How low should you go with LDL cholesterol lowering in secondary prevention?: clinical efficacy andsafety in the era of PCSK9 inhibitors

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ABSTRACT:

The aim of the paper is a justification for lowering of LDL cholesterol (LDL-C) to very low levels in secondary prevention patients. There is a strong evidence that the lower LDL-C the lower risk of cardiovascular (CV) events. The evidence on validity of this hypothesis comes from epidemiological, genetic and clinical studies. The hypothesis " the lower the better" has been strongly supported by the results of secondary prevention trias with PCSK9 inhibitors. The combination PCSK9 and statins has resulted in achieving verylow LDL-C levels and additional reduction of CV events in secondary prevention. However despite of clinical benefit the safety of aggressive LDL-C lowering should be taken into consideration. So far the serious adverse events associated with achieving very low LDL cholesterol levels or intensive drug therapy have not been noted. The possitive clinical effects have been reflected in recent ESC/EAS Guidelines for dyslipidaemia management. The experts strongly recommended " lowering LDL-C to levels that have been achieved in trials of PCSK9 inhibitors".

Introduction:

The question ‘how low should you go with low-density lipoproteincholesterol (LDL-C)?’is particularly pertinent in light of recent observations that further reduction of cardiovascular events can be achievedin primary but especially insecondary prevention when very low LDL-C concentrations are achieved by adding proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors to baseline lipid-lowering therapy (1, 2,3). The approval of two PCSK9 inhibitors, evolocumab and alirocumab expanded our therapeutic options but also raised some questions on safety concerns in relation to the low (<50 mg/dL, 1.3 mmol/L) and very low (<15-20 mg/dL, 0.4-0.5 mmol/L) concentrations of LDL-C achieved during therapy. To answer the question raised in the title of the present article, it is necessary to understand whether human organs and tissues requireLDL-C, and if so, how much?The answers to these questions are informed by findings from population studies and the study of subjects with genetically determined low or very low LDL-C levels([4-7). Equally important (to those with PCSK9 inihbitors) are the safety and efficacy findings of clinical trials using statins and other lipid-lowering drugs that achieved very low lipid levels, and the meta-analyses of these studies. This review summarises the relevant clinical evidence and explains how it has been used to inform the choice of LDL-C targets in clinical practice guidelines.

Cholesterol is essential for normal physiological function

Recent advances in lipid-lowering therapy allow ever-lower LDL-C concentrations to be reached, especially when drugs are used in combination. Consequently, the safety of extremely-low cholesterol levels has become a pertinent issue because cholesterol is known to be an essential component of cells. In general, cells have two sources of cholesterol: LDL and intrinsic synthesis. Recently, Masana*et al.* proposed an intriguing “zero LDL hypothesis” and performed a comprehensive review to evaluate it (8,9).This issue has also been discussed by Olsson *et al.* in an article with the insightful title ”*Can LDL cholesterol be too low?”* (10).

The physiological role of LDL is to transport cholesterol to peripheral tissues *via* LDL receptors (LDLR). All nuclear cells are capable of synthesizing cholesterol and regulating its production; however, cholesterol uptake from LDL is energetically more efficient compared to its de novo synthesis from acetate. Most cells are not in direct contactwith the plasma,but are surrounded by interstitial fluid, in which the concentration of LDL-C is 5-fold lower than it is in the bloodstream (11,12). Studies of LDLR binding kinetics indicate that half-maximal binding occurs at an LDL-C concentration of 2.5 mg/dL (0.064 mmol/L) (13).Based on these

data, Olsson *et al.* concluded that a plasma LDL-C level of about 12.5 mg/dL (0.32 mmol/L) would be sufficient for adequate cellular cholesterol influx (10).

Organs with a particularly important requirement for cholesterol include the adrenalcortex, gonads and liver(9)].Dividing cells also require cholesterol. The highest cellular receptor concentration is seen in organs that synthesize steroid hormones from cholesterol, i.e., adrenals and gonads (14,15).However, the synthesis of adrenal and gonadal steroid hormones appears to be unaffected in patients with very low LDL-C levels, such as those with hypobetalipoproteinaemia, abetalipoproteinaemia, and also in patients with homozygous familial hypercholesterolaemia (HoFH) who have acomplete lack of LDL receptors(10)However, the response to adrenocorticotropic hormone (ACTH) may be reducedin these patients. These three genetic disorders demonstratethe existence of alternative pathways by which cholesterol can be obtained for adrenal steroid synthesis (10). These pathways include cholesterol ester uptake from high-density lipoproteins (HDL) by scavenger receptors class B type 1 (SRB1) (8).

The liver also requires substantial quantities of cholesterol. Hepatocytes abundantly express LDL receptors, and the liver catabolizes two-thirds of these lipoproteins(10)Hepatocytes also synthesize cholesterol from acetate. Thus, cholesterol in hepatocytes may have a dual source, LDL (and also intermediate-density lipoproteins [IDL]) and intrinsic synthesis. Cholesterol and triglycerides (TG) are assembled into very-low-density lipoprotein (VLDL) particles, which areLDL precursors. In addition, cholesterol is a substrate for bile acid synthesis, and it is partially excreted with bile. However, bile-acid synthesis is not impaired in patients without LDL-receptors (HoFH) or with HDL-deficiencies (10).

In order to discuss further the issue of very low LDL-C levels as an effect of the therapy, it is important to ask whether genetically-determined extremely low circulating concentrations of LDL leads to adverse effects. The answer depends on the cause of the condition. If the underlying causes are geneticmutations of intrahepatocyte microsomal triglyceride transfer protein (MTTP) or apolipoprotein (apo) B, adverse effects may occur. These include liver steatosis, neurological manifestations (progressive neuropathy), and ophthalmological manifestations (including retinopathy) (10,16).Liver steatosis results from the accumulation of triglycerides in hepatocytesbecause of the lack of synthesis and release of VLDL(triglyceride- and cholesterol transporting lipoproteins). Neuropathy and retinopathy are associated with impaired absorption of lipid-soluble vitamins A and E,which occurs as a result of impaired chylomicron formation in the intestinal epithelium (10).Thus, absent or

impaired LDL formation (abetalipoproteinaemiaand homozygous hyperlipoproteinaemia) due to mutations of the above mentioned MTTP and apoB genes is associated with severe early abnormalities. However,the prevalence and risk of cardiovascular disease (CVD) are very low (4).

The situation is entirely different in subjects with *loss-of-function* PCSK9 gene mutations, which may also lead to lifelong very low LDL-C levels but do not result in adverse effects (10,16) and confer protection from coronary heart disease (CHD) (5). These observations prompted research to develop therapeutic inhibitors of PCSK9 and led to the development of the monoclonal antibody inhibitors (PCSK9Is) - alirocumab and evolocumab.

Rapidly dividing cells show an increased requirement for cholesterol. Thus, fetal development in the absenceof LDL-C is an important consideration. In women with HoFH, fetal development is normal during the period of highest cellular proliferation and cell membrane synthesis, and according to Masana*et al*, this suggests that influx of cholesterol via LDL receptors is not essential for normal development (9). This needs to be further investigated and confiemed that based on available observations there is also no evidence of vitamin deficiencies, hormone deficits, impaired central nervous system function, or abnormal sexual maturation in subjects with HoFH (17,18).

The braincontains 25% of the total cholesterol pool of the body and requires a constant concentration of cholesterol(19)Cerebral cholesterol metabolism is autonomous, i.e., independent of blood cholesterol level, as cholesterol is locally synthesized in the brain. The blood-brain barrier prevents the influx of cholesterol from the blood and thereby minimizes variations of cholesterol concentration (20). Nevertheless, hypercholesterolaemiacan occur in the brain and is associated with poor outcomes(10). Hypercholesterolaemia may be a risk factor for Alzheimer's disease (21), and therefore the described positive effects of statins/lipid lowering therapy in thisgroup of patients((22). A possible culprit is a cholesterol metabolite, 27-hydroxycholesterol, which penetrates from the blood to the brain, inhibits glucose uptake (23), and reduces the level of the activity-regulated cytoskeleton-associated (Arc) memory protein in the hypothalamus (24). It is not known, however, whether the accumulation of 27-hydroxycholesterol is the cause or an effect of the disease.

Epidemiological studies

Mendelian Randomization (MR), prospective population and retrospective studies provided early evidence that total cholesterol (TC) and LDL-C levels are risk factors for CHD(25). The year 2019 marked the 70th anniversary of the initiation of the Framingham

Heart Study (FHS)(26). The association between TC and CHD was confirmed in the large prospective Multiple Risk Factor Intervention Trial (MRFIT) intrapopulation study in 361,622 middle-aged men (27). Of note, the MRFIT study did not identify any baseline threshold concentration of TC, below which no risk would be present. Later, the Atherosclerosis Risk In Communities (ARIC) study showed that the risk of CHD was related to LDL-C level when data were adjusted for age and race (28). Thus, these early epidemiological studies demonstrated that "*lower is better*", although LDL-C levels below 80 mg/dL (2 mmol/L) and TC levels below 140 mg/dL (3.6 mmol/L) were rarely observed in these studies (4). This gap in evidence was filled by epidemiological studies focusing on hereditary low LDL-C levels which are discussed below.

Low LDL-C levels due to PCSK9 gene mutations – clinical cases and population studies

Case reports of individuals with*loss-of-function*mutations of thePCSK9 gene confirmed that the resultant low LDL-C levels do not lead to any adverse effects.These subjects are healthy, physically active and fertile, despite having circulating LDL-C concentrations as low as 0.4-0.8 mmol/L (14-29 mg/dL) (6,7). In the Dallas Heart Study (12,887 individuals, including 3363 Afroamericans and 9524 Caucasians), the authors showed that PCSK9 gene mutations wereassociated with significantly lower LDL-C levels compared to individuals without mutations. PCSK9 mutations were associated with a lower rate of CHD over 15 years of follow-up (5). In Afroamericans, a 28% inLDL-C levels was associated with an 88% lower rate of CVD, and in Caucasians,a 15%reduction of LDL-C was associated with a 47% lowered risk of CVD. Because the cause of low LDL-C was genetic, these individuals had low exposure to LDL-C throughout life. Neither the risk of haemorrhagic stroke nor cancer were increased. Loss-of-function PCSK9 gene mutations are rare (1-3%) (5).

Genetically determined low LDL-C levels – Meta-analyses

MR studies have demonstrated that the risk of atherosclerotic CVD is lower inindividuals with genetically determined lower LDL-C levels (29-32).The same studies confirmed the rules that need to be especially met now based onnumerous data available, that "*the lower the better*" cannot exist alone, it should be always companied with the "*earlier the better*" (on LDL-C goal of therapy), and "*the longer the better*", preferably long-life (25,29-33).

Particularly valuable information was provided by a recently published comprehensive Mendelian randomization analysis which evaluated the relationship between genetically determined lower LDL-C levels, (i.e., lifelong exposure to lower LDL-C levels), and the risk

of CHD (32). The relationship between nine polymorphisms in six different genes associated with lower LDL-C levels and the risk of CHD was evaluated. Subsequently, a meta-analysis of the results of these nineMendelian randomization studies was performed to allow more precise estimation of the effect of chronic exposure to lower LDL-C levels and to compare it with the clinical benefit of the same magnitude of LDL-C lowering during statin therapy. The meta-analysis of the genetic studies included 312,821 participants, and the meta-analysis of 26 statin trials included 169,138 participants (32). The authors showed that chronic exposure to a 1.0 mmol/L (about 40 mg/dL) lower concentration of LDL-Cwas associated with a 54.5% reduction in the risk of CHD (odds ratio [OR] 0.46, 95% confidence interval [CI] 0.41-0.51, $p=2.15 \times 10^{-45}$). In contrast, the statin meta-analysis showed that drug-induced LDL-C reduction by 1.0 mmol/L was associated with a 24% lower risk of CHD (OR 0.76, 95% CI 0.74-0.78). Thus, CHD risk reduction in individuals with genetically determined LDL-C level lower by 1.0 mmol/L was over two times higher compared to patients who initiated statin therapy later in life. These findings indicate that to achieve a risk reduction of the same magnitude that is associated with a naturally lower LDL-C level by 1.0 mmol/L, LDL-C would need to be reduced by 3.0 mmol/L in patients initiating statin therapy later in life (32).In addition, the results of this study (32) demonstrated that all9genetic causes of low LDL-C levels [LDLRgene mutations, PCSK9 gene mutations, and hydroxymethylglutaryl-coenzyme A reductase (HMGCR)gene mutations] were independently associated with low CHD risk. Based upon these findings, the authors suggested that LDL-C lowering is probably much more effective CHD primary prevention strategy than previously thought. They noted that a healthy diet and physical activity (as well as body weight reduction) initiated early in life might be effective in the prevention of CHD (32,34,35). If these measures are not sufficient to maintain low LDL-C level, it is reasonable to consider lipid-lowering therapy in early adulthood.

Of note, in MR studies in was shown that more than 50 genes associated with lower LDL-C levels were related to a lower CHD incidence (30).

Clinical trials as the basis for lower LDL-C targets in patients with acute coronary syndromes (ACS)

The history of how LDL-C targets in acute coronary syndromes (ACS) have been incrementally reduced in response to the results of randomized clinical trials (RCTs) has been recently summarized by Quamar and Libby (36). Except for the ODYSSEY OUTCOMES study with alirocumab, no other clinical trial evaluated risk with the therapy targeted to

achieve a specific LDL-C target(2). However, clinical trials evaluating statin monotherapy and combination therapy in the secondary prevention of CVD showed thatthe achievement of lower LDL-C levels was associated with a lower rate of cardiovascular events (30). This observation was consistent in the Scandinavian Simvastatin Survival Study (4S) study (simvastatin) (37), the IMProved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT) study (simvastatin and ezetimibe) (38),the Further Cardiovascular Outcomes Research with PCSK9 inhibiton in Subiects with Elevated Risk (FOURIER) (statins with or without ezetimibe and evolocumab) and ODYSSEY OUTCOMES (statins with or without ezetimibe and alirocumab) (1,2). A concentration-dependent relationship between LDL-C and CV risk was observed, with the lowest risk in secondary prevention observed in patients with LDL-C levels <50-55mg/dL (1.3-1.4 mmol/L).

The initial therapeutic target for LDL-C in secondary prevention was <100 mg/dL (39), and it was set based on the results of the Cholesterol and Recurrent Events (CARE) study with pravastatin 40 mg/d versus placebo in patients after myocardial infarction (MI) (40). The study included 4159 patients with baseline LDL-C level 115-174 mg/dL (mean 139 mg/dL/3.6 mmol/L). The duration of follow-up was fiveyears. Pravastatin reduced LDL-C level by 32%, from 139 mg/dL to 97-98 mg/dL (2.5 mmol/L). This was associated with a 24% reduction of coronary death and myocardial infarction in the intervention group (hazard ratio [HR] 0.76, 95% CI 0.64-0.91) compared with the control group. Next, the target LDL-C level for post-ACS patients was reduced to <70 mg/dL (<1.8 mmol/L)(36,41,42)based on the results of the Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis In Myocardial Infarction 22 (PROVE IT TIMI 22) (43,44). This study included 4192 patients with ACS and a median baseline LDL-C of 106 mg/dL. The study compared the effects of intensive (atorvastatin 80 mg/d) and moderate-intensity (pravastatin 40 mg/d) statin therapy. At 2 years, median LDL-C level was 62 mg/dL (1.6 mmol/L) (interquartile range 50-79 mg/dL) in the intensive treatment group versus 92 mg/dL (2.4 mmol/L) (interquartile range 79-113 mg/dL) in the moderate-intensity treatment group.Compared to moderate-intensity statin therapy, intensive treatment reduced the combined endpoint (death, myocardial infarction, unstable angina requiring hospitalization, coronary revascularization, and stroke) by 16% (HR 0.84, 95% CI 0.84-0.95). Also, patients who achieved LDL-C concentrations<70 mg/dL (1.8 mmol/L) had a lower risk of recurrent MI or coronary death compared to those with LDL-C level ≥70 mg/dL(1.8 mmol/L) (2.7% vs 4.0%/100 person-years) (44). A posthoc analysis of the PROVE IT TIMI 22 provided the first evidence that lower LDL-C levels also resulted in reduced recurrent CV events(45). Compared to the patients with LDL-C>80-100

mg/dL (reference), the risk in patients who achievedLDL-C concentrations of 60-80 mg/dL, >40-60 mg/dL, and ≤40 mg/dL (1 mmol/L) was lower by, respectively, 20% (HR 0.80, 95% CI 0.59-1.07), 33% (HR 0.67, 95% CI 0.50-0.92), and 39% (HR 0.61, 95% CI 0.40-0.91). The last two results were statistically significant. In addition, no adverse effects of achieving LDL-C levels as low as <40 mg/dL (1 mmol/L), or of intensive statin therapy were observed. This study was the first to show that lowering LDL-C to <40 mg/dL(1 mmol/L) continues to be beneficial in the highest risk patients with ACS (44).

Finally, the current target LDL-C level of <55 mg/dL (<1.4 mmol/L)for the highest risk patients, including those with ACS and/or CVD (46,47) was decided based on the results of three abovementioned clinical trials, i.e., IMPROVE-IT (38,48), FOURIER (1,49) and ODYSSEY OUTCOMES (2) and meta-analyses of RCTs data (50-52).The IMPROVE-IT study included 18,444 patients with ACS and LDL-C level 50-100 mg/dLon statin therapy or 50-125 mg/dLwithout statin therapy (38). The patients were randomized to simvastatin 40 mg/d + ezetimibe 10 mg/d or simvastatin 40 mg/d alone. The duration of follow-up was more than 7 years. The weighted median LDL-C level during combined therapy was 54 mg/dLversus 69 mg/dL during monotherapy. The rate of CV events was 6.4% lower in patients treated with simvastatin and ezetimibe (P=0.016). The primary endpoint included CV death, non-fatal MI, unstable angina requiring hospitalization, coronary revascularization, and non-fatal stroke. In patients with baseline LDL-C level 50-100 mg/dL (6.4%), LDL-C lowering to <30 mg/dL was associated with a 21% lower cardiovascular event rate (HR 0.79, 95% CI 0.69-0.91) over six years compared to the achieved LDL-C level of ≥70 mg/dL (48). The IMPROVE-IT study was the first trial that showed a clinical benefit of combined lipid-lowering therapy and a significant risk reduction with a non-statin drug. In addition, it provided strong data in favour of the concept“*the lower, the better*”, and thus supported the causal association between LDL-C and the risk of atherosclerotic CVD. Atthe same time, the authors showed that the higher risk was at the baseline, the higher CVD benefits (=higher reduction) were associated with the intervention of the combination therapy. No adverse effects were noted in the IMPROVE-IT study, importantly including those with the achieved LDL-C level of <30 mg/dL (0.8 mmol/L) (53,54)

The results of the FOURIER (1,49) and ODYSSEY OUTCOMES (2) studies strongly influenced the decision to adopt a new therapeutic target for LDL-C in the most recent European guidelines (46). The FOURIER study included 27,546 patients with atherosclerotic CVD and baseline LDL-C level ≥70 mg/dL (81% after myocardial infarction) who were treated with statins (1). They were randomized to evolocumab (140 mg every two weeks, or

420 mg every four weeks subcutaneously) or placebo. Compared to placebo, LDL-C level was reduced by 59%, from the median of 92 mg/dL(2.4 mmol/L) to 30 mg/dL (0.8 mmol/L), and this was associated with a 15% reduction in the risk of cardiovascular events (CV death, MI, stroke, hospitalization due to angina, coronary revascularization) at 48 weeks (HR 0.85,95% CI 0.79-0.92). LDL-C level <20 mg/dL (0.5 mmol/L) was achieved in 10% of patients, 20 to <50 mg/dLin 31%, 50 to <70 mg/dLin 13%, 70 to <100 mg/dLin 29%, and ≥100 mg/dLin 17% (49). A progressive reduction in the CV event rate was observed with lower achieved LDL-C levels. Those patients who achieved LDL-C level < 20 mg/dL(0.5 mmol/L) had the lowest risk of cardiovascular death, myocardial infarction, and stroke compared to patients with LDL-C ≥100 mg/dL(2.5 mmol/L) (HR 0.69,95% CI 0.56-0.85).

As noted above, the ODYSSEY OUTCOMES was designed to evaluate the effect ofLDL-C reduction to 25-50 mg/dL on cardiovascular risk in patients with a history of ACS (1-12 months after the event). Eligible patients hadLDL-C ≥70 mg/dL, (1.8 mmol/L) non-HDL-C ≥100 mg/dL (2.5 mmol/L), or apo B level ≥80 mg/dL and were treated with the maximally tolerated statin dose (2). Overall, 18,924 patients were randomized to receive alirocumab (75 mg every twoweeks subcutaneously) or placebo. The dose was then adjusted to achieve an LDL-C concentration in the target range. The mean achieved LDL-C level at four weeks was 40 mg/dL (1 mmol/L) in the alirocumab group, compared to 94 mg/dL in the placebo group. Alirocumabwas associated with a 15% reduction in cardiovascular events (the combined rate of coronary death, non-fatal MI, fatal and non-fatal ischaemic stroke, and unstable angina requiring hospitalization) over the median 2.8 years of follow-up (HR 0.85, 95% CI 0.78-0.93). In this study, in contrast to the FOURIER study, patients with higher baseline LDL-C levels benefited more than those with lower LDL-C levels(≥100 mg/dL vs <100 mg/dL (2.5 mmol/L)). This study also showed that alirocumab therapy was associated with the significant 15% reduction of all-cause mortality, and this effect was even higher in patients at baseline higher risk (e.g. in those with LDL-C levels >100 mg/dL/2.5 mmol/L), and in those treated over 3 years. This was one of the most important studies to confirm the thesis presented above, that not only the lower the better, but the earlier on LDL-C target, the better (mean level 40 mg/dL after 4 weeks) and finally the longer the better(3,55)

These trials demonstrated that a progressive reduction of atherosclerotic cardiovascular events could be achieved by reducing LDL-C levels using intensivecombined lipid-lowering therapy,and thus informed the move towards lower LDL-C targets in recent guidelines.

Meta-analyses of clinical trials as the basis for lower LDL-C targets in secondary prevention

In addition to the results of the clinical trials described above, meta-analyses (50-52) have contributed to the move towards lower LDL-C targets in secondary prevention.In 2014,Boekholdt*et al.* published a meta-analysis of8 statin trials, with a total of 38,153 participants (50). This analysis aimed to evaluate the individual variation in LDL-C, non-HDL-C and ApoB level reduction, the proportion of patients who did not achieveLDL-C level <70 mg/dL(1.8 mmol/L) and, importantly, the association between very-low levels of atherogenic lipoproteins in treated patients, and the risk of cardiovascular events.The study confirmed a substantial inter-individual variation in the atherogenic lipoprotein level reduction in response to a fixed dose of a statin. This was also shown for patients (n=18,677) receiving intensive statin therapy. The mean achieved LDL-C level in this group was 69.4±27 mg/dL. An LDL-C level of <70 mg/dL(1.8 mmol/L) was not achieved by 40.4% of patients, <100 mg/dL(2.5 mmol/L) by 12.7%, and <50 mg/dL(1.3 mmol/L) by 78.3%. A non-HDL-C level of <130 mg/dL(3.4 mmol/L) or <100 mg/dL(2.5 mmol/L) was not achieved by 11.7% and 33.7%of patients, respectively, and apo B level of <100 mg/dLor <80 mg/dLwas not achieved by 14.7% and 35.7%of patients, respectively (50). Most importantly, it was shown that the risk of CVD events (combined rate of fatal or non-fatal MI, other fatal CHD cases, hospitalization due to unstable angina, and fatal or non-fatal stroke) was progressively reduced as lower LDL-C concentrations were achieved with treatment. Compared to patients with LDL-C level >175 mg/dL (>4.5 mmol/L), the adjustedHR for a CV event in those with achieved LDL-C level75 to <100 mg/dL (1.9 to < 2.5 mmol/L), 50 to <75 mg/dL (1.3 to 1.9 mmol/L), and <50mg/dL (< 1.3 mmol/l) was 0.56 (95% CI 0.46-0.67), 0.51 (95% CI 0.42-0.62), and 0.44 (95% CI 0.35-0.55), respectively. The appropriateness of the therapeutic target LDL-C level of <55 mg/dL (1.4 mmol/L) (in secondary prevention) is attested to by a significantly lower risk of major cardiovascular events in patients with the achieved LDL-C level of <50 mg/d (1.3 mmol/L) compared to those with the achieved LDL-C level of 75-100 mg/dL (1.9-2.5 mmol/L) (adjusted HR 0.81,95% CI 0.70-0.95). A similar relationship between the achieved LDL-C concentration, and CHD risk was noted. Despite these findings, the authors did not suggest a target LDL-C level of <55-50 mg/dL (1.4 mmol/L-1.3 mmol/L);instead, this came about in light of the results of the IMPROVE-IT (48), FOURIER (49), and ODYSSEY OUTCOMES (2) studies. A practically important observation from the meta-analysis by Boekholdt*et al.*(50) is the fact that 78.3% of patients receiving intensive

statin therapy did not achieve an LDL-C level of <50 mg/dL(1.3 mmol/L), indicating the widespread need for combination lipid-lowering therapy(25)

Another meta-analysis of statin and non-statin trials, published by Sabatine*et al.* in 2018, sought to answer the questionwhether the benefit of LDL-C lowering preserved in patient populations starting with LDL-C levels averaging 1.8 mmol/L (70 mg/dL) or less, and is LDL-C lowering safe in such patients?(51). For this purpose, the meta-analysis included patients from the intervention groups in statin trials included in the Cholesterol Treatment Trialists (CTT) Collaboration meta-analysis and patients from the IMPROVE-IT, FOURIER and Randomized EValuation of the Effects of Anacetrapib Throuth Lipid-modification (REVEAL) (statin + anacetrapib) studies who achieved the mean LDL-C level of CTTCollaboration meta-analysis (no data available), 55 mg/dl (1.4 mmol/L) in IMPROVE-IT,20mg/dl (0.5 mmol/L)in FOURIER and 55 mg/dl (1.4 mmol/L) in REVEAL and compared to the control groups with LDL-C levels of, respectively 67,70,67,and 62 mg/l (1.7, 1.8, 1.7 and 1.6 mmol/L). In all studies, a similar reduction in major cardiovascular events per 38.5mg/dl (1 mmol/l) was observed. This risk reduction was 22% (relative risk [RR] 0.78,95% CI 0.65-0.94)in CTTC, 21% (RR 0.79,95% CI 0.67-0.93)in IMPROVE-IT, 20% (RR 0.80,95% CI 0.61-1.04)in FOURIER, and 23% (RR 0.77,95% CI 0.63-0.96)in REVEAL. The overall result was a 21% reduction in the major cardiovascular event risk (RR 0.79,95% CI 0.71-0.87). A reduction was also noted in the risk of specific CV event types, i.e., coronary deaths, MIs, ischaemic strokes, and coronary revascularization. No significant differences were noted between the intervention and control groups in the rates of adverse events, i.e. any adverse events, myalgia and myopathy, aminotransferase elevation, new diabetes, haemorrhagic stroke, and malignancies (51).The authors concluded that there is a consistent RR reduction in major vascular events per further reduction in LDL-C in patient populations starting as low as a median of 1.6 mmol/L (63 mg/dL) and achieving levels as low as a median of 0.5 mmol/L (21 mg/dL), with no observed offsetting adverse effects (51). Moreover, the authors noted that the clinical benefit per LDL-C level reduction by 40 mg/dL (1.0 mmol/L) was virtually identical regardless of the lipid-lowering treatment used. However, these drugs exert variable effects on other risk factors, such as lipoprotein(a) level (PCSK9 inihbitors) (56,57) This observation underscores the great importance of LDL-C reduction to prevent cardiovascular events.

The aim of a 2020 meta-analysis by Wang *et al.* was to evaluate the effect of intensive LDL-C-lowering treatment beyond current recommendations on the risk of cardiovascular events and the safety of treatments(52). The meta-analysis included 52 RCTs with a total of

327,037 participants, including 31 studies of statin treatment vs placebo, tenstudies of intensive vs moderate-intensity statin treatment, 2 studies of ezetimibe + statin treatment vs placebo, 2 studies of ezetimibe vs placebo vs usual care, and 7 studies of a PCSK9 inhibitor vs placebo. It was shown that each 1.0 mmol/L reduction of LDL-C was associated with a 19% reduction in the risk ofmajor cardiovascular events (RR 0.81,95% CI 0.78-0.84). Over a mean follow-up of 3.7 years, a similar risk reduction per by 1.0 mmol/L LDL-C reduction was noted regardless of the baseline LDL-C level (<2.6 mmol/L, 2.61-3.40 mmol/L, 3.41-4.10 mmol/L, and >4.10 mmol/L) or the type of treatment (statin, ezetimibe, PCSK9 inhibitor). In addition, when evaluating the appropriateness of the current lower target LDL-C level in secondary prevention, it is important to note that a further reduction in the risk of major cardiovascular events occurred in patients with low baseline LDL-C levels, i.e., in patients with baseline LDL-C level <2.0 mmol/L in CTTCollaboration, <2.04 mmol/L in IMPROVE-IT, <1.8 mmol/Lin FOURIER, and <2.07 mmol/L in ODYSSEY OUTCOMES - by 21% (RR 0.79, 95% CI 0.65-0.96), 20% (RR 0.80,95% CI 0.73-1.11), 19% (RR 0.81,95% CI 0.62-1.06) and 18% (RR 0.66-1.01), respectively. This difference was significant in CTTCollaboration analysisand was observed as a clear (but not statistically significant) trend for risk reduction in the other studies. A meta-analysis of all the studies showed a significant 17% reduction in the risk of major cardiovascular events(HR 0.83,95% CI 0.75-0.92) (52).The clinical efficacy of further LDL-C lowering in patients with a low baseline value was consistent among subgroups, with no differences between women and men, diabetic and non-diabetic subjects, and patients with or without chronic kidney disease (52).The safety parameters evaluated in the meta-analysis by Wang *et al.* included any adverse events, major adverse events, treatment withdrawal, aminotransferase elevation, creatine kinase (CK) elevation, rhabdomyolysis, malignancies, myalgia and new diabetes cases. Of these, a significant increase was noted for aminotransferase elevation (twice the upper reference limit; RR 1.67,95% CI 1.35-2.05,P<0.0001) and CK elevation(three times the upper reference limit;RR 1.7,95% CI 1.05-2.79,P=0.031) among patients receiving intensive statin treatment compared to moderate-intensity treatment or no therapy (52).The authors highlighted in their discussion that the extent of LDL-C reduction was the strongest independent predictor ofthe reduction in risk of major vascular events. They criticized the recommendations based on percentage LDL-C level reduction, as even a 50% reduction may not provide the maximum treatment benefit. In fact it might have been one of the reasons that the the recommendations were finally changed and now not only reduction to target is necessary and at least 50% reduction (instead of or) (46) No identifiable lower limit has yet been identified below which,

further LDL-C reduction does not drive further cardiovascular benefit. Therefore, guidelines should focus on the absolute reductions of LDL-C risk reductions, combined with even lower target LDL-C concentrations (52).

Findings of intravascular ultrasound imaging studies as justification forvery low target LDL-C levels

Serial intravascular ultrasound of the coronary arteries enables evaluation of the relationship between concentrations LDL-C achieved with lipid-lowering therapy and the volume ofatheroma. Lower LDL-C levels are associated with greater plaque regression when patients are treated with intensive statin therapy or combination lipid-lowering therapy (58-62). In fact, the studies on statinsalready showed a significant effect of this therapy on atheroma plaque. Banach *et al.*in their systematic review and meta-analysis investigated the impact of statin therapy on plaque volume and its composition using virtual histology intravascular ultrasound (VH-IVUS). Based on data from 9 studies the authors showed that there was a significant effect of statin therapy in reducing plaque volume, external elastic membrane volume, fibrous plaque volume, and an increase of dense calcium volume, with no significant effect on other parameters of atheroma plaque morphology(63)We have similar data also in patients receiving combined lipid-lowering therapy, incluingthe Plaque Regression with Cholesterol absorption Inhibitor or Synthesis inhibitor Evaluated by IntraVascular Ultrasound (PRECISE IVUS) (atorvastatin + ezetimibe vs atorvastatin) (61) and the Global Assessment of Plaque reGRession With PCSK9 antibOdy as Measured by intraVascular Ultrasound (GLAGOV) (statin + evolocumab vs statin) studies (62)..

The PRECISE IVUS study included 202 patients. The duration of treatment was 9-12 months (61). In patients receiving combined lipid-lowering therapy, the change in total atheroma volume was greater (-1.4%; 95% CI -3.4% to -0.1%) than that in patients receiving monotherapy (-0.3%; 95% CI -1.9% to 0.9%). In addition, plaque regression was noted in a higher proportion of patients receiving combined lipid-lowering therapy (79% vs 58%,P=0.004). The mean LDL-C level in patients treated with atorvastatin and ezetimibe was 63.2±16.3 mg/dL compared to 73.3±20.03 mg/dLin the atorvastatin group (P<0.001).

Particularly robust evidence in favour of reducing LDL-C level to very low values was recently provided by the results of the GLAGOV study that included 968 patients with angiographically proven CHD who were treated with evolocumab (420 mg/month) and statin vsplacebo and statin for 76 weeks (62). The reduction of coronary plaque volume was associated with on-treatment LDL-C levels. The mean LDL-C level in patients treated with

evolocumab + statin was 36.6 mg/dLcompared to 93 mg/dLin the statin monotherapy group. The atheroma volume increased by 0.05% with placebo and reduced by 0.95% with evolocumab. The greatest plaque regression was noted in patients who achieved LDL-C concentrations of ≤20 mg/dL. In the next analysis based on the GLAGOV data, the authors investigated the effect of evolocumab on atheroma plaque composition (based on data of 331 patients with evaluable radiofrequency analysis of the ultrasound backscatter signal(64) The authors showed even higher regression of percent atheroma volume(PAV) in those treated with evolocumab (-1.2% vs. +0.17%; $p < 0.0001$) as well as total atheroma volume (-3.6 mm^3 vs. -0.8 mm^3; $p = 0.04$). No difference was observed between the evolocumab and placebo groups in all other investigated parameters of atheroma plaque morphology, however an inverse correlation was observed between changes in LDL-C and plaque calcification ($p < 0.001$).The authors observed that at mean level of LDL-C of about 60-65 mg/dl the lines presenting PAVand calcium volume are crossing, what means that below this level there is the largest reduction of atheroma plaque volume with its highest stabilization(64)

Safety of using intensive lipid-lowering therapy to achieve low LDL-C

The most important issues related to the safety of intensive lipid-lowering therapy and low LDL-Cinclude the effect on muscle symptoms, steroid hormone levels, neurocognitive function, and the occurrence of incident diabetes, malignancies and haemorrhagic stroke(65)

In the PROVE IT TIMI 22 study, no significant differences were noted in the rates of myalgia, myositis, or CK elevation to ≥3 x upper reference limit between patients receiving intensive (atorvastatin 80 mg/d) and moderately intensive (pravastatin 40 mg/d) statin therapy (43). In the Treating to New Targets (TNT)study (atorvastatin 80 mg/dvsatorvastatin 10 mg/d), there were no significant differences in the rates of muscle symptoms between the quintiles of achieved LDL-C concentration(66).

In the meta-analysis by Wang*et al.*, the rate of myalgia and myopathy was not increased in patients receiving intensive statin therapy compared to those receiving less intensive or no lipid-lowering therapy (52). However, aminotransferase and CK elevation were significantly more common during intensive statin therapy. No significant differences in the rates of all these adverse effects were noted between ezetimibe and PCSK9 inhibitor treatment vs placebo.

Particularly valuable data on the effect of achieving very low LDL-C levels on steroid hormone levels were provided by studies with most potent lipid-lowering drugs, i.e. PCSK9

inhibitors. In patients who achieved as low LDL-C levels as 15 mg/dl (0.39 mmol/L), no changes in cortisol, aldosterone, androgen, oestrogen, and progestagen levels, and in cortisol response to ACTH were observed (67,68).

A meta-analysis of 5 statin clinical trials that included 32,752 patients without diabetes at baseline, found that incident diabetes occurred in 2749 of these patients, and a cardiovascular event occurred in 6684 (69). Intensive statin therapy was associated with a 20% higher risk of incident diabetes (OR 1.20,95% CI 1.04-1.22) and a 16% lower risk of cardiovascular events (OR 0.84,95% CI 0.75-0.94). However, the number needed to harm was 498, and the number needed to treat for cardiovascular disease was 155 (3.2 x less). In the abovementioned meta-analysis by Wang *et al.*, a trend for an increased rate of incident diabetes in patients receiving intensive therapy was noted in 4 statin trials, which provided data on incident diabetes cases (RR 1.07,95% CI 1.00-1.17,P=0.058) (52).

In the FOURIER study, adding evolocumab to intensive statin therapy did not increase the rate of incident type 2 diabetes overall or in patients with prediabetes. Control of diabetes was not worsened(1, 70). In a pooled analysis of ten alirocumab ODYSSEY studies, no significant increase in the rate of incident diabetes was noted in patients who achieved as low LDL-C levels as 30 mg/dL (0.78 mmol/L) (71). Similarly, treatment withalirocumab in the ODYSSEY OUTCOMES study did not lead to an increased rate of incident diabetes (2). In a meta-analysis of six PCSK9 inhibitor studies, Wang *et al.* did not find an increased rate of incident diabetes compared to placebo (52).

Currently, available data do not suggest that achieving low LDL-C levels with statins results in an increased risk of cancer (4). In the same meta-analysis by Wang *et al.*(including 18 studies) which showed the RR of cancer was 0.97 (95% CI 0.93-1.02) in patients receiving intensive statin treatment compared to controls (52). Lipid-lowering therapy with ezetimibe (2 studies) was not also associated with an increased risk of cancer (RR 0.98,95% CI 0.80-1.19) (52).

In landmark trials of PCSK9 inhibitors, i.e., FOURIER and ODYSSEY OUTCOMES studies, no data on cancer incidence or mortality were reported among the evaluated adverse effects (1, 2). In one PCSK9 inhibitor study which reported data on cancer incidence and which was included in the meta-analysis by Wang *et al*., no significant difference was noted compared to placebo [47 cases (1.9%) vs 34 cases (2.7%), respectively] (52). The meta-analysis of 7 randomized statin trials by Boeckholdt*et al.* showed a modest increase in the risk of haemorrhagic stroke in patients who achieved very low LDL-C levels compared to those with moderately low LDL-C levels. However, the number of haemorrhagic strokes was very

small (50).In the meta-analysis by Wang et al., no information was provided on the rates of specific types of cardiovascular events, including haemorrhagic strokes (52). In the largest and most recent meta-analysis by Banach *et al*. the authors systematically evaluated the impact of LDL-C levels and lipid lowering agents on the different types of stroke(72)Based on the data from 11 observational studies with 355,591 participants and 18 RCTs with 165,988 individuals the authors showed that despite the participants at highest LDL-C category had a lower risk for of hemorrhagic stroke (RR: 0.91, 95%CI: 0.85–0.98) compared with the lowest category, lipid lowering therapy significantly decreased the risk of all types of strokes for those who achieved LDL-C<1.8 mmol/L (<70 mg/dL; RR=0.88, 95%CI: 0.80–0.96, ARR: 0.7%, NNT: 143). They also showed no significant effect of lipid lowrring therapy regardless the achieved level of the LDL-C on the risk of hemorrhagic stroke(72) Adding evolocumab or alirocumab to statin therapy and achieving very low LDL-C levels in the FOURIER (1) and ODYSSEY OUTCOMES (2)studies was not associated with an increased risk of haemorrhagic stroke compared to statin + placebo treatment.

It should be stressed that in ODYSSEY OUTCOMES 2.8 years alirocumab therapy added to statin treatment in acute syndrome patients resulted in any stroke and ischemic stroke reduction, respectively by 28% (HR 0.72, 95%Cl 0.59 - 0.91) and 27% (HR 0.73, 95% Cl 0.57 - 0.93),without increasing hemorrhagic stroke (HR0.83, 95% Cl 0.42 - 1.65) (73). This possitive treatment effect was independent on baseline LDL cholesterol levels <80, 80 to 100, and >100mg/dl. The percentageof patients who achieved LDL-C concentration <25, 25 to <50, 50 to <70, and>=70 mg/dl at 4 months was respectively as follow, 32.9 ,39.6 ,11.5 and 12.4. It is worth to mention that similar benefit appeared in patients with previous cerebro-vascular event and without of cerebral disease .

Some concern was raised by the meta-analysis of 17 randomized trials of PCSK9 inhibitors which showed an increased rate of neurocognitive disturbances compared to placebo (OR 2.34,95% CI 1.11-4.93,P=0.02) (74). However, these observations were not confirmed in either the FOURIER (1) or the ODYSSEY OUTCOMES (2) studies. To provide more data in this regard, the Evaluating PCSK9 BINDING antiBody Influence oN coGnitive HeAlth in High Cardiovascular Risk Subjects (EBBINGHOUS) study was performed in a subset of 1974 participants from the FOURIER study (75). The duration of follow-up was

approximately 19 months. During this period, the Cambridge Neuropsychological Test was administered to the study participants at 6, 12, and 24 months. No differences in neurocognitive function were noted between the statin + evolocumab and statin + placebo groups despite very low LDL-C levels achieved in the former (75,76).Recently, cerebral cognitive function (including memory) was evaluated at 2.2 years of evolocumab therapy in the FOURIER study in patients who achieved LDL-C level < 20 mg/dL(0.5 mmol/L) and no difference was shown compared to patients with LDL-C level ≥100 mg/dL (2.5mmol/L) (3.8% vs 4.5%, P= 0.57) (77). It is important to note thatPCSK9 inhibitors do not cross the blood-brain barrier.

Summary

Loss-of-function mutations of the PCSK9 gene lead to very-low LDL-C levels and are associated with a low CVD risk without adverse effects. Clinical trials of intensive statin therapy and of adding ezetimibe to statin have demonstrated that the lower LDL-C is reduced on therapy, the rate of cardiovascular events. This observation in respect of LDL-C has been dubbed "*the lower, the better*". Two monoclonal antibodies against PCSK9, evolocumab and alirocumab, when added to statin therapy in secondary prevention patients with a baseline LDL-C level of about 90 mg/dL(2.25 mmol/L) ,potently reduced LDL-C level to very low values < 30 mg/dL (0.78 mmol/L), and this was associated with a significant reduction in the recurrent cardiovascular event rate. These drugs are well tolerated and appear to be safe, based on currently available evidence. It should be noted, however, that we have much more clinical experience and long-term follow up of patients with statins than we do for PCSK9 inhibitors.

These observations and early prospective epidemiological studies confirm that LDL-C is the main driver for atherogenesis. Cellular and genetic studies and observations of individuals with loss-of-functionPCSK9 gene mutations have demonstrated that the body's requirement for LDL-C is low. Thus, to what levels and in whom should we intensively lower LDL-C level? This is addressed in the current guidelines. The recent 2019 European Society of Cardiology and European Atherosclerosis Society guidelines as well as polish Society of Laboratory Diagnostics and Polish Lipid Association Guidelines recommend a target LDL-C level of <1.4 mmol/L (55 mg/dL) in very high-risk patients and < 1.0 mmol/L (40 mg/dL) in extremely high-risk patients (despite it was limited only to the definition of one group with 2 vascular events in last 2 years) (46,78)). A lower threshold has not been set. An interesting proposal in this respect is the *zero-LDL hypothesis*(8).The authors of this emphasized they

were not recommending achieving a zero-LDL level, but rather advisedthat extremely-low LDL plasma concentrations due to increased LDLR activity should have not been considered harmful. They also convinced that it is not a rare situation as LDL concentrations <0.4 mmol/L (15 mg/dL) are frequently seen, and no adverse effect have been reported, only benefits (8). Maybe, it is hard to agree that LDL-C levels <0.4 mmol/L are frequently seen, but if they result from highly efficient LDL-removal processes(loss-of-functionPCSK9 gene mutations or the ultimate effect of potent lipid-lowering therapy), they are associated with low cardiovascular risk without adverse effects. It should be noted, however, that long-term safety data are not yet available for PCSK9 inhibitors that may reduce LDL-C to such low levels.

However, based on above, we would like to suggest, that having so strong data on efficacy and safety of low and very low LDL-C levels according to the rule of "*the lower, the better*" we should now equally focus on the effective implementation to guidelines and clinical practice the rule of "*the earlier the better*" to be on LDL-C target as quickly as possible, with double or triple combination therapy even during the hispitzalitaion for very high and extremely high ACS patients (25,79), as well as the rule of "*the longer the better*" – if possible lifelong, as we have still had a large problems with therapy adherence and being on the goal with our patients for a long time (80).

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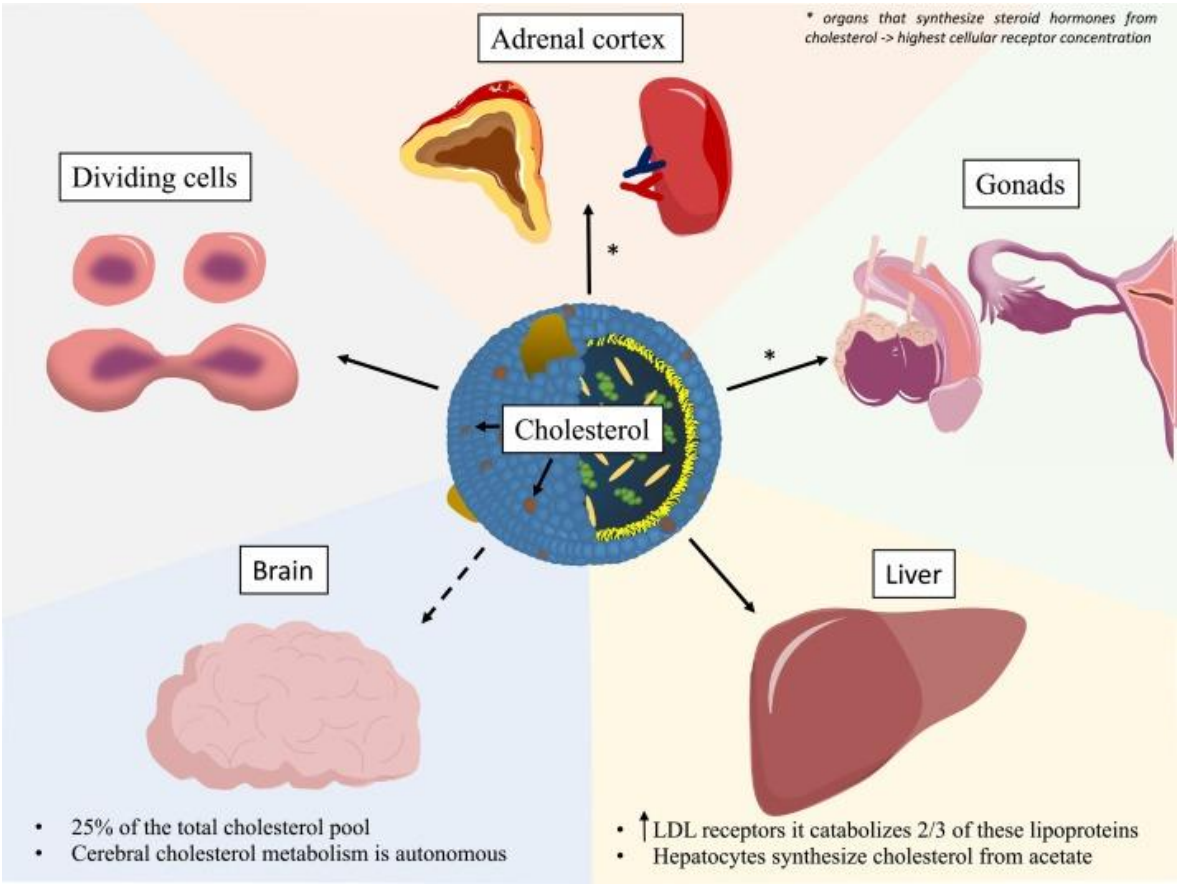

Fig. 1. The physiological role of cholesterol in the human body.

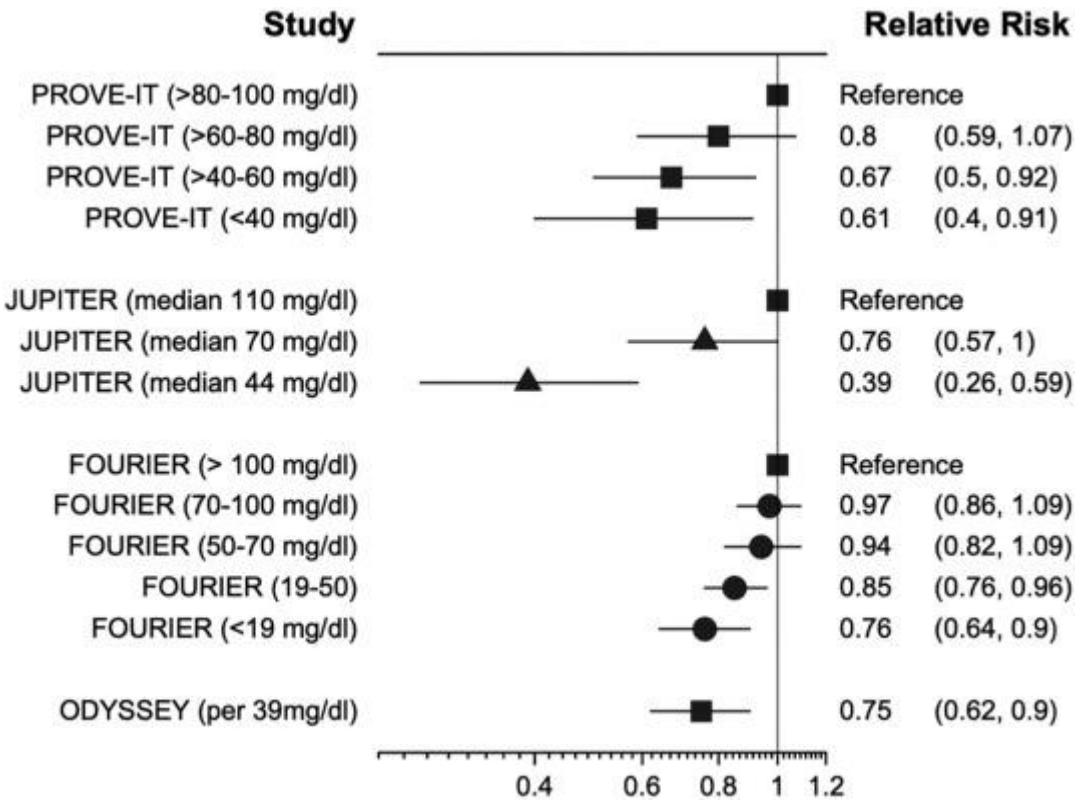

Fig. 2. Relative risk of ASCVD outcomes among subgroups, based upon achieved LDL-C in major lipid-lowering trials.

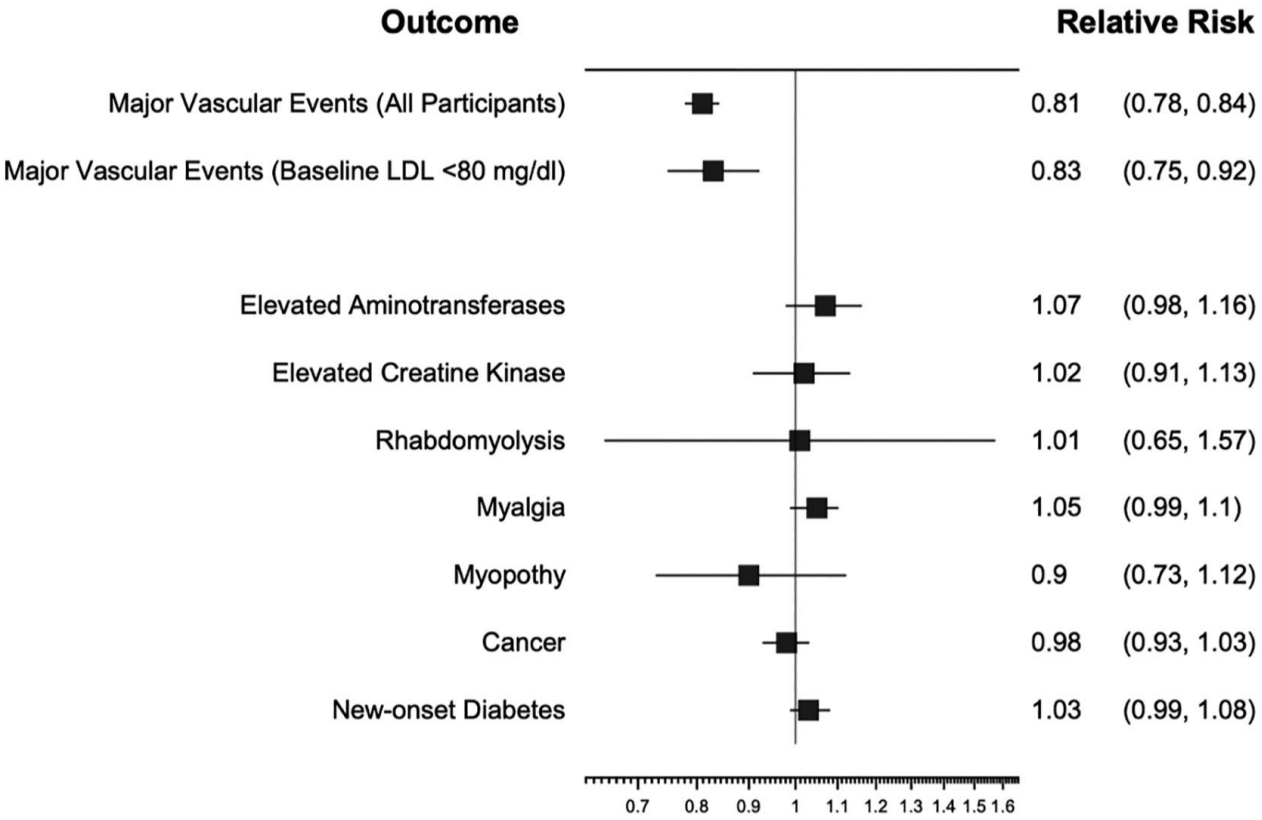

Fig. 3. Relative risk of major vascular events (in all participants, and those with baseline LDL-C <80 mg/dL) and adverse effects in patients using intensive lipid-lowering therapy. Data from Wang et al. doi:https://doi.org/10.1016/S2213-8587(19)30388-2.

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