Ezetimibe and vascular endothelial function

Satoshi Ikeda, MD, PhD, and Koji Maemura, MD, PhD.

Department of Cardiovascular Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Corresponding author: Koji Maemura, MD, PhD

Department of Cardiovascular Medicine
Nagasaki University Graduate School of Biomedical Sciences
1-7-1 Sakamoto, Nagasaki 852-8501, Japan
Tel: +81-95-819-7288; Fax: +81-95-819-7290
E-mail: maemura@nagasaki-u.ac.jp
Abstract

Hypercholesterolemia is a major risk factor for cardiovascular diseases that has been managed mostly with 3-hydroxy-3-methyl glutaryl coenzyme A reductase inhibitors (statins) that suppress de novo cholesterol synthesis in the liver. Statins also have beneficial pleiotropic effects on the atherosclerotic process that are independent of their ability to lower lipid values. However, the levels of low-density lipoprotein cholesterol (LDL-C) in most hypercholesterolemic patients at high risk for cardiovascular disease do not reach the goals proposed by guidelines even when prescribed with statins. Ezetimibe is a new lipid-lowering agent that blocks the intestinal absorption of dietary and biliary cholesterol and reduces LDL-C levels, especially when combined with statins. However, its effect on cardiovascular events remains unknown. We reviewed the effects of ezetimibe on cardiovascular diseases, in particular, vascular endothelial function, which is initially impaired during the atherogenetic process and an important predictor of cardiovascular events. Increasing evidence suggests that ezetimibe improves endothelial function and nitric oxide availability, and reduces inflammation as well as oxidative stress. However, this mechanism has not been clarified and limited, large trials and cohort studies have not shown that this agent prevents cardiovascular events. Ezetimibe has just recently become commercially available, which might explain the paucity of evidence regarding its benefits and effects on cardiovascular morbidity and mortality.

Key words

nitric oxide, C-reactive protein, oxidative stress, cardiovascular disease, atherosclerosis
Introduction

Cardiovascular diseases based on atherosclerosis comprise one of the most frequent causes of worldwide mortality [1]. The initiation of atherosclerotic disease is closely related to prolonged dyslipidemia, and reducing levels of low-density lipoprotein cholesterol (LDL-C) is a cornerstone in minimizing cardiovascular events in high-risk patients. Guidelines from the National Cholesterol Education Program (NCEP) Adult Treatment Program (ATP) III in the United States [2] and the Joint Task Force of European and other Societies on Cardiovascular Disease Prevention in Clinical Practice in Europe [3] continue to identify elevated LDL-C as the primary target for lipid-modifying therapy. Furthermore, the subsequent ATP-III update in 2004 suggested that the optimal LDL-C level in high-risk individuals might be lower (< 70 mg/dl) than that of current treatment guidelines (< 100 mg/dl) [4]. Despite these guidelines, many hypercholesterolemic patients on lipid-lowering therapy do not achieve the recommended LDL-C goals [5,6]. This treatment gap is particularly evident among high-risk patients with coronary heart disease (CHD). Foley et al. [7] demonstrated that 52% of high-risk hyperlipidemic patients did not achieve the LDL-C goal with an initial dose of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins), and that 86% of these patients had still not reached the goal after 6 months. Dembowski et al. showed that only 18% of these patients achieved the LDL-C treatment goal with statin monotherapy [8]. One reason is that the initiation dose of statins is most effective and doubling the dose achieves only a 6% additional reduction in LDL-C levels [9].

Statins lower cholesterol through inhibiting cholesterol synthesis. However, the intestinal absorption of dietary and biliary cholesterol is also a major source of
cholesterol that affects lipid profiles [10]. Therefore, the inhibition of cholesterol absorption in the intestine is considered to be a potentially complementary component of lipid management. Ezetimibe was discovered as a potential inhibitor of acylcoenzyme A: cholesterol acyltransferase and it is the first cholesterol absorption inhibitor to actually inhibit biliary and dietary cholesterol absorption from the small intestine. This agent specifically blocks the cholesterol transporter, Niemann-Pick-like protein 1, which is enriched in the brush border membrane of the small intestine. The inhibition of cholesterol absorption consequently decreases cholesterol delivery to the liver, reduces hepatocyte cholesterol stores, increases liver expression of LDL receptors and increases serum LDL production and clearance, resulting in a fall in serum LDL-C levels [11,12]. Ezetimibe reduces cholesterol absorption by 40 to 50% [13], and it reduces LDL-C levels by about 18% [14]. Co-administration of ezetimibe and statin significantly reduces LDL-C levels by 18 to 25% compared to statin monotherapy [15]. This synergistic effect can help more patients with hyperlipidemia to achieve LDL-C targets. Gagne et al. [16] reported that only 19% of patients reach treatment goals on statin monotherapy whereas adding ezetimibe increases this proportion to 72%. This might result in a decrease in the incidence of future cardiovascular events.

Impaired vascular endothelial function is one initial step in the progress of atherosclerosis, which leads to the development of cardiovascular disease. Therefore, endothelium dysfunction is an important predictor of cardiovascular events. This chapter reviews the effects of ezetimibe on endothelial function.

Endothelial function

The vascular endothelium is a continuous layer of cells that separates blood from
vessel walls and it plays an important role in many physiological functions including the regulation of vasomotor tone and the maintenance of vascular homeostasis [17-25]. These processes involve the production of vasoconstrictive and vasodilating factors from endothelial cells. Endothelium-derived relaxing factors comprise nitric oxide (NO), prostacyclin and endothelium–derived hyperpolarizing factor. Nitric oxide is very important and it is mediated by the endothelium isoform of NO synthase (endothelial NO synthase, eNOS). The production of NO derived via eNOS protects the endothelium from vascular damage by inhibiting vascular smooth muscle cell proliferation, platelet and leukocyte activation and adhesion, oxidative stress and the degranulation of proinflammatory vesicles. In addition, NO inhibits both the release and action of endothelin-1, a potent vasoconstrictive peptide in the endothelium, which facilitates a proatherogenic phenotype [24-30]. Thus, NO plays a pivotal role in maintaining endothelial structural integrity and functional activity, which subsequently protects against the development of atherosclerotic vascular disease. Despite the importance of the intact endothelium and the maintenance of endothelial integrity, the regenerative capacity of mature endothelial cells is limited. Interest in endothelial progenitor cells (EPCs) is increasing [31-34], because they are precursors of mature endothelial cells originating from the bone marrow, replenish damaged endothelial cells, and migrate to sites of ischemia where they participate in neovascularization and collateral development [35-39], processes that also involve NO. An experimental study has shown that NO derived from bone marrow is pivotal to EPC mobilization from this source. Moreover, eNOS activation is thought to mediate vascular endothelial growth factor (VEGF)-induced EPC mobilization [40-42]. These results indicate that eNOS-derived NO production is crucial for maintaining endothelial health.
The bioavailability of vasodilators, especially NO is reduced, whereas that of endothelial-derived contracting factors is increased in endothelial dysfunction. This imbalance predisposes the vasculature to vasoconstriction, leukocyte adhesion, platelet activation, pro-oxidation, thrombosis, inflammation and atherosclerosis [23,25,43]. Endothelial dysfunction probably precedes the clinical manifestations of cardiovascular disorders such as hypertension, dyslipidemia, and diabetes. Cholesterol levels have an intimate and linear relationship with coronary artery disease risk. Endothelium-dependent vascular relaxation is impaired and vascular superoxide production is increased in hypercholesterolemic apoE-knockout mice. This is due to a deficiency of tetrahydrobiopterin (BH4), an essential cofactor for NOS in the aortas of these knockout mice compared to wild-type mice, but dietary BH4 supplementation appears to reduce superoxide production and restore NO synthesis [44]. Reduced levels of BH4 induce “eNOS uncoupling”, with the resultant production of superoxide, leading to endothelial dysfunction [25,45]. Moreover, increased superoxide generation might also promote increased LDL oxidation, which exerts further endothelial damage mediated by the generation of peroxynitrite and hydroxy radicals [46]. Endothelial progenitor cells are also impaired and their numbers inversely correlate with these conditions. Oxidized LDL dose-dependently decreases EPC survival and its adhesive, migratory, and tube-formation capacities [47]. Endothelial dysfunction is associated with future cardiovascular events and is clinically detectable as blunted vasodilation in response to acetylcholine or hyperemia, both of which produce eNOS-dependent vasodilation. Hedblad et al. [48] discovered that lower pulse-wave amplitude during reactive hyperemia in the calf muscle with venous occlusion determined by plethysmography is associated with a higher probability of cardiac events and all-cause
Studies of forearm arterial responses to acetylcholine infusion or hyperemia have revealed that impaired endothelial function is an independent predictor of cardiovascular events [49-51]. All of these findings indicate that endothelial dysfunction is a key early step in atherogenesis and that it can serve as a predictor of cardiovascular morbidity and mortality.

Endothelial dysfunction is reversible, and strategies for reducing cardiovascular risk factors, such as cholesterol lowering [52], blood pressure lowering [53], smoking cessation [54], physical exercise [55], and estrogen replacement therapy for post menopausal women [56] can improve endothelial function.

Hypercholesterolemia is one of the most important risk factors for cardiovascular disease because LDL-C, especially oxidized LDL, contributes to the initiation of plaque formation from the early stage of atherogenesis [57,58]. During this process, protein kinase C and nuclear factor kinase-kB (NF-kB) are initially activated in cells, where they up-regulate the local production of angiotensin II, as well as the expression of enzymes that produce oxidative stress as well as endothelial cell surface adhesion molecules [59]. These play key roles in the development of endothelial dysfunction. Lipid lowering per se is associated with improved endothelial function regardless of the choice of therapeutic strategy; for example, LDL apheresis improves endothelial-dependent vasodilation [60].

Statins are potent lipid-lowering agents and considerable evidence shows that statins have beneficial pleiotropic effects on cardiovascular diseases that are independent from their lipid-lowering effects [61-64]. These involve improved endothelial function, attenuation of the inflammatory process and normalization of the coagulation system, leading to a reduction in cardiovascular disease morbidity and
mortality [64]. The anti-inflammatory properties of statins that are independent of cholesterol-lowering have recently been announced as the primary objective of the Justification for the Use of Statins in Prevention and Intervention Trial Evaluating Rosuvastatin (JUPITER). This trial enrolled 17,802 healthy individuals without hyperlipidemia but with elevated high-sensitivity CRP (hs-CRP) levels and followed cardiovascular events for a median of 1.9 years (maximum 5.0 years). The results showed that rates of a major cardiovascular event and death from any cause were significantly reduced among participants who received rosuvastatin as compared to those who received a placebo [65]. Although the mechanisms of the pleiotropic effects by statins are not fully understood, the following have been considered. The enzyme HMG-CoA reductase plays a pivotal role not only in cholesterol synthesis but also in the synthesis of isoprenoids such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate. These isoprenoids serve through prenylation as important lipid attachments for the posttranslational modification of many proteins. The prenylation of small GTP-binding proteins such as Rho, Rac and Ras is involved in the pathophysiology of atherosclerosis; for example, the prenylation of Rho proteins down-regulates eNOS expression [66]. This posttranslational modification promotes membrane-protein–protein interactions and influences several intracellular signaling pathways [67,68]. Statins diminish the formation of isoprenylated and geranylgeranylated proteins. Thus, the statin-induced inhibition of isoprenoid formation on Rho proteins might increase eNOS expression. Caveolin is a scaffolding protein on lipid rafts in the plasma membrane that blocks eNOS access to its cofactor and substrate, resulting in reduced NO production, which is reversed by statins [69,70]. The beneficial effects of statins are also associated with less reactive oxygen species
(ROS) being produced in the vasculature through inhibiting the activation of oxidant system NADPH oxidase [71,72]. The inhibition of Rac-1 prenylation appears to mediate the down-regulation of NADPH oxidase [73].

Ezetimibe is a novel lipid lowering agent, which unlike statins, selectively inhibits biliary and dietary cholesterol absorption from the small intestine. Increasing evidence has shown that this agent, when applied alone or in combination with statins, decreases LDL-C levels and the probability of cardiovascular events [74,75]. The following paragraph reviews the effects of ezetimibe on clinically detectable endothelial function.

**Clinical effect of ezetimibe on endothelial function**

Endothelial dysfunction is an early event in the pathogenesis of atherosclerosis, and it predicts cardiovascular complications in patients with coronary artery disease (CAD) [76,77]. Therefore, the assessment of vascular endothelial function is clinically important. Historically, endothelial-dependent vasomotion induced by the pharmacological or physiological stimulation of NO release from endothelial cells has been the most popular method of such assessment. For example, infusing the coronary arteries with acetylcholine can detect epicardial coronary arterial spasm, because it can dilate or constrict blood vessels with or without an intact endothelium, respectively [78-82]. However, this method is invasive and requires coronary angiography. Over the past decade, several new techniques have been developed to assess endothelium-dependent vascular function in the forearm arteries, among which, vasodilator responses in the brachial artery to an infusion of endothelium-dependent vasodilators such as acetylcholine measured by strain gauge plethysmography after venous occlusion is important [43]. Another is flow-mediated vasodilation (FMD).
with high-resolution ultrasonography [83]. Forearm blood flow is influenced mainly by the endothelial function of resistant vessels, whereas FMD reflects mainly that of the conduit arteries [84]. Although these techniques are not significantly associated, endothelial-dependent vasodilation with either of them is related to endothelial function in coronary arteries [85,86]. Thus, these techniques allow the minimally- or non-invasive assessment of endothelial function in humans, and thus alter the morbidity or mortality of patients with atherosclerosis.

Several studies have examined whether ezetimibe has effects other than cholesterol-lowering or improves endothelial function measured as forearm vasodilator responses and/or FMD. Bulut et al. [87] showed that switching from atorvastatin 40 to 10 mg/day combined with ezetimibe 10 mg/day, induced a significant additional reduction in cholesterol and improved endothelial function in patients with metabolic syndrome. Olijhoek et al. [88] demonstrated that post-fat load FMD was preserved in patients with metabolic syndrome taking simvastatin and ezetimibe (both 10 mg/day), findings that were contrary to those generated by 80 mg of simvastatin. Settergren et al. [89] showed that FMD after 6 weeks increased from 4.3 to 5.5% in patients given simvastatin and ezetimibe (both 10 mg/day) and from 4.3 to 5.2% in those given simvastatin (80 mg/day). However, the increase in FMD between the groups did not significantly differ. These findings suggest that low-dose statin combined with ezetimibe is more (or similarly) effective on endothelial function than high-dose statin. In contrast, monotherapy with statin, but not ezetimibe, improves endothelial function in patients with congestive heart failure [90,91]. Moreover, low-dose statin plus ezetimibe was not superior to high-dose statin monotherapy in terms of improving peripheral endothelial function in patients with CAD [92,93]. The effects of ezetimibe
on endothelial function measured as forearm vasodilator responses or FMD are summarized in Table 1.

Collectively, ezetimibe monotherapy is less effective than statin monotherapy in improving endothelial function, but the effect of combining ezetimibe with low-dose statin is greater than, or similar to that of high-dose statin. Ezetimibe might be more effective in the primary prevention of CAD, such as in avoiding metabolic syndrome. Whether the effects of ezetimibe on endothelial function are dependent or independent of cholesterol-lowering remain unknown. However, that improvements in endothelial function are direct effects of ezetimibe on vascular endothelial cells is unlikely because only a little ezetimibe is absorbed into the blood circulation.

**Effect of ezetimibe on NO**

Ezetimibe, especially in combination with statin, might exert a favorable effect on endothelial function in the clinical setting. Ezetimibe might modulate the pathway of NO mediated by eNOS, which is a key factor in maintaining the integrity of the vascular endothelium. However, few reports have described an association between ezetimibe and NO.

One study using an animal model has shown that ezetimibe reduces plaque progression by 47 and 27% in male and female apoE knockout mice, respectively, and by 34 and 26% in male and female apoE/eNOS double-knockout mice, respectively, compared to animals of the same genotype fed with a western diet [94]. Furthermore, the same study showed that ezetimibe did not influence eNOS protein expression or NO production in blood vessels of apoE knockout mice. Thus, the anti-atherosclerotic effect of this agent seems largely eNOS independent, although the effects might be
partially mediated by the eNOS pathway because plaque areas were more reduced among male apoE knockout, than in male apoE/eNOS double-knockout mice. On the other hand, the expression of vascular cell adhesion molecule-1 (VCAM-1) protein was significantly reduced in both genotypes. Thus, ezetimibe might reduce VCAM-1 expression dependently upon the LDL-C lowering effect, but not upon eNOS function, although the reduction of VCAM-1 expression by statins is considered to be dependent on the eNOS pathway [95].

Birnbaum et al. have demonstrated differential effects on myocardial infarction among high-dose statin, low-dose statin, ezetimibe and ezetimibe plus low-dose statin [96]. In rats orally pretreated with high-dose atorvastain and simvastatin (both 10 mg/kg/day), the sizes of myocardial infarctions induced by ischemia-reperfusion injury were decreased whereas activity levels of calcium-dependent NOS including eNOS were significantly increased compared to sham treated rats. In contrast, infarction size was not reduced in rats pretreated with low-dose simvastatin (2 mg/kg/day) alone, ezetimibe (1 mg/kg/day) alone or a combination of the two. Calcium-dependent NOS activity was reduced by low-dose monotherapy with either simvastatin or ezetimibe, and slightly increased by a combination of both. These findings suggest that ezetimibe alone or in combination with low-dose statin has no effect on the eNOS bioavailability that can protect against myocardial infarction in rats.

In contrast, Nakagami et al. [97] recently demonstrated that ezetimibe significantly reduced LDL-C levels in ApoE-deficient mice on a high-fat diet and up-regulated the expression of eNOS mRNA and protein. Furthermore, ezetimibe suppressed the expression of interleukin-6 (IL-6), an inflammatory cytokine, as well as oxidative stress assessed as dihydroethidium staining in the aorta. Ezetimibe
eventually prevented the progression of lipid plaque and hepatic lipid accumulation. Lipid-lowering mediates these favorable effects of ezetimibe on atherosclerosis as well as direct and/or indirect vascular protective effects.

An association between ezetimibe with NO in humans has also been described. Asymmetric dimethylarginine (ADMA), an endogenous NO synthase inhibitor, is considered a novel emerging risk factor for cardiovascular disease in patients with chronic kidney disease [98,99]. Six months of ezetimibe significantly decreased ADMA levels independently of cholesterol in patients with early stage chronic kidney disease and dyslipidemia [100]. That study also showed that ezetimibe reduced urinary excretion levels of 8-hydroxy deoxyguanosine (8-OHdG), an oxidative stress marker. Thus, the mechanism of ADMA reduction might be mediated by its own anti-oxidative properties. Indeed, oxidative stress inactivates dimethylarginine dimethyl aminohydrolase, a rate-limiting enzyme of ADMA degradation, and increases ADMA levels [101]. These suggest that ezetimibe improves NOS activity through effects that are independent of cholesterol lowering. The effect of ezetimibe on NO requires more detailed investigation.

**Effect of ezetimibe on endothelial progenitor cells**

Interest in EPCs that purportedly function in maintenance of endothelial integrity, function and postnatal neovascularization is increasing [33]. Several studies have demonstrated that the number and function of EPCs are affected by cardiovascular risk factors such as dyslipidemia, hypertension, diabetes mellitus and smoking [34,102,103]. Levels of LDL-C negatively correlate with numbers of EPCs and impair their migration, proliferation, vasculogenic capacity and other properties [103,104]. Elevated
cholesterol levels in sera decrease the number of colony forming units of EPCs [105].

Oxidation alters the native properties of LDL and scavenger receptors incorporate it into macrophages, through which it deteriorates various vascular wall functions such as the inhibition of endothelial nitric oxide (NO) production, endothelial apoptosis and the proliferation of smooth muscle cells [106]. Oxidized LDL (oxLDL) detrimentally affects the number and activity of EPCs, inhibits EPC differentiation and induces EPC senescence [107-109]. The mechanism of these effects involves a decrease in Akt phosphorylation and eNOS protein as well as in mRNA expression within EPCs [110]. Indeed, circulating levels of ADMA inversely correlate with the number of EPCs and down-regulate EPC function [111]. A lectin-like oxidized LDL receptor (LOX-1), identified predominantly in endothelial cells where it mediates the biological effects of oxLDL, is also involved in the mechanism through interaction with eNOS. Thus, eNOS-derived NO plays a key role in the mobilization and function of EPCs [47,108].

Statins increase the numbers of circulating EPCs, inhibit their apoptosis and enhance their proliferation. These effects are considered to be mainly mediated by endothelial NO availability. Statins activate the phosphorylation of Akt, a serine-threonine kinase, through phosphatidylinositol 3-kinase (PI3K), leading to eNOS phosphorylation at serine 1177 [112,113]. Li et al. [114] recently demonstrated that lovastatin-enhanced EPC differentiation is mediated, at least in part, by AMP-activated protein kinase via eNOS phosphorylation. Furthermore, atorvastatin affects micro RNA (miR)-221/-222 expression, which might control the proliferation and differentiation of CD34-positive hematopoietic progenitor cells in EPCs and increase the number of EPCs via an eNOS-dependent pathway in patients with CAD [115].
A few investigators have examined the effects of ezetimibe on EPCs. Landmesser et al. [91] identified an obvious increase in the number of functionally active EPCs after 4 weeks of treatment with simvastatin, but not with ezetimibe. This suggests that ezetimibe alone does not exert a better effect on the number of EPCs compared to statin monotherapy. Westerweel et al. [42] showed that obese men with metabolic syndrome but without diabetes or manifest cardiovascular disease have lower levels of circulating EPCs than age-matched controls without metabolic syndrome. On the other hand, they found that the effects on EPC levels in this population were similar between low-dose simvastatin plus ezetimibe and high-dose simvastatin that achieve similar LDL-C levels. This suggests that LDL-C reduction is more important than the pleiotropic effects of statins. Thus, ezetimibe combined with a statin might result in a synergistic effect on EPC functions.

**Effect of ezetimibe on inflammation**

C-reactive protein (CRP), mainly produced by hepatocytes in response to IL-6 and then secreted into the systemic circulation, is an acute-phase reactant in humans [116]. Elevated CRP levels are related to impaired endothelial function and comprise a powerful predictor of future cardiovascular events. Furthermore, studies *in vitro* have shown that CRP modulates the activity and expression of several factors implicated in atherogenesis; for example CRP down-regulates eNOS and decreases NO release, which facilitates endothelial dysfunction [117].

Studies that have examined the effect of statin alone and a statin-ezetimibe combination on CRP are listed in Table 2. Pearson et al. [118] reported the effects of ezetimibe alone or in combination with statins on CRP and LDL-C determined from
two pooled analyses of randomized, placebo-controlled trials in patients with hypercholesterolemia. The investigation included six studies of ezetimibe monotherapy and seven of ezetimibe added to ongoing statin therapy. Ezetimibe monotherapy induced a mean 1% decrease in CRP after 12 weeks; the mean difference from placebo was 6% (p = 0.09). In contrast, ezetimibe added to baseline statin resulted in a significant additional reduction in CRP (mean treatment difference was 10%, P < 0.001) after 6–8 weeks. Thus, although adding ezetimibe to statin obviously enhances CRP reduction, the role of ezetimibe monotherapy is less well defined. Efrati et al. [119] reported that adding ezetimibe to 40 mg of simvastatin reduced CRP to levels below that achieved by doubling the statin dose. However, ezetimibe combined with rosuvastatin significantly reduced CRP levels compared to rosuvastatin alone [120], whereas a single tablet comprising ezetimibe/simvastatin induced a greater reduction in LDL-C levels relative to rosuvastatin but a similar change in hs-CRP levels in both groups [121]. Although combination therapy seems to be superior to statin monotherapy in terms of lowering LDL-C, a superior effect on CRP remains contentious. Even studies that demonstrated a favorable effect of combination therapy on CRP also found a significantly larger reduction in LDL-C. Therefore, whether the effect of ezetimibe on CRP is dependent or independent of its cholesterol-lowering effect remains unknown. To understand the differences in CRP reduction among therapies that achieve similar levels of LDL reduction is important because clinicians may choose lipid-lowering therapy based not only on a reduction in LDL, but also in CRP.

Thus, ezetimibe might have synergistic effects on levels of CRP as well as of LDL-C when combined with statins, although whether the effect on CRP is dependent
or independent of its cholesterol-lowering effect remains undetermined.

**Effect of ezetimibe on oxidative stress**

Oxidative stress caused by enhanced production of reactive oxygen species (ROS) and/or decreased antioxidant levels plays an important role in the pathophysiology of vascular endothelial dysfunction, resulting in abnormal vasorelaxation. Several reports describe the effect of ezetimibe on oxidative stress.

Animal studies have shown that ezetimibe suppresses ROS production in the aorta [97] and liver [122]. Ezetimibe monotherapy exerted the highest increase in the hepatic alpha-tocopherol/maleic dialdehyde ratio (means antioxidant/oxidant ratio), compared to monotherapy with other insulin-sensitizing agents, such as rosiglitazone, metformin and valsartan in a methionine choline-deficient diet rat model of non-alcoholic fatty liver disease [123]. This suggests that ezetimibe has antioxidant effects. Hussein et al. [124] demonstrated that three months of ezetimibe (10 mg/day) prolonged the lag time to LDL oxidation from 144 ± 18 to 195 ± 16 min, and that add-on simvastatin induced further prolongation of LDL oxidation and decreased the malondialdehyde content in LDL compared to simvastatin monotherapy in hypercholesterolemic patients. Ezetimibe for six months reduced urinary excretion levels of 8-OHdG as well as protein in patients with non-diabetic chronic kidney disease and dyslipidemia [100]. Ezetimibe significantly decreased the levels of oxidized LDL in patients with rheumatoid arthritis, although to a lesser extent than simvastatin [125]. In contrast, Landmesser at al. [91] demonstrated that the activity of endothelium-bound extracellular superoxide dismutase, a major antioxidant enzyme, released by a bolus injection of heparin is increased by simvastatin, but not by ezetimibe. A comparison
of atorvastatin (80 mg) and atorvastatin (10 mg) + ezetimibe (10 mg) in patients with CAD found no difference in levels of urinary 8-iso-prostaglandin F2α, a metabolite in the arachidonic cascade regarded as an oxidative stress marker in vivo [92]. These findings indicated that ezetimibe improves oxidative status in animal models. In contrast, the improvement by ezetimibe in humans remains controversial. It might be due to small number of subjects, and differences as well as stabilities of oxidative stress markers in the previous studies.

**Effect of ezetimibe on cardiovascular events in clinical trials**

Ezetimibe, especially in combination with statin, might ameliorate endothelial dysfunction. However, whether ezetimibe impacts morbidity and mortality in patients with cardiovascular diseases remains obscure. Two large, long-term trials of ezetimibe, in which the primary outcome was designated as a change in carotid intima-media thickness (CIMT), have recently been published: the Ezetimibe and Simvastatin in Hypercholesterolemia Enhances Atherosclerosis Regression (ENHANCE) [126] and Stop Atherosclerosis in Native Diabetics Study (SANDS) trials [127]. The former trial of 720 patients with heterozygous familial hypercholesterolemia found that simvastatin (80 mg) combined with ezetimibe (10 mg) for 24 months significantly reduced levels of LDL-C and hs-CRP compared to simvastatin (80 mg) alone. However, CIMT changes did not differ between the two groups (simvastatin vs simvastatin+ezetimibe: +0.0033 vs +0.00182 mm; p = 0.15). This might mean that ezetimibe does not exert additional effects on CIMT progression. However, the results might be affected by various factors, such as the study subjects having a low level of CIMT at baseline and 81% of them taking statins before the study. The latter was a randomized, open-label, 3-year
trial that examined the effects of aggressive goals for LDL-C ($\leq 70$ mg/dl), non-HDL-C ($\leq 100$ mg/dl) and blood pressure ($\leq 115/75$ mm Hg) reduction versus standard goals of $\leq 100$ mg/dl, $\leq 130$ mg/dl and $\leq 130/80$ mm Hg, respectively, in 499 individuals with type 2 diabetes. In 223 patients in the aggressive treatment group, 69 required ezetimibe added to statin, and CIMT similarly regressed from baseline in both subgroups with or without ezetimibe (-0.025 vs -0.012 mm, for subgroups with or without ezetimibe, respectively). On the other hand, CIMT progressed in the group that received standard treatment (0.039 mm). In addition, multivariate analysis revealed that changes in LDL-C levels, but not use of ezetimibe, independently correlated with changes in CIMT. These results indicate that aggressive cholesterol lowering therapy results in CIMT regression regardless of ezetimibe. However many patients required ezetimibe to achieve the aggressive goal. The Simvastatin and Ezetimibe in Aortic Stenosis (SEAS) study showed that simvastatin and ezetimibe neither inhibited the progression of aortic stenosis nor reduced major cardiovascular events in 1873 patients with asymptomatic, mild-to-moderate aortic stenosis during a median follow-up of 52.2 months [128]. Limited cohort studies have not yet shown additional and beneficial effects of ezetimibe on cardiovascular events in the clinical setting. Large cohort studies are required to examine the anti-atherosclerotic effects of ezetimibe in the setting of primary or secondary prevention.

**Conclusions**

Statin alone is currently insufficient for patients with hypercholesterolemia, especially those at high risk for cardiovascular disease, to achieve guideline-defined LDL-C goals. Ezetimibe is a potent LDL-C lowering agent, and, when combined with
statins, it might induce synergistic effects not only on lipid profiles but also on surrogate markers of future cardiovascular events, such as endothelial function, CRP and oxidative stress (Figure 1). The simultaneous inhibition of cholesterol synthesis by statin and of cholesterol absorption by ezetimibe might retard the atherogenetic process. These effects are considered to be mainly mediated by lipid lowering. However, further studies should elucidate the mechanism of the anti-atherosclerotic effects induced by ezetimibe; for instance, whether or not it directly affects atherogenesis independently from its lipid-lowering effects.
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Figure 1. Effects of ezetimibe on vascular endothelium.

Elevated low-density lipoprotein cholesterol (LDL-C) induces impaired eNOS (endothelial nitric oxide synthase)-derived NO availability, inflammation and oxidative stress, leading to vascular endothelial dysfunction. Inhibition of cholesterol synthesis using statins and of cholesterol absorption using ezetimibe agents improves these effects in endothelial cells.
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<td>Switch</td>
<td>14 patients with Mets</td>
<td>62.4 ± 10.7 years Male: 100%</td>
<td>Atorvastatin 40 mg/day; &gt; 4 weeks ↓ Atorvastatin 10 mg/day + ezetimibe 10 mg/day 8 weeks</td>
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<td>Olijhoek et al. [88]</td>
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<td>19 patients with Mets</td>
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<td>Gounari et al. [90]</td>
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<td>20 patients with RA</td>
<td>58 ± 12 years, Male 20%</td>
<td>Simvastatin 20 mg/day Ezetimibe 10 mg/day 6 weeks each arm</td>
<td>The improvement in FMD by simvastatin was greater, but not significant, than that by ezetimibe.</td>
</tr>
<tr>
<td>Trial</td>
<td>Methods</td>
<td>Population</td>
<td>Protocol, Demographics</td>
<td>Endothelial function</td>
<td></td>
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<tr>
<td>Ostad et al. [92]</td>
<td>R, DB FMD</td>
<td>49 patients with CAD</td>
<td>Atorvastatin 80 mg/day (24 patients)</td>
<td>The improvement in FMD by atorvastatin was greater than the combination therapy.</td>
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<td></td>
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<td>66 ± 9 years, Male 79%</td>
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<td></td>
<td>Atorvastatin 10 mg/day + ezetimibe 10 mg/day (25 patients)</td>
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<td></td>
<td>64 ± 10 years, Male 76%</td>
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<td></td>
<td>8 weeks</td>
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<tr>
<td>Settergren et al. [89]</td>
<td>R, DB FMD</td>
<td>39 patients with CAD and IGT/DM</td>
<td>Simvastatin 80 mg/day (20 patients)</td>
<td>There was no difference in the increase of FMD between the two treatment groups.</td>
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<td></td>
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<td>70 years, Male 75%</td>
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<td></td>
<td>Simvastatin 10 mg/day + ezetimibe 10 mg/day (19 patients)</td>
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<td></td>
<td></td>
<td>74 years, Male 58%</td>
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<td></td>
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<td></td>
<td>6 weeks</td>
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<tr>
<td>Fichtlscherer et al.</td>
<td>R FBF</td>
<td>60 patients with CAD</td>
<td>Ezetimibe 10 mg/day de novo (15 patients)</td>
<td>Only atorvastatin monotherapy improved FBF</td>
<td></td>
</tr>
<tr>
<td>[93]</td>
<td></td>
<td></td>
<td>53.2 ± 4.3 years, Male: 100%</td>
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<td></td>
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<td></td>
<td>Ezetimibe 10 mg/day added on simvastatin 20 mg/day (15 patients)</td>
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<td></td>
<td>55.2 ± 6.6 years, Male: 67%</td>
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<td></td>
<td>Atorvastatin 10 mg/day → 40 mg/day (15 patients)</td>
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<td></td>
<td>56.5 ± 3.9 years, Male: 80%</td>
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<td></td>
<td>Atorvastatin 40 mg/day de novo (15 patients)</td>
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<td></td>
<td>56.5 ± 2.8 years, Male: 100%</td>
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<td>4 weeks</td>
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<tr>
<td>Landmesser et al. [91]</td>
<td>R</td>
<td>FDD</td>
<td>Simvastatin 10 mg/day (10 patients) 59 ± 3 years, Male 80% Ezetimibe 10 mg/day (10 patients) 59 ± 4 years, Male 70%</td>
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<tr>
<td></td>
<td>20 patients with CHF</td>
<td>4 weeks</td>
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</tbody>
</table>

The treatment of simvastatin, but not ezetimibe, improved FDD.

FMD, flow-mediated vasodilatation in brachial artery; FDD, flow-dependent dilation in radial artery; FBF, forearm blood flow responded by acetylcholine. R, randomized; DB, double-blind; CO, cross-over; Mets, metabolic syndrome; CHF, congestive heart failure; RA, rheumatoid arthritis; CAD, coronary artery disease; IGT, impaired glucose tolerance; DM, diabetes mellitus.
Table 2. Comparison of effect on CRP of statin monotherapy and of statins combined with ezetimibe.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Patients</th>
<th>Period</th>
<th>Medicine</th>
<th>Changes in LDL-C</th>
<th>Changes in CRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malmström et al. [129]</td>
<td>32 patients with coronary artery disease</td>
<td>6 weeks</td>
<td>Sim 80 mg</td>
<td>3.0 ± 1.0 → 1.4 ± 0.5 mM</td>
<td>3.8 ± 3.2 → 3.2 ± 2.9 mg/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sim 10 mg + Ez 10 mg</td>
<td>3.2 ± 0.6 → 1.7 ± 0.7 mM</td>
<td>4.8 ± 4.5 → 4.0 ± 4.0 mg/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ator 80 mg</td>
<td>-60 ± 11%</td>
<td>-1.9 ± 1.9%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ator 10 mg + Ez 10 mg</td>
<td>-54 ± 18%</td>
<td>-1.8 ± 2.1%</td>
</tr>
<tr>
<td>Ostad et al. [92]</td>
<td>58 patients with coronary artery disease</td>
<td>8 weeks</td>
<td>Ator 80 mg</td>
<td>-35.2%</td>
<td>1.9 ± 1.9 → 1.9 ± 2.2 mg/L</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ator 10 mg + Ez 10 mg</td>
<td>-49.9%*</td>
<td>2.6 ± 2.3 → 2.1 ± 1.9 mg/L</td>
</tr>
<tr>
<td>Alvarez-Sala et al.</td>
<td>82 patients with hypercholesterolemia</td>
<td>12 weeks</td>
<td>Flu 80 mg</td>
<td>-46 ~ 57%</td>
<td>-22.2%</td>
</tr>
<tr>
<td>[130]</td>
<td></td>
<td></td>
<td>Flu 80 mg + Ez 10 mg</td>
<td>-52 ~ 61%*</td>
<td>-25.0%</td>
</tr>
<tr>
<td>Catapano et al. [121]</td>
<td>2,959 patients with hypercholesterolemia</td>
<td>6 weeks</td>
<td>Rosu 10, 20 mg</td>
<td>-45.3%</td>
<td>-25.1%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sim 20, 40 mg + Ez 10 mg</td>
<td>-53.4%*</td>
<td>-24.8%</td>
</tr>
<tr>
<td>Ballantyne et al. [131]</td>
<td>1,902 patients with hypercholesterolemia</td>
<td>6 weeks</td>
<td>Ator 10, 20, 40, 80 mg</td>
<td>-36.5 ~ 39.4%</td>
<td>-16.8 ~ 22.4%</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Sim 20 mg + Ez 10 mg</td>
<td>-49.6%*</td>
<td>-17.2%</td>
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<td></td>
<td></td>
<td></td>
<td>Ator 40 mg</td>
<td>-46.0%</td>
<td>-30.0%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sim 40 mg + Ez 10 mg</td>
<td>-53.9%*</td>
<td>-27.6%</td>
</tr>
<tr>
<td>Robinson et al. [132]</td>
<td>1,128 patients with hypercholesterolemia and metabolic syndrome</td>
<td>6 weeks</td>
<td>Ator 40 mg → 80 mg (+40 mg)</td>
<td>-11%</td>
<td>-11%</td>
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<td></td>
<td></td>
<td></td>
<td>Ator 40 mg + Ez 10 mg</td>
<td>-27%*</td>
<td>-18%</td>
</tr>
<tr>
<td>Leiter et al. [133]</td>
<td>579 patients with hypercholesterolemia</td>
<td>6 weeks</td>
<td>Ator 20 mg → 40 mg (+20 mg)</td>
<td>-31%*</td>
<td>-7%</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Ator 20 mg + Ez 10 mg</td>
<td>-31%*</td>
<td>-7%</td>
</tr>
<tr>
<td>Study</td>
<td>Patients with hypercholesterolemia</td>
<td>Duration</td>
<td>Treatment 1</td>
<td>Treatment 2</td>
<td>LDL Reduction</td>
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<tr>
<td><strong>Statin monotherapy &gt; Combination therapy with statin and ezetimibe</strong></td>
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<tr>
<td>Efrati et al. [119]</td>
<td>40 patients with hypercholesterolemia</td>
<td>3 months</td>
<td>Sim 40 mg</td>
<td>Sim 40 → 80 mg</td>
<td>-40 ± 4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ez 10 mg</td>
<td>Ez 10 mg</td>
<td>-18 ± 13%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sim 40 mg + Ez 10 mg</td>
<td></td>
<td>-36 ± 17%</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>2.8 ± 2.5 → 1.6 ± 1.5 mg/L</td>
</tr>
<tr>
<td><strong>Combination therapy with statin and ezetimibe &gt; Statin monotherapy</strong></td>
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</tr>
<tr>
<td>Ballantyne et al. [120]</td>
<td>469 patients with hypercholesterolemia</td>
<td>6 weeks</td>
<td>Rosu 40 mg</td>
<td>Rosu 40 mg + Ez 10 mg</td>
<td>-57%</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>-14.3%</td>
</tr>
<tr>
<td>Sager et al. [135]</td>
<td>1,089 patients with hypercholesterolemia</td>
<td>12 weeks</td>
<td>Sim 10, 20, 40, 80 mg</td>
<td>Sim 10, 20, 40, 80 mg + Ez 10 mg</td>
<td>-37.5%</td>
</tr>
<tr>
<td>Goldberg et al. [136]</td>
<td>887 patients with hypercholesterolemia</td>
<td>12 weeks</td>
<td>Sim 10, 20, 40, 80 mg</td>
<td>Sim 10, 20, 40, 80 mg + Ez 10 mg</td>
<td>-38.5%</td>
</tr>
<tr>
<td>Bays et al. [137]</td>
<td>1,528 patients with hypercholesterolemia</td>
<td>12 weeks</td>
<td>Sim 10, 20, 40, 80 mg</td>
<td>Sim 10, 20, 40, 80 mg + Ez 10 mg</td>
<td>-39.0%</td>
</tr>
<tr>
<td>Ballantyne et al. [14]</td>
<td>628 patients with hypercholesterolemia</td>
<td>12 weeks</td>
<td>Ator 10, 20, 40, 80 mg</td>
<td>Ator 10, 20, 40, 80 mg + Ez 10 mg</td>
<td>-42.4 ± 0.95%</td>
</tr>
<tr>
<td>Gagné et al. [16]</td>
<td>769 patients with hypercholesterolemia</td>
<td>8 weeks</td>
<td>Statins + placebo</td>
<td>Statins + Ez 10 mg</td>
<td>-3.7%</td>
</tr>
</tbody>
</table>

Ez, Ezetimibe; Sim, Simvastatin; Ator, Atorvastatin; Flu, Fluvastatin; Ros, Rosuvastatin. *p < 0.001; †p < 0.01, ‡p < 0.05.