

A Critical Review of the Consensus Statement from the European Atherosclerosis Society Consensus Panel 2017

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Keywords

Consensus Statement from the European Atherosclerosis Society Consensus Panel 2017 · Critical interpretation · Low-density lipoprotein · Atherosclerotic cardiovascular disease · Mendelian randomization study · Statin · Warfarin · Vegetable oil · Randomized controlled trial · Prospective cohort study

Abstract

Background: The Consensus Statement from the European Atherosclerosis Society (EAS) Consensus Panel 2017 concludes on the basis of 3 different types of clinical studies that low-density lipoprotein (LDL) causes atherosclerotic cardiovascular disease (ASCVD). In Mendelian randomization studies, rare genetic mutations affecting LDL receptor function were found to cause higher or lower LDL-C levels, which are associated with correspondingly altered ASCVD risk. In prospective cohort studies and randomized controlled trials (RCTs) of statins, a remarkably consistent log-linear association was demonstrated between the absolute magnitude of LDL-C exposure and ASCVD risk. The EAS Statement proposes that any mechanism of lowering plasma LDL concentration should reduce the risk of ASCVD events proportional to

the absolute reduction in LDL-C and the cumulative duration of exposure to lower LDL-C. However, as we explain, we do not find this conclusion acceptable. **Summary:** Our review points out that different interpretations are possible for the results of Mendelian randomization studies. As for prospective cohort studies, many inconsistent reports on the association of LDL-C and ASCVD were disregarded when drafting the Statement, reports with and without genetic factors related to LDL receptor function should be analyzed separately, and the term ASCVD in the Statement is used inappropriately because myocardial infarction and cerebral infarction differ in their association with LDL-C. As for RCTs, clinical reports on statins published before and after the implementation of new regulations affecting clinical trials (2004/2005) should not both be included in meta-analyses because the evaluated efficacy of statins changed markedly, and the irreversible adverse effects of statins need to be evaluated more rigorously now that their mechanisms have been elucidated. **Key Messages:** Apart from the EAS hypothesis that LDL causes ASCVD, recent pharmacological/biochemical studies, as summarized in this review and elsewhere, have revealed that atherosclerosis is caused by statins taken to lower LDL-C, as well as by warfarin and some types of vegetable fats and oils, in the absence of significantly elevated

LDL-C levels. Thus, the promotion of statin treatment by the Statement is rather risky and we do not feel that the conclusions are justified for the prevention of ASCVD.

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Introduction

Based on 1-month intervention trials performed in the 1950s, the Keys equation and Hegsted equation were developed to predict plasma cholesterol levels as a function of dietary saturated fatty acids, cholesterol, and polyunsaturated fatty acids (essentially, linoleic acid). When taken together with the observation that arterial plaque is rich in cholesterol, the so-called cholesterol hypothesis, cholesterol myth, or lipid-diet hypothesis of atherosclerosis spread widely. The hypothesis was applied in the agricultural world by, for example, producing oilseed and oilseed meals as feed for livestock, poultry, and cultured fish, while vegetable oil byproducts were used in the human food supply. Although the Keys and Hegsted equations predict changes in plasma cholesterol levels on a short-term basis (months), they do not predict the consequences of longer intervention periods (several years), as shown in the MRFIT study [1] and Helsinki Businessmen study [2]. Moreover, intervention trials based on the cholesterol hypothesis resulted in increased cardiovascular disease (CVD) [2–4], and pharmacological and biotechnological studies have revealed mechanisms by which an increased dietary ratio of polyunsaturated (linoleic acid) to saturated fat leads to increased CVD [5–9]. Despite such progress in the field of evidence-based medicine, even now some scholars and organizations, including the World Health Organization, recommend increased intake of vegetable oils with high ratios of linoleic acid to α -linolenic acid ($\omega 6/\omega 3$). It seems that these groups have allowed the maintenance of current industrial structures to take precedence over benefit to human health [10].

Coronary heart disease (CHD) is a major cause of death in many Western countries, and the development of statins in the 1980s was welcomed because they are very effective for lowering low-density lipoprotein cholesterol (LDL-C) levels. No adverse effects of statins were apparent within several years of their use, despite the fact that their carcinogenic, teratogenic, and immunosuppressive activities were revealed in early clinical studies with chronic use [10].

Randomized controlled trials (RCTs) of statins, performed mainly in the 1990s, reported a roughly 30% decrease in LDL-C and CHD events, which led to the phi-

losophy of “the lower the better.” However, practicing clinicians have claimed that the benefits of statins were overstated, and many scientists have noted the potential problems associated with clinical reports published by industry-supported groups, which have been discussed openly in established medical journals. For example, as the Vioxx scandal unfolded, the innate tendency of industry actors to improperly pursue their own interests within a lawful but shady range was legally criticized. In this context, new and more stringent regulations for clinical trials came into force in the EU and US in 2004/2005. The evaluated effectiveness of statins changed substantially after these new regulations took effect, and no significant benefits of statins have since been reported for the reduction of CHD mortality or all-cause mortality; indeed, little if any benefit has been noted for the prevention of CHD even when less objective composite end points were assessed [10–12].

RCTs conducted by industry-supported scientists have often been terminated within several years, before adverse effects become more apparent. Moreover, they tend to explain little about irreversible adverse effects such as teratogenicity, carcinogenesis, immune suppression, cognitive dysfunction, kidney failure, and even endocrine-disrupting activity, the pharmacological/biochemical mechanisms of which have been elucidated [10]. However, statin-associated risks have gradually become recognized in the field of evidence-based medicine. Therefore, the Consensus Statement from the European Atherosclerosis Society (EAS) Consensus Panel 2017 (hereafter, the EAS Consensus Statement 2017) with its conclusion that “LDL is the cause of atherosclerotic CVD (ASCVD)” [13] was unexpected.

This review is based not on a formal systematic survey of clinical trial data, but rather on a restricted number of papers, including those from basic studies, that met the following criteria. To assess the efficacy of treatments for the prevention of ASCVD, mortality rates (especially all-cause mortality) were considered the strongest objective end points, and composite end points, including hospitalization, revascularization, and stroke events were considered less objective. Statin RCTs published before 2004/2005 and any meta-analyses that included them were considered to be less reliable, for the reasons noted above [14]. In this review, we critically evaluate the EAS Consensus Statement 2017, emphasize the risks of statin medications, and propose new causes of ASCVD that do not involve elevated LDL-C levels. Following the style of the Statement, we first summarize our views compared with those of the EAS Consensus Panel (Table 1).

Table 1. LDL and ASCVD: EAS criteria for causality and our critical evaluation

EAS consensus statement 2017		Critical evaluation by the present authors
1. Plausibility	LDL and other apoB-containing lipoproteins are directly implicated in the initiation and progression of ASCVD; experimentally induced elevations in plasma LDL and other apoB-containing lipoproteins lead to atherosclerosis in all mammalian species studied.	a. Older subgroups among general populations exhibit no significant positive associations, but often inverse associations between LDL and ASCVD. b. It is generally understood that in animal models such as the Watanabe heritable hyperlipidemic rabbit and apolipoprotein knockout mouse, fatty streaks are produced at the lumen side of the endothelial cell layer; these are likely different from the irreversible atherosclerotic plaques that develop at the intima layer of human blood vessels. c. No evidence from animal models has been presented to indicate that LDL-lowering medications are effective in preventing ASCVD mortality.
2. Strength	Monogenic and polygenic-mediated lifelong elevations in LDL lead to markedly higher lifetime risk.	a. No association or even exposure-dependent inverse associations are seen between the absolute magnitude of exposure to LDL and the objective measure of ASCVD mortality. These are the opposite findings against those with less objective measure of ASCVD events; hence, the association is not consistent with the outcome (see 6. Consistency) and not strong. Moreover, the association is rather inversely strong. b. In FH cases, elevation of LDL is almost synonymous with suppression of the uptake of LDL-carried fats, a major energy source for cardiac muscles. The lifetime restriction of energy supply, rather than high LDL levels per se, is more likely to be the major cause of ASCVD because the impact of LDL on ASCVD decreases with age; indeed, inverse associations between LDL and ASCVD events have even been noted in older general populations. c. The Statement overlooks lifetime restriction of fat energy as a potential cause of ASCVD, the mechanism of which is inconsistent with current LDL-lowering medications. The 2 consequences of impaired LDL receptor function—elevated LDL and suppressed intake of LDL-carried lipids—require entirely different medical treatments. Even in the case of FH, no evidence has been presented to indicate that lowering LDL is effective for reducing CVD mortality.
3. Biological gradient	Monogenic lipid disorders, prospective cohort studies, Mendelian randomization studies, and randomized intervention trials uniformly demonstrate a dose-dependent, log-linear association between the absolute magnitude of exposure to LDL and risk of ASCVD.	a. The LDL receptor gene consists of 18 exons, some of which encode sequences similar to coagulation factors, complement c9, and the EGF precursor. Mutations in or near the LDL receptor allele could be associated with coagulability, inflammation, and endothelial lability, which may be more important for arterial pathology than high plasma LDL-C per se. ASCVD is not a monogenic lipid disorder, and genetic confounding factors must be considered in deducing causality. b. Biological mechanisms have been revealed whereby statins stimulate atherosclerosis while simultaneously lowering LDL, and warfarin and some types of vegetable fats and oils can cause ASCVD through vitamin K2-osteocalcin pathways without significant LDL elevation.
4. Temporal sequence	Monogenic lipid disorders and Mendelian randomization studies demonstrate that exposure to elevated LDL precedes the onset of ASCVD.	a. In the case of LDL receptor mutations, lifetime exposure to decreased energy supply leading to ischemic conditions is likely the major cause of ASCVD. Genetic confounders associated with LDL receptor mutations are likely risks for increased coagulability, TNF- α -mediated inflammation, and EGF-mediated endothelial lability. Hence, other confounders (or true risk factors) can precede arterial pathologic changes. b. Statins and some types of vegetable fats and oils share a common mechanism for impairing vitamin K2 and bone hormone (osteocalcin) activity, leading to vascular calcification and ischemic conditions without involving LDL.
5. Specificity	Mendelian randomization studies and randomized intervention trials both provide unconfounded randomized evidence that LDL is associated with ASCVD independent of other risk factors.	There are many genetic confounders associated with Mendelian randomization studies, as explained above. Randomized statin intervention trials that use composite endpoints that include less objective measures (e.g., coronary revascularization and nonfatal cardiovascular events) are used in the Statement. No significant suppressive effects of statins on more objective measures (e.g., CVD mortality and all-cause mortality) have been demonstrated since the 2004/2005 enactment of new clinical trial regulations in the EU and US.

Table 1. (continued)

	EAS consensus statement 2017	Critical evaluation by the present authors
6. Consistency	Over 200 studies involving more than 2 million participants with over 20 million person-years of follow-up and more than 150,000 cardiovascular events consistently demonstrate a dose-dependent, log-linear association between the absolute magnitude of exposure to LDL and risk of ASCVD.	a. Association is not consistent with the outcomes: apparent positive associations were seen by the less objective measure of ASCVD events and negative or even inverse associations were seen with more objective outcome measure of mortality (ASCVD and all-cause mortality: see 10. All-cause mortality). b. The enormous scale of a meta-analysis of RCTs does not necessarily guarantee that its conclusion is valid. We point out the presence of relatively large-scale follow-up studies with results that are inconsistent with the huge meta-analysis in the Statement. Many inconsistent reports among the 3 types of studies listed in the Statement were excluded from consideration. c. The relationship between LDL and ASCVD events is not continuous; although a small number of patients with extremely high LDL-C levels, including FH cases, exhibit high ASCVD event rates, CVD outcomes among the majority of the general population are not substantially affected by LDL-C d. To deduce a log-linear association, odds ratios and hazard ratios are exponentiated and converted to percentages, which are then shown on a non-uniform scale. However, both increased and decreased mortality rates have been associated with statin treatment, indicating that the method used to assess log-linear relationships may include fundamental defects.
7. Coherence	Monogenic lipid disorders, prospective cohort studies, Mendelian randomization studies, and randomized intervention trials all show a dose-dependent, log-linear association between the absolute magnitude of exposure to LDL and risk of ASCVD.	ASCVD risk in the Statement was determined using less objective composite endpoints, including coronary revascularization and nonfatal ASCVD events. Using the more objective endpoint of ASCVD mortality, both positive and inverse associations have been reported in prospective cohort studies. In order for the Statement to maintain coherence, the risk of cardiovascular mortality should decrease with the absolute magnitude of exposure to LDL.
8. Reduction in risk with intervention	More than 30 randomized trials involving over 200,000 participants and 30,000 ASCVD events evaluating therapies specifically designed to lower LDL (including statins, ezetimibe, and PCSK9 inhibitors) consistently demonstrate that reducing LDL cholesterol (LDL-C) reduces the risk of ASCVD events proportional to the absolute reduction in LDL-C.	a. Thus far, no significant suppressive effects on ASCVD mortality by cholesterol-lowering medications have been demonstrated since the 2004/2005 enactment of new clinical trial regulations in the EU and US; in fact, some studies have even found increased mortality. Hence, it is incorrect to imply that risk reduction due to interventions has been conclusively demonstrated. b. Although no accurate RCTs have been performed, there is accumulating evidence that raising the $\omega 3/\omega 6$ ratio of highly unsaturated fatty acids (with a chain length of ≥ 20 and the number of double bond of ≥ 3) in dietary and tissue lipids is effective for preventing ASCVD regardless of plasma LDL-C levels.
9. ASCVD (item added by the present authors)	In the Statement, ASCVD is confined to atherosclerotic cardiovascular disease, but the general definition of ASCVD includes both cardiovascular and cerebrovascular diseases.	a. We present evidence that the heart and brain respond differently to altered LDL levels and genetic lesions of the LDL receptor because of differences in the dependence of energy metabolism on the LDL receptor at the vascular endothelium. Most prospective cohort studies describe no positive associations of LDL with stroke. b. Mortality from cardiovascular disease is higher but that from cerebrovascular disease is lower in the US than in Japan, which cannot be accounted for by differences in LDL levels alone.
10. All-cause mortality (item added by the present authors)	No comment made.	All-cause mortality is the most important endpoint for cholesterol-related health issues. In many prospective cohort studies, high LDL levels are associated with low all-cause mortality due to low rates of cancer, stroke, and infectious disease, and high LDL-C is a predictor of longevity.

Columns 1 and 2 are from Ference et al. [13], and column 3 represents our view. We added items 9 and 10 to this table. "Statement" refers to the Consensus Statement from the European Atherosclerosis Society Consensus Panel 2017.

Fig. 1. The LDL receptor gene: a mosaic of exons shared with different proteins. Data from Südhof et al. [15]. See text for an explanation.

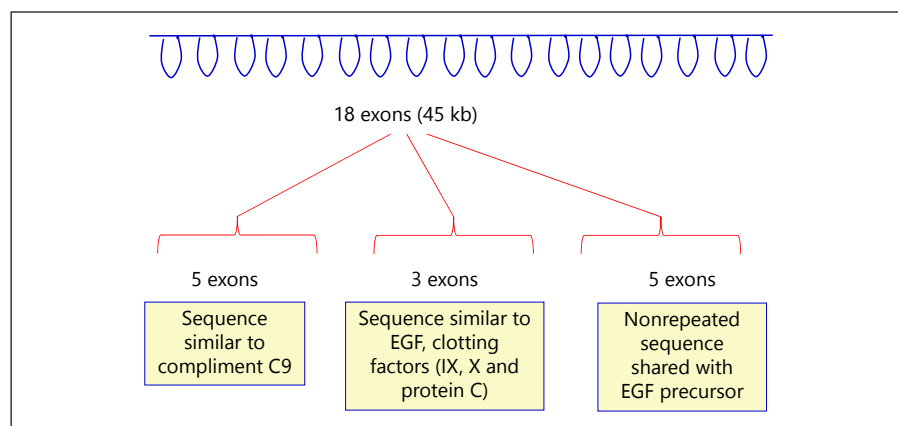
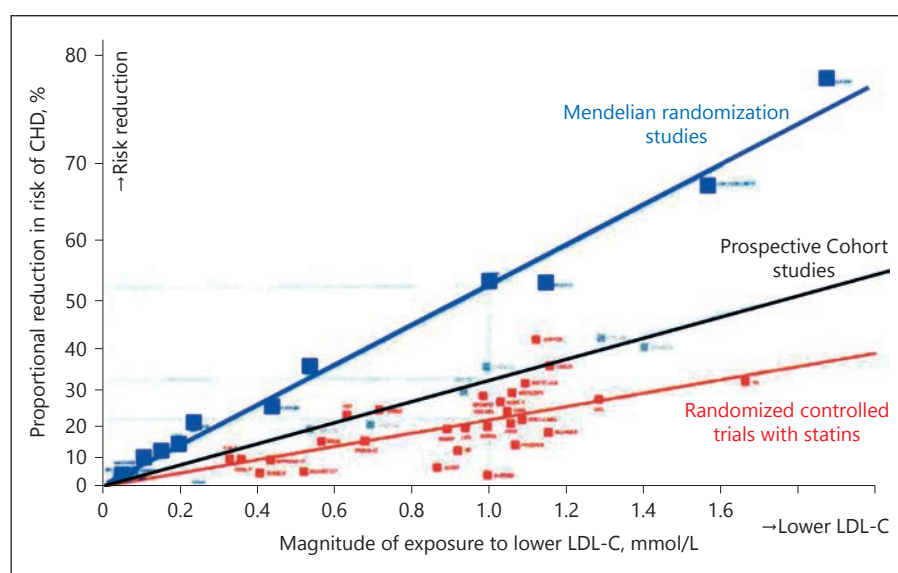


Fig. 2. Log-linear association of per-unit change in LDL-C and CVD risk. Data reproduced with permission from Figure 2 in Ference et al. [13]. The y-axis is calculated as 1-relative risk (as estimated by the hazard ratio) on a log scale, then exponentiated and converted to a percentage. The difficulty of interpreting the y-axis is noted in the text. The x-axis is shown as per-unit change in LDL-C. Synonymous titles added to the x-axis in italics are explained in section II in the text. Synonymous titles of the X-axis for the Mendelian randomization studies. Magnitude of exposure to: (a) increased supply of LDL-carried lipids to artery tissues; (b) lower thrombotic tendency associated with coagulation factors; and (c) lower inflammatory tone related to TNF α and other chemokines.



EAS Consensus Statement 2017 Based on Mendelian Randomization Studies

Mendelian randomization studies are based on the fact that genotypes are randomly assigned at meiosis, are independent of non-genetic confounding factors, and are not modified by disease processes. Single nucleotide polymorphisms (SNPs) of genes related to markers of disease are often analyzed. However, genetic confounders must be taken into account. For example, the LDL receptor gene (*LDLR*) is known to be a mosaic of exons shared with different proteins [15]. Most of its 18 exons correlate with functional domains defined at the protein level, and some of them encode sequences similar to those of other proteins (Fig. 1). Therefore, genetic mutations in or near *LDLR* are expected to affect not only LDL receptor func-

tion, but also blood coagulability [16] through the coagulation factors IX, X, and protein C, inflammatory tone through tumor necrosis factor (TNF)- α , epidermal growth factor (EGF)-related physiology, and infectivity through complement C9. SNPs can cause gain or loss of function. Moreover, the process of ASCVD development is affected by lifestyle-related factors even though the genetic factors (SNPs) themselves are not altered. In fact, in the past when infection was the major cause of death, people with familial hypercholesterolemia (FH)-related genes lived longer than average because LDL-C competitively inhibits the infection process (Chapter 1 in [17]). Thus, Mendelian randomization studies essentially involve multiple confounders.

In the Mendelian randomization studies on which the Statement was based (Fig. 2), a log-linear association is

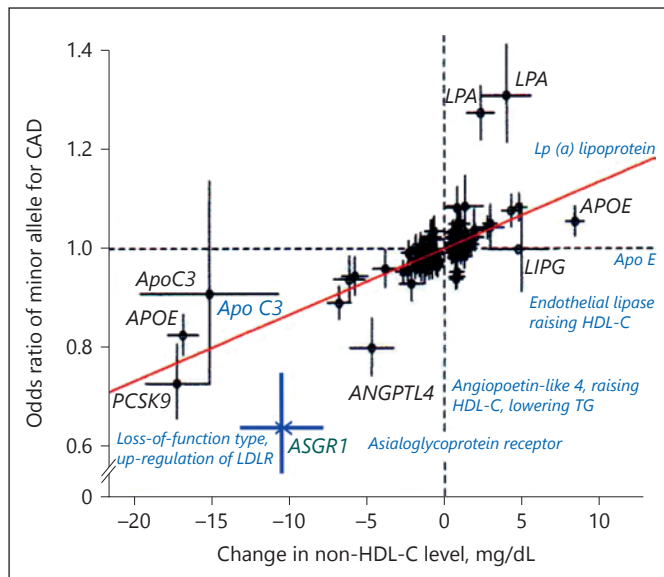


Fig. 3. Relationship between the effect of sequence variants on non-HDL cholesterol and the risk of CAD. Slightly modified from Figure 3 in Nioi et al. [18]. PCSK9, proprotein convertase subtilisin/kexin type 9, which modifies LDLR processing. Although “Non-HDL” is used for the x-axis, the association of CAD events with non-HDL-C was qualitatively similar to that with LDL-C, the factor for non-HDL/LDL-C being 1.06–1.09 (Okamura [19]).

beautifully demonstrated. The proportional risk reduction (y-axis) is calculated as 1-relative risk (as estimated by the odds ratio [OR] or hazards ratio) on the log scale, then exponentiated and converted to a percentage. However, it is not easily understandable how the y-axis on the log scale starts at 0% with increasing intervals toward 80%. Provided that the mathematical manipulations used in the Figure 2 are reliable, we point out the following problems: (a) the slopes of the 3 regression curves differ, indicating that factors other than LDL-C are involved, (b) the y-axis values are explained in the text to be exponentiated, then the 0 value is impossible, (c) some clinical trials (e.g., JUPITER, POST-CABG, IDEAL, SEARCH) reported minus values for the risk reduction of CHD but are treated here as plus values, and (d) the ERFC trial is included, but this article does not include values for LDL-C. This kind of complicated data manipulation (y-axis) successfully serves to confuse the reader and should be avoided in evidence-based medicine.

A similar but more readily understandable dataset was presented by Nioi et al. [18] (Fig. 3). In this study, a loss-of-function mutation in the asialoglycoprotein receptor gene (*ASGR1*) was found to lower non-HDL-C by 15.3 mg/dL (0.4 mmol/L) and the risk of coronary artery dis-

ease by 34%. Although the hazards ratio of the minor allele for CHD on the y-axis was positively associated with change in LDL-C, giving a straight regression line without logarithmic transformation, the data for *ASGR1* appear to deviate significantly from the regression line. The Mendelian regression line shows that for each reduction of LDL-C by 20 mg/dL (0.52 mmol/L), risk of CHD decreases approximately by 30% (y-axis; Fig. 2). The reduction is greater than in RCTs with statins (12%).

It should be noted that the SNPs located at y-axis >1 and x-axis >0 cannot be included in Figure 2 because logarithmic transformation of changes defined by this area is not possible because of negative values.

SNPs of genes known to raise LDL-C levels are summarized in Table 2. Known gene functions and the changes caused by each mutation are noted. All 10 SNPs that are reported to raise LDL-C levels are associated with increased risk of myocardial infarction (MI) [20]. Analysis of the data in Table 2 revealed that increased LDL-C was highly positively associated with increased MI risk, with a regression coefficient of 0.89 and a 10-mg/dL decrease in LDL-C corresponding to a 48% decrease in MI risk. An association of this strength has never been reported for cholesterol-lowering medications.

Among the 11 SNPs listed in Table 2, at least 9 are known to be related to LDL and LDL receptor functions, that is, recognition of LDL-apolipoproteins by the LDL receptor, processing of the LDL-LDL receptor complex at the plasma membrane, internalization of the LDL-LDL receptor complex, intracellular release of LDL-associated lipids, and scavenging of the LDL receptor protein. Mutation of proteins involved in these processes can either lower or raise plasma LDL-C levels.

LDL-C-lowering SNPs and their associations with CAD and type 2 diabetes mellitus (DM) are shown in Figure 4 [22].

Cholesterol-lowering SNPs were associated with a lower hazards ratio for CAD (consistent with Figure 3), but a higher hazards ratio for DM. The impact on DM of these cholesterol-lowering SNPs is not prominent compared with results from statin intervention trials, which have shown a 1.5-fold increased risk of DM [23, 24] and up to a 2.6-fold increased risk in users of statins compared with non-users [25].

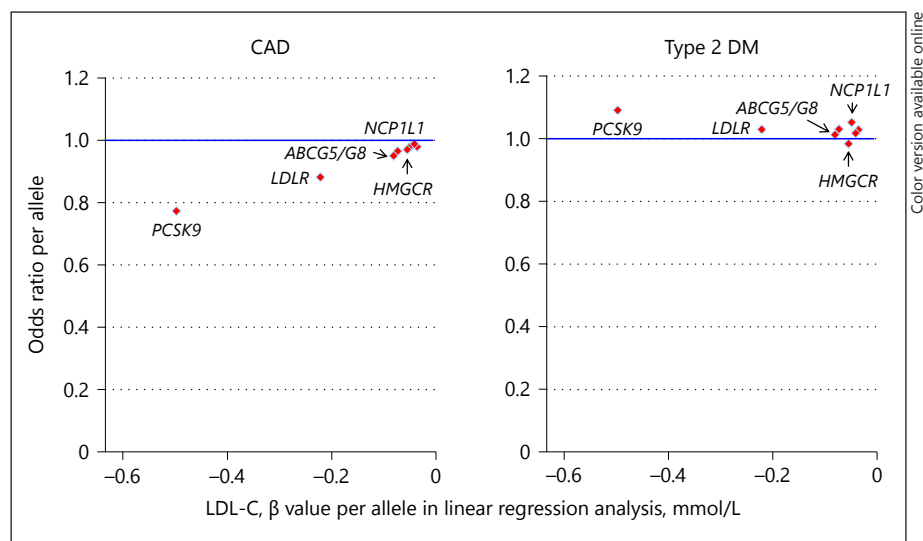
Although the “good” cholesterol (HDL-C) and “bad” cholesterol (LDL-C) hypothesis had long been widely embraced, its basis has been completely undermined. Treatment with statins or statins plus inhibitors of cholesteryl ester transport protein effectively lowered the LDL-C/HDL-C ratio but was ineffective for reducing

Table 2. Gene SNPs associated with increased LDL-C and their association with the incidence of MI

Genes of interest	Allele (frequency)	LDL-C, mg/dL	Increase in MI risk, %	Related functions of genes	SNP rs ID
<i>CELSR2, PSRC1, Sort1</i>	T,C (0.21)	7.8	16	EGF-like 2, cadherin family. Intracellular protein transport	646776
<i>LDLR</i>	G,T (0.10)	8.9	23	LDL receptor (6 types). Uptake of LDL-carried lipids	6511720
<i>PCSK9</i>	T,C (0.17)	1.9	13	Protease. Turnover of LDL receptor. Target of evorocumab. Gain-of-function type mutation brings about FH-like phenotype.	11206510
<i>LPA</i>	T,C (0.02)	14.0	72	Lp (a) lipoprotein forming complex with LDL, decreasing affinity to LDL receptor.	3798220
<i>APOB</i>	G,A (0.20)	5.4	8	ApoB recognizes LDL receptor. Affinity to LDL receptor reduces by the deletion, raising LDL-C.	562338
<i>ABCG8</i>	C,T (0.32)	5.1	8	ATP-binding cassette G8. Role in intestinal fat absorption. Elevated VLDL to LDL-apo Bconversion. Downregulation of LDL receptor.	6544713
<i>HNF1A</i>	A,G (0.44)	2.7	5	Hepatocyte nuclear factor 1 α , modifying insulin and GLUT 2 gene expression in the pancreas.	7953249
<i>APOEA-POC1-APOC4-APOC2</i>	T,G (0.33)	4.7	4	Binding to LDL receptor. Mutation of apoE and apoC II causes hyperlipidemia.	10402271
<i>HMGCR</i>	C,T (0.38)	2.3	4	HMG-CoA reductase. Downregulation of LDLR in Loss-of-function mutations	3846663
<i>TIMD4-HAVCR1</i>	C,G (0.37)	2.3	3	T-cell signal transduction	1501908
<i>ARH (LDLAP1)</i>	↑			Role as adaptor protein in LDLR internalization. FH-like phenotype	603813 (OMIM)

Data from Voight et al. [20]. Gene function explanations have been added. The last line of the table is from Harada-Shiba et al. [21].

Fig. 4. LDL-C-lowering SNPs and their association with CAD and DM. Data from Lotta et al. [22]. NPC1L1 is the gene for the LDL-C transporter Niemann-Pick C1-like 1, which is presumed to mediate intestinal absorption of cholesterol and vitamin K and is the target of ezetimibe. *HMGCR* is the gene for HMG-CoA reductase, the target of statins. PCSK9 (proprotein convertase subtilisin/kexin type 9) is the gene for processing of the LDL receptor, loss-of-function mutations of which lower LDL-C. ABCG5/G8 is the gene involved in intake and efflux of cholesterol.



CHD mortality; in fact, some studies even found these treatments to be associated with increased all-cause mortality [26, 27]. Mendelian randomization studies of the 14 SNPs known to raise HDL-C also revealed no significant positive association between changes in HDL-C levels and MI risk [20]. Moreover, mutations at the HDL-C receptor (scavenger receptor, *SR-B1*) were found to raise HDL-C but were associated with a 1.79-fold higher CHD risk compared with the control [28].

Critical Interpretation of the Statement Based on Mendelian Randomization Studies

We are relatively new to the field of Mendelian randomization studies and would find it challenging to evaluate the methodology and results in detail. Therefore, we have first summarized above what we learned from the publications, together with associated problems (Fig. 1–4; Tables 1, 2). Although the EAS Consensus Panel concluded that LDL causes ASCVD on the basis of Mendelian randomization studies, different interpretations of causality are possible as described in the inserted square (Fig. 2) and (1)–(5) in this chapter.

High LDL-C Could be a Surrogate Marker of Impaired Supply of LDL-Carried Lipids to Arterial Cells in the Case of the SNPs Listed in Table 2

SNPs that raise or lower LDL-C were shown to be associated log-linearly (Fig. 2) or linearly (Fig. 3) with hazards ratio for CHD. However, the “magnitude of exposure to lower LDL-C” shown by the x-axis in Figure 2 could be a surrogate marker of or a synonym of “magnitude of exposure to increased supply of LDL-carried lipids to arterial tissues.” The basis for this interpretation is as follows. Most, if not all, SNPs that were found to be correlated with CHD (Table 2) are related to steps involved in LDL-apolipoprotein recognition of the LDL receptor, LDL-LDL receptor processing at the membrane, internalization and lysosome fusion, and reutilization of the LDL receptor. When these steps are altered by mutation of the related genes, LDL-C levels rise, the supply of LDL-carried lipids to arterial tissues decreases, and CHD events increase – or conversely, decreased LDL-C leads to increased supply of LDL-carried lipids and subsequently to decreased CHD events. This new interpretation of FH pathology raises a serious question as to whether it is high LDL-C per se or the restricted supply of LDL-carried lipids – such as triacylglycerol (TG), cholesterol, lipophilic vitamins, and eicosanoid precursors – that causes athero-

sclerosis, requiring an almost opposite medical treatment. The cardiovascular system relies heavily on TG as an energy source; 50–70% of energy used by cardiac muscle is known to depend on fatty acid oxidation [29]. Hence, a lifetime of continuously restricted supply of LDL-carried lipids, particularly fats that are used for energy, causes ischemic stress and arterial damage in patients with FH. This interpretation has arisen mainly from the observations of Dr. Walter Hartenbach, former professor of pathology at München University [30]. Analyzing thousands of clinical samples from FH cases, Hartenbach found that atherosclerotic plaques consist of connective tissue-like fibrous cells with a cholesterol content of up to 1%. Cholesterol deposition and systemic degeneration of the arterial intima are observed well before plaques appear on the luminal surface of the endothelium. While Hartenbach focused on the restricted supply of cholesterol to cells, we have focused more on the impact of restricted supply of energy from fats, as described above.

Among the LDL-C-lowering SNPs listed in Table 2, the mutation affecting proprotein convertase subtilisin/kexin type 9 (PCSK9) exhibited the strongest effect on CAD risk reduction (Fig. 3). PCSK9 is known to modify LDL receptor processing [31]. Its expression in the EGF domain of the *LDLR* locus is regulated by sterol regulatory element-binding proteins 1a and 2, and it binds to the EGF domain of the cell surface LDL receptor. PCSK9 exists in an LDL-apolipoprotein-bound form in the bloodstream and also in an intracellular form. Low intracellular cholesterol upregulates its expression and stimulates apolipoprotein B (ApoB) synthesis and lysosomal processing of the LDL receptor. A gain-of-function PCSK9 mutation leads to elevated LDL-C and increased CVD, while a loss-of-function mutation lowers ApoB synthesis and LDL-C levels by increasing endocytic processing of the LDL-LDL receptor complex.

The effectiveness of a monoclonal antibody to PCSK9 was recently reported (Table 3) [32].

The authors of the FOURIER study found that evolocumab on a background of statin therapy lowered LDL-C to a median of 30 mg/dL and reduced the risk of cardiovascular events, concluding that patients with ASCVD derive benefit from lowering LDL-C to levels below current targets [32]. However, our critical interpretation of these findings is as follows.

1. LDL-C levels were 60 mg/dL lower in the evolocumab group than in the placebo group at the first laboratory examination and thereafter during the study period, which would allow investigators to easily guess a patients

Table 3. Clinical trial of evolocumab, a monoclonal antibody to PCSK9 (FOURIER trial)

	Evolocumab (<i>n</i> = 13,784), <i>n</i> (%)	Placebo (<i>n</i> = 13,780), <i>n</i> (%)	Δ	Hazards ratio	<i>p</i> value
Primary end point	1,344 (9.8)	1,563 (11.3)	−219	0.85 (0.79–0.92)	<0.001
Coronary angioplasty	759 (5.5)	965 (7.0)	−206	0.78 (0.71–0.86)	<0.001
MI	468 (3.4)	639 (4.6)	−171	0.73 (0.65–0.82)	<0.001
Acute MI mortality	25 (0.18)	30 (0.22)	−5	0.84 (0.49–1.42)	
CVD mortality	251 (5.9)	240 (7.4)	11	1.05 (0.88–1.25)	0.62
All-cause mortality	444 (3.2)	426 (3.1)	18	1.05 (0.91–1.19)	0.54

Patients with ASCVD (*n* = 27,564) under statin treatment were randomized to placebo or evolocumab. The primary end point was the composite of cardiovascular death, MI, stroke, hospitalization for unstable angina, or coronary angioplasty. The trial, performed at 1,242 centers in 49 countries, was terminated prematurely at 2.2 years (vs. 4 years by design). The Δ value in the table represents the balance of the evolocumab group and the placebo group (shown in bold figures). Sabatine et al. [32].

study group allocation; thus, double blinding could have been compromised.

2. The composite end point included measures that were less objective; hence, the accuracy of these measures as used across 49 different countries could have been biased, with the exception of mortality. For example, troponin level is known to be elevated after coronary angioplasty, leading to more frequent diagnosis of MI in the placebo group.

3. The termination of treatment at 2.2 years was premature because the difference in cumulative incidence between the 2 groups is decreasing compared with that in earlier periods.

4. Problematically, ASCVD mortality and all-cause mortality tended to be lower in the placebo group.

5. There may be serious reporting bias for adverse events in the FOURIER trial. In the many phase II studies of evolocumab and allirocumab, infections and neurocognitive events and injuries were more frequently reported in the PCSK9 inhibitor groups than in the placebo groups; however, these events were not well documented in the FOURIER trial [33–38].

We cannot assess the validity of the less objective end points that were reported, such as hospitalization, stroke events, and angioplasty. However, we speculate that the reported data do not substantiate the conclusions made by the original authors, and we recommend against accepting their conclusions as an endorsement of PCSK9 inhibitors.

Apart from the clinical effectiveness of PCSK9 monoclonal antibodies, it is important to note that some PCSK9 SNPs lower LDL-C levels below the control value, which is associated with reduced CVD mortality (Fig. 3, 4). Lowered LDL levels resulting from increased LDL-LDL re-

ceptor processing are consistent with the interpretation that an increased supply of LDL-carried lipids is beneficial for artery physiology when the supply of lipids is insufficient to meet the requirements of arterial tissues, which will be discussed in section III–2. In the case of the *LDLR* SNPs shown in Table 1, “lowered LDL” can be interpreted as qualitatively synonymous with “increased supply of LDL-carried lipids.” In the latter case, use of cholesterol-lowering medications is not supported.

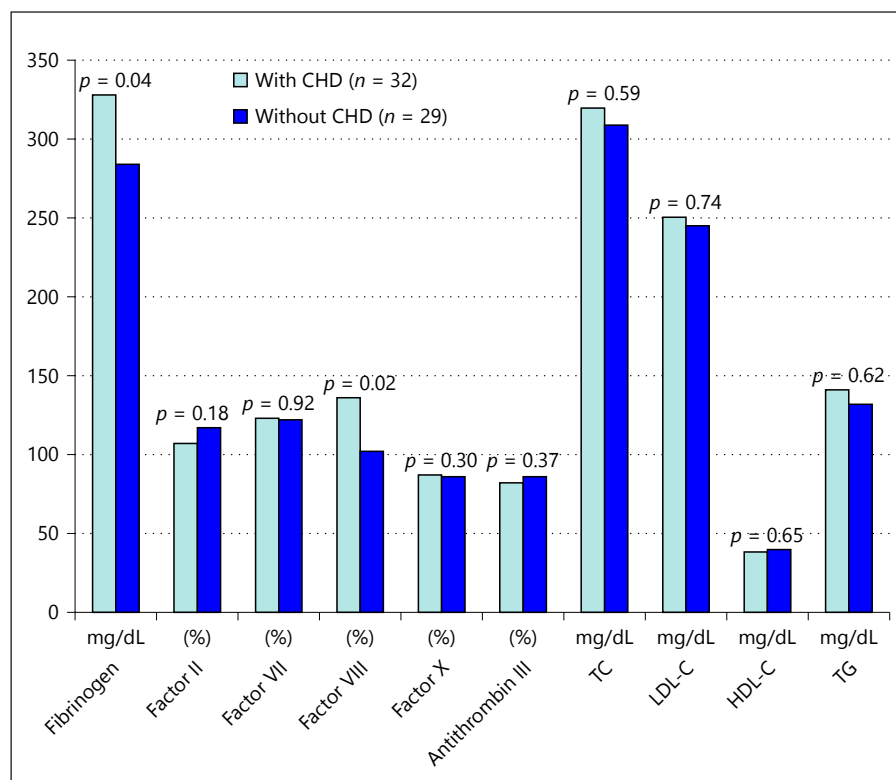
SNPs in or Near the LDLR Locus Are Likely to be Associated with TNF- α and Other Inflammatory Mediators

Inflammation is involved in atherosclerosis, and increased chemokine levels have been shown in heterozygous FH. In children with FH, selective upregulation of the chemokine RANTES (regulated on activation, normal T cell expressed and secreted) has been observed [39]. These children also have higher serum levels of TNF- α and lower levels of soluble TNF receptor (sTNFR), which results in an increased TNF- α /sTNFR ratio and potentially reflects enhanced TNF- α activity [40].

The arachidonate 5-lipoxygenase-activating protein (*ALOX5AP*) gene has been found to contribute to CHD risk in patients with FH [41]. This emphasizes the important role of leukotrienes (inflammatory mediators) in the pathogenesis of early CHD, particularly in patients with more severe elevation of LDL cholesterol levels.

These observations indicate that the x-axis in Figure 2 could also be equivalent to the “magnitude of exposure to anti-inflammatory tone related to TNF- α , other chemokines, and eicosanoids” or, at the very least, the impact of these factors must be evaluated and adjusted for before concluding that LDL is the cause of ASCVD.

Fig. 5. Hemostatic variables in patients with familial hypercholesterolemia with and without CHD. Data based on Surgrue et al. [16]. CHD was associated with significantly increased levels of fibrinogen and coagulation factor VIII but not with increased levels of classical risk factors such as LDL-C, HDL-C, and TG.



SNPs Related to Blood Coagulation, EGF, Complement C9, and Other Factors

Genes for the blood coagulation factors IX, X, and protein C (coagulation factor XIV) are in the *LDLR* locus, and these proteins are activated by a vitamin K2 (VK2)-dependent enzyme. An earlier comparison of FH cases with and without CHD [16] found no significant differences in levels of plasma cholesterol and TG; however, levels of fibrinogen and coagulation factor VIII were significantly higher in the group with CHD. Among heterologous FH cases, high plasma lipid levels were not associated with CHD but hypercoagulability was a likely cause of CHD pathogenesis (Fig. 5).

Needless to say, enhanced blood coagulability would lead to increased thrombotic disease while suppressed coagulability is likely to lead to enhanced hemorrhagic disease. The mechanisms by which inhibitors of VK2-dependent processes lead to atherosclerosis, DM, chronic kidney disease, and decreased bone mineral density have been reviewed elsewhere [16, 42], and will be addressed in section V below. Thus, the alternative measures predicted from Mendelian randomization studies (x-axis; Fig. 2) have in part been substantiated (Fig. 2, 5).

EGF is involved in growth and proliferation of endothelial cells, the integrity of which is essential for the pre-

vention of blood coagulation. The impact of SNPs in the *EGF* gene must be evaluated, and the x-axis of Figures 2 and 3 should be adjusted as well.

SNPs Related to DM

As noted above (Fig. 4), several SNPs of the LDL receptor gene were not only linked to a positive association of LDL-C with the OR for CAD but to an inverse association of LDL-C with the OR for DM [22]. A relatively good correlation was observed when the ORs for CAD and DM were plotted against each other, with a correlation coefficient of 0.71 (Fig. 6). This means that the x-axis of the Mendelian randomization study of LDL receptor SNPs cannot be confined to LDL-C but may also apply to DM-related markers.

The Term ASCVD Is Inappropriate When Discussing Atherosclerotic Disease

Although the term ASCVD by definition encompasses both cardiovascular and cerebrovascular disease, the Statement appears confined to the former. As the heart and the brain differ, MI and cerebrovascular infarction also differ significantly in their dependency on circulating LDL-C and LDL receptor functionality (see details in sec-

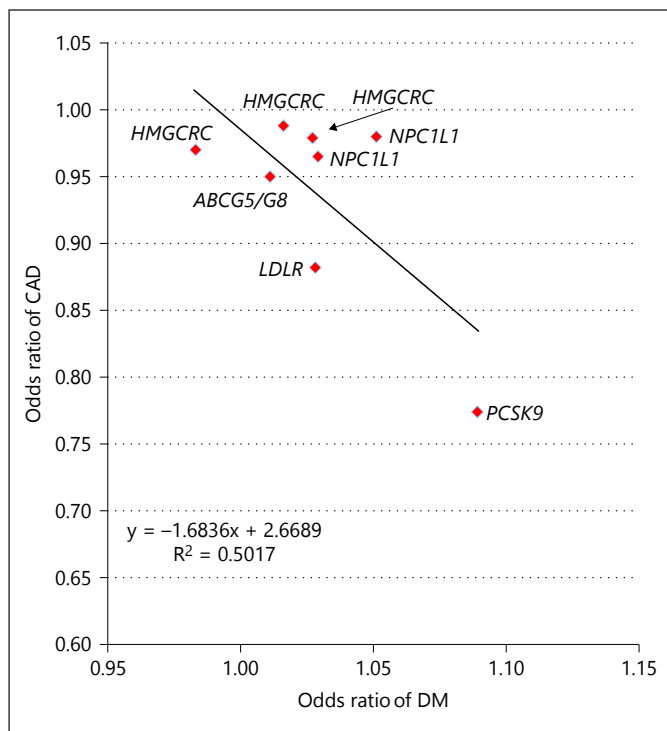


Fig. 6. Correlation of odds ratios for CAD and DM. Data replotted from those reported by Lotta et al. [22].

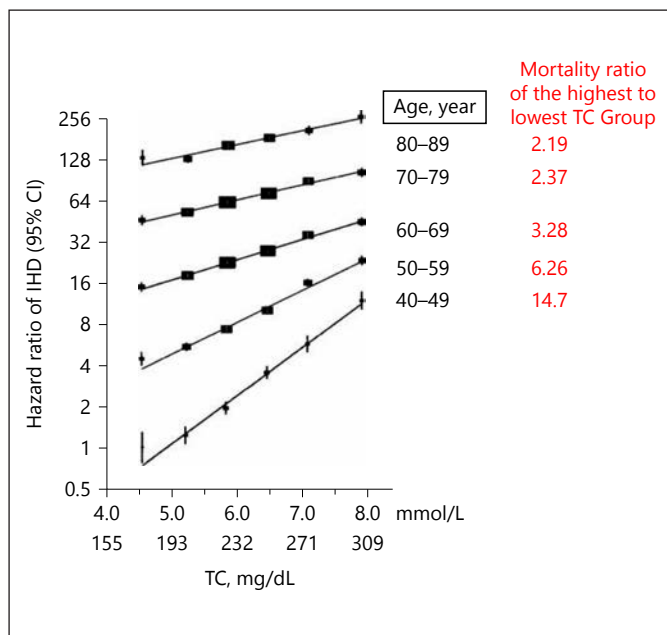


Fig. 7. Relationship between TC values and IHD mortality in different age groups. Prospective Studies Collaboration, Lewington et al. [43]. See text for detailed critical interpretation. Mortality ratios of the highest to lowest TC divisions were calculated from the figure.

tion III). Relatively recently, medical groups promoting statin medication have begun referring to cardiovascular and cerebrovascular diseases in combination as ASCVD.

As explained in items (1) through (5) above, the x-axis label “lower LDL” could be replaced by “increased uptake (supply) of LDL-carried lipids to the artery.” Anti-inflammatory tone, blood coagulability, and markers of DM could also be correlated with LDL and the OR for ASCVD. Until these factors are taken into account, the conclusion that LDL causes ASCVD is not justified and could well be wrong. The observations described below (sections III–V) clearly indicate that it is not LDL per se but rather other factors that are critical causatives of ASCVD, raising serious questions as to the validity of statin medications and the EAS Consensus Statement 2017.

Critical Review of the EAS Consensus Statement 2017 Based on Prospective Epidemiologic Studies

Continuous Log-Linear versus Discontinuous Associations between Plasma LDL-C and ASCVD Risk

Large-scale meta-analyses of prospective observational studies have reported continuous log-linear associations between plasma levels of total cholesterol (TC) or LDL-C and the risk of ASCVD. A typical example of such studies is that from the Clinical Trial Support Unit at Oxford University, which is managed with substantial contributions from the pharmaceutical industry (Fig. 7) [43].

This meta-analysis was performed using 61 reports that included 11.6 million person-years (ages 40–89 years) and 55,000 deaths from ischemic heart disease (IHD). A log-linear association of LDL-C with IHD mortality is clearly demonstrated. Because of the huge scale of the analysis, the conclusion from the analysis seems to have been unconditionally accepted by the Japan Atherosclerosis Society (JAS) in their recent guidelines (JAS GL 2010) and some other organizations. However, there are several points to be raised. The y-axis of Figure 7 is expressed in base-2 log scale, and we calculated hazards ratios (the ratio of mortality at the highest TC quantile to that at the lowest) as shown in Table 4. When these data were compared with those from other relatively large-scale cohort studies, noteworthy discrepancies were revealed between the data shown in Table 4 (absolute values of hazards ratios) and those not included in the meta-analysis:

(a) The absolute hazards ratios vary greatly among the populations examined; and (b) the hazards ratios decrease with aging in all listed studies, while most guidelines for CVD prevention set stricter cholesterol targets

Table 4. Hazards ratio (the ratio of mortality at the highest quantile of the TC group to that at the lowest) in large-scale follow-up studies

Age group	Oxford PSC study Age at 40–89 years. 11.6 million person-years	US MRFIT study Age at 35–57 years, 8 million person-years	Framingham study Age at 31–65 years 0.13 million person-years	Austrian study Age at 20–95 years 2.2 million person-years	
				male	female
30'		4.9	1.6		
40'	14.7	3.9		1.3	1.02
		3.1	1.5	1.2	1.07
50'	6.26	2.8	1.1		
		2.1	1.2		
60'	3.28			1.1	0.70
70'	2.37				
80'	2.19				
Population Ref.	General population prospective studies collaboration, 2007	Selected population Kannel, 1986	General population Anderson, 1987	General population Ulmer, 2004	

Data from Prospective Studies Collaboration, Lewington et al. [43]; Kannel et al. [44]; Anderson et al. [45]; and Ulmer et al. [46].

for older age groups. The important fact is that while the incidence of CVD increases with age, the impact of high TC on IHD decreases.

So far, we have found no rational explanations given by other scientists for (a) and (b) noted above; however, we have interpreted the data using the proportion of FH cases and those with similar genetic factors in the group or subgroup as the critical factor, which are as follows.

(a') Samples initially selected from a high-TC population (e.g., the MRFIT study) would include greater proportions of FH than would general populations (e.g., the Framingham study and Austrian Vorarlberg study), and hence would have greater hazards ratios.

(b') Some genetic factors (e.g., FH) are associated with shortened lifespan (Ulmer H, 2004; Mabuchi H, 1986), which is reflected in hazards ratios that decrease with age (Table 4). In contemporary society, the mean survival time of patients with FH is shorter than that of patients without FH [46–48]. Sex differences in CHD mortality have not been fully explained, but it should be noted that inverse associations are often observed between TC and CHD mortality in groups that are all or predominantly female [46, 49].

Data from the Oxford Prospective Studies Collaboration [43] (Table 4) do not conform with our generaliza-

tion, in that the hazards ratios are much higher than those from other studies, although the participants are described as being from general populations; the calculated hazards ratios were 14.7 for the fourth decade of life and 2.19 for the eighth decade. We have tried unsuccessfully to find at least one report among the 61 listed that describes a hazards ratio as high as those shown in Table 4.

In meta-analysis, the scale of study (number of participants, follow-up years, and number of cases) is important, and we tend to believe that “the larger the scale, the higher the accuracy.” However, specialized smaller scale analyses are sometimes buried in massive figures from a huge meta-analysis, such as those shown in Figure 7 and Table 4. We would like to emphasize that of multiple prospective follow-up studies performed in many countries and regions, none have produced hazards ratio values similar to those in Figure 7.

Log-Linear versus Discontinuous Association of CHD Mortality with TC Levels

The association of TC with CHD mortality in NIPPON DATA80 (Fig. 8), in which a hazards ratio as high as 4 is described [50], was the major basis for the Cholesterol

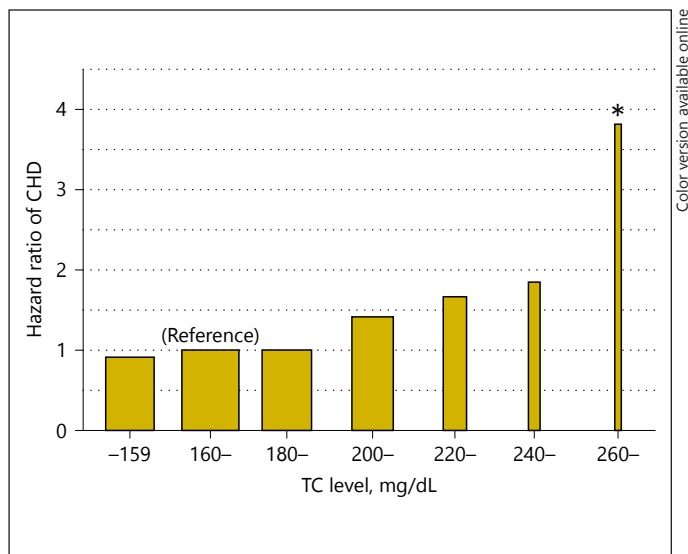


Fig. 8. Association of TC with CHD mortality-in NIPPON DATA80 (17.3-year study). Participants from 300 areas of Japan (men and women >30 years of age, $n = 9,216$) were followed for 17.3 years beginning in 1980. Data from Okamura et al. [50]. Column width was made proportional to the number of participants. Only the highest TC group (*) exhibited a significantly increased hazard ratio for CHD.

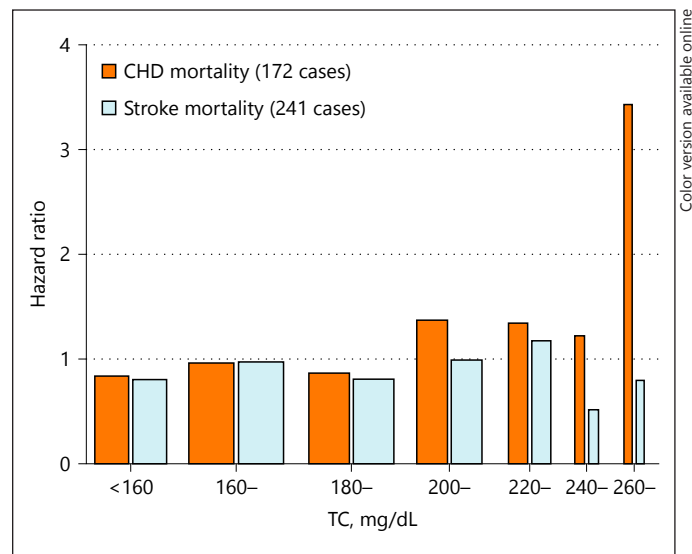


Fig. 9. Association of TC with CHD mortality and stroke mortality in NIPPON DATA80 (24-year study). Data from Sugiyama et al. [51]. Men and women >30 years of age ($n = 9,209$) were followed for 24 years. Column width was made proportional to the number of participants. Note that the relationship is not linear. All-cause mortality was not reported.

Guidelines from the JAS (GL 2007–2012). A log-linear relationship may be deduced by logarithmic transformation of the ORs shown in Figure 8. One problem with the TC-mortality relationship is that the number of participants in each column (subgroup) differs. If the top 2 TC levels were to be combined to make the number of participants roughly equal in each column, the hazards ratio would be much smaller than 4. Rather than the log-linear relationship claimed by the Consensus Statement 2017, our interpretation is that a small number of participants with the highest TC levels exhibited a significantly higher hazards ratio for CHD compared with the majority of the population.

When the follow-up period was extended from 17.3 to 24 years, the discontinuous association of TC with CHD mortality became clearer (Fig. 9) [51]. Thus, the subgroup with the highest TC levels, which is likely to include the majority of individuals with FH, exhibited a strikingly high hazards ratio for CHD compared with the majority of participants, while no significant positive associations were detected among the other TC subgroups. It has been suggested that the NIPPON DATA80 study population included a higher than average proportion of FH cases, with a prevalence ranging from 1.5-fold higher (for women) to 3-fold higher (for men) than in the general population (0.2%) [17]. Thus, we need to clarify the relationship

between plasma cholesterol level and CHD mortality. It is not continuous, and only the subgroup with the highest TC levels and highest proportion of FH cases exhibits a significantly higher OR for CHD compared with the majority of the general population.

Recently, larger scale follow-up studies from Japan (Fig. 9) and Korea [52] have been reported for which similar clarification is applicable. Discontinuous relationships between TC level and CHD mortality have been observed in other Japanese studies [53–55] and Korean studies (Fig. 10) [52, 56].

Given the findings of these large-scale general population follow-up studies, we emphasize that the association of TC levels with CHD mortality is not log-linear as was reported in the EAS Consensus Statement 2017 (Fig. 2, 7) but is discontinuous; the small number of participants in the highest TC group, to which individuals with FH are generally confined, exhibits a significantly higher OR for CHD compared with the majority of participants (Fig. 9, 10 and the abovementioned studies).

MI and Cerebral Infarction Have Different Associations with Plasma LDL-C Levels

Recently, medical organizations strongly promoting statin medication began using the term ASCVD, which en-

Fig. 10. Discontinuous relationship between TC levels and CHD mortality. In the Japan Collaborative Cohort study (a), a general-population sample of men and women aged 40–79 years ($n = 39,242$) was followed for 10 years (Cui et al. [53]). In a Korean study (b), men aged 35–65 years ($n = 482,472$) were followed for 6.4 years. All-cause mortality was inversely associated with TC (data not shown) [52, 56].

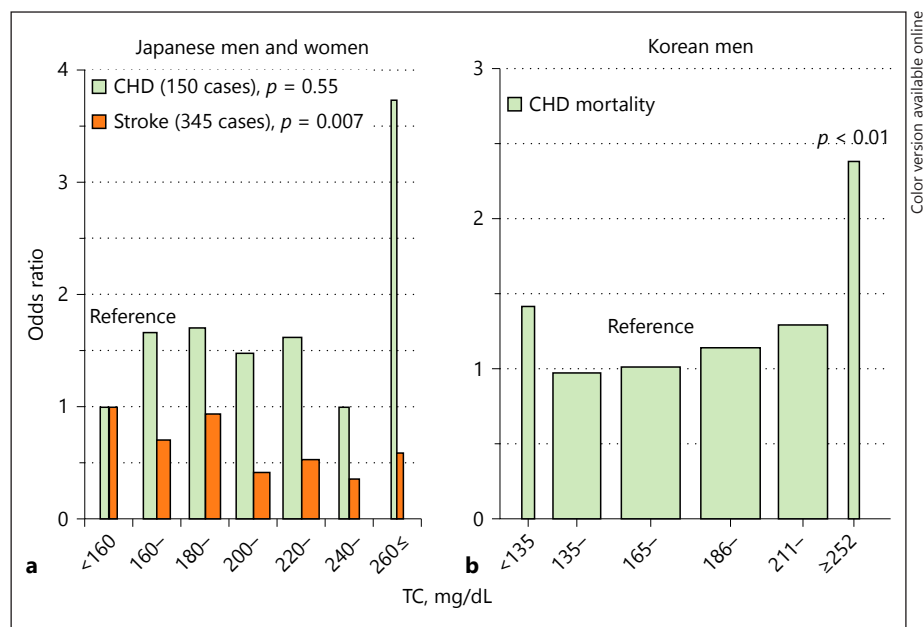
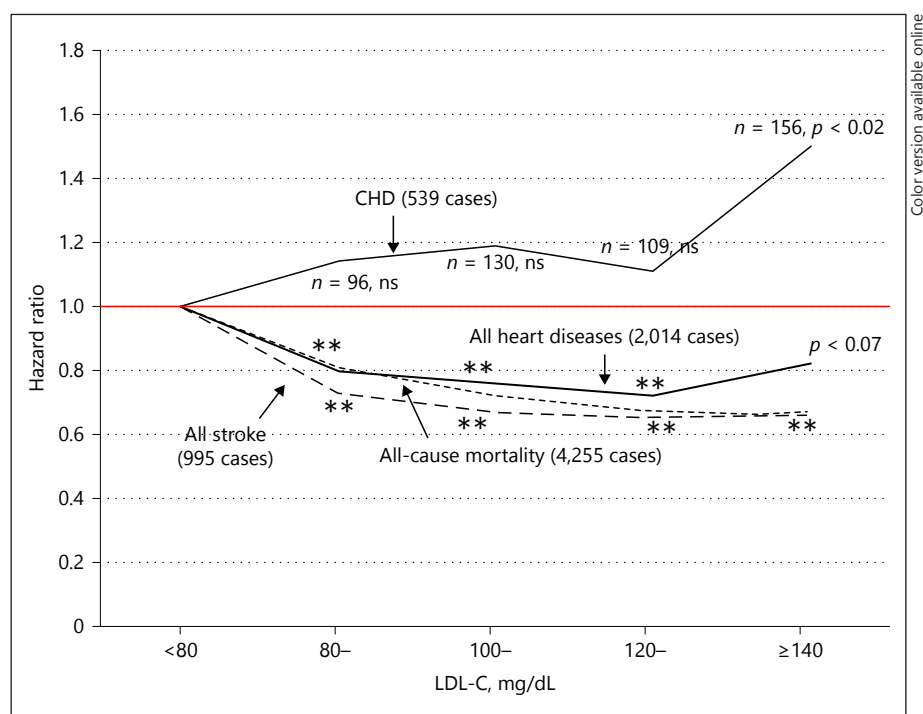


Fig. 11. Association of plasma LDL-C with hazards ratio for causes of death among Ibaraki Prefecture residents. Men and women aged 40–79 years ($n = 91,219$) were followed for 10.3 years beginning in 1993 (0.94 million person-years). Noda et al. [54]. ** $p < 0.001$.



compasses both cardiovascular and cerebrovascular disease (JAS Guidelines). However, these conditions must be differentiated because their associations with LDL-C differ significantly, even though the atherosclerotic pathology involved is likely similar. CHD mortality in Japan is roughly one-third that in the US. Stroke mortality has dropped

rapidly in Japan over the past several decades, as in many other countries; however, in Japan, it has stopped decreasing and remained 2-fold higher than in the US, presenting an important opportunity for epidemiological studies.

One of the largest scale follow-up studies was performed among Ibaraki Prefecture residents (Fig. 11) [54].

The association of LDL-C with CHD mortality does not appear to be continuous, with only the highest LDL-C subgroup exhibiting a significantly higher hazards ratio, as described above. However, inverse associations were observed for mortality from all heart diseases, all stroke, and all causes. Similarly, stroke mortality was inversely associated with TC level (Fig. 10), although no significant association had been observed in the 24-year NIPPON DATA80 study (Fig. 9). Another large-scale follow-up study performed among Isehara citizens [57] revealed that, in both men and women, annual cerebrovascular mortality was highest in the groups with the lowest TC (Fig. 12).

When stroke subtypes were compared (Fig. 13), the association of LDL-C with ischemic stroke tended to be inverse but was not statistically significant [54]. Incidence rates for both all stroke and hemorrhagic stroke were inversely associated with LDL-C levels.

In a follow-up study of a sample of Moriguchi citizens that was predominantly female (76%) and received regular medical checkups, mortality for cardiovascular and cerebrovascular diseases both tended to be inversely associated with TC (Fig. 14) [49]. The relative risk of ischemic stroke death was consistently and significantly lower for the highest TC group than in all other cholesterol groups after adjustment for clinical risk factors.

Another relatively large-scale general population follow-up study reported a significantly increased hazards ratio for ischemic stroke only among men in the highest TC group, while hazards ratios for all stroke and hemorrhagic stroke were not associated with TC in men (Fig. 15) [58]. Among women, no significant association with TC was observed for these stroke types. When cerebral infarction was classified into large artery occlusive (atheromatous), lacunar (occlusion of small penetrating arteries), and cardioembolic types, a significant positive association with TC was observed only among men in the highest TC group, but there was no association with other cerebral infarction subtypes.

Similar observations were reported in the Health Survey for England; high TC was protective for stroke, but IHD mortality was not affected by TC levels (Fig. 16) [59].

In a large-scale Norwegian study, no significant association with TC was observed for CVD and IHD (Fig. 17) [60], raising a serious question about the validity of including cholesterol as a component of mortality risk algorithms in clinical guidelines.

Consistent with the differential associations of TC with CHD and stroke [58], no significant positive asso-

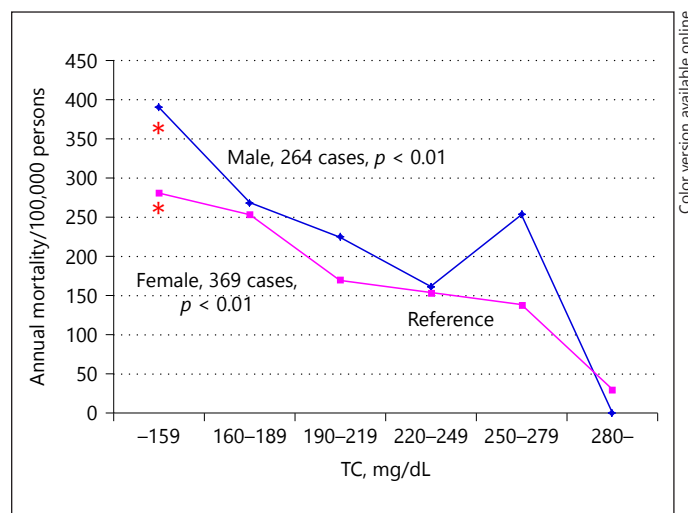


Fig. 12. Association of cerebrovascular mortality with TC in the Isehara cohort study. Isehara citizens (male, $n = 8,575$, 63.9 ± 9.5 years of age; female, $n = 13,751$, 61.0 ± 11.8 years of age) were followed for 7.1 years (156,000 person-years) [57].

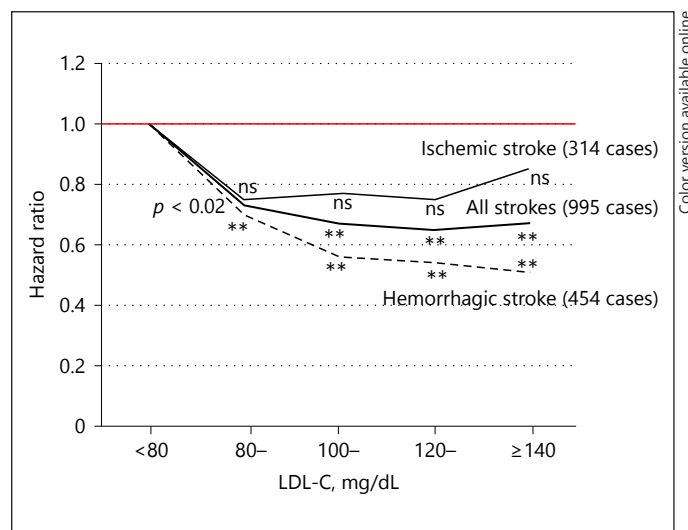


Fig. 13. Association of plasma LDL-C with hazard ratios for incidence of stroke subtypes among Ibaraki Prefecture residents. Data taken from Noda et al. [54]. ** $p < 0.001$.

ciation was observed when the relationship between TC and ASCVD (including CHD and stroke) was analyzed (Fig. 9) [51], and U-shaped relationships were even noted in a Korean population [61].

The scale of the Ibaraki Prefecture health study (0.94 million person-years) [54], the JPHC study [58], and several other studies were much larger than those of

Fig. 14. Relationship between TC and mortality for cardiovascular and cerebrovascular diseases in the Moriguchi follow-up study. Moriguchi citizens aged 15 years or older who received annual health examination ($n = 16,462$, 24% male) were followed for 10.9 years. Column width is roughly proportional to the number of participants. Tsuji [49].

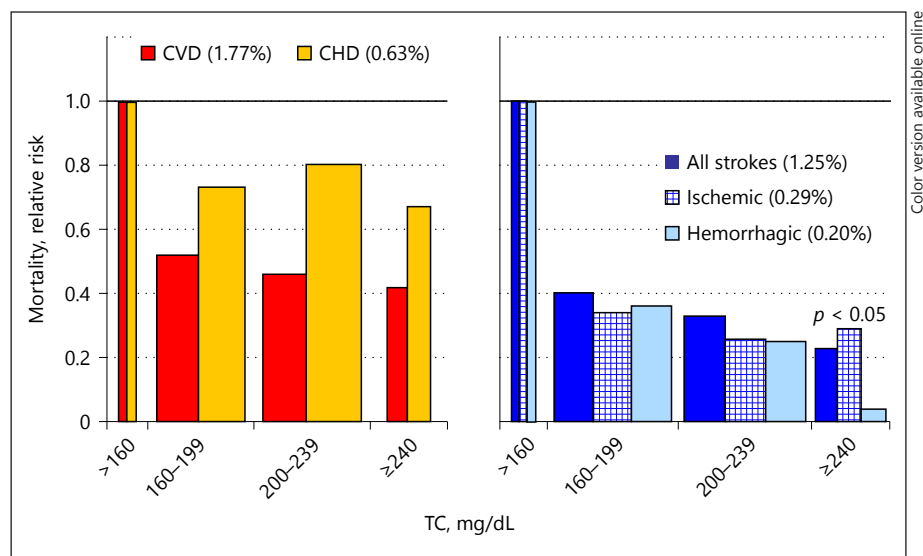
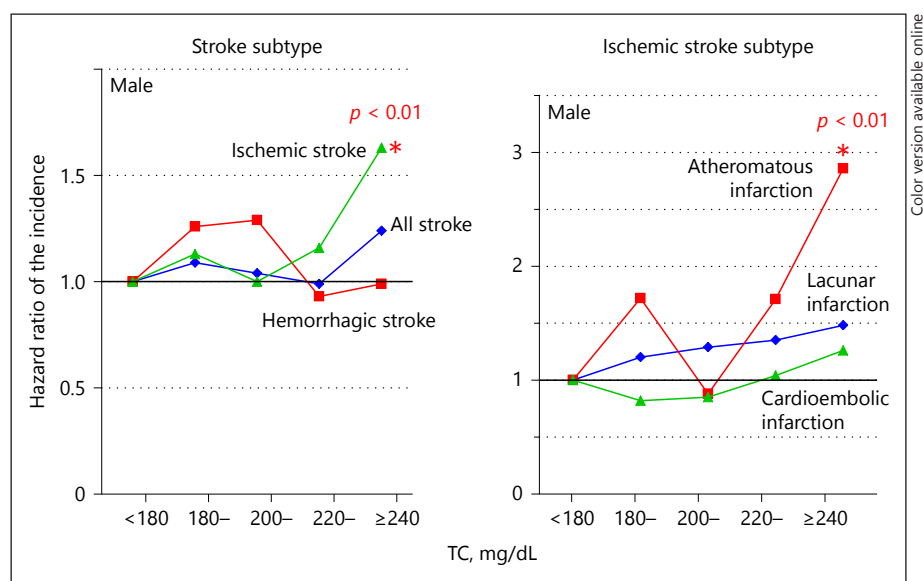


Fig. 15. Association of TC with mortality from stroke subtypes and ischemic stroke subtypes in the general population Japan Public Health Center study. Men ($n = 11,727$) and women ($n = 21,742$) aged 40–49 years were followed for a median of 12 years, with ischemic stroke (612 cases), lacunar infarction (293 cases), large-artery occlusive infarction (107 cases), and embolic infarction (168 cases) noted (Cui et al. [58]).



NIPPON DATA80 (0.22 million person-years) [51] and the Suita study (0.06 million person-years) [19, 62]. Yet, the JAS Guidelines curiously adopted only the data from NIPPON DATA80 and the Suita study [62], disregarding the differences in positive and inverse associations of LDL-C with disease mortality observed in other studies [6]. The relatively large-scale follow-up studies listed here should be enough, we believe, to convince the authors of the EAS Consensus Statement 2017 that a log-linear relationship between LDL and ASCVD incidence, as well as the data shown in Figure 7 [43], is inconsistent with many

other publications (Fig. 8–17). Hence, we judge that the Statement is a result of disregarding many inconsistent reports and is not evidence based.

Dietary Cholesterol and Animal Fats (Saturated Fats) Are Protective against Ischemic Stroke

Inconsistent with the so-called cholesterol hypothesis, raising the dietary polyunsaturated/saturated ratio and reducing cholesterol intake were found to be ineffective for lowering LDL levels after several years of intervention [1, 63], as reviewed [6, 63, 64]. Moreover, the groups in-

Fig. 16. Comparison of risk factors for fatal stroke and ischemic heart disease: a prospective follow-up of the Health Survey for England. Participants ($n = 82,380$, mean age 55.4 ± 14.2 years) were followed for an average of more than 8 years (Hamer et al. [59]).

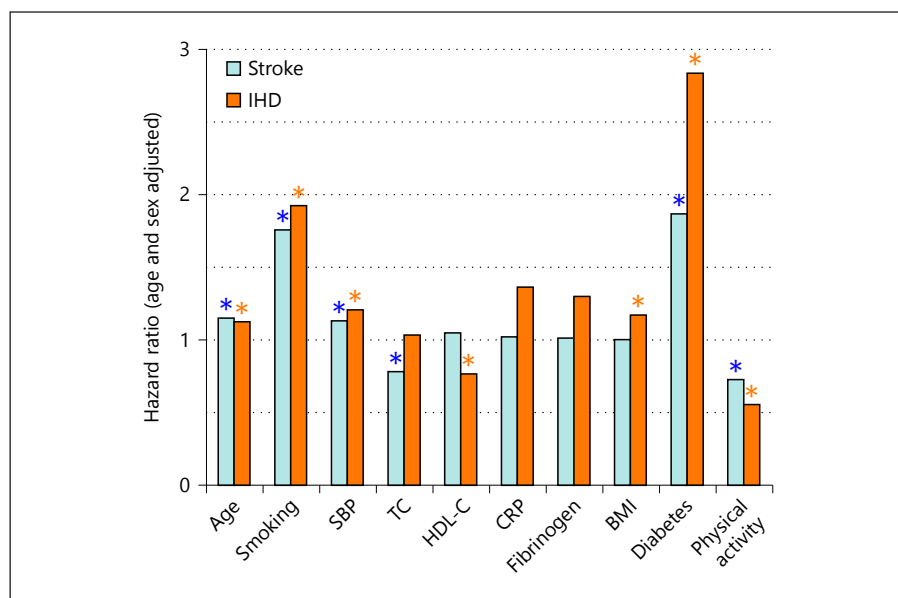
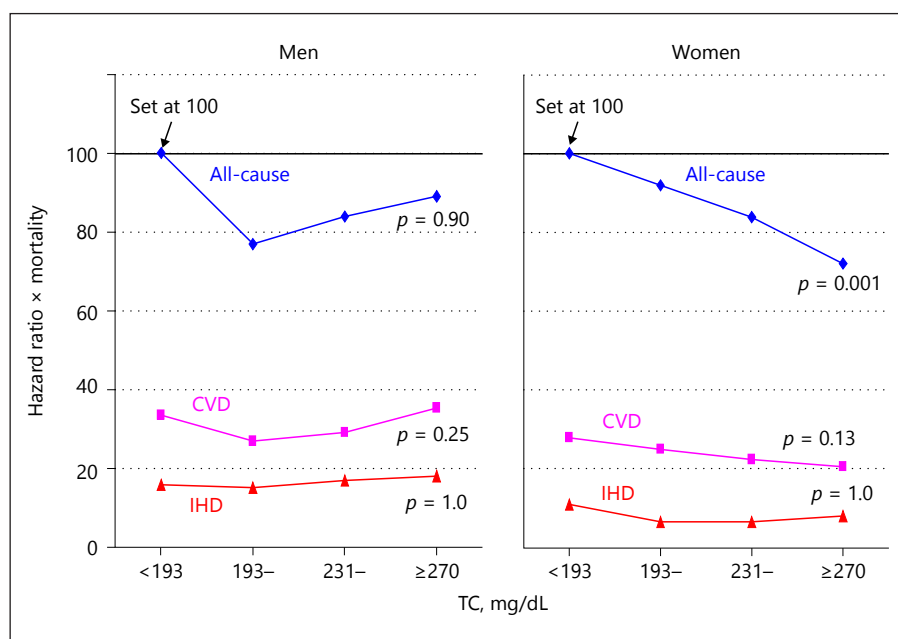


Fig. 17. Is the use of cholesterol in mortality risk algorithms in clinical guidelines valid? Norwegians aged 20–74 years ($n = 52,087$) were followed for 10 years. Data taken from Petursson et al. [60].



gesting greater amounts of animal fats (rich in saturated fatty acids) and cholesterol, as well as animal protein, exhibited lower mortality from ischemic stroke (Fig. 18) [65]. In another study, the intake of saturated fatty acids was inversely associated with ischemic stroke (Fig. 19) [66]. Stroke mortality was previously very high in north-eastern Japan but fell rapidly over the past several decades, which has been attributed to increased intake of

animal foods rather than to decreased salt intake. In stroke-prone spontaneously hypertensive (SHRSP) rats, supplementation with cholesterol was found to be effective for prolonging survival [67].

A similar conclusion was reached for US citizens in the Framingham study (Fig. 20) [68]. Intake of saturated and monounsaturated fatty acids was inversely associated with the incidence of cerebral infarction, but the impact

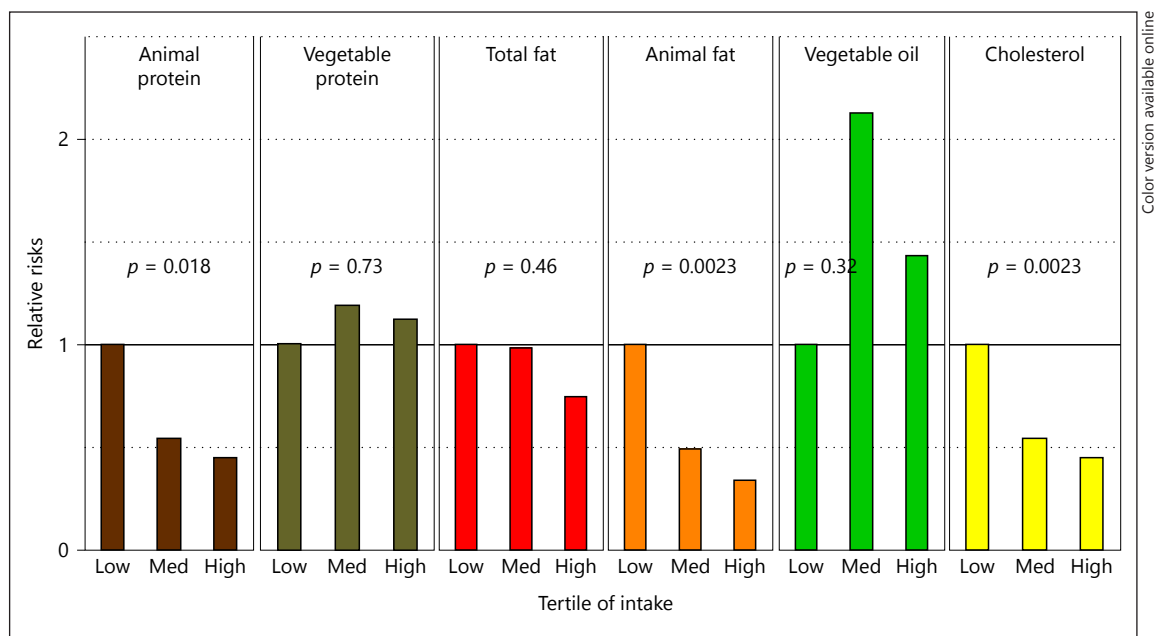


Fig. 18. Intake of animal protein, animal fat, and cholesterol as beneficial factors for cerebral infarction mortality in the Adult Health Study. Healthy Japanese men and women ($n = 3,731$) aged 35–89 years were followed for 17 years (Sauvaget et al. [65]).

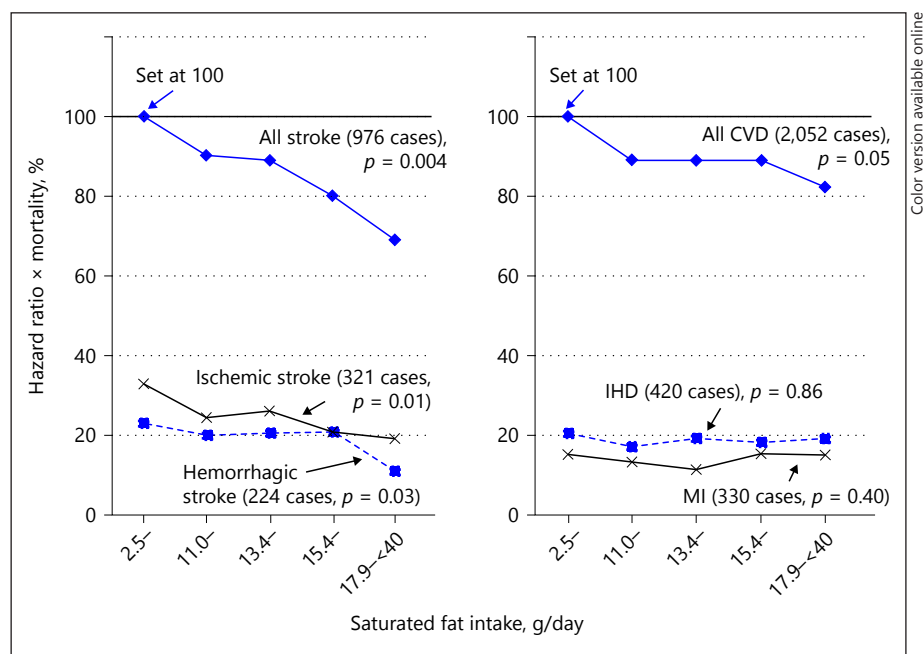


Fig. 19. Association of saturated fat intake with cause-specific mortality (JACC study). Japanese men ($n = 23,024$) and women ($n = 35,429$) aged 40–79 years were followed for 14 years. Data taken from Yamagishi et al. [66].

of polyunsaturated fatty acid intake was not significant ($p = 0.4$). It should be noted that animal fat intake among US citizens is known to be greater than that in many other countries (e.g., Japan), but these results suggest that higher than average intake of animal fats by US citizens is

beneficial for the prevention of stroke. In fact, stroke mortality in the US is half that in Japan. In 1996, we discussed the apparent paradoxes observed in Israel, India, and Japan (Okinawa) where dietary shifts from animal fats to polyunsaturated oils were associated with increased CHD

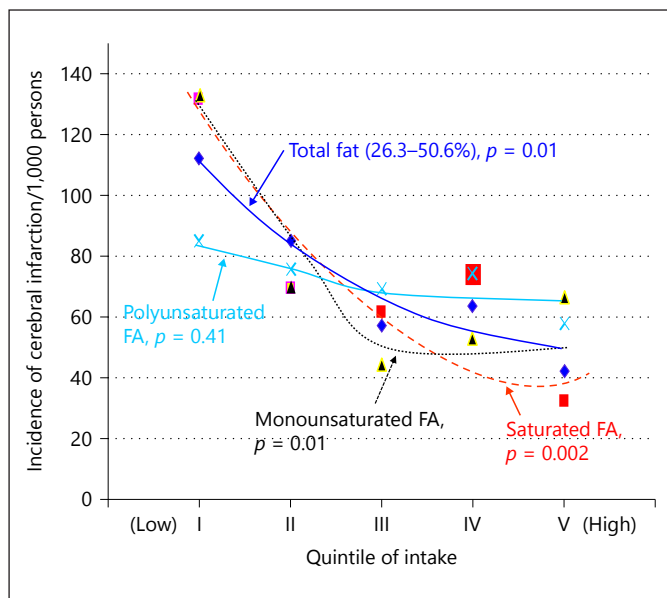


Fig. 20. Association of fatty acid intake and incidence of cerebral infarction. Framingham citizens (men aged 45–65 years, $n = 832$) were followed for 20 years. Data taken from Gillman et al. [68].

mortality [69]. It is not until now, however, that the problems associated with intake of saturated fats and polyunsaturated oils are being more widely discussed [70, 71].

Plasma Lipid Status and Mortality of Patients Hospitalized for Acute Stroke

Among patients hospitalized for acute stroke, those diagnosed with hyperlipidemia exhibited significantly lower mortality at hospital discharge (Fig. 21a) [72]. Similarly, in Sweden (Fig. 21b) [73], the 5-year survival rate of patients hospitalized for acute stroke was higher in the high-TC group than in the low-TC group.

These data obviously suggest that there are mechanisms by which dietary animal fats and cholesterol, as well as high plasma TC levels, suppress cerebral infarction and improve stroke prognosis. Consistently, decreasing the intake of these dietary components has been shown to lead to increased incidence of MI, as explained above and elsewhere [6, 17].

The Brain and Heart Differ in Their Dependency on LDL-C and the LDL Receptor System

As noted above, the associations of TC and LDL-C with MI and cerebral infarction differ. The integrity of myocardial cells depends heavily on LDL-carried fats taken up through the LDL receptor, and these fats serve as a major energy source for myocardial function; thus, ge-

netic lesions of the LDL-LDL receptor system, as in FH (Table 1), lead inevitably to damage to coronary artery physiology over time. In contrast, brain cholesterol is primarily derived by de novo synthesis and the blood-brain barrier prevents the uptake of lipoprotein cholesterol from the circulation [74, 75], because of the absence of LDL receptor in most parts of the brain blood vessel endothelial layer, apart from LDLR-related protein-1 with different functions [76, 77]. Hence, cerebral infarction is unassociated or is less positively associated with plasma TC and LDL-C.

The amounts of saturated and monounsaturated fatty acids synthesized in the brain may not be sufficient to meet requirements because increased dietary supply of saturated fatty acids and cholesterol was found to be inversely associated with cerebral infarction, as noted above (Fig. 18–20). Recently, a pathway has been proposed whereby small HDL carries lipids across the blood-brain barrier (Fig. 22) and astrocytes produce HDL-apolipoprotein E to carry lipids to the neurons [78]. The HDL receptor (scavenger receptor) and very low-density lipoprotein receptor are also expressed in the brain endothelial layer, and it is likely that they supply fats and cholesterol to cerebral arteries to prevent ischemic stroke.

Apart from the biochemical aspects of cholesterol metabolism in the brain, we are unable to determine a scientific basis to justify the use of cholesterol-lowering medications for both cardiovascular and cerebrovascular infarction under the name ASCVD. Currently available evidence supports recommendations to increase the intake of animal foods and dairy products (as a source of VK2) and cholesterol, while reducing intake of certain vegetable fats and oils for the prevention of stroke, which will be explained below (section V).

All-Cause Mortality Is the Most Important Endpoint to be Adopted in Cholesterol Guidelines

All-cause mortality is the most objective and reliable endpoint, particularly for possibly lethal diseases (National Cancer Institute: Comprehensive Cancer Information, <https://www.cancer.gov/>). Earlier, we noted that all-cause mortality was inversely associated with plasma TC or LDL-C among the general population over 40 years of age [6, 48]. One exception is NIPPON DATA80 where the highest TC group exhibited higher all-cause mortality, with the TC-mortality curve exhibiting a mirror J-shape [17]. We propose that the mirror J-shape is observed only when the proportion of FH is high [6, 48]. Lower all-cause mortality rates at higher TC levels have been accompanied by similar associations of TC with

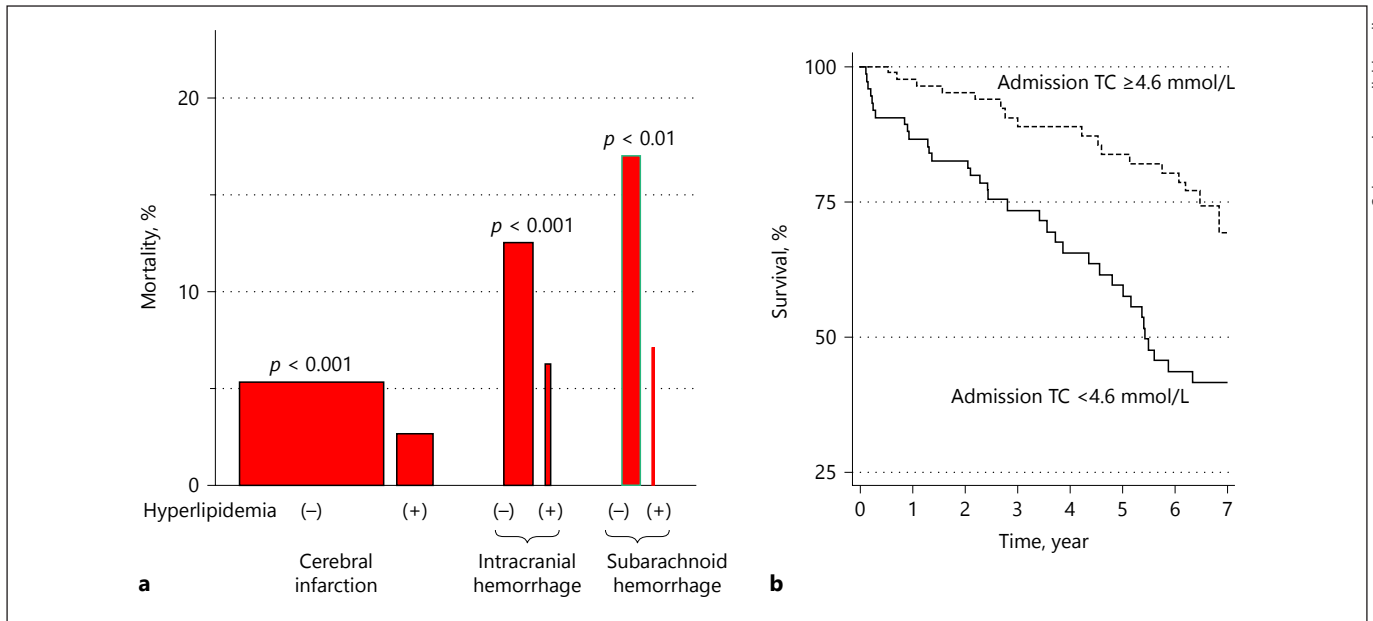
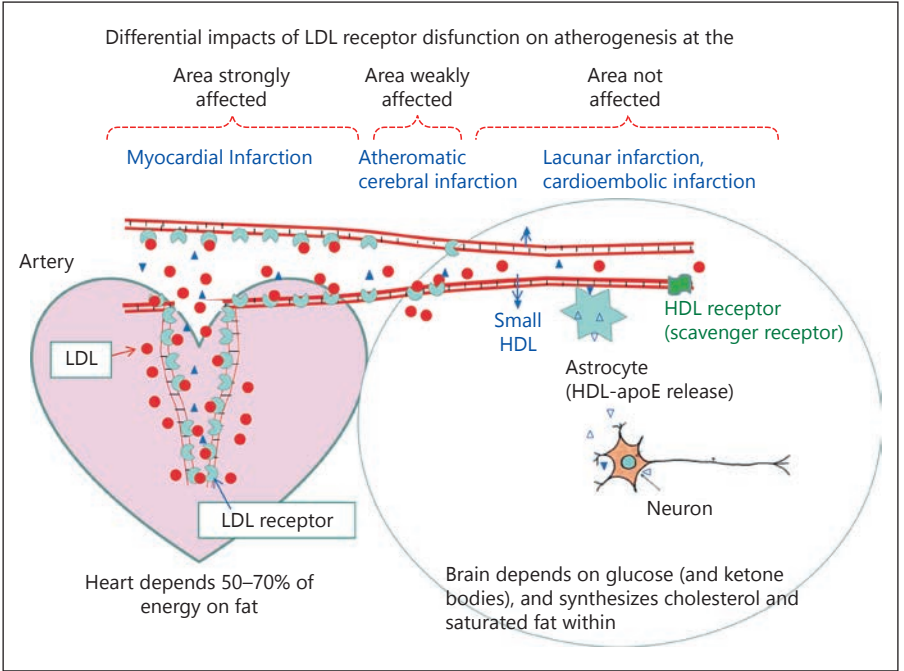


Fig. 21. Correlation of lipid status of patients hospitalized for acute stroke with subsequent mortality. **a** Mortality at hospital discharge of patients hospitalized for acute stroke (Ogushi et al. [72]). **b** Survival of patients hospitalized for acute stroke (Markaki et al. [73]).

Fig. 22. Impact of LDL receptor mutations differs between MI and cerebral infarction: a tentative explanatory diagram. Based on Orth and Bellosta [74]; Hanaka et al. [75]; and Wang and Eckel [78]. Cholesterol is synthesized de novo in neurons, astrocytes, and microglial cells. Astrocytes are known to excrete HDL and apolipoprotein E, which is assumed to carry lipids (DHA, cholesterol etc.) to neurons. See text for the observation that increased intake of cholesterol and saturated fat is associated with decreased ischemic stroke mortality and better prognosis after acute stroke (Fig. 18–21).



cancer and stroke mortality [6, 48], shown in part in Figures 12, 17, and 19. An inverse association has also been observed in many follow-up studies conducted in Western countries, as shown in a systematic survey of individuals over 60 years of age (Fig. 23) [79, 80].

The findings of another long-term follow-up study, performed before the introduction of statins, should also be considered [45]. Framingham citizens were first followed for 14 years and classified into 3 groups: one with decreasing TC, one with no significant change in TC, and

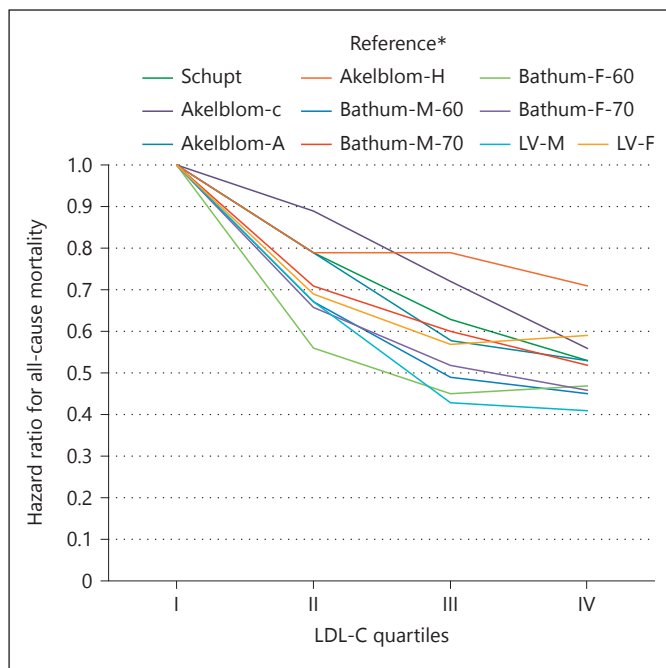


Fig. 23. Inverse association of all-cause mortality with LDL-C. From Ravnskov et al. [79]. General population cohort studies investigating LDL-C and all-cause mortality and/or cardiovascular mortality among individuals aged ≥ 60 years were identified via a PubMed search. * References for each curve are listed in the original article [79]. In one of our previous review, several studies of general Japanese populations over 40 years of age that report inverse associations between all-cause mortality and TC were listed [80].

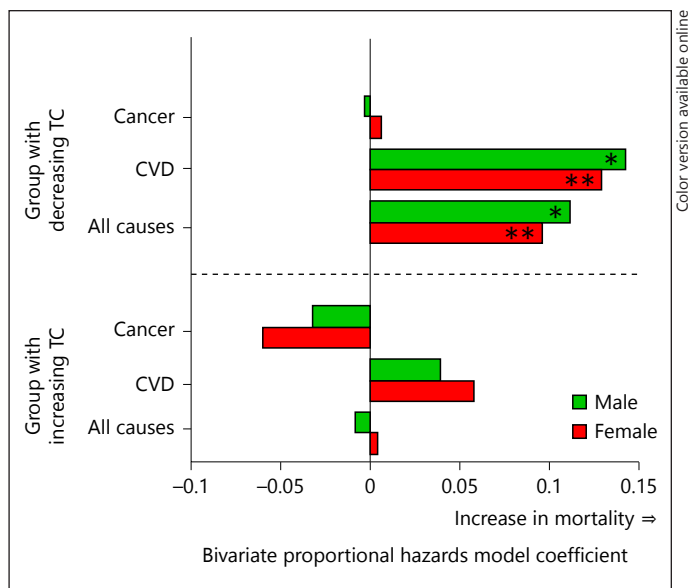


Fig. 24. Changes in TC over 14 years and mortality during the subsequent 18 years in the Framingham study. Data taken from Anderson et al. [45]. * $p < 0.05$, ** $p < 0.01$.

one with increasing TC. Participants were then followed for an additional 18 years (Fig. 24). The group with decreasing TC for the initial 14 years exhibited significantly higher mortality rates for CVD and all causes during the subsequent 18 years.

Another finding of this study was that for participants under the age of 50 years, cholesterol levels were directly related to 30-year overall and CVD mortality; for each 10 mg/dL decrease in LDL-C, the overall death rate and CVD death rate increased by 5 and 9%, respectively. After age 50 years, there was no increase in overall mortality with either high or low serum cholesterol. A younger patient group with high cholesterol is likely to include a higher proportion of FH than an older group, which is consistent with the observation from the Austrian Vorarlberg study [46] that only among patients aged in their 30s and 40s did those who die of CVD have significantly higher cholesterol levels than survivors.

The EAS Consensus Panel seems to have prepared its Consensus Statement 2017 without regard to the following observations.

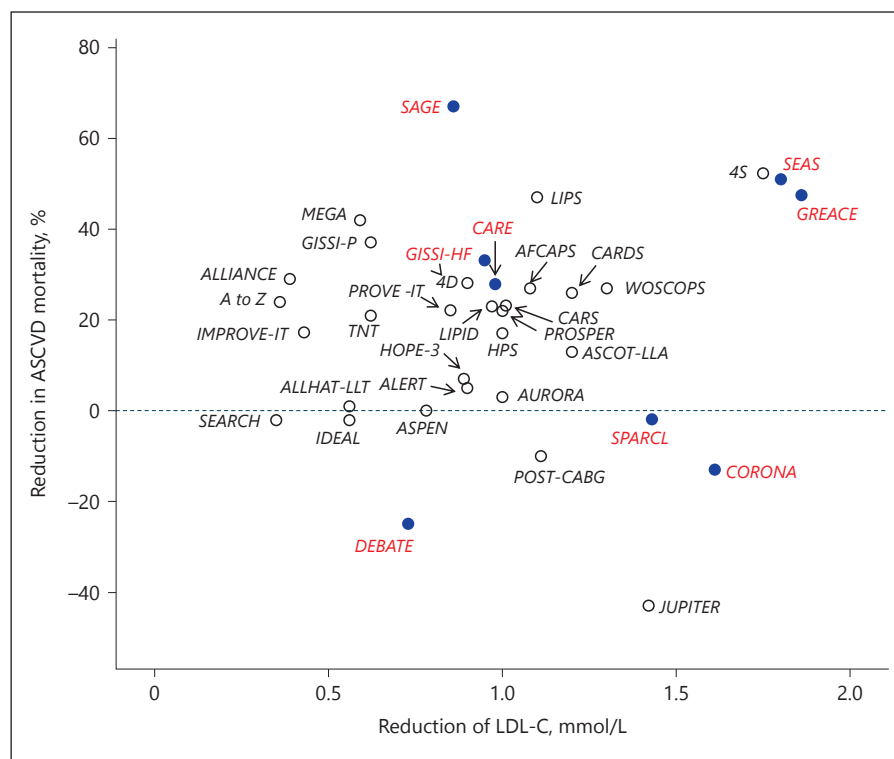
1. The hazards ratios of high cholesterol levels decrease with age (Table 1).
2. Men and women exhibit significantly different hazards ratios (Table 3; Fig. 17).
3. The LDL-CHD relationship is discontinuous, which is proposed to be associated with FH-related genetic factors (Fig. 8–10).
4. The impact of LDL-LDL receptor system impairment on ischemic diseases differs between the heart and brain (Fig. 9, 10, 12, 17, 19, 22).
5. All-cause mortality is inversely associated with TC or LDL-C levels among most of the general population.

When we published new cholesterol guidelines for longevity [48, 80], we sent an open letter to the JAS asking why clinical scientists emphasize the importance of lowering plasma cholesterol when all-cause mortality is inversely associated with TC levels and MI mortality is less than 10% of all-cause mortality; however, we did not receive a reply. The EAS Consensus Panel should have taken into consideration the facts pointed out in this section, which are inconsistent with their Statement.

Critical Review of the Consensus Statement 2017 Based on Statin RCTs

In the case of statin medications, double-blind RCTs are theoretically but not actually feasible, because the physicians in charge can easily tell whether patients are

Fig. 25. Relationship between reduction of ASCVD mortality and reduction of LDL-C by statin treatments. The statin trials with open circles were included in the meta-analysis by Ference et al. [13]. Trials with closed circles were added by the present authors and those with open circles were listed elsewhere by Ravnskov et al. [79]. No significant association was detected when ASCVD mortality was plotted as a function of LDL-C reduction.



receiving placebo or statin. End points such as re-hospitalization, cardiac events, and non-fatal stroke would then be less objective than mortality rates for CVD or all causes. As pointed out previously [11, 81], the apparent effectiveness of statins for the prevention of ASCVD as reported in the literature changed drastically after new clinical trial regulations came into effect in 2004/2005 in the EU and US. After this point, no significant beneficial effects of statins for lowering objective end points have been reported [11, 81]. There are groups of scientists who claim that statins are effective for the prevention of ASCVD, which is based on meta-analyses including RCT reports published before 2004 – mainly in the 1990s. However, it is difficult for us to accept meta-analyses that include reports published before the new regulations were issued.

What the Consensus Statement 2017 Claimed Based on Statin RCTs, and What We Cannot Accept

The Statement claims that “a statin leads to dose-dependent reduction in the risk of major cardiovascular events that is proportional to the absolute magnitude of the reduction in LDL-C.” This claim is based on a meta-analysis of individual participant data from 26 statin trials including almost 170,000 individuals; treatment with

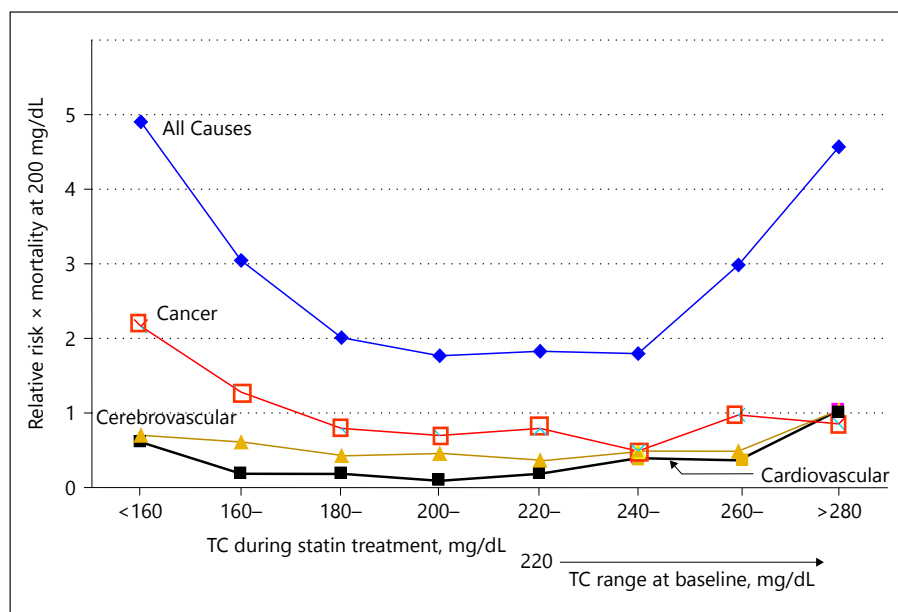
a statin was associated with a log-linear 22% proportional reduction in the risk of major cardiovascular events per mmol/L reduction in LDL-C over a median of 5 years of treatment (Fig. 2). Furthermore, intravascular ultrasound studies of coronary atherosclerosis involving statin-treated patients have consistently demonstrated that progression of coronary atherosclerotic plaque volume can be substantially arrested at LDL-C levels of ~1.8 mmol/L (70 mg/dL), as shown in Figure 7 [43].

In the abovementioned report, the end points were major coronary events (i.e., non-fatal MI or coronary death), stroke, and coronary revascularization. When instead of composite end points we plotted reduction in ASCVD mortality against LDL-C reduction, we found no significant association (Fig. 25). Similarly, no positive association was observed when absolute 5-year risk reduction was plotted against LDL-C reduction in a meta-analysis of 12 trials included in a report by Ference et al. [13] and 21 trials that were not included [79].

Variation in the objectiveness of the end points clearly may have brought about critical differences in the conclusions deduced (Fig. 2 vs. Fig. 25).

Given these observations, we conclude that statins’ effectiveness (if any) for suppressing objective end points

Fig. 26. The first large-scale statin therapy trial performed in Japan (J-LIT). Data taken from Matsuzaki et al. [83]. Participants aged 35–70 years ($n = 41,801$) were treated with low-dose simvastatin for 6 years; results were reported as a trial with no control group.



such as CHD mortality is not associated with LDL-C reduction. As noted above (Table 1), we must be careful not to be confused by analyses conducted by industry-supported organizations using non-objective end points, because even now the veracity of clinical trial publications is a hot topic [82].

Statin Trials Performed in Japan

There have been 3 relatively large-scale statin trials performed in Japan, 2 of which were originally RCTs—MEGA and KLIS (the latter had been planned as an RCT but failed in randomization). However, these studies did not conclusively demonstrate that statins are effective for prevention of CHD; details have been critically analyzed elsewhere [48]. The third study (J-LIT) was reported as a trial with a low dose of simvastatin but no control group [83, 84]. Participants were divided into subgroups according to TC levels during treatment (Fig. 26).

Mortality rates for ASCVD, cancer, and all causes increased with decreasing TC values from 200 mg/dL, which is consistent with the results of many general population prospective follow-up studies; that is, there is an inverse association of mortality from cancer, stroke, and all causes with TC or LDL-C (Fig. 11 and those reviewed in [80]). Mortality rates in the highest TC groups remained high even after statin treatment.

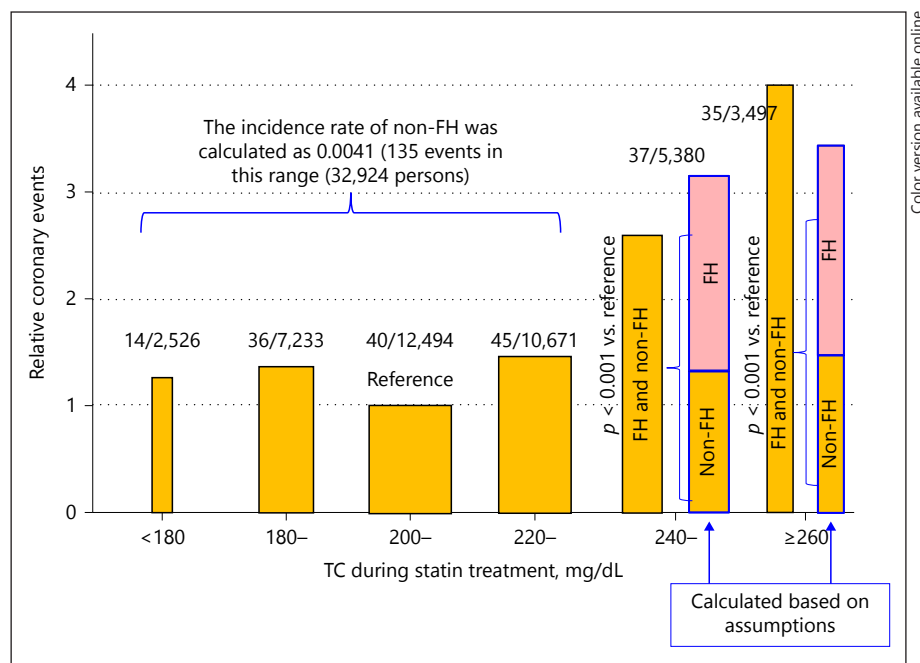
This population was initially selected from those with TC levels of ≥ 220 mg/dL ($n = 41,801$) and is reported to

include a higher proportion of FH cases ($n = 1,045$, 2.5%) compared with the general population (0.2%). Although statins lower LDL-C levels in patients with FH as well, it is likely that FH cases are confined to the highest TC groups during statin treatment because of their decreased LDL receptor function. We attempted to calculate the contribution of FH cases to coronary events, assuming that TC divisions below 240 mg/dL during statin treatment include a negligible proportion of FH cases (which are mostly confined to the top 2 divisions ≥ 240 mg/dL) and that FH cases develop CVD with a frequency approximately 10 times that of non-FH cases [47] (Fig. 27). Column width is roughly proportional to the number of participants, and the calculated event rates for FH and non-FH cases were attached to the top 2 divisions of TC levels during treatment with simvastatin.

An important observation from these calculations (Fig. 27) is that the rate of coronary events is roughly the same regardless of LDL-C levels during statin treatment, which is consistent with findings from several observational studies of the Japanese general population and other populations in which the proportion of FH cases are, or are assumed to be, relatively small. However, the validity of the above calculations must be confirmed when the results of the J-LIT study are made open after anonymization.

Although the J-LIT study was performed by major members of JAS, its results are not reflected in the JAS

Fig. 27. Calculated relative contribution of FH to coronary events in the J-LIT study. It was assumed that the TC divisions <240 mg/dL during statin treatment include a negligible proportion of FH cases (which are mostly confined to the top two divisions (≥ 240 mg/dL) and that patients with FH develop CVD at a frequency approximately 10 times that of non-FH cases [47]. The rate of coronary events for non-FH cases (the lower 4 divisions) was calculated to be 0.0041 (135 events per 32,924 persons), and that for FH cases to be 0.041. For the fifth and sixth divisions, the relative contributions of FH and non-FH cases to coronary events are shown in adjacent columns. Column width is proportional to the number of participants in each division. The figures above each column represent the number of cases divided by the number of participants.



Guidelines, which continue to advocate the approach of “the lower, the better.” Similarly, 12-month follow-up of patients with intracerebral hemorrhage has revealed that LDL-C levels among those who die are significantly lower than those of survivors. Moreover, no benefit has been observed for pre-stroke statin use [85].

Statins Stimulate Atherosclerosis and Cause Various Adverse Effects: Pharmacological/Biochemical Mechanisms

We have discussed this subject elsewhere [14, 42, 81] and expand on the broader aspects of statins’ adverse effects here.

Statins are toxic to mitochondria because essential components of the electron transport chain, such as co-enzyme Q10 and heme A, are derived from prenyl intermediates of cholesterol biosynthesis. Statin-mediated impairment of the synthesis of ATP and ketone bodies has been demonstrated in humans [86], and as ATP is used as energy currency, chronic statin use may cause cellular energy depletion and ischemia. Rhabdomyolysis and/or muscle pains are highly likely a consequence of statin mitochondrial toxicity. Because the heart depends heavily on mitochondrial ATP, it will inevitably be affected by this action of statins; in fact, artery calcification has been shown to be stimulated by statin treatment [87]. Selenoprotein synthesis is also impaired by statins, because the tRNA that carries selenocysteinyl

residues to the site of protein synthesis requires a prenyl intermediate to form isopentenyl-adenine as a minor base. One of the several known types of selenoproteins comprises the glutathione peroxidases, which serve to suppress peroxidative injury. Any tissue damage, whether noninfectious or derived from a pathogen, may trigger an inflammatory response that helps repair damaged tissues, but over time can promote the development of many diseases, including atherosclerosis [81].

A prenyl intermediate is required in various tissues for VK2 synthesis from ingested VK1, and statins lead to tissue VK2 deficiency by impairing the prenylation process. The consequences of inhibiting VK2 synthesis are very diverse (Fig. 28). VK2 regulates the activation (γ -carboxylation) of coagulation factors, osteocalcin (bone Gla protein), and matrix Gla protein. VK2 also acts on gene expression through the steroid and xenobiotic receptor (also known as the pregnane X receptor) to regulate the production of osteocalcin [14]. Understanding of the role of osteocalcin as a hormone secreted from bone marrow and targeting various organs has been increasing rapidly (Fig. 28).

Teratogenicity and Endocrine-Disrupting Activity of Statins

In the early years after statins were introduced into clinical use, miscarriage, premature birth, and teratoge-

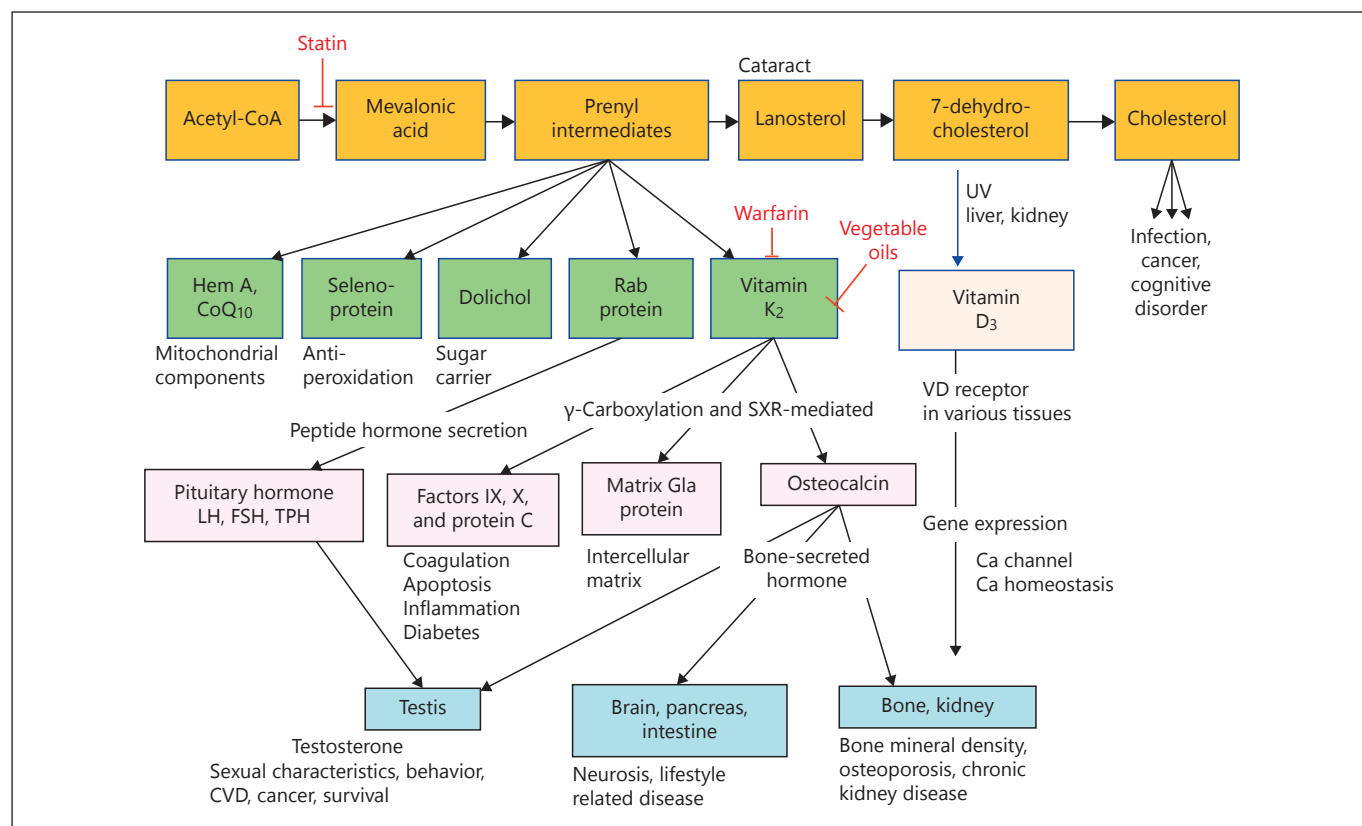


Fig.28. Statins inhibit an early step of cholesterol biosynthesis, resulting in inhibition of diverse metabolic pathways and leading to various diseases. Some types of vegetable fats and oils, as well as warfarin, lead to various organ disorders by inhibiting VK2-dependent processes,

as described in detail elsewhere [10, 14, 88]. Prenylation of Rab protein is necessary for the secretion of peptide hormones (Binnington et al. [89]), and dolichols serve as carriers of carbohydrate moieties to form glycoproteins and glycolipids (Leconte et al. [90]).

nicity were frequently observed, and statins were designated as inappropriate for use in women who may become pregnant. One candidate target through which statins may cause these problems is the signal peptide sonic hedgehog, which is activated by peptide cleavage and covalent binding of cholesterol. An inhibitor isolated from lilies growing on a livestock farm was found to cause monophthalmia (cyclopia) in sheep offspring by inhibiting sonic hedgehog signal transduction. Statins are likely also to inhibit this process, which requires cholesterol [91]. Sonic hedgehog protein is expressed in postnatal and adult hippocampal neurons, where it is involved in determining presynaptic terminal size and ultrastructure as well as the functions of hippocampal neurons [92]. Involvement of statins and dolichols (polyisoprenoids) in abnormal embryonic development has also been noted [93, 94].

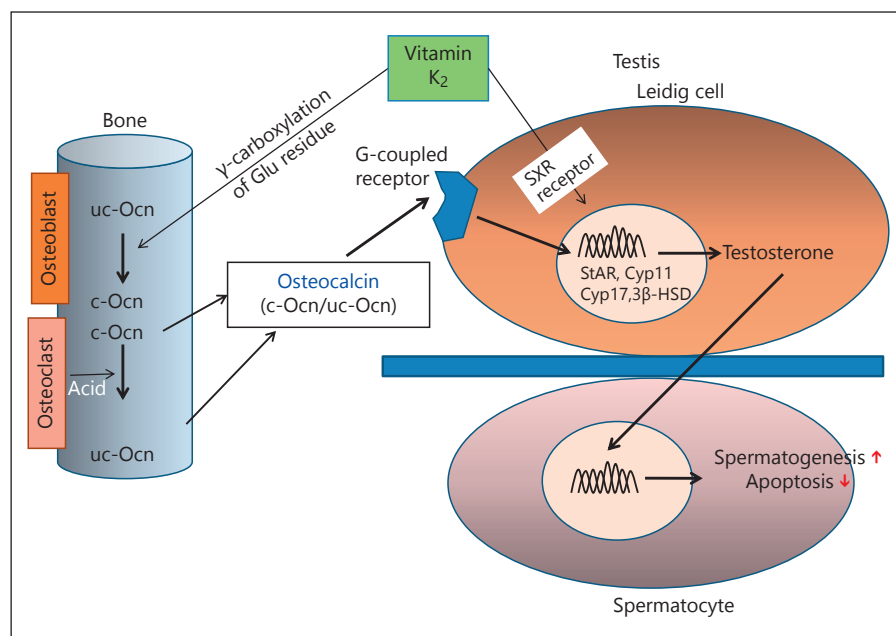
Understanding of the physiological roles of VK2 has been increasing rapidly. It serves as a cofactor for

γ -carboxylation of glutamyl residues in some proteins and also regulates gene expression through steroid and xenobiotic receptor (reviewed by Hashimoto and Okuyama [42]). Statins reduce osteocalcin levels in various tissues by inhibiting VK2 formation. Osteocalcin stimulates testosterone production in the Leydig cells of the testis [95, 96] (Fig. 29). Thus, statins exhibit endocrine disrupting activity, which is likely to contribute to teratogenicity, behavioral change, and metabolic syndrome [97, 98].

Carcinogenic and Anti-Carcinogenic Activity of Statins

Cholesterol is required for the proliferation of cytotoxic T-cells and their recognition of mutated cells, which constitutes part of the mechanism underlying statin-induced carcinogenesis. Although the carcinogenic activity of statins in clinical use is still being evaluated, all tested statins have exhibited carcinogenic activity in rodents at

Fig. 29. Regulation of male fertility by a bone-derived hormone, osteocalcin (Ocn). Based on data presented by Oury et al. [95, 96]. Canola oil and hydrogenated soybean oil suppress tissue testosterone levels [99], affecting the survival of male rat pups (Tatematsu et al. [100]).



doses comparable to those used clinically [101]. Consistently, in the J-LIT study with a low dose of simvastatin, cancer mortality was progressively increased in the subgroups with decreased TC levels from the basal level (Fig. 26). Moreover, high LDL-C levels are beneficial for suppressing viral infection; for example, hepatitis C virus competes with LDL at the LDL receptor for entry into hepatocytes, and distinct inverse associations of hepatic cancer mortality with TC or LDL-C have been noted (reviewed by Hamazaki et al. [17]). Statins also suppress thioredoxin disulfide reductase [102] and glutathione reductase, resulting in elevated peroxide tone (reactive oxygen species and lipid peroxides), which is a major risk factor for carcinogenesis. Because thioredoxin is essential for the synthesis of deoxyribonucleotides and DNA, the inhibition of which could stimulate pro- and anti-carcinogenicity at the same time, it was critically evaluated by Ravnskov et al. [103].

Cholesterol is required by all cells and is synthesized in many types of cells. Because statins cause changes in many aspects of cellular metabolism by affecting intermediates of cholesterol biosynthesis, we are unable to recommend their chronic use aimed at preventing ASCVD.

Cognitive and Behavioral Disorders

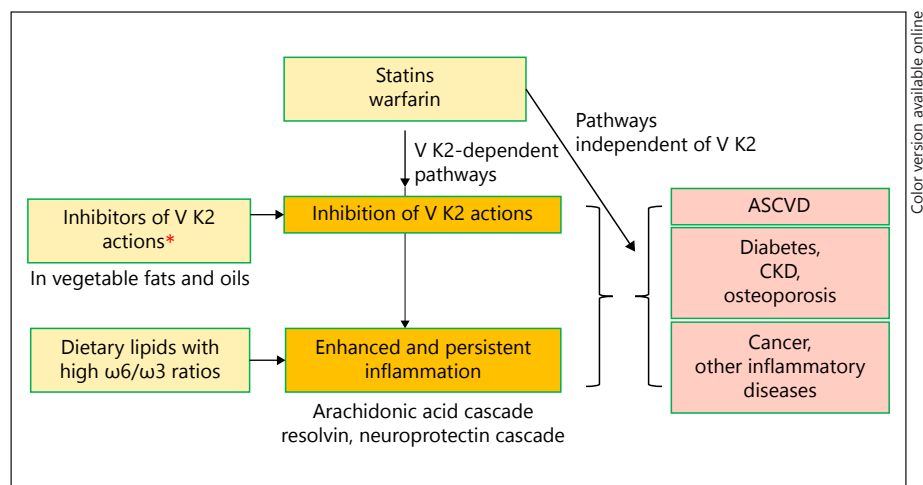
The adverse effects of statins on the central and peripheral nervous system have been suspected but no RCTs have proved or disproved their associations clearly

[104]. However, it should be noted that basic studies revealed the mechanisms by which statins lead to altered brain and testis functions [14, 95, 96]. In a mouse model devoid of synthesizing osteocalcin (Ocn), both c-Ocn (carboxylated) and uc-Ocn (undercarboxylated) were transferred to embryonic and offspring brains through the blood-brain barrier. Compared with Ocn-knockout mice, the group maternally supplemented with c-Ocn or uc-Ocn exhibited improved learning and memory performance and decreased anxiety and depression behavior, while elevating the monoamine/GABA ratio. Statins would decrease c-Ocn levels by inhibiting K2 synthesis, leading to altered brain function (cognitive and behavioral disorders).

The brain contains more cholesterol than any other organ in the body, and its supply depends mostly on biosynthesis within the brain. The brain also synthesizes sex hormones in the hippocampus, which is the memory center. Statin inhibition of an early step of cholesterol synthesis (hydroxymethylglutaryl-CoA reductase) leads to reduced levels of prenyl intermediates, vitamin K2, Ocn, and testosterone, which is expected to affect sexual appetite as well as all of the biochemical steps shown in Figure 28.

Polyneuropathy is not generally discussed in relation to statin use. In a case-control study, patients treated with statins for 2 or more years exhibited a dose-dependent increase in the incidence of polyneuropathy and the OR was as high as 26.4 [105]. It is important to have intention

Fig. 30. Onset of ASCVD and other life-style-related diseases in the absence of elevated LDL-C levels. * Fats and oils containing hydrogenated (dihydro) vitamin K1 and/or unidentified factors with similar VK2-inhibitory activities include canola oil and hydrogenated soybean oil. Olive oil, corn oil, palm oil, evening primrose oil, high-oleic safflower oil, and hydrogenated canola oil are presumed to have similar VK2-inhibitory activities because they have been found to stimulate stroke and shorten survival in stroke-prone SHR rats [108–110]. Perilla oil, linseed oil, butter, and lard are safe in this model.



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to evaluate adverse effects, and the results of basic studies would help motivate clinicians to pay attention to subtle but serious adverse effects on central and peripheral nervous systems.

Chronic Kidney Disease and DM

The prevalence of chronic kidney disease and DM began to increase around 1980. Because these conditions have been recognized as major risk factors of ASCVD, the causal relationships among them are of serious concern. We have recently pointed out that the inhibition of VK2-related pathways is the common cause of these diseases, and that several types of vegetable fats and oils as well as statins and warfarin inhibit these pathways [10].

Bone Homeostasis

Despite increasing intake of calcium in Japan over the past several decades, osteoporosis among aged individuals has become a major topic. Astronauts develop osteoporosis during stays in space possibly due to decreased physical activity and tensile load exerted on bone tissue, and age-related osteoporosis is likely to share the same mechanism. In statin users, skeletal muscle lesions leading to rhabdomyolysis and/or muscle pains reduce the ability to perform the physical activity necessary to protect against bone fracture [86, 106].

Statin reduction of levels of 7-hydroxy-cholesterol, a precursor of vitamin D, should also be addressed. The inhibition of VK2-dependent processes by vegetable fats and oils is associated with impaired bone homeostasis [10, 42], in which vitamins D3 and K2 play essential roles [88, 107]. This will be explained in more detail in the next section (section V).

Atherosclerosis in the Absence of Elevated LDL-C Levels

Statins lower LDL-C levels but stimulate atherosclerosis, as described above (section IV-2) and elsewhere [81]. Some types of vegetable fats and oils also stimulate atherosclerosis without elevating LDL-C levels, as we will now summarize (Fig. 30).

Atherosclerosis through Inhibition of VK2-Dependent Pathways

Some types of vegetable fats and oils are known to shorten survival of stroke prone SHR (SHRSP) rats at a dose as low as 6% of total energy. The action of phytosterols was proposed to underlie this shortened survival [111], but their activity is not enough to account for the effects observed with canola oil and hydrogenated soybean oil. Decreased platelet counts, kidney injury, enhanced cerebral hemorrhage, and behavioral change have been observed in SHRSP rats fed canola oil or hydrogenated soybean oil compared with those fed soybean oil. Thus, minor components other than phytosterols and fatty acids in these fats and oils are presumed to shorten survival [10, 14].

In one study, mice were fed a diet supplemented with canola oil, hydrogenated soybean oil, or soybean oil, then a sustained-release capsule containing bone morphogenetic protein (BMP) was implanted, and ectopic bone formation was estimated [88]. Four-fold greater amounts of bone were formed in the canola oil-fed and hydrogenated soybean oil-fed groups compared with the soybean oil-fed group, indicating that the activities of Ocn (bone Gla protein) and/or matrix Gla protein had

been impaired. In fact, a decreased ratio of the active form to the inactive form of Ocn was detected in the canola oil and hydrogenated soybean oil groups, suggesting that ectopic bone formation and tissue calcification had been accelerated by inhibition of VK2-dependent activation of Ocn.

Partial hydrogenation of vegetable oils produces not only trans-fat but also hydrogenated (dihydro) VK1; in human studies the latter is known to impair the conversion to and activity of VK2 [112, 113]. These observations led us to conclude that the activity of hydrogenated VK1 underlies inhibition of VK2-dependent activation of Ocn. Canola oil does not contain hydrogenated VK1, but is presumed to contain 1 or more minor components with properties similar to those of hydrogenated VK1. The breakdown products of glucosinolates and their related physiological activities have been clarified in animals, although not all are lipophylic; for example, isothiocyanates (hemorrhage, hepatic injury, epithelial cell injury); dimethyl disulfide (hemolysis, jaundice due to hepatic injury); thiocyanates (polioencephalomalacia [“rape blindness”], psychosis, locomotive disorder); indole carbinol (endocrine disruption); and oxazolidinethione (disruption of iodine uptake by the thyroid) as reviewed by Schmid and Schmid [114], and Okuyama et al. [10].

A question may be raised that the correlation between the results of basic studies with stroke-prone rats and human ASCVD is not clear. However, the stroke-stimulating activities of some types of vegetable fats and oils were ascribed mechanistically to their inhibition of VK2 and decreased cOcn level as explained above. The impaired VK2-Ocn link has been proved clinically to cause elevated mortality from CHD [115], cancer [116], DM [117] and all causes [115] as well as of decreased bone mineral density [118].

In some counties in the US, intake was restricted of industrial trans-fats produced by partial hydrogenation of vegetable oils, but not of trans-fats of ruminant animal origin. Within a decade after this restriction, a significantly lower rate of MI plus stroke events was reported in restricted counties compared with non-restricted counties [119]. A widely accepted study by the Harvard School of Public Health [120] concluded that industrial trans-fats raise the LDL-C/HDL-C ratio and thereby ASCVD mortality. However, the cholesterol-elevating activity of ruminant trans-fat was shown clinically to be substantially higher [121]. Moreover, LDL-C/HDL-C balance has little to do with ASCVD, as noted above and elsewhere [81, 114].

These inconsistent observations regarding the effects of trans-fat could suggest that industrial trans-fat is a surrogate marker of hydrogenated (dihydro) VK1 and has VK2-inhibiting activity (Fig. 28). On the other hand, ruminant trans-fat, but not industrial trans-fat, has been shown to effectively suppress DM onset [122]. Here, we suggest that ruminant trans-fat is a surrogate marker of VK2, because both ruminant trans-fat and VK2 are plentiful in animal foods, poultry products, and dairy products [10].

Stimulated Arachidonic Acid Cascade and High ω 6/ ω 3 Ratio of Highly Unsaturated Fatty Acids in Tissue as Major Risk Factors of ASCVD

The ω 6 and ω 3 fatty acids compete with each other at several steps of their metabolism: elongation and desaturation, incorporation into and release from phospholipids [5, 69], conversion to eicosanoids, and binding to receptors [123]. The ω 6/ ω 3 balance of dietary fatty acids is reflected in the fatty acid composition of membrane phospholipids [124]. In opportunistic infection, for example, arachidonic acid (an ω 6 fatty acid) is released to produce various eicosanoids with potent and diverse physiological activities related to functions such as atherosclerosis, allergy, inflammation, and blood pressure. Eicosapentaenoic acid (ω 3 fatty acid) is also released to form analogous eicosanoids, but its rates of conversion are generally lower and/or physiological activities weaker than those of arachidonic acid products [6, 123].

Basic and clinical studies have led to the understanding that lowering the ω 6/ ω 3 ratio of dietary and membrane lipids is beneficial for prevention of allergic-inflammatory diseases, atherosclerosis, and hypertension [5, 6]. In fact, classical (but incorrect) dietary interventions based on the so-called cholesterol hypothesis have been found to result in increased mortality rates for ASCVD, cancer, and/or all causes [2–4, 63, 125]. The roles of hydroxyl derivatives of the ω 3 fatty acids eicosapentaenoic acid and docosahexaenoic acid have been revealed to serve as anti-inflammatory mediators that mitigate persistent inflammation [126–128]. Anti-inflammatory activities of docosahexaenoic acid-derived neuroprotectin have also been clarified [129].

These observations are consistent with our earlier proposal that it is not cholesterol but the ω 6/ ω 3 balance that is the major risk factor for CHD [5, 6, 17]. Comparisons among Japanese residents of Japan, Japanese Americans in Hawaii, and whites in the US revealed that the serum lipid ω 6/ ω 3 fatty acid balance is associated with coronary artery calcification and intima-media thickness, indepen-

Fig. 31. Comparison of atherosclerosis markers among Japanese residents of Japan, Japanese Americans, and white Americans: a cross-sectional study (ERA-JUMP). Data from Sekikawa et al. [130].

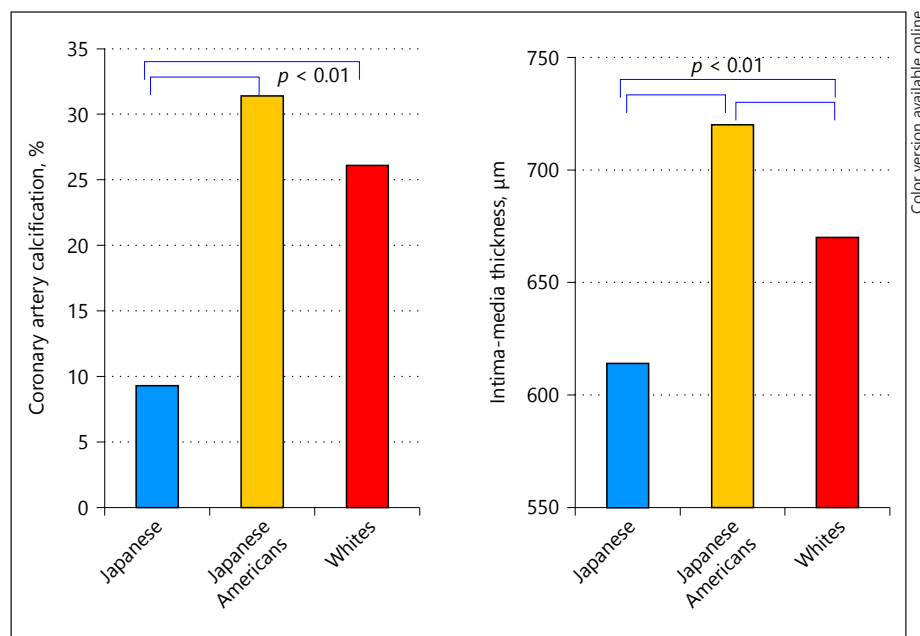
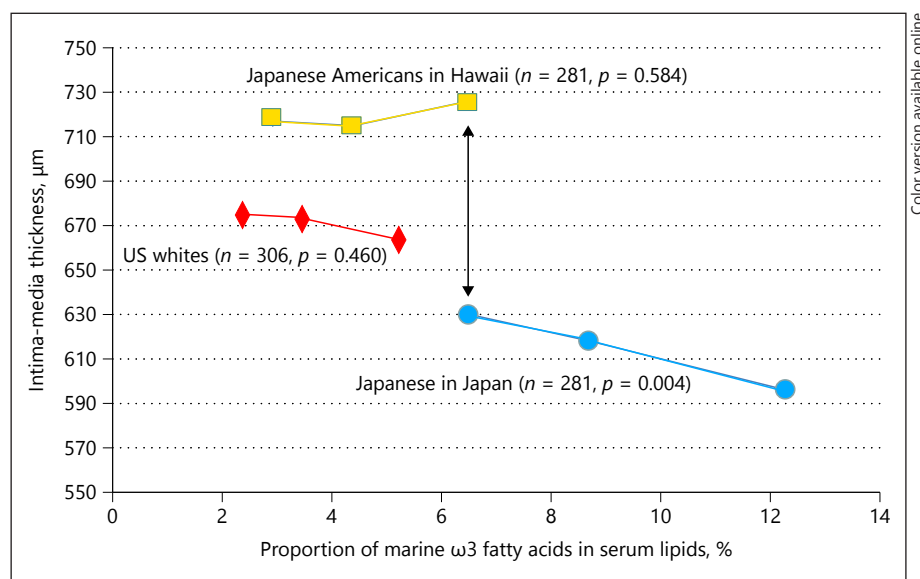


Fig. 32. Association of marine $\omega 3$ fatty acids in serum lipids and intima-media thickness. Data from Sekikawa et al. [130]. The range of $\omega 3$ fatty acids ingested by white Americans and Japanese Americans in Hawaii is likely too small to exert suppressive activity on atherosclerosis markers. The discrepancy in intima-media thickness between the top tertile of Japanese Americans and the bottom tertile of Japanese residents of Japan suggests the involvement of other factors, such as the types and amounts of dietary vegetable fats and oils.



dent of classical lipid markers such as LDL and HDL and are not likely due to genetic factors (Fig. 31) [130]. The proportion of marine $\omega 3$ fatty acids in serum lipids was inversely associated with intima-media thickness for Japanese residents, but there were no significant associations for Japanese Americans and US whites, in whom the proportions of $\omega 3$ fatty acids were much lower than in the Japanese (Fig. 32). Another interesting feature apparent

in Figure 32 is that the relationship appears to be incongruous between Japanese Americans and Japanese residents of Japan, as shown by the arrow (†). Factors other than $\omega 6$ and $\omega 3$ fatty acids are likely to be involved, and we propose that the types of vegetable fats and oils consumed, such as soybean oil, canola oil, and hydrogenated oils, is one factor affecting ASCVD, as described above (section V).

Our Overall Commentary on the EAS Consensus Statement 2017

Mendelian Randomization Studies

By analyzing *LDLR*-related SNPs, the EAC Panel concluded that LDL causes ASCVD [13], using Mendelian randomization studies as one of the strongest bases of evidence. The merit of Mendelian randomization studies is based on the understanding that genotypes are randomly assigned at meiosis, are independent of non-genetic confounding factors, and are not modified by disease processes.

However, such studies overlook the genetic confounders introduced by a mosaic of exons shared with different proteins (Fig. 1) [15]. Genetic confounders in FH include genes related to blood coagulability, inflammatory tone through TNF- α and other cytokines, and EGF-related endothelial lability. Moreover, in the case of FH with SNPs in the LDL receptor allele, high plasma LDL-C is likely to be a reflection of impaired uptake of LDL-carried lipids. Serious questions then arise about whether ASCVD is due to elevated LDL-C level per se or to decreased uptake of LDL-carried lipids. It has long been assumed that high LDL-C leads to increased production of oxidized LDL that is taken up by macrophages, which constitutes an early step of atherosclerosis. We do not accept this interpretation for the following reasons: (a) there are many populations in which LDL-C levels are not positively associated with ASCVD; (b) statin therapy or combination therapy with a statin plus a cholesteryl ester transport protein inhibitor or inhibitor of intestinal cholesterol absorption have failed to demonstrate beneficial effects on objective indices such as CHD mortality and/or all-cause mortality; (c) mechanisms by which statins stimulate atherosclerosis have been elucidated; (d) SNPs of the *PCSK9* gene have been associated with decreased ASCVD events (Fig. 3), and a monoclonal antibody to *PCSK9* was found to effectively lower LDL-C levels without decreasing mortality (Table 2); (e) some kinds of vegetable fats and oils can cause atherosclerosis without significantly increasing LDL levels [14, 81]; and (f) an increased ratio of polyunsaturated to saturated dietary lipids increases CVD mortality either with decreased LDL-C [3, 4] or without it [2]. Pathological observations by a specialist are consistent with our interpretation [30].

In one Mendelian randomization study (Fig. 3), some SNPs were associated with lower LDL-C and the OR was less than 1 compared with the control group; this indicates that lower-than-average LDL-C levels either suppress the onset of ASCVD or reflect increased cellular uptake of LDL-carried lipids. The latter interpretation is

consistent with the observation that greater dietary intake of cholesterol and saturated fats is associated with lower incidence of cerebral infarction (Fig. 18–20, 22). Increased uptake of LDL (non-HDL)-carried lipids might be proven in the case of individuals with the SNPs shown in Figure 3 (*PCSK9*, *APOE*, *APOC3*, and *ASG1*). Regarding this connection, we have pointed out that understanding how lipid uptake from circulating lipoproteins occurs in the brain is increasing rapidly (Fig. 22).

Prospective Follow-Up Studies

Inverse associations of TC or LDL-C with ASCVD have been noted repeatedly, but a meta-analysis of large-scale follow-up studies (Fig. 7) included many inconsistent reports. The meta-analysis thus includes a “black box” that is inaccessible to readers. We believe that the implausibly large ORs in Figure 7 could well be erroneous, because we were unable to locate any published general population studies with similar findings. In contrast, many relatively large-scale reports that contradict Figure 7 have been identified in this review (Fig. 9–17) and elsewhere [6, 17]. The Consensus Statement 2017 is fatally flawed in that studies reporting no positive association and/or inverse association of plasma TC, LDL-C, or non-HDL-C with ASCVD risk were excluded from consideration, and is inconsistent with our systematic analysis showing the inverse association of LDL-C with all-cause mortality [79].

Statin RCTs

The EAS asserted the effectiveness of statins for ASCVD risk reduction by displaying a beautiful log-linear association between CHD risk reduction and lower LDL-C (Fig. 2), contradicting the conclusions derived data shown in Figures 25 and 26 and also reported elsewhere [80, 81]. The discrepancies among these reports are attributable in part to the use of different end points. CVD mortality is the end point in Figure 25 and our reports, whereas a composite end point comprising less objective measures is used in the EAS Statement (Fig. 2). If less objective composite end points were reliable, studies using these end points could be regarded as support for the clinical utility of statins. However, the findings of such studies tend to be highly biased because physicians' decisions on composite endpoints may be affected by patients' cholesterol levels.

No significant beneficial effects of statins for the reduction of CVD mortality have been reported since 2004/2005. Given the history of statin therapy studies over the past 3 decades, it is extremely difficult for us to rely entirely on the CVD events and risks used in Figure 2.

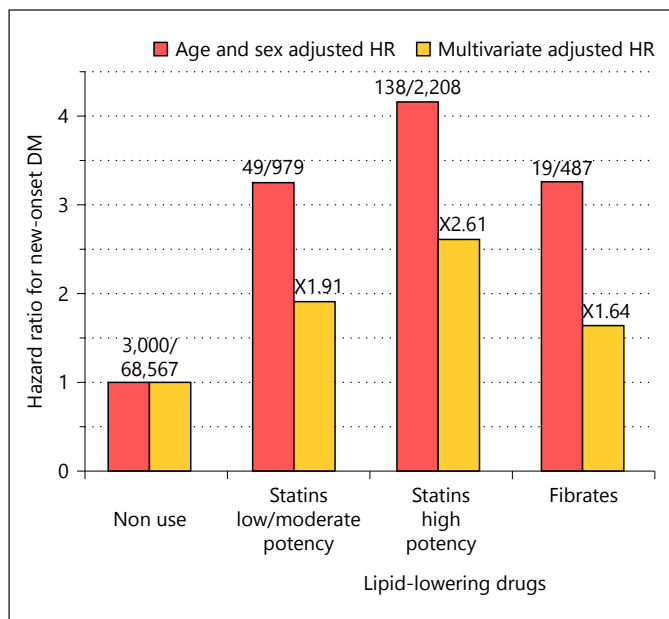


Fig. 33. Lipid-lowering drugs and risk of new-onset DM: a retrospective cohort study. Data from Ooba et al. [25]. Japanese employees of large corporations and their dependents, aged 20–74 years, were analyzed. Those diagnosed with dyslipidemia were followed until the day of treatment initiation with a lipid-lowering drug (mean follow-up period, 1.96 years). The figures above each column represent the number of cases divided by the number of participants.

Biochemists/pharmacologists have revealed the mechanisms underlying statin-mediated stimulation of atherosclerosis, heart failure, and many kinds of adverse effects, and clinical evidence consistent with these mechanisms has been reported, as reviewed briefly above and elsewhere [14, 17, 81]. It is widely accepted that statins cause DM [131], and a recent report described a 2.6-fold greater incidence of DM with high-potency statin use over a mean follow-up of 2 years (Fig. 33) [25]. Informed consent that includes the adverse effects of statins de-

scribed above does not appear to be provided adequately, at least in Japan. This is happening even with the Hippocratic Oath including a phrase akin to “First, do no harm.”

In the US, the number of statin prescriptions has increased steadily (US Department of Health and Human Services Data Brief 177, Dec 2014). Despite this, the American Heart Association predicts that the prevalence of IHD and stroke will continue to rise through 2035 [132]. In fact, no medicines developed thus far appear to effectively prevent ASCVD and reduce its associated complications effectively. However, we would like to point out that authoritative organizations and/or governmental policy makers are reluctant to accept new perspectives on lipid nutrition even though they have adopted the term “lifestyle-related diseases.”

We have been proposing new lipid nutrition guidelines for the prevention of lifestyle-related diseases, and responsible organizations should revise their guidelines to reflect the progress being made in the lipid nutrition field [6, 17, 69, 81]. In particular, the reported adverse effects of vegetable fats and oils must be scientifically evaluated and should not be overlooked [10, 14, 133].

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Disclosure Statement

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