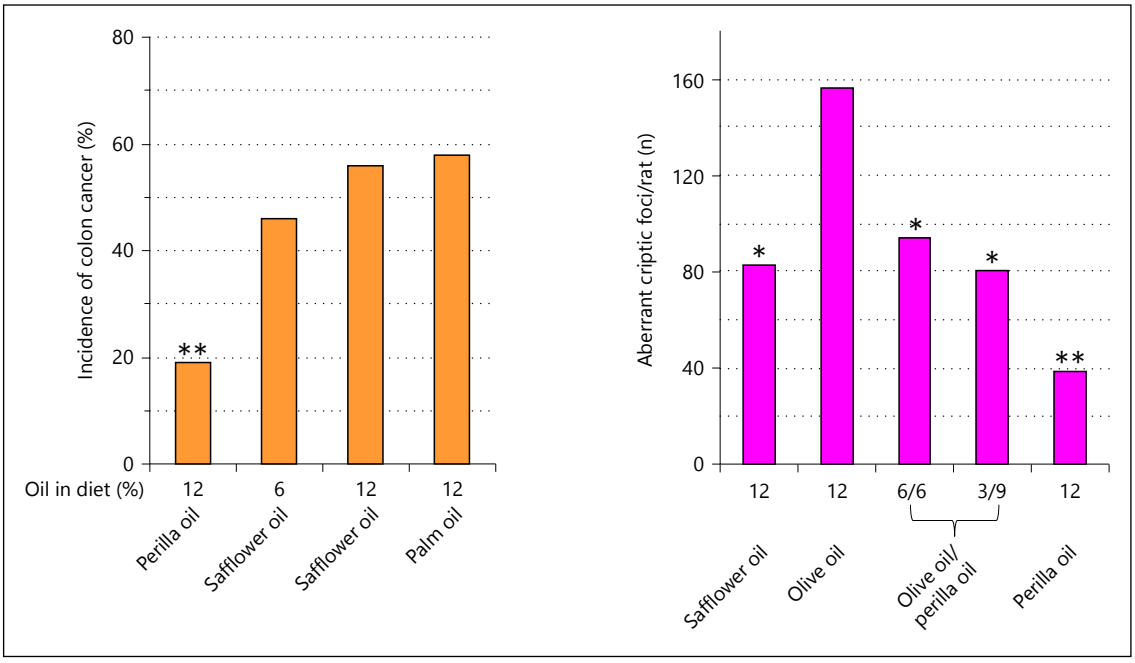

Fig. 31. Unusual cancer-promoting activities of palm oil and olive oil in animal experiments. Data for palm oil (left) are from Narisawa et al. [61] and those for olive oil (right) are from Onogi et al. [62]. Refer to figure 22 for the fatty acid composition of vegetable oils used. In the left figure, ** $p < 0.01$ vs. other groups. In the right figure, * $p < 0.05$ and ** $p < 0.01$ vs. olive oil group.

Anti-Thrombotic Activity vs. Hemorrhagic Activity of Rapeseed Oil and Olive Oil

In the Mediterranean area, CHD mortality is lower than in northern Europe despite the high intake of dietary cholesterol and saturated fat; this is the so-called French paradox [66, 67]. Olive oil and rapeseed oil are common in the Mediterranean area, and the high proportion of oleic acid and the high α-linolenic/linoleic ratio of these vegetable oils have been postulated to be beneficial for the prevention of CHD [68]. However, both of these oils induced the onset of cerebral hemorrhage in stroke-prone SHRSP rats (fig. 23), and inhibitory activity toward the vitamin K_2-dependent processes was demonstrated at least in rapeseed oil (fig. 24). Also, the protective effects of these oils for CHD were significant within a couple of years of intervention, a period which is too short for dietary fatty acids to exert protective effects. In the Helsinki Businessmen Study, the risks of increasing the polyunsaturated to saturated ratio became apparent after several years [69]. Therefore, it is possible that olive oil and rapeseed oil exerted protective effects on CHD by warfarin-like activity to inhibit vitamin K metabolism. The impact of these 2 oils on hemorrhagic disease is yet to be evaluated.

6. N-6/n-3 Balance as a Critical Factor for the Prevention of Lifestyle-Related Diseases

The metabolism of major fatty acids can be classified into 3 pathways (fig. 32).

(1) Saturated and monounsaturated fatty acids are synthesized in our body from carbohydrates and proteins, and they serve as major components of phospholipids and depot fats.

(2) Linoleic acid (n-6 or ω6) is an essential fatty acid synthesized mainly in plants, and it is stored in many kinds of seeds (grains) and nuts. When ingested, it is converted to dihomo-γ-linolenic acid (DGLA) and then to ARA. Both DGLA and ARA are precursors of eicosanoids with diverse physiological activities.

(3) Alpha-linolenic acid (n-3 or ω3) is another essential fatty acid synthesized in plants. When ingested, it is converted to EPA and docosahexaenoic acid (DHA). DHA is relatively rich in brain, retina, testis and muscle and is known to be essential for the maintenance of these organs.

It should be noted that no interconversion occurs in our body among the fatty acids in different pathways (fig. 32), although enzymes catalyzing retro-conversion are present. For example, DHA is converted to EPA and then to α-linolenic acid, and vice versa.

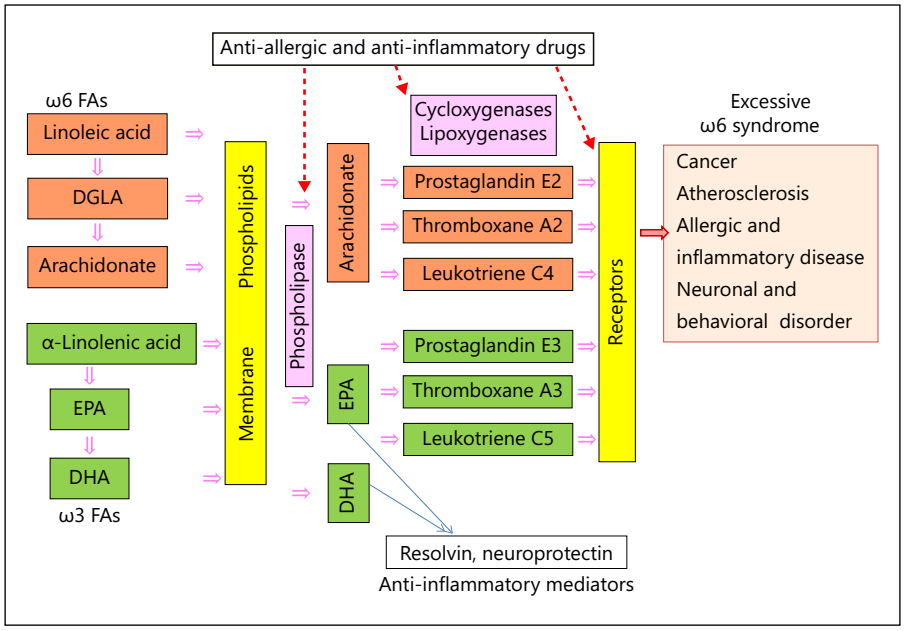

Fig. 32. Three metabolic pathways of major fatty acids in our body.

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Although the production of eicosanoids from linoleic and ARA is essential to maintain normal growth, reproductive physiology and healthy skin, over-production and unbalanced production of n-6 eicosanoids are associated with increased incidence of adenocarcinoma-type cancers, atherosclerotic diseases, allergic and inflammatory diseases (atopic dermatitis, asthma, pollen allergy), neuronal and behavioral disorders and others [70–72].

Eicosanoids of n-3 type are also synthesized from EPA in our body, but the conversion rates are much lower than those from ARA, and the physiological activities of n-3 eicosanoids are much lower than those of n-6 eicosanoids. Moreover, n-6 and n-3 fatty acids compete with each other at the steps of incorporation into and release from membrane phospholipids, conversion to eicosanoids through cyclooxygenases, lipoxygenases and cytochrome p-450, and binding to receptors. Moreover, anti-inflammatory lipid mediators such as resolvins and neuroprotectin are synthesized from EPA and DHA [73, 74]. Because the amount of membrane phospholipids is kept relatively constant, the ARA/EPA ratio and ARA/DHA ratio of phospholipids are modifiable by changing the n-6/n-3 balance of dietary lipids, which is deeply involved in the onset and severity of many diseases, as shown in figure 32.

The summary above has been proven using different techniques:

(1) By nutritional means to examine the effects of diets with different proportions of n-6/n-3 fatty acids on animal physiology.

(2) By pharmacological means to reveal the mechanisms of action of some medicines. For example, steroidal anti-inflammatory drugs exert their effects by inhibiting the release of ARA from phospholipids and cyclooxygenase induction. Aspirin, indomethacin and ibuprofen used as analgesics exert their effects by inhibiting cyclooxygenase, and more than 95% of anti-allergic drugs used in Japan exert their effects by inhibiting the production of eicosanoids and inhibiting their binding to receptors.

(3) By gene technology means. Knockout of the genes encoding phospholipase to release ARA, as well as those of eicosanoid receptors, was effective in suppressing carcinogenesis. More recently, a genetically manipulated mouse model was produced in which a gene encoding an enzyme synthesizing n-3 fatty acids was transduced, and the mutant mouse (*fat-1*) was able to synthesize EPA (n-3) and DHA (n-3) from dietary linoleic acid (n-6). As compared with the wild type, the various diseases listed in figure 32 were suppressed in the *fat-1* model [75, 76]. We are now in the position where it has been established that lowering the n-6/n-3 ratio of phospholipids – either by nutritional means through using oils with a low n-6/n-3 ratio or by using EPA and DHA as drugs or supplements – is effective for suppressing the diseases and symptoms listed in figure 32.

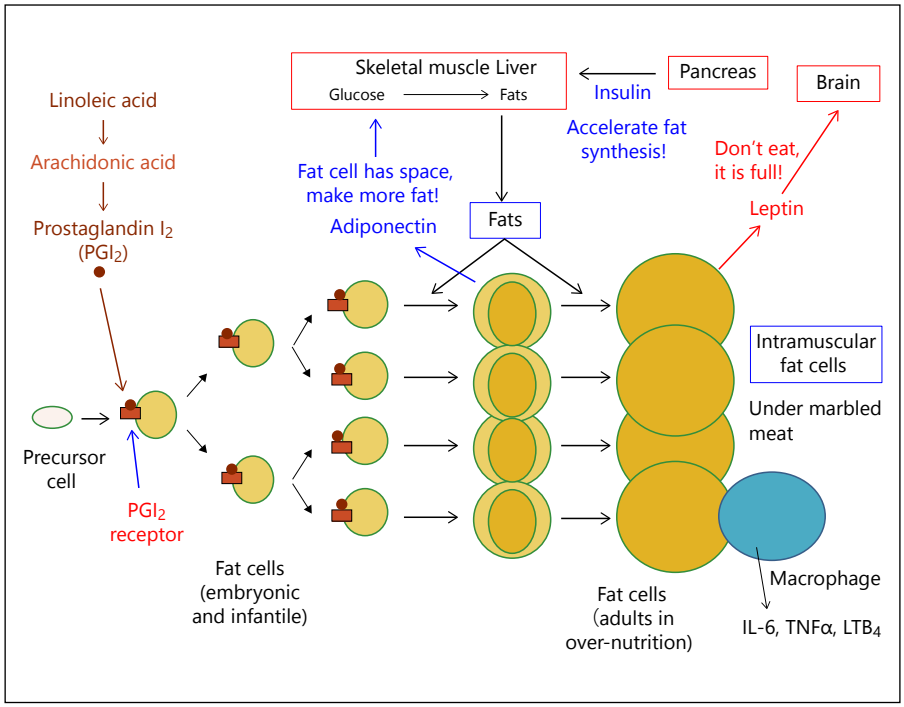

Fig. 33. The number and size of adipocytes regulated by dietary fatty acids. Data based on Massiera et al. [77].

Dietary n-6 and n-3 Fatty Acids in DM

Adipocytes proliferate actively during embryonic and infantile development and prostaglandin I_2 serves as a ligand for its receptor to activate the proliferation of small adipocytes (fig. 33). In fact, the increase in body weight and fat weight of offspring from high-linoleic corn oil-fed dams was higher than when corn oil was partly replaced with perilla oil rich in α-linolenic acid [77].

In adulthood, adipocytes store fat and increase in size. Small adipocytes with capacity to store further fat release adiponectin to accelerate the synthesis of fat from blood glucose, a process that is stimulated by insulin. On the other hand, large adipocytes filled with fat can no longer accumulate fat and release the hormone leptin to suppress appetite and metabolism so as to convert glucose to fat. We hypothesize that the adipocytes filled with fat do not proliferate, or at least the proliferation of large adipocytes filled with fat is the rate-limiting step in the development of insulin resistance and DM.

Among a group of medicines that improve insulin resistance, pioglitazone (Actos) regulates transcription factor PPAR γ to produce more small adipocytes and it improves insulin sensitivity. However, it has serious adverse effects such as severe hepatic failure and teratogenicity, and it is suspected to be carcinogenic. Lifestyle interventions are effective in changing large adipocytes to smaller adipocytes and in improving insulin sensitivity.

When adipocytes become large, macrophages are recruited and they produce inflammatory cytokines such as interleukin (IL)-6, tumor necrosis factor alpha (TNFα) and leukotriene B_4 (LTB_4). The roles of IL-6 and TNFα appear not to be simple, but LTB_4 synthesized from ARA (n-6) was found to be positively associated with DM markers [78]. In the LTB_4 receptor knockout mouse (Ltb4r1-KO), the LTB_4 contents in muscle, liver and adipose tissue were greater in a high-fat diet group (lard as the major source) compared with those of the standard diet group (fig. 34). Fasting insulin level and blood glucose level in the oral glucose test were both lower in the knockout mouse than in the wild type. Combined with the results of experiments using an LTB receptor inhibitor, elevated levels of tissue LTB_4 were positively correlated with the markers of DM.

Correlation of Plasma Lipid Fatty Acid Composition with DM

There have been many papers reporting possible correlations between ingested fatty acids and DM prevalence in different countries. However, we could not deduce any simple conclusions from the data (data not shown), probably due to the fact that none of the food frequency questionnaires distinguish between the intake of different types of oil, for example, soybean oil and canola rapeseed oil, despite their differential effects on DM (fig. 25).

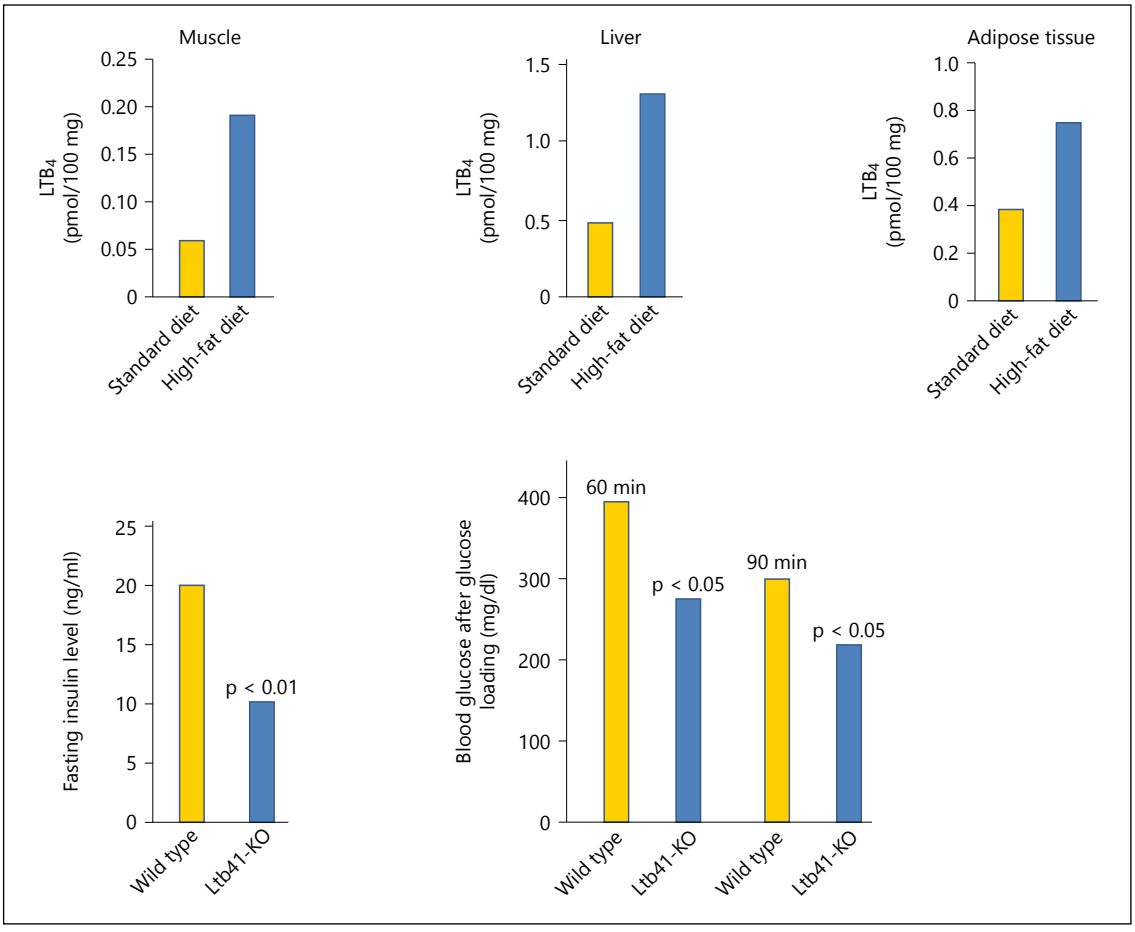

Fig. 34. High tissue LTB_4 levels induced by a high-fat diet are positively correlated with markers of DM. Data taken from Li et al. [78].

Although the tissue fatty acid composition could have been measured more accurately, the proportion of DGLA, but not that of ARA, in plasma phospholipid fatty acids was found to be positively associated with DM prevalence, and that of linoleic acid and *trans*-fatty acids was found to be inversely associated with DM prevalence (fig. 35) [79]. Similarly, the proportion of DGLA in plasma total lipids was found to be positively associated with DM prevalence [80].

As DGLA is synthesized from linoleic acid by ⊿6-desaturase and chain elongation enzyme, and then is converted to ARA by ⊿5-desaturase, these changes in plasma lipid fatty acid composition are consistent with increased ⊿6-desaturase and decreased ⊿5-desaturase activities [81]. These DM-associated changes in the proportion of eicosanoid precursors in plasma lipids may represent a part of our body's adaptive mechanism, because PGE_1 from DGLA is vasodilative and PGE_2 from ARA is vasoconstrictive. The involvement of eicosanoids in the onset and development of DM has not been fully elucidated.

IV. Endocrine Disruption and Behavioral Disorders Induced by Inhibitors of Vitamin K_2-Dependent Processes

1. Ocn May Be Involved in Behavioral Disorders

Ocn is synthesized and carboxylated in osteoblasts to form c-Ocn, which is stored in the matrix phase of the bone. c-Ocn is decarboxylated under the acid conditions produced by osteoclasts. Both c-Ocn and uc-Ocn are released from the bone into the bloodstream. In studying the transport of Ocn across the blood–brain barrier, Oury et al. [7, 8] group demonstrated in Ocn–/– mice that both types are taken up and stored as c-Ocn in the brain. Both

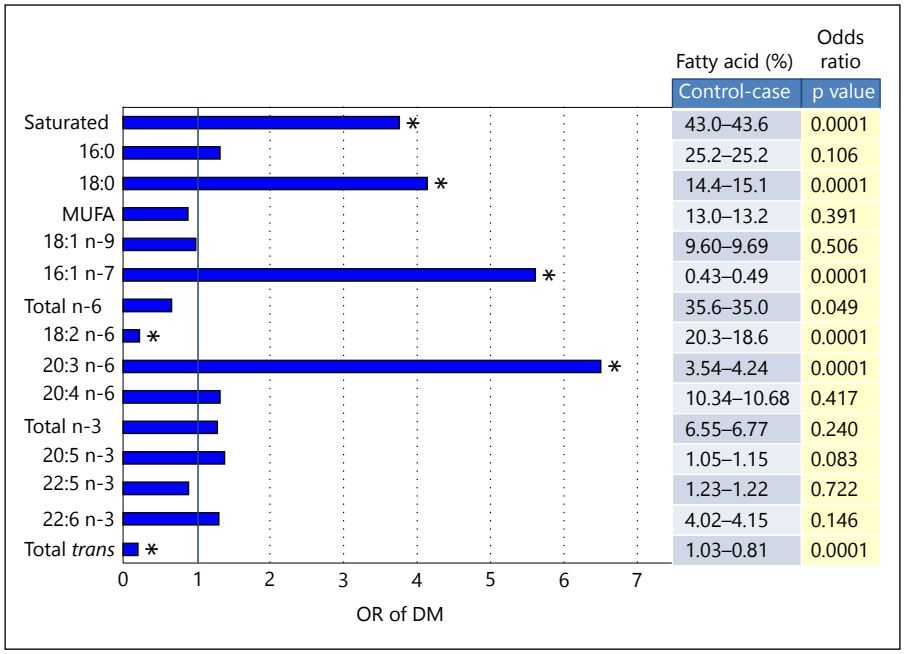

Fig. 35. Relationship between plasma phospholipid fatty acids and DM prevalence: Melbourne Collaborative Research. Data from Hodge et al. [79]. In a prospective case-cohort study of 3,737 adults aged 36–72 years, plasma phospholipid fatty acids (%) were measured at baseline, and diabetes incidence was assessed by self-report 4 years later. The OR for DM represents the ratio of DM prevalence of the highest to lowest quintile of percent fatty acid. Fatty acids are shown by the ratio of chain length to number of double bonds, and the position of the first double bonds numbered from the methyl terminus are shown as n-9, n-7, n-6 or n-3. MUFA, monounsaturated fatty acids.

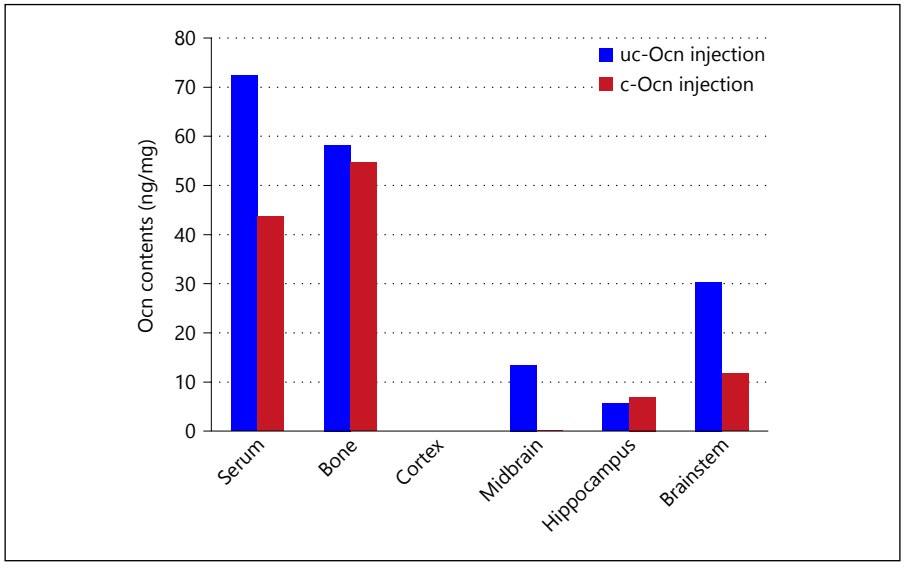

Fig. 36. Tissue Ocn contents in Ocn–/– mice receiving either uc-Ocn or c-Ocn s.c. over 7 days. Data from Oury et al. [8]. No significant amounts of Ocn were detected in Ocn–/– mice without Ocn infusion.

types are equally incorporated into the hippocampus, but uc-Ocn was incorporated into the midbrain and brain stem more efficiently than c-Ocn (fig. 36).

Using the Ocn–/– mice, the effects of infusion of c-Ocn or uc-Ocn on neurotransmitter contents and behavioral patterns were evaluated (fig. 37). Compared with the Ocn–/– group, the infusion of Ocn resulted in an increased level of monoamine and decreased level of GABA (γ-aminobutyric acid), which was associated with elevated memory and learning ability and with decreased anxiety and depression in behavioral tests.

Medicines for CVD, statins and warfarin, and vegetable oils such as canola and hydrogenated soybean oil were shown to interfere with vitamin K_2-dependent processes (fig. 20, 24); hence, they are likely to affect behavioral patterns through altered Ocn metabolism (fig. 37). In fact, statins have been reported to cause memory loss and decreased libido in both sexes. In mice, the canola oil group

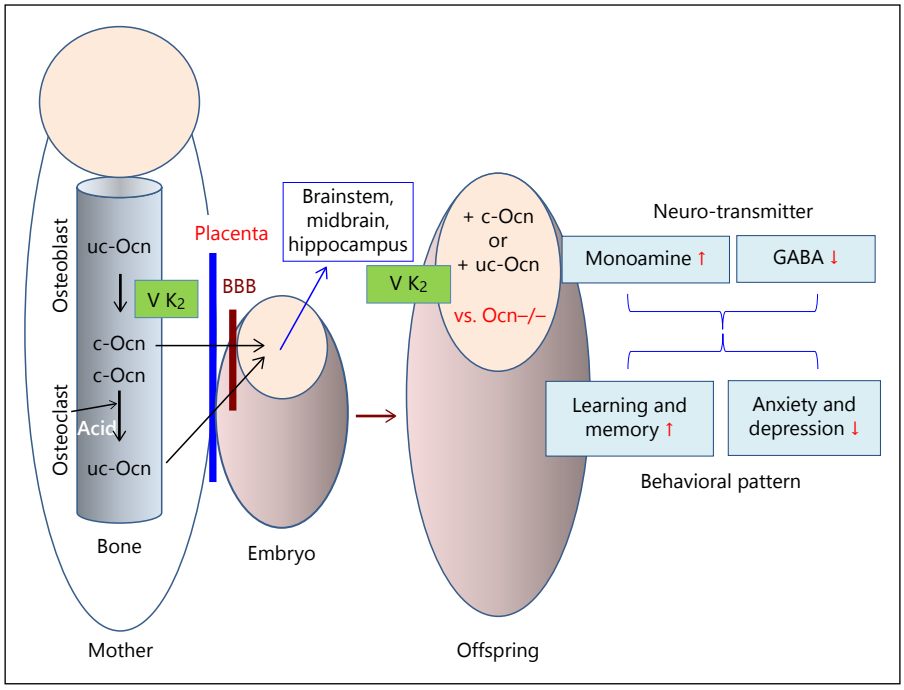

Fig. 37. Maternal and offspring pools of Ocn influence brain development and function. Based on Oury et al. [8]. In the Ocn–/– mouse model, Ocn was shown to be transported to the embryo across the blood–brain barrier and affect the behavior of the offspring.

exhibited extremely high spontaneous locomotor activity, higher ambulation and rearing activities in an open-field test and a lower pain threshold compared with mice fed other vegetable oils [82]. These findings could not be accounted for by differences in the n-6/n-3 ratio of fatty acids, another dietary factor affecting animal behavior [83]. It is likely that these behavioral disorders were induced at least in part by decreased testosterone content, as will be explained in the next section.

2. Endocrine Disruption by Vegetable Oils and Crucial Seminal Fluid Status in Humans and Other Mammals

Gene expression related to steroidal hormone metabolism in the rat testis, as measured by microarray analysis, was significantly different between the group fed a canola oil-supplemented diet and the group fed a soybean oil-supplemented diet [84]. Such genes as *StAR*, *Cyp11*, *Cyp17* and *3β-HSD* were downregulated in the canola oil group. Plasma and testis testosterone levels were significantly higher in the soybean oil group (fig. 38). The antiperoxidative enzymes in red cells and plasma LDL-C levels were lower in the canola oil group than in the soybean oil group [52].

Dioxins are the most powerful endocrine-disrupting substances known so far. However, there is more than a 200-fold difference between the dioxin dose that inhibits testosterone production in animal experiments (161 pg TEQ/kg/day, toxicity equivalency quantity) and the average daily intake of Japanese people (0.6 pg TEQ/kg/day in 2012). Rapeseed oil also exhibited endocrine-disrupting activity in the SHRSP rat, but the dose that decreased the tissue testosterone level was comparable to the average intake of Japanese people (6–11% of energy). Therefore, these vegetable oils may be the strongest endocrine-disrupting substances in today's environment.

Testosterone biosynthesis in the Leidig cells was under the control of Ocn (c-Ocn, uc-Ocn), and their binding to a presumed receptor leads to upregulation of related genes (fig. 39) [7]. Testosterone and derived dihydrotestosterone are known to stimulate spermatogenesis and suppress apoptosis in adjacent spermatocytes.

We noted above that statins, warfarin, canola oil and hydrogenated soybean oil inhibit the vitamin K_2-dependent processes and decrease testosterone production in animals. Currently, 2 facets of reproductive physiology are important issues in Japan. First, the rate of successful insemination of cattle by registered Domestic Animal Inseminators reached almost 60% in 1985, but this dropped gradually to below 50% in 2000 in Japan's northernmost prefecture. The specialist inseminators argue that the sexual cycle of cattle might be abnormal. Second, tendencies for decreased sperm concentration and fewer normal sperm have been noted over the last quarter of a century in Japanese men as well as in the men of Western coun-

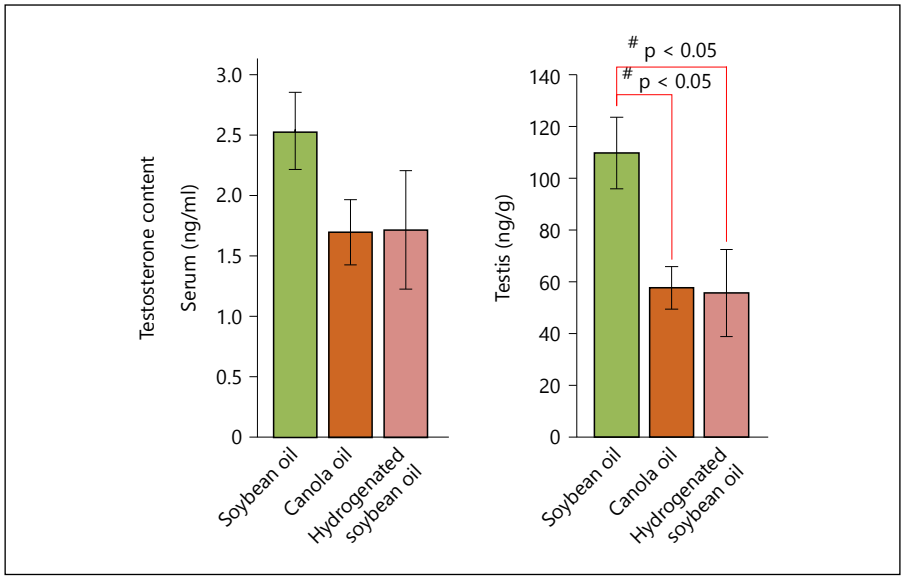

Fig. 38. Comparison of tissue testosterone levels in the SHRSP rat fed a soybean oil-supplemented diet, a partially hydrogenated soybean oil-supplemented diet or a canola oil-supplemented diet. Data from Okuyama et al. [84]. After repeating similar experiments 3 times, the differences in the serum testosterone levels of the pairs of groups became significant ($p < 0.05$; data not shown).

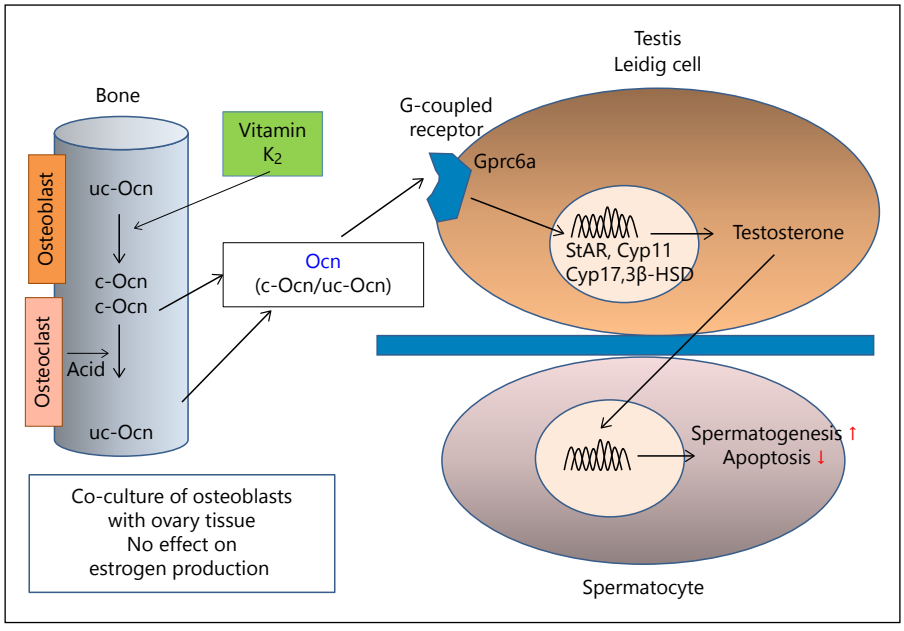

Fig. 39. Regulation of male fertility by the bone-derived hormone Ocn. Data from Oury et al. [7] and others are shown schematically.

tries, and the current situation in Japan is worse than that in European countries (fig. 40) [85].

Because of the relatively young age of the participants, and due to the fact that statins began to be used widely after 1990, the proportion of those taking statins or warfarin and the impact of these medications on the semen properties would be relatively small during the period after 1990, when the changes in semen properties began to be recognized. On the other hand, concentrated feed made of grain meal began to be given to cattle and extracted vegetable oils to humans from 1960 to 1970 in these countries. There may be a 20-year lag before the decrease in semen quality in fertile men was first seen. In the veterinary field, it is known that upper limits were set for the daily supply of rapeseed meal for cows, chickens and pigs to avoid adverse effects, whereas no upper limit was set for soybean meal, which reflects the results of animal experiments described above. Compared with soy-

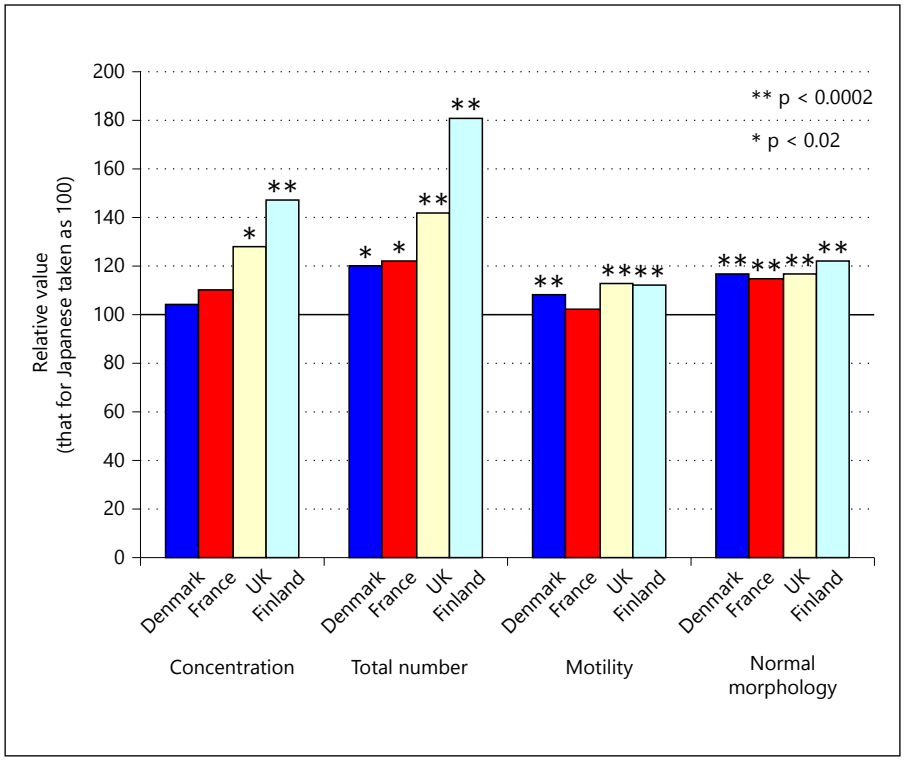

Fig. 40. Comparison of semen quality in fertile Japanese and European men. Data taken from Iwamoto et al. [85].

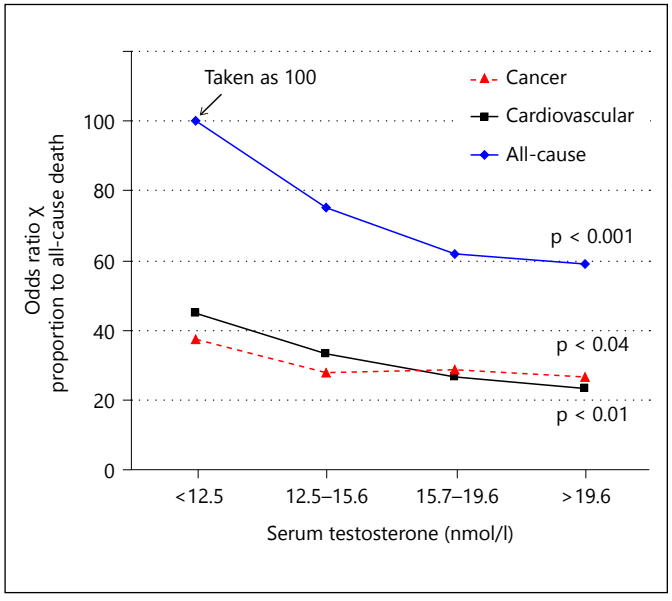

Fig. 41. A case-control study on mortality and serum testosterone level: EPIC-NORFOLK Study. Data from Khaw et al. [88]. The participants (men aged 40–79 years) were followed for 7 years on average, and those who died (n = 825) and those who were alive (n = 1,489) were compared. On the vertical axis, all-cause mortality at the lowest testosterone level was taken as 100.

bean meal, rapeseed meal caused testis hyperplasia in boars [86].

To close this section, we would like to emphasize that the increased supply of meals with residual oils and extracted vegetable oils from such grains as rapeseed, corn and cottonseed could be one of the major factors resulting in decreased tissue testosterone levels that is affecting the related male physiology. Testosterone is also synthesized in females and is involved in the development of female characteristics. In an editorial, Andersson et al. [87] pointed out the 'adverse trends in male reproductive health: we may have reached a crucial 'tipping point''.

3. Testosterone Levels Related to CVD and DM

CVD mortality is generally higher in men than in women; so it has been postulated that a low androgen/estrogen balance is beneficial for the prevention of CVD. However, the EPIC-Norfolk Study revealed that the mortality rates for cancer, CVD and all causes were inversely associated with serum testosterone levels [88] (fig. 41).

In a clinical RCT using 2% testosterone gel for hypogonadal men with DM and/or MetS, testosterone supplement was effective in suppressing insulin resistance, but HbA1c level did not decrease over 12 months of treat-

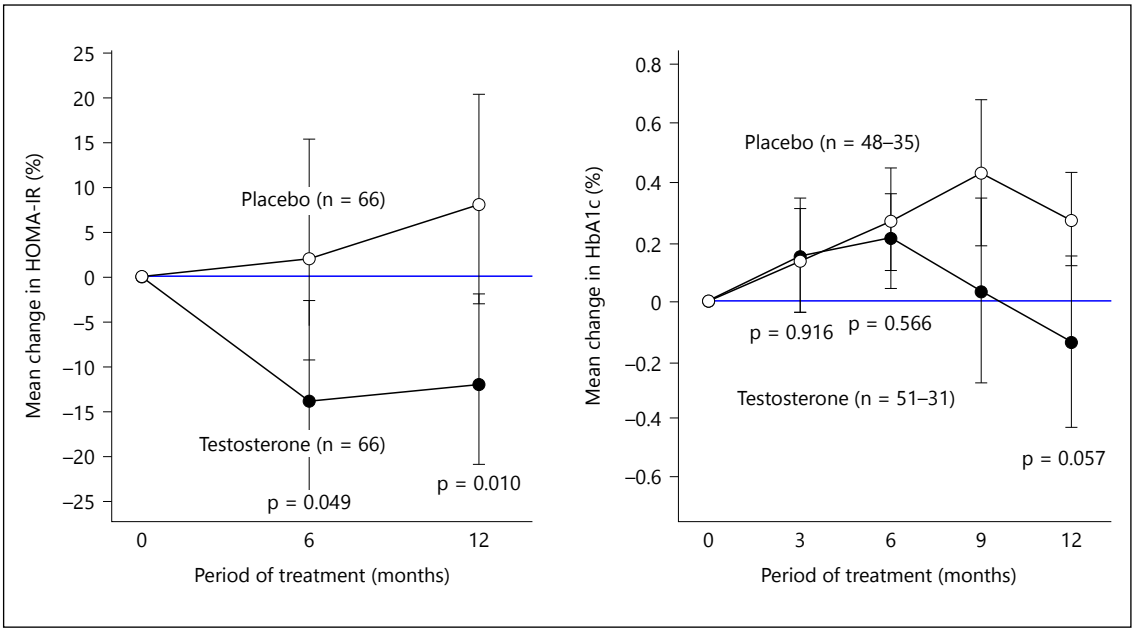

Fig. 42. Effect of testosterone supplementation for hypogonadal men with DM and/or MetS. Data from the TIMES2 Study. Jones et al. [89]. The efficacy, safety and tolerability of a novel transdermal 2% testosterone gel was evaluated over 12 months in 220 hypogonadal men with DM and/or MetS in a multicenter, prospective, randomized, double-blind, placebo-controlled study.

ment [89] (fig. 42). The authors concluded that over a 6-month period, transdermal treatment was associated with beneficial effects on insulin resistance, total and LDL-C, Lp(a) and sexual health in hypogonadal men with DM and/or MetS. These observations emphasize the importance of maintaining testis physiology and appropriate tissue testosterone levels, and of avoiding environmental factors affecting tissue testosterone levels as much as possible.

V. New Recommendations for the Prevention of CVD, DM and Other Lifestyle-Related Diseases: Section Summary and Perspectives

In this section, first, we summarize our evaluation of the medications currently used for the prevention of CVD and DM. Then, we propose new directions in lipid nutrition for the prevention of these and other elderly onset diseases, disregarding the cholesterol hypothesis. The endocrine-disrupting activities of medications for CVD and some commonly consumed vegetable fats and oils are emphasized. Finally, we would like to emphasize the need for scientists disregarding socioeconomic pressures and instead focus on promoting health for the majority of people.

1. Medications That Can Cause CVD and DM

In this review, we have discussed statins, warfarin and medications for DM. The latest cholesterol guidelines from ACC/AHA (NHLBI) 2013 recommend statins for the control of blood cholesterol level. We are in a position to accept the results of clinical trials performed after 2004 once the new penal regulations came into effect in the EU, but the conclusions of meta-analyses that include clinical trials performed before 2004 cannot be taken as reliable.

Statins and CETP Inhibitor

Clinical RCT studies performed after 2004 failed to demonstrate the effectiveness of statins for the prevention of CVD although all were successful in lowering blood LDL-C levels. The combination of a statin and CETP inhibitor significantly decreased LDL-C levels and significantly increased HDL-C levels, but this combination therapy was not effective in preventing CVD events and in fact increased all-cause mortality. The pharmacological mechanisms of statins to stimulate atherosclerosis and heart failure have been revealed, at least in part, elsewhere [3] and discussed in this review. New RCT studies would be impossible to perform now because the mechanisms of statins that lead to diverse and irreversible adverse effects have been reported and gaining informed consent for them would naturally be difficult and inadvisable.

Warfarin

Warfarin's mechanisms of action have been known from the outset, but the diverse and essential roles of vitamin K_2 have been revealed only relatively recently. The short-term beneficial effect of warfarin to lower thrombotic tendency is obvious, but the long-term adverse effects of inhibiting vitamin K_2-dependent processes have become apparent over time. Clinical doctors should pay special attention to the long-term adverse effects of warfarin.

Medications for DM

Several types of medication to control DM-related markers have been developed. However, these medical interventions have been unsuccessful in reducing complications (e.g., CVD, mortality), as have lifestyle modification programs.

Thus, factors other than the known risk factors for CVD and DM need to be taken into account, and here we propose statins, warfarin and some vegetable fats and oils as causative factors for these diseases.

2. Paradigm Shift in Lipid Nutrition for the Prevention of Lifestyle-Related Diseases

From the Cholesterol Hypothesis to the n-6/n-3 Balance

Lipid nutrition has long been developed based on Ancel Keys' equation relating to dietary fatty acids and blood cholesterol. Short-term dietary manipulation to raise the polyunsaturated to saturated ratio of foods results in a transient decrease in blood cholesterol levels as Keys' equation predicts. However, long-term intervention based on Keys' equation results in no significant changes in blood cholesterol but increases mortality from cancer, violent death and all causes as reviewed earlier [39, 58, 70] and those pointed out recently [90–92]. Moreover, the intake of dietary animal fats (saturated fatty acids) and cholesterol was shown to be inversely associated with ischemic stroke, and no significant increase in CHD mortality has been demonstrated by increasing saturated fat and cholesterol intake [93].

High blood cholesterol was demonstrated to be a predictor of longevity; the higher the cholesterol level, the lower the mortality rates for cancer, infectious diseases and all-cause mortality among general populations, particularly in aged populations; thus, it is not the saturated to polyunsaturated ratio but the n-6/n-3 ratio of dietary lipids that is crucial for the prevention of CVD, DM and several other elderly onset diseases [39, 58, 70] as Bibus and Lands [94] recently reviewed. Currently, people in industrialized countries generally ingest several-fold more linoleic acid than is essential (0.5% of energy) and the intake of n-3 fatty acids is relatively deficient. The more than 3-fold higher CHD mortality in the US as compared to Japan is likely to be due to the higher n-6/n-3 balance of ingested foods in the US.

Minor Components Other Than Fatty Acids and Phytosterols of Oils

Another important aspect of lipid nutrition is that some vegetable oils and hydrogenated oils contain minor components that are detrimental to animal physiology. Canola and hydrogenated soybean oil accelerate the onset of cerebral hemorrhage and kidney injury to shorten the survival of SHRSP rats at a dose comparable to that ingested by the average Japanese person. Some other vegetable oils including olive oil, corn oil, high oleic safflower oil and evening primrose oil share common properties that are known to induce stroke and shorten survival in SHRSP rats. Animal fats such as butter and lard are relatively safe in this animal model, and increased intake of cholesterol and saturated fatty acids is beneficial for the prevention of ischemic stroke. Nutritional evaluation of fats and oils must be made based on both the fatty acid composition and the minor components.

Trans-Fats

Generally accepted interpretations of the risk of *trans*-fats need to be revised based on the scientific evidence available. Long-term feeding of *trans*-fats (e.g., for several years) is unlikely to elevate the LDL-C/HDL-C ratio. Even if it were to elevate it, lowering the LDL-C/HDL-C ratio would not be beneficial for the prevention of CVD because treatment with statins or statin + CETP inhibitor has failed to show any benefits. High plasma LDL-C is a predictor of longevity in general populations aged ≥40–50 years. With respect to the reported correlations between the intake of *trans*-fat and CVD or DM, we propose industrial *trans*-fat as a surrogate marker for dihydro-vitamin K_1, as it interferes with the vitamin K_2-dependent processes to increase CVD and all-cause mortality, and ruminant *trans*-fat as a surrogate marker for increased vitamin K_2 intake, which is beneficial for the prevention of DM.

3. Endocrine Disruption by Certain Medications and Vegetable Fats and Oils

Here we would like to highlight 3 main points regarding the endocrine disruption caused by certain medica-

tions and vegetable fats and oils. First, statins and warfarin lead to tissue vitamin K_2 deficiency and endocrine disruption. Ocn synthesized in bone is γ-carboxylated by a vitamin K_2-dependent enzyme and stored in the matrix phase of bone. The bone-derived Ocn regulates the synthesis of testosterone that controls spermatogenesis as well as the development of sexual characteristics.

Second, partially hydrogenated soybean oil and rapeseed oil decrease tissue testosterone levels compared with soybean oil in SHRSP rats partly through inhibition of the vitamin K_2-dependent activation of Ocn. Several other vegetable oils with survival-shortening activity in SHRSP rats are suspected to have testosterone-lowering activity similar to that of rapeseed oil. In evaluating the impact of observed endocrine-disrupting activity, dose-dependency is an important criterion. There is a >200-fold difference between the amount of dioxins to lower testosterone levels in animal experiments and the amount currently ingested by the average Japanese person, but the 2 amounts are comparable in the case of vegetable fats and oils.

And third, emphasis has been placed on the importance of considering causal relationships among the trends seen in increased vegetable oil intake, decreased semen properties the decreased rate of successful insemination in cattle and the increased incidence of behavioral disorders in younger generations. Testosterone (androgen) is converted to estrogens, and the production of these steroid hormones in different stages of development in different tissues is required for proper development of male and female characteristics, including behavioral performance.

4. Placing Emphasis on Human Health by Overcoming the Pressures of Current Industrial and Socioeconomic Structures

Rapeseed, soybean and corn have been used as feed materials for stock raising and poultry farming because of their good amino acid scores, energy content and apparent (short-term) safety. Oils are extracted from these grains to produce meal for cattle, while vegetable oils rich in linoleic acid have been extracted for human use. In every country every year, the amounts of different vegetable oils produced need to be consumed in order to be economical. Keys' equation allowed industry to convince consumers that linoleic-rich vegetable oils were good for their health. Even after Keys' equation was found not to be applicable for the prevention of chronic diseases, and excessive intake of linoleic acids was found to increase cancer, CVD and all-cause mortality, certain countries exporting these grains would not accept the risks associated with linoleic-rich vegetable oils.

In the health food sector in Japan, more than 10 kinds of food were claimed to be effective in lowering plasma cholesterol, and their labeling as 'Food for Special Dietary Use' was approved by the Consumer Affairs Agency of the Ministry of Internal Affairs and Communications. On television, information on these foods appears hourly and full-page advertisements are often seen in major newspapers, successfully convincing the majority of people to accept the discredited hypothesis that a high cholesterol level is bad for the health and linoleic-rich vegetable oils are good. Needless to say, statins are a huge financial interest to the pharmaceutical industry.

In this era, when big industry exerts enormous influence over the media, and nutritional and treatment guidelines are issued by professional societies in favor of industry, we seem to have largely lost our way in the promotion of human health. However, despite the pressures from current industrial and socioeconomic structures, many scientists in medical and nutritional fields working in evidence-based research have begun to raise their voices and we join them in unison because the impact of increasing the intake of some vegetable oils on human nutrition seems to be much more severe than what we previously thought.

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