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Review

Lipids and lipoproteins in cardiovascular diseases: a classification

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Lipids and lipoproteins, their metabolism, and their transport are essential contributing factors of cardiovascular disease (CVD) as they regulate plasma cholesterol concentration, enhancing cholesterol uptake by macrophages, leading to foam cell formation and ultimately resulting in plaque formation and inflammation. However, lipids and lipoproteins have cardioprotective functions as well, such as preventing oxidation of proatherogenic molecules and downregulating inflammatory proteins.

This review gives an update about our current knowledge of the mechanisms of lipid and lipoprotein subclasses impacting CVD and 'classifies' them on this basis as 'lipids and lipoproteins enhancing CVDs' or 'lipids and lipoproteins with a conditional impact on CVDs' or 'lipids and lipoproteins with no known effect on CVDs'. Low and dysfunctional high-density lipoprotein cholesterol (HDL-C), high low-density lipoprotein cholesterol (LDL-C), and elevated triglycerides (TGs) are the most common targets for reducing CVD risk, and therefore, the mechanisms by which they impact cardiovascular health are emphasised.

Lipids have recently emerged as targeted delivery agents for therapeutic drugs and as imaging probes because they are localised within plaques, facilitating the targeted application of the drugs and probes. Recent studies show that liposomes and HDL-mimicking nanoparticles (HDL-NPs) have promising results in animal models and are now being extended to human trials. This review summarises our knowledge about the impact of lipids and lipoproteins on CVD genesis, progression, diagnosis, and treatment.

Highlights

A new classification of lipids and lipoproteins based on their mechanistic impact on cardiovascular disease (CVD) and not only on their intrinsic function is urgently needed to discover new therapeutic opportunities, focusing on lipid classes impacting CVDs and not individual lipid subclasses.

Lipids and lipoproteins classified under 'lipids and lipoproteins enhancing CVDs' lead to similar outcomes of CVD, and their synergistic effect should be targeted for CVD treatment.

Lipids and lipoproteins are emerging as targeted drug delivery systems for drugs such as urokinase and tanshinone II.

Lipids and lipoproteins are ideal imaging probes for atherosclerotic plaques and as *in vivo* trackers for diagnosis of CVDs.

The impact of lipids and lipoproteins on CVDs

CVDs are the leading causes of mortality worldwide [1]. Lipids and lipoproteins have a major impact on the genesis and progression of CVD by means of their cellular synthesis, transportation, assembly, degradation, oxidation, and plasma concentrations [2,3].

Traditionally, lipids are classified based on their function as 'storage lipids' [e.g., fatty acids (FAs), TGs, and sterols] or 'structural lipids' [e.g., phospholipids (PLs), glycolipids, and ceramides] (Figure 1A) because lipids are essential for various physiological processes supporting biological life. A third group comprises the lipoproteins, subclassified into five types based on their density and size: chylomicrons, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), LDL, and HDL [4] (Table 1).

Blood cholesterol level was identified as the first direct link between circulating lipids and CVD by the Framingham Heart Study (FHS) [5] and the Multiple Risk Factor Intervention Trial (MRFIT) [6]. Increased cholesterol levels are associated with an increased 10-year risk of cardiovascular death from 3.8% to almost 19.6% in men with a pre-existing CVD. This risk increases from 1.7% to 4.9% in patients without CVD but with elevated cholesterol levels [7].

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Follow-up studies on the FHS identified elevated plasma LDL levels as a risk factor for cardiovascular events [8,9]. Patients with atherosclerotic plaques correlate with 45% higher plasma oxidised LDL (oxLDL) concentrations as compared with control subjects [10]. In addition, patients with high total LDL particles of 1.935–3.560 nmol/L have a 3.7 times higher risk of coronary artery calcification than those with lower LDL particles of 620–1530 nmol/L [11].

Follow-up analysis of the FHS in the Coronary Primary Prevention Trial (CPPT) and MRFIT, with a focus on HDL, showed that an increase of 1 mg/dL HDL was associated with a significant coronary heart disease risk reduction of 2% in men (FHS, CPPT, and MRFIT) and 3% in women (FHS) [12]. In the Multi-Ethnic Study of Atherosclerosis, individuals with primary low HDL-C correlated to adjusted chronic heart failure and CVD hazard ratios of 2.25 and 1.93, respectively, compared with individuals with an optimal lipid profile of HDL-C ≥40 mg/dL for men or ≥50 mg/dL for women and TGs and LDL-C <100 mg/dL [13].

Recent epidemiological studies also correlate the ratios of other lipids with CVD risk, demonstrating that increased plasma TG levels are associated with a 14% increase of CVD risk in men and a 37% increase in CVD risk in women, respectively, which is attributed to a higher incidence of myocardial infarction, stroke, and total mortality [14]. Subjects with a TG/HDL ratio >3.5 have an unadjusted hazard risk of CVD mortality of 1.62 (1.43–1.84) [15]. The correlation of the TG/HDL ratio and cardiovascular risk compared with serum HDL levels is more accurate than individual values because it correlates with the negative impact of TGs and the positive impact of HDL on CVD.

To assess CVD risk, specific lipids and lipoprotein concentrations in plasma are measured. Usually, LDL-C, non-HDL-C, and apolipoprotein B (ApoB) concentrations are correlated; hence, only LDL-C is the commonly quantified parameter, but it might underestimate the concentrations of the other lipids and lipoproteins, and therefore, a total lipid analysis accurately assessing CVD risk is highly recommended [16].

Because imbalances of lipids and lipoproteins play a major role in CVD genesis and progression, there is an increasingly evident need to classify them based on their mechanistic impact on CVD in order to find novel CVD prediction and treatment options. Therefore, there is an urgent demand for a new classification of lipids based on their impact on CVD.

Classification of lipids and lipoproteins based on their impact on genesis and progression of CVD

The synergistic effect of lipids with similar mechanistic outcomes is of high relevance for identifying potential therapeutic biomolecules for CVD prevention and treatment. Classification of lipids is necessary based on the mechanistic impact of lipids and lipoproteins on CVD, instead of the traditional classification of lipids that is based on their essential biological functions. Therefore, we suggest classifying lipids and lipoproteins into three novel categories: lipids and lipoproteins (i) enhancing CVDs, (ii) with a conditional impact on CVDs, and (iii) with no known effect on CVDs due to a lack of evidence (Figure 1B). This classification, which is in line with the current CVD treatment guidelines [16], reflects the current mechanistic knowledge of lipids affecting CVD discussing the major lipid classes that are vital for survival but are pathological only under altered concentrations (Figure 1A). In addition, HDL increase [17], LDL decrease [18], and TG decrease [19] are common therapeutic interventions for the prevention and treatment of CVDs and have therefore been studied extensively compared with other lipid subclasses.

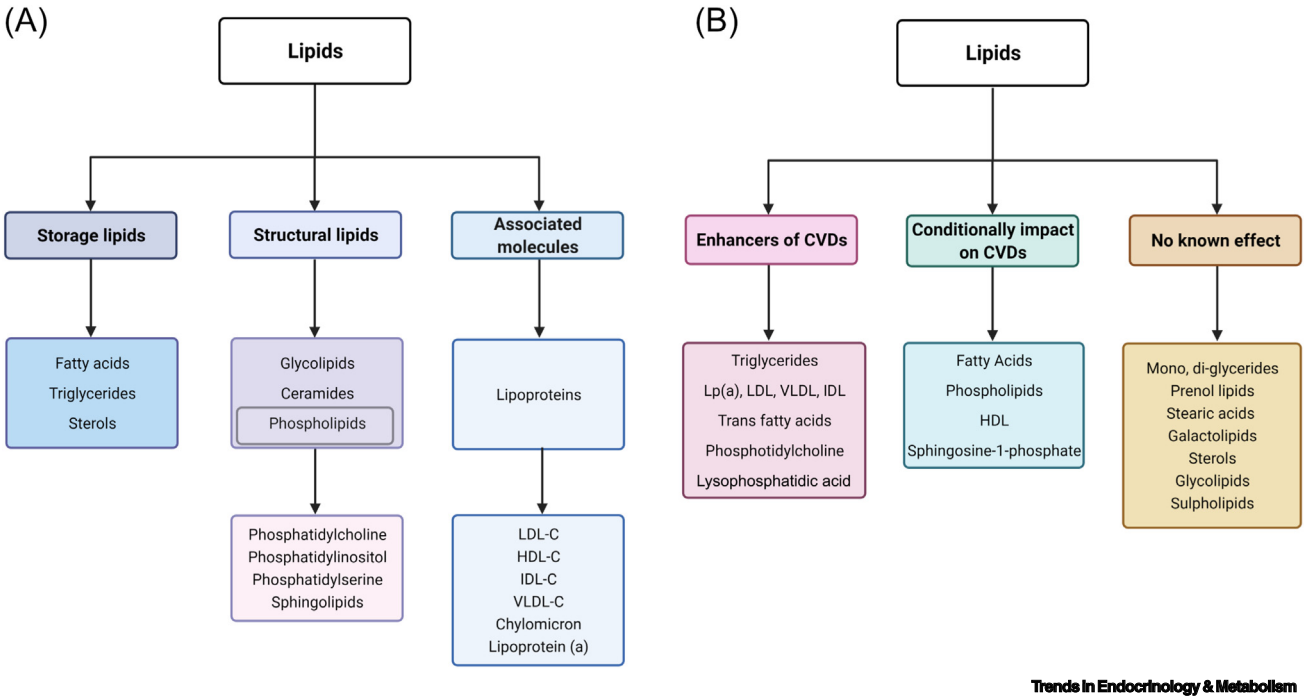


Figure 1. (A) Classification of lipids based on the function of lipids. (B) Classification of lipids based on their role in cardiovascular disease (CVD) development as enhancers, with a conditional impact or with no known effect on CVDs. Abbreviations: HDL-C, high-density lipoprotein cholesterol; IDL-C, intermediate-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol. Images were created using BioRender software (www.biorender.com).

Table 1. Types of lipoproteins and their properties

	Size	Density (g/cm^3)	Apolipoproteins	Surface composition (protein/phospholipids/free cholesterol in %)	Core composition (triacylglycerols/cholesteryl esters in %)	Function
Chylomicrons	75–100	<0.95	A-I, A-II, A-IV, B-48, C-I, C-II, C-III, E	2/7/3	85/3	Transport of dietary exogenous core substances from the intestine to the tissues
Very low-density lipoprotein	30–70	<1.006	B-100, C-I, C-II, C-III, E	8/17/8	55/12	Transport of endogenous core substances from the liver to the tissues
Intermediate-density lipoprotein	25–40	1.006–1.019	B-100, C-I, C-II, C-III, E	17/22/9	22/30	Transport of endogenous core substances from the liver to the tissues
Low-density lipoprotein	17–25	1.019–1.063	B-100	22/17/8	9/44	Transport of endogenous core substances from the liver to the tissues
High-density lipoprotein	2–10	1.063–1.210	A-I, A-II, C-I, C-II, C-III, D, E	50/28/4	6/12	Transport of endogenous cholesterol from the tissues to the liver

Lipids and lipoproteins enhancing CVDs

LDL, VLDL, lipoprotein (a) [Lp(a)], TGs, trans-fatty acids (TFAs), phosphatidylcholine, and lysophosphatidic acid (LPA) have a strong impact on thrombosis and atherosclerosis.

LDL, VLDL, and Lp(a) lipoproteins

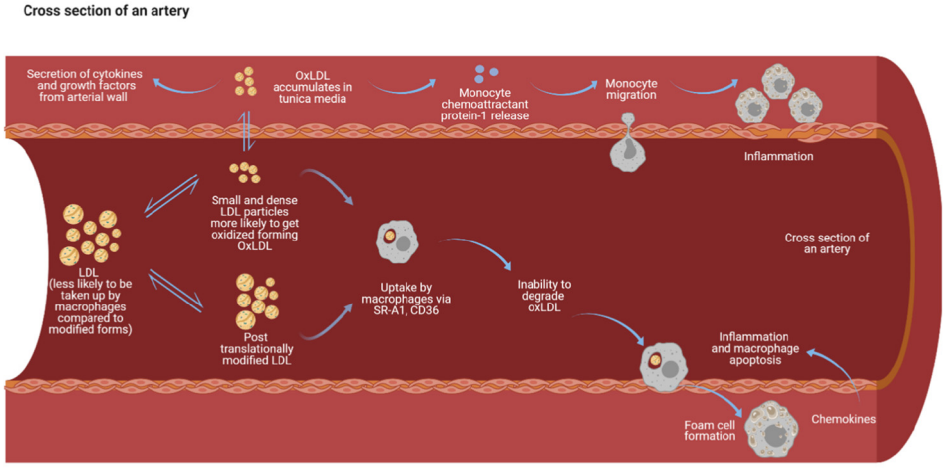
High plasma concentrations of LDL, VLDL, IDL, and Lp(a) enhance CVD by promoting thrombus and plaque formation [20,21]. Native LDL and its post-translationally oxidised form, oxLDL, are the driving factors of the development of atherosclerotic plaques [22]. Macrophages within atherosclerotic lesions possess scavenger receptors (SRs) such as SR-A1, CD36, and 'lectin-like oxLDL receptor-1', which recognise oxidation-specific epitopes, such as ApoB-100 modification, cholesteryl ester modification, and PL oxidation on oxLDL, and facilitate its uptake, but not native LDL [23]. The susceptibility of LDL particles to oxidation depends on specific density: small and dense LDL particles are more likely than large ones to be oxidised. The underlying mechanism of *in vivo* LDL oxidation are, to date, not fully understood; however, it is suggested that LDL is oxidised in the presence of a proinflammatory stage [24]. The macrophage phagocytosed LDL and modified LDL are converted to free cholesterol by the liposomal acid lipase and further to cholesterol esters by acetyl-coenzyme A acetyltransferase, which are converted to free cholesterol in the endoplasmic reticulum and are exported out of the cells by cholesterol transporters [23]. The atherogenicity of modified LDL results from its inability to be degraded by macrophages, thereby leading to foam cell formation [23]. oxLDL and aggregates of LDL intensify atherosclerosis by triggering the secretion of inflammatory cytokines and growth factors from the arterial wall [25]. Furthermore, oxLDL causes the production of asymmetric dimethylarginine by activating S-adenosylmethionine-dependent methyltransferases, which deteriorate the vascular tone by decreasing nitric oxide production through endothelial nitric oxide synthase inhibition [26]. LDL and oxLDL promote CVDs by enhancing foam cell formation, inflammation, and plaque formation (Figure 2A).

VLDL particles promote intercellular leukocyte migration by interaction with VLDL receptors on endothelial cells and fibrin, aiding foam cell formation and thereby promoting inflammation [27]. The presence of Lp(a) in combination with low oxidised lipid levels causes deposition of calcium and phosphate salts, resulting in plaque formation [28]. Lp(a) accelerates the calcification process *in vitro* by increasing the alkaline phosphatase activity and activating the p38 mitogen-activated protein kinase (MAPK), glycogen synthase kinase 3 beta (GSK3β), and Wingless-related integration (Wnt) signal pathways [28].

Triglycerides

Hypertriglyceridaemia leads to reduced activity of hepatic TG lipase and/or glycosylphosphatidylinositol-anchored HDL-binding protein 1, thereby leading to modified lipoprotein uptake by the liver, which increases the concentration of TGs in plasma [29,30]. TG-rich lipoproteins produce inflammatory proteins derived from endothelial cells and macrophages, containing monocyte chemoattractant protein-1, intercellular adhesion molecule-1 (ICAM-1), E-selectins, metalloproteases [31], platelet endothelial cell adhesion molecule-1, and interleukin (IL)-6, which are released through the activity of transcription factors nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and cAMP response element-binding protein [32]. TG-rich lipoproteins migrate into the arterial wall and lead to an accumulation of remnant cholesterol-aiding plaque formation, whereas remnant cholesterol remains in atherosclerotic plagues, triggering foam cell formation [33]. Elevated levels of TGs activate endothelial cells to produce hydroxylated linoleates 9-hydroxyoctadecadienoic acid (HODE) [34] and 13-HODE by lipoprotein lipase (LPL) lipolysis upregulation of reactive oxygen species (ROS), ICAM, and tumour necrosis factor-α (TNFα), promoting inflammatory responses, plaque formation, and

(A)



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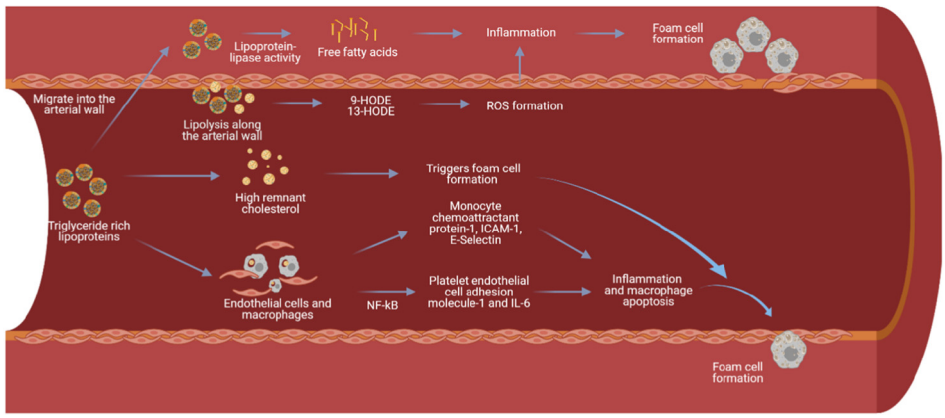

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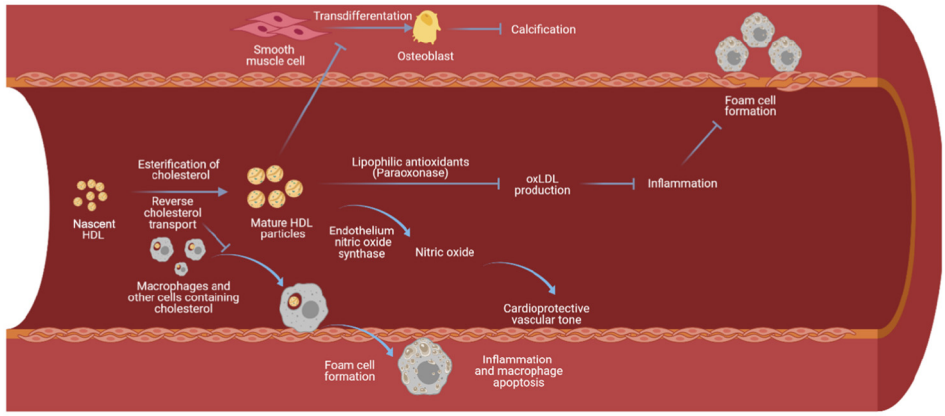

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atherosclerosis [35,36]. 9-HODE and 13-HODE interact with cells through peroxisome proliferator-activated receptors and surface receptors for FAs, enhancing CD36 expression as an atherogenic SR [37]. TGs cause inflammation along the arterial wall by promoting the release of inflammatory molecules such as 9-HODE, 13-HODE, ROS, ICAM, and TNFα (Figure 2B).

Trans-fatty acids

TFAs cause systemic inflammation by increasing IL-6, TNF receptor densities, and monocyte chemoattractant protein-1, causing atherosclerosis [38]. Increased TFA intake causes endothelial dysfunction by increasing soluble ICAM-1, promoting transmigration of leukocytes from blood vessels to tissues [39]. TFAs induce the release of vascular cell adhesion molecule-1 (VCAM-1) by acting as a scaffold for leukocyte migration and promoting endothelial signal transduction, stimulating inflammation [40].

PLs (phosphatidylcholine)

Phosphatidylcholine leads to foam cell formation, attributed to the increase in phosphatidylcholine biosynthesis during free cholesterol uptake by macrophages. The increase in phosphatidylcholine biosynthesis maintains the free cholesterol/phosphatidylcholine ratio in the cell, a prerequisite to cell survival, and is achieved by free cholesterol activating the choline-phosphate cytidylyltransferase enzyme [41]. Phosphatidylcholine is metabolised by intestinal microbes, and the resulting metabolites, choline and betaine, upregulate macrophage SRs. Choline and betaine are metabolised by gut microflora into trimethylamine before it is converted to trimethylamine N-oxide (TMAO) by hepatic flavin monooxygenase. There is a dose-dependent relationship between TMAO levels and atherosclerotic plaque burden. High phosphatidylcholine intake leads to higher TMAO production and contributes to CVD risk in patients with end-stage renal disease [42].

Lysophosphatidic acid

LPA accumulates in atherosclerotic plaques, promoting both platelet activation and thrombogenesis, as a cofactor alongside ICAM-1 or VCAM-1 and collagen type I or III. LPA triggers proinflammatory cytokines, growth factors, and coagulation factors by inducing the expression of early growth response factor-1 [43], promoting mineralisation and osteogenic differentiation of valve interstitial cells by interacting with the LPA receptor-1 and Ras homolog family member A (RhoA)/NF-κB pathways [44]. LPA promotes CVD progression by stimulating tissue factor expression on smooth muscle cells, resulting in macrovascular thrombosis [45]. LPA suppresses autophagy, leading to cardiac dysfunction and hypertrophy by activating the LPA receptor-3 and the protein kinase B/mammalian target of rapamycin signalling pathway [46].

Lipids and lipoproteins with a conditional impact on the genesis and/or progression of CVD

The lipids of this subclass have a profound effect on CVDs; however, the positive impact of this lipid subclass is conditional based on factors such as oxidation, presence of cofactors, and plasma concentration of other lipids. This section characterises these lipids, their impact on CVD, and the conditions that negate their cardioprotective effects.

Figure 2. (A) Role of low-density lipoprotein cholesterol (LDL-C) in cardiovascular disease (CVD) development. LDL-C promotes inflammation, plaque formation, and atherosclerosis by various mechanisms, as shown. (B) Role of triglycerides in CVD development. Triglycerides enhance CVD by inducing inflammation and foam cell formation by the depicted mechanisms. (C) Role of high-density lipoprotein cholesterol (HDL-C) in CVD development. HDL-C transports cholesterol, preventing its accumulation in plaques; acts as an anti-inflammatory molecule; and prevents foam cell formation. Images were created using BioRender software (www.biorender.com).

High-density lipoprotein

Remodelling of nascent HDL particles to mature HDL through the esterification of cholesterol by the enzyme lecithin-cholesterol acyltransferase, as well as by the transfer of additional cellular cholesterol to HDL by the cell surface transporter ATP-binding cassette subfamily G member 1, and the action of SR-B1, is essential for the prevention of atherosclerosis [47,48] (Figure 2C). Lipid-poor pre-β-HDL particles remove abundant cholesterol and oxLDL from macrophages via reverse cholesterol transport, which otherwise transforms them into foam cells having a proatherosclerotic effect [49]. HDL increases the production of the atheroprotective signalling molecule nitric oxide, which is triggered by the Src- and phosphatidylinositol 3-kinase-mediated signalling, along with Akt and MAPK signalling [50]. In addition, HDL inhibits the formation of oxLDL because it contains enzymes with antioxidant-like activities such as paraoxonase and platelet-activating factor acetylhydrolase [51]. HDL prevents plaque formation by inhibiting the transdifferentiation of smooth muscle cells to osteoblast-like cells by reducing the osteogenic activity of inflammatory cytokines IL-1β, IL-6, and minimally oxLDL [52].

High plasma levels of HDL are caused by a mutation on the endothelial type lipase G (LIPG) by a loss-of-function SNP [47]. The function of HDL changes with its composition, such as differences in PL or unsaturated lipid content; percentage of esterified lipids; inflammation or glycation of apolipoprotein A-I (ApoA-I), myeloperoxidase, and paraoxonase; and modification of the serum lipid levels [53]. Oxidised cargo molecules, glycated ApoA-I, and high glucose reduce the anti-inflammatory activity of HDL particles and promote HDL dysfunction [54]. A comparison between the cardiovascular risk-predicting potential of HDL-related analytes such as ApoA-I, HDL subfractions HDL2 and HDL3, and the HDL particle number (HDL-P) results in HDL-P emerging as a better predictor of incident events [55,56]. Thus, HDL functions, such as its ability to promote cholesterol efflux from cells and mediate reverse cholesterol transport, are essential to reduce cardiovascular risk in patients (Figure 2B).

Fatty acids

Polyunsaturated FAs omega-3 and omega-6 are counteractive in terms of inflammation, with omega-3 reducing inflammation and omega-6 nullifying the effect of omega-3 [57]. Omega-3 FAs from fish oils, such as the 'eicosapentaenoic acid' (EPA) and 'docosahexaenoic acid' (DHA) [58], reduce plasma TGs up to 50%, thereby lowering cardiovascular risk [59]. Furthermore, omega-3 FAs improve endothelial function and reduce blood pressure, increase nitric acid production, improve vasodilatory responses, and have antithrombotic effects [60]. However, clinical trials and meta-analyses show that EPA and DHA have little to no protective effect on cardiovascular events [61–63], and omega-3 FAs cause lipid peroxidation in predialysis patients [64]. These contrasting findings are attributed to low dosage, absence of cofactors of EPA and DHA, and not targeting patients with high TG levels.

In addition, a high linoleic acid level, an omega-6 FA, is associated with a 7% risk reduction in cardiovascular events [65]. However, omega-6 FAs are known to promote TNF-mediated endothelial cell injury, leading to endothelial dysfunction, which reverses the anti-inflammatory effect of omega-3 FAs by enhancing cyclooxygenase-2 expression, resulting in a proinflammatory microenvironment [57]. Compilation of different trials did not show a reduction in the risk of CVD events after supplementation with omega-6 [relative risk 0.86 (0.69–1.07)] or an association with cardiovascular mortality [odds ratio 0.89 (0.74–1.06)] [66,67]. Therefore, the impact of omega-6 FAs on inflammation, atherosclerosis, and CVD is currently under scrutiny.

Phospholipids

In vitro studies show phosphatidylserine internalises microvesicles into endothelial cells and regulates coagulation and inflammation by downregulating the production of IL-6, IL-8, prostaglandin E_2, and vascular endothelial growth factor [68]. *In vivo* it has been shown that dietary PLs reduce liver lipid levels by interfering with neutral sterol absorption in the intestinal lumen and downregulating bile acids and cholesterol secretion [69]. *In vivo* post-translational oxidation, leading to the formation of oxidised PLs, enhances uptake by macrophages causing proinflammatory and proatherogenic effects [70].

Sphingolipids

In vivo study shows that the reduction in sphingosine-1-phosphate (S1P)/ceramide levels in the case of myocardial infarction leads to apoptosis [71]. S1P is involved in cell survival and resistance to apoptosis, proliferation, angiogenesis, and cell growth by binding to SIP receptor 1-5 G protein-coupled receptors [72]. S1P promotes leukocyte rolling by interacting with the S1P3 receptor and the Gα-subunit, which mobilises P-selectin, enabling it to assist in monocyte–platelet aggregate formation [73]. Even though S1P promotes cardioprotective cellular mechanisms, they have not yet been explored as translational CVD treatment options.

Lipids with an unknown effect on genesis and progression of CVD

Due to lack of evidence in existing literature proving their impact on CVD risk or events, biologically active lipids such as mono- and diglycerides, prenol lipids, stearic acids, galactolipids, sterols, glycolipids, and sulpholipids are placed in this category, implying that these lipids are essential for biological functions but do not have known impact on the onset or progression of CVD [74].

LDL, VLDL, IDL, Lp(a), and TGs determine the plasma cholesterol concentration, and their imbalance enhances plaque formation; TFAs lead to systemic inflammation; PLs, specifically phosphatidylcholine, enhance the expression of SRs on macrophages; and LPA enhances thrombus formation. Consequently, they have a cumulative impact on the enhancement of CVD development (Figure 3A).

The cardioprotective properties of HDL are conditional and change with the oxidation of cargo molecules and cholesterol efflux capacity. Omega-3 FAs were initially shown to reduce CVDs, but large-scale human trials failed to prove these cardioprotective effects. Similarly, PLs show cardioprotective properties such as reduced cholesterol secretion but cause CVD enhancing outcomes when oxidised. S1P causes cardioprotective effects that are not yet supported by clinical studies. While certain properties of these lipid subclasses suggest an impact on cardiovascular health, this has not yet been confirmed by clinical studies (Figure 3B).

Lipids and lipoproteins: clinical application for prevention and treatment of CV

Treatment of CVD targeting lipids and lipoproteins

Current recommendations to reduce CVD risk first aim to improve plasma profile by adopting a healthy lifestyle to control body weight, alcohol, smoking, and diet [16]. When these recommendations are not enough, medicinal treatments are used to counteract the negative effects of lipids and lipoproteins on CVDs, with the main focus on lowering LDL, TGs, or Lp(a) and raising HDL [75], although they do not focus on the complete lipid profile [16]. Several trials showed that lipids and lipoproteins are targeted by specific diets aimed at decreasing cardiovascular risk [34,76]. Statins are currently the most used drugs in clinics to lower plasma concentration of LDL, and they show a reduced risk of CVD events in clinical trials [75]. Drugs such as ezetimibe and bile acid sequestrants are used in the clinic to reduce LDL by increasing intestinal absorption of

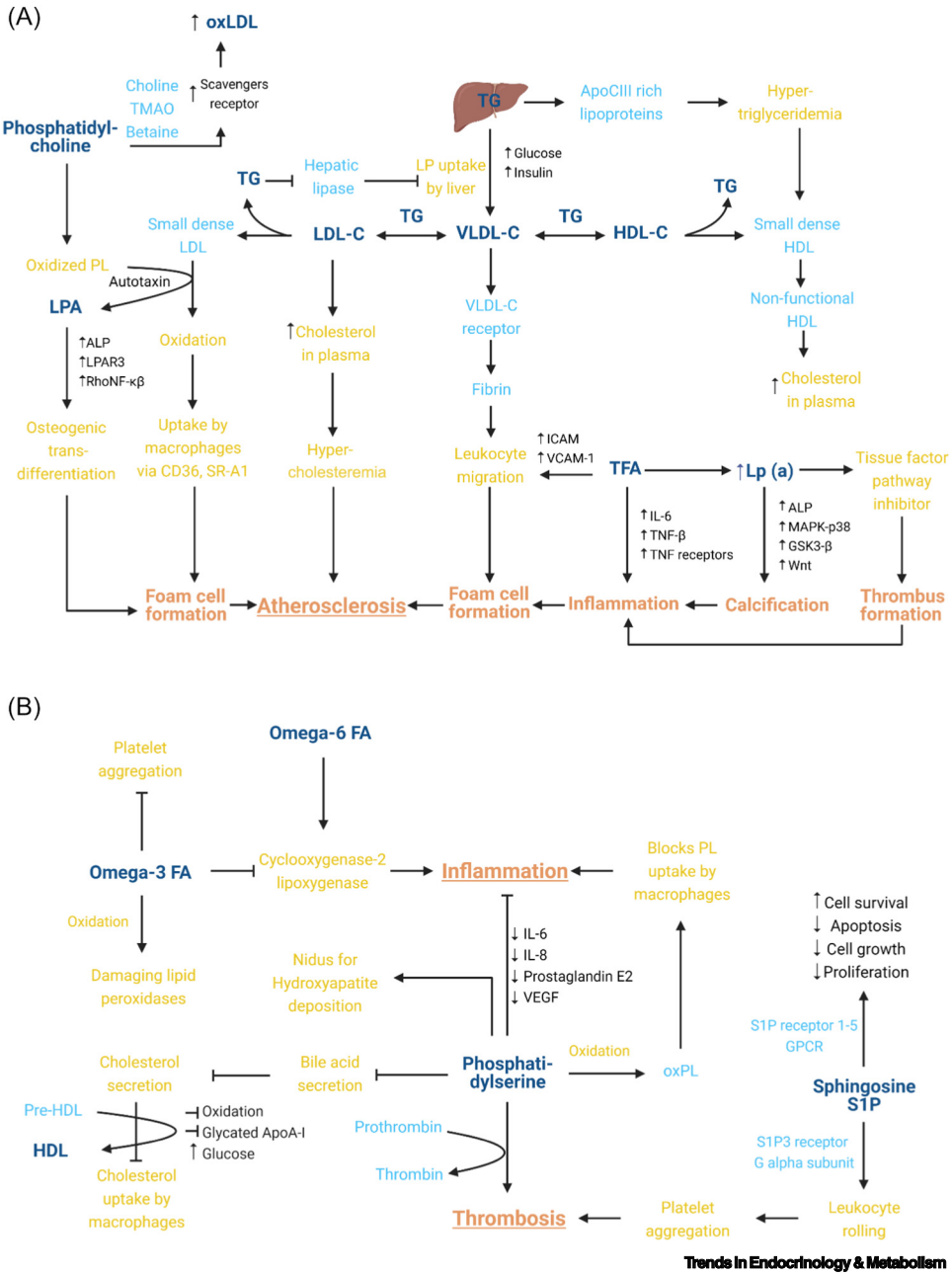

Figure 3. (A) Cumulative impact of lipids and lipoproteins in enhancing cardiovascular diseases (CVDs). Very low-density lipoprotein (VLDL), which is released from the liver, changes to low-density lipoprotein (LDL) or high-density lipoprotein (HDL) based on its triglyceride (TG) concentration. Small, dense LDL and HDL particles are nonfunctional and lead to high plasma cholesterol, which directly leads to atherosclerosis. High TG concentrations block the activity of the hepatic lipase enzyme, which is essential for lipoprotein uptake by the liver. Phosphatidylcholine is metabolised into atherogenic molecules, leading to an increase of scavenger receptors that facilitate the uptake of oxidised LDL (oxLDL). Lysophosphatidic acid (LPA) is formed by autotaxin acting on oxidised phospholipids, leading to osteogenic transdifferentiation of cells and promoting foam cell formation. Dark blue: main lipoproteins and lipids that enhance CVDs; light blue: intermediate molecules that enhance CVDs; yellow: processes that lead to atherosclerosis development; orange: processes with a direct effect on atherosclerosis and CVDs; black: signalling pathways modified by lipids and

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cholesterol and bile acids [16,75]. The coadministration of ezetimibe with statins has shown a reduction in CVD events in patients with CKD and is recommended by the European Society of Cardiology [16]. The serine-protease proprotein convertase subtilisin/kexin type 9 (PCSK9) binds to the LDL receptor, inhibiting its recycling and leading to increased circulating levels of LDL-C. Therefore, approaches using antibodies and siRNA against PCSK9 show an increase in LDL receptors and a subsequent decrease in LDL. Only two fully monoclonal antibodies against PCSK9, evolocumab and alirocumab, are approved to be used in the clinic [16]. A combination of PCSK9 inhibitors with statins and ezetimibe is highly recommended to increase the reduction of LDL-C [16,75]. Studies with siRNA against PCSK9 are still in Phase I, but they have shown promising results regarding lowering LDL-C and CVD events [77]. Experimental drugs to prevent the formation of LDL particles by interfering with the synthesis of ApoB-containing lipoprotein particles are currently under investigation, and their effect on CVD is still not known [16,75].

Studies to increase HDL plasma levels by inhibiting ATP synthase β-chain to avoid HDL endocytosis, or inhibition of cholesteryl ester transfer protein to prevent the transfer of HDL to other lipoproteins, have failed and have even shown an increase in CVD events [75]. Recent approaches to increase HDL plasma concentration are based on the infusion of ApoA-I and HDL mimetics [75]. ApoA-I infusion decreases plaque burden and markers of inflammation in plasma in small-scale clinical trials [78], but the general effect on CVD is still unknown [16]. The first clinical trial with HDL mimetic infusions has not shown an improvement in CVD events [79]. In addition, fibrates reduce major cardiovascular events in different trials, although their effect is less strong than that of the statins, by decreasing the production of VLDL in the liver and enhancing the removal of TGs from blood [80]. Because increased LPL activity decreases TG levels, antisense oligonucleotide and monoclonal antibodies against the LPL inhibitors apolipoprotein C-III and angiopoietin-like 3 proteins have been used in some trials [81,82]. These treatments decrease TG levels in plasma, but the direct benefit for CVD is currently not completely understood [16].

Treatment of CVD using lipids: lipid–drug conjugates

The application of lipids has recently gained increasing interest to improve the properties of drugs and enhance the efficiency of drug delivery systems in CVD [83]. Lipid–drug conjugates include emulsions and micelles within others, but liposomes are the most studied ones. They prevent premature hydrolysis of the drug and enhance penetration into the organs, thanks to their bilayer structure (Figure 4) [83,84].

In vitro and *in vivo* data look promising for the use of liposomes as drug deliverers in CVDs [85–88]. The atheroprotective effects of polyethylene glycol (PEG)-liposomes containing anti-inflammatory drugs such as prednisolone phosphate have been evaluated in *in vivo* studies showing reduced vessel wall inflammation and decreased atherosclerosis progression after drug release [85]. Phosphatidylserine liposomes reduce atherosclerosis progression *in vivo* by mimicking apoptotic cells and targeting B1a cell activation [86]. Cyclic aminoacidic liposomes encapsulating urokinase, a drug that triggers plasminogen synthesis, reduces thrombolysis *in vivo* [87]. The first clinical trial using liposomes encapsulating prednisolone confirmed an

lipoproteins. (B) Cumulative impact of lipids and lipoproteins with a conditional impact on CVDs. Dark blue: main lipoproteins and lipids with conditional impact on CVDs by leading to inflammation, endothelial dysfunction, or thrombosis; light blue: intermediate molecules with conditional impact on CVDs; yellow: processes that will end in cardiovascular events; orange: processes with a direct effect on CVDs; black: signalling pathways modified by lipids and lipoproteins. Images were created using BioRender software (www.biorender.com).

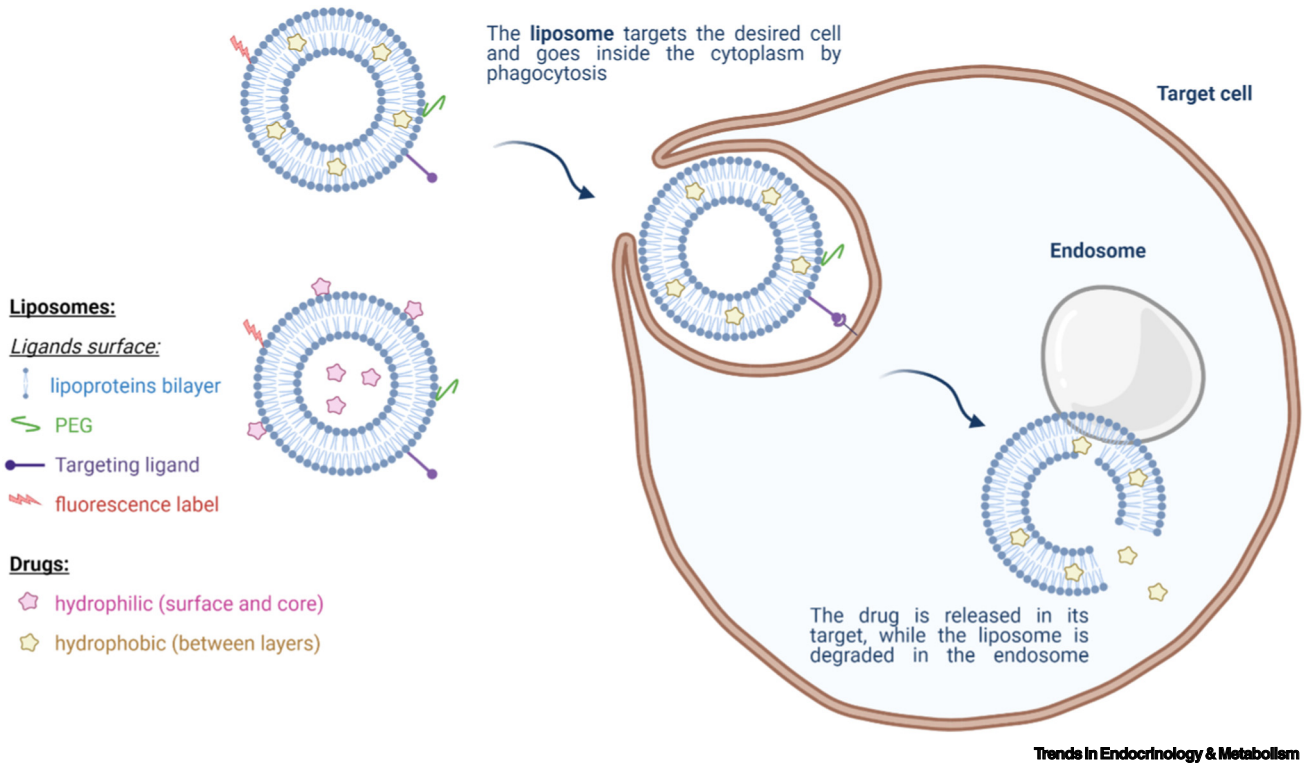

Figure 4. Liposome targeting the desired cell. The liposome can be loaded with hydrophilic (pink) or hydrophobic (yellow) drugs. On the surface, it can be loaded with different molecules such as polyethylene glycol (PEG) [55], fluorescent labelling (red) to track it, and/or with the targeting ligand (purple) to bind to the desired cell. Images were created using BioRender software (www.biorender.com).

improved pharmacokinetic profile compared with the application of the nonencapsulated drug. However, liposomes did not show anti-inflammatory effects in the atherosclerosis plaques [88].

Until now, one liposome formulation, Liprostin (Endovasc, Montgomtery, USA), which encapsulates prostaglandin E_1 to act as a vasodilator, has shown a potential benefit against CVDs, such as restenosis or angioplasty, and is in Phase III of clinical trials [89].

Liposomes are also used as a vaccination vehicle. In atherosclerotic mice vaccinated with derived phosphoglycerol liposomes, which encapsulate ApoB100-derived peptide from oxLDL, the liposomes activate T regulatory cells via complement component 1q; thus, T regulatory cell activation reduces plaque formation and cholesterol concentration in atherosclerotic events [90].

The use of labelled liposomes predicts the accumulation of the drug at target sites [91]. As for the drug delivery system, diagnosis of CVDs with liposomes has been done only in *in vivo* models showing that the combination of IL-10 and fluorescent labelling on liposomes works as an imaging probe for atherosclerotic plaques in apolipoprotein E (ApoE)-deficient mice [92,93].

Lipoprotein as a drug carrier: HDL-NPs targeting macrophages in atherosclerotic events

The lipoproteins LDL, HDL, VLDL, and chylomicrons are used as drug carriers or imaging probes. Because HDL has an intrinsic affinity to macrophages, HDL is suitable for diagnostics and therapy

in CVD. HDL-NPs carry contrast agents for the detection of vulnerable plaques or act as drug transporters to atherosclerotic lesions [58,94–96] (Figure 5).

Targeting atherosclerotic lesions with HDL-NPs loaded with the drug tanshinone II led to an accumulation of HDL-NPs in lesions and reduced the development of atherosclerosis *ex vivo* and *in vivo* [94]. Furthermore, HDL-NPs coated with simvastatin reduce macrophage proliferation in ApoE-deficient mice by inhibiting the mevalonate pathway [95]. Hybrid polymer–HDL-NPs coated with lipids and ApoA-I have biological characteristics similar to HDL, demonstrating the capacity to act both as diagnostic nanoparticles and drug delivery system to target atherosclerosis plaque macrophages *in vivo* [96]. In ApoE-knockout mice, HDL-NPs loaded with statins inhibit atherosclerotic plaque inflammation [97], and HDL-NPs coated with gold localise macrophages in the atherosclerotic lesions [98].

Even with the promising *in vitro*, *ex vivo*, and *in vivo* results, before liposomes are approved to be used in the clinic, their efficacy in clinical trials has to be confirmed because they can trigger an innate immune response. This limits the use of liposomes as drugs in the clinic. Current cardiovascular research most likely will lead to the development of liposomes as drug carriers and/or imaging probes in patients with CVD.

LDL-lowering strategies are the most often used therapy to decrease CVD risk, but their efficacy and tolerance have not been definitively clarified to date. The direct administration of lipids and lipoproteins, the so-called lipid–drug conjugates, to treat or diagnose CVD is increasingly emerging as a possible new treatment strategy. Liposomes and HDL-NPs have been well explored, with promising results in animal models.

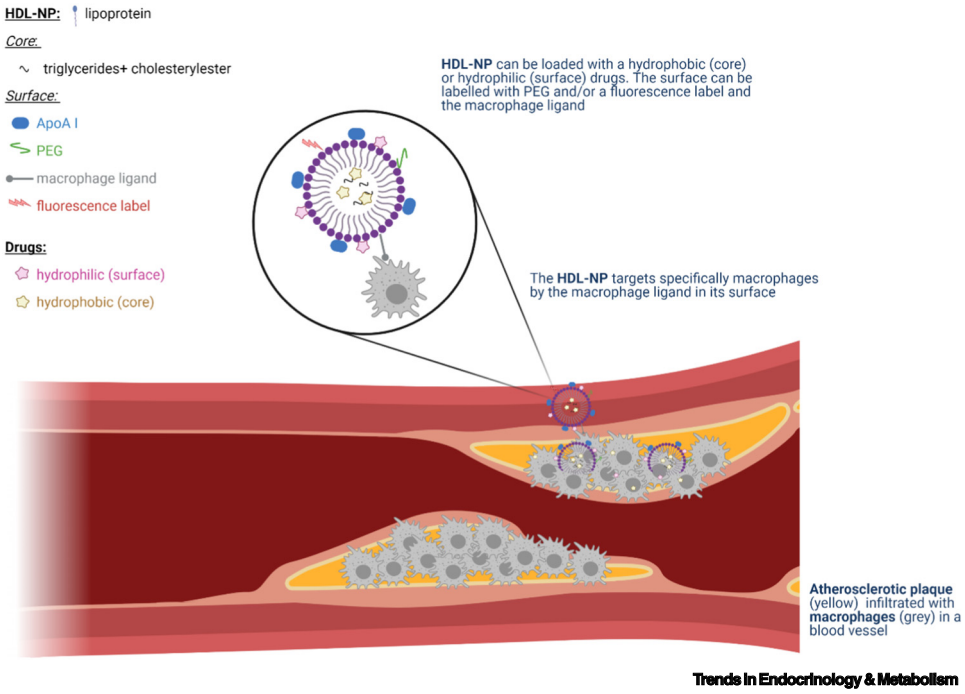

Figure 5. Atherosclerotic plaque infiltrated with macrophages in a blood vessel. Due to the intrinsic affinity of high-density lipoprotein cholesterol (HDL-C) particles for macrophages, HDL-mimicking nanoparticles (HDL-NPs) target especially macrophages, delivering the drug in the atherosclerotic plaque or carrying the contrast agent to track it. Images were created using BioRender software (www.biorender.com).

Concluding remarks

Lipids have essential functions in the progression of CVDs; therefore, a new classification of lipids regarding their effect as enhancers of CVDs, lipids with a conditional impact of CVDs or with no effect on CVDs, is essential.

Lipids that enhance CVDs are LDL, VLDL, Lp(a), TGs, TFAs, phosphatidylcholine, and LPA. Lipids with a conditional impact on CVDs are FAs, HDL, PLs, and sphingolipids. Finally, lipids with no known effect are stearic acids or glycolipids within others, because no clear information is known about them.

Lipoproteins and TGs have an important impact on CVDs and are current targets for treatment. LDL and oxLDL promote CVDs by aggravating plaques, enhancing inflammation and facilitating foam cell formation; meanwhile, HDL has a protective function, transporting cholesterol from plasma to the liver, leading to reduced plaque formation.

As lipids are localised in plaques, these are now being used as targeted delivery agents for therapeutic drugs as well as imaging probes. Liposomes and HDL-NPs have been well explored with promising results in animal models. The analysis of lipids with regard to CVD is vital to understand the various mechanisms through which they propagate (see Outstanding questions).

Outstanding questions

The identification of the intersecting mechanisms by which lipids that enhance CVDs cause genesis and progression of CVDs is important to discover new therapeutic avenues.

There is an insistent need to focus on the impact of lipids with a conditional impact on CVDs and outline clear *in vivo* mechanisms by which these lipids conditionally lead to CVDs.

While LDL, HDL, and TG levels play an important role in mitigating CVD risk, the underlying mechanisms are not completely understood. Moreover, other lipid subclasses need to be explored as potential CVD therapy.

Lipid–drug conjugates are being used to treat or diagnose CVD, but their efficacy and efficiency need to be studied in large-scale randomised trials.

Liposomes and HDL-NPs have been well explored with promising results in animal models but need to be studied in humans.

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Declaration of interests

The authors have no interests to declare.

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