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# Can LDL cholesterol be too low? Possible risks of extremely low levels

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**Abstract.** Olsson AG, Angelin B, Assmann G, Binder CJ, Björkhem I, Cedazo-Minguez A, Cohen J, von Eckardstein A, Farinaro E, Müller-Wieland D, Parhofer KG, Parini P, Rosenson RS, Starup-Linde J, Tikkanen MJ, Yvan-Charvet L (Linköping University, Linköping; Karolinska Institutet and Karolinska University Hospital Huddinge, Stockholm, Sweden; University of Münster, Münster, Germany; Medical University of Vienna & Center for Molecular Medicine of the Avustrian Academy of Sciences, Vienna, Austria; Karolinska Institutet and Karolinska University Hospital Huddinge, Stockholm; Karolinska Institutet Huddinge, Sweden; UT Southwestern Medical Center, Dallas, TX, USA; University of Zürich, Zürich, Switzerland; University of Naples, Naples, Italy; Klinik II und Poliklinik für Innere Medizin der Universität zu Köln, Köln, Germany; Ludwig-Maximilians-University of Munich, Munich Germany; The Mount Sinai Hospital, New York, USA; University of Aarhus, Aarhus, Denmark; University of Helsinki, Helsinki, Finland; University of Nice Sophia Antipolis, Nice, France). Can LDL cholesterol be too low? Possible risks of extremely low levels (Review Symposium). *J Intern Med* 2017; **281**: 534–553.

Following the continuous accumulation of evidence supporting the beneficial role of reducing low-density lipoprotein cholesterol (LDL-C) levels in the treatment and prevention of atherosclerotic cardiovascular disease and its complications, therapeutic possibilities now exist to lower LDL-C to very low levels, similar to or even lower than those seen in newborns and nonhuman species. In addition to the important task of evaluating potential side effects of such treatments, the question arises whether extremely low LDL-C levels *per se* may provoke adverse effects in humans. In this review, we summarize information from studies of human cellular and organ physiology, phenotypic characterization of rare genetic diseases of lipid metabolism, and experience from clinical trials. Specifically, we emphasize the importance of the robustness of the regulatory systems that maintain balanced fluxes and levels of cholesterol at both cellular and organismal levels. Even at extremely low LDL-C levels, critical capacities of steroid hormone and bile acid production are preserved, and the presence of a cholesterol blood–brain barrier protects cells in the central nervous system. Apparent relationships sometimes reported between less pronounced low LDL-C levels and disease states such as cancer, depression, infectious disease and others can generally be explained as secondary phenomena. Drug-related side effects including an increased propensity for development of type 2 diabetes occur during statin treatment, whilst further evaluation of more potent LDL-lowering treatments such as PCSK9 inhibitors is needed. Experience from the recently reported and ongoing large event-driven trials are of great interest, and further evaluation including careful analysis of cognitive functions will be important.

**Keywords:** abetalipoproteinaemia, adverse effects, hypocholesterolaemia, low-density lipoprotein, safety.

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## Introduction

An elevated plasma level of low-density cholesterol (LDL-C) has been firmly established as a major risk factor for coronary heart disease (CHD). Data from epidemiological studies, genetic analyses and clinical trials have provided compelling evidence that high plasma levels of LDL-C, irrespective of the underlying cause, are strongly associated with CHD and cardiovascular mortality. In cross-sectional comparisons amongst different countries, those populations with the highest levels of cholesterol invariably had high rates of CHD. Prospective studies have yielded similar results. The striking increase in risk of CHD in individuals with diverse forms of genetic hypercholesterolaemia, and the success of LDL-lowering studies indicate that elevated LDL-C plays a causal role in CHD [1].

With the advent of powerful new LDL-lowering drugs, it is now possible to decrease LDL-C to levels at the lower margin of the distribution seen in healthy populations. In particular, the treatment option of inhibition of proprotein convertase subtilisin/kexin 9 (PCSK9) activity can now lead to an additional 50–70% decrease in LDL-C in statin-treated individuals, resulting in levels below those seen in the newborn infant, that is <1.5–1.8 mmol $L^{-1}$ (60–70 mg $dL^{-1}$) [2, 3]. Even if the target level of LDL-C will not be formulated to such low levels, it has become important to consider potential adverse effects of extremely low LDL-C levels in their own right, in addition to the obvious need to document possible side effects from the agents used in therapy.

Against this background, we examined the present knowledge regarding potential untoward effects that could be caused by extremely low levels of LDL-C. In this review, we summarize clinical findings associated with very low levels of LDL-C that occur naturally or during therapeutically induced hypocholesterolaemia. Special attention has been paid to disturbances of cholesterol fluxes that may influence the function of physiological pathways predicted to be critically dependent on cholesterol availability.

## The cellular cholesterol balance and its relation to LDL-C

Cholesterol is an essential component of all cell membranes and is critical to the maintenance of normal cell functions. Some cells, such as those synthesizing steroid hormones (e.g. adrenals, ovaries, testicles), secreting lipoprotein particles (hepatocytes and intestinal cells) or excreting bile acids and cholesterol (hepatocytes), have a higher requirement for cholesterol as have cells undergoing rapid division, including some tumour cells. In order to maintain an adequate cholesterol level, each cell in the body harbours a complex machinery that regulates the *de novo* synthesis of cholesterol from acetate, the uptake of lipoprotein cholesterol from its surroundings via surface receptors such as the LDL receptor (LDLR), the storage of cholesterol in its esterified form and the export of excess cholesterol through various transfer processes.

The details of how this complex machinery is controlled at the cellular level by sensing of intracellular cholesterol concentration are gradually being understood [1] (Fig. 1).

A central element of this regulatory system includes the steroid response element-binding proteins (SREBPs) that belong to a family of transcription factors that regulate the expression of genes involved in lipid homeostasis and glucose metabolism [1, 4, 5]. An essential feature of SREBPs is that they are embedded in the membrane of the cellular endoplasmic reticulum (ER), where they are bound to SREBP cleavage-activating protein (Scap). When the cholesterol is lower than 5 mol% of total lipids present in the ER membranes, Scap binds to cytosolic coat protein complex II (COP II) proteins. This leads to incorporation of SREBP/Scap into COP II-coated vesicles which bud off and move into the Golgi apparatus. After sequential cleavage by two proteases in the Golgi, mature forms of SREBP translocate into the cell nucleus where they activate target genes like the LDLR, HMG CoA reductase, PCSK9 and many others. SREBP-2 mainly targets genes involved in cholesterol homeostasis, whereas SREBP-1 isoforms also stimulate genes involved in, for example, fatty acid synthesis. In this way, each cell can regulate the activity of HMG CoA reductase and the expression of LDLRs in response to its immediate need for cholesterol, providing it with multiple pathways to maintain cellular cholesterol levels within a very narrow range.

Uptake of preformed cholesterol from circulating lipoproteins is a convenient way to provide cells with their needs for cholesterol (Fig. 2).

Due to their long half-life (2–3 days), LDL particles represent a major fraction of circulating cholesterol,

 

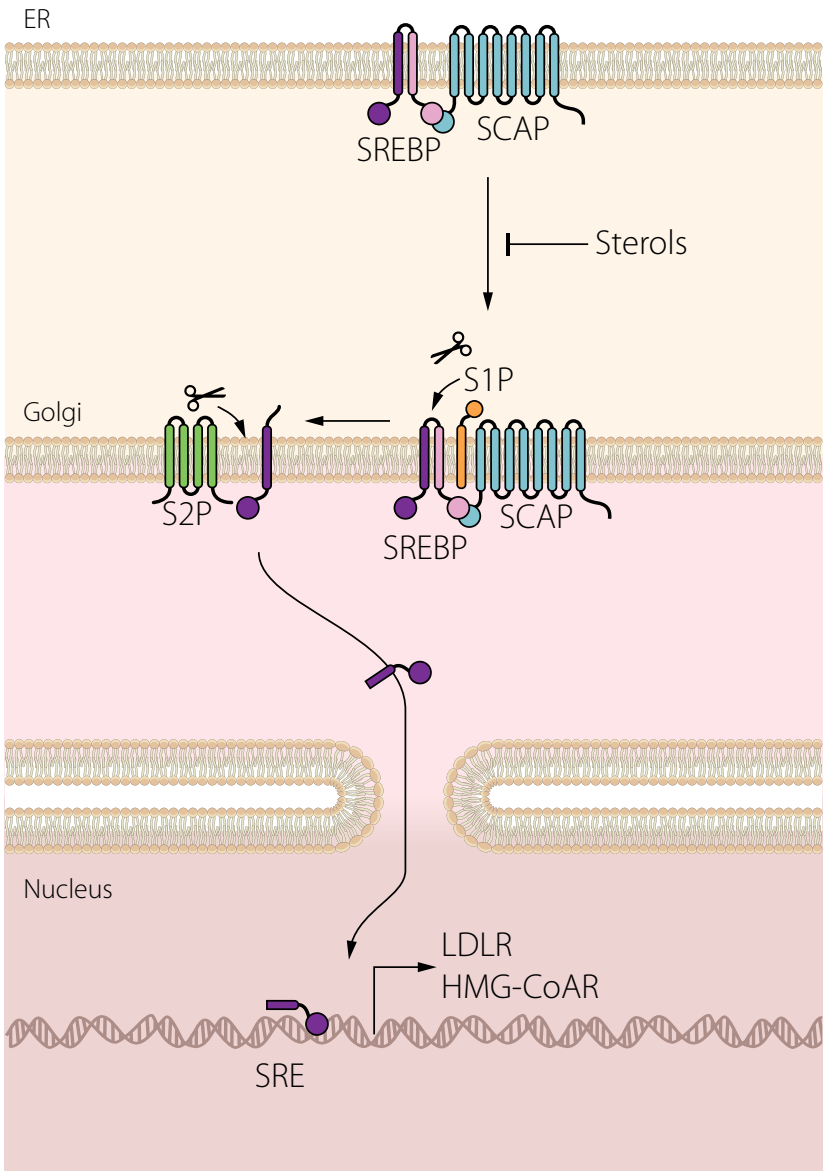


**Fig. 1** *Simplified scheme of intracellular control of cholesterol homeostasis (ref. 1). SREBP (sterol regulatory element-binding protein) is embedded in the membrane of the cellular endoplasmic reticulum (ER). When cholesterol content of ER membranes is reduced, the integral membrane protein SCAP (SREBP cleavage-activating protein) binds to COP II (coat protein complex II) proteins which incorporate the SCAP/SREBP complex into vesicles which are transferred to the Golgi apparatus. Cleavage of SREBP by proteases S1P and S2P allows it to enter the cell nucleus where it activates genes that increase cholesterol synthesis and uptake, such as HMG CoA reductase and LDLR.*

whereas the daily flux of cholesterol (in mmoles $d^{-1}$) through the chylomicron/chylomicron remnant (exogenous) pathway is larger than that of the very-low-density lipoprotein (VLDL)/intermediate-density lipoprotein (IDL)/LDL (endogenous) pathway. The role of reverse cholesterol transport (from peripheral cells to the liver) through interaction with HDL particles is also important for maintaining whole body cholesterol balance.

When discussing the potential risks of markedly reduced circulating LDL-C levels, it is important to consider how the LDLR pathway may contribute to the demand for cholesterol in various tissues. The saturation kinetics of the specific, high-affinity binding of LDL to its receptor indicates that half-maximal binding occurs already at a ligand concentration of about 0.064 mmol $L^{-1}$ (2.5 mg $dL^{-1}$) of LDL-C [1]. However, it should be taken into consideration that most cells in the body are not in direct contact with plasma but surrounded by interstitial fluid where the concentration of LDL is only ~20% of that in plasma [6, 7].

 

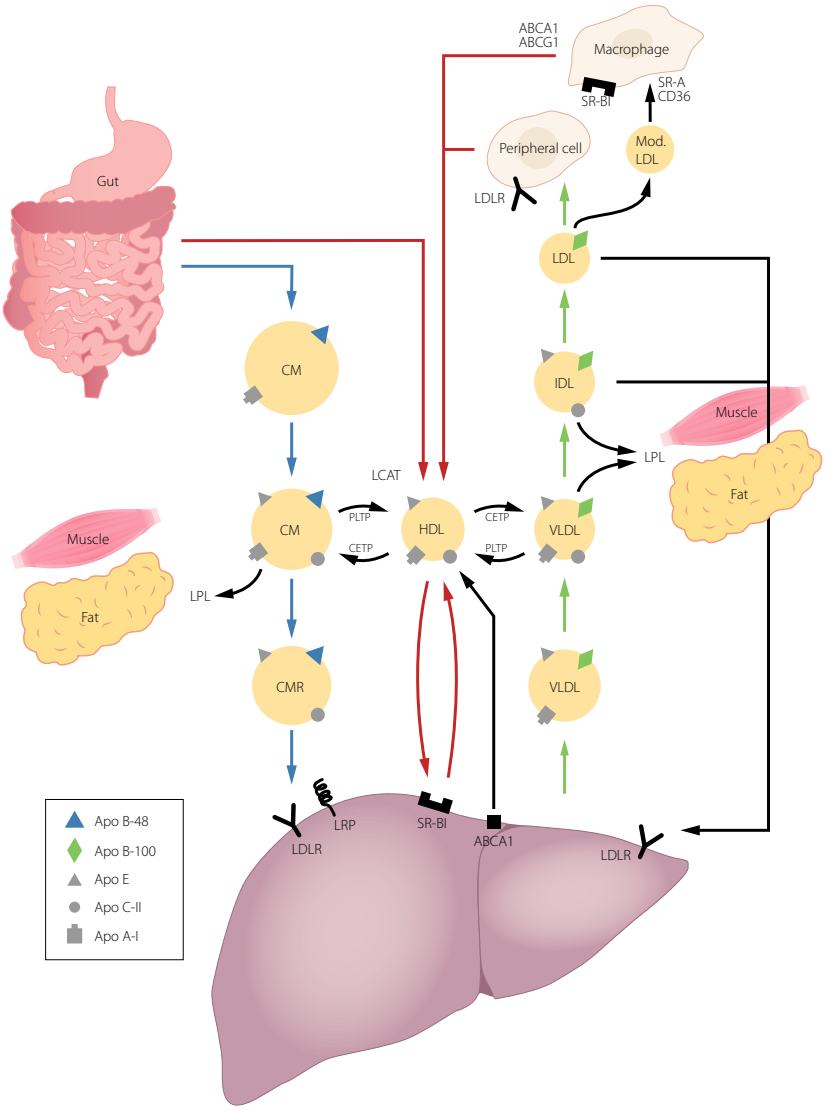


**Fig. 2** *Simplified scheme of normal human lipoprotein cholesterol metabolism [106]. Blue – exogenous cholesterol transport; green – endogenous cholesterol transport; red – reverse cholesterol transport. Apo, apolipoprotein; CM, chylomicron; CMR, chylomicron remnant; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; LPL, lipoprotein lipase; HL, hepatic lipase; CETP, cholesteryl ester transfer protein; PLTP, phospholipid transfer protein; LCAT, lecithin-cholesteryl acyltransferase; ABC-1, ATP-binding cassette transporter 1; LDLR, LDL receptor; LRP, LDLR-related protein; SR-BI, scavenger receptor class B type I; SR-A, scavenger receptor class A.*

This indicates that a plasma concentration of LDL-C of ~0.32 mmol $L^{-1}$ (12.5 mg $dL^{-1}$) would still provide adequate amounts of LDL-C to ensure an ample uptake of cholesterol. Furthermore, cells with potentially high demand for cholesterol, such as steroid hormone-producing cells or hepatocytes, are exposed to higher levels of LDL due to the presence of fenestrated endothelial cells in the vasculature of the organs.

When considering which physiological functions are most vulnerable to situations of extremely low LDL-C, it is also relevant to recognize which organs express the highest number of LDLRs. Although information from humans is limited, two independent sets of data may be of some interest. First, when the specific binding of radiolabelled LDL particles was assayed in human tissues from a number of organs obtained during surgical procedures [8], it was evident that steroid hormone-producing organs, particularly the adrenal cortex, had the highest LDLR expression/cell (Fig. 3a). The liver also had a relatively high LDLR expression, and considering its size, it is obvious to assume that this organ may contribute as much as two-thirds of body LDL catabolism. Secondly, when the distribution of radioactivity in various organs was examined after the injection of LDLs carrying

 

radiolabelled sucrose [9], the pattern was quite similar (Fig. 3b).

Based on this information, it is reasonable to predict that the steroid hormone-producing tissues, particularly the adrenal cortex, would be sensitive to situations in which LDL-C levels are extremely low (or LDLR activity is markedly reduced). Even if the compensatory mechanisms of cholesterol provision discussed above may balance, an inherent deficiency should be possible to detect either from simultaneous measurements of steroid hormones and the pituitary hormones that regulate their secretion, or from characterizing the degree of steroid hormone responsiveness following challenge by administration of the stimulatory pituitary hormones. Individuals with very low concentrations of LDL-C, such as hypobeta- or abetalipoproteinemia (see below), as well as patients with homozygous FH lacking functional LDLRs, seem to maintain a normal basal production of adrenal steroid hormones. The response to stimulation with ACTH may, however, be submaximal in these situations [10–12]. The production of steroid hormones by the ovaries and testes seems to be maintained in the corresponding situations, suggesting that supplementary pathways of cholesterol delivery (i.e. uptake of HDL-C or stimulation of cholesterol synthesis) can ensure an adequate hormone production also following stimulation of these organs.

Maintaining a normal secretion of bile acids into the bile is critical for the ability to absorb fat and fat-soluble vitamins from the intestine (Fig. 4). Production of bile acids in the liver, controlled by the activity of the rate-limiting enzyme, cholesterol 7-alpha-hydroxylase (CYP7A1), requires access to adequate levels of the precursor free cholesterol in the ER [13]. In situations with very low LDL-C, such as abetalipoproteinemia or homozygous hypobetalipoproteinemia (see below) as well as in HDL deficiencies, there is no indication of a reduced bile acid synthesis. Also complete LDLR deficiency (homozygous FH) is not associated with reduced bile acid production [14]. Inhibition of hepatic cholesterol synthesis by statin treatment does not result in deficient bile acid production [15]. When the production of bile acids is markedly stimulated by pharmacological or surgical interruption of their enterohepatic circulation (i.e. following treatment with cholestyramine or creation of a bile fistula), bile acid pool size is upheld through a pronounced increase in cholesterol production. In such situations, the additional LDL-lowering by treatment with statin or LDL apheresis therapy results in a

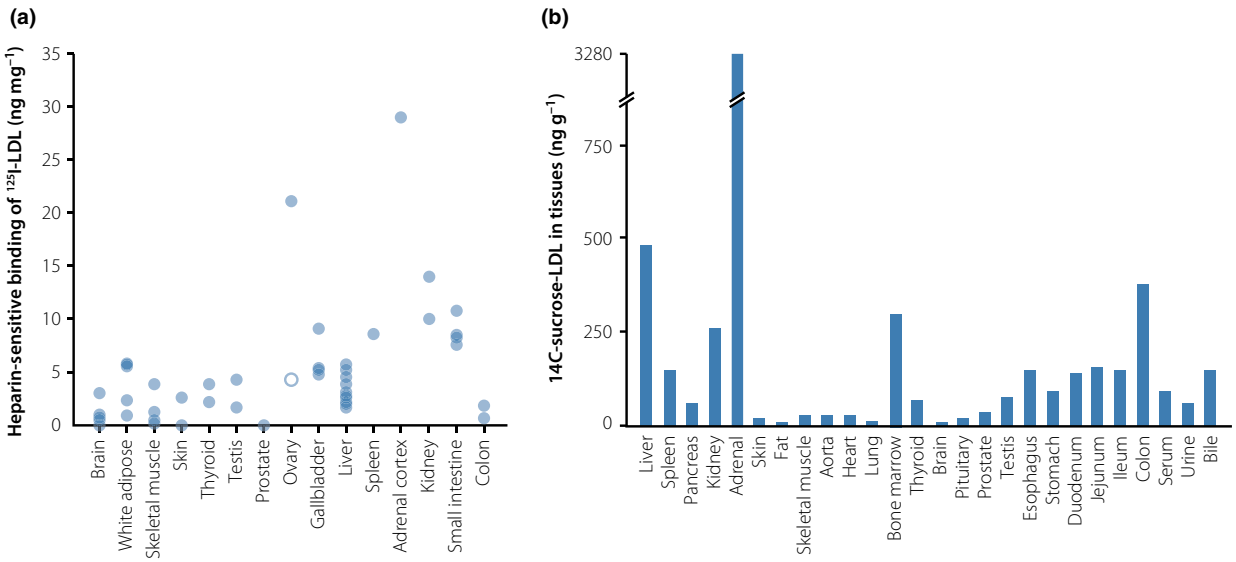


**Fig. 3** *LDL-receptor distribution in human tissues. (a) Heparin-sensitive binding of 125I-LDL to homogenate particulates (ng mg$^{-1}$) of various tissues obtained at surgery [8]. Ovarian tissue from postmenopausal woman indicated by open circle. (b) Postmortal content of 14C-sucrose-LDL in various organs (ng g$^{-1}$) of a leukaemic patient one week after injection of 14C-sucrose [9].*

 

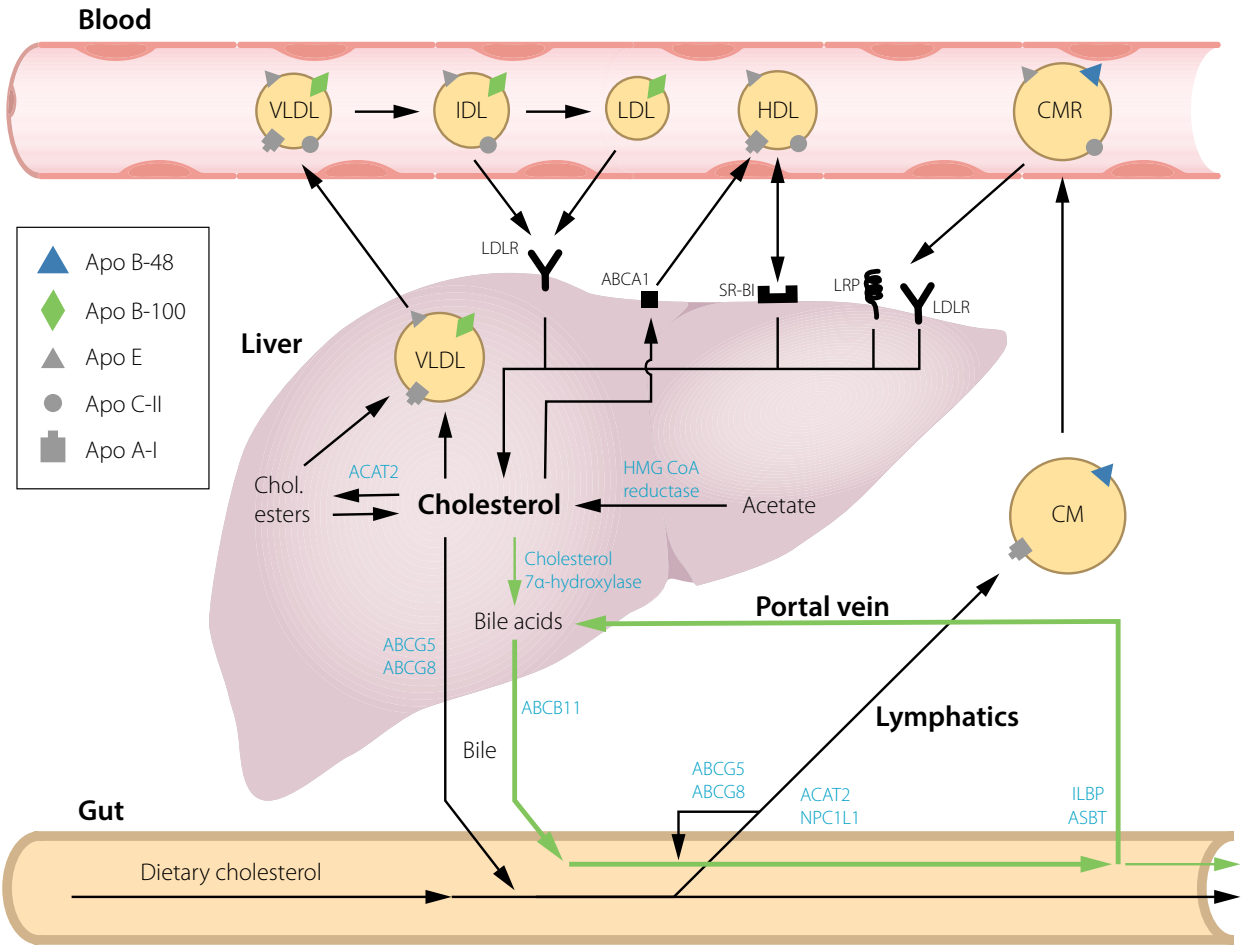


**Fig. 4** *Schematic representation of hepatic cholesterol and bile acid metabolism [106]. Apo, apolipoprotein; CM, chylomicron; CMR, chylomicron remnant; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; LDLR, LDL receptor; LRP, LDLR-related protein; SR-BI, scavenger receptor class B type I; ACAT, acyl CoA-cholesteryl acyl transferase; HMG CoA reductase, 3-hydroxy-3-methylglutaryl CoA reductase; ASBT, apical sodium-dependent bile acid transporter; ILBP, ileal lipid binding protein; ABC, ATP-binding cassette; NPC1L1, Niemann-Pick C1-like 1.*

reduced production of bile acids [16, 17]. Hence, it is evident that the liver can generally maintain essentially normal production of bile acids despite very low levels of circulating LDL-C.

### Inherently low LDL-C: experience from abeta- and hypobetalipoproteinemias

Until the mid-1990s, evidence that low LDL-C confers protection from CHD was less robust. CHD is uncommon in populations with low average levels of circulating LDL, but these populations also tend to have lower levels of other CHD risk factors, particularly obesity-associated disorders such as diabetes, sedentary lifestyle and hypertension. Rare genetic mutations that cause very low levels of LDL were less informative than those that cause severe hypercholesterolaemia because the phenotype associated with LDL-lowering mutations (i.e. the absence of coronary disease in an elderly individual) is not unusual, whereas the presence of severe disease in a child, as it occurs in homozygous familial hypercholesterolaemia, is extremely rare. Accordingly, demonstrating an atheroprotective effect of genetically low LDL-C requires a cohort of affected individuals that is

 

large enough to support a statistical argument. The assembly of such cohorts was precluded by the very low prevalence of the two classical Mendelian forms of hypocholesterolaemia, hypobetalipoproteinemia and abetalipoproteinemia, and by the fact that mutations that confer protection from disease are less likely to come to clinical attention. Moreover, anecdotal reports of severe atherosclerosis in individuals with genetic hypobetalipoproteinemia suggested that a low plasma level of LDL-C does not invariably confer protection against CHD.

Hypobetalipoproteinemia and abetalipoproteinemia are associated with a variety of adverse sequelae. Abetalipoproteinemia is caused by mutations in the microsomal triglyceride transfer protein (MTTP), which plays a role in lipidation of apolipoprotein B (apoB) in the early stages of VLDL formation [18]. This disorder is inherited as an autosomal-recessive trait and occurs with an estimated frequency of 1 per million. Affected individuals have very low plasma levels of apoB-containing lipoproteins and frequently suffer from fat malabsorption and hepatic steatosis. They may also develop neurological and ophthalmological symptoms due to deficiency of fat-soluble vitamins (A and E). Hypobetalipoproteinaemia is caused by mutations in APOB, the gene encoding apoB. Heterozygotes are present in most populations at a frequency of ~1:1000 and usually have plasma levels of LDL-C below the 5th percentile. Homozygous hypobetalipoproteinaemia is associated with fat malabsorption and hepatic steatosis, which progresses to cirrhosis and hepatocellular carcinoma in some patients. Hepatic steatosis has also been reported in several patients with heterozygous hypobetalipoproteinemia [19]. In both hypobetalipoproteinemia and abetalipoproteinemia, the primary metabolic defect is a disruption of triglyceride-rich lipoprotein secretion by the liver and gut. Therefore, the clinical symptoms of these disorders may reflect a failure to secrete lipoproteins with a consequent intracellular lipoprotein accumulation, rather than a low level of LDL *per se*.

### Inherently low LDL-C: experience from PCSK9 mutations

In 2003, a new Mendelian form of hypercholesterolaemia was identified [20]. The culprit gene, proprotein convertase subtilisin kexin type 9 (PCSK9), was initially described as a neural proprotein convertase, but was soon associated with hypercholesterolaemia through classical linkage analysis. In an elegant study of two families with autosomal-dominant hypercholesterolaemia that did not segregate with *LDLR*, Abifadel *et al.* [20] mapped the mutant gene to a locus on chromosome 1. Sequencing of candidate genes in the linked locus revealed that both families had missense mutations in PCSK9. Mechanistic studies using recombinant adenoviruses to overexpress PCSK9 in the livers of mice revealed markedly decreased expression of hepatic LDLRs and increased plasma levels of LDL-C in these animals [21–23]. Expression of the mutant isoforms of PCSK9 that caused hypercholesterolaemia in humans yielded similar results, indicating that the mutations conferred a gain-of-function, rather than a loss of function [21]. The observation that gain-of-function mutations in PCSK9 caused hypercholesterolaemia led to the hypothesis that loss-of-function mutations in the gene would increase LDLR expression and decrease LDL-C levels (Fig. 5).

This hypothesis was confirmed by sequencing the coding regions of PCSK9 in individuals from the Dallas Heart Study who had plasma levels of LDL-C in the lower 5th percentile of that population. Two loss-of-function mutations (Y142X and C692X) were found in 2% of African Americans, but were very rare in other ethnic groups. No homozygotes or compound heterozygotes were observed in the cohort. A less severe mutation (R46L) was found in 3% of European American participants (and at lower frequencies in African Americans). This variant was associated with a 20% reduction in LDL-C.

Subsequent studies confirmed that sequence variants that impair PCSK9 function, particularly the R46L substitution, are consistently associated with decreased plasma levels of LDL-C and a greater-than-expected reduction in CHD. In the Dallas Heart Study, these mutations were associated with a 28 per cent reduction in mean LDL cholesterol and an 88 per cent reduction in the risk of CHD ($P = 0.008$ for the reduction; hazard ratio, 0.11; 95 per cent confidence interval, 0.02–0.81; $P = 0.03$) [24]. The apparently disproportionate decrease in CHD risk associated with an LDL-lowering allele is not peculiar to PCSK9. Variants in *NPC1L1*, *APOB*, *SORT1* and *LDLR* also confer a higher-than-expected level of protection from CHD for a given level of LDL-C reduction [25, 26]. The discrepancy between pharmacological and genetic LDL-lowering may reflect the fact that individuals who inherit PCSK9- or other LDL-lowering mutations have lower LDL levels from birth on, whereas statin

 

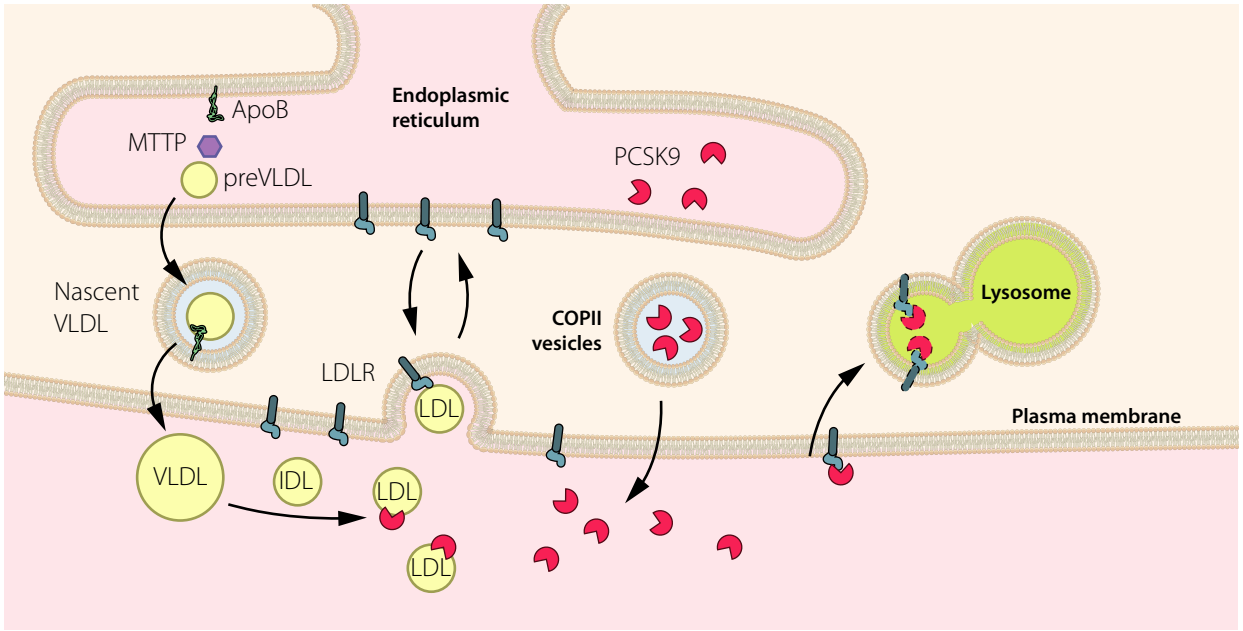


**Fig. 5** *Interaction between PCSK9 and LDL receptor in hepatocytes. PCSK9 is transported from endoplasmic reticulum to cell surface via COP II (coat protein complex II) vesicles. By binding to LDLRs, circulating PCSK9 prevents their normal recirculation to the cell surface resulting in a reduced number of LDLRs.*

treatment is typically initiated in middle age, when the disease process is usually already well established [27, 28]. These findings have important clinical implications. LDL is the cardinal risk factor for CHD, and whilst the importance of other risk factors should not be ignored, LDL-lowering is likely to remain the cornerstone of CHD prevention. The increased protection associated with LDL-lowering genotypes suggests that CHD risk is a function of LDL concentration combined with duration of exposure. Current American recommendations delay therapeutic intervention until the absolute risk of CHD is 7.5% over the next 10 years [29]. The recent European guidelines recommend lifestyle changes and statins in patients with SCORE absolute mortality risks of 5% or 10% over the next 10 years [30]. Typically, patients are at least 50 years of age when lipid-lowering is initiated [29]. Taken together, the genetic data suggest that earlier initiation might produce greater reductions in CHD than later and more conservative interventions.

The observation that loss-of-function mutations in PCSK9 confer protection against CHD established the protein as a therapeutic target for LDL lowering and CHD prevention. Heterozygotes for nonsense mutations in PCSK9 appeared healthy and had no obvious sequelae other than low levels of LDL. Detailed clinical examination of one compound heterozygote revealed no adverse effects of PCSK9 deficiency. She is an apparently healthy, fertile, normotensive, physically active, college-educated woman with normal liver and renal function tests (including urinalysis) [31]. A follow-up examination performed six years after ascertainment verified that she continued to enjoy good health after nearly 40 years of total absence of PCSK9 and extremely low LDL-C at 0.4–0.8 mmol $L^{-1}$ (14-29 mg $dL^{-1}$). Subsequently, a 21-year-old Zimbabwean woman who presented at a postnatal clinic was found to be homozygous for the C679X allele [32]. Few clinical details of this patient have been reported, but her plasma level of LDL-C was extremely low at 0.4 mmol $L^{-1}$ (16 mg $d^{-1}$). Whereas the mean LDL-C of the clinic population was low [~2.2 mmol $L^{-1}$ (85 mg $dL^{-1}$)], the homozygote had the lowest LDL-C level of 653 subjects studied. This finding suggests that inhibition of PCSK9 will further reduce LDL-C even in individuals whose levels are already low.

Cariou *et al.* [33] described a 54-year-old diabetic man with striking hypolipidaemia [LDL-C = 0.4–

0.6 mmol $L^{-1}$ (16–24 mg $dL^{-1}$)] who was heterozygous for a PCSK9 allele with two mutations: R104C and V114A. No mutation was found on his other PCSK9 allele, but he had no detectable PCSK9 in his plasma. Those authors proposed that the double-mutant allele is a dominant negative, although the proband's daughter and sister who shared the same mutation had readily detectable circulating PCSK9 levels in the low-normal range and more moderate reductions in LDL-C [33]. Whether the R104C/V114A results in complete or partial PCSK9 deficiency remains to be determined, but taken together the available data indicate that PCSK9 deficiency is not strongly associated with adverse sequelae and suggest specific inhibitors of PCSK9 are likely to be benign.

#### Inherently low LDL-C: experience from ANGPLT3 mutations

Angiopoietin-like 3 (ANGPTL3) regulates lipoprotein metabolism by inhibiting lipoprotein lipase and endothelial lipase. Loss-of-function mutations in the ANGPTL3 gene cause familial combined hypolipidemia (FHBL2): homozygotes, who have total deficiency of ANGPTL3, have a 60–70 per cent reduction in plasma levels of LDL-C and triglycerides. Evaluation of 14 individuals who were homozygotes or compound heterozygotes revealed no sign of adverse clinical sequelae [34]. According to one review, some patients with ANGPL3 mutations presented with fatty liver [19].

#### Extremely low LDL-C: experience from lipid-lowering trials

Effective LDL-C-lowering therapy remains a challenge in certain high-risk patients despite treatment with high-intensity statins [35]. Amongst patients with elevated LDL-C levels whilst taking maximum tolerated statins, certain nonstatin cholesterol-lowering medications provide incremental reductions in LDL-C. These therapies include ezetimibe, bile acid sequestrants, niacin and PCSK9 inhibitors [36]. Other nonstatin agents approved for LDL-C lowering in homozygous familial hypercholesterolaemia include lomitapide and mipomersen [37].

LDL-C levels less than 0.65 mmol $L^{-1}$ (25 mg $dL^{-1}$) on two or more consecutive measurements have been considered the lowest threshold for protocol-mandated discontinuation of cholesterol-lowering therapy in some randomized clinical trials [38]. However, a more conservative lower limit for LDL-C of <1.04 mmol $L^{-1}$ (40 mg $dL^{-1}$) was recommended by the American College of Cardiology/American Heart Association Cholesterol Guideline [29].The recent European guidelines for prevention of cardiovascular disease in clinical practice recommend a LDL-C goal of 1.8 mmol $L^{-1}$ (70 mg $dL^{-1}$) or 50% lowering if LDL-C is 1.8–3.5 mmol $L^{-1}$ (70–135 mg $dL^{-1}$) [30]. Extremely low LDL-C levels are uncommon with statin therapy except when the baseline LDL-C is low and the LDL-C lowering potency is high. Amongst the 16 304 participants enrolled in the Justification for the Use of statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial, treatment with rosuvastatin 20-mg daily lowered LDL-C levels to <0.78 mmol $L^{-1}$ (30 mg $dL^{-1}$) in 767 subjects. As compared with the 7387 subjects with LDL-C levels ≥0.78 mmol $L^{-1}$, LDL-C levels <0.78 mmol $L^{-1}$ were associated with more physician-reported type 2 diabetes, haematuria, hepatobiliary disorders and insomnia. This may have been due to a selection phenomenon, as, for example, the prevalence of metabolic syndrome was higher in those with low baseline LDL-C. However, reports of insomnia and haematuria were not different in JUPITER participants with LDL-C < 1.295 mmol $L^{-1}$ (50 mg $dL^{-1}$) vs. ≥1.295 mmol $L^{-1}$ (50 mg $dL^{-1}$). No differences in myalgia were reported for JUPITER participants with on-trial LDL-C levels <0.78 mmol $L^{-1}$ (30 mg $dL^{-1}$) or <1.295 mmol $L^{-1}$ (50 mg $dL^{-1}$). Haemorrhagic strokes have been a concern with low LDL-C levels. However, haemorrhagic strokes were numerically less in JUPITER participants with very low LDL-C levels [39, 40].

Anti-PCSK9 antibody therapy has been investigated in short-term studies of patients with LDL-C levels >1.81 mmol $L^{-1}$ (70 mg $dL^{-1}$) [2, 3]. In phase II and IIIA trials, eligible participants were randomized (2:1) to active treatment with anti-PCSK9 antibody or placebo. LDL-C reductions with anti-PCSK9 antibodies average 61% [3] to 62% [2] in pooled analyses, with many individuals achieving LDL cholesterol <0.65 mmol $L^{-1}$ (25 mg $dL^{-1}$). Due to anticipated reductions in LDL cholesterol <0.65 mmol $L^{-1}$ (25 mg $dL^{-1}$), regulatory agencies required enhanced monitoring of treatment emergent adverse events and laboratory studies of special interest.

Two fully human monoclonal antibodies to circulating PCSK9, alirocumab and evolocumab, have been approved by regulatory agencies [2, 41]. In pooled

 

analyses from phase II and IIIA trials, treatment with fully human monoclonal anti-PCSK9 antibody lowered LDL-C levels to <0.65 mmol $L^{-1}$ (25 mg $dL^{-1}$) in 37.0% (575/1550) of alirocumab-treated [3], and 26.0% (773/2976) of evolocumab-treated patients [2, 42]. Levels of LDL-C < 0.39 mmol $L^{-1}$ (15 mg $dL^{-1}$) were reported in 9.4% of alirocumab-treated participants.

Treatment with the partially humanized monoclonal anti-PCSK9 antibody bococizumab for 28 days lowered LDL-C to <0.65 mmol $L^{-1}$ (25 mg $dL^{-1}$) in 28.7% (72/251) [42]. Further development of bococizumab has, however, recently been stopped because of attenuating LDL-C lowering effect over time, unanticipated higher level of immunogenicity (production of antidrug antibodies) and higher rate of injection-site reactions with this drug than shown for the other agents in this class of compounds (message to all SPIRE investigators on 1 November 2016 from James H. Revkin, Clinical bococizumab lead).

Two of the authors (AGO and RR) have served in the Data Monitoring Committee (DMC) of the phase 2 and 3a development stages of alirocumab. A special routine was developed to monitor treatment-emerging adverse events, see Table 1.

The 12-month safety of alirocumab and evolocumab and 24-month safety of alirocumab have been reported for the entirety of participants allocated to anti-PCSK9 therapy and placebo. Odyssey long-term included 1550 participants in the alirocumab group and 788 participants in the placebo group [3] Table 2.

Adverse events were similar in both treatment groups except for a higher incidence of myalgia that was mainly reported from Odyssey Alternative that enrolled participants intolerant to statins due to adverse muscle events [45]. Osler I and II included 2976 participants in the evolocumab groups and 1489 in the standard therapy group. Neurocognitive events were more commonly reported in the evolocumab group than in the placebo group (27/2976 [0.9 per cent] versus 4/1489 [0.3%]) [2]. After 24 months, there were more cataracts reported in alirocumab-treated participants [43]. Neurocognitive events were recorded as delirium (including confusion), cognitive and attention disorders and disturbances, dementia and amnestic conditions, disturbances in thinking and perception, and mental impairment disorders. In a network meta-analysis that comprised 17 randomized clinical trials that included 13 083 participants (8250 PCSK9 inhibitor, 3957 placebo, 846 ezetimibe), treatment with PCSK9 inhibitors was associated with a higher incidence of neurocognitive adverse events (odds ratio 2.34 [95% confidence interval: 1.11–4.93) [46]. In a recent meta-analysis including two larger clinical outcome PCSK9 inhibitor studies, the latter ones showed a significant more than twofold higher risk of neurocognitive events amongst participants being treated with PCSK9 inhibition [47]. The absolute figures of neurocognitive events were low, only around 1 or 2 per cent of the treated populations. The authors conclude that there should be a close monitoring for the increased risk of neurocognitive

**Table 1** *Monitoring of adverse Events of Special Interest as requested by FDA in Patients Treated With PCSK9 Inhibitors Who Achieve LDL-C Levels < 0.65 mmol $L^{-1}$ (25 mg $dL^{-1}$).*

| |
|---|
| General Allergic Adverse Events |
| Neuromuscular Adverse Events |
| Neurocognitive Disorders |
| Musculoskeletal Events |
| Creatine kinase levels >fivefold above normal range |
| Neurological Events |
| Psychiatric Disorders |
| Depression, suicide |
| Diabetes |
| New diagnosis |
| Worsening glycaemic status |
| Diabetic complications |
| Endocrine Deficiencies |
| Sex steroids |
| Adrenal corticoid deficiencies |
| Fat-soluble vitamin Deficiencies |
| Vitamin A, D, E and K levels |
| Disorders associated with deficiencies of fat-soluble vitamins |
| Hepatic Disorders |
| ALT/AST >threefold above normal range with and without elevated bilirubin |
| Ophthalmologic Events |
| Cataracts |
| Retinopathies |
| Infections |
| Cancers |

**Table 2** *Adverse events (%) in clinical trials of alirocumab- and evolocumab-treated patients [2, 43, 44].*

| | Alirocumab group (total) | Standard of Care | Alirocumab LDL-C < 0.65 mmol $L^{-1}$ on 2 occasions | Evolocumab Group | Standard of Care | Evolocumab LDL-C < 0.65 mmol $L^{-1}$ on 2 occasions |
|---|---|---|---|---|---|---|
| Any | 81.0 | 82.5 | 75.7 | 69.2 | 64.8 | NR |
| Local site reaction | 5.9 | 4.2 | 3.8 | 4.3 | NR | NR |
| Muscle-reported | 6.4 | 6.0 | NR | 6.4 | 6.0 | 4.9 |
| Neurocognitive | 1.2 | 0.5 | 0.5 | 0.9 | 0.3 | 0.5 |
| Cataracts | 1.0 | 1.0 | 1.9 | NR | NR | NR |
| Alanine aminotransferase /aspartate aminotransferase or both | 1.8, 1.4 | 2.1, 2.3 | NR | 1.0 | 1.2 | 0.9 |
| Creatine kinase >fivefold above normal | 3.7 | 4.9 | NR | 0.61 | 1.0 | 0.4 |

NR, Not reported.

events in ongoing outcome studies and postmarketing surveillance. In shorter-term trials with humanized monoclonal antibodies, neurocognitive-related adverse events were reported in 1.9% of LY3015014-treated participants and 3.4% of placebo-treated participants [48] [13]. Amongst subjects receiving bococizumab, 0.8% experienced adverse events of memory loss [42]. Prospective assessments of neurocognitive function have been incorporated into the phase III clinical outcomes trials FOURIER (Evaluating PCSK9 Binding antibody Influence oN cognitive HeAlth in High cardiovascular Risk Subjects [EBBINGHAUS; *ClinicalTrials.gov NCT02207634*]). As a part of the prospective FOURIER study[1,2], comprising almost 2000 participants, the EBBINGHAUS subgroup tested the PCSK9 inhibitor evolocumab in statin treated patients with regard to cognitive function. After a follow-up of 20 months no differences between evolocumab and placebo were noted regarding a battery of cognitive tests, patient reported every day cognition or adverse cognitive events reported by their doctors. No evidence of differences in cognitive tests by achieved nadir LDL-C, even <0.65 mmol $L^{-1}$ were seen.

In a safety analysis of very low LDL-C levels in a pooled sample of 14 studies of alirocumab, 839 participants reached two consecutive LDL-C levels below 0.65 mmol $L^{-1}$ (25 mg $L^{-1}$) and 314 below 0.40 mmol $L^{-1}$ (15 mg $dL^{-1}$) [44]. Attaining these LDL-C levels were not associated with an increase in overall treatment emergent adverse event rates or neurocognitive events, although cataract incidence appeared to be increased in the group achieving LDL-C level below 0.65 mmol $L^{-1}$.

Many of the safety and efficacy issues of very low LDL-C levels are addressed in the FOURIER (Further Cardiovascular OUtcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk) [49]. FOURIER is a multinational phase 3 double-blind, randomized, placebo-controlled trial of evolocumab in approximately 27 500 patients who had either a myocardial infarction, an ischaemic stroke or symptomatic peripheral artery disease and an LDL ≥1 mmol $L^{-1}$ or a non-HDL-C ≥ 2.6 mmol $L^{-1}$ on optimized statin therapy. On 2 February 2017, the sponsor (Amgen) announced that the FOURIER trial met its primary composite end-point (cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, hospitalization for

[1]Sabatine MS, Giugliano RP, Keech AC, et al. Evolocumab and Clinical Outcomes in Patients with Cardiovascular Disease. New England Journal of Medicine. 2017; https://doi.org/10.1056/NEJMoa1615664.
[2]Giugliano R, Mach F, Zavitz K, et al. EBBINGHAUS: -A Cognitive Study of Patients Enrolled in the FOURIER Trial. American College of Cardiology; Washington DC 2017.

unstable angina or coronary revascularization) and the key secondary composite end-point (cardiovascular death, nonfatal MI or nonfatal stroke). No new safety issues were observed.

Fat-soluble vitamin concentrations (A,D,E,K) were measured as part of the prespecified safety analysis in ODYSSEY long-term [3]. Vitamin E levels were lower in both alirocumab-treated and evolocumab-treated participants; however, the results were nonsignificant after adjustment for LDL-C [3, 50]. Vitamin E in red cell membranes was unchanged from baseline to 52 weeks in both absolute and LDL-C normalized levels [15].

Tests of reduced adrenal cortical function included serum cortisol levels that were followed by ACTH stimulation if these levels were low. Amongst these participants, no differences were noted (0.6% for alirocumab group vs. 0.6% for placebo group) [10]. In evolocumab-treated subjects, the cortisol: ACTH ratio remained <3.0 (nmol $pmol^{-1}$) in both treatment groups [50].

Gonadal hormones were unchanged with evolocumab therapy from baseline to 52 weeks. In men, testosterone levels were unchanged from baseline. Follicle-stimulating hormone (FSH) levels decreased significantly, but luteinizing hormone (LH) levels remained unchanged after normalization for postbaseline LDL-C levels [50]. There were no correlations between changes in LDL-C and testosterone levels. In women, oestradiol levels were unchanged, but FSH and LH levels increased. There were no associations between LDL-C change and change in oestradiol for evolocumab-treated participants. As discussed above, these findings are consistent with the minimal involvement of LDL particles in the production of steroid hormones [51].

Currently, 52-week data from double-blind, controlled trials do not demonstrate changes in levels of vitamin E, cortisol: ACTH levels or sex steroids [3, 50]. Future reports are pending on the longer-term safety of PCSK9 inhibitor therapy and particularly amongst the subset of participants with LDL-C levels <0.65 mmol $L^{-1}$ (25 mg $dL^{-1}$). Safety measures will be further assessed in study participants who achieve LDL-C levels <0.39 mmol $L^{-1}$ (15 mg $dL^{-1}$) and ≥0.39 to 0.65 mmol $L^{-1}$ (≥15–25 mg $dL^{-1}$).

In the recently published GLAGOV study [52], where the effect of evolocumab on coronary atheroma was assessed by intravascular ultrasound in 968 patients with CHD, the actively treated group reached a mean LDL-C of 0.94 mmol $L^{-1}$ (36.6 mg $dL^{-1}$) against 2.4 mmol $L^{-1}$ (93 mg $dL^{-1}$). The treatment was associated with a reduction in per cent atheroma volume for evolocumab but not for placebo. The reduction in coronary plaque volume was associated with on-trial LDL-C levels with lowest reported value of 0.52 mmol $L^{-1}$ (20 mg $dL^{-1}$). Evolocumab was well tolerated, and no differences were seen in myalgia, neurocognitive events or new diagnosis of diabetes mellitus.

### LDL-C and risk of type 2 diabetes

The association of LDL cholesterol with diabetes has been less obvious in epidemiological studies, but some evidence is accumulating in genetic studies. A score of 130 single nucleotide polymorphisms (SNPs) explaining 7.9% of the variance in LDL cholesterol levels revealed an inverse association of genetically defined LDL cholesterol levels and risk of diabetes [53]. Likewise mutations in PCSK9 and HMGCR, which decrease LDL cholesterol levels and CHD risk, were found to increase the risk of diabetes [54, 55]. Conversely, familial hypercholesterolaemia was found associated with reduced risk of diabetes [56]. Several statin trials as well as meta-analyses thereof revealed increased risk of diabetes in statin-treated patients, especially if they showed components of the metabolic syndrome [57]. By contrast to the genetic data, meta-analysis of rather short-term trials (maximal follow-ups 18 months) with the PCSK9 inhibitor alirocumab revealed no diabetogenic effects. The hazard ratio (HR; 95% confidence interval) for diabetes-related treatment emergent adverse events in alirocumab versus placebo was 0.64 (0.36–1.14) [58]. This finding has recently been confirmed also to apply to patients reaching LDL-C levels below 0.65 mmol $L^{-1}$ in a meta-analysis of 14 studies of alirocumab [44].

The association between statin therapy and the incidence of type 2 diabetes in a possible drug potency-related manner has induced a complex discussion about possible mechanisms [57, 59, 60]. The first question was whether this observation is related to the direct mechanism of action of statins or rather an off-target effect. Genetic data indicate that individuals with variations in the HMG CoA reductase gene, which are associated with reduced enzyme, activity have an

increased risk for type 2 diabetes [61]. In line with this observation, in vitro experiments indicate that the interference with sterol synthesis disturbs insulin secretion by pancreatic beta-cells as well as insulin sensitivity in skeletal muscle and adipocytes [60]. However, reduced sterol synthesis will also increase LDL-receptor expression and hence influx of LDL. The reduced prevalence of diabetes in patients with familial hypercholesterolaemia and the increased incidence of diabetes in probands carrying loss-of-function mutations in PCSK9 support such a diabetogenic role of LDL-receptor-mediated LDL uptake into either insulin-producing beta-cells or insulin-sensitive cells. Experiments in islets of wild-type and LDLR-knockout mice as well as comparative studies of hyperlipidaemic mice with either LDLR-knockout or apoE knockout revealed that LDLR-mediated LDL uptake into beta-cells interferes with glucose-stimulated insulin secretion. [62, 63].

Can activation of SREBPs in liver drive diabetes manifestation? Transgenic overexpression of the constitutively active trans-domain of SREBPs in liver of mice induces fatty liver disease, and it has been shown that selective overexpression of SREBP-1 in liver is not only associated with induction of fatty liver disease, but also with the development of severe obesity, ectopic lipid accumulation in skeletal muscle, and insulin resistance [64, 65]. This development of clinical features in these transgenic mice corresponding to the metabolic syndrome was not related to increased food consumption, which was comparable between transgenic and control mice. Therefore, it appears that activation of SREBPs might increase gene regulatory susceptibility for the development of the metabolic syndrome and possibly type 2 diabetes.

Four weeks of treatment with atorvastatin (80 mg $d^{-1}$) did not influence hepatic SREBP2 expression, whilst it reduced hepatic TGs and the expression of SREBP1c, glucokinase and ANGPLT3. Hepatic expression of glucose-6-phosphatase was increased [66, 67]. Thus, it is possible that statins may modify peripheral rather than hepatic insulin sensitivity.

A recent comprehensive review on the safety of statin therapy showed that adverse events definitely to be caused by statin therapy are myopathy (specifically defined as muscle pain or weakness combined with large increases in blood concentrations of creatine kinase) and diabetes, although it is likely that the risk of haemorrhagic stroke is also increased [68].

From the clinical perspective, it is important to emphasize that the incidence of diabetes is defined by glycaemia. Like other intermediate phenotypes such as elevated blood pressure or lipid concentrations in plasma, diabetes becomes clinically relevant by its clinical complications, that is CHD, nephropathy, retinopathy and neuropathy. Cardiovascular event rates are lowered by statins also in patients with manifest or latent diabetes, and post hoc analyses of trial data and registries indicate protective rather than harmful effects of statins on microvascular events. Overall, the risk/benefit ratio hence argues in favour of intensive LDL-C lowering despite the risk of increasing diabetes. A more personalized treatment strategy should also take into account that the low HDL-C/hypertriglyceridemia syndrome, rather than LDL-hypercholesterolaemia, is the prominent dyslipidemia of diabetic subjects.

### LDL-C, infections and the immune system

Observational studies revealed association of low LDL-C with increased mortality in the general population [69] and specifically in elderly humans [70, 71] as well as in patients with acute myocardial infarction [72] or community-acquired pneumonia [73]. A low cholesterol concentration in sepsis patients has been suggested as a marker for a bad prognosis [74].

In the JUPITER study, rosuvastatin treatment was associated with a lower incidence of pneumonia (adjusted hazard ratio 0.82, 95% CI 0.68–0.99) [75]. The authors attribute this effect to the statin treatment and not the low LDL-C level reached by the rosuvastatin treatment.

The question is if a low LDL-C in acute diseases including sepsis represents a pathogenic mechanism or if this finding is an example of reverse causation, that is the septic condition per se has caused the low LDL-C. The latter is suggested by the observation that PCSK9 decreases the clearance of pathogenic lipids, such as endotoxin, carried in LDL. PCSK9 inhibition improves survival in septic mice. Similarly, humans who carry loss-of-function variants of the PCSK9 gene have increased survival in sepsis [76].

 

Conversely, uptake of cholesterol, for example from LDL, by immune cells may have an activating effect on the immune response and could therefore be protective against acute inflammatory diseases, but a disadvantage for chronic inflammatory processes. This could be relevant, not only to atherosclerosis but also to chronic infections such as HIV, or to autoimmune disorders such as rheumatoid arthritis and psoriasis.

Because the proinflammatory effect of cholesterol loading may have beneficial effects on the ability of the organism to combat acute inflammation by infection or wounding, it has been suggested that cholesterol loading of myeloid cells may have evolved to support host defence [77]. On the other hand, HDL-mediated cholesterol efflux may be important to resolve inflammation. In this context, cholesterol efflux and reverse cholesterol transport (RCT) could represent a physiological adaptation to limit or resolve inflammatory responses of macrophages but also other cells [77]. If so, extreme lowering of LDL-C may be disadvantageous for acutely diseased patients who are in need of inflammatory responsiveness. These hypotheses must, however, be confirmed in clinical data. Possibly the ongoing hard outcome studies on PCSK9 inhibitors may cast light on this important question.

### LDL-C and malignant neoplasms

The results of prospective studies of lipids in relation to cancer development are contradictory. In a recent meta-analysis, no significant relations between LDL-C levels and breast cancer in women were found [78]. Fifteen prospective cohort studies involving 1 189 635 participants and 23 369 breast cancer cases were included in the meta-analysis. No relation between LDL-C and breast cancer was noted.

It is, however, well known that some haematologic malignancies are associated with low cholesterol levels [9]. The Copenhagen City Heart Study also observed an increased incidence of cancers in Danes with LDL cholesterol levels below 2.6 mmol $L^{-1}$ (100 mg $dL^{-1}$) [79]. LDL-C levels below the 10th percentile were associated with a 43% increase in the risk of cancer. However, a Mendelian randomization analysis of these data revealed that LDL-C lowering mutations in PCSK9, ABCG8 or APOE do not increase the risk of cancer. Similarly, Folsom *et al.* [80] reported that PCSK9 variants were not associated with increased risk of cancer in African American or in whites in the ARIC study. These findings suggest that low LDL-C levels per se do not cause cancer. More likely the association of low LDL-C with cancer is an example of reverse causation, the malignant cells requiring more cholesterol for their proliferation.

A word of caution has been expressed regarding possible cancerogenic effects of statin treatment in primary prevention, that is when treatment is given to patients who have not developed clinical manifestations of atherosclerosis. In an evaluation of individual statin trials, there were trends towards more cancer promotion [81]. In the PROspective Study of Pravastatin in the Elderly at Risk (PROSPER) [82], total cancer incidence was significantly increased ($P = 0.02$) in those randomized to pravastatin. A recently published 16-year follow-up of the LIPID study has shown no increase in cancer incidence and mortality, which had been suspected in a previous *post hoc* analysis [83]. The authors conclude that the results provide reassurance on the long-term safety of statins in relation to non-CVD causes of death, cancer incidence and cancer mortality.

Several other long-term interventional studies with statins have not shown any increased incidence of cancer [84, 85]. The few observations of increased cancer rate on statin therapy therefore may be an effect of chance. However, vigilance should be exercised regarding the possibility of untoward effects of therapies that are meant for lifelong treatment.

### LDL-C and bone metabolism

The information on a possible influence of LDL-C levels on bone metabolism is very limited. Low LDL-C has been associated with an increased risk of fractures in patients with type 2 diabetes [86]. However, further research with larger cohorts is needed to investigate a possible relationship between low LDL-C and bone metabolism.

### Hypocholesterolaemia and brain function

The brain is the most cholesterol-rich organ, containing about 25% of total body cholesterol. The many roles of cholesterol in the brain include that it is needed in the myelin for isolation of the axons and that it is critical for synaptogenesis [for a review, see reference [87]]. A normal brain

 

function requires constant levels of cholesterol. Nature has met this need by the evolution of a blood–brain barrier that efficiently prevents uptake of cholesterol from the circulation. This means that the large pool of cholesterol in the brain is formed in situ and is isolated from all other pools of cholesterol in the body. The size of the pool of brain cholesterol is regulated by mechanisms that are somewhat different from the regulation of the extracerebral pools of cholesterol [87]. Given the integrity of the blood–brain barrier, high or low levels of cholesterol in the circulation are not likely to have direct effects on the brain. If effects of changes in circulating levels of cholesterol are observed in the brain, such effects are likely to be mediated by other factor(s) than cholesterol itself, for example cholesterol-induced effects on intracerebral microcirculation or metabolites of cholesterol.

A simplified scheme of the flux of oxysterols across the blood–brain barrier is given in Fig. 6 [87]. The oxysterol 27-hydroxycholesterol (27OHC) is fluxing from the circulation into the brain where it is efficiently metabolized by the enzyme CYP7B1. Further metabolism in the brain results in formation of a steroid acid, 7 alpha hydroxy-3-oxo-4-cholestenoic acid (7a-OH-3 = O-4-acid). This acid fluxes from the brain into the circulation. Cholesterol in the brain is metabolized by the enzyme CYP46 into the oxysterol 24S-hydroxycholesterol (24OHC). This oxysterol fluxes from the brain into the circulation and represents the major pathway for elimination of cholesterol from the brain. All the different oxysterols reaching the circulation are further metabolized to bile acids in the liver.

In spite of the fact that there is no direct flux of cholesterol from the circulation into the brain, there are a number of negative effects of hypercholesterolaemia on brain function. Hypercholesterolaemia in mid-life is a known risk factor for Alzheimer's disease [88]. Dietary cholesterol induces generation of beta-amyloid in experimental animals [89] and induces memory defects in mice [90]. Most of these effects of cholesterol may be caused by a metabolite of cholesterol, 27-hydroxycholesterol, that is able to pass the blood–brain barrier. There is a close relation between cholesterol and 27-hydroxycholesterol levels in the circulation [91]. Thus, high plasma cholesterol leads to high plasma levels of 27-hydroxycholesterol and increased flux of this oxysterol into the brain. Increased flux of 27-hydroxycholesterol into the brain leads to a number of negative effects including reduced uptake of glucose by the brain [6,92], reduced levels of the 'memory protein' Arc (activity regulated cytoskeleton associated protein) in hippocampus [90], negative effects on spatial memory, and upregulation of the brain renin–angiotensin system [93]. Patients with Alzheimer's disease have an accumulation of 27-hydroxycholesterol in the brain [94], although we do not know with certainty if this accumulation is a primary process or secondary to the neuronal degeneration.

According to the above results, demonstrating negative effects on hypercholesterolaemia, reduction in circulating levels of plasma cholesterol should be beneficial. Such treatment can thus be expected to lead to increased glucose uptake by the brain, increased levels of the 'memory protein' in hippocampus, downregulation of the renin–angiotensin system, and reduced production of beta-amyloid. It is important to emphasize, however, that most of the above beneficial effects of hypocholesterolaemia are predicted, and not yet experimentally confirmed.

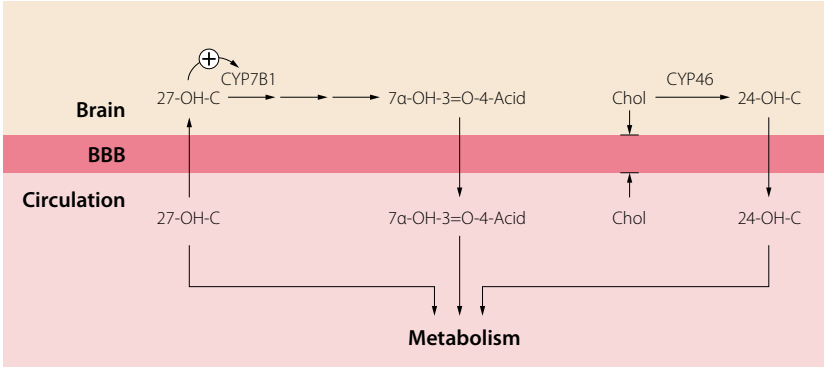


**Fig. 6** *Flux of oxysterols across the blood–brain barrier [87]. See text.*

 

Accordingly, treatment with statins could be expected to have some preventive effect on development of Alzheimer's disease. Although some studies have shown a beneficial effect, two large prospective studies could not confirm this [95]. Treatment with lipophilic statins like simvastatin would be expected to lead to some flux of the drug across the blood–brain barrier with a direct effect on cholesterol synthesis in the brain [96]. According to studies on mice, this can be expected to lead to negative effects on the memory function [97]. Memory loss has been observed in patients treated with simvastatin[98], although its frequency is very low. In their interpretation of the evidence for the efficacy of statin therapy, Collins & al gives a comprehensive outline of the effects of statins on memory and other aspects of cognition [68]. Large randomized trials with masked control groups have provided evidence that allocation to statin therapy is not associated with an excess of memory loss or adverse effects on other aspects of cognitive function. The authors conclude that given the weight of evidence against adverse effects of statin therapy on memory or other aspects of cognition, it would now be appropriate for regulatory authorities to consider their removal from lists of potential side effects on the drug labels.

There is a possible negative effect of hypocholesterolaemia on mood and behaviour that needs to be considered. In a recent analysis of data from 24 216 postmenopausal women between 50 and 79 years participating in the Women's Health Study, an LDL-C level below 3 mmol $L^{-1}$ did not show any association with the presence of depression, but there was an association with new-onset of depression at both 7 years and 11 years of follow-up [99]. In a recent review, it was concluded that the majority of the studies reviewed suggest a relationship between reduced levels of cholesterol and suicide [100]. The included studies were small and regarded selected groups. At least part of these observations may be attributed to reverse causality. On the other hand, particularly during the last years, relationships between serum cholesterol and suicide risk were challenged on the basis of some recent studies that have not found any correlation [101, 102]. Studies on a larger sample of patients are needed to further clarify this important issue [100].

The possibility of risk of cholesterol-lowering treatment on mood and behaviour has been negated in a meta-analysis [103].

The serotonergic system is strongly recognized as being linked to suicidality and impulsive and aggressive behaviour. Lower concentrations of 5-hydroxyindoleacetic acid (5-HIAA) in the cerebrospinal fluid (CSF) in suicide victims and suicide attempters were found in several studies. Jokinen *et al.* found a significant positive correlation between serum total cholesterol and level of CSF 5-HIAA in suicide attempters that remained significant after correction for age, gender, BMI and comorbid substance abuse [104]. Attempts to demonstrate a direct effect of a lipid-lowering therapy on levels of 5-HIAA in CSF from healthy volunteers have failed, however [105]. Interestingly, in the latter study, the statin treatment led to increased levels of the anxiogenic cholecystokinin tetrapeptide in CSF. Whether or not this effect was due to statin itself or its cholesterol-lowering effect could, however, not be established.

In conclusion, mainly based on work with experimental animals, lowering of plasma cholesterol can be expected to have beneficial effects on brain function. Although the possibility that a small fraction of subjects with low cholesterol may have an increased risk for depression and suicide cannot be excluded, further studies on larger cohorts are needed to further clarify this.

## Concluding remarks

Reaching very low levels of LDL-C further decreases the progression and may lead to regression of atherosclerosis. The rapidity of such changes (GLAGOV) may indicate that intensive LDL-C lowering could be efficient also when applied during a limited time period. The FOURIER study will be a game changer with regard to cholesterol treatment in patients with CHD. Understanding the potential risks that may occur as a response to the treatment modalities used in order to reach such levels is of vital importance, and continued evaluation through long-term follow-up of treated cohorts will remain an important task for the future. The detailed control of cholesterol metabolism at the cellular level, and the redundancy of mechanisms that secure cholesterol availability in pathways of critical importance provide a great robustness against potential risks that could emerge from extremely low LDL-C levels in the circulation. So far, data from human physiology and rare genetic diseases indicate that important functions such as steroid hormone production, maintaining an intact enterohepatic circulation

 

of bile acids, and protecting neuronal cell function are not disturbed as a consequence of extremely low circulating LDL-C. The information soon emerging from clinical studies where very low LDL-C levels will be maintained for several years, such as those using PCSK9 inhibition, will be of great interest not only regarding the potential to further reduce complications of atherosclerosis, but also to scrutinize for possible side effects.

### Conflicts of interest

AGO has received support for clinical trials from Amgen, AstraZeneca, MSD, Pfizer, Roche, Sanofi-Aventis and consultation fees from AstraZeneca, Merck, Pfizer, Roche.

BA receives grant support from the Swedish Research Council and Heart-Lung Foundation, the Knut and Alice Wallenberg and Leducq Foundations, Karolinska Institutet/Stockholm County Council (ALF) and AstraZeneca (ICMC). Dr Angelin is a minor shareholder in KaroBio AB; no other conflict of interest is reported.

AvE received honoraria from Amgen, Merck-Shape & Dohme and Sanofi-Aventis for lectures or consultancy.

KGP has received honoraria for presentations, advisory board activities or DMC activities by Aegerion, Amgen, Berlin-Chemie, Boehringer Ingelheim, Isis, Merck Sharp & Dohme, Regeneron, Sanofi and Servier. I have received research support by Genzyme, Merck Sharp & Dohme, Regeneron and Sanofi.

MJT, received consultation fees from Amgen, Aegerion, Sanofi and from advisory board of Aegerion.

JC received consultation fees and honoraria from Amgen, Merck, Pfizer and Regeneron.

DMW is the speaker of Amgen, AstraZeneca, Boehringer Ingelheim, Lilly, MSD, Novartis, NovoNordisk, Sanofi-Aventis, and advisor to AstraZeneca, Amgen, Boehringer Ingelheim, MSD, NovoNordisk.

No conflicts of interest were declared for the other authors.

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