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REVIEW

Biochemical risk factors of atherosclerotic cardiovascular disease: from a narrow and controversial approach to an integral approach and precision medicine

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ABSTRACT

Introduction: Guidelines of management of dyslipidemias and prevention of cardiovascular disease (CVD) are based on firm scientific evidence obtained by randomized controlled trials (RCTs). However, the role of elevated low-density lipoprotein-cholesterol (LDL-C)as a risk factor of CVD and therapies to lower LDL-C are frequently disputed by colleagues who disagree with the conclusions of the RCTs published. This review focuses on this dispute, and evaluates the current approach of management of dyslipidemias and CVD prevention to find modern alternatives for more precise diagnosis and therapy of dyslipidemic patients.

Areas covered: Recent interest in lipoprotein(a) (Lp(a)) and remnants lipoproteins and in therapies that do not influence LDL-C levels primarily, such as anti-inflammatory drugs and icosapent ethyl, has revitalized our concern to optimize the care for patients with increased CVD risk without focusing simply on reduction of LDL-C by therapy with statins, ezitemibe, and proprotein convertase subtilisin-kexin type 9 (PCSK9) inhibitors.

Expert opinion: The limited characterization of study populations by measurement of total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C) and triglycerides (TG) followed by measurement or calculation of LDL-C should be extended by a more integral approach in order to realize precision diagnostics and precision medicine, for the sake of personalized patient care.

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1. Introduction

In numerous reviews, the lipid risk factors of atherosclerotic cardiovascular disease (CVD) and therapies of this disease have been documented. A landmark paper by Ference et al. elegantly demonstrated that firstly the low-density lipoprotein-cholesterol (LDL-C) concentration is a risk factor of CVD; and secondly a therapeutic reduction of LDL-C is associated with reduction of the risk of CVD [1]. These achievements of state-of-the-art medical science have resulted in (revised) guidelines to diagnose and treat individuals with atherosclerotic CVD (e.g. ref [2]). Although these guidelines have been implemented and used world-wide, we notice occasionally a paper [e.g. Ravnskov et al. [3]) that refutes the scientific basis of these guidelines, or calls our attention to exceptions to the conclusions. In this review, we refer to several of these 'opposing' papers which cast doubt on the scientific basis of the conclusions drawn by Ference et al. [1], Collins et al. [4], Silverman et al. [5], and many others. Ravnskov et al. question the cholesterol hypothesis since the randomized controlled trials (RCTs) with statins lack an '*exposure-response*,' and they claim that the benefits of statins are exaggerated and that adverse effects of statins are underreported [3]. DuBroff analyzed 29 major RCTs of cholesterol reduction published after 2004, the year in which new trial regulations became effective, and claimed that in only 10 of these RCTs a cardiovascular benefit was reported [6]. How can we explain these allegations that are in contradiction with existing practice, coined in guidelines, on the basis of scientific achievements? Or do they disseminate disinformation deliberately? This review tries to connect the different directions, in an independent way, by looking at the details, and finally formulate conclusions and recommendations.

2. Do the LDL-C data in RCT studies represent the true LDL-C concentration?

In most papers on LDL-C as the main risk factor of atherosclerotic CVD and the factor to be reduced by statin therapy the actual LDL-C has not been measured, but calculated according to the formula of Friedewald et al., being LDL-C = total cholesterol – high-density lipoprotein-cholesterol – (triglyceride/2.2), provided that triglyceride concentration is <4.0 mmol/L [7]. Doing so, the concentration of lipoprotein (a) (Lp(a)) is ignored. The Lp(a) concentration may vary among individuals between 1 and 1000 nmol/L, and Lp(a) levels >90 nmol/L are considered to be a risk factor of CVD, aortic valve calcification, and aortic stenosis [8]. With other words, the calculated LDL-C data are 'contaminated' by an uncertain contribution of Lp(a). Moreover, the presence of remnant particles that are a cause of ischemic heart disease [9] is easily missed using estimated (Friedewald) LDL-C data. Contaminations like these disturb a clear discussion about the role of (too) high 'LDL-C' as a CVD risk factor.

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Article highlights

- elevated LDL-cholesterol (LDL-C) is only one of the risk factors of cardiovascular disease
- in many (if not most) publications the presented LDL-C data are estimated LDL-C values
- in studies on therapeutic efficacy, the Number-Needed-to-Treat should be mentioned
- in the management of patients with cardiovascular disease the measurement of total cholesterol, HDL-cholesterol and total triglyceride levels should be extended with measurement of Lp(a), remnant lipoproteins and true LDL-C
- one should seriously reconsider the use of cholesterol-lowering drugs for individuals aged older than 65 years in primary prevention of cardiovascular disease.

3. Is LDL-C a risk factor of CVD in all age groups?

People aged >70 y with elevated LDL-C (no atherosclerotic CVD, no diabetes, no statins at baseline) had the **highest** absolute risk of myocardial infarction (MI) and atherosclerotic CVD and the lowest estimated number-needed-to-treat (NNT) in 5 years to prevent one event, compared to younger healthy individuals [10]. The JUPITER and HOPE-3 trial results demonstrated that primary prevention of the elderly (≥70 years of age) is supported by statin therapy [11]. Likewise, in symptomatic patients aged ≥75 years LDL-C lowering reduced the risk of cardiovascular death, MI, stroke, and coronary revascularization and was as effective in reducing cardiovascular events as it was in patients aged <75 years [12]. In a Mendelian randomization study Postmus et al. demonstrated that individuals without CVD who had a genetic risk score for high LDL-C had higher risk (by 13%) of mortality than age-matched individuals with a favorable LDL genetic risk score. A genetic predisposition to high LDL-C contributed to mortality throughout life, including in the oldest old. The beneficial LDL genetic risk score was associated with familial longevity [13].

However, other reports mentioned that the CVD risk of elevated LDL-C levels declines with age. Iversen et al. studied the CVD risk of elevated cholesterol levels in a healthy population in Copenhagen City Heart Study. They found that the older the individual, the higher the cholesterol level above which the risk of CVD was increased, and concluded: '*The risk of incident CHD associated with plasma-cholesterol declines with age*.' [14]. The risk of cardiovascular events associated with apoB particles is greater in younger compared to older individuals [15]. Bathum et al. investigated a group of individuals (n = 118,160) without diabetes, **without CVD** and without statins at baseline, aged >50 years. Their study shows that **high** total cholesterol (TC) and LDL-C levels in the elderly (having TC >5 mmol/L and/or LDL-C > 3 mmol/L) are associated with a **lower** all-cause mortality compared with the group with the recommended low TC or LDL-C levels [16]. The Cholesterol Treatment Trialists' Collaboration studied the use of statin therapy for primary prevention in patients >75 y (without evidence of occlusive vascular disease) and showed no significant reduction of risk of vascular events per 1.0 mmol/L lowering of LDL-C [17].

4. Is LDL-C a risk factor of CVD for men and women alike?

In a study including 67,413 men and 82,237 women who had been followed up for many years, TC was weakly associated with CHD mortality for men, except for those between age 50 and 64 years. For women, TC was weakly associated with CHD mortality among those <50 years, and no association was present after that age. Ulmer et al. concluded ' *The role of high cholesterol in predicting risk of premature heart disease could be confirmed in men of all ages and in women under the age of 50.*' These authors also found a role of **low** cholesterol in predicting risk of premature heart disease in men aged <50 years, but not in women aged <50 years [18].

5. Is LDL-C a risk factor of CVD in all racial groups?

Several Japanese studies have found that LDL-C is **not** a risk factor for CHD mortality in women of any age. Hamazaki et al. concluded: '*The theory that the lower the cholesterol levels are, the better is completely wrong in the case of Japan – in fact, the exact opposite is true*' and '*it seems clear that high cholesterol levels should not be considered unhealthy especially in elderly people*.' [19]. Zhou et al. studied data of middle-aged and elderly participants collected in the China Health and Retirement Longitudinal Study. The 4-years follow-up of 4,981 male and 5,529 female respondents, not on statin therapy, was analyzed. For men aged 45–60 y high LDL-C (>3.54 mmol/L) was not a risk factor for all-cause mortality, but **low LDL-C** (≤2.17 mmol/l) was. For women aged 45–60 y neither high LDL-C, nor low LDL-C was a risk factor for all-cause mortality. Both for men and women aged ≥60 y neither high LDL-C, nor low LDL-C was a risk factor for all-cause mortality. The authors questioned whether low LDL-C should be a target for a healthy life [20]. So it seems that **racial differences** exist with regard to high cholesterol and its association with CHD risk in East-Asian populations, at least in healthy individuals. However, a recent study in Chinese statin-naïve patients who underwent percutaneous coronary intervention (PCI) showed that LDL-C has better predictive value for cardiovascular outcomes than non-LDL-C lipid parameters [21].

6. Is the relation between LDL-C and CVD risk influenced by the presence of comorbidities?

In individuals with type 1 diabetes (DM) LDL–C was **not a good predictor** of CVD. Furthermore, Hero et al. found no support for an LDL–C cutoff point of 2.6 mmol/l. When considering primary prevention in type 1 DM patients, TC/HDL–C appeared more reliable as a marker for CVD risk than LDL-C [22]. In patients with type 2 DM, the dyslipidemia is characterized by increased number of TG-rich particles, increased postprandial concentrations of TG-rich particles, increased number of LDL particles, small dense LDL particles, decreased HDL particle numbers, several changes in particle composition of HDL [23] and elevated levels of remnant-like particle cholesterol levels [24].

7. Does reduction of CVD risk by LDL-C lowering translate into reduction of mortality?

DuBroff published a commentary that questioned the practice of LDL-C reduction as LDL-C reduction – if it would reduce CVD risk – does not translate into reduction of mortality. From 29 major RCTs of cholesterol reduction, published after 2004, only two studies reported a mortality benefit [6]. Also Kristensen et al. concluded that statin treatment (both in primary and secondary prevention) results in a surprisingly small average gain in overall survival within the trials' running time. For patients whose life expectancy is limited or who have adverse effects of treatment, withholding statin therapy should be considered [25]. Particularly in meta-analyses on the major RCTs, reduction of CVD risk should include reduction of mortality, but there are several factors active that obscure this conclusion, such as selection of trials, definition of end-points and others (see below).

8. Selection of trials to be analyzed in a meta-analysis

Ravnskov and coworkers [3] accused the international lipid societies which organize the RCTs together with the pharmaceutical industry from selection of papers and use of soft endpoints (including composite endpoints). Furthermore, these authors claim that statistically nonsignificant findings in favor of the cholesterol hypothesis were inflated, and unsupportive results were quoted as if they were supportive. In scientific publications, subjective judgments should be avoided. In each paper with results from RCT or meta-analysis the criteria for inclusion in the study should be mentioned in detail in a way that reviewers can assess each study for correct inclusion criteria.

9. Endpoints

Utilizing combined endpoints may lead to an exaggeration of perceived benefit by assigning equal importance to disparate clinical events such as a hospital admission for angina and death from a heart attack. In meta-analyses there is a combination of different types of CHD events from diverse studies into one endpoint even though each study defines CHD events differently [26].

10. Factors that influence the effect of LDL-C on CVD risk

We believe that much of the opposition is caused by factors that – if present – may disturb the causal relation between LDL-C reduction and CVD risk reduction. Below we address several of these factors.

10.1. Target levels

In the clinical guidelines for lipid lowering in the secondary prevention of CVD events Brown et al. noticed **considerable variations in the target levels of TC and LDL-C**. As to the long-term follow-up they found **considerable variations in the recommendations** for the interval of testing (only annually, or at intervals of 3 to 12 months) [27]. Therefore, we believe that target levels should be defined for age and gender groups separately. But in what time interval should these target levels be reached? Dubroff et al. analyzed the recommendations to define new cholesterol target levels for four groups of patients with moderate-to-high risk of CVD: 1. patients who already sustained a CVD event; 2. adult diabetic patients; 3. individuals with LDL-C ≥ 4.9 mmol/L; 4. individuals with an estimated 10 year risk of CVD event ≥ 7.5%. Whether target levels of LDL-C were achieved or not did not confer any additional benefit, as they concluded from 35 RCTs. Secondly, these recommendations would lead to unnecessary treatment of low-risk individuals [28].

10.2. Is a low LDL-C level always beneficial and when are elevated levels of LDL-C beneficial?

In the Japan Lipid Intervention Trial, Matzuzaki and coworkers found that hyperresponders on simvastatin therapy (to TC levels <4.13 mmol/L) had a higher relative risk of death from malignancies than other patient groups [29]. Low cholesterol levels (TC < 2.84 mmol/L) in patients with postoperative abdominal infections predicted higher mortality rates than in patients with TC ≥ 2.84 mmol/L: 30.3% vs 12.7%, respectively ($p < 0.001$) [30]. Hospitalization death of patients with severe and critical COVID-19 was worse the lower the LDL-C (measured after admission), particularly if LDL-C < 1.83 mmol/L, and the lower the LDL-C, the higher the risk of cardiac injury [31]. Wei et al. reported that LDL-C and TC levels (measured after admission) of COVID-19 patients were inversely correlated with C-reactive protein (CRP) and interleukin-6 (IL-6), and positively correlated with the number of lymphocytes [32]. With other words, in infected patients a high LDL-C appears to have a beneficial effect on outcome. In $LDLR^{-/-}$ mice, hypercholesterolemia protected against lethal endotoxemia and severe Gram-negative infections [33]. Sijbrands et al. studied the pedigrees of three carriers of an familial hypercholesterolemia (FH) mutation in a genealogic way, and found a survival advantage of having a FH mutation (and corresponding elevated cholesterol levels) in a time when infectious diseases were prevalent [34].

10.3. Composition of the study group

Is it always clear who is included in the study, such as a RCT? Healthy individuals (without or with selection of health indexes, such as obesity, smoking habits, sedentary life style), individuals with mild cardiovascular complaints, or patients who have developed a major cardiovascular event (e.g. MI)? The conclusion may be that RCTs and meta-analyses of RCTs mix up a series of variations to produce a 'general' result (or artificial result) that may be true for the 'average' patient. For any conclusion to be drawn and generalized from RCTs and meta-analyses the population that is studied should be well described and homogeneous in its composition.

10.4. Differences between trial study designs

The RCTs and meta-analyses that underlie the 'cholesterol hypothesis,' show several differences, as to (1) selection of the trial participants, including age, race, gender, lifestyle, disease symptoms; (2) duration of the study period, (3) whether the target levels have been reached, or not? (4) the end points used (particularly in the composite end points), (5) statistical methods to correct for various covariate factors, and (6) the statins that have been used in trials, lipophilic or hydrophilic statins, in different concentrations, and different maximally tolerated doses.

11. Statins

For over 20 years, the benefits of this pharmacological family of HMG-CoA reductase inhibitors, in terms of health effects, side-effects, adherence, and toxicity, are praised as well as questioned.

Diamond & Ravnskov noted that the directors of clinical trials make an effort of minimizing the significance of numerous adverse effects of statin treatment. That is caused by the policy of **withdrawing** a large number of eligible subjects from a study during the run-in period, probably because these subjects did not tolerate the adverse effects of the statin therapy [35]. As an example, they mentioned the British Heart Protection Study that was published in 2002 [36]. In this study, 26% of all eligible subjects were withdrawn from the study after being on simvastatin for 1 month before the formal initiation of the study. The major reason to withdraw was occurrence of muscle pain. In his Anitschkov lecture read during the congress of the European Atherosclerosis Society in 2021 Collins elegantly showed that inclusion or exclusion of eligible subjects from RCTs did not change the conclusion about the health effects of the statin being tested [37].

In individuals with 5-year risk of major vascular events <10%, each 1 mmol/L reduction in LDL cholesterol produced an absolute reduction in major vascular events of ≈11 per 1000 over 5 years, which result led to the conclusion '*This benefit greatly exceeds any known hazards of statin therapy*' [38]. However, the number of individuals needed to treat to prevent one major vascular event is 91, a benefit that is further curtailed by the side-effects of statins. Three years later the same collaborative research group, the Cholesterol Treatment Trialists' Collaboration, published a large meta-analysis that showed that in men and women having an equivalent risk of CVD, statin therapy is similarly effective for the prevention of major vascular events [39]. In 2020, the TIMI Study Group demonstrated that the reduction in major vascular events induced by statin therapy occurred irrespective of age [12].

But statin therapy may be less beneficial in the elderly. Results from RCTs support the use of statins in older patients at high or very high risk of atherosclerotic CVD, although there is less direct evidence of benefits in the primary prevention setting among patients aged ≥75 years [40]. The use of statins in older individuals with no previous cardiovascular disease or statin prescription is also under debate. Gitsels et al. carried out a longitudinal study using **primary care** records and found support for the use of statins in those aged ≥75 years [41]. So, this issue is not solved yet. The groups of elderly individuals who are to have statin therapy for primary prevention and the groups of elderly patients who are to have statin therapy for secondary prevention should be selected in more detail to obtain optimal effect of cholesterol lowering in those who benefit from this treatment.

11.1. Statin resistance

The resistance to statins is a genuine phenomenon, and has been associated with polymorphisms in a series of genes, including the genes encoding HMG-CoA reductase, apolipoprotein E (apoE), PCSK9, LDR-receptor, and lipoprotein(a) (apo(a)) [42]. The transport of statins into the hepatocyte is regulated by the solute carrier organic anion transporter 1B1 (*SLCO1B1*) gene which encodes the organic anion transporter polypeptide OATP1B1. Several polymorphisms of *SLCO1B1* are associated with statin-associated muscle symptoms, which effects are statin specific [43–45]. Zubiaur and coworkers reported that the best predictor for atorvastatin's pharmacokinetic variability is the phenotype of SLCO1B1 and they recommended that prescription of atorvastatin should be adjusted based on it [46].

11.2. Adherence to statin therapy

Numerous studies have documented high rates of non-adherence to statins [47]. It is estimated that ≈50% of the patients discontinue statin therapy within the first year of treatment, with further decreases in adherence over time [48]. Adherence to statin therapy for primary prevention in individuals ≥60 years was only 25% after 2 years [49,50]. In their investigation of long-term persistence with statin treatment in Israel, Chodick et al. found that ≥75% of patients discontinued therapy within 2 years of initiation [51]. Besides, high out-of-pocket costs as well as misleading claims in the media contribute to limited adherence to statin therapy in the long term. Non-adherence to statins may be hazardous: it is associated with an increased mortality risk [52]. Recently, May et al. demonstrated that in atherosclerotic CVD patients with ≥5 years of continuous pharmacy, long-term adherence to statins was associated with decreased number of major adverse cardiovascular events (MACE) in a linear-fashion [53]. In Scottish, patients with diabetes long-term adherence to statin therapy is poor, especially among those with few other cardiovascular risk factors. Most of the drop in adherence occurred in the first 6 months of therapy. Only 41% of patients were still taking >80% of their statin therapy after 5 years [54]. These studies emphasize the need to motivate the patients to adhere to long-term statin therapy, and to carefully watch occurrence and treatment of side-effects that are the primary causes of non-adherence. Another cause of non-adherence is statin intolerance. There are alternative therapeutic options if statin intolerance emerges, such as lowering statin dose, switching to a different statin, prescribing intermittent dosages and combining a statin with other lipid-lowering drugs, like ezetimibe, PCSK9 inhibitors, and bempedoic acid, beyond diet and lifestyle measures [55]. In a recent

expert opinion paper on statin adherence Drexel et al. listed a number of factors leading to non-adherence and formulated possible solutions, including educational support from the health-care provider, use of combi-pills, and increased availability of medical support [56].

11.3. Side-effects of statins

The high rates of non-adherence to statins should be noticed in light of undesired side-effects of this therapy. The American Heart Association has published a scientific document on statin safety and associated adverse effects and concluded that particularly in patients with high risk of CVD prevention and/or treatment of side-effects is a priority, as the benefits of statin therapy outweigh the risks [57].

A number of side-effects have been described, but only the muscle-related side-effects occur quite frequently.

11.3.1. Muscle-related adverse effects (incl. myopathy, rhabdomyolysis)

Symptoms include muscle aches or myalgia, weakness, stiffness, and cramps. The statin-associated muscle symptoms are one of the principal reasons for statin non-adherence and/or discontinuation, contributing to adverse cardiovascular outcomes [58]. It is estimated that statin-associated muscle symptoms occur in ≈5–10% of statin-treated individuals [59,60]. Diamond & Ravnskov noticed that dividing muscular symptoms into 11 categories resulted in a low incidence of adverse effects per category, which is -to their opinion- a misleading trick to obtain categories of adverse effects that contain low numbers of patients [35].

11.3.2. Cancer

A meta-analysis compiled from 28 RCTs that included ≈150,000 participants demonstrated that statin therapy had no effect at any age on cancer death or cancer incidence [12]. From the publication of PROSPER, a statin trial with **elderly** people [61], Ravnskov et al. recalculated the data on cancer, nonfatal, and fatal combined, and found significantly more cancer (by 23%) in the pravastatin group [62].

11.3.3. Diabetes

Almost 20 years after the start of statin therapy, it was found that the use of statins is associated with an increased risk of type 2 DM [63]. A Mendelian randomization study using genetic variants coding for HMG-CoA reductase demonstrated that these variants were associated with an increase in type 2 diabetes [64,65]. New-onset type 2 DM, induced by statin therapy, occurs only in ≈0.2% per year of treatment [57]. Culver et al. found that the use of statin medication in postmenopausal women is associated with an increased risk (by 46–48%) for new-onset type 2 DM, and that this effect is independent of whether the women suffered from CVD (at baseline) or not [66]. These results suggest a medication class effect, not related to potency or to individual statin. Klimentidis et al. suggested that statin-induced **low** circulating LDL-C may be a risk factor for type 2 DM, but is this effect specific for statins? They identified a collection of genetic variants that may provide insight into the mechanisms underlying the diabetogenic risk of **low** circulating LDL-C and of lipid-lowering medications, and underlying the relatively low type 2 DM risk among individuals with familial hypercholesterolemia [67]. Attempts to make statins more specific and thereby reduce off-target effects will not avoid the increased risk of diabetes [68].

11.3.4. Cerebrospinal dysfunction

Diamond & Ravnskov noted that cerebrospinal adverse effects of statins are characterized as rare, perhaps because they are classified into many different subgroups. According to the FDA Adverse Event Reporting System, adverse effects from cerebrospinal dysfunctions are classified in 23 separate terms (suicidal attempt, suicidal ideation, suicidal behavior, aphasia, balance disorders, and 18 others). The incidence of statin-related side effects in the many different subcategories is present at a low rate, but if all of them were to be combined the total number of adverse events may be substantial [35]. Recently, Kim et al. reported that in Koreans the use of statins showed an association with a significant reduction of risk of Alzheimer's disease by 5%. This risk was lowest for women ≥75 years and individuals with a low risk of Alzheimer's disease development [69]. As to cognitive function, qualitative and quantitative systematic reviews of available evidence from RCTs have not found evidence of any adverse effects of exposure to statin therapy on a wide range of different cognitive measures [4].

11.3.5. Hepatotoxicity

Elevations of hepatic transaminases occurred significantly more frequently (4.3 cases per 1000 patients treated, excluding use of cerivastatin) with statins than with placebo, but progression to liver failure is exceedingly rare. These elevations are usually reversible with reduction of dose or termination of therapy [70].

11.3.6. Hemorrhagic stroke

Treatment of 10,000 patients for 5 years with an effective statin regimen would cause 5 to 10 hemorrhagic strokes [4].

11.3.7. Pleiotropic effects

Although usually not described as a side-effect, statins have a number of pleiotropic effects that are cardiovascular protective effects independent of LDL-C lowering. Due to their HMG-CoA reductase inhibition, the production of isoprenoid intermediates is inhibited, and post-translational prenylation of small GTP-binding proteins is inhibited. In cell culture and animal studies the expression of endothelial nitric oxide synthase is stimulated, and there are favorable effects on stability of atherosclerotic plaques, pro-inflammatory intermediates, platelet reactivity, cardiac hypertrophy, and cardiac fibrosis [71].

12. Treatment of hyperlipidemia by other drugs than statins

12.1. Ezetimibe

Ezetimibe inhibits the absorption of cholesterol by targeting a sterol transporter called Niemann-Pick C1-like 1, which is

localized at the brush border cells of the small intestine and involved in the uptake of cholesterol [72]. Monotherapy with ezetimibe has demonstrated a mean percentage reduction of 18% in LDL-C from baseline, TC fell by 13.5%, HDL-C fell by 3%, and TG fell by 8% when compared with placebo [73,74]. Often ezetimibe is prescribed to patients whose LDL-C did not decrease sufficiently with statin alone, or are intolerant for statins. When added to statins, ezetimibe's LDL-C lowering effects and degree of risk reduction are additive.

12.2. PCSK9 inhibitors

Proprotein convertase subtilisin-kexin type 9 (PCSK9) inhibitors are monoclonal antibodies that bind to the PCSK9 protein. The binding of PCSK9 to the LDL receptors causes degradation of the receptor through intracellular pathways. PCSK9 inhibitors prevent the binding of PCSK9 to the LDL receptors, and consequently increase LDL-receptor recycling, increase LDL receptor density, increase uptake of circulating LDL, and decrease of the LDL-C concentration in blood. After subcutaneous delivery, the PCSK inhibitor is rapidly absorbed. Within days, LDL-C reductions of ≈60% from the baseline value is observed and the effect is sustained for about 2 weeks at lower doses [75]. With higher doses of PCSK9 inhibitor injection, the duration of the LDL-C lowering effect is extended [76]. One of the PCSK9 inhibitors, alirocumab, is studied in the Odyssey Outcomes Trial in patients who were treated with a high-intensity or maximally tolerated dose of a statin [77,78]. This trial provided evidence that alirocumab has additive effects on both the LDL-C reductions and cardiovascular benefits when combined with high-intensity statins. In the FOURIER trial, the PCSK9 inhibitor used was evolocumab, which reduced LDL-C by 59%, the risk of primary end point by 15%, and the risk of secondary endpoint by 20% [79]. Generally, the two PCSK9 inhibitors mentioned have a favorable safety profile and the injections appear to be well tolerated. Their potent LDL-C reducing effects come with a price, but the costs have dropped in recent years. To date, these PCSK9 inhibitors are therapy of choice in patients who do not reach target LDL-C levels despite maximal statin dose in combination with ezetimibe.

12.3. Bempedoic acid (BDA)

BDA is a once daily prodrug that requires activation by the enzyme very-long-chain-acyl-CoA synthetase A [80]. The active metabolite of BDA inhibits ATP citrate lyase, an enzyme upstream of HMG-CoA reductase. Inhibition of ATP citrate lyase prevents *de novo* cholesterol synthesis in hepatocytes, and thereby increase LDL receptor density, increase uptake of circulating LDL, and decrease of the LDL-C concentration in blood. Therapy with BDA reduced LDL-C levels by 15–16%, compared to placebo [81,82]. Concomitent use of BDA with simvastatin doses >20 mg or pravastatin doeses >40 mg is not recommended, but no dosage adjustments are required with atorvastatin or rosuvastatin [83]. The introduction of BDA in the medical arena is largely hampered by high costs.

12.4. Inclisiran

Inclisiran is a long acting, double-stranded, siRNA molecule. It inhibits the production of PCSK9 in the liver by cleaving mRNA required for PCSK9 production, thereby preventing the interaction of PCSK9 with the LDL-receptors, leading to upregulation of LDL-receptors, increased uptake of circulating LDL, and reduced levels of LDL-C in blood [84]. Inclisiran is administered subcutaneously at day 0, 90, and every 6 months thereafter. Twice-yearly injections of inclisiran reduced LDL-C by 56%, compared to an increase of 1% with placebo [84]. The FDA did not granted the new drug application, so this drug is not (yet) on the market for clinical purposes.

13. Other therapies than LDL-C lowering therapies

The incidence of CHD is related to -besides lipid/lipoprotein factors, like LDL, Lp(a) and remnants- metabolic (incl. diabetes) and inflammatory factors. Therapies that address non-lipid mechanisms and demonstrate effectiveness by other mechanisms than reducing cholesterol levels are dietary measures, healthy lifestyle factors, anti-inflammatory measures, and daily intake of high doses of fish oil. These therapies have proven to be efficacious in primary and secondary prevention of CHD, and deserve, in our opinion, much more awareness, in combination with current therapies.

13.1. Mediterranean diet

DuBroff & De Lorgeril recommended the Mediterranean diet for individuals with increased CVD risk [26]. This diet has also been advocated to function for primary prevention of CVD, without changing TC and LDL-C levels [85]. In elderly people the Mediterranean diet was found to be associated with reduced expression of proinflammatory and proatherogenic genes, independent of LDL-C levels [86]. In an initially healthy female cohort study that ran for 20 years, the Mediterranean diet was associated with a 30% reduction of relative risk to develop type 2 DM, which could be mediated through insulin resistance, adiposity, lipoprotein metabolism, and inflammation [87].

13.2. Healthy lifestyle

It is generally known that healthy lifestyle factors are associated with a lower risk of CVD, in men and women alike. These factors include healthy diet, physical activity and no-smoking [88,89]. In Chinese individuals, Lv et al. found that the healthier the lifestyle factors (i.e. no-smoking, light-to-moderate alcohol consumption, healthy diet, physical activity, and a healthy body weight without central adiposity), the lower is the CVD risk (prevention of two-thirds of major coronary events over a period of ≈10 years) [90]. In Taiwanese patients with CAD who underwent a percutaneous coronary intervention (PCI) Yang et al. investigated whether lifestyle factors post-PCI could improve CVD risk. They found that a healthy diet, no-smoking, and exercise lowered the CVD risk by 50%. The more lifestyle factors the patients adopted, the lower the concentrations of inflammatory markers, CRP,

LDL-C and the ratio of TC/HDL, and the lower the waist circumference. These benefits were particularly seen in young and male patients, in patients treated with statins, in patients with HDL-C < 1.03 mmol/L, and in patients with left ventricular ejection fraction <50% [91].

13.3. Anti-inflammatory drugs

Anti-inflammatory drug studies, using interleukin (IL)-1β inhibitors [92], IL-6 ligand antibodies [93], and colchicine [94], were shown to reduce CVD risk in patients with a history of cardiovascular events. These potent effects of anti-inflammation therapies warrant further studies to add those therapies to the standard protocols and guidelines for treatment of CVD.

13.4. Icosapent ethyl

Instead of supplementation of the diet with docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), intake of icosapent ethyl (2 g twice daily), a highly purified and stable EPA ethyl ester, in patients with elevated TG levels despite the use of statins (30% primary prevention and 70% secondary prevention) reduced the risk of ischemic events, including cardiovascular death by 25% in 4.9 years. These beneficial effects were independent of the reduction of TG levels, but the mechanism behind the risk reduction is not yet elucidated [95]. Recently, Lakshmanan reported for the EVAPORATE Trial that in patients with elevated TG levels despite the use of statins by taking icosapent ethyl (2 g twice daily) the total atherosclerotic plaque burden was reduced by 55% in 18 months, compared with those in the placebo group [96]. Icosapent ethyl offers a new opportunity for substantially reducing persistent CVD risk in statin-treated patients with TG > 1.7 mmol/L and either established atherosclerotic CVD or diabetes with at least one additional risk factor [97]. In a recent review, Mason and Eckel approved with the TG-lowering effects of omega-3 fatty acids, but found no evidence that TG lowering itself is an effective strategy for reducing risk of CVD. These authors stipulated that the reduction of cardiovascular events by icosapent ethyl in the REDUCE-IT and JELIS trials is not correlated with TG lowering, and considered the improved CVD risk to be associated with pleiotropic effects of high EPA levels, such as lipid/lipoprotein (per)oxidation, inflammation, endothelial function, free radical scavenging, and platelet aggregation [98]. In a recent meta-analysis Fan et al. compared the addition of omega-3 supplements to statin therapy versus statin therapy alone on coronary artery plaques. The combination treatment delayed the progression of total plaque volume and fiber content, and increased fibrous cap thickness of the plaque, more than did statin therapy alone [99].

Are these beneficial interventions – that do not necessarily reduce LDL-C levels – beneficial because these interventions exert an anti-oxidative action of lipoproteins and their associated apolipoproteins? Already in 1989 Steinberg et al. published about **modifications** of LDL that increase its atherogenicity, as the concentration of TC and/or LDL-C could '*by no means be the only causative factor*' for CHD. They proposed an oxidative modification of LDL to be causative for CVD [100]. As oxLDL has decreased affinity for the LDL-receptor and increased affinity for scavenger receptors of subendothelial macrophages, oxLDL promotes the formation of foam cells, leading to advanced vascular lesions [101]. Increased levels of malondialdehyde-modified LDL are associated with plaque expansion in statin-treated patients with coronary artery disease [102]. We argue that much of the controversies as to high cholesterol is a risk factor of CVD and low cholesterol levels should be the target of statins (and newer cholesterol-lowering drugs) is caused by the treatments cited above that do not necessarily alter cholesterol levels, but do lower CVD risk. Question is who will benefit from what therapy, and in the coming years we should study therapies that include diet, lifestyle factors, anti-inflammatory and anti-oxidative measures, omega-3 supplements, and drugs that specifically reduce LDL, Lp(a) or remnant lipoproteins. These remnant lipoproteins are frequently observed in combination with low HDL-C, elevated TG, insulin resistance, and small dense LDL (sdLDL). In 2006, Packard et al. described the sdLDL concentration as well as the particle size of sdLDL as a risk factor of CVD [103] and recently Balling et al. added that high levels of sdLDL-C are associated with higher risk of MI and atherosclerotic CVD [104]. However, the role of sdLDL as an independent predictor of cardiovascular disease, that is independent of HDL-C and TG levels, and the laborious assay of plasma sdLDL concentrations have prohibited its introduction in standard clinical diagnostic tests.

14. Residual risk of cardiovascular events

Despite current standards of care aimed at achieving targets for LDL-C, blood pressure and glycemia, dyslipidemic patients remain at high residual risk of vascular events. Atherogenic dyslipidemia, specifically elevated TG and low levels of HDL-C, often with elevated apoB and non-HDL-C levels, is common in patients with established CVD, type 2 DM, obesity or metabolic syndrome and is associated with macrovascular and microvascular residual risk. The Residual Risk Reduction Initiative (R^3I) was established to address this important issue [105]. They defined residual cardiovascular risk as '*the risk of cardiovascular events that persist in people despite achievement of treatment goals for LDL-C, blood pressure, and glycemia according to current standards of care*.' As an example, these authors discussed the Treating to New Targets (TNT) study, which demonstrated that patients treated with high-dose atorvastatin had a residual risk of a major cardiovascular event in the first 6 years of 8.7%. High blood pressure, lifestyle factors (including exercise, smoking, and diet), inflammatory factors and metabolic factors (including DM) are implicated in this residual vascular risk, with or without combination with remnant lipoproteins, Lp(a) and oxLDL. By addressing these factors in each patient, residual vascular risk can be reduced specifically, and that is what we mean with precision medicine, for the sake of personalized patient care. In high-risk patients with type 2 DM presenting with high TG levels and/or low HDL-C, Reiner promoted the addition of fenofibrate to statin treatment [106]. Several years later, in 2016, Ferrari and coworkers addressed atherogenic dyslipidemia and residual cardiovascular risk in detail [107]. While addressing lifestyle optimization, they included 5–10% weight reduction and monitoring of sleep pattern. As to potential markers of atherogenic

dyslipidemia they include Lp(a) and mention lipoprotein apheresis to reduce LDL-C and Lp(a) levels. As markers of proper treatment, these authors promote measurement of non-HDL-C, that '*gives an assessment of the levels of atherogenic molecules, including LDL-C and TG-containing particles*.' Later on, non-HDL-C was replaced by apoB. Marston and coworkers who sought for lipid parameters that are associated with risk of myocardial infarction (MI), reported that risk of MI was captured best by the number of apoB-containing lipoproteins, independent from lipid content (cholesterol or TG) or type of lipoprotein (LDL or TG-rich lipoproteins). Lowering the concentration of all apoB-containing lipoproteins should be the focus of therapeutic strategies [108]. This paper was accompanied by an editorial written by Sniderman and coworkers who included in the title of their contribution: '*The debate is over*' [109].

15. Familial hypercholesterolemia (FH)

Individuals with heterozygous and homozygous familial hypercholesterolemia (HeFH and HoFH, respectively) have elevated serum levels of LDL-C on the basis of mutations in genes encoding the LDL-receptor, apolipoprotein B_{100}, or PCSK9. Patients with untreated heterozygous FH tend to experience a first coronary event ≥20 years earlier than the general population (mean age 42 vs 64 y) [110]. The guidelines of treatment of FH patients advocate statin therapy to reduce LDL-C levels and thereby reduce risk of CVD [2]. In spite of statin therapy, FH patients in a Norwegian registry had a standardized CVD mortality ratio of 2.29 [111], a result quite similar to that of a FH population in an UK cohort study [112]. Patients with FH were shown to have an inflammatory phenotype, despite long-term intensive cholesterol lowering treatment [113]. Ravnskov et al. elaborated that FH patients should not be treated as having only high LDL-C, as LDL-C of FH patients without CVD is almost as high as LDL-C of FH patients with CVD. Results of 9 RCTs with FH patients did not show any significant effects of LDL-C lowering therapy on CHD events, nor total mortality [114]. In the SAFEHEART cohort it was shown that plasma Lp(a) levels were higher in FH patients (by 12%) than in relatives without FH, and that the high Lp(a) levels in patients with FH independently predicted the risk of CVD [115]. Ravnskov et al. promoted screening for inborn coagulation disorders being more important CVD risk factors than LDL-C [114]. Earlier, Jansen et al. studied the contribution of classical risk factors for CVD in a large cohort of FH patients and demonstrated that <20% of the variation in occurrence of CVD could be explained by the classical risk factors alone [116]. In a search for candidate genes to CVD risk they found that a polymorphism in the prothrombin gene of patients with FH was significantly associated with an increased risk of CVD [117]. This polymorphism was shown to be a significant risk factor for MI at a young age [118]. These findings should lead to improvements of therapy of FH patients to prevent CVD, to replace simple targeting of LDL-C reduction [119].

16. Commercial independence

Diamond & Ravnskov have accused writers of papers with the results of RCTs to publish what the pharmaceutical industry demands as they (or their departments) are sponsored by that industry. They demand that the industry should make the clinical trial data available, but that request is most often denied. They accuse the RCT publications for '*deceptive approaches*' and '*statistical deception*' to maximize small therapeutic effects into significant efficacy of CVD therapies, by presenting relative risk reduction data for therapy effect and absolute risk reduction data for undesired side effects [35]. The ties of the writers and study participants with the industry are listed separately, in long sections of '*declaration of interests*,' of these RCT publications that clearly show that these ties with the industry are intense. The British Medical Journal stated that clinical education articles should be authored by experts without financial ties to industry. Chew et al. formulated this as '*Our aims are to preserve and enhance readers' trust in the journal's content and to help to shape a new relationship between journals and industry, rather than perpetuate the perception of medical journals as the marketing arm of commercial interests*.' [120].

17. Recommendations and conclusions

Papers that publish 'opposing' theories about the LDL hypothesis, that question its universal validity and scientific basis, should keep the medical profession sharp and critical. Members of the medical profession should analyze what population is selected in the papers they read. Is LDL-C measured or calculated? What outcome is studied? What does the outcome mean in terms of Number Needed To Treat to prevent one case? And any physician should think: What are the downstream consequences for patients? However, the **average** patient does not exist. We should diagnose the risk factors (lifestyle, genetics, & race) of the individual patient more precisely, to find out which patient would benefit from what therapy. DuBroff expressed this belief as follows: '*Our LDL-C-centric approach to cardiovascular disease prevention may have distracted us from investigating other pathophysiologic mechanisms and treatments*.' And as to who will benefit from lipid-modifying therapy DuBroff stated '*the real question is how to identify them*.' [6]. Calculated LDL-C and TG are completely **insufficient** quantities to judge the patients' CVD risk as they capture only part of the atherosclerotic profile, thereby causing controversial decisions. Moreover, the total errors for direct HDL-C and direct LDL-C methods are huge [104]. Thus, TG-rich lipoproteins, Lp(a) and apoB should all be included in the clinical workup as they represent risk factors of CVD beyond LDL-C [8,121–125]. Then, we should make an effort to identify all the risk factors of that patient, and to start specific interventions, but not without general adaptations to lifestyle and diet. This search for the particular patient's risk factors is an element of a molecular and holistic definition of health and disease, to improve the professional efforts from imprecision to precision medicine. Nevertheless, critical papers keep the medical profession sharp, by paying attention to specific elements of public health which should result in proper treatment to the individuals involved.

18. Expert opinion

Opponents as well as proponents of the cholesterol hypothesis published sound arguments within the narrow approaches that they considered. Therefore, we should focus on characterizations of study population by including the well-known risk factors, adapt specific study designs, and develop a more

integral approach in order to realize precision diagnostics and precision medicine, for the sake of improved patient care. In fact, atherogenicity is caused by those lipoproteins that can enter the vessel wall. So, not plasma cholesterol levels are relevant but the number of circulating lipoproteins that can invade into the vessel wall. We hence need biomarkers that can better reveal the number of circulating atherogenic (=apoB-containing) lipoprotein particles. ApoB is a good candidate to do so as it includes LDL, VLDL, IDL, remnant lipoproteins, small dense LDL, and Lp(a), and apoB can be measured with high accuracy [126,127]. Moreover, as to biochemical risk factors of atherosclerotic CVD, diagnosis should also include assessment of (pre)diabetes, and healthy lifestyle, including exercise and diet. Other risk factors, like hypertension, smoking and genetic risk score, should be taken in account as well. Most importantly, in primary as well as secondary prevention of atherosclerotic CVD, the workflow to check and maintain preventive measures should be intensified, thereby increasing the chances that clinical problems are prevented. As in current clinical practices most specialists are too short in time to do so, 'prevention assistants' may assess how well the individual is protected against future signs and symptoms of atherosclerotic CVD. The same applies to pharmacotherapy; the goals, target levels, and side-effects should be monitored regularly to decide whether doses are to be maintained or changed, or pharmacotherapy is to be stopped because of lack of effects, or unwanted effects. All members of the medical team involved in primary or secondary therapy of persons with, or suspected to have, atherosclerotic CVD, should stop with (1) treatment of the 'average' patient, and/or (2) uncoordinated treatment by a cardiologist, family doctor, dietician, and physiotherapist. Instead, the medical profession should be encouraged to prepare a personalized therapy plan that includes all disciplines needed to serve all aspects of the patients' health. We see a future in which the 'residual risk,' i.e. the risk of atherosclerotic CVD despite pharmacotherapy, is handled in a personalized way that includes therapy of all risk factors of that particular patient to realize precision diagnostics and precision medicine.

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