Clinical and pathophysiologic evidence supporting the safety of extremely low LDL levels—The zero-LDL hypothesis

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Abstract: While the impact of very low concentrations of low-density lipoprotein cholesterol (LDL-C) on cardiovascular prevention is very reassuring, it is intriguing to know what effect these extremely low LDL-C concentrations have on lipid homoeostasis. The evidence supporting the safety of extremely low LDL levels comes from genetic studies and clinical drug trials. Individuals with lifelong low LDL levels due to mutations in genes associated with increased LDL receptor (LDLR) activity reveal no safety issues. Patients achieving extremely low LDL levels in the IMPROVE-IT and FOURIER, and the PROFICIO and ODYSSEY programs seem not to have an increased prevalence of adverse effects. The main concern regarding extremely low LDL-C plasma concentrations is the adequacy of the supply of cholesterol, and other molecules, to peripheral tissues. However, LDL proteomic and kinetic studies reaffirm that LDL is the final product of endogenous lipoprotein metabolism. Four of 5 LDL particles are cleared through the LDL-LDLR pathway in the liver. Given that mammalian cells have no enzymatic systems to degrade cholesterol, the LDL-LDLR pathway is the main mechanism for removal of cholesterol from the body. Our focus, therefore, is to review, from a physiological perspective, why such extremely low LDL-C concentrations do not appear to be detrimental. We suggest that extremely low LDL-C levels due to increased LDLR activity may be a surrogate of adequate LDL-LDLR pathway function.

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Introduction

The proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors have changed the paradigm for lipid-lowering therapy to prevent cardiovascular disease. Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk (FOURIER), the first completed outcome study with a PCSK9 inhibitor, showed the clinical benefit of lowering low-density lipoprotein cholesterol (LDL-C) below the current goal, extending the data obtained by the IMPROVE-IT study. In the FOURIER trial, 42% of patients achieved LDL-C levels <0.65 mmol/L (25 mg/dL). While the impact of very low LDL-C concentration on cardiovascular prevention is very reassuring, it is intriguing to know what effect these extremely low LDL-C concentrations have on lipid homoeostasis.

Our focus, therefore, is to review, from a physiological perspective, why such extremely low LDL-C concentrations do not appear to be detrimental. We suggest that as the LDL-LDL receptor (LDLR) pathway is the main mechanism for removal of circulating cholesterol from the body, extremely low LDL-C levels due to increased LDLR activity may be a surrogate of LDL-LDLR pathway optimization. (Complete references list in online supplementary material)

Clinical evidence for the safety of extremely low LDL-C levels

Setting the stage: Evidence from trials

The direct correlation between LDL-C levels and cardiovascular events shown by epidemiological studies is indisputable. Added to this, there is a wealth of data from randomized controlled trials with statin therapy showing that lowering LDL-C levels drives cardiovascular risk reduction underpinning “the lower is better” concept. The Cholesterol Treatment Trialists’ Collaboration showed a 22% relative risk reduction per unit LDL-C mmol/L reduction with statin therapy. IMPROVE-IT (Examining Outcomes in Subjects With Acute Coronary Syndrome: vytorin [ezetimibe/simvastatin] vs simvastatin) and FOURIER have extended the scientific evidence for the cardiovascular benefit of LDL-C reduction to <1.29 mmol/L (50 mg/dL) and <0.78 mmol/L (30 mg/dL), respectively; therefore, the lower LDL-C threshold for benefit has not been defined yet (Fig. 1). In parallel, imaging studies provide added support in GLAGOV (GLocal Assessment of Plaque reGression With a PCSK9 antibOdy as Measured by intraVascular Ultrasound), patients with baseline LDL-C levels <1.8 mmol/L (70 mg/dL) attained greater atheroma regression with evolocumab against a background of high-intensity statin therapy than those with higher baseline LDL-C levels, thus reaffirming the linear correlation between LDL-C lowering and reduction in the burden of coronary atherosclerosis. Indeed, current evidence shows that cardiovascular benefit is dependent on the absolute magnitude of LDL-C reduction regardless of the LDL-lowering therapy, provided that it increases LDLR activity. What, then, is the evidence that these VLDL-C levels are safe?

Living with genetically driven extremely low LDL-C levels

A number of lines of evidence offer insights into this question. LDL-C levels at birth are very low. Ethnic groups that maintain a hunter-gatherer culture have been shown to have lifelong LDL-C levels below 50 mg/dL, with no evidence of health concerns. Added to this, genetic hypercholesterolemia provides a unique opportunity to evaluate the effects of lifelong exposure to low LDL-C levels. Interestingly, those genetic conditions leading to serious defects in lipoprotein formation, such as abetalipoproteinemia and classical (apoB gene defects) homozygous hypobetalipoproteinemia, are associated with clinical symptoms, including fat malabsorption, and digestive, hematological, and neurological symptoms, as well as ectopic fat deposition in the liver and other organs; therapies developed to block apolipoproteins (apo) B synthesis, such as mipomersen, or microsomal triglyceride transfer protein, such as lomitapide, have side effects that mirror these effects. In contrast, genetic mutations resulting in accelerated LDL removal, even those leading to very low LDL levels (eg, PCSK9 loss of function mutations compound heterozygous and IDOL loss of function), are asymptomatic even at levels <0.39 mmol/L (15 mg/dL) (Fig. 2).

Individuals with variants in genes associated with increased LDLR activity (HMGCoA R, NPC1L1, PCSK9, LDLR, ABCG7/ABCG8) have moderate but lifelong low LDL-C levels with no evidence of any safety issues.

Mendelian randomization studies have shown that the benefit of lifelong exposure to low LDL-C levels on cardiovascular risk is greater than that observed with...
lipid-lowering therapy, a reflection of the exposure duration, while, there is no evidence of myopathy, liver toxicity, or cognitive changes.

Mendelian randomization studies have, however, indicated an increased risk for diabetes. Different hypotheses have been proposed to explain the underlying mechanism(s), including effects on the liver mediated by NPC1L1, or an increase of intracellular cholesterol concentration due to a higher LDLR activity in beta cells based on findings for PCSK9, HMGCoR, and LDLR gene variants. HMGCoR variants were also associated with increased body weight.

It should be noted that neither IMPROVE-IT (with ezetimibe) nor FOURIER (with evolocumab) has detected an increase in new-onset diabetes after 7- and 2-year follow-up, respectively; in addition, in the ODYSSEY clinical trial program, very low LDL-C levels attained with alirocumab did not impact on glucose control. In any case, it appears that low circulating LDL-C levels do not have a direct role in the pathophysiology of lipid-lowering therapy associated with new-onset diabetes.

**Figure 2** Different pathophysiological mechanisms associated with genetically driven VLDL cholesterol levels. Panel A shows the usual metabolic situation; panel B shows low LDL due to increased lipoprotein clearance. A normal apo B-rich lipoprotein metabolism axis is maintained; panel C shows low LDL due to reduced lipoprotein synthesis. Because of apo B formation inhibition, there is an alteration in fat secretion from the liver (and intestine) producing fatty liver, malabsorption and impacting all lipoprotein cascade. LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; LDLSR, LDL-LDL receptor; apo B, apolipoprotein B; IDL, intermediate-density lipoprotein; LOF, loss of function; GOF, gain of function.

Living with pharmacologically driven extremely low LDL-C levels

Emerging clinical data indicate the safety of very low LDL-C levels achieved with nonstatin therapy, as shown by analyses from IMPROVE-IT and FOURIER of patients with VLDL-C levels (<0.78 mmol/L and <0.65 mmol/L, respectively). In a prespecified analysis of FOURIER data, patients achieving LDL-C values below 0.3 mmol/L, assessed by ultracentrifugation to overcome the underestimation of Friedewald-calculated LDL at this very low levels, showed no additional side effects, on the contrary, they had less cardiovascular events, although these observations should be confirmed at the long term. Added to this, data from the ODYSSEY program show that 60% of patients with baseline Friedewald-calculated LDL-C levels <2.6 mmol/L achieved levels <0.65 mmol/L, and 28% < 0.39 mmol/L (15 mg/dL) with alirocumab. Although long-term, real-world data are clearly needed, the available trial data do not suggest
adverse effects such as myopathy, deterioration in cognition, or changes in hormone or vitamin status at these extremely low LDL-C levels.\textsuperscript{23,24} Indeed, the lack of detrimental effect on cognitive function is consistent with mechanistic evidence that cholesterol metabolism in the central nervous system (CNS) is autonomous as only discoid apo A-I containing high-density lipoprotein (HDL) particles may enter the CNS via scavenger receptor class B member 1–mediated uptake, with maturation and enrichment with glia-derived apo E mediating uptake by CNS cells through LDLR.\textsuperscript{25} The relationship between cataracts and lipid lowering therapy has been a matter of controversy for a long time. An increased incidence of cataracts was observed in dogs treated with statins while human studies did not confirm this effect. The lens needs a high cholesterol concentration to maintain its membrane transparency and synthesizes its own cholesterol independently of circulating LDL. An increase in cataracts in patients achieving an LDL-C \(<0.39\) mmol/L on alirocumab was tentatively suggested; however, the calculated low LDL levels in the ODYSSEY program could be overestimated and patients were probably misclassified in the different subgroups. Moreover, both in the FOURIER and SPIRE 1 and 2, the prespecified analyses on VLDL levels show no increase of cataracts, rather the contrary. On the other hand, as the lens has a slow lifelong growth, it is highly improbable that this is a real effect at the short time.\textsuperscript{26} Concerns have been raised about 2 issues: the risk for diabetes (as discussed above) and the potential link between very low LDL-C levels and increased susceptibility to hemorrhagic stroke, based on findings from epidemiological studies and meta-analyses suggestive of an inverse association.\textsuperscript{27} In the Stroke Prevention by Aggressive Reduction in Cholesterol Levels study, despite a significant decrease in stroke incidence in patients with a history of cerebrovascular events (stroke or transient ischemic attacks) treated with atorvastatin \(80\) mg/d, the number of hemorrhagic strokes also increased; however, this was not associated with the magnitude of LDL-C reduction.\textsuperscript{28,29} Moreover, the “Cholesterol Treatment Trialsists’ Collaboration”\textsuperscript{15} data show a nonsignificant association between low LDL and hemorrhagic stroke. Moreover, patients participating in the IMPROVE-IT and PCSK9 inhibitors trials achieving extremely low LDL-C concentrations showed no increased hemorrhagic stroke risk. These data have been further confirmed by the FOURIER study in which patients on evolocumab, achieving extremely low LDL levels, had no increase in hemorrhagic stroke.\textsuperscript{1,20,21}

In summary, therefore, the evidence with novel lipid-lowering therapies such as PCSK9 inhibitors offer the reality of extremely low LDL-C levels with risk-benefit ratio within the confines of clinical trial data. What is the mechanistic evidence to support the safety of VLDL-C levels?

**Mechanistic evidence for the safety of extremely low LDL concentrations**

**What is an LDL particle?**

Before discussing this question, a background on LDL physiology is appropriate. LDL particles comprised lipid (~80%, predominantly cholesterol as cholesteryl ester) and protein. The triglyceride content of LDL is relatively low under normal conditions (5%–10%) but increases with diabetes or obesity (Fig. 3A).

LDL transports cholesterol in the blood and extracellular fluids, and this is largely controlled by the interactions of surface proteins with lipid transport proteins, enzymes, and cell membrane receptors. Apo B100 acts as the ligand for LDL receptors, and contributes to the interaction between LDL and the arterial wall proteoglycans, thus promoting subendothelial retention of atherogenic LDL in early atherosclerosis.\textsuperscript{30} Compared to HDL, LDL particles contain relatively few proteins, representing only about 15% of the non-apo B100 protein content of LDL (Fig. 3B).\textsuperscript{31} This implies that LDL composition is mainly associated with its own metabolism than with peripheral protein supply.

**LDL metabolism—The LDLR pathway**

LDL is the final product of endogenous lipoprotein metabolism mediated by the action of lipoprotein lipase on very low-density lipoprotein (VLDL) secreted by the liver and of hepatic lipase on intermediate-density lipoprotein and VLDL remnants.\textsuperscript{15} The main function of LDL is to transport cholesterol and other minor components to peripheral tissues, although all cells also have the ability to synthesize cholesterol.

Kinetic studies performed with radiolabeled apo B show that the LDLR-dependent pathway is responsible for removal from the plasma of about 80% of LDL, with the remaining 20% cleared by non-LDLR–dependent pathways, mainly nonsaturable scavenger receptors in vascular wall macrophages and the mononuclear phagocytic system.\textsuperscript{32,33} Although suprarenal glands have the highest relative concentration of LDLR, the liver expresses the largest absolute number of LDLR and thus has a unique role in maintaining circulating LDL-C levels.\textsuperscript{34} This is further supported by evidence of normalization of LDL-C levels in homozygous familial hypercholesterolemia (FH) patients following a liver transplant.\textsuperscript{35,36}

The intracellular cholesterol pool is the main source of biliary cholesterol, with less than 1% derived from de novo synthesis.\textsuperscript{37} Given that mammalian cells have no enzymatic systems to degrade cholesterol, the LDL-LDLR pathway is, along with HDL reverse transport, the main mechanism for removal of cholesterol from the body. Thus, the main consequence of a defective LDL-LDLR pathway is an increase in circulating LDL, leading to accumulation in the
artery wall and other peripheral tissues (Fig. 4A, B). In contrast, the main metabolic effect of statins and PCSK9 inhibitors is an increase in the LDL fractional catabolic rate, thus confirming that enhancing the LDL-LDLR pathway is a safe and efficient cholesterol reduction mechanism.

The role of LDL in plasma lipoprotein metabolism

In plasma, there is a continuous interchange of lipids and proteins among different lipoprotein particles and between lipoproteins and cells that is facilitated by specialized transport proteins. Phospholipid transfer protein is involved in the interchange of phospholipids, while cholesterol ester transfer protein mediates cholesterol ester-triglyceride interchange mainly between HDL and apo B-containing lipoproteins, including LDL, resulting in a reduction in the cholesteryl ester content of the HDL core and net cholesterol enrichment of atherogenic lipoproteins, which is balanced by LDLR-mediated particle removal. In the presence of very low LDL particle plasma concentrations, cholesterol ester transfer protein activity is reduced, thereby increasing HDL cholesterol available for heteroexchange with VLDL or removal by the liver.

Other LDL functions

Transporting liposoluble vitamins

Among the liposoluble vitamins, only vitamin E (z-tocopherol) is strongly associated with LDL metabolism. Vitamin E is absorbed via chylomicrons, and also
HDL, in enterocytes and its transport in the blood largely follows that of cholesterol within lipoprotein metabolism, with subsequent transport into the liver by chylomicron remnants through LRP1, and VLDL and LDL by the LDLR pathway. One of the main physiological roles of vitamin E is linked to LDL protection because of its antioxidant activity. No specific vitamin E transport protein, an alternative to lipoprotein cascade, has yet been described. Data from IMPROVE-IT, the ODYSSEY program with alirocumab, and the PROFICIO phase III studies with evolocumab show that while the total amount of vitamin E is reduced with LDL-C lowering, the vitamin E/cholesterol rate is not modified even in patients achieving extremely low LDL-C levels, thus maintaining its protective role. On the other hand, by improving the LDL-LDLR pathway, vitamin E peripheral delivery should not be reduced. In fact, in the PROFICIO study, vitamin E levels in red blood cells, a valid assessment of vitamin E tissue content, were unchanged despite apparent reductions in LDL-C plasma levels.

**Providing cholesterol to synthesize steroid hormones**

Several specialized tissues, including the suprarenal cortex and gonadal glands, have an increased cholesterol requirement because of steroid hormone synthesis, evidenced by the highest LDLR concentration per unit of tissue in the body. However, these tissues also have alternative pathways to capture cholesterol, notably via scavenger receptor class B member 1, which is responsible for uptake of cholesterol esters from HDL. This may explain why cholesterol supply to these organs is not altered by LDLR functional abnormalities as in FH patients.

Interestingly, patients on PCSK9 inhibitors attaining LDL-C levels <0.39 mmol/L show no changes in cortisol, cortisol response to adrenocorticotropic hormone, aldosterone, androgens, estrogens, or progestogens, thus maintaining a normal follicle-stimulating hormone-luteinizing hormone axis. Low LDL-C levels driven by therapies increasing LDLR activity should therefore not alter cholesterol peripheral delivery.

**Lipophilic toxins disposal**

Circulating lipopolysaccharide (LPS) can be found free, bound to transfer proteins, or linked to lipoproteins. Free circulating LPS can be translocated into lipoproteins (25%–52% to LDL) through the action of transfer proteins, buffering the inflammatory response. Although concerns have been raised about the impact of low LDL-C concentrations on the endotoxin buffering action, LDL-bound LPS is cleared by hepatocytes, mainly through the LDL-LDLR pathway.

Experimental studies show that increasing LDLR activity results in accelerated LPS plasma clearance, suggesting a better sepsis prognosis.

**Lessons from homozygous FH**

The main concern about very low LDL-C plasma concentrations is that cholesterol and other molecules transported by LDL will not be properly supplied to peripheral organs and tissues. Homozygous FH (HoFH), which is characterized by absent or defective LDLR
function, provides an opportunity to evaluate the impact of the lack of LDLR-mediated peripheral delivery of cholesterol and other molecules associated with LDL.

Systematic studies of liposoluble vitamin or steroid hormone synthesis in HoFH individuals are lacking, although there is evidence that levels are normal in heterozygous FH children, despite maintenance on low-fat diets. HoFH individuals have normal fetal development, precisely when there are the highest cell proliferation and membrane synthesis, suggesting that LDL-mediated cholesterol acquisition is negligible. There is also no evidence of vitamin deficiency or deficits in hormone levels, CNS function, or changes in maturation. Reports of pregnancy in adult HoFH females had a normal outcome. Beyond the evidence of markedly accelerated cardiovascular disease, HoFH is also characterized by extravascular cholesterol ester depots at the corneal level, in tendons and articulations, and in macrophages in the mononuclear phagocyte system. This ectopic depot probably represents a magnification of the LDL-independent LDL clearance pathway that, under normal conditions, accounts for less than 20% of LDL particles. Thus, HoFH is a model of extreme LDL-LDLR pathway derangement, providing further support that the main function of this metabolic pathway is to control the amount of cholesterol by facilitating its excretion through the liver (Fig. 4C).

Conclusion

In conclusion, the clinical and physiological evidence makes the case that extremely low LDL-C plasma concentrations mediated by increased LDLR activity are not associated with significant adverse effects, within the limitations of the available clinical trial data. Given that the main function of LDL metabolism is cholesterol excretion, therapies that increase LDLR activity could provide optimization of this physiological role. In other words, extremely low LDL-C concentrations due to increased receptor-mediated clearance should be interpreted as a marker of adequate LDL-LDLR pathway function.

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Supplementary material: Additional references (distributed according to article sections)

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**The role of LDL in plasma lipoprotein metabolism**


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