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REVIEW ARTICLE

Oxidative Stress and Early Atherosclerosis: Novel Antioxidant Treatment

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Abstract Atherosclerotic lesions initiate in regions characterized by low shear stress and reduced activity of endothelial atheroprotective molecules such as nitric oxide, which is the key molecule managing vascular homeostasis. The generation of reactive oxygen species from the vascular endothelium is strongly related to various enzymes, such as xanthine oxidase, endothelial nitric oxide synthase and nicotinamide-adenine dinucleotide phosphate oxidase. Several pharmaceutical agents, including angiotensin converting enzyme inhibitors, angiotensin receptors blockers and statins, along with a variety of other agents, have demonstrated additional antioxidant properties beyond their principal role. Reports regarding the antioxidant role of vitamins present controversial results, especially those based on large scale studies. In addition, there is growing interest on the role of dietary flavonoids and their potential to improve endothelial function by modifying the oxidative stress status. However, the vascular-protective role of flavonoids and especially their antioxidant properties are still under investigation. Indeed, further research is required to establish the impact of the proposed new therapeutic strategies in atherosclerosis.

Keywords Atherosclerosis · Endothelial function · Oxidative stress · Nitric oxide · Reactive oxygen species

Introduction

Atherosclerosis is the underlying condition in most cardiovascular diseases (CVDs), which are the leading cause of death in Western societies. It is a chronic systemic disease affecting the entire arterial tree, associated with impaired inflammatory status [1, 2]. Moreover, endothelial dysfunction (ED) due to decreased nitric oxide (NO) bioavailability and activity plays a pivotal role in the initiation and progression of atherosclerosis [3]. Accordingly, ED is now considered an important early event in the development of atherosclerosis as it seems to precede atherosclerotic lesions in coronary vessels, and even occurs in offspring with a positive history for CVDs [4].

More specifically, NO is a key molecule managing endothelial function and vascular homeostasis [5], while reduced production of NO or increased production of reactive oxygen species (ROS) promotes ED. Thus, oxidative stress plays a crucial role in the pathogenesis and development of CVD [6].

Consequently, a range of antioxidant strategies have been tested with the aim to improve ED [7, 8]. Novel antioxidants have shown encouraging results. However, the available data are inadequate. Recently, a growing body of evidence has indicated the role of dietary modification. Particularly, dietary flavonoids appear to have the potential to restore endothelial function by decreasing oxidative stress status, though the suggested mechanisms mediating their effects are not fully evaluated [9, 10].

In the present review article, we aim to provide an overview of the physiological pathways involved in oxidative stress and in the pathophysiology of atherosclerosis. Further, we will focus on the available therapeutic strategies for targeting redox signaling in vascular endothelium.

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Vascular Endothelium

The vascular endothelium consists of a thin semipermeable layer of cells covering the internal surface of vessels and forming a boundary between the vessel wall and blood flow. Its structural and functional integrity is vital for the protection of the vessel wall and circulatory function. Endothelial cells (ECs) have several functions, exerting significant autocrine, paracrine and endocrine actions and influencing smooth muscle cells (SMCs), platelets and circulating leucocytes [11].

It is worth noting that there is a considerable phenotypic deviation between ECs in different parts of the vascular system, expressing different surface antigens and receptors and in turn generating different responses to the same stimulus. Similarly, ECs' in vivo responses may differ from in vitro responses seen in cultures of ECs' lines used in many studies [12].

Over the last decade, the vascular endothelium has emerged mostly as a paracrine organ responsible for the secretion of several beneficial substances with anti-atherogenic effects. It regulates body homeostasis and affects thrombosis, thrombolysis, platelet adherence, vascular tone and blood flow. It is involved in many disease processes, including atherosclerosis, arterial and pulmonary hypertension, sepsis and inflammatory syndromes which are related to endothelial injury, dysfunction and activation [13].

NO bioavailability is well-known to exert multiple actions on the vascular endothelium (Table 1). It is capable of reversing constrictive effects of acetylcholine, leading to vasorelaxation and maintaining the balance against various endothelium-derived contracting factors, such as endothelin-1 and thromboxane A2, thereby modulating vascular tone. Beyond this role, decreased NO bioavailability crucially participates in atherothrombosis, given the NO antithrombotic, antiapoptotic, anti-inflammatory and antioxidant effects. Thus, several pathophysiological conditions such as accumulation of ROS, oxidative stress, inflammation, increased adhesion molecules, insulin resistance, decreased shear stress etc., participate in the induction of ED, mainly through a decrease in endothelium-dependent vasodilation [14, 16, 17].

Indisputably, loss of the functional integrity of the endothelium and of its anti-atherogenic effects causes a shift of the action of the endothelium towards reduced vasodilation, inflammation and thrombosis [18] (Fig. 1) and plays a major role in all stages of atherosclerosis, from plaque formation to rupture [19, 20].

Furthermore, several studies have extensively explored ED with respect to its clinical utility as a biomarker in risk prediction [21, 22]. In particular, ED as estimated by the existing invasive and non-invasive techniques could serve as a useful diagnostic and potentially prognostic tool in coronary artery disease (CAD) [23]. Regarding non-invasive techniques, peripheral vascular endothelial function can be assessed by strain gauge venous plethysmography, which evaluates forearm blood flow changes in response to direct intra-arterial administration of agonists or during reactive hyperemia [24]. However, another technique assessing endothelium-dependent flow-mediated dilation (FMD) of the brachial artery using high resolution ultrasound in the brachial circulation, has been widely accepted as a low-cost and easily to perform test [25]. FMD has been found to predict cardiac events in subjects with stable CAD, peripheral arterial disease and in post-myocardial infarction (MI) patients [26–28].

Table 1 Physiological role of nitric oxide [14, 15]

- Relaxing factors (particularly NO) predominate over constrictive factors in normal endothelium
- It promotes endothelium-dependent vasodilation
- It decreases the vasoconstrictive effect of increased Ca^{2+} in smooth muscle cells in normal endothelium
- Its release may participate in the regulation of basal systemic and coronary tone, especially at the level of the arterioles
- It inhibits the production of a variety of immunomodulatory cytokines by macrophages
- It diminishes endothelial permeability, acting predominantly as an anti-inflammatory agent
- It plays a critical role in reducing leukocyte adherence to the endothelium
- It decreases platelet aggregation and adherence

Oxidative Stress and Atherosclerosis Pathophysiology

Atherosclerosis is well known as a disease of the large and medium-sized arteries. It develops progressively, starting at the beginning of life and leading to the formation of atherosclerotic plaques [29]. ED, which is considered an early step in the progression of atherosclerosis, is linked to low NO bioavailability. Low NO bioavailability is due either to decreased production by the endothelium or increased NO inhibition by ROS [30].

The second step in the pathophysiology of atherosclerosis is the passage of plasma LDL into the arterial wall. The strong association between hyperlipidemia and atherosclerosis has been previously recognized and lipid-laden macrophages or foam cells are documented as a hallmark of the disease [31]. More specifically, plasma LDL is transported across the endothelium and is trapped in the subendothelial space where it is oxidized to produce highly oxidized LDL. These molecules are potent inducers of inflammatory molecules which stimulate inflammatory signaling by ECs, through releasing

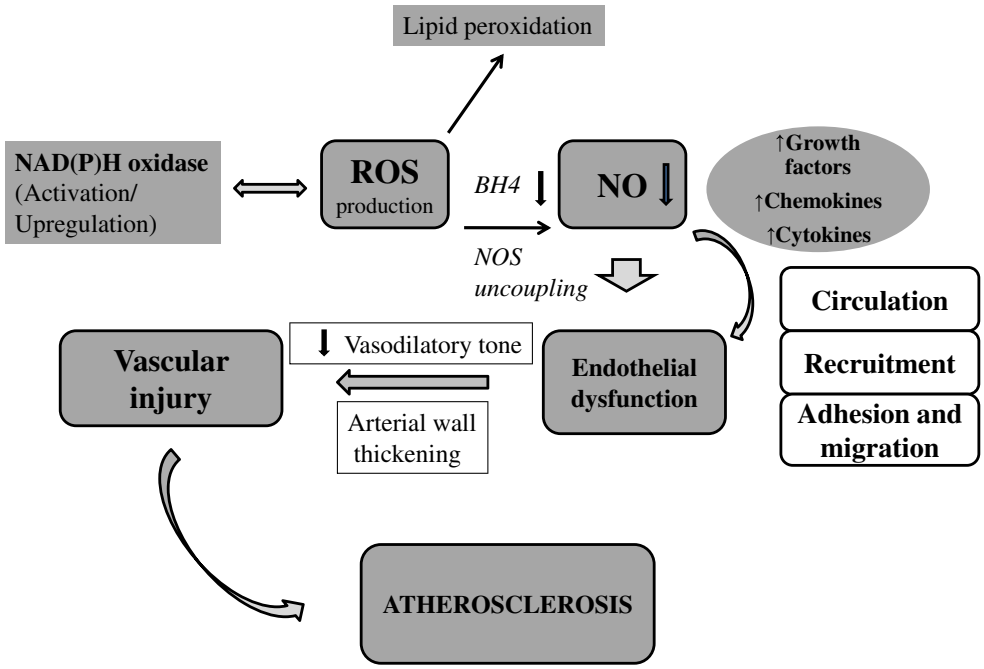

Fig. 1 Pathophysiological mechanisms contributing to atherosclerosis. Abbreviations: *NADPH*, Nicotinamide adenine dinucleotide phosphate-oxidase; *ROS*, Reactive oxygen species; *BH4*, Tetrahydrobiopterin; *NOS*, Nitric oxide synthase; *NO*, Nitric oxide

chemotactic proteins and growth factors, and by further recruiting monocytes into the arterial wall [32].

Concurrently, monocytes entering into the arterial wall differentiate into macrophages incorporating cholesterol from lipoproteins and remain in the subendothelial space. The oxidized LDL particles are taken up by macrophages which then can induce a local inflammatory response [32]. Chemokines, which are produced in response to oxidized lipoproteins, participate in the transendothelial migration of adherent monocytes. Accordingly, oxidative stress status alters the expression of adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1). Moreover, the expression of a number of proinflammatory cytokines and other inflammation-induced molecules like CD40 ligand are strongly related to redox-sensitive factors such as the nuclear factor-kB [33, 34].

The final consequence of the evolution of atherosclerosis is the formation of plaques with thin fibrous caps which are the result of increased collagen breakdown by matrix metalloproteinases and decreased collagen synthesis by dysfunctional or apoptotic SMCs [35]. Finally, the significant contribution of macrophages to the rupture of the thin fibrous cap has been well-recognized, as plaques are likely to rupture at sites of increased macrophage content [36].

Obviously, these observations are of major importance, as they have provided not only mechanistic links between lipoproteins and cell biology of atherosclerosis, but have also provided concepts for potential therapeutic interventions.

Therapeutic Interventions Targeting Oxidative Stress

As it is widely established that ED leads to atherosclerosis, significant effort has been devoted to improving clinical outcome by modulating vascular redox state. There are various therapeutic strategies intending to improve or restore ED targeting oxidative stress. Some of them are effective while others are promising or under investigation (Table 2).

Angiotensin Converting Enzyme Inhibitors and Angiotensin Receptor Blockers

According to clinical and experimental data, angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) seem to exert beneficial anti-atherosclerotic and anti-ischemic effects [44–46]. Growing data indicate that ACE inhibitors and ARBs are capable of reversing ED, atherosclerosis and vascular inflammation and may decrease the burden of CVDs [47–50] . Moreover, from data based on vascular response to acetylcholine, on B-mode ultrasound evaluation of atherosclerotic progression in the carotid artery and on the progression of CAD using quantitative coronary angiography, it is evident that ACE inhibitors and ARBs may increase plaque stability and reduce cardiac events [51–53].

Disparity exists concerning the endothelial protective effects of different anti-hypertensive regimes. In particular, calcium channel antagonists improve endothelial function in the microcirculation while ACE inhibitors and ARBs mostly do so in conduit arteries [54]. Interestingly, a sulfhydryl group confers to ACE inhibitors some additional properties to those of ARBs, such as scavenging of superoxide anion, greater

Table 2 Established and novel treatments of oxidative stress in atherosclerosis

Author	Treatment	Comments
Desideri G et al. 2008 [37]	Zofenoprilat/Captopril	In HUVECs zofenoprilat and, to a lesser extent, captopril reduced oxidative stress
Pöss J te al. 2010 [38]	Aliskiren	In apolipoprotein E deficient mice aliskiren reduced atherosclerotic plaque area in parallel with reduced vascular NADPH oxidase activity
Kobayashi N et al. 2010 [39]	Eplerenone	Eplerenone in rats after hind limp ischemia significantly decreased the NADPH oxidase p22, p47, gp91
Noda K et al. 2012 [40]	Spironolactone/Olmesartan	In rats spironolactone in combination with olmesartan suppressed myocardial lipid peroxidation, in association with an attenuation of NADPH
Oelze M et al. 2006 [41]	Nebivolol	In angiotensin II-treated rats nebivolol inhibited upregulation of the activity and expression of the vascular NADPH oxidase
Antoniades C et al. 2011 [17]	Atorvastatin	In CAD patients undergoing bypass surgery oral atorvastatin directly improves vascular NO bioavailability and reduces vascularsuperoxide
Hermanz R et al. 2012 [42]	Pioglitazone	In spontaneous hypertensive rats pioglitazone abolished the increased vascular ROS production and NOX-1 levels
Martin A et al. 1997 [8]	Vitamin C	Enrichment of human vascular endothelial cells with vitamin C lowers their capacity to oxidize LDL
Tsai KL et al. 2012 [43]	Coenzyme Q10	In HUVECs coenzyme Q10 attenuated the generation of ROS and improved the antioxidant capacity
Wind S et al. 2010 [7]	Triazolo pyrimidines	Specific NADPH oxidase inhibitors (triazolo pyrimidines) consistently inhibited NADPH oxidase activity in low micromolar concentrations

HUVECs, Human vascular endothelial cells; *NADPH*, nicotinamide adenine dinucleotide phosphate-oxidase; *CAD*, Coronary artery disease; *NOX-1*, NADPH oxidase 1; *LDL*, Low density lipoprotein; *ROS*, reactive oxygen species

protection against LDL oxidation and nuclear factor kappa-light-chain-enhancer of activated B cells activation, leading to more pronounced favorable effects on NO balance in vascular ECs [55].

More specifically, zofenoprilat (zofenopril prodrug) and to a less significant extent captopril, have been demonstrated to decrease generation of ROS induced by tumor necrosis-alpha (TNF-α) in human umbilical vein ECs [37].

Moreover, ACE inhibitors, beyond their decreasing effect on angiotensin II levels, also decrease the degradation of bradykinin. Elevated bradykinin levels oppose the negative actions of angiotensin II with parallel antiapoptotic actions. As Oeseburh et al. have shown, by adding bradykinin in cultured bovine aortic endothelial cells, bradykinin protects against oxidative stress-induced endothelial cell senescence [56]. The endothelial protective and anti-oxidative role of bradykinin was further confirmed by Kobayashi et al. who found that treatment of hypertensive rats with quinapril and a bradykinin B2 receptor antagonist did not achieve up-regulation of eNOS and down regulation of lectin-like oxidized LDL receptor-1 as was the case in rats treated only with quinapril [57].

Importantly, evidence from the EUROPA study in CAD patients has shown that blood pressure reduction with perindopril by itself does not explain the observed benefits [58]. In addition, treatment with perindopril resulted in up regulation of eNOS protein expression and activity [59]. Similar results concerning the role of perindopril on bradykinin increase and in endothelial function improvement were also reported from sub-studies of EUROPA [60, 61]. Interestingly, we have to notice that the effect of ACE inhibitors on bradykinin may vary between ACE inhibitors and may depend on the degree of tissue affinity of specific ACE inhibitors, with perindopril having the highest selectivity for the bradykinin binding sites [62, 63].

Olmesartan, an ARB, has also exhibited beneficial properties regarding progression of coronary atherosclerosis (OLIVUS) [52, 53], reduction of inflammatory biomarkers in hypertension (EUTOPIA) [64] and improvement of intima-media thickness (IMT) in patients with diagnosed atherosclerosis [65, 66]. Of note, valsartan compared to amlodipine has exhibited an enhanced vasodilatory response after co-infusion of acetylcholine and L-NG-monomethyl Arginine in a double-blind, crossover trial [67]. Even though the underlying physiological mechanisms have not been elucidated yet, this trial supported that valsartan reversed ED through both NO-dependent and independent pathways, while amlodipine had only a partial effect on NO bioactivity.

Conclusively, although ARBs appear to have some vasoprotective effects, in clinical trials only ACE inhibitors have proven their efficacy in reducing total mortality in subjects with heart failure [68], vascular disease [48], or diabetes [69]. However, not all clinical trials have demonstrated that ACE inhibitors reduce all-cause mortality in patients with atherosclerosis and preserved left ventricular function [70]. ARBs can be used instead of ACE inhibitors but no clinical

outcome studies have shown a beneficial effect of ARB in stable CAD [71].

Renin Inhibitors

Aliskiren is the only direct renin inhibitor used in clinical practice for its antihypertensive properties. Animal studies suggest that aliskiren down-regulates pro-atherogenic cells and reduces atherogenesis and aortic plaque areas in atherogenic mice partly through attenuation of vascular nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity [38]. In line with this, Yamamoto et al. reported that in NOS deficient mice the combination of aliskiren and valsartan exerts synergistic organ-protective effects through synergistic attenuation of oxidative stress [72]. Despite the theoretical advantages of aliskiren in experimental studies, which have raised expectations, the neutral or disappointing results regarding clinical outcome [73] warrant careful extrapolations and further investigation.

Aldosterone Antagonists

Mineralocorticoid receptor blockers are mostly used in hypertension and heart failure. Aldosterone, apart from regulating salt homeostasis, also enhances fibrosis, inflammation and oxidative stress. Recently, numerous experimental studies have focused on the antioxidative potential of mineralocorticoid receptor blockers. Indeed in post-myocardial infarction rats, spironolactone and angiotensin II increased NADPH oxidase-dependent and mitochondrial superoxide production in myocytes, and the combination of an angiotensin II receptor blocker and spironolactone resulted in a synergistic attenuation of cardiac oxidative stress [40]. Moreover, in rats after himdlimb ischemia, eplerenone significantly decreased the NADPH oxidases in parallel with an improvement in the proliferation and function of endothelial progenitor cells, introducing a possible novel and effective therapeutic strategy for the repair of atherosclerotic cardiovascular diseases [39].

Beta-Blockers

Third-generation beta-blockers have been shown not only to avoid increased peripheral resistance, but also to stimulate vasodilation via various mechanisms. In particular, nebivolol which has the highest beta(1)-receptor affinity among beta-blockers, has been associated with improvement of endothelial function via its strong stimulatory effects on the activity of eNOS and its antioxidative effects on cellular superoxide dismutase and dimethylarginine levels [74, 75]. In particular, it has been found that nebivolol, beyond improving blood pressure levels, improved FMD and increased erythrocyte cellular superoxide dismutase levels indirectly, suggesting a beneficial effect mediated by increased NO bioavailability [76]. Furthermore, nebivolol has been shown to significantly affect the amount of ROS released from human ECs under oxidative stress. Particularly this effect may be mediated, at least in part, by the inhibition of endothelial NADPH oxidase and also by the direct ROS scavenging effect of the drug [41, 76, 77]. Interestingly, in patients with slow coronary flow nebivolol treatment for 6 months reduced the levels of malondialdehyde, superoxide dismutase (SOD) and erythrocyte catalase [78]. Furthermore, recent studies have documented the beneficial effects of nebivolol on arterial stiffness to a greater extent than previous agents, which may have significant clinical implications for its use in the treatment of CVD [55].

Statins

The 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA) inhibitors or statins are the most commonly used lipid-lowering agents. Recent compelling evidence suggests that their beneficial effects may include not only cholesterol lowering effects, but also cholesterol-independent or so-called pleiotropic effects. These effects are mediated by concomitant inhibition of protein isoprenylation, a process responsible for a variety of cellular responses downstream the mevalonate pathway [17]. Importantly, ex vivo experiments in internal mammary arteries incubated with either atorvastatin or atorvastatin with mevalonate have shown that in the presence of mevalonate atorvastatin failed to achieve a reduction in oxygen free radicals confirming the key role of mevalonate inhibition in the restoration of arterial redox state achieved with statins [17]. Through these pleiotropic effects, statins are directly involved in restoring or improving endothelial function, attenuating vascular remodeling, inhibiting vascular inflammatory response, and, perhaps, stabilizing atherosclerotic plaques [79, 80]. Also, they may ameliorate ED through their antioxidant properties, since they seem to attenuate Ang-II-induced free radical production in SMCs by inhibiting Rac1-mediated NADPH oxidase activity and down-regulating Ang-II type 1 receptor expression [81]. Moreover, there is evidence that fluvastatin may exert a superoxide or hydroxyl radical scavenging activity and diminish susceptibility to oxidation, while atorvastatin has been shown to reduce vascular mRNA expression of essential NADPH oxidase subunits p22phox and NADPH oxidase 1, increasing at the same time catalase expression both in vitro and in vivo [55, 82]. Further, in 12 dyslipidemic patients with ischemic heart disease who had received organic nitrates for a long period, fluvastatin reduced anti-oxidized LDL antibody titer and serum 8-hydroxydeoxyguanosine levels, while it attenuated nitrate tolerance in subjects under organic nitrites for a long-term period [83].

In addition, evidence suggests that statins exhibit beneficial properties in endothelial function and arterial stiffness in CVD

[84]. Importantly, it has been shown recently that intensive therapy with rosuvastatin delayed progression of the mean-IMT within 12 month in hypercholesterolemic patients [85]. Likewise, dual lipid-lowering therapy has been linked with atherosclerosis regression, even though it has not been clarified whether it contributes to significant changes in plaque composition [86].

Thus, it seems reasonable that several data from observational and randomized studies have indicated favorable effects of statin therapy early after the onset of acute coronary syndrome (ACS). In secondary prevention of CVD, the MIRACL (Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering) study, randomly assigned 3,086 patients with unstable angina or non-Q-wave acute MI to atorvastatin 80 mg or placebo for 16 weeks. Lipid-lowering therapy reduced recurrent ischemic events, and mostly recurrent symptomatic ischemia requiring rehospitalization [87]. Of note, recent studies have provided evidence that statins may be administered as adjunctive therapy in ACS and may also improve clinical outcomes [88, 89].

Peroxisome Proliferator-Activated Receptors Agonists

Peroxisome proliferator-activated receptors (PPARs) agonists (fibrates, thiazolidinediones or glitazones) have been shown to exert a broad spectrum of antiatherogenic effects in vitro, in animal models of atherosclerosis and in humans. These agents, which are widely used in the clinical setting, antagonise Ang-II effects and have been shown to exert antioxidant and anti-inflammatory effects [90]. Interestingly, emerging data have suggested that they may augment endothelial NO release [91], decline NADPH-dependent O_2^- production in human umbilical vein endothelial cells and also reduce relative mRNA levels of the NADPH oxidase subunits. Further, PPAR-γ ligands induce both activity and expression of Cu/Zn-SOD [92]. Moreover, in hypertensive rats pioglitazone reduces vascular ROS production and Nox1 levels while it does not affect eNOS expression [42]. Interestingly, novel thiazolidinediones (SF23) under investigation offer superior antioxidant effects compared to rosiglitazone, preventing ROS generation and the expression of NADPH oxidase subunits, Nox1 and Nox2 [93]. Unlike rosiglitazone, these effects are independent of nuclear factor erythroid 2-related factor 2. Although more data are required on the effects of PPARγ agonist on cardiovascular events, due to their interference with key processes of atherogenesis, they present additional properties to improve cardiovascular risk beyond glycemic control in patients with DM [94].

Xanthine Oxidase Inhibitors

Xanthine oxidase catalyzes the oxidation of hypoxanthine to xanthine and finally to uric acid. Xanthine oxidase is a source of ROS with detrimental effects on the progression of atherosclerosis. Experimental studies have also shown that xanthine oxidase binds to endothelial cells and inactivates NO [95].

Taking into consideration the physiological role of xanthine oxidase, inhibition of its activity may be beneficial for endothelial function. Several studies have examined the impact of xanthine oxidase inhibitors on endothelial function [96]. Xanthine oxidase inhibition has been tested in patients at increased cardiovascular risk [97], in patients with CAD [98] and with heart failure [99], with favorable effects on endothelial function. Moreover, Yiginer et al. concluded that in patients with metabolic syndrome allopurinol reduces oxidative stress, improves endothelial function and ameliorates myeloperoxidase, further confirming the pathophysiology of improvement of endothelial function. Although there is a lack of a large randomized trials, a recent meta-analysis supported the positive impact of such an approach [96].

Strategies to Increase Tetrahydrobiopterin

Tetrahydrobiopterin (BH4) is a pteridine that is known to have a plethora of cofactor and antioxidant roles in pathological conditions associated with cardiovascular and endothelial dysfunction, monoamine neurotransmitter formation, the immune response, and pain sensitivity [100]. In order for eNOS to transport electrons to L-arginine and synthesize NO, it has to be bound to BH4 (“coupling” of eNOS) [101]. Decreased levels of BH4 in endothelial cells can result in eNOS “uncoupling” and generation of oxygen radicals causing oxidative damage [102, 103].

Given the key pathophysiological role of BH4 in vascular function it is not surprising that redox disequilibrium can be achieved by increasing BH4 levels. Experimentally, incubation of isolated vessels (human coronary arteries) with BH4 results in an improvement of endothelial function [104]. Moreover, oral administration of BH4 to ensure continuous BH4 availability in hypercholesterolemic apolipoprotein E-knockout mice improves endothelial dysfunction and attenuates increased mRNA expression of NADPH oxidase components [105]. Similarly, in hypercholesterolaemic patients endothelial dysfunction and oxidative stress can be reversed by chronic oral treatment with BH4. Thus, oral supplementation with BH4 may provide a rational therapeutic approach to maintain NO synthesis and low levels of free oxygen radicals and to prevent cardiovascular disease [106]. Interestingly, statins can also increase BH4 levels. In patients with CAD atorvastatin not only increases BH4 levels with a parallel improvement in NO bioavailability, but also decreases oxygen radicals [17]. Moreover, in diabetic rats telmisartan prevented down regulation of BH4, eNOS uncoupling and the increase in NADPH oxidase and ROS with a parallel restoration of endothelial function. These findings provide evidence how

telmisartan can restore endothelial dysfunction in diabetic models through up-regulation of BH4 [107].

Antioxidant Vitamins

Even though oxidative stress plays a vital role in the evolution of atherosclerosis, and antioxidants have been enthusiastically used in the treatment and prevention of cardiovascular disease, the role of antioxidant vitamins is a matter of long debate, as the results of prospective, randomized and clinical studies have not been as encouraging as expected [108]. Several data have illustrated positive responses of vitamin C and vitamin E. Specifically, they were considered as inhibitors of LDL oxidation by ROS scavenging and as mediators that increase NO bioavailability. Moreover, vitamin C has been shown to improve endothelial NO synthase (eNOS) coupling by scavenging ROS, while vitamin E suppresses eNOS expression (protein kinase C- dependent) [109, 110]. Moreover, several data have demonstrated positive responses of these vitamins in hypertensive patients, as combined treatment significantly improved ED and arterial stiffness, effects which were associated with changes in plasma markers of oxidative stress [111, 112]. Additionally, favorable effects of combined administration of vitamins C and E on endothelial function, inflammatory process and thrombosis/fibrinolysis have also been revealed in chronic smokers [113, 114]. In terms of CVD endpoints, a few studies using different combinations of antioxidant vitamins have reported encouraging results [108].

However, a large meta-analysis including over 77,000 subjects has shown neutral effects concerning the clinical outcome [115]. Similarly, vitamin supplementation has also been unconvincing in the progression of atherosclerosis [116]. Moreover, neither vitamin E nor vitamin C supplementation reduced the risk of major CVDs in the long-term [117], and likewise, in females at high risk for CVDs, no beneficial effects have been observed for ascorbic acid or β-carotene on cardiovascular events [118]. Thus, despite the encouraging data from basic-science studies using several vitamin supplements, antioxidant treatment with vitamins has not been proven an ideal strategy to reduce cardiovascular risk. Further, the effects of vitamins in some occasions have turned to be even detrimental, given that oral vitamin E supplementation may actually have pro-oxidant effects [109].

Folic Acid

Folic acid and its circulating metabolite 5-methyltetrahydrofolate (5-MTHF) have been shown to exert effects on vascular function, independently of the effects on oxidized or reduced plasma homocysteine [119, 120]. 5-MTHF, via improved eNOS activity and coupling as well as scavenging of peroxynitrite radicals, has been considered to improve endothelial NO bioavailability and decrease vascular superoxide production both in vivo and ex vivo, leading to a remarkable improvement of vascular tetrahydrobiopterin bioavailability [121, 122]. However, large randomized data derived from prospective folate supplementation revealed no beneficial effects of folic acid, B6 and B12 vitamins on cardiovascular risk [119, 123].

Omega-3 Polyunsaturated Fatty Acids

The omega-3 polyunsaturated fatty acids (omega-3 PUFAs) eicosapentaenoic acid and docosahexaenoic acid are present mainly in oily fish and commercially available supplements, which are available either over the counter (as fish oils) or as concentrated pharmaceutical preparations. The use of omega-3 PUFAs has been associated with substantial cardiovascular benefits in subjects with diabetes mellitus, heart failure and cardiovascular risk factors [124–126]. Omega-3 PUFAs also improve endothelial function and arterial elastic properties in subjects with metabolic syndrome and in healthy smokers [127, 128]. The beneficial effects of omega-3 PUFAs on endothelial function are attributed to reduced production of inflammatory cytokines, reduced levels of adhesion molecules and suppression of thromboxane production [129, 130]. Experimental data have also shown that omega-3 PUFAs exert favorable effects on lipid metabolism and on the oxidant/antioxidant status of offspring of diabetic rats [131]. Similarly, supplementation of human aortic endothelial cells with omega three fatty acids decreases ROS formation. Therefore it seems that this fatty acid acts as an indirect anti-oxidant in vascular endothelial cells, hence diminishing inflammation and, in turn, the risk of atherosclerosis and cardiovascular disease [132]. Moreover, 2 month treatment with omega-3 PUFAs in patients with atherosclerotic lesions significantly decreased malondialdehyde [133]. Recently, eicosapentaenoic acid and docosahexaenoic acid supplementation was shown to lower plasma lipoperoxide concentrations in mild cognitive impairment patients, further establishing the antioxidant role of omega-3 PUFAs [134].

Polyphenols-Flavonoids

It is evident that lifestyle factors such as smoking, alcohol consumption and obesity are risk factors for the development of impaired endothelial function. There is significant evidence that emphasizes the importance of a variety of strategies to use lifestyle modification to improve endothelial function and cardiovascular status. In this direction, many epidemiological research studies have shown that dietary factors, such as red wine, Greek coffee and tea consumption, rich in polyphenols, have been associated with a reduced risk of CVDs and beneficial effects on endothelial function [135, 136]. Polyphenols consist of a large number of molecules, further divided according to their chemical structure into the subcategories of phenolic acids, flavonoids, stilbenes, and lignans [137].

Even though the mechanisms by which polyphenols act as antioxidants are not fully elucidated, it seems that they are reducing agents or hydrogen atom-donating molecules, thus playing a role as free radical scavengers. In particular, several free hydroxyl groups participate in the scavenging effects of flavonoids, while hydroxyl groups in an ortho position of ring B is a key structure [138]. It has become evident that the most effective antioxidant actions of flavonoids are dependent on indirect mechanisms that inhibit enzymes such as xanthine oxidase, lipoxygenases, and NADPH oxidases which produce ROS [139]. Also, polyphenols exert antioxidant activities via inhibition of NADPH oxidase, 15-lipoxygenase, cytochrome p450 and myeloperoxidase, while further evidence advocates that they may prevent LDL oxidation by their binding to LDL, or act via alterations in gene expression resulting in changes in cell communication [140].

Many in vivo trials have assessed the effects of a variety of fruit polyphenols on different CVD risk factors, including ED [141]. Interestingly, the majority of data have indicated that grape products might produce hypotensive, hypolipidemic and anti-atherosclerotic effects, potentially through ameliorating oxidative stress as measured in terms of oxidation biomarkers and maintenance of endothelial function [10, 142, 143]. Randomized intervention studies in humans have presented additional evidence of a causal relationship between vascular health outcomes and flavonoid intake. A meta-analysis which included 133 human studies with polyphenol interventions showed that cocoa significantly increased acute and chronic FMD of the brachial artery [144]. The intake of polyphenol-rich sources (red wine, cocoa, green tea and berries) has been shown to favor cardiovascular health via an improved lipid profile, anti-atherosclerotic, anti-hypertensive and anti-inflammatory effects, as well as direct actions on ECs [145]. Indeed, green tea supplementation decreased several cardiovascular risk factors, including body composition, dyslipidemia, inflammatory status, and antioxidant capacity, in rats fed an atherogenic diet [146]. More evidence concerning the favorable effect of tea in endothelial function is provided by a recent meta-analysis of nine studies [147]. The effect of tea on FMD is constant and independent of the population and type of tea and can be attributed to the effect of dietary flavonoids in reducing SOD mediated NO breakdown [148] and the inhibition of NADPH oxidase activity [149].

Another source of phenolic compounds is olive oil, with hydroxytyrosol and oleuropein as the most representative [150]. The Mediterranean diet and olive oil consumption were associated with decreased risk factors and lower LDL cholesterol levels [151]. Moreover, olive oil consumption was associated with improved endothelial function [152, 153]. The ATTICA study documented that adherence to Mediterranean diet and increased olive oil consumption is associated with increased total antioxidant capacity in healthy subjects [154]. Further insights into the mechanisms of atherosclerosis protection by olive oil consumption are provided by the documentation of the inhibitory effect of hydroxytyrosol in superoxide anion and F2-isoprostanes production [155, 156]. Oleuropein inhibits also oxidation of LDL and superoxide production [157].

Despite the ample experimental and epidemiological data, there are only a few studies examining the effect of flavonoids on clinical endpoints in atherosclerosis [158].

Coenzyme Q10

Coenzyme Q10 is essential for mitochondrial oxidative phosphorylation and adenosine triphosphate production and is located mostly in the mitochondria but also in lysozomes, Golgi and plasma. It exerts antioxidant activities either by directly reacting with free radicals or by regenerating tocopherol and ascorbate from their oxidized state. Coenzyme Q10 is either synthesized in tissue or is obtained from diet [159–161]. In human umbilical vein endothelial cell cultures treated with oxidized LDL, coenzyme Q10 protects endothelial cells from oxidative stress-induced injury by up-regulation of eNOS and down-regulation of inducible nitric oxide synthase [43]. The antioxidant properties of coenzyme Q10 can possibly explain the improvement in endothelial function of subjects at risk, as documented in a recent metanalysis of five trials [162].

Endocannabinoid System- Cannabinoid Receptors Antagonists

Recent data suggests that the endocannabinoid system contributes to the progression of atherosclerosis and can modulate oxidative stress and endothelial dysfunction. Despite the fact that cannabinoid receptors antagonists are not in use in cardiovascular practice, several experimental studies have documented favorable effects. Tiyerilli et al. concluded that inhibition of the cannabinoid receptor one with rimonabant in apolipoprotein E deficient mice leads to decreased NADPH oxidase activity and ROS production together with endothelial protective effects [163]. Beneficial effects were also reported with rimonabant in subjects with metabolic risk factors and early atherosclerosis raising expectations for a novel and efficacious treatment of patients at risk [164]. Nevertheless, adverse psychiatric effects reported in clinical studies prevent cannabinoid receptors antagonists from being used in clinical practice.

NADPH Oxidase Inhibitors

As previously described, NADPH oxidases are key molecules in vascular oxidative stress. Consequently, approaches to inhibit their action have gained attention, as potential

modification of NADPH oxidase can restore endothelial function and ameliorate the progression of atherosclerosis.

Diphenylene iodonium was one of the first oxidase inhibitors [165]. Data suggest that diphenylene iodonium abolished NADPH oxidase-mediated ROS formation, but also inhibited other flavo-enzymes such as NO synthase (NOS) and xanthine oxidase which may induce toxicity [7] .

Apocynin, is another direct NADPH oxidase inhibitor which has only minor efficacy due to the fact that it is a prodrug requiring metabolic activation [7] . Nevertheless, Kinkade et al., in an atherosclerotic mice model, concluded that apocynin attenuates the progression of atherosclerosis, potentially by its ability to inhibit generation of superoxide by NADPH oxidase through its ability to inhibit translocation of the subunit p47phox (subunit of the NADPH oxidase) [166]. Liu et al. also found that in mice inhibition of NADPH oxidase by apocynin reduced endotheial NOS activation and platelet adhesion, which are likely responsible for the arrest of plaque growth and improvement of vascular mechanical properties [167]. Similarly, 7 days treatment with apocynin reduced endothelial cell adhesion molecule expression in atherosclerotic mice but without a detectable change in oxidative burden [168].

Several other direct NADPH inhibitors have also been studied with limited efficacy and specificity [169]. Favorable results have also been reported with selective NADPH oxidase inhibitors which selectively bind to the p47phox subunit of NADPH oxidase inhibited Nox1 and Nox2 but not Nox4 [170].

Interestingly, the novel NADPH oxidase inhibitor VAS3947, used in low micromolar concentrations, consistently inhibited NADPH oxidase activity, but did not inhibit xanthine oxidase or endothelial NOS activities. Nevertheless, the mechanisms of actions of triazolo pyrimidines such as VAS3947 are unclear and we cannot exclude the possibility that VAS3947, in addition to inhibiting NADPH oxidases, also interferes with alternative sources of ROS that have not yet been elucidated, such as the mitochondrial electron chain [7].

Despite the preliminary positive results regarding the use of direct NADPH oxidase inhibitors we have to notice that data are based on experimental studies. There are no clinical data yet and a major efforts have to be made before the possible use of this agents in clinical practice.

Conclusions

The endothelium is considered of major importance in maintaining vascular homeostasis while oxidative stress has a central role in the progression of endothelial dysfunction. Several established treatments, with proven cardiovascular utility, such as angiotensin converting enzyme inhibitors and angiotensin receptor blockers, beta-blockers and statins, beyond their main action, have antioxidant effects and can restore endothelial function. Additionally, novel strategies such as antioxidant supplementation, peroxisome proliferator-activated receptors agonists and dietary flavonoids may also have a role against oxidative stress and endothelial dysfunction, exerting anti-inflammatory and antithrombotic actions at the same time. Nevertheless, their impact on clinical outcome has not been established yet and further studies are required to establish their clinical usefulness.

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References

1. Okazaki S, Sakaguchi M, Miwa K, et al. Association of interleukin-6 with the progression of carotid atherosclerosis: a 9-year follow-up study. Stroke; J Cereb Circ. 2014;45:2924–9.
2. Tousoulis D, Kampoli AM, Papageorgiou N, et al. Pathophysiology of atherosclerosis: the role of inflammation. Curr Pharm Des. 2011;17:4089–110.
3. Gutstein DE, Fuster V. Pathophysiology and clinical significance of atherosclerotic plaque rupture. Cardiovasc Res. 1999;41:323–33.
4. Sandoo A, van Zanten JJ, Metsios GS, Carroll D, Kitas GD. The endothelium and its role in regulating vascular tone. Open Cardiovasc Med J. 2010;4:302–12.
5. Guzik TJ, Sadowski J, Kapelak B, et al. Systemic regulation of vascular NAD(P)H oxidase activity and nox isoform expression in human arteries and veins. Arterioscler Thromb Vasc Biol. 2004;24:1614–20.
6. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. Circulation. 2007;115:1285–95.
7. Wind S, Beuerlein K, Eucker T, et al. Comparative pharmacology of chemically distinct NADPH oxidase inhibitors. Br J Pharmacol. 2010;161:885–98.
8. Martin A, Frei B. Both intracellular and extracellular vitamin C inhibit atherogenic modification of LDL by human vascular endothelial cells. Arterioscler Thromb Vasc Biol. 1997;17:1583–90.
9. Pusparini, Dharma R, Suyatna FD, Mansyur M, Hidajat A. Effect of soy isoflavone supplementation on vascular endothelial function and oxidative stress in postmenopausal women: a community randomized controlled trial. Asia Pac J Clin Nutr. 2013;22:357–64.
10. Siasos G, Tousoulis D, Kokkou E, et al. Favorable effects of concord grape juice on endothelial function and arterial stiffness in healthy smokers. Am J Hypertens. 2014;27:38–45.
11. Binder RL, Gallagher PM, Johnson GR, et al. Evidence that initiated keratinocytes clonally expand into multiple existing hair follicles during papilloma histogenesis in SENCAR mouse skin. Mol Carcinog. 1997;20:151–8.
12. Galley HF, Webster NR. Physiology of the endothelium. Br J Anaesth. 2004;93:105–13.
13. Ignarro LJ. Biosynthesis and metabolism of endothelium-derived nitric oxide. Annu Rev Pharmacol Toxicol. 1990;30:535–60.

14. Esper RJ, Nordaby RA, Vilarino JO, Paragano A, Cacharron JL, Machado RA. Endothelial dysfunction: a comprehensive appraisal. Cardiovasc Diabetol. 2006;5:4.
15. Wennmalm A. Endothelial nitric oxide and cardiovascular disease. J Intern Med. 1994;235:317–27.
16. Breton-Romero R, Acin-Perez R, Rodriguez-Pascual F, et al. Laminar shear stress regulates mitochondrial dynamics, bioenergetics responses and PRX3 activation in endothelial cells. Biochim Biophys Acta. 1843;2014:2403–13.
17. Antoniades C, Bakogiannis C, Leeson P, et al. Rapid, direct effects of statin treatment on arterial redox state and nitric oxide bioavailability in human atherosclerosis via tetrahydrobiopterin-mediated endothelial nitric oxide synthase coupling. Circulation. 2011;124:335–45.
18. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med. 2005;352:1685–95.
19. Pirs M, Jug B, Erzen B, et al. Relationship between markers of endothelial dysfunction and inflammation and subclinical atherosclerosis in HIV-infected male patients below 55 years of age. Acta dermatovenerologica Alpina, Pannonica, et Adriatica. 2014;23:49–52.
20. Choi BJ, Matsuo Y, Aoki T et al. Coronary Endothelial Dysfunction Is Associated With Inflammation and Vasa Vasorum Proliferation in Patients with Early Atherosclerosis. Arterioscler Thromb Vasc Biol 2014.
21. Martin BJ, Anderson TJ. Risk prediction in cardiovascular disease: the prognostic significance of endothelial dysfunction. Can J Cardiol. 2009;25(Suppl A):15A–20A.
22. Halcox JP, Schenke WH, Zalos G, et al. Prognostic value of coronary vascular endothelial dysfunction. Circulation. 2002;106:653–8.
23. John S, Schmieder RE. Potential mechanisms of impaired endothelial function in arterial hypertension and hypercholesterolemia. Curr Hypertens Rep. 2003;5:199–207.
24. Schlaich MP, John S, Langenfeld MR, Lackner KJ, Schmitz G, Schmieder RE. Does lipoprotein (a) impair endothelial function? J Am Coll Cardiol. 1998;31:359–65.
25. Deanfield J, Donald A, Ferri C, 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.
26. Brevetti G, Silvestro A, Schiano V, Chiariello M. Endothelial dysfunction and cardiovascular risk prediction in peripheral arterial disease: additive value of flow-mediated dilation to ankle-brachial pressure index. Circulation. 2003;108:2093–8.
27. Chan SY, Mancini GB, Kuramoto L, Schulzer M, Frohlich J, Ignaszewski A. The prognostic importance of endothelial dysfunction and carotid atheroma burden in patients with coronary artery disease. J Am Coll Cardiol. 2003;42:1037–43.
28. Guazzi M, Reina G, Gripari P, Tumminello G, Vicenzi M, Arena R. Prognostic value of flow-mediated dilatation following myocardial infarction. Int J Cardiol. 2009;132:45–50.
29. Ross R. Atherosclerosis–an inflammatory disease. N Engl J Med. 1999;340:115–26.
30. McGorisk GM, Treasure CB. Endothelial dysfunction in coronary heart disease. Curr Opin Cardiol. 1996;11:341–50.
31. Pepine CJ. Why vascular biology matters. Am J Cardiol. 2001;88:5K–9K.
32. Khalil MF, Wagner WD, Goldberg IJ. Molecular interactions leading to lipoprotein retention and the initiation of atherosclerosis. Arterioscler Thromb Vasc Biol. 2004;24:2211–8.
33. Tousoulis D, Antoniades C, Stefanadis C. Assessing inflammatory status in cardiovascular disease. Heart. 2007;93:1001–7.
34. Olejarz W, Bryk D, Zapolska-Downar D, Malecki M, Stachurska A, Sitkiewicz D. Mycophenolic acid attenuates the tumour necrosis factor-alpha-mediated proinflammatory response in endothelial cells by blocking the MAPK/NF-kappaB and ROS pathways. Eur J Clin Invest. 2014;44:54–64.
35. Martinet W, De Meyer GR. Autophagy in atherosclerosis: a cell survival and death phenomenon with therapeutic potential. Circ Res. 2009;104:304–17.
36. Kolodgie FD, Narula J, Burke AP, et al. Localization of apoptotic macrophages at the site of plaque rupture in sudden coronary death. Am J Pathol. 2000;157:1259–68.
37. Desideri G, Grassi D, Croce G, et al. Different effects of angiotensin converting enzyme inhibitors on endothelin-1 and nitric oxide balance in human vascular endothelial cells: evidence of an oxidant-sensitive pathway. Mediators Inflamm. 2008;2008:305087.
38. Poss J, Werner C, Lorenz D, Gensch C, Bohm M, Laufs U. The renin inhibitor aliskiren upregulates pro-angiogenic cells and reduces atherogenesis in mice. Basic Res Cardiol. 2010;105:725–35.
39. Kobayashi N, Fukushima H, Takeshima H, et al. Effect of eplerenone on endothelial progenitor cells and oxidative stress in ischemic hindlimb. Am J Hypertens. 2010;23:1007–13.
40. Noda K, Kobara M, Hamada J, et al. Additive amelioration of oxidative stress and cardiac function by combined mineralocorticoid and angiotensin receptor blockers in postinfarct failing hearts. J Cardiovasc Pharmacol. 2012;60:140–9.
41. Oelze M, Daiber A, Brandes RP, et al. Nebivolol inhibits superoxide formation by NADPH oxidase and endothelial dysfunction in angiotensin II-treated rats. Hypertension. 2006;48:677–84.
42. Hernanz R, Martin A, Perez-Giron JV, et al. Pioglitazone treatment increases COX-2-derived prostacyclin production and reduces oxidative stress in hypertensive rats: role in vascular function. Br J Pharmacol. 2012;166:1303–19.
43. Tsai KL, Huang YH, Kao CL, et al. A novel mechanism of coenzyme Q10 protects against human endothelial cells from oxidative stress-induced injury by modulating NO-related pathways. J Nutr Biochem. 2012;23:458–68.
44. Yang S, Li R, Tang L, et al. TLR4-mediated anti-atherosclerosis mechanisms of angiotensin-converting enzyme inhibitor–fosinopril. Cell Immunol. 2013;285:38–41.
45. Clancy P, Koblar SA, Golledge J. Angiotensin receptor 1 blockade reduces secretion of inflammation associated cytokines from cultured human carotid atheroma and vascular cells in association with reduced extracellular signal regulated kinase expression and activation. Atherosclerosis. 2014;236:108–15.
46. Komers R, Simkova R, Kazdova L, Ruzickova J, Pelikanova T. Effects of ACE inhibition and AT1-receptor blockade on haemodynamic responses to L-arginine in type 1 diabetes. J Renin-Angiotensin-Aldosterone Syst: JRAAS. 2004;5:33–8.
47. Schmieder RE, Delles C, Mimran A, Fauvel JP, Ruilope LM. Impact of telmisartan versus ramipril on renal endothelial function in patients with hypertension and type 2 diabetes. Diabetes Care. 2007;30:1351–6.
48. Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The heart outcomes prevention evaluation study investigators. N Engl J Med. 2000;342:145–53.
49. Pitt B, O'Neill B, Feldman R, et al. The QUinapril ischemic event trial (QUIET): evaluation of chronic ACE inhibitor therapy in patients with ischemic heart disease and preserved left ventricular function. Am J Cardiol. 2001;87:1058–63.
50. Schlaifer JD, Wargovich TJ, O'Neill B, et al. Effects of quinapril on coronary blood flow in coronary artery disease patients with endothelial dysfunction. TREND investigators. Trial on reversing endothelial dysfunction. Am J Cardiol. 1997;80:1594–7.
51. Pepine CJ. Improved endothelial function with angiotensin-converting enzyme inhibitors. Am J Cardiol. 1997;79:29–32.
52. Hirohata A, Yamamoto K, Miyoshi T, et al. Impact of olmesartan on progression of coronary atherosclerosis a serial volumetric

intravascular ultrasound analysis from the OLIVUS (impact of OLmesarten on progression of coronary atherosclerosis: evaluation by intravascular ultrasound) trial. J Am Coll Cardiol. 2010;55:976–82.
53. Hirohata A, Yamamoto K, Miyoshi T, et al. Four-year clinical outcomes of the OLIVUS-Ex (impact of Olmesartan on progression of coronary atherosclerosis: evaluation by intravascular ultrasound) extension trial. Atherosclerosis. 2012;220:134–8.
54. Ghiadoni L, Taddei S, Virdis A. Hypertension and endothelial dysfunction: therapeutic approach. Curr Vasc Pharmacol. 2012;10:42–60.
55. Briasoulis A, Tousoulis D, Androulakis ES, Papageorgiou N, Latsios G, Stefanadis C. Endothelial dysfunction and atherosclerosis: focus on novel therapeutic approaches. Recent Pat Cardiovasc Drug Discov. 2012;7:21–32.
56. Oeseburg H, Iusuf D, van der Harst P, van Gilst WH, Henning RH, Roks AJ. Bradykinin protects against oxidative stress-induced endothelial cell senescence. Hypertension. 2009;53:417–22.
57. Kobayashi N, Honda T, Yoshida K, et al. Critical role of bradykinin-eNOS and oxidative stress-LOX-1 pathway in cardiovascular remodeling under chronic angiotensin-converting enzyme inhibition. Atherosclerosis. 2006;187:92–100.
58. Remme WJ, Deckers JW, Fox KM, et al. Secondary prevention of coronary disease with ACE inhibition–does blood pressure reduction with perindopril explain the benefits in EUROPA? Cardiovasc Drugs Ther Sponsored Int Soc Cardiovasc Pharmacother. 2009;23:161–70.
59. Ferrari R, Fox K. Insight into the mode of action of ACE inhibition in coronary artery disease: the ultimate 'EUROPA' story. Drugs. 2009;69:265–77.
60. Simoons ML, Vos J, de Feyter PJ, et al. EUROPA substudies, confirmation of pathophysiological concepts. European trial on reduction of cardiac events with perindopril in stable coronary artery disease. Eur Heart J. 1998;19:J56–60.
61. Bots ML, Remme WJ, Luscher TF, et al. ACE inhibition and endothelial function: main findings of PERFECT, a sub-study of the EUROPA trial. Cardiovasc Drugs Ther Sponsored Int Soc Cardiovasc Pharmacother. 2007;21:269–79.
62. Ceconi C, Francolini G, Bastianon D, Gitti GL, Comini L, Ferrari R. Differences in the effect of angiotensin-converting enzyme inhibitors on the rate of endothelial cell apoptosis: in vitro and in vivo studies. Cardiovasc Drugs Ther Sponsored Int Soc Cardiovasc Pharmacother. 2007;21:423–9.
63. Remme WJ. Secondary prevention of coronary artery disease and the choice of the ACE inhibitor why EUROPA and not PEACE. Cardiovasc Drugs Ther Sponsored Int Soc Cardiovasc Pharmacother. 2007;21:405–7.
64. Fliser D, Buchholz K, Haller H. Olmesartan EUTo, Pravastatin in I, Atherosclerosis I. Antiinflammatory effects of angiotensin II subtype 1 receptor blockade in hypertensive patients with microinflammation. Circulation. 2004;110:1103–7.
65. Mason RP. Optimal therapeutic strategy for treating patients with hypertension and atherosclerosis: focus on olmesartan medoxomil. Vasc Health Risk Manag. 2011;7:405–16.
66. Stumpe KO, Agabiti-Rosei E, Zielinski T, et al. Carotid intima-media thickness and plaque volume changes following 2-year angiotensin II-receptor blockade. The multicentre olmesartan atherosclerosis regression evaluation (MORE) study. Ther Adv Cardiovasc Dis. 2007;1:97–106.
67. Tzemos N, Lim PO, MacDonald TM. Valsartan improves endothelial dysfunction in hypertension: a randomized, double-blind study. Cardiovasc Ther. 2009;27:151–8.
68. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. The SOLVD Investigators. N Engl J Med 1991;325:293–302.
69. Patel A, Group AC, MacMahon S, et al. Effects of a fixed combination of perindopril and indapamide on macrovascular and microvascular outcomes in patients with type 2 diabetes mellitus (the ADVANCE trial): a randomized controlled trial. Lancet. 2007;370:829–40.
70. ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group. The antihypertensive and lipid-lowering treatment to prevent heart attack trial. Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs diuretic: The antihypertensive and lipid-lowering treatment to prevent heart attack trial (ALLHAT). JAMA. 2002;288:2981–97.
71. Task Force M, Montalescot G, Sechtem U, et al. 2013 ESC guidelines on the management of stable coronary artery disease: the task force on the management of stable coronary artery disease of the European society of cardiology. Eur Heart J. 2013;34:2949–3003.
72. Yamamoto E, Kataoka K, Dong YF, et al. Aliskiren enhances the protective effects of valsartan against cardiovascular and renal injury in endothelial nitric oxide synthase-deficient mice. Hypertension. 2009;54:633–8.
73. Harel Z, Gilbert C, Wald R, et al. The effect of combination treatment with aliskiren and blockers of the renin-angiotensin system on hyperkalaemia and acute kidney injury: systematic review and meta-analysis. BMJ. 2012;344:e42.
74. Maffei A, Lembo G. Nitric oxide mechanisms of nebivolol. Ther Adv Cardiovasc Dis. 2009;3:317–27.
75. Pasini AF, Garbin U, Stranieri C, et al. Nebivolol treatment reduces serum levels of asymmetric dimethylarginine and improves endothelial dysfunction in essential hypertensive patients. Am J Hypertens. 2008;21:1251–7.
76. Merchant N, Searles CD, Pandian A, et al. Nebivolol in high-risk, obese African Americans with stage 1 hypertension: effects on blood pressure, vascular compliance, and endothelial function. J Clin Hypertens (Greenwich). 2009;11:720–5.
77. Evangelista S, Garbin U, Pasini AF, Stranieri C, Boccioletti V, Cominacini L. Effect of DL-nebivolol, its enantiomers and metabolites on the intracellular production of superoxide and nitric oxide in human endothelial cells. Pharmacol Res. 2007;55:303–9.
78. Akcay A, Acar G, Kurutas E, et al. Beneficial effects of nebivolol treatment on oxidative stress parameters in patients with slow coronary flow. Turk Kardiyol Dern Ars. 2010;38:244–9.
79. Gao WQ, Feng QZ, Li YF, et al. Systematic study of the effects of lowering low-density lipoprotein-cholesterol on regression of coronary atherosclerotic plaques using intravascular ultrasound. BMC Cardiovasc Disord. 2014;14:60.
80. Tousoulis D, Oikonomou E, Siasos G, et al. Dose-dependent effects of short term atorvastatin treatment on arterial wall properties and on indices of left ventricular remodeling in ischemic heart failure. Atherosclerosis. 2013;227:367–72.
81. Wassmann S, Nickenig G. Interrelationship of free oxygen radicals and endothelial dysfunction–modulation by statins. Endothelium. 2003;10:23–33.
82. Ostadal P. Statins as first-line therapy for acute coronary syndrome? Exp Clin Cardiol. 2012;17:227–36.
83. Inoue T, Takayanagi K, Hayashi T, Morooka S. Fluvastatin attenuates nitrate tolerance in patients with ischemic heart disease complicating hypercholesterolemia. Int J Cardiol. 2003;90:181–8.
84. Milionis HJ, Liberopoulos EN, Elisaf MS, Mikhailidis DP. Analysis of antihypertensive effects of statins. Curr Hypertens Rep. 2007;9:175–83.
85. Nohara R, Daida H, Hata M, et al. Effect of intensive lipid-lowering therapy with rosuvastatin on progression of carotid intima-media thickness in Japanese patients: justification for atherosclerosis regression treatment (JART) study. Circ J. 2012;76:221–9.
86. Kovarnik T, Mintz GS, Skalicka H, et al. Virtual histology evaluation of atherosclerosis regression during atorvastatin and ezetimibe administration: HEAVEN study. Circ J. 2012;76:176–83.

87. Schwartz GG, Olsson AG, Ezekowitz MD, et al. Effects of atorvastatin on early recurrent ischemic events in acute coronary syndromes: the MIRACL study: a randomized controlled trial. JAMA. 2001;285:1711–8.
88. Kim JS, Kim J, Choi D, et al. Efficacy of high-dose atorvastatin loading before primary percutaneous coronary intervention in ST-segment elevation myocardial infarction: the STATIN STEMI trial. JACC Cardiovasc Interv. 2010;3:332–9.
89. Ostadal P, Alan D, Vejvoda J, et al. Fluvastatin in the first-line therapy of acute coronary syndrome: results of the multicenter, randomized, double-blind, placebo-controlled trial (the FACS-trial). Trials. 2010;11:61.
90. Jackson SM, Parhami F, Xi XP, et al. Peroxisome proliferator-activated receptor activators target human endothelial cells to inhibit leukocyte-endothelial cell interaction. Arterioscler Thromb Vasc Biol. 1999;19:2094–104.
91. Calnek DS, Mazzella L, Roser S, Roman J, Hart CM. Peroxisome proliferator-activated receptor gamma ligands increase release of nitric oxide from endothelial cells. Arterioscler Thromb Vasc Biol. 2003;23:52–7.
92. Hwang J, Kleinhenz DJ, Lassegue B, Griendling KK, Dikalov S, Hart CM. Peroxisome proliferator-activated receptor-gamma ligands regulate endothelial membrane superoxide production. Am J Physiol Cell Physiol. 2005;288:C899–905.
93. Faine LA, Rudnicki M, Cesar FA, et al. Anti-inflammatory and antioxidant properties of a new arylidene-thiazolidinedione in macrophages. Curr Med Chem. 2011;18:3351–60.
94. van Wijk JP, Rabelink TJ. Impact of thiazolidinedione therapy on atherogenesis. Curr Atheroscler Rep. 2005;7:369–74.
95. Houston M, Estevez A, Chumley P, et al. Binding of xanthine oxidase to vascular endothelium. Kinetic characterization and oxidative impairment of nitric oxide-dependent signaling. J Biol Chem. 1999;274:4985–94.
96. Higgins P, Dawson J, Lees KR, McArthur K, Quinn TJ, Walters MR. Xanthine oxidase inhibition for the treatment of cardiovascular disease: a systematic review and meta-analysis. Cardiovasc Ther. 2012;30:217–26.
97. Mercuro G, Vitale C, Cerquetani E, et al. Effect of hyperuricemia upon endothelial function in patients at increased cardiovascular risk. Am J Cardiol. 2004;94:932–5.
98. Baldus S, Koster R, Chumley P, et al. Oxypurinol improves coronary and peripheral endothelial function in patients with coronary artery disease. Free Radic Biol Med. 2005;39:1184–90.
99. Tousoulis D, Andreou I, Tsiatas M, et al. Effects of rosuvastatin and allopurinol on circulating endothelial progenitor cells in patients with congestive heart failure: the impact of inflammatory process and oxidative stress. Atherosclerosis. 2011;214:151–7.
100. Werner ER, Blau N, Thony B. Tetrahydrobiopterin: biochemistry and pathophysiology. Biochem J. 2011;438:397–414.
101. Cunnington C, Channon KM. Tetrahydrobiopterin: pleiotropic roles in cardiovascular pathophysiology. Heart. 2010;96:1872–7.
102. Vasquez-Vivar J, Kalyanaraman B, Martasek P. The role of tetrahydrobiopterin in superoxide generation from eNOS: enzymology and physiological implications. Free Radic Res. 2003;37:121–7.
103. Bendall JK, Douglas G, McNeill E, Channon KM, Crabtree MJ. Tetrahydrobiopterin in cardiovascular health and disease. Antioxidants Redox Signaling. 2014;20:3040–77.
104. Tiefenbacher CP, Bleeke T, Vahl C, Amann K, Vogt A, Kubler W. Endothelial dysfunction of coronary resistance arteries is improved by tetrahydrobiopterin in atherosclerosis. Circulation. 2000;102:2172–9.
105. Hattori Y, Hattori S, Wang X, Satoh H, Nakanishi N, Kasai K. Oral administration of tetrahydrobiopterin slows the progression of atherosclerosis in apolipoprotein E-knockout mice. Arterioscler Thromb Vasc Biol. 2007;27:865–70.
106. Cosentino F, Hurlimann D, Delli Gatti C, et al. Chronic treatment with tetrahydrobiopterin reverses endothelial dysfunction and oxidative stress in hypercholesterolaemia. Heart. 2008;94:487–92.
107. Wenzel P, Schulz E, Oelze M, et al. AT1-receptor blockade by telmisartan upregulates GTP-cyclohydrolase I and protects eNOS in diabetic rats. Free Radic Biol Med. 2008;45:619–26.
108. Pashkow FJ. Oxidative stress and inflammation in heart disease: do antioxidants have a role in treatment and/or prevention? Int J Inflam. 2011;2011:514623.
109. Carr AC, Zhu BZ, Frei B. Potential antiatherogenic mechanisms of ascorbate (vitamin C) and alpha-tocopherol (vitamin E). Circ Res. 2000;87:349–54.
110. Padayatty SJ, Katz A, Wang Y, et al. Vitamin C as an antioxidant: evaluation of its role in disease prevention. J Am Coll Nutr. 2003;22:18–35.
111. Paravicini TM, Touyz RM. NADPH oxidases, reactive oxygen species, and hypertension: clinical implications and therapeutic possibilities. Diabetes Care. 2008;31(2):S170–80.
112. Plantinga Y, Ghiadoni L, Magagna A, et al. Supplementation with vitamins C and E improves arterial stiffness and endothelial function in essential hypertensive patients. Am J Hypertens. 2007;20:392–7.
113. Tousoulis D, Antoniades C, Tentolouris C, et al. Effects of combined administration of vitamins C and E on reactive hyperemia and inflammatory process in chronic smokers. Atherosclerosis. 2003;170:261–7.
114. Antoniades C, Tousoulis D, Tentolouris C, et al. Effects of antioxidant vitamins C and E on endothelial function and thrombosis/fibrinolysis system in smokers. Thromb Haemost. 2003;89:990–5.
115. Stephens NG, Parsons A, Schofield PM, Kelly F, Cheeseman K, Mitchinson MJ. Randomised controlled trial of vitamin E in patients with coronary disease: cambridge heart antioxidant study (CHAOS). Lancet. 1996;347:781–6.
116. Bleys J, Miller 3rd ER, Pastor-Barriuso R, Appel LJ, Guallar E. Vitamin-mineral supplementation and the progression of atherosclerosis: a meta-analysis of randomized controlled trials. Am J Clin Nutr. 2006;84:880–7. quiz 954–5.
117. Sesso HD, Buring JE, Christen WG, et al. Vitamins E and C in the prevention of cardiovascular disease in men: the physicians' health study II randomized controlled trial. JAMA. 2008;300:2123–33.
118. Cook NR, Albert CM, Gaziano JM, et al. A randomized factorial trial of vitamins C and E and beta carotene in the secondary prevention of cardiovascular events in women: results from the women's antioxidant cardiovascular study. Arch Intern Med. 2007;167:1610–8.
119. Bonaa KH, Njolstad I, Ueland PM, et al. Homocysteine lowering and cardiovascular events after acute myocardial infarction. N Engl J Med. 2006;354:1578–88.
120. Till U, Rohl P, Jentsch A, et al. Decrease of carotid intima-media thickness in patients at risk to cerebral ischemia after supplementation with folic acid, vitamins B6 and B12. Atherosclerosis. 2005;181:131–5.
121. Antoniades C, Antonopoulos AS, Tousoulis D, Marinou K, Stefanadis C. Homocysteine and coronary atherosclerosis: from folate fortification to the recent clinical trials. Eur Heart J. 2009;30:6–15.
122. Antoniades C, Shirodaria C, Warrick N, et al. 5-methyltetrahydrofolate rapidly improves endothelial function and decreases superoxide production in human vessels: effects on vascular tetrahydrobiopterin availability and endothelial nitric oxide synthase coupling. Circulation. 2006;114:1193–201.
123. Toole JF, Malinow MR, Chambless LE, et al. Lowering homocysteine in patients with ischemic stroke to prevent recurrent stroke, myocardial infarction, and death: the vitamin intervention for stroke prevention (VISP) randomized controlled trial. JAMA. 2004;291:565–75.

124. Gissi HFI, Tavazzi L, Maggioni AP, et al. Effect of n-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): a randomized, double-blind, placebo-controlled trial. Lancet. 2008;372:1223–30.
125. Wang Q, Liang X, Wang L, et al. Effect of omega-3 fatty acids supplementation on endothelial function: a meta-analysis of randomized controlled trials. Atherosclerosis. 2012;221:536–43.
126. Miller 3rd ER, Juraschek SP, Anderson CA, et al. The effects of n-3 long-chain polyunsaturated fatty acid supplementation on biomarkers of kidney injury in adults with diabetes: results of the GO-FISH trial. Diabetes Care. 2013;36:1462–9.
127. Tousoulis D, Plastiras A, Siasos G, et al. Omega-3 PUFAs improved endothelial function and arterial stiffness with a parallel antiinflammatory effect in adults with metabolic syndrome. Atherosclerosis. 2014;232:10–6.
128. Siasos G, Tousoulis D, Oikonomou E, et al. Effects of Omega-3 fatty acids on endothelial function, arterial wall properties, inflammatory and fibrinolytic status in smokers: a cross over study. Int J Cardiol. 2013;166:340–6.
129. Das UN. Long-chain polyunsaturated fatty acids interact with nitric oxide, superoxide anion, and transforming growth factor-beta to prevent human essential hypertension. Eur J Clin Nutr. 2004;58:195–203.
130. Saravanan P, Davidson NC, Schmidt EB, Calder PC. Cardiovascular effects of marine omega-3 fatty acids. Lancet. 2010;376:540–50.
131. Guermouche B, Soulimane-Mokhtari NA, Bouanane S, Merzouk H, Merzouk S, Narce M. Effect of dietary N - 3 polyunsaturated Fatty acids on oxidant/antioxidant status in macrosomic offspring of diabetic rats. BioMed Res Intern. 2014;2014:368107.
132. Richard D, Kefi K, Barbe U, Bausero P, Visioli F. Polyunsaturated fatty acids as antioxidants. Pharmacol Res: Off J Ital Pharmacol Soc. 2008;57:451–5.
133. Hassan Eftekhari M, Aliasghari F, Babaei-Beigi MA, Hasanzadeh J. Effect of conjugated linoleic acid and omega-3 fatty acid supplementation on inflammatory and oxidative stress markers in atherosclerotic patients. ARYA atherosclerosis. 2013;9:311–8.
134. Lee LK, Shahar S, Rajab N, Yusoff NA, Jamal RA, Then SM. The role of long chain omega-3 polyunsaturated fatty acids in reducing lipid peroxidation among elderly patients with mild cognitive impairment: a case–control study. J Nutr Biochem. 2013;24:803–8.
135. Mukamal KJ, Conigrave KM, Mittleman MA, et al. Roles of drinking pattern and type of alcohol consumed in coronary heart disease in men. N Engl J Med. 2003;348:109–18.
136. Ivey KL, Lewis JR, Prince RL, Hodgson JM. Tea and non-tea flavonol intakes in relation to atherosclerotic vascular disease mortality in older women. Br J Nut. 2013;110:1648–55.
137. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2009;2:270–8.
138. Okawa M, Kinjo J, Nohara T, Ono M. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of flavonoids obtained from some medicinal plants. Biol Pharm Bull. 2001;24:1202–5.
139. Park YC, Rimbach G, Saliou C, Valacchi G, Packer L. Activity of monomeric, dimeric, and trimeric flavonoids on NO production, TNF-alpha secretion, and NF-kappaB-dependent gene expression in RAW 264.7 macrophages. FEBS Lett. 2000;465:93–7.
140. Mackenzie GG, Carrasquedo F, Delfino JM, Keen CL, Fraga CG, Oteiza PI. Epicatechin, catechin, and dimeric procyanidins inhibit PMA-induced NF-kappaB activation at multiple steps in Jurkat T cells. FASEB J. 2004;18:167–9.
141. Nicholson SK, Tucker GA, Brameld JM. Physiological concentrations of dietary polyphenols regulate vascular endothelial cell expression of genes important in cardiovascular health. Br J Nutr. 2010;103:1398–403.
142. Auclair S, Chironi G, Milenkovic D, et al. The regular consumption of a polyphenol-rich apple does not influence endothelial function: a randomized double-blind trial in hypercholesterolemic adults. Eur J Clin Nutr. 2010;64:1158–65.
143. Gollucke AP. Recent applications of grape polyphenols in foods, beverages and supplements. Recent Pat Food Nutr Agric. 2010;2:105–9.
144. Hooper L, Kroon PA, Rimm EB, et al. Flavonoids, flavonoid-rich foods, and cardiovascular risk: a meta-analysis of randomized controlled trials. Am J Clin Nutr. 2008;88:38–50.
145. Andriantsitohaina R, Auger C, Chataigneau T, et al. Molecular mechanisms of the cardiovascular protective effects of polyphenols. Br J Nutr. 2012;108:1532–49.
146. Bornhoeft J, Castaneda D, Nemoseck T, Wang P, Henning SM, Hong MY. The protective effects of green tea polyphenols: lipid profile, inflammation, and antioxidant capacity in rats fed an atherogenic diet and dextran sodium sulfate. J Med Food. 2012;15:726–32.
147. Ras RT, Zock PL, Draijer R. Tea consumption enhances endothelial-dependent vasodilation; a meta-analysis. PLoS One. 2011;6:e16974.
148. Fitzpatrick DF, Hirschfield SL, Ricci T, Jantzen P, Coffey RG. Endothelium-dependent vasorelaxation caused by various plant extracts. J Cardiovasc Pharmacol. 1995;26:90–5.
149. Schewe T, Steffen Y, Sies H. How do dietary flavanols improve vascular function? A position paper. Arch Biochem Biophys. 2008;476:102–6.
150. Visioli F, Galli C. Biological properties of olive oil phytochemicals. Crit Rev Food Sci Nutr. 2002;42:209–21.
151. Gimeno E, Fito M, Lamuela-Raventos RM, et al. Effect of ingestion of virgin olive oil on human low-density lipoprotein composition. Eur J Clin Nutr. 2002;56:114–20.
152. Tousoulis D, Papageorgiou N, Antoniades C, et al. Acute effects of different types of oil consumption on endothelial function, oxidative stress status and vascular inflammation in healthy volunteers. Br J Nut. 2010;103:43–9.
153. Papageorgiou N, Tousoulis D, Psaltopoulou T, et al. Divergent anti-inflammatory effects of different oil acute consumption on healthy individuals. Eur J Clin Nutr. 2011;65:514–9.
154. Pitsavos C, Panagiotakos DB, Tzima N, et al. Adherence to the Mediterranean diet is associated with total antioxidant capacity in healthy adults: the ATTICA study. Am J Clin Nutr. 2005;82:694–9.
155. Salami M, Galli C, De Angelis L, Visioli F. Formation of F2-isoprostanes in oxidized low density lipoprotein: inhibitory effect of hydroxytyrosol. Pharmacol Res: Off J Ital Pharmacol Soc. 1995;31:275–9.
156. Khurana S, Venkataraman K, Hollingsworth A, Piche M, Tai TC. Polyphenols: benefits to the cardiovascular system in health and in aging. Nutrients. 2013;5:3779–827.
157. Masella R, Vari R, D'Archivio M, et al. Extra virgin olive oil biophenols inhibit cell-mediated oxidation of LDL by increasing the mRNA transcription of glutathione-related enzymes. J Nutr. 2004;134:785–91.
158. Hollman PC, Cassidy A, Comte B, et al. The biological relevance of direct antioxidant effects of polyphenols for cardiovascular health in humans is not established. J Nutr. 2011;141:989S–1009S.
159. Kalen A, Appelkvist EL, Dallner G. Age-related changes in the lipid compositions of rat and human tissues. Lipids. 1989;24:579–84.
160. Ochiai A, Itagaki S, Kurokawa T, Kobayashi M, Hirano T, Iseki K. Improvement in intestinal coenzyme q10 absorption by food intake. Yakugaku zasshi: J Pharm Soc Japan. 2007;127:1251–4.
161. Lee BJ, Tseng YF, Yen CH, Lin PT. Effects of coenzyme Q10 supplementation (300 mg/day) on antioxidation and anti-inflammation in coronary artery disease patients during statins therapy: a randomized, placebo-controlled trial. Nutr J. 2013;12:142.
162. Gao L, Mao Q, Cao J, Wang Y, Zhou X, Fan L. Effects of coenzyme Q10 on vascular endothelial function in humans: a meta-analysis of randomized controlled trials. Atherosclerosis. 2012;221:311–6.

163. Tiyerili V, Zimmer S, Jung S, et al. CB1 receptor inhibition leads to decreased vascular AT1 receptor expression, inhibition of oxidative stress and improved endothelial function. Basic Res Cardiol. 2010;105:465–77.
164. Van Gaal LF, Scheen AJ, Rissanen AM, Rossner S, Hanotin C, Ziegler O. Long-term effect of CB1 blockade with rimonabant on cardiometabolic risk factors: two year results from the RIO-Europe study. Eur Heart J. 2008;29:1761–71.
165. O'Donnell BV, Tew DG, Jones OT, England PJ. Studies on the inhibitory mechanism of iodonium compounds with special reference to neutrophil NADPH oxidase. Biochem J. 1993;290(1):41–9.
166. Kinkade K, Streeter J, Miller FJ. Inhibition of NADPH oxidase by apocynin attenuates progression of atherosclerosis. Int J Mol Sci. 2013;14:17017–28.
167. Liu Y, Davidson BP, Yue Q, et al. Molecular imaging of inflammation and platelet adhesion in advanced atherosclerosis effects of antioxidant therapy with NADPH oxidase inhibition. Circ Cardiovasc Imaging. 2013;6:74–82.
168. Khanicheh E, Qi Y, Xie A, et al. Molecular imaging reveals rapid reduction of endothelial activation in early atherosclerosis with apocynin independent of antioxidative properties. Arterioscler Thromb Vasc Biol. 2013;33:2187–92.
169. Schramm A, Matusik P, Osmenda G, Guzik TJ. Targeting NADPH oxidases in vascular pharmacology. Vasc Pharmacol. 2012;56:216–31.
170. Rey FE, Cifuentes ME, Kiarash A, Quinn MT, Pagano PJ. Novel competitive inhibitor of NAD(P)H oxidase assembly attenuates vascular O(2)(−) and systolic blood pressure in mice. Circ Res. 2001;89:408–14.