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

mortality. 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, metoformin 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 (≤ 70 mg/dl), non–HDL-C (≤ 100 mg/dl) and blood pressure (≤ 115/75 mm Hg) reduction versus standard goals of ≤ 100 mg/dl, ≤ 130 mg/dl and ≤ 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 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.

References

1. Summary of the second report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). *Jama* 1993, 269:3015-3023.
2. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *Jama* 2001, 285:2486-2497.
3. De Backer G, Ambrosioni E, Borch-Johnsen K, Brotons C, Cifkova R, Dallongeville J, et al.: European guidelines on cardiovascular disease prevention in clinical practice. Third Joint Task Force of European and Other Societies on Cardiovascular Disease Prevention in Clinical Practice. *Eur Heart J* 2003, 24:1601-1610.
4. Grundy SM, Cleeman JI, Merz CN, Brewer HB, Jr., Clark LT, Hunninghake DB, et al.: Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. *Circulation* 2004, 110:227-239.
5. Andrews TC, Ballantyne CM, Hsia JA, Kramer JH: Achieving and maintaining National Cholesterol Education Program low-density lipoprotein cholesterol goals with five statins. *Am J Med* 2001, 111:185-191.
6. Pearson TA, Laurora I, Chu H, Kafonek S: The lipid treatment assessment project (L-TAP): a multicenter survey to evaluate the percentages of dyslipidemic patients receiving lipid-lowering therapy and achieving low-density lipoprotein cholesterol goals. *Arch Intern Med* 2000, 160:459-467.
7. Foley KA, Simpson RJ, Jr., Crouse JR, 3rd, Weiss TW, Markson LE, Alexander CM: Effectiveness of statin titration on low-density lipoprotein cholesterol goal attainment in patients at high risk of atherogenic events. *Am J Cardiol* 2003, 92:79-81.

8. Dembowski E, Davidson MH: Statin and ezetimibe combination therapy in cardiovascular disease. *Curr Opin Endocrinol Diabetes Obes* 2009, 16:183-188.
9. Jones PH, Davidson MH, Stein EA, Bays HE, McKenney JM, Miller E, et al.: Comparison of the efficacy and safety of rosuvastatin versus atorvastatin, simvastatin, and pravastatin across doses (STELLAR* Trial). *Am J Cardiol* 2003, 92:152-160.
10. Dujovne CA, Ettinger MP, McNeer JF, Lipka LJ, LeBeaut AP, Suresh R, et al.: Efficacy and safety of a potent new selective cholesterol absorption inhibitor, ezetimibe, in patients with primary hypercholesterolemia. *Am J Cardiol* 2002, 90:1092-1097.
11. Knopp RH, Gitter H, Truitt T, Bays H, Manion CV, Lipka LJ, et al.: Effects of ezetimibe, a new cholesterol absorption inhibitor, on plasma lipids in patients with primary hypercholesterolemia. *Eur Heart J* 2003, 24:729-741.
12. Altmann SW, Davis HR, Jr., Zhu LJ, Yao X, Hoos LM, Tetzloff G, et al.: Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption. *Science* 2004, 303:1201-1204.
13. Sudhop T, Lutjohann D, Kodal A, Igel M, Tribble DL, Shah S, et al.: Inhibition of intestinal cholesterol absorption by ezetimibe in humans. *Circulation* 2002, 106:1943-1948.
14. Ballantyne CM, Houri J, Notarbartolo A, Melani L, Lipka LJ, Suresh R, et al.: Effect of ezetimibe coadministered with atorvastatin in 628 patients with primary hypercholesterolemia: a prospective, randomized, double-blind trial. *Circulation* 2003, 107:2409-2415.
15. Genest J: Combination of statin and ezetimibe for the treatment of dyslipidemias and the prevention of coronary artery disease. *Can J Cardiol* 2006, 22:863-868.

16. Gagne C, Bays HE, Weiss SR, Mata P, Quinto K, Melino M, et al.: Efficacy and safety of ezetimibe added to ongoing statin therapy for treatment of patients with primary hypercholesterolemia. *Am J Cardiol* 2002, 90:1084-1091.

17. Vita JA, Keaney JF, Jr.: Endothelial function: a barometer for cardiovascular risk? *Circulation* 2002, 106:640-642.

18. Ross R: The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature* 1993, 362:801-809.

19. Schechter AN, Gladwin MT: Hemoglobin and the paracrine and endocrine functions of nitric oxide. *N Engl J Med* 2003, 348:1483-1485.

20. Furchgott RF, Zawadzki JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980, 288:373-376.

21. Forstermann U, Munzel T: Endothelial nitric oxide synthase in vascular disease: from marvel to menace. *Circulation* 2006, 113:1708-1714.

22. Govers R, Rabelink TJ: Cellular regulation of endothelial nitric oxide synthase. *Am J Physiol Renal Physiol* 2001, 280:F193-206.

23. Bonetti PO, Lerman LO, Lerman A: Endothelial dysfunction: a marker of atherosclerotic risk. *Arterioscler Thromb Vasc Biol* 2003, 23:168-175.

24. Gonzalez MA, Selwyn AP: Endothelial function, inflammation, and prognosis in cardiovascular disease. *Am J Med* 2003, 115 Suppl 8A:99S-106S.

25. Grover-Paez F, Zavalza-Gomez AB: Endothelial dysfunction and cardiovascular risk factors. *Diabetes Res Clin Pract* 2009, 84:1-10.

26. Moncada S: Nitric oxide in the vasculature: physiology and pathophysiology. *Ann N Y Acad Sci* 1997, 811:60-67; discussion 67-69.

27. Marletta MA: Nitric oxide: biosynthesis and biological significance. *Trends Biochem*

Sci 1989, 14:488-492.

28. Li H, Wallerath T, Munzel T, Forstermann U: Regulation of endothelial-type NO synthase expression in pathophysiology and in response to drugs. *Nitric Oxide* 2002, 7:149-164.

29. Feron O, Balligand JL: Caveolins and the regulation of endothelial nitric oxide synthase in the heart. *Cardiovasc Res* 2006, 69:788-797.

30. Vanhoutte PM: Endothelial dysfunction: the first step toward coronary arteriosclerosis. *Circ J* 2009, 73:595-601.

31. Caplan BA, Schwartz CJ: Increased endothelial cell turnover in areas of in vivo Evans Blue uptake in the pig aorta. *Atherosclerosis* 1973, 17:401-417.

32. Dimmeler S, Zeiher AM: Vascular repair by circulating endothelial progenitor cells: the missing link in atherosclerosis? *J Mol Med* 2004, 82:671-677.

33. Rafii S, Lyden D: Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. *Nat Med* 2003, 9:702-712.

34. Shantsila E, Watson T, Lip GY: Endothelial progenitor cells in cardiovascular disorders. *J Am Coll Cardiol* 2007, 49:741-752.

35. Fadini GP, Agostini C, Avogaro A: Characterization of endothelial progenitor cells. *Biochem Biophys Res Commun* 2005, 336:1-2.

36. Khakoo AY, Finkel T: Endothelial progenitor cells. *Annu Rev Med* 2005, 56:79-101.

37. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, et al.: Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997, 275:964-967.

38. Hristov M, Erl W, Weber PC: Endothelial progenitor cells: mobilization, differentiation, and homing. *Arterioscler Thromb Vasc Biol* 2003, 23:1185-1189.

39. Mohler ER, 3rd, Shi Y, Moore J, Bantly A, Hamamdzic D, Yoder M, et al.: Diabetes

reduces bone marrow and circulating porcine endothelial progenitor cells, an effect ameliorated by atorvastatin and independent of cholesterol. *Cytometry A* 2009, 75:75-82.

40. Aicher A, Heeschen C, Mildner-Rihm C, Urbich C, Ihling C, Technau-Ihling K, et al.: Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells. *Nat Med* 2003, 9:1370-1376.
41. Aicher A, Heeschen C, Dimmeler S: The role of NOS3 in stem cell mobilization. *Trends Mol Med* 2004, 10:421-425.
42. Westerweel PE, Visseren FL, Hajer GR, Olijhoek JK, Hoefer IE, de Bree P, et al.: Endothelial progenitor cell levels in obese men with the metabolic syndrome and the effect of simvastatin monotherapy vs. simvastatin/ezetimibe combination therapy. *Eur Heart J* 2008, 29:2808-2817.
43. Anderson TJ: Assessment and treatment of endothelial dysfunction in humans. *J Am Coll Cardiol* 1999, 34:631-638.
44. Laursen JB, Somers M, Kurz S, McCann L, Warnholtz A, Freeman BA, et al.: Endothelial regulation of vasomotion in apoE-deficient mice: implications for interactions between peroxynitrite and tetrahydrobiopterin. *Circulation* 2001, 103:1282-1288.
45. Alp NJ, Channon KM: Regulation of endothelial nitric oxide synthase by tetrahydrobiopterin in vascular disease. *Arterioscler Thromb Vasc Biol* 2004, 24:413-420.
46. O'Brien SF, Watts GF, Playford DA, Burke V, O'Neal DN, Best JD: Low-density lipoprotein size, high-density lipoprotein concentration, and endothelial dysfunction in non-insulin-dependent diabetes. *Diabet Med* 1997, 14:974-978.

47. Ma FX, Zhou B, Chen Z, Ren Q, Lu SH, Sawamura T, et al.: Oxidized low density lipoprotein impairs endothelial progenitor cells by regulation of endothelial nitric oxide synthase. *J Lipid Res* 2006, 47:1227-1237.

48. Hedblad B, Ogren M, Janzon L, Isacsson SO, Lindell SE: Low pulse-wave amplitude during reactive leg hyperaemia: an independent, early marker for ischaemic heart disease and death. Results from the 21-year follow-up of the prospective cohort study 'Men born in 1914', Malmo, Sweden. *J Intern Med* 1994, 236:161-168.

49. Perticone F, Ceravolo R, Pujia A, Ventura G, Iacopino S, Scozzafava A, et al.: Prognostic significance of endothelial dysfunction in hypertensive patients. *Circulation* 2001, 104:191-196.

50. Heitzer T, Schlinzig T, Krohn K, Meinertz T, Munzel T: Endothelial dysfunction, oxidative stress, and risk of cardiovascular events in patients with coronary artery disease. *Circulation* 2001, 104:2673-2678.

51. Gokce N, Keaney JF, Jr., Hunter LM, Watkins MT, Menzoian JO, Vita JA: Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. *Circulation* 2002, 105:1567-1572.

52. Vogel RA: Cholesterol lowering and endothelial function. *Am J Med* 1999, 107:479-487.

53. Taddei S, Virdis A, Ghiadoni L, Sudano I, Salvetti A: Effects of antihypertensive drugs on endothelial dysfunction: clinical implications. *Drugs* 2002, 62:265-284.

54. Celermajer DS, Sorensen KE, Georgakopoulos D, Bull C, Thomas O, Robinson J, et al.: Cigarette smoking is associated with dose-related and potentially reversible impairment of endothelium-dependent dilation in healthy young adults. *Circulation* 1993, 88:2149-2155.

55. Hambrecht R, Wolf A, Gielen S, Linke A, Hofer J, Erbs S, et al.: Effect of exercise on coronary endothelial function in patients with coronary artery disease. *N Engl J Med* 2000, 342:454-460.

56. Vita JA, Keaney JF, Jr.: Hormone replacement therapy and endothelial function: the exception that proves the rule? *Arterioscler Thromb Vasc Biol* 2001, 21:1867-1869.

57. Libby P, Ridker PM, Maseri A: Inflammation and atherosclerosis. *Circulation* 2002, 105:1135-1143.

58. Murohara T, Kugiyama K, Ohgushi M, Sugiyama S, Ohta Y, Yasue H: LPC in oxidized LDL elicits vasocontraction and inhibits endothelium- dependent relaxation. *Am J Physiol* 1994, 267:H2441-2449.

59. Ohgushi M, Kugiyama K, Fukunaga K, Murohara T, Sugiyama S, Miyamoto E, et al.: Protein kinase C inhibitors prevent impairment of endothelium-dependent relaxation by oxidatively modified LDL. *Arterioscler Thromb* 1993, 13:1525-1532.

60. Tamai O, Matsuoka H, Itabe H, Wada Y, Kohno K, Imaizumi T: Single LDL apheresis improves endothelium-dependent vasodilatation in hypercholesterolemic humans. *Circulation* 1997, 95:76-82.

61. Arnaud C, Mach F: Pleiotropic effects of statins in atherosclerosis: role on endothelial function, inflammation and immunomodulation. *Arch Mal Coeur Vaiss* 2005, 98:661-666.

62. Calabro P, Yeh ET: The pleiotropic effects of statins. *Curr Opin Cardiol* 2005, 20:541-546.

63. Lahera V, Goicoechea M, de Vinuesa SG, Miana M, de las Heras N, Cachofeiro V, et al.: Endothelial dysfunction, oxidative stress and inflammation in atherosclerosis: beneficial effects of statins. *Curr Med Chem* 2007, 14:243-248.

64. Athyros VG, Kakafika AI, Tziomalos K, Karagiannis A, Mikhailidis DP: Pleiotropic effects of statins--clinical evidence. *Curr Pharm Des* 2009, 15:479-489.

65. Ridker PM, Danielson E, Fonseca FA, Genest J, Gotto AM, Jr., Kastelein JJ, et al.: Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein. *N Engl J Med* 2008, 359:2195-2207.

66. Goldstein JL, Brown MS: Regulation of the mevalonate pathway. *Nature* 1990, 343:425-430.

67. Zhang FL, Casey PJ: Protein prenylation: molecular mechanisms and functional consequences. *Annu Rev Biochem* 1996, 65:241-269.

68. Casey PJ: Protein lipidation in cell signaling. *Science* 1995, 268:221-225.

69. Ju H, Zou R, Venema VJ, Venema RC: Direct interaction of endothelial nitric-oxide synthase and caveolin-1 inhibits synthase activity. *J Biol Chem* 1997, 272:18522-18525.

70. Mason RP, Walter MF, Jacob RF: Effects of HMG-CoA reductase inhibitors on endothelial function: role of microdomains and oxidative stress. *Circulation* 2004, 109:II34-41.

71. Haendeler J, Hoffmann J, Zeiher AM, Dimmeler S: Antioxidant effects of statins via S-nitrosylation and activation of thioredoxin in endothelial cells: a novel vasculoprotective function of statins. *Circulation* 2004, 110:856-861.

72. Maack C, Kartes T, Kilter H, Schafers HJ, Nickenig G, Bohm M, et al.: Oxygen free radical release in human failing myocardium is associated with increased activity of rac1-GTPase and represents a target for statin treatment. *Circulation* 2003, 108:1567-1574.

73. Wassmann S, Laufs U, Baumer AT, Muller K, Konkol C, Sauer H, et al.: Inhibition of

geranylgeranylation reduces angiotensin II-mediated free radical production in vascular smooth muscle cells: involvement of angiotensin AT1 receptor expression and Rac1 GTPase. *Mol Pharmacol* 2001, 59:646-654.

74. Bays HE, Neff D, Tomassini JE, Tershakovec AM: Ezetimibe: cholesterol lowering and beyond. *Expert Rev Cardiovasc Ther* 2008, 6:447-470.

75. Al Badarin FJ, Kullo IJ, Kopecky SL, Thomas RJ: Impact of ezetimibe on atherosclerosis: is the jury still out? *Mayo Clin Proc* 2009, 84:353-361.

76. Schachinger V, Britten MB, Zeiher AM: Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease. *Circulation* 2000, 101:1899-1906.

77. Suwaidi JA, Hamasaki S, Higano ST, Nishimura RA, Holmes DR, Jr., Lerman A: Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. *Circulation* 2000, 101:948-954.

78. Ludmer PL, Selwyn AP, Shook TL, Wayne RR, Mudge GH, Alexander RW, et al.: Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. *N Engl J Med* 1986, 315:1046-1051.

79. Furchgott RF: Role of endothelium in responses of vascular smooth muscle. *Circ Res* 1983, 53:557-573.

80. Vanhoutte PM, Rimele TJ: Role of the endothelium in the control of vascular smooth muscle function. *J Physiol (Paris)* 1982, 78:681-686.

81. Werns SW, Walton JA, Hsia HH, Nabel EG, Sanz ML, Pitt B: Evidence of endothelial dysfunction in angiographically normal coronary arteries of patients with coronary artery disease. *Circulation* 1989, 79:287-291.

82. Horio Y, Yasue H, Rokutanda M, Nakamura N, Ogawa H, Takaoka K, et al.: Effects

of intracoronary injection of acetylcholine on coronary arterial diameter. *Am J Cardiol* 1986, 57:984-989.

83. Deanfield J, Donald A, Ferri C, Giannattasio C, Halcox J, Halligan S, et al.: Endothelial function and dysfunction. Part I: Methodological issues for assessment in the different vascular beds: a statement by the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension. *J Hypertens* 2005, 23:7-17.

84. Wu HD, Katz SD, Beniaminovitz A, Khan T, DiTullio MR, Homma S: Assessment of endothelium-mediated vasodilation of the peripheral circulation by transcutaneous ultrasonography and venous occlusion plethysmography. *Heart Vessels* 1999, 14:143-148.

85. Lerman A, Zeiher AM: Endothelial function: cardiac events. *Circulation* 2005, 111:363-368.

86. Lind L, Fors N, Hall J, Marttala K, Stenborg A: A comparison of three different methods to evaluate endothelium-dependent vasodilation in the elderly: the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study. *Arterioscler Thromb Vasc Biol* 2005, 25:2368-2375.

87. Bulut D, Hanefeld C, Bulut-Streich N, Graf C, Mugge A, Spiecker M: Endothelial function in the forearm circulation of patients with the metabolic syndrome--effect of different lipid-lowering regimens. *Cardiology* 2005, 104:176-180.

88. Olijhoek JK, Hajer GR, van der Graaf Y, Dallinga-Thie GM, Visseren FL: The effects of low-dose simvastatin and ezetimibe compared to high-dose simvastatin alone on post-fat load endothelial function in patients with metabolic syndrome: a randomized double-blind crossover trial. *J Cardiovasc Pharmacol* 2008, 52:145-150.

89. Settergren M, Bohm F, Ryden L, Pernow J: Cholesterol lowering is more important than pleiotropic effects of statins for endothelial function in patients with dysglycaemia and coronary artery disease. *Eur Heart J* 2008, 29:1753-1760.

90. Gounari P, Tousoulis D, Antoniades C, Kampoli AM, Stougiannos P, Papageorgiou N, et al.: Rosuvastatin but not ezetimibe improves endothelial function in patients with heart failure, by mechanisms independent of lipid lowering. *Int J Cardiol* 2009.

91. Landmesser U, Bahlmann F, Mueller M, Spiekermann S, Kirchhoff N, Schulz S, et al.: Simvastatin versus ezetimibe: pleiotropic and lipid-lowering effects on endothelial function in humans. *Circulation* 2005, 111:2356-2363.

92. Ostad MA, Eggeling S, Tschentscher P, Schwedhelm E, Boger R, Wenzel P, et al.: Flow-mediated dilation in patients with coronary artery disease is enhanced by high dose atorvastatin compared to combined low dose atorvastatin and ezetimibe: results of the CEZAR study. *Atherosclerosis* 2009, 205:227-232.

93. Fichtlscherer S, Schmidt-Lucke C, Bojunga S, Rossig L, Heeschen C, Dimmeler S, et al.: Differential effects of short-term lipid lowering with ezetimibe and statins on endothelial function in patients with CAD: clinical evidence for 'pleiotropic' functions of statin therapy. *Eur Heart J* 2006, 27:1182-1190.

94. Kuhlencordt PJ, Padmapriya P, Rutzel S, Schodel J, Hu K, Schafer A, et al.: Ezetimibe potently reduces vascular inflammation and arteriosclerosis in eNOS-deficient ApoE ko mice. *Atherosclerosis* 2009, 202:48-57.

95. Tsunekawa T, Hayashi T, Kano H, Sumi D, Matsui-Hirai H, Thakur NK, et al.: Cerivastatin, a hydroxymethylglutaryl coenzyme a reductase inhibitor, improves endothelial function in elderly diabetic patients within 3 days. *Circulation* 2001, 104:376-379.

96. Birnbaum Y, Lin Y, Ye Y, Merla R, Perez-Polo JR, Uretsky BF: Pretreatment with high-dose statin, but not low-dose statin, ezetimibe, or the combination of low-dose statin and ezetimibe, limits infarct size in the rat. *J Cardiovasc Pharmacol Ther* 2008, 13:72-79.

97. Nakagami H, Osako MK, Takami Y, Hanayama R, Koriyama H, Mori M, et al.: Vascular protective effects of ezetimibe in ApoE-deficient mice. *Atherosclerosis* 2009, 203:51-58.

98. Vallance P, Leiper J: Cardiovascular biology of the asymmetric dimethylarginine:dimethylarginine dimethylaminohydrolase pathway. *Arterioscler Thromb Vasc Biol* 2004, 24:1023-1030.

99. Ueda S, Yamagishi S, Matsumoto Y, Fukami K, Okuda S: Asymmetric dimethylarginine (ADMA) is a novel emerging risk factor for cardiovascular disease and the development of renal injury in chronic kidney disease. *Clin Exp Nephrol* 2007, 11:115-121.

100. Nakamura T, Sato E, Fujiwara N, Kawagoe Y, Ueda Y, Suzuki T, et al.: Ezetimibe decreases serum levels of asymmetric dimethylarginine (ADMA) and ameliorates renal injury in non-diabetic chronic kidney disease patients in a cholesterol-independent manner. *Pharmacol Res* 2009.

101. Lin KY, Ito A, Asagami T, Tsao PS, Adimoolam S, Kimoto M, et al.: Impaired nitric oxide synthase pathway in diabetes mellitus: role of asymmetric dimethylarginine and dimethylarginine dimethylaminohydrolase. *Circulation* 2002, 106:987-992.

102. Edelberg JM, Tang L, Hattori K, Lyden D, Rafii S: Young adult bone marrow-derived endothelial precursor cells restore aging-impaired cardiac angiogenic function. *Circ Res* 2002, 90:E89-93.

103. Vasa M, Fichtlscherer S, Aicher A, Adler K, Urbich C, Martin H, et al.: Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. *Circ Res* 2001, 89:E1-7.

104. Chen JZ, Zhang FR, Tao QM, Wang XX, Zhu JH: Number and activity of endothelial progenitor cells from peripheral blood in patients with hypercholesterolaemia. *Clin Sci (Lond)* 2004, 107:273-280.

105. Hill JM, Zalos G, Halcox JP, Schenke WH, Waclawiw MA, Quyyumi AA, et al.: Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. *N Engl J Med* 2003, 348:593-600.

106. Steinberg D: Low density lipoprotein oxidation and its pathobiological significance. *J Biol Chem* 1997, 272:20963-20966.

107. Imanishi T, Hano T, Matsuo Y, Nishio I: Oxidized low-density lipoprotein inhibits vascular endothelial growth factor-induced endothelial progenitor cell differentiation. *Clin Exp Pharmacol Physiol* 2003, 30:665-670.

108. Imanishi T, Hano T, Sawamura T, Nishio I: Oxidized low-density lipoprotein induces endothelial progenitor cell senescence, leading to cellular dysfunction. *Clin Exp Pharmacol Physiol* 2004, 31:407-413.

109. Wang X, Chen J, Tao Q, Zhu J, Shang Y: Effects of ox-LDL on number and activity of circulating endothelial progenitor cells. *Drug Chem Toxicol* 2004, 27:243-255.

110. Fulton D, Gratton JP, McCabe TJ, Fontana J, Fujio Y, Walsh K, et al.: Regulation of endothelium-derived nitric oxide production by the protein kinase Akt. *Nature* 1999, 399:597-601.

111. Thum T, Tsikas D, Stein S, Schultheiss M, Eigenthaler M, Anker SD, et al.: Suppression of endothelial progenitor cells in human coronary artery disease by the

endogenous nitric oxide synthase inhibitor asymmetric dimethylarginine. *J Am Coll Cardiol* 2005, 46:1693-1701.

112. Llevadot J, Murasawa S, Kureishi Y, Uchida S, Masuda H, Kawamoto A, et al.: HMG-CoA reductase inhibitor mobilizes bone marrow--derived endothelial progenitor cells. *J Clin Invest* 2001, 108:399-405.

113. Dimmeler S, Aicher A, Vasa M, Mildner-Rihm C, Adler K, Tiemann M, et al.: HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase/Akt pathway. *J Clin Invest* 2001, 108:391-397.

114. Li X, Han Y, Pang W, Li C, Xie X, Shyy JY, et al.: AMP-activated protein kinase promotes the differentiation of endothelial progenitor cells. *Arterioscler Thromb Vasc Biol* 2008, 28:1789-1795.

115. Minami Y, Satoh M, Maesawa C, Takahashi Y, Tabuchi T, Itoh T, et al.: Effect of atorvastatin on microRNA 221 / 222 expression in endothelial progenitor cells obtained from patients with coronary artery disease. *Eur J Clin Invest* 2009, 39:359-367.

116. Rattazzi M, Puato M, Faggin E, Bertipaglia B, Zambon A, Pauletto P: C-reactive protein and interleukin-6 in vascular disease: culprits or passive bystanders? *J Hypertens* 2003, 21:1787-1803.

117. Venugopal SK, Devaraj S, Yuhanna I, Shaul P, Jialal I: Demonstration that C-reactive protein decreases eNOS expression and bioactivity in human aortic endothelial cells. *Circulation* 2002, 106:1439-1441.

118. Pearson TA, Ballantyne CM, Veltri E, Shah A, Bird S, Lin J, et al.: Pooled analyses of effects on C-reactive protein and low density lipoprotein cholesterol in placebo-controlled trials of ezetimibe monotherapy or ezetimibe added to baseline

statin therapy. *Am J Cardiol* 2009, 103:369-374.

119. Efrati S, Averbukh M, Dishy V, Faygenzo M, Friedensohn L, Golik A: The effect of simvastatin, ezetimibe and their combination on the lipid profile, arterial stiffness and inflammatory markers. *Eur J Clin Pharmacol* 2007, 63:113-121.

120. Ballantyne CM, Weiss R, Moccetti T, Vogt A, Eber B, Sosef F, et al.: Efficacy and safety of rosuvastatin 40 mg alone or in combination with ezetimibe in patients at high risk of cardiovascular disease (results from the EXPLORER study). *Am J Cardiol* 2007, 99:673-680.

121. Catapano AL, Davidson MH, Ballantyne CM, Brady WE, Gazzara RA, Tomassini JE, et al.: Lipid-altering efficacy of the ezetimibe/simvastatin single tablet versus rosuvastatin in hypercholesterolemic patients. *Curr Med Res Opin* 2006, 22:2041-2053.

122. Nomura M, Ishii H, Kawakami A, Yoshida M: Inhibition of Hepatic Neiman-Pick C1-Like 1 Improves Hepatic Insulin Resistance. *Am J Physiol Endocrinol Metab* 2009.

123. Assy N, Grozovski M, Bersudsky I, Szvalb S, Hussein O: Effect of insulin-sensitizing agents in combination with ezetimibe, and valsartan in rats with non-alcoholic fatty liver disease. *World J Gastroenterol* 2006, 12:4369-4376.

124. Hussein O, Minasian L, Itzkovich Y, Shestatski K, Solomon L, Zidan J: Ezetimibe's effect on platelet aggregation and LDL tendency to peroxidation in hypercholesterolaemia as monotherapy or in addition to simvastatin. *Br J Clin Pharmacol* 2008, 65:637-645.

125. Maki-Petaja KM, Booth AD, Hall FC, Wallace SM, Brown J, McEniery CM, et al.: Ezetimibe and simvastatin reduce inflammation, disease activity, and aortic stiffness

and improve endothelial function in rheumatoid arthritis. *J Am Coll Cardiol* 2007, 50:852-858.

126. Kastelein JJ, Akdim F, Stroes ES, Zwinderman AH, Bots ML, Stalenhoef AF, et al.: Simvastatin with or without ezetimibe in familial hypercholesterolemia. *N Engl J Med* 2008, 358:1431-1443.

127. Fleg JL, Mete M, Howard BV, Umans JG, Roman MJ, Ratner RE, et al.: Effect of statins alone versus statins plus ezetimibe on carotid atherosclerosis in type 2 diabetes: the SANDS (Stop Atherosclerosis in Native Diabetics Study) trial. *J Am Coll Cardiol* 2008, 52:2198-2205.

128. Rossebo AB, Pedersen TR, Boman K, Brudi P, Chambers JB, Egstrup K, et al.: Intensive lipid lowering with simvastatin and ezetimibe in aortic stenosis. *N Engl J Med* 2008, 359:1343-1356.

129. Malmstrom RE, Settergren M, Bohm F, Pernow J, Hjemdahl P: No effect of lipid lowering on platelet activity in patients with coronary artery disease and type 2 diabetes or impaired glucose tolerance. *Thromb Haemost* 2009, 101:157-164.

130. Alvarez-Sala LA, Cachofeiro V, Masana L, Suarez C, Pinilla B, Plana N, et al.: Effects of fluvastatin extended-release (80 mg) alone and in combination with ezetimibe (10 mg) on low-density lipoprotein cholesterol and inflammatory parameters in patients with primary hypercholesterolemia: A 12-week, multicenter, randomized, open-label, parallel-group study. *Clin Ther* 2008, 30:84-97.

131. Ballantyne CM, Abate N, Yuan Z, King TR, Palmisano J: Dose-comparison study of the combination of ezetimibe and simvastatin (Vytorin) versus atorvastatin in patients with hypercholesterolemia: the Vytorin Versus Atorvastatin (VYVA) study. *Am Heart J* 2005, 149:464-473.

132. Robinson JG, Ballantyne CM, Grundy SM, Hsueh WA, Parving HH, Rosen JB, et al.: Lipid-altering efficacy and safety of ezetimibe/simvastatin versus atorvastatin in patients with hypercholesterolemia and the metabolic syndrome (from the VYMET study). *Am J Cardiol* 2009, 103:1694-1702.

133. Leiter LA, Bays H, Conard S, Bird S, Rubino J, Hanson ME, et al.: Efficacy and safety of ezetimibe added on to atorvastatin (40 mg) compared with uptitration of atorvastatin (to 80 mg) in hypercholesterolemic patients at high risk of coronary heart disease. *Am J Cardiol* 2008, 102:1495-1501.

134. Conard SE, Bays HE, Leiter LA, Bird SR, Rubino J, Lowe RS, et al.: Efficacy and safety of ezetimibe added on to atorvastatin (20 mg) versus uptitration of atorvastatin (to 40 mg) in hypercholesterolemic patients at moderately high risk for coronary heart disease. *Am J Cardiol* 2008, 102:1489-1494.

135. Sager PT, Capece R, Lipka L, Strony J, Yang B, Suresh R, et al.: Effects of ezetimibe coadministered with simvastatin on C-reactive protein in a large cohort of hypercholesterolemic patients. *Atherosclerosis* 2005, 179:361-367.

136. Goldberg AC, Sapre A, Liu J, Capece R, Mitchel YB: Efficacy and safety of ezetimibe coadministered with simvastatin in patients with primary hypercholesterolemia: a randomized, double-blind, placebo-controlled trial. *Mayo Clin Proc* 2004, 79:620-629.

137. Bays HE, Ose L, Fraser N, Tribble DL, Quinto K, Reyes R, et al.: A multicenter, randomized, double-blind, placebo-controlled, factorial design study to evaluate the lipid-altering efficacy and safety profile of the ezetimibe/simvastatin tablet compared with ezetimibe and simvastatin monotherapy in patients with primary hypercholesterolemia. *Clin Ther* 2004, 26:1758-1773.

Figure legends

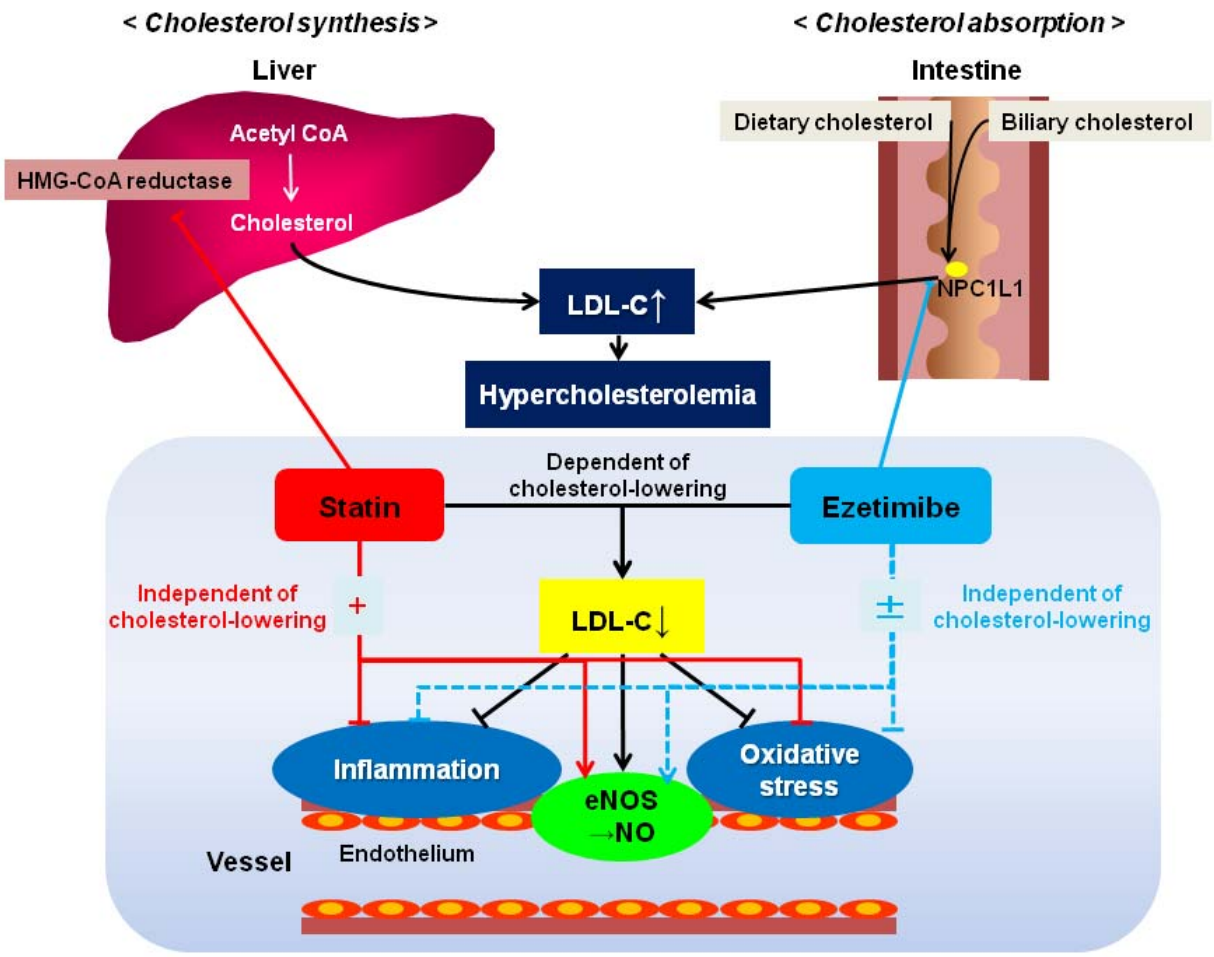

Figure 1. Effects of ezetimibe on vascular endothelium.

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.

Table 1. Effects of ezetimibe on clinical evaluation of endothelial function.

Trial	Methods	Population	Demographics (Age, %male)	Intervention (Medicines, Period)	Findings and conclusions
Bulut et al. [87]	Switch FBF	14 patients with Mets	62.4 ± 10.7 years Male: 100%	Atorvastatin 40 mg/day; > 4 weeks ↓ Atorvastatin 10 mg/day + ezetimibe 10 mg/day 8 weeks	Switching from atorvastatin. monothrapy to atorvastatin and ezetimibe enhanced the increase of FBF.
Olijhoek et al. [88]	R, DB, CO FMD	19 patients with Mets	54 ± 7 years Male: 100%	Simvastatin 80 mg/day Simvastatin 10 mg/day + ezetimibe 10 mg/day 6 weeks each arm	The combination of simvastatin and ezetimibe did not decrease post-fat load FMD, contrary to simvastatin monotherapy.
Gounari et al. [90]	R, DB, CO FMD	22 patients with CHF	60.6 ± 2.6 years, Male 91%	Rosuvastatin 10 mg/day Ezetimibe 20 mg/day 4 weeks each arm	The treatment of rosuvastatin, but not ezetimibe, improved FMD.
Mäki-Petäjä et al. [125]	R, DB, CO FMD	20 patients with RA	58 ± 12 years, Male 20%	Simvastatin 20 mg/day Ezetimibe 10 mg/day 6 weeks each arm	The improvement in FMD by simvastatin was greater, but not significant, than that by ezetimibe.

Trial	Methods	Population	Protocol, Demographics	Endothelial function
Ostad et al. [92]	R, DB FMD	49 patients with CAD	Atorvastatin 80 mg/day (24 patients) 66 ± 9 years, Male 79% Atorvastatin 10 mg/day + ezetimibe 10 mg/day (25 patients) 64 ± 10 years, Male 76% 8 weeks	The improvement in FMD by atorvastatin was greater than the combination therapy.
Settergren et al. [89]	R, DB FMD	39 patients with CAD and IGT/DM	Simvastatin 80 mg/day (20 patients) 70 years, Male 75% Simvastatin 10 mg/day + ezetimibe 10 mg/day (19 patients) 74 years, Male 58% 6 weeks	There was no difference in the increase of FMD between the two treatment groups.
Fichtlscherer et al. [93]	R FBF	60 patients with CAD	Ezetimibe 10 mg/day *de novo* (15 patients) 53.2 ± 4.3 years, Male: 100% Ezetimibe 10 mg/day added on simvastatin 20 mg/day (15 patients) 55.2 ± 6.6 years, Male: 67% Atorvastatin 10 mg/day→40 mg/day (15 patients) 56.5 ± 3.9 years, Male: 80% Atorvastatin 40 mg/day *de novo* (15 patients) 56.5 ± 2.8 years, Male: 100% 4 weeks	Only atorvastatin monotherapy improved FBF

Landmesser et al. [91]	R FDD	20 patients with CHF	Simvastatin 10 mg/day (10 patients) 59 ± 3 years, Male 80% Ezetimibe 10 mg/day (10 patients) 59 ± 4 years, Male 70% 4 weeks	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.

Trial	Patients	Period	Medicine	Changes in LDL-C	Changes in CRP
Statin monotherapy = Combination therapy with statin and ezetimibe					
Malmström et al. [129]	32 patients with coronary artery disease	6 weeks	Sim 80 mg Sim 10 mg + Ez 10 mg	3.0 ± 1.0→1.4 ± 0.5 mM 3.2 ± 0.6→1.7 ± 0.7 mM	3.8 ± 3.2→3.2 ± 2.9 mg/L 4.8 ± 4.5→4.0 ± 4.0 mg/L
Ostad et al. [92]	58 patients with coronary artery disease	8 weeks	Ator 80 mg Ator 10 mg + Ez 10 mg	-60 ± 11% -54 ± 18%	-1.9 ± 1.9% -1.8 ± 2.1%
Alvarez-Sala et al. [130]	82 patients with hypercholesterolemia	12 weeks	Flu 80 mg Flu 80 mg + Ez 10 mg	-35.2% -49.9%*	1.9 ± 1.9→1.9 ± 2.2 mg/L 2.6 ± 2.3→2.1 ± 1.9 mg/L
Catapano et al. [121]	2,959 patients with hypercholesterolemia	6 weeks	Rosu 10, 20 mg Sim 20, 40 mg + Ez 10 mg	-46 ~ 57% -52 ~ 61%*	-22.2% -25.0%
Ballantyne et al. [131]	1,902 patients with hypercholesterolemia	6 weeks	Ator 10, 20, 40, 80 mg Sim 10, 20, 40, 80 mg + Ez 10 mg	-45.3% -53.4%*	-25.1% -24.8%
Robinson et al. [132]	1,128 patients with hypercholesterolemia and metabolic syndrome	6 weeks	Ator 10, 20 mg Sim 20 mg + Ez 10 mg Ator 40 mg Sim 40 mg + Ez 10 mg	-36.5 ~ 39.4% -49.6%* -46.0% -53.9%*	-16.8~22.4% -17.2% -30.0% -27.6%
Leiter et al. [133]	579 patients with hypercholesterolemia	6 weeks	Ator 40 mg → 80 mg (+40 mg) Ator 40 mg + Ez 10 mg	-11% -27%*	-11% -18%
Conard et al. [134]	196 patients with hypercholesterolemia	6 weeks	Ator 20 mg → 40 mg (+20 mg) Ator 20 mg + Ez 10 mg	-11% -31%*	-9% -7%

Statin monotherapy > Combination therapy with statin and ezetimibe					
Efrati et al. [119]	40 patients with hypercholesterolemia	3 months	Sim 40 mg Sim 40 → 80 mg Ez 10 mg Sim 40 mg + Ez 10 mg	-40 ± 4%‡ -17 ± 19% -18 ± 13% -36 ± 17%‡	2.8 ± 2.5 →1.6 ± 1.5 mg/L‡ 1.98 ± 2.2 →1.2 ± 0.8 mg/L 2.4 ± 1.2 →2.7 ± 2.2 mg/L 2.1 ± 1.8 →1.57 ± 1.2 mg/L
Combination therapy with statin and ezetimibe > Statin monotherapy					
Ballantyne et al. [120]	469 patients with hypercholesterolemia	6 weeks	Rosu 40 mg Rosu 40 mg + Ez 10 mg	-57% -70%*	-29% -46%*
Sager et al. [135]	1,089 patients with hypercholesterolemia	12 weeks	Sim 10, 20, 40, 80 mg Sim 10, 20, 40, 80 mg + Ez 10 mg	-37.5% -52.3%†	-14.3% -33.3%†
Goldberg et al. [136]	887 patients with hypercholesterolemia	12 weeks	Sim 10, 20, 40, 80 mg Sim 10, 20, 40, 80 mg + Ez 10 mg	-38.5% -53.2%*	-8.7% -33.3%*
Bays et al. [137]	1,528 patients with hypercholesterolemia	12 weeks	Sim 10, 20, 40, 80 mg Sim 10, 20, 40, 80 mg + Ez 10 mg	-39.0% -53.0%*	-16.7% -31.0%*
Ballantyne et al. [14]	628 patients with hypercholesterolemia	12 weeks	Ator 10, 20, 40, 80 mg Ator 10, 20, 40, 80 mg + Ez 10 mg	-42.4 ± 0.95% -54.5 ± 0.94%†	-31% -41%†
Gagné et al. [16]	769 patients with hypercholesterolemia	8 weeks	Statins + placebo Statins + Ez 10 mg	-3.7% -25.0% *	0% -9.7%‡

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