Does reversal of oxidative stress and inflammation provide vascular protection?

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Chronic inflammation is a pathogenic feature of atherosclerosis and cardiovascular disease mediated by substances including angiotensin II, proinflammatory cytokines, and free fatty acids. This promotes generation of reactive oxygen species in vascular endothelial cells and smooth muscle cells, which mediate injury through several mechanisms. Reciprocal relationships between endothelial dysfunction and insulin resistance as well as cross-talk between hyperlipidaemia and the renin–angiotensin–aldosterone system (RAAS) at multiple levels contribute importantly to a variety of risk factors. Therefore, combination therapy that simultaneously addresses multiple mechanisms for the pathogenesis of atherosclerosis is an attractive emerging concept for slowing progression of atherosclerosis. Combined therapy with statins, peroxisome proliferator-activated receptors, and RAAS blockade demonstrates additive beneficial effects on endothelial dysfunction and insulin resistance when compared with monotherapies in patients with cardiovascular risk factors due to both distinct and interrelated mechanisms. These additive beneficial effects of combined therapies are consistent with laboratory and recent clinical studies. Thus, combination therapy may be an important paradigm for treating and slowing progression of atherosclerosis, coronary heart disease, and co-morbid metabolic disorders characterized by endothelial dysfunction and insulin resistance.

KEYWORDS
Inflammation; Oxidative stress; Atherosclerosis; Insulin resistance; Combination therapy

1. Introduction
Systemic hypertension, hypercholesterolaemia, and diabetes are associated with endothelial dysfunction that promotes inflammation, oxidation of lipoproteins, smooth muscle proliferation, extracellular matrix deposition or lysis, accumulation of lipid-rich material, platelet activation, thrombus formation, and insulin resistance. All of these consequences of endothelial dysfunction and insulin resistance may contribute to development and clinical expression of atherosclerosis.1–3 Chronic inflammation and oxidative stress play crucial roles in endothelial dysfunction, insulin resistance, and atherosclerosis.3,4 In this review, we discuss the role of inflammation and oxidative stress to link endothelial dysfunction and insulin resistance in cardiovascular disease. Moreover, we evaluate therapeutic interventions with cardiovascular drugs, insulin sensitizers, and combination therapies that have important antioxidant and anti-inflammatory actions.

2. Role of NOX family enzymes in reactive oxygen species production
Reactive oxygen species (ROS) have been implicated in the initiation and progression of atherosclerosis. ROS can oxidize lipoproteins, limit the vascular availability of anti-atherosclerotic nitric oxide (NO), and promote vascular expression of cytokines and adhesion molecules. Nox proteins of the NADPH oxidase family are prominent sources of vascular ROS, and Nox protein-dependent ROS production has been linked to atherogenesis. Six homologues of the cytochrome subunit of the phagocyte NADPH oxidase were found: NOX1, NOX3, NOX4, NOX5, DUOX1, and DUOX2. Together with the phagocyte NADPH oxidase itself (NOX2/ gp91phox), the homologues are now referred to as the NOX family of NADPH oxidases. These enzymes share the capacity to transport electrons across the plasma membrane and to generate superoxide and other downstream ROS. Members of the Nox1-4 subfamily in animals form a stable heterodimer with the membrane protein p22phox, which functions as a docking site for the SH3 domain-containing regulatory proteins p47phox, p67phox, and p40phox; the small

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3. Inflammation and oxidative stress in endothelial dysfunction and insulin resistance

Endothelial dysfunction is characterized by decreased bioavailability of NO. This may be due, in part, to enhanced NO catabolism secondary to increased superoxide anion production. Endothelial dysfunction is also characterized by increased synthesis and secretion of endothelin-1 from endothelial cells. One mechanism underlying this is oxidative stress, which generates a strong stimulus for increased expression of endothelin-1 in vascular smooth muscle and endothelial cells, resulting in enhanced vasoconstrictor tone and release of proinflammatory proteins. Transcription of many proinflammatory proteins is regulated by the nuclear transcription factor NF-κB. Plausible mechanisms for these actions include various substances, such as Ang II, proinflammatory cytokines, and free fatty acids, which promote ROS generation in vascular endothelial cells and smooth muscle cells in response to injury through several mechanisms. Oxygen-derived free radicals activate NF-κB to stimulate transcription of proinflammatory genes in the nucleus. This results in synthesis of protein products such as cell adhesion molecules, cytokines, and chemokines. In transgenic rats, increased Ang II type I (AT1) receptor/NADPH oxidase activation/ROS contribute to vascular insulin resistance, endothelial dysfunction, apoptosis, and inflammation. Under these conditions, the endogenous NO synthase inhibitor asymmetrical dimethylarginine reduces insulin sensitivity, consistent with previous observations that NO plays a role in insulin sensitivity. Importantly, elevated levels of free fatty acids associated with insulin resistance, obesity, diabetes, and the metabolic syndrome cause endothelial dysfunction by activating innate immune inflammatory pathways upstream of NF-κB. Thus, inflammation and oxidative stress contribute to endothelial dysfunction and insulin resistance while endothelial dysfunction and insulin resistance promote oxidative stress and inflammation.

4. Pharmacological interventions to reverse oxidative stress and inflammation

Hypercholesterolaemia and hypertension are major risk factors for atherosclerosis. Their coexistence is associated with an increased incidence of cardiac events. Common mechanisms related to both hypercholesterolaemia and hypertension increase ROS production and decrease antioxidant capacity, leading to increased oxidative stress that may adversely affect vascular function. Further, hypercholesterolaemia and hypertension have synergistic deleterious effects on coronary endothelial function. With both of these conditions present, the increase in oxidative stress is more pronounced when compared with each risk factor alone. Chronic antioxidant supplementation with vitamin C and E significantly improves coronary artery vasoreactivity in pigs.

4.1 Renin–angiotensin–aldosterone system blockades

4.1.1 Experimental evidence

Ang II produces ROS such as superoxide anions, hydrogen peroxide, and hydroxyl radicals, which exert multiple effects on the cardiovascular system including hypertension and cardiovascular hypertrophy. Ang II-induced hypertension accelerates atherosclerosis to a greater extent than either atherogenic diet or standard diet and Ang II in apo E-deficient mice. Oxidized low-density lipoprotein (LDL) upregulates AT1 receptor (but not AT2 receptor) mRNA and protein in human coronary artery endothelial cells. Oxidized LDL activates NF-κB. Treatment of cells with the antioxidant α-tocopherol attenuates oxidized LDL-mediated activation of NF-κB and inhibits upregulation of AT1 receptor mRNA and protein. Incubation of cells with both oxidized LDL and Ang II increases cell injury and lactic dehydrogenase release when compared with either oxidized LDL or Ang II alone. α-Tocopherol as well as candesartan attenuates cell-injurious effects of oxidized LDL. AT1 receptor blocker (ARB) diminishes intracellular production of superoxide anions via reduced activity of Ang II-dependent oxidases in the endothelium and vascular smooth muscle. This protects NO from oxidative degradation to biologically inert or toxic molecules. Inhibition of the production of superoxide anions may also limit oxidation of LDL. This will contribute to increased NO bioactivity by enhancing NO synthesis and limiting oxidative degradation of NO. In this regard, angiotensin-converting enzyme (ACE)-inhibitors and ARBs inhibit LDL oxidation and attenuate atherosclerosis independent of lowering BP. In addition to improving vasomotor tone, increased NO bioactivity within the vasculature may prevent activation of proinflammatory transcription factors and thus reduce synthesis of inflammatory chemokines.

4.1.2 Clinical evidence

ACE-inhibitor therapy with quinapril improves endothelium-dependent vasodilator responsiveness by increasing NO bioactivity in vascular smooth muscle in patients with coronary artery disease. Under these conditions, NO release is stimulated via bradykinin and superoxide anion production is decreased by the inhibition of the NADPH oxidase. Indeed, ACE-inhibitor reduces Ang II-induced oxidant stress.
within the vessel wall and protects NO from oxidative inactivation.\textsuperscript{27} In hypertensive patients, Ang II infusion leads to a rapid increase in plasma intercellular adhesion molecule-1 via AT1 receptor-dependent mechanisms.\textsuperscript{28} We investigated the effects of candesartan in hypertensive patients.\textsuperscript{29,30} Candesartan therapy significantly reduces plasma levels of malondialdehyde levels, improves percent flow-mediated dilator response to hyperaemia, and reduces plasma levels of monocyte chemoattractant protein-1, tumour necrosis factor-\(\alpha\), and soluble CD40 ligand. Of interest, there are no significant correlations between these changes and reduction of systolic and diastolic BP.\textsuperscript{30} Our studies demonstrate that inhibition of renin–angiotensin–aldosterone system (RAAS) improves oxidative stress and inflammatory markers independent of BP-lowering effects. Different antihypertensive drugs have distinct effects. Losartan therapy significantly decreases collagen volume fraction.\textsuperscript{31} The effects of losartan and atenolol were investigated on resistance artery abnormalities in patients with essential hypertension.\textsuperscript{32} Both treatments for 1 year attenuated. Cerivastatin may act by inhibiting prenylation, membrane anchoring, and subsequent activation of Rac proteins.\textsuperscript{41} FOXO (Forkhead O) transcription factors are a direct target of phosphatidylinositol-3 kinase/Akt signalling in skeletal and smooth muscle and regulate the expression of the Cip/Kip family of cyclin kinase inhibitors in other cell types. Lovastatin specifically recruits the forkhead box FoxO3a transcription factor to the cell cycle inhibitor p21 promoter, mediating transcriptional transactivation of the p21 gene as analysed in isolated primary cardiomyocytes. Lovastatin also stimulates protein kinase B/Akt kinase activity, and Akt-dependent phosphorylation promotes translocation of p21 into the cytoplasm, leading to inhibition of Rho-kinases. This contributes to suppression of cardiomyocyte hypertrophy, p21 function is a downstream target of FoxO3a that helps to mediate anti-hypertrophic responses to statins.\textsuperscript{42}

Therefore, combined therapy with statins and RAAS blockade may have additive beneficial effects on inhibition of oxygen-derived free radical production, inhibition of NF-\(\kappa\)B and inflammatory protein production, and improvement in NO bioactivity. Combined therapy with statins and RAAS blockade may have additive beneficial effects on endothelial function, insulin resistance, and atherosclerosis.\textsuperscript{43,44} Indeed, in apolipoprotein E null mice fed with a high-cholesterol diet, neither valsartan nor fluvastatin had any effect on BP or cholesterol concentration. However, therapy with both drugs together decreases plaque area and lipid deposition after 10 weeks. Similar inhibitory effects of valsartan or fluvastatin on expressions of nicotinamide-adenine dinucleotide/nicotinamide-adenine dinucleotide phosphate oxidase subunits p22phox and p47phox, production of superoxide anion, expression of monocyte chemoattractant protein-1, and intercellular adhesion molecule-1 expression are observed.\textsuperscript{44} These results suggest that concomitant statin and ARB treatment blunts oxidative stress and inflammation independent of BP or cholesterol-related effects.

### 4.2 Cross-talk between statins and renin–angiotensin–aldosterone system

#### 4.2.1 Experimental evidence

LDL induces expression of AT1 receptor in hypercholesterolaemic rabbits where enhanced vascular expression of AT1 receptors mediates increased activity of Ang II.\textsuperscript{34,35} Hypercholesterolaemia increases Ang II-induced BP elevation. AT1 receptor expression is significantly enhanced in hypercholesterolaemic individuals when compared with normocholesterolaemic men. Cholesterol-lowering treatment with statins to reverse the elevated BP response to Ang II infusion is accompanied by downregulated AT1 receptor density.\textsuperscript{36} Simvastatin may have a beneficial effect on cardiovascular alterations through its antioxidant action in experimental Ang II-infused rats. Simvastatin prevents superoxide anion and hydrogen peroxide production and increases the heart weight index and carotid cross-sectional area associated with Ang II infusion.\textsuperscript{37} RAAS blockade inhibits binding of Ang II to AT1 receptors. This results in decreased production of oxygen-derived free radicals. Statins inhibit the expression of AT1 receptor upregulation. Further, statins inhibit the production of oxygen-derived free radicals by reducing LDL, increasing NO synthesis, and through antioxidant effects.\textsuperscript{1} Statins also have an indirect NOX inhibitory action through inhibition of Rac isoprenylation.\textsuperscript{38} Small GTP-binding proteins, in particular Rac1, may play a key role in the development of cardiac hypertrophy. Statins block the isoprenylation and activation of members of the Rho family, such as RhoA and Rac1. Rac1 also regulates NADPH oxidase, which is a major source of ROS in cardiovascular cells. Statins attenuate oxidative stress through inhibition of Rac1.\textsuperscript{39} Atorvastatin blunts the expression of membrane subunit gp91phox and prevents the translocation of cytoplasmic subunit p47phox to the membrane in the penumbra 2 h after reperfusion. Consequently, cerebral infarct volume is significantly reduced in atorvastatin-treated rats. These results indicate that atorvastatin protects against cerebral infarction via inhibition of NADPH oxidase-derived superoxide in transient focal ischaemia.\textsuperscript{40}
4.2.2 Clinical evidence
We reported outcomes of vascular and metabolic responses to either statin and RAAS blockade alone or in combination in hypertensive, hypercholesterolaemic patients. Losartan alone, simvastatin alone, or combined therapy significantly improves flow-mediated dilator response to hyperaemia and decreases plasma oxidant stress and inflammation relative to baseline measurements. However, these parameters are changed to a greater extent with combined therapy when compared with simvastatin or losartan alone (Figure 1A). Of interest, combined therapy or losartan alone significantly increases plasma adiponectin levels and insulin sensitivity relative to baseline measurements. These changes are significantly greater than those observed in the group treated with simvastatin alone (Figure 1B). This study demonstrates that simvastatin combined with losartan improves endothelial function, reduces inflammatory markers, and improves insulin sensitivity to a greater extent than monotherapy with either drug in hypercholesterolaemic, hypertensive patients. In another study, additive beneficial effects of combined therapy with statin and ACE-inhibitor, ramipril, were demonstrated in hypercholesterolaemic patients and patients with type 2 diabetes. Ramipril alone, simvastatin alone, or combined therapy treatment arms significantly improve flow-mediated dilator response to hyperaemia and reduce plasma levels of malondialdehyde relative to baseline measurements. However, these parameters were changed to a greater extent with combined therapy when compared with simvastatin or ramipril alone. When compared with simvastatin or ramipril alone, combined therapy significantly reduces high-sensitivity C-reactive protein levels. Interestingly, combined therapy or ramipril alone significantly increases plasma adiponectin levels and insulin sensitivity relative to baseline measurements. These changes are significantly greater than in the group treated with simvastatin alone.

Figure 1 (A) Percent change in malondialdehyde levels from respective pre-treatment values after treatment with simvastatin alone, combined therapy, and losartan alone. Percent change in flow-mediated dilation (FMD) from respective pre-treatment values after treatment with simvastatin alone, combined therapy, and losartan alone. The combined therapy with simvastatin and losartan significantly reduced malondialdehyde levels and improved FMD when compared with simvastatin and losartan alone. (B) Percent change in adiponectin levels from respective pre-treatment values after treatment with simvastatin alone, combined therapy, and losartan alone. The combined therapy with simvastatin and losartan significantly increased adiponectin levels and QUICKI when compared with simvastatin and losartan alone. SEM is identified by bars. Reproduced from Koh et al.45
Our studies are confirmed by others. Twenty type 2 diabetic patients took atorvastatin, irbesartan, or both for 1 week. High-fat load and glucose alone produced a decrease in endothelial function and increase in inflammation. These effects were more pronounced when high-fat load and glucose were combined. Short-term atorvastatin and irbesartan treatments significantly counterbalanced these phenomena, and their combination was more effective than either therapy alone. On-pump coronary artery bypass graft surgery is associated with an intense systemic inflammatory response that is almost completely prevented by early treatment with high doses of ACE-inhibitors and statins.

4.3 Peroxisome proliferator-activated receptors

4.3.1 Experimental evidence

Fibrin acid is a synthetic ligand of peroxisome proliferator-activated receptor (PPAR) α, a nuclear receptor activated by fatty acids and its derivatives. PPARα regulates expression of key proteins involved in all stages of atherogenesis, including vascular inflammation, plaque instability, and thrombosis. Fibrin acid improves endothelial function via stimulation of NO synthase activity and mediates antioxidant effects that result in enhanced NO bioactivity. Fenofibrate reduces myocardial fibrosis and development of diastolic dysfunction in deoxycorticosterone acetate-salt hypertensive rats. Moreover, in rats, fenofibrate significantly suppresses inflammatory gene expression associated with NF-κB (interleukin-6, cyclooxygenase-2, vascular cell adhesion molecule-1, and monoocyte chemoattractant protein-1). Fenofibrate lowers abdominal and skeletal adiposity and improves insulin sensitivity in rats. The expression of visfatin and adiponectin mRNA in visceral fat deposits is elevated by rosiglitazone or fenofibrate treatments when compared with untreated rats. In contrast, tumour necrosis factor-α mRNA is downregulated by these drugs. In obese diabetic mice, PPARα agonist, Wy-14,643, decreases adipocyte size and reduces the expression of macrophage-specific genes. This suggests that activation of PPARα prevents adipocyte hypertrophy and inflammation. Wy-14,643 treatment upregulates the expression of the adiponectin receptor in white adipose cells. This may improve obesity-induced insulin resistance.

PPARγ regulates gene expression of key proteins involved in lipid metabolism, vascular inflammation, and proliferation, contributing to atherogenesis and post-angioplasty restenosis. The thiazolidinedione PPARγ agonists not only improve insulin resistance in patients with type 2 diabetes but also exert a broad spectrum of antiatherogenic effects in vitro, in animal models of atherosclerosis, and in humans. PPARγ ligands increase endothelial NO release without altering endothelial NO synthase expression. Superoxide anion radical (O₂⁻) decreases NO bioavailability. NADPH oxidase produces O₂⁻, thereby contributing to NO catabolism in endothelial cells. PPARγ ligands decrease NADPH-dependent O₂⁻ production in human umbilical vein endothelial cells and also reduce relative mRNA levels of the NADPH oxidase subunits, nox-1, gp91phox (NOX2), and NOX4. PPARγ ligands also stimulate both activity and expression of Cu/Zn-SOD. These data suggest that in addition to any direct effects on NO production in endothelial cells, PPARγ ligands may enhance NO bioavailability, in part, by altering endothelial O₂⁻ metabolism through suppression of NADPH oxidase and induction of Cu/Zn-SOD. These findings illuminate additional molecular mechanisms by which PPARγ ligands may directly alter vascular endothelial function.

4.3.2 Clinical evidence

Fenofibrate significantly changes lipoprotein levels, improves endothelial function, reduces markers of inflammation and haemostasis, and raises adiponectin levels in patients with hypertriglyceridaemia. Fenofibrate treatment reduces oxidized fatty acids and lowers soluble vascular cell adhesion molecule-1 and intercellular adhesion molecule-1. In non-diabetic patients with elevated LDL cholesterol and metabolic syndrome, pioglitazone significantly raises high-density lipoprotein cholesterol and favourably affects lipoprotein particle size, markers of inflammation, and adipokines without changes in triglycerides, LDL cholesterol, or weight. Pioglitazone retards carotid intima–media thickness progression in patients with type 2 diabetes mellitus.

4.4 Cross-talk between peroxisome proliferator-activated receptors and renin–angiotensin–aldosterone system

4.4.1 Experimental evidence

Recent studies demonstrate cross-talk between PPARα or PPARγ and Ang II. Interestingly, Ang II, through activation of NF-κB, stimulates proinflammatory gene expression and downregulation of PPARα and PPARγ. This promotes vascular inflammation and acceleration of atherosclerosis in apolipoprotein E knockout mice. Ang II is a potent endogenous vasoconstrictor, whereas PPARα activators attenuate the development of hypertension, correct structural abnormalities, and improve endothelial dysfunction induced by Ang II. Further, the effect of fenofibrate to reverse the elevated BP response to Ang II infusion is accompanied by decreased oxidative stress and inflammation in the vascular wall. PPARγ ligands reduce AT1 receptor messenger RNA and protein. Thus, PPARγ ligands may inhibit Ang II-induced cell growth and hypertrophy in vascular smooth muscle cells by suppressing AT1 receptor expression. In addition, ARBs induce PPARγ activity, thereby promoting PPARγ-dependent differentiation in adipocytes. Activation of PPARγ provides a potential mechanism for insulin-sensitizing/antidiabetic effects of ARBs. Therefore, combined therapy with PPARα or PPARγ and RAAS blockades may have additive beneficial effects on inhibition of oxygen free radical production, inhibition of NF-κB and inflammatory protein production, and improvement of NO bioactivity. Combined therapy with PPARα or PPARγ and RAAS blockades may have additive beneficial effects on endothelial function, insulin resistance, and atherosclerosis. Indeed, candesartan or pioglitazone protects against hypertensive cardiovascular damage without lowering BP, whereas combination therapy exerts greater beneficial effects than monotherapy with either drug on hypertensive cardiovascular injury by suppressing ROS to a greater extent.

4.4.2 Clinical evidence

We investigated vascular and metabolic responses to either fenofibrate 200 mg or candesartan 16 mg alone or in
Combined therapy significantly decreases plasma malondialdehyde, high-sensitivity C-reactive protein, and soluble CD40 ligand levels and improves flow-mediated dilator response to hyperaemia to a greater extent than monotherapy (Figure 2). Fenofibrate combined with candesartan improves endothelial function and reduces inflammatory markers to a greater extent than monotherapy in hypertriglyceridemic, hypertensive patients. Fenofibrate, combined therapy, and candesartan significantly increase plasma adiponectin levels and insulin sensitivity relative to baseline measurements. Thus, there is a strong and growing scientific rationale for recommending a combination of PPAR\(\alpha\) or PPAR\(\gamma\) and ARBs to slow progression of atherosclerosis and coronary heart disease.

A recent meta-analysis of 42 trials comparing rosiglitazone with placebo or active comparators in more than 27 000 patients with diabetes suggests that treatment with rosiglitazone is associated with an increased risk of myocardial infarction and cardiovascular death. The Prospective Pioglitazone Clinical Trial in Macrovascular Events (PROactive) studied more than 5000 patients with diabetes at high risk for macrovascular complications and reported that treatment with pioglitazone did not significantly reduce risk for coronary and peripheral vascular events. As a secondary endpoint, a composite of death, myocardial infarction, or stroke was significantly improved. However, pioglitazone increases the incidence of congestive heart failure (although not of mortality associated with heart failure). Moreover, it is unclear whether findings among patients in PROactive can be extrapolated to lower-risk populations of diabetic patients without established vascular disease. A recent meta-analysis evaluated effects of pioglitazone on incidence of ischaemic cardiovascular complications from 19 randomized controlled trials of pioglitazone in diabetes mellitus. Pioglitazone is associated with a significantly lower risk of death, myocardial infarction, or stroke among a diverse population of patients with diabetes. Serious heart failure is increased by pioglitazone, although without an associated increase in mortality.

Therefore, randomized trials are needed to gain additional insight into the extent that combination therapy may be superior to monotherapy alone because large prospective studies have more positive predictive power than small uncontrolled clinical trials.

### 4.5 Cross-talk between statin and PPAR\(\alpha\)

#### 4.5.1 Experimental evidence

Fibrates and statins synergistically increase transcriptional activities of PPAR\(\alpha\)/RXR\(\alpha\) and decrease transactivation of NF-\(\kappa\)B. Cerivastatin, fluvastatin, and pitavastatin synergistically and dose-dependently increase transcriptional activation of PPAR\(\alpha\)/RXR\(\alpha\) induced by bezafibrate. Moreover, concomitant administration of statins and fibrates also decreases transactivation of NF-\(\kappa\)B. Activation of NF-\(\kappa\)B by mitogen-activated protein kinase also decreases transactivation of PPAR\(\alpha\)/RXR\(\alpha\). Statin-induced inhibition of Rho-signalling activates PPAR\(\alpha\) and induces high-density lipoprotein apoA-I. Co-treatment with statins and fibrates activates PPAR\(\alpha\) in a synergistic manner. Statin-induced inhibition of PPAR\(\alpha\) mediated anti-inflammatory effects of simvastatin in vivo in models of acute inflammation. Inhibitory effects of statins on lipopolysaccharide-induced inflammatory response genes are abolished in PPAR\(\alpha\)-deficient macrophages and neutrophils. Moreover, simvastatin inhibits PPAR\(\alpha\) phosphorylation by lipopolysaccharide-activated protein kinase C (PKC) alpha. A constitutively active form of PKCalpha inhibits NF-\(\kappa\)B transrepression by PPARalpha, whereas simvastatin enhances transrepression activity of wild-type PPAR\(\alpha\), but not of PPAR\(\alpha\) mutated in its PKC phosphorylation sites. These data indicate that acute anti-inflammatory effects of simvastatin occur via PPAR\(\alpha\) using a mechanism involving inhibition of PKCalpha inactivation of PPAR\(\alpha\) transrepression activity. These observations provide a molecular basis for combination treatment with statins and fibrates in coronary heart disease. Therefore, combined therapy with statins and fenofibrate may have beneficial effects on endothelial function, insulin resistance, and atherosclerosis.

![Figure 2](image-url)
4.5.2 Clinical evidence
We compared vascular and metabolic responses (and adverse responses) to statin and fenofibrate therapies alone or in combination in patients with combined hyperlipidaemia. Flow-mediated dilator response to hyperaemia and plasma high-sensitivity C-reactive protein and fibrinogen levels are changed to a greater extent with combined therapy when compared with atorvastatin or fenofibrate alone. The effects of combined therapy or fenofibrate alone on plasma adiponectin levels and insulin sensitivity are significantly greater than those of atorvastatin alone. This study demonstrates that fenofibrate combined with simvastatin improves endothelial function and reduces inflammatory markers to a greater extent than monotherapy in patients with combined hyperlipidaemia. No patients were withdrawn from the study as the result of serious adverse effects. Combination therapy is safe and has beneficial additive effects on endothelial function in patients with combined hyperlipidaemia.78

Although combination therapy may increase the risk of myopathy, with an incidence of ~0.12%, this small risk of myopathy rarely outweighs the established morbidity and mortality benefits of achieving lipid goals. Combination therapy with statins and gemfibrozil is more likely to be accompanied by severe myopathy.79 This may be due to the fact that gemfibrozil has significant pharmacokinetic interactions with statins that lead to increased plasma levels of statins.80 This limitation is not observed with fenofibrate, bezafibrate, or ciprofibrate and no significant side effects have been observed with combination treatment with statins and fibrates.78,81,82–84 Studies evaluated differences in the rate of myotoxicity between fenofibrate and gemfibrozil in combination with statins.83 Data from the United States Food and Drug Administration’s Adverse Event Reporting System have been reviewed to determine how many adverse events are reported from patients treated concomitantly with statins and fibrates. Fenofibrate results in fewer reports of rhabdomyolysis than gemfibrozil when used in combination with any statin medication. Of the total number of reports of rhabdomyolysis for combined fibrates and statin therapies, only 2.3% (14 of 606) are associated with fenofibrate/cevastatin therapy, whereas 88% (533 of 606) are associated with gemfibrozil/cevastatin therapy. Similarly, the number of reports of rhabdomyolysis per million prescriptions dispensed is ~33 times lower for fenofibrate than for gemfibrozil when used in combination with cerivastatin. Indeed, a recent randomized clinical trial reports combination therapy with statins and fenofibrate is safe in patients with type 2 diabetes over 5-year follow-up.84

4.6 Calcium channel blockers
4.6.1 Experimental evidence
Calcium channel blockers reduce oxidative stress in the vasculature and improve endothelium-dependent vasodilatation in Ang II-infused rats.85 The potential mechanism by which calcium channel antagonists exert their beneficial activity on endothelial dysfunction may not involve direct calcium antagonism at the level of the endothelium since endothelial cells do not express voltage-sensitive calcium channels to a significant extent. Rather, calcium channel antagonists may exert antioxidant effects that enhance basal NO formation through increasing the expression of endothelial NO synthase, resulting in improved endothelial function.85–87

4.6.2 Clinical evidence
Efonidipine therapy significantly reduces biomarkers of oxidant stress and improves the percent flow-mediated dilator response to hyperaemia in patients with hypertension. Importantly, these improvements in flow-mediated dilation are inversely correlated with changes in plasma levels of malondialdehyde as an independent predictor. Specific calcium channel blockers have differential benefits with respect to reversal of endothelial dysfunction. The effects of amloidipine on endothelium-dependent vasodilatation are controversial. Some studies demonstrate that amloidipine improves endothelium-dependent vasodilatation,88 whereas others do not.89,90 Efonidipine has distinct properties when compared with other calcium channel blockers. When properties of efonidipine and nifedipine (both are dihydropyridine analogues) are evaluated using recombinant T- and L-type Ca^{2+} channels expressed separately in mammalian cells, only efonidipine has a high affinity for T-type Ca^{2+} channels.91 Moreover, efonidipine has a larger effect to improve endothelial function in patients with hypertension when compared with nifedipine even though the decrease in mean BP in each group is similar.92 Finally, urinary excretion of both 8-hydroxy-2′-deoxyguanosine and serum malondialdehyde-modified LDL is decreased by efonidipine, but not nifedipine, therapy.

5. Discrepancy of vitamin C and E therapies in animal models and human diseases
A wealth of previous experimental and epidemiological data suggests that excess LDL oxidation may be partly responsible for the development of atherosclerosis. Because vitamin E and C inhibit LDL oxidation ex vivo, a logical strategy to reduce oxidative stress may include the administration of antioxidants such as vitamin E and C. Acute intra-arterial administration of pharmacological doses of vitamin C improves endothelial dysfunction in subjects with diabetes93 and hypertension,94 and chronic oral vitamin C therapy improves endothelial function in children with hyperlipidaemia.95 Vitamin E has been shown to protect LDL from oxidation, prevent PKC activation by oxidized lipoproteins,96 and improve endothelium-dependent relaxation.97–99 The use of vitamin E supplements is also associated with decreased risk of cardiovascular events in the Nurses’ Health Study100 and the Cambridge Heart Antioxidant Study.101

In contrast to experimental studies, many randomized clinical trials demonstrate that long-term vitamin E supplementation does not prevent cancer or major cardiovascular events and may increase the risk for heart failure in patients with vascular disease or diabetes mellitus102 or in healthy women.103 Moreover, vitamin C supplementation does not prevent cardiovascular events among women at high risk for cardiovascular diseases104 or in middle-aged and older men.105 One plausible reason for these discrepancies is that although vitamin E effectively scavenges lipid peroxyl radicals, it has limited activity against other oxidants such as superoxide, peroxynitrite, and hypochlorous
acid, which have been implicated in atherosclerosis.\textsuperscript{106} Experimental evidence from both animals and patients suggests that lipid peroxidation does proceed in the vascular wall even in the presence of vitamin E.\textsuperscript{107} Attempts to increase the effectiveness of vitamin E with higher doses have met with worsening atherosclerosis and vascular function in experimental models.\textsuperscript{108} High-dose oral vitamin C 800 mg/day therapy, resulting in incomplete replenishment of vitamin C levels, is ineffective at improving endothelial dysfunction and insulin resistance in type 2 diabetes.\textsuperscript{109}

In contrast, ACE-inhibitors, ARBs, and statins effectively limit the production of oxidants at the source. This is in contrast with antioxidant therapy with vitamin E, which is limited to scavenging lipid-soluble oxidants and may therefore be considered more of a ‘symptomatic’ rather than a causal treatment for vascular oxidative stress. Such marked differences in the targets for these treatment strategies may help to explain why antioxidant therapies in humans have failed to successfully influence morbidity and prognosis in patients with cardiovascular disease. Thus, a single-agent antioxidant strategy may not completely reduce vascular oxidative stress and may leave other important processes, such as smooth muscle proliferation and impaired vascular function, unaffected.\textsuperscript{106}

6. Summary and clinical prospects

Chronic inflammation is a pathogenic feature of atherosclerosis and cardiovascular disease mediated by various substances including Ang II, proinflammatory cytokines, and free fatty acids. Moreover, additional mechanisms contributing independently to both insulin resistance and endothelial dysfunction include glucotoxicity, lipotoxicity, and inflammation together with oxidative stress (Figure 3).\textsuperscript{3,110} Cross-talk between inflammatory signalling pathways and insulin signalling pathways causes both metabolic insulin resistance and endothelial dysfunction, which synergize to predispose to cardiovascular disorders.\textsuperscript{3,111} Decreased production of NO mediated by endothelial dysfunction and insulin resistance contributes to accelerated atherosclerosis by multiple mechanisms including cross-talk between hyperlipidaemia and RAAS at multiple levels.

Controlling a variety of risk factors causing inflammation and oxidative stress with combination therapy may simultaneously address multiple mechanisms underlying the pathogenesis of atherosclerosis. This emerging therapeutic paradigm for slowing the progression of atherosclerosis has advantages over currently recommended treatment regimens. In this regard, combined therapy with statins, PPARs, and RAAS blockade demonstrates additive beneficial effects on endothelial dysfunction and insulin resistance when compared with monotherapies in patients with cardiovascular risk factors due to both distinct and interrelated mechanisms. Adapted from Koh et al.\textsuperscript{3,4}

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