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REVIEW ARTICLE

Current Treatment of Dyslipidemia: Evolving Roles of Non-Statin and Newer Drugs

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Abstract Since their introduction, statin (HMG-CoA reductase inhibitor) drugs have advanced the practice of cardiology to unparalleled levels. Even so, coronary heart disease (CHD) still remains the leading cause of death in developed countries, and is predicted to soon dominate the causes of global mortality and disability as well. The currently available non-statin drugs have had limited success in reversing the burden of heart disease, but new information suggests they have roles in sizeable subpopulations of those affected. In this review, the status of approved non-statin drugs and the significant potential of newer drugs are discussed. Several different ways to raise plasma high-density lipoprotein (HDL) cholesterol (HDL-C) levels have been proposed, but disappointments are now in large part attributed to a preoccupation with HDL quantity, rather than quality, which is more important in cardiovascular (CV) protection. Niacin, an old drug with many antiatherogenic properties, was re-evaluated in two imperfect randomized controlled trials (RCTs), and failed to demonstrate clear effectiveness or safety. Fibrates, also with an attractive antiatherosclerotic profile and classically used for hypertriglyceridemia, lacks evidence-based proof of efficacy, save for a subgroup of diabetic patients with atherogenic dyslipidemia. Omega-3 fatty acids fall into this category as well, even with an impressive epidemiological evidence base. Omega-3 research has been plagued with methodological difficulties yielding tepid, uncertain, and conflicting results; well-designed studies over longer periods of time are needed. Addition of ezetimibe to statin therapy has now been shown to decrease levels of low-density lipoprotein (LDL) cholesterol (LDL-C), accompanied by a modest decrease in the number of CV events, though without any improvement in CV mortality. Importantly, the latest data provide crucial evidence that LDL lowering is central to the management of CV disease. Of drugs that inhibit cholesteryl ester transfer protein (CETP) tested thus far, two have failed and two remain under investigation and may yet prove to be valuable therapeutic agents. Monoclonal antibodies to proprotein convertase subtilisin/kexin type 9, now in phase III trials, lower LDL-C by over 50 % and are most promising. These drugs offer new ability to lower LDL-C in patients in whom statin drug use is, for one reason or another, limited or insufficient. Mipomersen and lomitapide have been approved for use in patients with familial hypercholesterolemia, a more common disease than appreciated. Anti-inflammatory drugs are finally receiving due attention in trials to elucidate potential clinical usefulness. All told, even though statins remain the standard of care, non-statin drugs are poised to assume a new, vital role in managing dyslipidemia.

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Key Points

There is great need for additional lipid-lowering agents beyond statin drugs. Fibrates and niacin may each have niches in subpopulations, and CETP inhibitors are still under investigation; currently, none have sufficient evidence-based support for general use.

In the IMPROVE-IT study, ezetimibe modestly reduced cardiovascular events, simultaneously confirming the "LDL hypothesis." The potent PCSK-9 monoclonal antibody inhibitors, may, like statin drugs, bring about a major change in cardiology practice.

Since inflammation contributes about half the attributable risk for atherosclerosis or thrombosis, current investigations may prove fruitful.

Nature never deceives us; it is always we who deceive ourselves.
–Jean Jacques Rousseau, 1754

1 Introduction

Changes in statin (HMG-CoA reductase inhibitor) allocation according to a new risk calculator included in the 2013 American College of Cardiology/American Heart Association (ACC/AHA) cholesterol and assessment guidelines (referred to as the new ACC/AHA guidelines), the elimination of low-density lipoprotein cholesterol (LDL-C) targets, and a lower threshold for the initiation of statin therapy based upon total cardiovascular (CV) risk are discussed elsewhere [1]. However, at the same time, limitations in the use of statin drugs have received more attention, particularly in view of the greater proportion of the population receiving these drugs.

Residual risk in statin users, ranging from 65 to 70 %, remains a problem that has not been adequately addressed. In high-risk patients, even intense statin therapy may not lower LDL-C to goals in up to 40 % of patients. Adverse reactions and statin intolerance have become better defined as important issues limiting management. Finally, there is greater appreciation of the under-diagnosis and under-treatment of familial hypercholesterolemia (FH). In view of the complex and changing conceptual and therapeutic environment for lowering CV risk, what non-statin drugs have been considered for use, what has been their fate, and what additional agents remain on the horizon to help patients with these predicaments?

2 High-Density Lipoprotein Cholesterol

Observational data from the Framingham Risk Score [Framingham Heart Study (FHS)] first indicated the strong inverse relationship between high-density lipoprotein (HDL) cholesterol (HDL-C) levels and CV risk and outcomes that were independent of LDL-C. The CV-protective actions of HDL, a widely heterogenous mixture of many molecules differing in composition and function, led to the name 'good cholesterol.' HDL-C values vary genetically and in response to medical, environmental, and lifestyle factors, generally rising with physical activity and alcohol intake, and falling with obesity, diabetes mellitus (DM), metabolic syndrome (MetSyn), inflammation, and tobacco use. The prevalence of low HDL-C levels is appreciable, affecting about one-third of the American population, and an HDL-C <1.03 mmol/L (40 mg/dL) in men or <1.29 mmol/L (50 mg/dL) in women is one criterion for the diagnosis of the MetSyn, which currently has a prevalence of nearly 40 % in the US population, 44 % in adults over 50 years, and 50 % in coronary heart disease (CHD) patients. The mean HDL-C level in patients with acute coronary syndrome (ACS) has fallen considerably in the past decade, again reflecting the high background levels of CV risk.

HDL-C is still regarded as a predictor of CV risk and hard CV endpoints, such as myocardial infarction (MI) and ischemic stroke, in the general population and in secondary prevention patients [2–4], with each 0.026 mmol/L (1 mg/dL) rise in HDL-C associated with a ~2–3 % reduction in major adverse cardiovascular events (MACE) [5]. Several properties of HDL particles are believed to contribute to an atheroprotective effect (Table 1). In successfully treated patients receiving statin drugs, the rate of events remained high when HDL-C levels were low [6, 7], leading to the HDL hypothesis: raising HDL-C levels might reduce total and residual CV risk. In order to reverse plaque progression, or cause regression, a meta-analysis using intravascular ultrasonography (IVUS) suggested that both a rise in HDL-C of >7.5 % and a fall in LDL-C <2.26 mmol/L (87.5 mg/dL) are necessary [8]. Importantly, in that study there was no change in the rate of clinical events. In part, the rationale was based upon the assumption that HDL-C values are surrogates for cholesterol efflux out of macrophages within arterial lesions. However, the amount of cholesterol released by peripheral macrophages during reverse cholesterol transport that is added to the total HDL-C pool is small: 3–5 % of the total HDL-C mass [9]. Genetic disorders associated with low HDL-C levels include variations in apolipoprotein (apo) A-I, adenosine triphosphate (ATP)-binding cassette protein A1 (ABCA1), and lecithin: cholesteryl acyltransferase (LCAT, the enzyme that esterifies cholesterol to become the core of mature HDL). In patients

Table 1 Some functions of high-density lipoprotein

Antiatherogenic properties	Protection and support of endothelium, through inhibition of monocyte chemotaxis, adhesion molecule expression, and enhanced NO production. Participation in reverse cholesterol transport, through the ABC transporters and additional mechanisms, is the key atheroprotective function. Peripheral cells cannot metabolize cholesterol, which needs to be carried back to the liver, otherwise it will accumulate. Macrophages have 4 ways of transporting free (unesterified) cholesterol to extracellular HDL: bi-directionally by passive diffusion or facilitated diffusion mediated by SR-BI, or actively and unidirectionally by either membrane lipid translocase ABCA1 to nascent HDL, or ABCG1 to mature HDL [12]. Efficient cholesterol efflux from macrophages impedes atherogenesis, but this process is related more to HDL function than HDL abundance i.e., HDL-C levels
	apoA-I, constituting $\approx$75 % of the protein content of HDL, facilitates randomized controlled trials. HDL particle subfractions are continuously exchanging moieties and interacting with other lipoproteins, lipolytic enzymes (hepatic and endothelial lipases), and transfer proteins (LCAT, phospholipid transfer protein). HDL particles are highly heterogenous and in a constant state of flux; changes in HDL functions follow, according to differing protein and lipid cargos. A major feature of HDL remodeling involves transfer of cholesteryl ester from cholesterol-rich HDL in exchange for triglycerides from apoB-containing cholesterol-acceptor particles, mediated by CETP
	HDL also promotes efflux of oxidized LDL from macrophages, and inhibits atherogenic remnant particle production by maintaining VLDL–triglyceride homeostasis
Antioxidative properties	Prevents LDL oxidation, involving 2 redox active methionine centers in apoA-I, PON-1 and paraoxonase, as well as other component antioxidant enzymes contained in HDL, such as glutathione peroxidase, and platelet-activating factor acetylhydrolase
Antiproliferative actions	Suppresses apoptosis mediated by oxidized LDL, TNF-α, and growth factor deficit
Antithrombotic properties	Lowers platelet activation and aggregation, suppresses thrombin and tissue factor, inhibits factors Va and VIIa and promotion of urokinase-dependent fibrinolysis, and inhibits Factor X. Augments protein S and protein C activities, needed for assembly of the anticoagulant complex on cell surfaces. HDL reproducibly raises activated protein C:protein S anticoagulant activity, consistent with the with the observation that low HDL levels are found in male venous thrombosis patients
Anti-inflammatory properties	Inhibits adhesion molecule expression, lowers neutrophil infiltration into injured endothelium, and reduces macrophage proinflammatory cytokine expression. HDL suppresses Toll-like receptor 4-mediated inflammation in macrophages, which are linked to unidirectional free cholesterol efflux through ABCA1 (to nascent HDL) and ABCG1 (to mature HDL). SR-BI mediates selective uptake of HDL cholesteryl ester, allowing cholesteryl ester cell uptake without endocytic uptake and degradation of the HDL particle itself. In addition, SR-BI enhances the bi-directional flux of free cholesterol between cells and lipoproteins [12]
Vasodilatory properties	Enhances availability of NO and augmentation of prostacyclin synthesis through activation of cyclooxygenase-2. Vascular protection associated with 17β-estradiol is related to enhanced HDL-induced endothelial NO synthase 3 activity to increase NO release
Endothelial support and repair properties	Improves re-endothelialization, reduces intimal hyperplasia, increases endothelial cell proliferation via a cell surface F(1)-ATPase, promotes endothelial cell migration (in part NO-dependent), augments recruitment of circulating endothelial progenitor cells, inhibits endothelial apoptosis, enhances vascular reactivity
Immunomodulatory properties	Participates in the innate immune system through component complement proteins. Teleologically involved with infection and removal of apoptotic cells from inflamed sites, contains more proteins involved with acute-phase response than lipid metabolism; exchange of proteins and lipid molecules between macrophages and HDL may regulate inflammation. Bacterial endotoxin lipopolysaccharide in humans downregulates the transporters ABCA1 and ABCG1, lowering their ability to efflux cholesterol by 73 %, demonstrating a putative proinflammatory role as an acute-phase reactant
Antidiabetic properties	Promotes glucose uptake and fatty acid oxidation, tempering insulin resistance by activating AMP-activated protein kinase in skeletal muscle. Upregulates pancreatic β cell insulin secretion, Inhibits pancreatic β cell apoptosis and promotes β cell survival, increases adiponectin levels
Endocrine functions	HDL transports miRs, carrying them through the blood between organs. These small non-coding molecules regulate intracellular gene expression and post-transcriptionally help maintain cholesterol homeostasis, including cellular cholesterol efflux. For instance, miR-33 suppresses expression of ABCA1 and ABCG1 and decreases HDL biogenesis. A decrease in miR-33 or inhibition increases circulating HDL-C levels

ABC adenosine triphosphate-binding cassette, *ABCA1* ABC protein A1, *ABCG1* ABC transporter G1, *AMP* adenosine monophosphate, *apoA-I* apolipoprotein A-I, *apoB* apolipoprotein B, *CETP* cholesteryl ester transfer protein, *HDL* high-density lipoprotein, *HDL-C* HDL cholesterol, *LCAT* lecithin:cholesteryl acyltransferase, *LDL* low-density lipoprotein, *miR* microRNA, *NO* nitric oxide, *PON-1* paraoxonase-1, *SR-BI* scavenger receptor class B, type 1, *TNF* tumor necrosis factor, *VLDL* very low-density lipoprotein

with these mutations, despite extremely low levels of HDL-C that led investigators to expect profound atherosclerosis, no consistent premature CHD was apparent [10, 11]. Although still incomplete, the lack of genetic evidence argues against the HDL hypothesis.

Of all agents, niacin is the most efficient in raising HDL-C levels, typically ~20 % (range 15–35 %), due to slowed catabolism of apoA-I, without a change in hepatic synthesis [13, 14]. Triglyceride (TG) values fall up to 50 % through decreased fatty acid mobilization from adipose, increased TG metabolism by skeletal muscle, and inhibition of hepatocyte diacylglycerol acyltransferase and TG synthesis, to increase intracellular apoB degradation and lower secretion of very LDL (VLDL) and LDL-C [13]. Falls in LDL-C are about 14 %, with a range of 5–25 %, and decreases in plasma non-HDL-C range from 8 to 23 %. In addition to enlarging LDL and HDL particle sizes, niacin also lowers the LDL particle (LDL-P) number by about 14 %. Finally, niacin lowers levels of lipoprotein a ('little a') [Lp(a)] up to 25 %, an independent, causal, risk factor for CVD and aortic stenosis, typically not adequately lowered by statin drugs.

Preclinical work has shown that niacin has antioxidative and anti-inflammatory properties [15], improves endothelial function independently of lipid effects [16], and stimulates the macrophage hydroxyl-carboxylic acid receptor to suppress proinflammatory cytokine expression. Niacin also retards progression of atherosclerosis in mice and humans, as detected by carotid intima-media thickness (cIMT) measurement and magnetic resonance imaging. In the pre-statin era, the Coronary Drug Project, using immediate-release niacin, reported a reduction in MACE, associated with significant falls in LDL-C levels [17]. Some studies found benefits of extended-release (ER) niacin when added to simvastatin, others showed inconsistent benefit on cIMT or angiographic outcomes as part of combination regimens. In order to determine the effects of niacin alone, the AIM-HIGH (Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High Triglycerides: Impact on Global Health Outcomes) trial randomized 3414 patients with CHD and atherogenic dyslipidemia to either extended-release niacin (1.0–2.0 g/day) and simvastatin, or placebo and simvastatin [18]. At 2 years, the niacin group had increased the median HDL-C value from 0.91 mmol/L (35 mg/dL) to 1.08 mmol/L (42 mg/dL), reduced the TG level from 1.85 mmol/L (164 mg/dL) to 1.38 mmol/L (122 mg/dL), and lowered LDL-C from 1.91 mmol/L (74 mg/dL) to 1.60 mmol/L (62 mg/dL). Due to a lack of efficacy, the trial was stopped after a mean follow-up of 3 years. The dose of simvastatin was adjusted to an LDL-C between 1.03 and 2.07 mmol/L (40–80 mg/dL), with ezetimibe 10 mg added if needed. The study was designed with 85 % power to demonstrate a 25 % reduction in the primary CVD outcome. Unfortunately, the group not receiving niacin experienced a higher HDL-C value than anticipated, LDL-C was not titrated closely, and the intergroup differences for HDL-C and LDL-C were close (about +4 and −5 mg/dL), leaving the study underpowered for the purpose. The discontinuation rate in the niacin group was ~25 % due to flushing. Since there was no difference between groups, the study was declared negative, even though conditions were not ideal.

The HPS2-THRIVE (Heart Protection Study 2-Treatment of HDL to Reduce the Incidence of Vascular Events) trial of 25,673 patients with vascular disease and/or DM compared ER niacin 2 g and laropiprant (a prostaglandin dopamine D_2 receptor-1 antagonist to reduce flushing) and simvastatin 40 mg versus statin alone [19, 20]. If required, ezetimibe 10 mg daily was also used to standardize LDL-C. There was no statistical difference between MACE in the niacin group (13.2 %) and the placebo group (13.7 %), but again lipid level differences between the two arms were small, with the niacin-treated group showing only a 0.155 mmol/L (6 mg/dL) rise in HDL-C levels. In this study, there were ~30 adverse drug events (ADEs)/1000 treated, including myopathy, DM, infections, and a number of hemorrhagic strokes. Even though the trial included some 10,000 Chinese patients, a population known to be intolerant to both niacin and intensive statin therapy, along with the other trial imperfections, niacin as an add-on to statin therapy was declared dead [21, 22], leaving a dark cloud in its wake. One criticism of the two negative niacin trials was they were not relevant to the real-world target patient population, which should be a consideration for lipid-lowering therapies. Nonetheless, niacin may still have a role in patients who cannot achieve lipid goals on maximally tolerated statin therapies.

Curiously, acipimox, a nicotinic acid-derived lipolysis inhibitor with the interesting property of raising leptin levels as it lowers plasma glucose, TG, free fatty acid (FFA), and insulin levels, remains available in the UK and EU [23, 274]. Acipimox decreases the production of TG by the liver and VLDL secretion, which indirectly leads to a modest reduction in LDL-C and increase in HDL-C. Adverse effects shared with niacin are myopathy, gastrointestinal disturbances, liver damage, flushing, pruritus, rash, and palpitation. After HPS2-THRIVE, the European Medicines Agency (EMA) recommended the recall of nicotinic acid and laropiprant across the EU, followed by further instructions by the EMA's Pharmacovigilance Risk Assessment Committee to limit use of acipimox to alternative or adjunct treatment for hypertriglyceridemia unresponsive to lifestyle changes and other agents [24]. The decision was made partly because acipimox is (1) less

potent than nicotinic acid as an agonist of the hydroxycarboxylic acid (nicotinic acid) receptor(2), yet (2) is efficacious in Fredrickson type IV and type IIb hyperlipoproteinemias to prevent non-cardiac complications, and (3) the potential confounding effect of laropiprant in HPS2-THRIVE precludes extrapolation to acipimox.

The niacin experience offers instructive insights into the perils of using time-honored agents for CV prevention, their re-evaluation, and practical limitations while doing so. To many physicians, niacin was ineffective, exposed patients to harm over a long period of time, and illustrates the pitfall of using surrogates in places of hard outcomes in trials. To others, niacin was never adequately tested. The former highlight the potential dangers of using agents clinically on the basis of favorable mechanisms, no matter how numerous or attractive, particularly to bypass other evidence. Despite an impressive profile, and multimechanistic actions expected to enhance reverse cholesterol transport, the largest niacin randomized controlled trial (RCT) showed an unacceptable risk to benefit ratio. Even though the two trials discussed above were defective, reality dictates the inability to continue clinical use and a lasting distaste among stakeholders to invest further. Clearly, assuming that raising HDL-C or lowering LDL-C alone will improve outcomes without clinical trials, using the specific agent of interest is no longer tenable. However, the opposite is also untrue—assuming that all HDL-based interventions are not viable is premature.

3 Cholesteryl Ester Transfer Protein Inhibitors

Cholesteryl ester transfer protein (CETP) catalyzes the exchange of cholesteryl esters from cholesterol-rich HDL to proatherogenic apoB-containing lipoproteins, LDL, intermediate-density lipoproteins (IDLs), and VLDL. Some of the cholesteryl esters transferred to these particles return to the liver to be degraded but also may be recirculated out to peripheral cells. Inhibition of CETP is associated with raised HDL-C levels and lower LDL-C levels and is considered antiatherogenic, although data are mixed. Beneficial effects are believed to be a lower cholesterol uptake and increased macrophage cholesterol efflux in plaques [25]. Additional support comes from the link between CETP loss-of-function genotypes with lower coronary risk in communities [26]. Four agents have been of interest: two that failed and two that remain under investigation. The first, torcetrapib, was able to raise HDL-C values 72 % and lower LDL-C 25 %, but this was accompanied by a 25 % rise in MACE and 58 % rise in mortality in the ILLUMINATE (Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events) study [27]. Off-target actions of this agent unrelated to CETP inhibition were caused by a small rise in aldosterone and cortisol secretion, resulting in hypertension, impaired endothelial function, and hypokalemia—without a decrease in atheroma volume [28, 29]. Dalcetrapib, a weaker CETP, which binds CETP differently than torcetrapib or anacetrapib (see below), raised HDL-C 30 % without much change in LDL-C. Using non-invasive multimodality imaging in dal-PLAQUE, insufficient improvement in plaque progression and inflammation resulted in termination of development due to futility [30, 31].

Anacetrapib binds to CETP with a 1:1 stoichiometry and completely inhibits cholesteryl ester transfers, efficiently increasing cholesterol efflux from foam cells. The DEFINE (Determining the Efficacy and Tolerability of CETP Inhibition with anacetrapib) trial of statin-treated patients showed a rise in HDL-C of 138 %, a fall in LDL-C of 40 %, and a 36 % drop in levels of Lp(a), with no rises in blood pressure [32]. Through 76 weeks, there was no significant ADE leading to drug discontinuance, including changes in blood pressure, electrolyte, or aldosterone levels with anacetrapib as compared with placebo. Of non-serious ADEs, headache has been the most frequent, and all have been transient. REVEAL (Randomized EValuation of the Effects of Anacetrapib Through Lipid-modification), a phase II study currently underway is administering anacetrapib 100 mg versus placebo to 30,624 patients with CVD or DM, for a composite outcome of CHD mortality, MI, or coronary revascularization, and is due to be completed in 2017 [33]. Evatracepib (a fourth CETP inhibitor) monotherapy produced a dose-dependent rise in HDL-C from 53.6 to 128.8 %, and a fall in LDL-C levels in the range of 13.6–35.9 %. When used in statin-treated patients in a small dose, LDL-C fell by ~50 %. When a submaximal dose was added to statin therapy, LDL-C fell about 50 % [34]. No signs of serious ADEs were noted. In ACCELERATE (A Study of Evacetrapib in High-Risk Vascular Disease), some 30,000 patients with high-risk CVD are being studied for the time to first occurrence of the composite endpoint of CV mortality, MI, stroke, coronary revascularization, or hospitalization for unstable angina; results are due in 2016 [35].

Piecing together HDL data has transformed the HDL hypothesis into the HDL function hypothesis: the benefits accruing from HDL properties vary according to its healthy status and function, the most important of which is reverse cholesterol transport [36]. An early and critical event in reverse cholesterol transport is the interaction of nascent and mature HDL, the cell membrane, and transporters ABCA1 and ABCG1 to accept cholesterol on its way back to the liver, a process that depends upon the integrity of apoA-I. HDL from healthy individuals likely preserves this and most other functions, such as prevention of LDL oxidation. As the CV risk burden rises, whether by low-grade inflammation in the obese, oxidative and glycemic stress in

DM, established atherosclerosis, immune stress in autoimmune diseases, age or multiple chronic diseases, the ability of HDL to perform physiological functions diminishes. ApoA-I or other HDL components may themselves be oxidized or modified, even becoming proinflammatory. Such HDL is referred to as 'dysfunctional,' wherein the ability of HDL to promote cholesterol efflux may vary widely, even though levels of HDL-C and apoA-I are similar. For instance, one mechanism through which HDL may become dysfunctional is mediated by enhanced myeloperoxidase (MPO) expression, upregulated by macrophage cytokines during inflammation. MPO generates hypochlorous acid which converts tyrosine to 3-chlorotyrosine and also oxidizes methionine moieties in apoA-I, impairing the ability of ABCA1 to transport excess cholesterol. Quantification of site-specific oxidation of apoA-I and ABCA1 cholesterol efflux capacity shows that HDL from patients with ACS and CHD is less able to accept cholesterol than from controls [37].

A means of measuring macrophage reverse cholesterol transport in vivo is necessary to fully explore and understand this concept and carry it forward. Among the proposed methods, cholesterol acceptor activity of human apoB-depleted serum in cultured macrophages has been used as a surrogate index of HDL function [38, 39]. Using this method, Khera et al. [40] found that cholesterol acceptor activity was not strongly correlated with HDL-C in CHD patients, and cholesterol acceptor activity was an inverse predictor of CHD independently of HDL-C. The explosion in HDL information and need for re-examination of HDL biology in the light of HDL function was the theme of a recent issue of *Cardiovascular Research* [41]. The availability of a reliable test of HDL function that correlates inversely with CHD outcomes would add a welcome dimension to advance HDL research.

Other HDL-based therapies have been used to augment reverse cholesterol transport. Use of apoA-I upregulators, such as RVX208 has been investigated using coronary atheroma volume as an endpoint. This agent uniquely affected the *apoA-I* gene through transcription machinery. Although studies were positive in primates, in humans there was no reduction of IVUS-imaged plaque size [42]. Another approach has been to use recombinant apoA-I or the molecule derived from human plasma and re-combine it with phospholipids to optimize pharmacokinetics. The infused product is envisioned to accept cholesterol from tissues and lower the volume, cholesterol content, and instability of plaques. Two positive IVUS studies in patients with CHD using wild-type apoA-I and apoA-I_{Milano} recombinant particles were reported, the latter finding a decrease in atheroma volume by intravascular ultrasound in ACS patients [43, 44]. ACS patients receiving reinfusion of delipidated HDL also showed some regression using IVUS [45]. Infusion of CSL-112 (Cerenis), human apoA-I reconstituted with phosphatidylcholine, robustly promotes cholesterol efflux from macrophages, and leads to increased levels of pre-β1-HDL when added to serum of volunteers [46]. These data are exciting, since large and rapid elevations of apoA-I (≥2-fold) can remove over 50 % of plaque cholesterol in a week, much faster than statins, fibrates, or niacin. Results are not uniform, evidenced by the failure of CER-100, another pre-β-HDL-mimick in a phase II trial that did not reduce atheroma volume, as assessed by IVUS and quantitative coronary angiography in CHI SQUARE (Can HDL Infusions Significantly Quicken Atherosclerosis Regression) in 500 ACS patients [47].

4 Fibrates and Triglyceride Reduction as a Target

The nuclear receptor family, ligand-activated peroxisome proliferator-activated receptors (PPARs) regulates aspects of intermediary metabolism, including adipocyte characteristics, glucose transportation and removal, insulin sensitivity, storage and catabolism of fatty acids, and inflammation. The net effects of activation are a function of the prior substrate, PPAR isoform, ligand, and tissue. Of the three isoforms, PPAR-α, expressed in the liver, skeletal muscle, kidney, and T cells, is primarily concerned with fatty acid oxidation and TG-rich lipoprotein (TRL) metabolism, whereas PPAR-γ governs insulin sensitivity, adipose cell maturation, and lipid storage [48]. Both PPARs are also expressed in macrophages, smooth muscle, endothelium, and the heart. Ligands for PPAR-α include fibrates (relatively weak), omega-3 polyunsaturated long-chain fatty acids (n-3 PUFA), and leukotriene B_4 [49, 50]. For PPAR-γ, ligands include FFAs, some eicosanoids, prostaglandins, and thiazolidinediones [50, 51].

Clinically, the main lipid effect of fibrates is a decrease in plasma TG levels, with small increases in HDL-C levels. The fall in TGs is due to higher uptake and hepatic oxidation of fatty acids, decreased hepatic production of apoC-III, and enhanced muscle expression of lipoprotein lipase (LPL) leading to enhanced TG clearance from lipoproteins [52]. Decreased VLDL synthesis is due to enhanced cellular fatty acid uptake and oxidation, together with lower FFA and TG production [53]. Hepatocyte production of apoA-I raises HDL-C, and ABCA1 and scavenger receptor class B, type 1 (SR-BI) are upregulated to promote reverse cholesterol transport. Fibrates also lower the expression of proinflammatory cytokines and inhibit proliferation and migration of vascular smooth muscle cells.

Fibrates increase affinity of LDL for the hepatic LDL receptor (LDL-R), and may lower LDL-C levels modestly. These pleiotropic actions of fibrates decrease plasma TG

Table 2 Outcomes of dyslipidemic groups in major fibrate randomized controlled trials

Trial (year, duration)	Subjects/prevalence of subgroup/other	Treatment (vs. control)	Subgroup criteria	MACE fibrate/control	RRR (95 % CI)	*P* value
HHS [55, 56] (1988, 5 years)	*n* = 4081 M/14 %/ primary prevention	Gemfibrozil	TG >204 mg/dL, LDL-C/ HDL-C >5.0, HDL-C ≤42 mg/dL	8/23 per 1000 patient-years	0.33 (0.16–0.77)	0.067
BIP [58] (2000, 6.3 years)	*n* = 3090 M and W/11 %/secondary prevention	Bezafibrate (resin used by some)	TG >200 mg/dL, HDL-C ≤35 mg/dL	13.0 %/ 22.3 %	0.58 (0.37–0.94	0.05
FIELD [64] (2005, 5 years)	*n* = 9795 M and W/21 %/prior CHD diagnosis in 22 %	Fenofibrate monotherapy (statin used by some)	TG ≥204 mg/dL, HDL-C <40 mg/dL (M), HDL-C <50 mg/dL (W)	13.5 %/ 17.8 %	0.73 (0.58–0.91)	0.053
ACCORD [65] (2010, 4.7 years)	*n* = 5518 M and W/17 %/prior CV events in 37 %	Fenofibrate + simvastatin vs. simvastatin	TG ≥204 mg/dL, HDL-C ≤34 mg/dL	12.3 %/ 17.3 %	0.69 (0.49–0.97)	0.057

Data for VA-HIT are omitted because the data were not comparable

CHD coronary heart disease, *CV* cardiovascular, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *M* men, *MACE* major adverse cardiovascular events, *n* patient number in original trial, *RRR* relative risk reduction, *TG* triglyceride, *W* women

and small dense LDL (sdLDL) particle levels, raise HDL-C levels, improve endothelial function, prevent myocardial ischemic injury, and are anti-inflammatory and atheroprotective. Endothelial function is enhanced due to increased expression and activity of nitric oxide synthase [54], inhibition of endothelin-1 expression, and in macrovascular endothelium, by interruption of signaling in the activator protein-1 and nuclear factor (NF)-κB pathways to quell inflammation. Non-lipid actions include promotion of fibrinolysis and a fall in uric acid and fibrinogen.

In hypertriglyceridemic patients, the average changes in lipid levels produced by fibrates are a (1) fall in TG levels of 20–50 % (to a greater extent when baseline levels are high); (2) rise in HDL-C of 9 % (range 10–20 %); (3) decrease in LDL-C of 8 % (range −5 to +20 %); and (4) drop in non-HDL-C (range 5–19 %).

Although several randomized trials have been conducted to delineate the clinical benefits of fibrates (Table 2), their precise roles in therapy remain unclear. The HHS (Helsinki Heart Study) was a 5-year, double-blind study in 4081 asymptomatic men with non-HDL-C ≥5.2 mmol/L (200 mg/dL) randomized to gemfibrozil 600 mg twice daily or placebo [55]. There was a reduction of 34 % in the incidence of CHD, but no difference in all-cause mortality was observed. An open-label, 18-year follow-up found a 23 % reduction in mortality. Moreover, patients with body mass index (BMI) and TG levels in the highest tertiles had a 71 % lower relative risk (RR) of CHD mortality, 33 % lower risk of all-cause mortality, and 36 % lower cancer-associated mortality [56].

VA-HIT (Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial) randomized 2531 men with CHD, HDL-C ≤1.0 mmol/L (40 mg/dL), and LDL-C ≤3.6 mmol/L (140 mg/dL) to either gemfibrozil 1200 mg/day or placebo, followed for 5.1 years. There was a reduction in RR of a MACE of 22 %, and a 24 % reduction in the combined outcome of death from CHD, non-fatal MI, and stroke in the treated arm without a change in LDL-C [57].

The BIP (Bezafibrate Infarction Prevention) study was a double-blind trial in 3090 patients with prior MI or stable ischemic heart disease randomized to receive either bezafibrate 400 mg daily or placebo, followed for 6.2 years [58]. A primary endpoint of reduction in fatal and non-fatal MIs or sudden death was not attained. A post hoc analysis of a subgroup with baseline TGs ≥2.26 mmol/L (200 mg/dL) found the cumulative probability of attaining the primary endpoint was 39.5 %. Bezafibrate is not available in the USA and produces a higher HDL-C and expresses additional PPAR-γ properties than fenofibrate. These include reducing glycosylated hemoglobin (HbA_{1c}) values, impeding the progression of impaired glucose tolerance to DM, and increasing adiponectin levels.

In diabetic patients, fibrates promote a change from sdLDL to larger particles with higher buoyancy that have greater affinity for the LDL-R [59]. Fenofibrate, not plagued with the safety issues associated with gemfibrozil [fenofibrate does not impair glucuronidation or organic anion transporting polypeptide 2 (OATP-C or OATP1B1) involved in statin metabolism], enabled improved lipid control in combination with atorvastatin in patients with mixed dyslipidemia [60]. Diabetic patients have inordinately higher risk at each level of LDL-C and therefore it appeared possible that statins and fibrates would have the potential to lower LDL-C and TG values while raising HDL-C, suppressing inflammation, and raising adiponectin levels and insulin sensitivity [61, 62], an impression supported by several studies [60, 62, 63].

The FIELD (Fenofibrate Intervention and Event Lowering in Diabetes) trial randomized 9795 participants with DM and TC < 6.5 mmol/L (251.3 mg/dL), randomized to either fenofibrate 200 mg or placebo [64]. Use of statins was permitted, though not mandatory. A primary endpoint of non-fatal MI and CHD mortality was not significantly changed after 5 years, although a microvascular benefit (albuminuria/retinopathy/neuropathy) was substantial.

The ACCORD-Lipid (Action to Control Cardiovascular Risk in Diabetes–Lipid) study was designed to see if fenofibrate and statins improved CV outcomes when given to diabetic patients [65]. Despite the expected fall in TG values in the treated group, there was no significant improvement in the primary outcome, a composite of the first occurrence of non-fatal MI, non-fatal stroke, or CV mortality. However, in a subgroup of participants with baseline values of TGs ≥2.3 mmol/L (204 mg/dL) and HDL-C ≤0.8 mmol/L (34 mg/dL), patients in the fenofibrate arm showed a 31 % reduction in MACE compared to the simvastatin group. About 17 % of the patients in ACCORD-Lipid were good candidates for fibrate therapy, in effect diluting the total results. Similar findings have been reported from post hoc subgroup analyses performed from the BIP, HHS, and FIELD studies. In addition, they support Adult Treatment Panel (ATP) III clinical guidelines in that fibrates should be reserved for statin-treated patients with high TG and low HDL-C levels, although the definitions differ, and are consistent with the view that TRLs may be responsible for residual risk in diabetic patients. A large body of literature now supports the belief that atherogenic dyslipidemia, highly prevalent in patients with DM or MetSyn, but also found in seemingly healthy individuals, contributes significantly to CV risk [66].

Additional data report a reduction in CV risk in patients with atherogenic dyslipidemia treated with statins and fibrates. One meta-analysis of RCTs concluded that fibrates reduced RR for CV events by 10 %, RR for coronary events by 13 %, RR for non-fatal coronary events by 19 %, and revascularization by 12 %, unaccompanied by a fall in cardiac or all-cause mortality and with no effect on stroke [67]. Another meta-analysis found an odds ratio of 0.85 for MACE in fibrate-treated subgroups whose TG level was ≥5.28 mmol/L (204 mg/dL) and HDL-C was ≤0.879 mmol/L (34 mg/dL) [68]; still another confirmed the higher RR correlated with elevated TG level among participants [69, 70]. A recent addition to the literature has since shown that statin treatment in patients with atherogenic dyslipidemia is in fact associated with high residual risk, manifested by a greater incidence of transient ischemic attack and stroke [66]. The data regarding use of fibrates in statin-treated diabetic patients with atherogenic dyslipidemia has been reviewed, and its use has been termed "essential" in reducing residual risk [71].

Since dyslipidemia and insulin resistance are linked to CVD in patients with DM, dual PPAR agonists (PPAR-α/γ) theoretically have the potential to improve macrovascular outcomes. However, a phase III trial, AleCardio, using the balanced PPAR-α/γ agonist aleglitazar in patients with ACS, has been halted prematurely due to futility in reaching endpoints and higher rates of fractures, heart failure, gastrointestinal bleeding, and reversible renal failure [72].

In the absence of a trial specifically designed to examine the effect of the combination of statins and fibrates in the DM subpopulation with high TG and low HDL-C levels on MACE and mortality, and supported by its Advisory Panel [73], the US FDA required one manufacturer of fenofibrate to proceed with such a study [74]. Although postmarketing evidence showed prescriptions for this agent were predominantly (and appropriately) in patients with low HDL and high TGs [73], the pattern and extent of fibrate utilization vis-a-vis the strength of the evidence has been questioned [75, 76].

Investigations of dual PPAR-α/γ, PPAR-δ, and stronger PPAR-α agonists for efficacy and safety are ongoing. Presently, the drug of choice for treating diabetic patients with atherogenic dyslipidemia is a statin. In patients who fail to reach targets, or have evidence of increased residual risk even though they have reached LDL-C targets, fibrates may be considered. This topic is but a part of the evolution of the vexing clinical conundrum of how HDL, TGs, and related disorders can be managed in the current practice environment [77].

5 Plasma Triglyceride Levels and Risk

The role of TGs as an independent risk factor for CVD has long been debated and remains unsettled, but has drawn greater interest recently [78–84]. Despite past clinical evidence that TGs alone contribute to CV risk [79], this relationship is attenuated sharply after adjustments for covariates [82], but is relevant because TG values in the population have increased, roughly in proportion to the collective BMI. TRLs, secreted in the liver and intestine, have been regarded as a link between TGs and raised risk [80–82]. Fasting TRL is composed of VLDL, IDL, and their remnants, but postprandial TRL also includes chylomicron remnants. TRLs initiate inflammation, activate endothelial cells, and lead to atherosclerosis. This process may be apoC-III-dependent, mediated by NF-κB and a specific protein kinase C pathway that initiates adhesion molecule expression and monocyte recruitment [85]. Hydrolysis of TRL by LPL may amplify inflammation by liberating non-esterified fatty acids, itself sufficient to cause Toll-like receptor expression and signaling mediated

by NF-κB and mitogen-activated kinase to decrease insulin sensitivity.

The *APOC3* gene encodes for TRL-associated apoC-III, a small protein that is normally a component of VLDL. ApoC-III decreases hepatic uptake of TRL, and inhibits both hepatic lipase and LPL, thereby increasing the plasma level of atherogenic TRL, including VLDL and chylomicrons. ApoC-III also promotes the assembly and release of TG-rich VLDL in the liver, and inhibition of apoC-III or loss-of-function or missense mutations in *APOC3* result in low TG levels. Conversely, rises in apoC-III are associated with hypertriglyceridemia. Gain-in-function mutations may produce non-alcoholic fatty liver disease. The TG level may therefore serve as a marker for both TRL and apoC-III [80]. In patients with TG values >4.52 mmol/L (400 mg/dL), the amount of cholesterol carried by TRL may exceed the amount in LDL-C or HDL-C [79]. Since atherogenic TRL remnants are not reported in the standard lipid profile, they can be a significant source of unrecognized residual risk in patients with obesity, DM, MetSyn, and chronic renal disease [82].

In 1998, a clinical association between TG levels and CVD was made in Copenhagen [86], and reports continue through the present, as TG levels are found to predict outcomes in DM patients with ACS [87]. Recent data tend to lend greater credence to the PROVE IT-TIMI (Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis In Myocardial Infarction) 22 trial, in which on-treatment TG levels >1.69 mmol/L (150 mg/dL) were independently associated with MACE post-ACS [88, 89]. In statin-treated ACS patients, fasting TG levels are strongly associated with both short- and long-term risk of MACE, independent of LDL, potentially raising risk by as much as 60 % for recurrent events [90]. Meta-regression analysis of clinical trial data also support clinical and genetic evidence (see below) that TG levels are predictive of CV events in primary prevention populations [91].

Plasma TG levels are heritable and may influence CV risk. A study by a National Heart, Lung, and Blood Institute (NHLBI) working group [92] sequenced 18,666 genes from 3734 individuals of European or African ancestry, and identified three loss-of-function mutations and one missense mutation in the *APOC3* gene. In heterozygous carriers of at least one mutation, TG levels were 39 % lower than in non-carriers, and the risk of CHD was 40 % lower. One proposed mechanism of atheroprotection was diminished life-long exposure to lower concentrations of atherogenic remnants. These observations were corroborated by a second paper from 75,725 participants in two Danish studies from the University of Copenhagen [93]. Individuals with TG levels <1 mmol/L (90 mg/dL) had a significantly lower incidence of CVD than those with TG ≥4.00 mmol/L (350 mg/dL). Participants with heterozygous loss-of-function *APOC3* mutations had a significant reduction of ~39 % in non-fasting TG levels, corresponding to falls in rates of ischemic vascular disease and ischemic heart disease of 41 and 36 %, respectively. Limitations in both studies are the number of changes in biomarkers, including low HDL-C values and low LDL-C levels that are associated with *APOC3* variations, although the HDL effects are clearly the most important. Despite the limitations, these data strengthen the view that TG levels do contribute to CV risk, cast new light upon the role of TRL and remnant cholesterol during atherogenesis [94], and suggest *APOC3* inhibition is worthy of further exploration. Clinically, the immediate translation of these results requires caution. Simultaneously, opportunities to reduce TG values in patients through apoC-III inhibition, n-3 PUFA, and *LPL* gene replacement appear more attractive. The development of antisense oligonucleotides that target apoC-III and TRL is a novel and welcome approach to the treatment of dyslipidemia. New agents in this class might find particular use in treating atherogenic dyslipidemia and to lower residual risk. One antisense drug in development which showed an acceptable safety profile and tolerability is ISIS-APOCIII$_{Rx}$, for patients with severe elevations in TG levels [95], which is capable of lowering TG and apoC-III levels by 44 and 78 %, respectively.

The advice given by guidelines on the management of hypertriglyceridemia, both mild-to-moderate [TGs higher than 1.69 mmol/L (150 mg/dL) but lower than 5.65 mmol/L (500 mg/dL)] and high (TGs >5.65 mmol/L), varies, particularly with respect to the former. Discordance between LDL-C values measured in the standard lipid profile and other indices of atherogenecity, such as LDL-P and apoB, widens as TG values increase between these two levels. On the basis of epidemiological, mechanistic, animal, and human clinical evidence, the AHA defined the optimum fasting level of TGs, <1.13 mmol/L (100 mg/dL), as an index of metabolic health [96]. The European Atherosclerosis Society concluded that both TRL and low HDL-C levels raised atherogenicity [97]. Intensive lifestyle change is generally favored with the option of adding n-3 PUFA, reserving drugs for TG levels >500 mg/dL [96]. Treatment for high TG levels to prevent pancreatitis is uniformly advised. Some guidelines direct attention to moderate elevations in TG during therapy [97–102], whereas others do not [96, 103, 104].

6 Omega-3 Polyunsaturated Long-Chain Fatty Acids

The 1936 report by Rabinowitch [105] was among the earliest publications describing the infrequency of CHD among the Canadian Inuit people, followed by a

Table 3 Potentially favorable properties of omega-3 polyunsaturated long-chain fatty acids

Lower blood pressure [107]
Decrease resting heart rate [108] and increase heart rate variability to lower arrhythmias [109]
Lower risk of sudden cardiac death in primary and secondary prevention patients [110]
Increase myocardial filling, improve function, and lower myocardial oxygen demand [109]
Lower ischemia-induced resting membrane depolarization [110]
Reduce risk of ischemia-related ventricular fibrillation [111–113]
High omega-3 levels retard development of heart failure and increase survival [114]
Incorporation in membranes increases fluidity [111]
Potent effects upon ion channels and signaling proteins [111, 112]
Modulate downstream metabolites that control inflammation, lower CRP [112, 113]
Lower cellular oxidative stress [115–117]
Precursors of prostaglandins, leukotrienes, and resolvins [111, 116]
Regulate gene expression mediated by nuclear receptors and transcription factors, including inhibition of synthesis of cytokines and mitogens [111]
Hypolipidemic actions—lower triglyceride and triglyceride-rich lipoprotein levels [111, 118, 119]
Raise adiponectin levels, may decrease insulin resistance (but dose-related), overall favorable impact in DM [116, 120]
Antithrombotic and antiplatelet actions [109, 116]
Improve endothelial and small arterial function, in part due to decreased adhesion molecule expression and increased availability of nitric oxide [118, 121]
Raise Treg modulation of Toll-like receptors to retard progression of atherosclerosis [122]
Correct relative deficiencies in n-3 PUFA levels in modern humans, an index of chronic disease, as compared to Paleolithic ancestors [123, 124]

CRP C-reactive protein, *DM* diabetes mellitus, *n-3 PUFA* omega-3 polyunsaturated long-chain fatty acids, *Treg* regulatory T cell

voluminous literature concerning dietary n-3 PUFA which has recently accelerated. Consumer interest in these compounds is high, but a full understanding of their properties remains elusive. Research has been marked by myths, controversy, and confusion, due in part to the lack of standardization in preparations and doses, the challenges in comparing studies and clinical trials with different methodologies (particularly times of administration, underpowering, limitations, etc.), unpredictable decay in potency over time, species differences when animals are involved, uneven adherence, background intake of omega-3 supplements and other environmental influences, and increased concomitant use of drugs that produce similar effects and/or change the internal milieu in which n-3 PUFA acts. The actions of n-3 PUFA differ with age, sex, and race or ethnicity. Further, remarkable inter-individual variation in responses to these agents has previously been underestimated. As many as 30 % of overweight and obese individuals fail to lower their plasma TG levels, a hallmark of n-3 PUFA action, after taking 5 g of concentrate daily [106]. Much of this variation in responsiveness is genetic, but non-genetic factors also apply. Average changes in plasma lipids produced by n-3 PUFA include a fall in TGs of 19–44 %, a fall in non-HDL-C of 5–14 %, a variation in HDL-C of −5 to +7 %, and a variation in LDL-C of −6 to +25 % [although decreases in LDL density improve the lipid profile, a rise in LDL-C in patients with high TG values is not seen when pure eicosapentaenoic acid (EPA), devoid of docosahexaenoic acid (DHA), is administered]. The many effects of n-3 PUFA are enumerated in Table 3.

Unfortunately, despite positive epidemiological and early studies, interventional arrhythmia n-3 PUFA trials and prevention of ventricular and atrial fibrillation have yielded neutral results, and appropriately designed trials using larger doses of omega-3 PUFA are needed. Regarding n-3 PUFA studies on CVD outcomes, no striking improvement in MACE or survival has consistently been reported, but researchers observe that some situations have not been properly investigated. Methodological difficulties have haunted all aspects of omega-3 research. Upon publication of any one study, flaws seem to be immediately apparent, and others follow leading to the opposite conclusion. Even the beneficial actions of n-3 PUFA in the Inuit population have been challenged [125]. Of the classical studies, CV benefits of n-3 PUFA have been reported in many [126–137] but not all [138, 139] trials. Of these, JELIS (Japan EPA Lipid Intervention Study) [128] distinguishes itself in that only pure EPA was used, and was given with a statin and administered to a population consuming high dietary amounts of n-3 PUFA. Over a 4.6-year period, JELIS reported a 19 % relative reduction in MACE. One double-blinded, placebo-controlled clinical trial randomized 6624 patients either at high risk of or having known CVD to 1 g of n-3 PUFA or olive oil,

followed for 1 year with a composite endpoint of death, non-fatal MI, and non-fatal stroke, at which time the endpoint was revised [140]. Adherence was limited by self-reporting, and the quality of the omega-3 was not specified. In addition to an unexpectedly low event rate and underpowering to detect a reduction in sudden cardiac death in a population prone to such events, and a small dose of n-3 PUFA in a Mediterranean cohort with a high background intake of these nutrients, the null results are not definitive. A very recent comparison of a positive study showing calcium artery calcification (CAC) to be lower in Japanese than in white American men, becoming non-significant after correcting for plasma omega-3 levels [141] is also imperfect. For example, multivariate-adjusted association of plasma n-3 PUFA levels with incident CAC within the two populations are not given [142]. In a systematic review and meta-analysis of n-3 PUFA [143], a 9 % reduction in cardiac death, 13 % reduction in sudden death, 11 % fewer MIs, and a 4 % drop in all-cause mortality were observed; it was concluded that no benefits from n-3 PUFA were evident. Again, methodological shortcomings in the 20 selected studies, including shortfalls in adherence, reliance upon estimation of n-3 PUFA intake rather than upon circulating or tissue levels of n-3 PUFA, absence of the ratio of EPA to DHA, differences between fish and mammalian sources, ill-defined effects of counseling, and lack of details regarding co-interventions, among others, seriously undermined the strength of the analysis. The duration of a modest intake of n-3 PUFA in this study was 2 years. In some included studies clinically insignificant doses of n-3 PUFA were consumed; use of larger doses may be associated with a greater fall in incident MIs, despite the often-repeated dose threshold of such actions. Comparing 2 years with the 20- to 40-year period during which CHD develops, a greater duration of exposure to this agent, if not a lifetime as seen in Inuit, Japanese, and Mediterranean people, at any dose might produce very different results.

In summary, a synthesis of preclinical data, including controlled physiological and mechanistic studies, observational data, and RCTs is that n-3 PUFA in moderate amounts does lower CHD mortality, although modestly [111]. The inverse relationship of sudden cardiac death with omega-3 therapy has endured, as has a low level of n-3 PUFA with heart failure.

One of three FDA-approved omega-3 preparations, Lovaza®, contains 840 mg of omega-3, composed of EPA 465 mg and DHA 375 mg, with a suggested dose for hypertriglyceridemia of 4 capsules daily. Both EPA and DHA are in the ethyl ester (EE) form, as opposed to the preformed TG. The EE form is composed of a single fatty acid esterified to one ethanol moiety; the preformed TG form is composed of three fatty acids conjugated to a glycerol moiety, which is how the oil exists in fish. The absorption of synthetic EE is slower, with a dose of EE 4 g completely incorporated into recipient TG and phospholipid pools within a week. Most clinical studies have used the EE form, which is the one approved for clinical use. The EE form, however, may be vulnerable to oxidative degradation. In practice, Lovaza® has enjoyed widespread off-label use for CVD for high-risk patients with atherogenic dyslipidemia. Earlier this year, the FDA approved a generic form of this drug [144].

Although the rise in LDL-C observed with the use of mixed EPA/DHA agents may not be accompanied by a precipitous rise in CV risk due to increases in LDL particle size, when TG levels are $\geq$5.66 mmol/L (500 mg/dL) the use of fibrates or n-3 PUFA may lead to such elevations, complicating efforts to attain goals. This effect may be avoided with the use of pure, EPA-only preparations [145]. A pharmaceutical-grade preparation of EPA was developed, with a phase III study, MARINE (the Multi-center, plAcebo-controlled, Randomized, double-blINd, 12-week study with an open-label Extension trial) showing efficacy and safety [146]. The MARINE study population had TG levels $\geq$5.65 mmol/L (500 mg/dL), and 25 % of them were taking statins. TG levels fell in the 4 g group by 33 % and in the 2 g group by 20 %. The drug significantly lowered the number of large VLDL (28 %), total LDL (16 %), HDL (7 %), and small LDL (26 %) particles, significantly reduced VLDL particle size (9 %), apoB concentrations, and in the 4 g/day dose also reduced lipoprotein-associated phospholipase A_2 (Lp-PLA_2) by 19 % and C-reactive protein (CRP) levels by 22 % [147, 148]. In this study there was no change in size of either LDL or HDL particles. The drug icosapent ethyl (Vascepa®) was approved for patients with high TG levels, being the first non-statin antilipid drug to lower TG levels without significantly raising LDL-C levels, although with FDA remarks that any effect on the risk for pancreatitis or CVD was unknown. Based on a special protocol assessment (SPA) agreement with the FDA that a large outcome study would not be necessary for approval, the company proceeded with ANCHOR, a phase III trial [149] enrolling 702 patients with mixed dyslipidemia and TG levels ranging from $\geq$2.26 mmol/L (200 mg/dL) to $<$5.65 mmol/L (500 mg/dL). All patients in this trial were taking statins titrated to an LDL-C of $<$2.59 mmol/L (100 mg/dL). At 12 weeks, both 2 and 4 g doses significantly lowered TGs, apoB, Lp-PLA_2, and VLDL-C levels. Non-HDL-C fell by 5.5 and 13.6 % at the 2 and 4 g doses, respectively. There were no interactions between EPA and statins.

In October 2013, the FDA denied an expanded indication for Vascepa® for patients with dyslipidemia, based on possible confounding by the placebo in ANCHOR, and the belief that a fall in TG levels may not translate into improved CV outcomes. Instead, they would await the results of REDUCE-IT (Reduction of Cardiovascular

Events With EPA—Intervention Trial), a CV outcomes trial, using icosapent ethyl in patients with either high-risk or established CVD, due in 2016. Soon thereafter, the special SPA was rescinded because of the doubt raised by negative fibrate and niacin studies, jeopardizing the completion of REDUCE-IT. In other words, the hypothesis that TG lowering significantly reduces CV risk in statin-treated patients with mixed dyslipidemia and residually high plasma TG levels of 2.26–5.63 mmol/L (200–499 mg/dL) now had to be proved. This was similar to the demand made on the fenofibrate manufacturer already discussed above, and constituted a formal statement of the agency's new requirements. However, the issue is important in lipidology research quite apart from any commercial interest [150]. A recent decision to proceed with the REDUCE-IT study has been welcomed by the cardiology community [151].

In the interim, the 2013 National Institute for Health and Care Excellence (NICE) secondary prevention guidelines have removed a recommendation of advising or offering patients n-3 PUFA supplements "to prevent another MI. If people choose to take n-3 fatty acid capsules or eat omega-3 fatty acid supplemented foods, be aware that there is no evidence of harm" [152]. Further, in more recent primary and secondary prevention guidelines, NICE does not recommend using n-3 PUFA, nicotinic acid, or acipimox for primary or secondary prevention of CVD, or for patients with chronic kidney disease (CKD), or either type of DM, particularly in primary care settings [153]. However, this view may be an artifact of using only trials with relatively short durations, yielding equivocal and low cost effectiveness. As such, lifestyle use of n-3 PUFA has never been adequately tested, for which observational data are available. Further, in a separate affirming Advisory [154], NICE proscribes eating *oily fish* along with a Mediterranean diet to achieve a specific rise in protecting against a future MI. Reasons given include an absence of evidence and the potential for omega-3 fatty acid-induced rises in LDL-C, discussed above with regard to preparations aimed at minimizing this phenomenon by lowering DHA content. Epanova® contains EPA and DHA as FFAs (omega-3-carboxylic acids) in a ratio of 50–60 % EPA to 15–25 % DHA, along with other potentially active n-3 PUFA, stored in a patented coated capsule to maximize bioavailability and tolerability (normally fish oil capsules have thick hulls). Since they are FFAs, they are directly absorbed in the intestine. In May 2014, this agent was approved in a 2 and 4 g dose for severe hypertriglyceridemia based on results from the EVOLVE (Epanova® for Lowering Very High Triglycerides) trial [155]. In this study, non-HDL-C, TC/non-HDL-C, VLDL, remnant-like particle cholesterol, apoC-III, Lp-PLA_2, and arachidonic acid (AA) levels were significantly lowered as compared with placebo, but LDL-C was also substantially increased. One advantage is that the availability of the FFA form is up to fourfold higher than from the EE form under low-fat dietary conditions [155–157]. In the Epanova combined with a Statin in Patients with hypeRglyerIdemia to reduce non-HDL cholesTerol (ESPRIT) trial, FFA omega-3 was administered to high-risk patients taking statins with TGs between 2.26 and 5.63 mmol/L (200–499 mg/dL); a dose of 2 g/day was found to be effective and well-tolerated for lowering non-HDL-C and TGs, as opposed to a higher dose of 4 g/day of other forms [157]. Two ongoing large CV outcomes trials, STRENGTH (STatin Residual risk reduction with Epa-Nova in hiGh cardiovascular risk paTients with Hypertriglyceridaemia) and REDUCE-IT will report whether n-3 PUFA added to statin therapy in high-risk patients improves CV outcomes.

No studies have yet identified any adverse interaction between statins and n-3 PUFA on a clinical level, and there has most certainly been widespread use of these agents together. Therefore, if a clinician chose to lower TG concentrations in particular patients after underlying factors were addressed, a 20–50 % reduction in TG levels could safely be produced using these well-tolerated agents. n-3 PUFA status, omega-6 (n-6) PUFA intake, and inflammatory states vary widely within populations, as do inter-individual variabilities. The suggestion has been made that by interfering with n-3 and n-6 metabolism, statins may tilt the balance to favor n-6 PUFA, which have very different properties. n-3 PUFA promote mitochondrial function, a determinant of myocardial preconditioning. Statin-induced detrimental changes in mitochondria, not only limited to coenzyme Q10 (CoQ10) depletion, may impede mitochondrial protective actions associated with n-3 PUFA [158]. In addition, n-3 PUFA generally increase insulin sensitivity and lower the risk of developing DM. The effects of statin drugs are the opposite, in part related to altered mitochondrial function, implicated in both pleitropic and adverse actions. These hypotheses deserve some consideration [158]. In this regard, Nozue et al. [159] have reported that pitavastatin lowered the serum DHA/AA ratio in CHD patients, whereas pravastatin did not. Neither statin had an effect on the EPA/AA ratio.

7 Ezetimibe

Ezetimibe selectively inhibits about 50 % of the activity of the Niemann-Pick C1 Like 1 (NPC1L1) transmembrane protein receptor located on apical enterocytes and canalicular membranes of hepatocytes, and is essential to facilitate cholesterol internalization [160]. A vesicle

complex within these cells translocates the cholesterol, with the help of myofilaments, to a storage endosome termed the endocytic recyclic compartment (ERC). When intracellular cholesterol is needed, NPC1L1 is liberated from the ERC and is trafficked back to the cell membrane [160, 161].

Ezetimibe prevents the uptake and absorption of dietary and recirculated (biliary) cholesterol and plant sterols in the small intestine without reducing the absorption of TGs, fat-soluble vitamins, or bile acids [162]. Biliary cholesterol provides nearly 70 % of cholesterol—800–1200 mg of unesterified cholesterol—in the gut, about 500 mg of which is represented to the liver. After ezetimibe blocks its receptor, there is a 54 % decrease in cholesterol absorption. Efficacy of ezetimibe is also a function of LDL-C levels, and may be impaired during potassium depletion. Pooled data from monotherapy studies using ezetimibe 10 mg report an 18.5 % (range 13–20 %) fall in LDL-C levels, a fall in non-HDL-C of 14–19 %, a 3–5 % rise in HDL-C, and an 8 % (range 5–11 %) reduction in TG values, as compared to placebo [163]. Synergy of ezetimibe and statins in lowering LDL-C may result in part from upregulation of cholesterol absorption with ezetimibe. The drug alone was approved by the FDA on 25 October 2002, and the ezetimibe–simvastatin combination was approved on 23 July 2004 on the basis of reduction of LDL-C levels as part of an overall profile considered similar to statins.

The first large clinical trial, ENHANCE (Ezetimibe and Simvastatin in Hypercholesterolemia Enhances Atherosclerosis Regression), found no difference in cIMT in patients with heterozygous FH (heFH) who were treated for 24 months with 80 mg daily of simvastatin either with placebo or with 10 mg daily of ezetimibe. Discontinuation due to ADEs of increased levels of alanine aminotransferase, aspartate aminotransferase, or both, and creatine kinase were similar in the two groups: 29:5 and 9.4 %, respectively, in the simvastatin-only group and 34.2 and 8.1 %, respectively, in the combined therapy group [164]. The SEAS (Simvastatin and Ezetimibe in Aortic Stenosis) trial (enrollment completed March 2004; follow-up completed April 2008) in 1873 patients with aortic stenosis randomized patients to either ezetimibe 10 mg and simvastatin 40 mg or placebo for 4 years [165]. The treatment group enjoyed a 61 % drop in LDL-C, but the combination was no better than placebo in reducing the primary composite endpoint of improving the course of aortic-valve disease and CV events. There was a 41 % drop in MACE in the treated group as part of the success in achieving the secondary endpoint, observed only in patients with less severe aortic stenosis. Information provided by SEAS to the aortic stenosis treatment database was substantial. However, since there was no simvastatin-only arm, a contribution of ezetimibe beyond that of statins was uncertain. In the SHARP (Study of Heart and Renal Protection) trial 9438 patients with CKD were randomized to ezetimibe 10 mg and simvastatin 20 mg or placebo [166]. After 5 years, those in the treatment arm enjoyed a significant 17 % reduction in major atherosclerotic events (in MACE, a 15.3 % reduction) compared to placebo. As for the secondary endpoint, there was no difference in progression to end-stage renal disease, with one-third of patients in both groups needing either dialysis or transplantation. At least one-third of patients discontinued the drug. Again, there was no statin-only arm, so any potential contribution of ezetimibe beyond that of simvastatin could not be discerned.

Objections to the ongoing clinical use of ezetimibe alone and with simvastatin grew in the years since their introduction until the recent study IMPROVE-IT (Improved Reduction of outcomes: Vytorin Efficacy International Trial). In the same issue of the journal in which ENHANCE was reported [164], an editorial highlighted the failure to show a difference in atherosclerotic lesions when the LDL-C level difference between treated and placebo groups was 1.32 mmol/L (51 mg/dL) [167]. The authors concluded that the steps in treatment should be achieving LDL-C goals first, and then turn to fibrates, n-3 PUFA, or niacin before considering ezetimibe. A second editorial commented that before such adjuvant therapy, a redoubling of efforts to improve diet and increase exercise would be preferable [168]. Thereafter, the number of prescriptions written for ezetimibe-based drugs from 2002 to 2006 in the USA were compared with the usage in Canada, where direct marketing of drugs to the public is prohibited [169]. The lag time to approval was later in Canada than in the USA; by 2006, 15 % of prescriptions for lipid-lowering drugs included ezetimibe in the USA versus 3 % in Canada, for a ratio of 26:1 to 5:1. A reappraisal of this question [170] placed the ezetimibe controversy in the setting of a new era of outcomes research wherein the unreliability of surrogates is recognized [171]. In the case of LDL-C and outcomes improvement, by that time it was apparent that it is not only lowering levels that matters, but also the path used to achieve them. This paper emphasized that the burden of proof of a treatment is on the intervention and its trials, as well as the need for additional safety data. Later analysis showed that ezetimibe use was indeed related to variability in formulary restrictiveness [172]. Through 2007–2010, 29.1 % of continuously eligible adults obtained at least one lipid-lowering medication [173]. Among them, 17.8 % were given ezetimibe and 95.3 % another agent, usually statins. Ezetimibe use was highest in January 2008, when 2.5 % of all adults were users, declining to 1.8 % by December 2010. In the interim, over 50 % of the patients who initiated ezetimibe did so without first using statins, and these figures remained similar before and after the ENHANCE trial. Overall,

while perceived misutilization continued, the ENHANCE results appeared to lower new ezetimibe initiations, and discontinuations rose.

Proponents of ezetimibe therapy maintained that the combination of the drug with statins caused atherosclerosis regression in patients whose LDL-C levels fell, which is strictly true as said. Nonetheless, both studies discussed above compared the combination to placebo only, so any effect of ezetimibe in addition to the statin remained to be demonstrated. IMPROVE-IT [174] randomized 18,141 patients with ACS during the prior 10 days to either simvastatin 40/80 mg or to simvastatin 40 mg with ezetimibe 10 mg. The primary endpoint consisted of the first occurrence of non-fatal MI, rehospitalization for unstable angina, coronary revascularization, stroke, or CV death. LDL-C was not controlled or matched in the two treatment groups to similar levels. Through design, the addition to ezetimibe to patients already at low LDL-C levels in IMPROVE-IT differed markedly from real-world practice, in which the addition is generally restricted to patients—about 40 % of those taking statin drugs—who fail to attain goals [175]. The trial was designed prior to 2005, amended, and randomized in 2010. A number of scenarios were considered in the prediction of results. In one, assuming a reduction of 11.1 % in LDL-C when ezetimibe was added to a statin [175], and allowing for withdrawals and crossover, the predicted LDL-C difference was about 8 % or 0.140 mmol/L (5.4 mg/dL), corresponding to a reduction in RR of 3.1 % [176]. The two main questions IMPROVE-IT was designed to answer were (1) whether further LDL-C lowering by combinations with statins can improve outcomes, essentially a confirmation of the lower-LDL-is-better hypothesis; and (2) whether or not the event reduction for each unit lowering of LDL-C is the same as simvastatin, linear, or even meaningful.

Results of IMPROVE-IT were presented at the AHA Scientific Session on 17 November 2014 [177], and were published in June the following year [178]. The primary endpoint was reached in 2742 patients (34.7 %) treated with simvastatin monotherapy (average LDL-C 1.78 mmol/L or 69 mg/dL), and in 2572 patients (32.7 %) treated with simvastatin and ezetimibe (average LDL-C 1.40 mmol/L or 54 mg/dL) ($p = 0.016$). There were 6.4 % fewer cardiac events (comprising the primary endpoint) in patients assigned to take ezetimibe with simvastatin—MIs were lowered by 13 % and stroke by 13 %. Over the 7-year follow-up, adding ezetimibe to simvastatin produced a statistically significant 7.6 % relative reduction in the primary endpoint, mainly driven by reductions in non-fatal endpoints. About 2 % of patients treated for 7 years avoided a heart attack or stroke, achieving a 7-year number needed to treat (NNT) of 50. Unfortunately, however, there was no statistical difference in deaths between the two groups. About 42 % of participants discontinued the combination of drugs before the end of the trial.

Of scientific importance, IMPROVE-IT was the first RCT to support (in part, since mortality was unchanged) voluminous prior evidence demonstrating that lower LDL is in fact better clinically using a non-statin drug. While ezetimibe was used in a population not ordinarily considered for prescribing an add-on, the beneficial effects did occur despite the fact that LDL-C was already well-controlled with simvastatin. On the other hand, critics pointed out that (a) there were no deaths prevented after 7 years of treatment; (b) even with a statistically valid report in a specific ACS population, efficacy post-MI or in primary prevention remains unknown, although the NNT for the latter is estimated at $\sim$350, and widespread use might not be justified; (c) cost effectiveness is low, although this will change soon as the drug patent expires; (d) simvastatin 40 mg is considered moderate-intensity therapy in the new ACC/AHA guidelines, and medical practice has changed considerably since IMPROVE-IT was designed, making it less relevant in this high-intensity statin era; (e) in ACS, greater mortality benefit may occur with high-intensity statins (usually providing $\sim$11 % greater RR reduction vs. moderate-intensity statins) than a moderate-intensity statin combined with exetimibe (providing 6 %); (f) the benefits of adding ezetimibe in populations are less than those of a meaningful change in lifestyle; (g) IMPROVE-IT did not address whether ezetimibe alone was effective; and (h) the drug should not be first-line therapy in any clinical scenario. On balance, most clinical observers concluded the combination drug is a useful clinical option and is safe.

Two related Mendelian genetic analyses examined individuals with *NPC1L1*-inactivating mutations [179, 180]. Heterozygous carriers of these genes had lifelong exposure to a mean LDL-C that was 0.31 mmol/L (12 mg/dL) lower than non-carriers, corresponding to a 53 % relative reduction in CHD. These new data lend further support to a causal connection between NPC1L1 inhibition (the mechanism of action of ezetimibe), a reduction in LDL-C, and improved CV risk, although other changes, such as a 12 % fall in TGs and 2 mg/dL rise in HDL, may have also contributed.

In summary, agreeing with the new ACC/AHA and NICE guidelines, a recent systematic review compared the effectiveness of add-on lipid-modifying therapy to statins, and concluded that evidence was insufficient to evaluate clinical outcome changes with fibrates, niacin, or n-3 PUFA [181]. However, using the same argument and disqualifying many non-RCT studies, the review tilted toward continuing the assumption that ezetimibe produced a similar degree of reduction in cardiac events as statin therapy, minimized the benefits of using fibrates in patients with statin-treated atherogenic dyslipidemia already at LDL-C

goals, and overlooked favorable evidence [111, 182, 183] and higher patient preference for n-3 PUFA rather than "drugs". The authors added "lower-intensity statin combined with bile acid sequestrant or ezetimibe may be alternatives to higher-intensity statin monotherapy among high-risk patients who are statin-intolerant or who have a less-than-anticipated LDL cholesterol response" [181]. Finally, one must acknowledge the important affirmation the IMPROVE-IT and Mendelian randomization studies gave to the links between hypofunction of *NPC1L1* and ezetimibe, respectively, with lower LDL-C levels and improved CHD outcomes.

8 Monoclonal Antibodies to Proprotein Convertase Subtilisin/Kexin 9 (PCSK9)

Proprotein convertase subtilisin/kexin 9 (PCSK9) is a glycoprotein primarily synthesized in the liver in an inactive form. After autocleavage of the blocking peptide moieties and molecular rearrangement, the active enzyme is generated, emerges from the endoplasmic reticulum, and is secreted. The enzyme then either binds to the nearby LDL-Rs and escorts them to intracellular degradation compartments, or enters the circulation. The best known function of PCSK9 is regulating the degradation of the LDL-R. PKSC9 binds directly to an extracellular domain of the LDL-R, followed by endocytic intracellular internalization [184]. This process lowers the LDL-R density on the surface of liver cells directing LDL to lysosomal/endosomal organelles, in which case the receptor is destroyed with the LDL particle, thus interrupting LDL-R recycling to the surface. Single nucleotide polymorphisms in the *PCSK9* gene may result in a gain of function, lower LDL-R levels, and slow LDL catabolism producing a phenotype similar to FH; or, more common loss-of-function nonsense mutations may produce a phenotype with about 28 % lower LDL-C levels and 88 % reduction in the risk of CHD [185]. Overexpression of PCSK9 in animals nearly doubles the LDL-C level; PCSK9 inhibition by antisense oligonucleotide antibodies, one of the several ways of inhibiting the PCSK9 pathway, lowers LDL-C levels from 50 to 70 %. Even a single injection of a viral vector encoded to induce a gain-in-function mutant *PCSK9* in laboratory animals is sufficient to induce atherosclerosis [186]. Statin drugs upregulate the *LDL-R* gene mediated by transcription factor sterol-regulatory element binding protein (SREBP)-2 and, along with the resulting fall in LDL-C, is accompanied by increased synthesis and dose-dependent oversecretion of PCSK9 by 14–47 %. The higher the blood PCSK9 levels are, the lower the number of LDL-R, and vice versa [187]. The development of monoclonal antibodies (mAbs) to PCSK9 is the best studied of all methods, and had been confirmed in a number of species before use in humans. These inhibitors bind to PCSK9 and prevent formation of the PCSK9/LDL-R complex, leading to more available LDL-Rs, higher receptor recycling, and increased LDL clearance. Phase I, II, and III studies report a 50–60 % further reduction in LDL-C levels when these agents are given to statin-treated patients, with corresponding falls in apoB, TG, and Lp(a) concentrations [187–190]. Pleiotropic actions of PCSK9 blockade include attenuated oxidized LDL-mediated activation of NF-κB and endothelial apoptosis, lower insulin levels, limitation of adipogenesis in murine models, and modulation of blood pressure. The agents are given subcutaneously every 2–4 weeks, using auto-injectors that minimize inconvenience. Notably, whether PCSK9 expression is low due to genetic mutation or is blocked by specific antibodies, LDL-C levels are reduced up to 50 %, and safety has not been an issue, at least short-term [184]. Therapeutic mAbs do not inhibit, nor are they metabolized by, cytochrome P450 isozymes or other transporters, and therefore do not clinically interfere with statin metabolism.

Of several agents, the three potent PSCK9 inhibitors that continue on accelerated investigation schedules are alirocumab (SAR236553/REGN727) by Sanofi and Regeneron, evolocumab (AMG145) by Amgen, and bococizumab (RN316/PF-04950615) by Pfizer. Alirocumab has been assigned an early action date of July 2015 by the FDA, and an approval decision for evolocumab is expected about a month later.

DESCARTES (Durable Effect of PCSK9 Antibody Compared with Placebo Study) [191] reported LDL-C lowering of 49–62 % in patients using evolocumab 420 mg or placebo every 4 weeks who were treated with diet, atorvastatin 10 or 80 mg, or atorvastatin 80 mg with ezetimibe. DESCARTES reported over 80 % of patients using evolocumab 420 mg monthly reached a target of LDL-C <1.81 mmol/L (70 mg/dL), with significant reductions in other apoB-containing lipoproteins, including Lp(a). RUTHERFORD-2 (Reduction of LDL-C with PCSK9 Inhibition in Heterozygous Familial Hypercholesterolemia Disorder Study-2 [192] randomized evidence-based treated patients with heFH to evolocumab or placebo, and found a 59–66 % further lowering of LDL-C in the treatment arm.

The GAUSS (Goal Achievement After Utilizing an Anti-PCSK9 Antibody in Statin Intolerant Subjects-2 trial [193, 194] evaluated evolocumab in patients intolerant to at least two, and in many cases three, different statins due to myopathy. Two doses of evolocumab were used and the comparators were ezetimibe or placebo. The median pre-study LDL-C was 4.99 mmol/L (193 mg/dL), which fell by 53–56 % after evolocumab therapy was given every 2 or

4 weeks, versus placebo. Over 80 % of moderate-risk patients and 75 % of high-risk patients attained an LDL <2.59 mmol/L (100 mg/dL) compared to 10 % in ezetimibe-treated patients. Myalgia was reported by 7.8 % in the evolocumab group but 17.6 % in the ezetimibe group. A blinded-statin re-challenge is planned in the GAUSS-3 study.

The FOURIER (Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk) trial [195] enrolled 22,500 patients with past MI, stroke, or symptomatic peripheral vascular disease already treated with statins and LDL-C ≥1.8 mmol/L (70 mg/dL) or non-HDL-C ≥2.6 mmol/L (101 mg/dL). Patients were randomized to either evolocumab or placebo on a 2- or 4-weekly basis, and will be followed for 5 years for a primary endpoint of non-fatal MI, non-fatal stroke, or transient ischemic attack, and CVD mortality. The study intends to determine whether reducing LDL-C in statin-treated patients by an additional ∼50 % will lower risk even more, with results expected in 2018.

The global phase III ODYSSEY program investigating alirocumab is anticipated to involve over 23,000 patients in about 12 clinical trials. ODYSSEY-MONO enrolled 103 subjects with LDL-C levels ranging from 2.59 to 4.91 mmol/L (100 to 190 mg/dL) and a 10-year fatal CVD risk <5 % who were randomized to either alirocumab 75 mg every 2 weeks or ezetimibe 10 mg daily. A lower dose of alirocumab, 75 mg, was able to lower LDL-C to <1.81 mmol/L (70 mg/dL), 47.2 % lower than baseline, and was comparable to, or better than, ezetimibe [196]. Patients with higher baseline values of LDL-C required up-titration to the higher, 150 mg dose of alirocumab [197].

At the European Society of Cardiology (ESC) 2014 Congress (30 August–3 September 2014; Barcelona, Spain), strikingly positive results were presented from several trials in the ODYSSEY program, which now includes 14 phase III studies. ODYSSEY Choice II, currently in progress, has enrolled patients not taking a statin drug with primary hypercholesterolemia, comparing alirocumab 75 mg every 2 weeks or 150 mg every 4 weeks [198]. The results will hopefully provide information about personalized treatment with up-titration and allow choice in conforming with guidelines, and is scheduled for completion May 2016. ODYSSEY Long Term [ODYSSEY Long-term Safety and Tolerability of Alirocumab (SAR236553/REGN727) Versus Placebo on Top of Lipid-Modifying Therapy in High Cardiovascular Risk Patients With Hypercholesterolemia]) studied the effects of alirocumab and subsequent MACE on 2341 high-risk and heFH patients who were receiving a maximally tolerated dose of statin but had LDL-C >1.81 mmol/L (70 mg/dL) [199]. After treatment, mean LDL-C was 61 % lower than baseline and 81 % achieved LDL-C <2.59 mmol/L (100 mg/dL) for moderate-risk patients and <1.81 mmol/L (70 mg/dL) for high-risk patients. Using a primary outcome of time to first CHD death, non-fatal MI, fatal and non-fatal ischemic stroke, or unstable angina requiring hospitalization, the absolute event rate of MACE was 1.4 % in the alirocumab arm compared with 3.0 % in the placebo arm, an RR reduction of 54 %. Reuters reported the remarkable results in the article "Cholesterol Drug Halves Heart Attack and Stroke in Early Test" [200]. Although the primary efficacy endpoint was the fall in LDL-C at 24 weeks, the study continues to follow patients. The phase III ODYSSEY Combo II [Efficacy and Safety of Alirocumab (SAR236553/REGN727) Versus Ezetimibe on Top of Statin in High Cardiovascular Risk Patients With Hypercholesterolemia] also tests alirocumab 75 mg [or 150 mg if LDL-C remained at 1.81 mmol/L (70 mg/dL) at the 8th week] in 720 high-risk patients with uncontrolled LDL-C levels while taking a maximally tolerated statin, as compared to ezetimibe 10 mg [201]. At the 24th week, 77 % of patients achieved an LDL-C goal of <1.81 mmol/L with alirocumab versus 45.6 % in the ezetimibe arm. The lower goal of LDL-C <1.3 mmol/L (50 mg/dL) was reached by 60.3 and 14.2 % of the two arms, respectively. The ongoing trial is expected to be completed in July 2015.

ODYSSEY OUTCOMES (Evaluation of Cardiovascular Outcomes after an Acute Coronary Syndrome During Treatment With Alirocumab), a secondary prevention trial, enrolled 18,800 patients within 4–52 weeks of an ACS with LDL-C >1.8 mmol/L (70 mg/dL) despite intensive or maximally tolerated statin therapy [202]. Patients were randomized to either alirocumab or placebo on a biweekly basis, and will be followed for about 4 years for a primary endpoint of non-fatal MI, ischemic stroke, unstable angina, or CHD mortality. The completion date of the study, which began in October 2012, is January 2018.

The ODYSSEY RCTs may be classified according to their three most likely applications (Table 4). All nine studies mentioned have met their primary efficacy endpoint of a greater percentage reduction from baseline in LDL-C at week 24 than placebo or active comparator.

Bococizumab (RN-316) is another agent in this category capable of producing over 50 % reduction in LDL-C levels over baseline [205] and in patients already being treated with statin drugs. The sponsor's enthusiastic program of five outcomes trials encompasses 22,000 patients over a broad risk range [188]. Patients in SPIRE-1 [206] and SPIRE-2 [207], together enrolling 18,300 high-risk patients receiving background lipid therapy with LDL-C levels between 1.8 mmol/L (70 mg/dL) and <2.6 mmol/L (100 mg/dL) (SPIRE-1) and LDL-C ≥2.6 mmol/L (100 mg/dL) (SPIRE-2), are being randomized to either bococizumab 150 mg or placebo. Participants will be followed for up to 5 years for the time to first event, which

Table 4 Application-oriented categories of members of the ODYSSEY family of randomized controlled trials using alirocumab

High or very high cardiovascular risk	ODYSSEY COMBO I, COMBO II, OPTIONS I, OPTIONS II and LONG TERM
Statin intolerance	ODYSSEY ALTERNATIVE, which randomized patients at moderate to very high risk with well-documented intolerance to at least 2 statins [203] to alirocumab 75 mg SC every 2 weeks, ezetimibe 10 mg/day, or atorvastatin 20 mg/day re-challenge. At 24 weeks, the primary endpoint was reached, the alirocumab group enjoying a reduction of 45 % in LDL-C, 40 % in non-HDL-C, 36 % in apoB, and 26 % in Lp(a), compared to the ezetimibe group, with 15 % reduction in LDL-C, 15 % in non-HDL-C, and 11 % in apoB. Rates of discontinuation due to adverse events between treatment groups were not statistically significant [204]
Heterozygous FH	ODYSSEY FH I, FH II, and HIGH FH

apoB apolipoprotein B, *FH* familial hypercholesterolemia, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *Lp(a)* lipoprotein a, *SC* subcutaneous

includes CV death, non-fatal MI, non-fatal stroke, and hospitalization for unstable angina needing urgent revascularization [208]. Both began in October 2013, and the completion date for both trials is August 2017.

Whether PCSK9 inhibitors will be able to lower rates of MACE in all circumstances and benefit a broad category of patients remains uncertain, and depends upon the efficacy of LDL-lowering per se; unlike statins, these drugs, with somewhat dissimilar pleiotropic benefits, may lack equal anti-inflammatory actions, particularly in vulnerable plaques. Positively, the profound PKSC9 inhibitor-induced falls in LDL-C appear to be able to improve plaque morphology and regress fixed stenoses. The hope is that PCSK9 inhibitors may bring about delipidation of atheromata and reductions in inflammatory cell activities in all lesions more completely than statins. Such shrinkage of total body atheroma volumes could have profound clinical implications. While additional long-term data from larger studies on CV outcomes are eagerly awaited, interval reports from ongoing trials continue. In one of these, although the numbers of events were limited, both evolocumab (Repatha®) and alirocumab (Praluent®), showed ~50 % relative reductions in composite CV events at 12–18 months versus standard therapy in a variety of high-risk patient populations.

However, some uncertainty must temper any enthusiasm. Much is unknown about the full consequences of ultra-low LDL-C levels, and information about PCSK9 inhibitor extralipid physiology is sparse. Before infusions of these agents, oral corticosteroids, histamine receptor antagonists, and acetaminophen have been administered. In the ODYSSEY Combo II trials, ADEs occurred in 67.2 % of the alirocumab group and 67.2 % of the ezetimibe group, resulting in medication discontinuance rates of 7.5 and 5.4 %, respectively. The ADEs included nasopharyngitis, upper respiratory infections, hypersensitivity pruritus, ophthalmological events, local injection-site reactions, and rare instances of elevation in creatine kinase levels. Specifically, concerns regarding neurocognitive ADEs of PCSK9 inhibitors have surfaced in an FDA communication to manufacturers, particularly since statins have had a label change warning about neurocognitive ADEs [209]. Neurocognitive events are a concern, and the agency requires rigorous assessments of these events, which are now incorporated in future trials. Collectively, a large number of patients enrolled in all PCSK9 inhibitor studies were without adverse incidents for up to 4 years, and, remarkably, few muscle symptoms have been reported, the discontinuation rate is low, and safety and efficacy have been established. Nonetheless, long-term safety, including the development of antidrug antibodies and non-hepatic actions of these drugs, remains to be established [210, 211].

Barring some currently unanticipated ADE, of all recent opportunities to lower CV risk, PCSK9 inhibition has the greatest potential; in view of the dramatic reductions in LDL-C and MACE associated with their use, these agents may, like statin drugs, bring about a major change in cardiology practice [188, 212]. Success rates have been most dramatic in FH patients, who may not reach desirable LDL-C levels even with multiple agents. Using a different, non-antibody method of interfering with PCSK9 function, an oral form of this drug is a future possibility. Practical issues regarding general use will include FDA regulations and modification of guidelines for prescribing, and cost–payers will undoubtedly insist on maximal use of other drugs beforehand and careful eligibility screening. The population in which their use may be of benefit is substantial, consisting of (a) patients intolerant to statin drugs (~10–18 % of statin-treated patients); (b) patients currently being treated according to guidelines who still remain far from goals; (c) statin-treated patients with atherogenic dyslipidemia (nearly 18 % of the statin-eligible population, as calculated from the 2013 AHA/ACC cholesterol and assessment guidelines and other sources [2, 103]) with high residual risk; (d) patients with FH, 90 % of whom remain undiagnosed, with the remainder undertreated; and (e) patients with especially high levels of Lp(a).

9 Mipomersen and Lomitapide

Mipomersen (ISIS 301012) is a short, single-stranded antisense oligonucleotide targeting a specific sequence on messenger RNA (mRNA) that binds to a base sequence coding for apoB-100. After binding to its target, translation of the mRNA is blocked, synthesis of ApoB-100 falls, and less VLDL is released by the liver, leading to sharp reductions in LDL-C [213–216]. In volunteers, a weekly dose of 400 mg/week subcutaneously for up to 4 weeks produced reductions in plasma LDL-C and apoB of 40 and 47 %, respectively; at a median dose of 200 mg, reductions were 27 and 42 %. Phase III trials in patients with either heFH or homozygous FH (hoFH) produced comparable changes in LDL-C, apoB, and Lp(a) of approximately 28–36 %, 26–36 %, and 21–33 %, respectively [217–219]. In four phase III trials, mipomersen-induced mean reductions in Lp(a), classically resistant to statin drugs, are particularly welcome [220]. There appears to be no interaction when mipomersen is used with statin drugs.

Adverse effects occur frequently, with nearly all patients developing erythema, pain, and/or pruritus at the injection sites. Other reactions are flu-like symptoms in 50 % of patients, and reversible elevations in hepatic enzymes in 15–20 %. Due to impaired VLDL secretion, fat accumulation in the liver is the most serious complication. In patients with an *APOB* gene mutation associated with synthesis of truncated apoB, lower lipidation and production of apoB-100 may also lower TG incorporation into VLDL, and TG accumulates within the liver. Patients with this form of heterozygous hypobetalipoproteinemia are clinically asymptomatic, and the hepatic steatosis that may result does not necessarily lead to insulin resistance. However, monitoring of liver status when this agent is used is advised, and long-term safety remains unclear [221, 222].

In January 2013, the FDA approved mipomersen as an orphan drug for hoFH, which has a prevalence about 1 in 1 million, with a Boxed Warning concerning progressive liver disease and other restrictions. It is administered as a weekly injection. The EMA has not granted approval for use in the EU.

Lomitapide is an inhibitor of microsomal TG transport protein (MTP), a molecule necessary to transfer TGs to apoB and for synthesis and release of VLDL in hepatocytes [213, 223, 224]. Phase I studies showed substantial dose-related decreases in LDL-C, but gastrointestinal symptoms were limiting at higher doses. An oral dose of 10 mg reduces LDL-C by 30 %, an effect that is synergistic with atorvastatin. After a first-pass effect in the liver, the half-life is about 29 h, reaching a pharmacokinetic steady state in 6 days. After 2 weeks of therapy, a plateau is seen in the LDL-C effect. Abetalipoproteinemia is a recessive disorder characterized by absence of functional MTP, absence of VLDL secretion by the liver, and absence of circulating apoB-containing lipoproteins, a situation akin to lomitapide-treated individuals.

A phase II study used a dose-escalation design in hoFH patients, and, at a maximal 1 mg/kg dose, plasma LDL-C, apoB, and TG levels were lowered by 51, 56, and 65 % respectively [225]. An open-label phase III study in 29 hoFH patients reported dose-related reductions in LDL-C, and established efficacy [226], with an extension study of 4.5 years to follow [223]. A transient fall in HDL-C has been consistently noted [225–228].

Nausea, flatulence, and diarrhea are ascribed to TG accumulation within enterocytes [226–228], and these reactions tend to abate with use. Vitamin E has been supplemented to avoid deficiency, since absorption of fat-soluble vitamins, chiefly transported in LDL, is decreased [226]. About half the subjects in the phase III study had elevations in hepatic enzymes $\geq$3 times the upper limit of normal, and hepatic fat rose by 8.3 % by the end of one study [226], which was rapidly reversible [225]. Changes in hepatic fat were inversely proportional to the reduction in LDL-C levels. Lomitapide is approved as an orphan drug for use in hoFH by both the FDA and EMA to minimize the use of apheresis, with a Boxed Warning and other restrictions. The medication is given orally, without food, at least 2 h after the evening meal, with fat-soluble vitamin and essential fatty acid supplements.

10 Anti-Inflammatory Drugs

Atherosclerosis not only involves lipid entrapment and accumulation within vascular walls, but also inflammation, which is essential for lesion formation, progression, and clinical complications at every step [85, 229–237]. The presence of modified LDL in the subendothelial space is a key event that initiates recruitment of monocyte-derived macrophages and T cells, along with complex interactions in both the innate and adaptive immune systems [232, 235, 236]. A number of insults may also initiate, modify, and perpetuate LDL-driven atherogenesis, such as smoking, [238], high BMI [239, 240], elevated TG [94, 240, 241], elevated remnant cholesterol [242, 243], plasma glucose [244], hypertension, and diet.

Aside from the sheer number of reactions involved [245], there are a number of challenges when targeting inflammatory and immune molecules with drugs. Both processes are highly conserved and necessary for survival, and are marked by redundancy and compensatory pathways. Favoring specificity may only result in compensation, with no meaningful desired change but unwanted

effects mediated by those alternative pathways. Targeting major pathways may cause life-threatening threats by raising susceptibility to infections and cancer. There are also less appreciated but important factors. Inflammation is a key determinant not only of atherogenesis but also plaque progression. Although MACE commonly arise from plaque rupture, one must be mindful that fewer than 5 % of thin-walled lipid-laden (vulnerable) plaques actually cause events, the disease is diffuse, and the numbers of plaques that exist in multiple arterial beds and do not rupture are vast [246]. Many become 'chronic' or undergo other fates, such as remodeling or healing, in part due to other factors, such as content thrombogenicity, lumen size, etc. Ongoing plaque activity and vulnerability depends on the balance between inflammation and lesion resolution within the plaques [247, 248]. Those with large necrotic cores are particularly dangerous, which expand as cells accumulate from (a) apoptosis and primary necrosis; and (b) defective removal of dead macrophages and smooth muscle-derived foam cells ('efferocytosis'). These processes induce more oxidative, mitochondrial, and endoplasmic reticulum stress, contributing to further inflammation, plaque progression, instability, and lack of resolution [249–251]. In coronary vessels, statins are effective in quelling plaque inflammation and reducing their size, but effects are frequently insufficient. Notably, inflammation plays a significant role in the pathogenesis of stroke, and statin drugs do lower risk for this disease more than anticipated from cholesterol-lowering alone.

'Upstream' cytokine targets in pertinent proinflammatory pathways include interleukin (IL)-1β, a gateway of inflammation [252], tumor necrosis factor (TNF)-α, IL-6, with 'downsteam' targets including the intercellular adhesion molecule (ICAM) type 1 (ICAM-1), vascular cellular adhesion molecule, CRP, and fibrinogen, among others. Although many cytokines have been correlated with CHD, the best studied and superior surrogate for inflammation is still CRP [253]. CRP is a useful predictor of CV events in the population [254, 255]; a 1 standard deviation (SD) rise in CRP levels is associated with a similar CV risk due to hyperlipidemia or blood pressure [256]. In particular, CRP adds as much to CV risk prediction as either total cholesterol or HDL-C [257, 258].

A recent study found that a 1 SD higher baseline level for each of IL-6, IL-18, and TNF-α is associated with an ~10–25 % higher risk of non-fatal MI or CHD mortality [259], which would likely be even more significant during a period of time beyond the length of this investigation. Additional support for the inflammation hypothesis comes from a Mendelian analysis of IL-6, suggesting a causal role in the development of CHD [260, 261]. IL-6, when activated, increases hepatic output of CRP, fibrinogen, and plasminogen activator type-1. Although these data are impressive, there is no RCT proving the inflammation hypothesis directly, or which answers the question of whether an anti-inflammatory agent actually improves hard CVD outcomes. In this regard, the large Justification for the Use of Statins in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) trial established that patients with CRP ≥2 mg/L and no elevation in LDL-C levels enjoyed a significantly lower risk of CHD events when treated with statins [262], and this study actually brought inflammation from the laboratory to the clinic.

For the cytokines mentioned above, there are drugs available to block the actions of IL-1β, such as anakinra and canakinumab, TNF-α, such as adalimumab or infliximab, and IL-6, such as methotrexate or tocilizumab [263–270]. Nucleotide-binding oligomerization domain receptors (NOD-like receptors or NLRs) are cytoplasmic pattern recognition receptors in the innate immune system that recognize molecular 'danger signals' and activate transcription factors, such as NF-κB. One of these, the inflammasome NLRP3, recognizes crystalline cholesterol and responds by activating caspase-1 to liberate active IL-1β from its inactive precursor [263]. Colchicine and canakinumab are drugs that inhibit NLRP3 within growing atheroma and prevent production of IL-1β [264]. Colchicine is an old drug, well-known for its prevention of inflammation in gout, which has also been studied as treatment for acute pericarditis, postoperative pericardial/pleural effusion, postpericardiotomy syndrome, and postoperative atrial fibrillation after cardiac surgery, all inflammatory conditions [265]. Preliminary data show a possible role in secondary prevention of CVD and an argument for possible use in ACS has also been made [266]. Another drug that interferes with the same mechanism is canakinumab, an anti-IL-1β mAb presently approved for rare pediatric genetic diseases in which IL-1β is overexpressed, among others. The drug interrupts the central IL-1β → TNF-α → IL-6 → CRP inflammatory pathway, and is being investigated in CANTOS (Canakinumab Anti-inflammatory Thrombosis Outcomes Trial) [267, 268]. Enrollment included 17,200 men and women post-MI (with any needed revascularization procedure completed) within 30 days of randomization, who were at high-risk as evidenced by a CRP ≥2 mg/L, and were already receiving usual care, including statins. The cohort was randomized to either canakinumab (50, 150, or 300 mg subcutaneously every 3 months) or placebo, and will be followed for ~4 years for a primary endpoint of recurrent MACE, defined as non-fatal MI, non-fatal stroke, or CV death. Due to similar proinflammatory mechanisms mediated by IL-1β in pancreatic β cells, this drug also has a modest anti-diabetic action. Canakinumab produces no changes in blood pressure, lipid levels, or the thrombotic cascade that might confound the outcomes. Completion is

anticipated by April 2017. Further details concerning molecular mechanisms and additional anti-inflammatory drugs are discussed elsewhere [269, 270].

Low-dose methotrexate, in a dosage of 10–30 mg/week, is commonly used for rheumatoid arthritis, psoriasis, and psoriatic arthritis, but has effects beyond folate antagonism and antiproliferative actions, which actually play a small part in its clinical benefits. Acting through the release of adenosine and binding to transmembrane-spanning adenosine surface receptor types A_{2a} and A_3, methotrexate inhibits TNF-α, decreases expression of ICAM-1, and modulates secretion of other cytokines, resulting in lower levels of IL-6 and CRP. Preclinical studies demonstrate that methotrexate prevents foam cell formation, and in a rabbit model retards the development of intimal lesions [266]. In patients treated with methotrexate for non-cardiac disease, there is a 21 % reduction in MACE compared with those treated with other agents. CIRT (Cardiovascular Inflammation Reduction Trial) is a randomized, double-blind, placebo-controlled, multicenter, event-driven trial funded by the NHLBI studying 7000 participants with DM and/or MetSyn who have had an MI or multivessel CHD on coronary angiography within the prior 5 years. Participants will be randomized to usual care and either methotrexate 15–20 mg/week or placebo, to be followed for up to 6 years for a primary endpoint of time to first MACE, a composite of CV death, non-fatal MI, and stroke [271, 272]. Completion of this ongoing trial is expected by December 2018.

Adverse reactions to methotrexate, although minimized with low doses, frequently include gastrointestinal symptoms, but may be more serious, such as pancytopenia or cirrhosis. Up to one-third of patients discontinue therapy because of an adverse effect. Contraindications are substantial and require pretreatment attention [273].

11 Conclusion

The effectiveness of statin drugs has significantly contributed to the transformation in the practice of cardiology over the last half-century. A major thrust in pharmacology research has been studying medications that can be added to statins, including fibrates, therapies such as niacin directed at raising HDL levels, n-3 PUFA, unique, refined HDL-based treatments, and ezetimibe. This quest has been accelerated by a need for more potent agents, greater appreciation for inter-individual responses to statin drugs, and recognition of some of their limitations, including the issue of residual risk. New evidence now provides greater understanding of the patient subpopulations in which existing non-statin drugs are likely to be of benefit. Additionally, a fresh smorgasbord of pharmaceuticals has now been investigated, featuring different mechanisms of action and properties that offer great potential. These include the CETP inhibitors, PCSK9 inhibitors, mipopersen, and lomitapide, with anti-inflammatory drugs on the horizon. Several are not only capable of reducing LDL-C to neonatal levels, but also can improve Lp(a) and ceramide profiles, allowing better control even in patients with extremely high risk. For instance, mipomersen and lomitapide may provide an alternative to LDL-apheresis in patients with severe FH.

Even though the reign of statins is far from over, the era of potent, targeted, and personalized therapies is at its beginning. Ongoing collaboration between researchers, clinicians, and industry now present a number of promising solutions, many with great appeal. Certainly, the future looks exciting and will further common goals of even greater successes in combatting the scourge of heart disease.

Compliance with Ethical Standards

Conflict of interest The authors have no conflicts of interest to declare.

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