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REVIEW

Oxidative stress and cardiovascular disease: new insights

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Abstract

The role of oxidative stress in the onset and progression of atherosclerosis and its impact on the development of cardiovascular events has been widely described.

Thus, increased oxidative stress has been described in several atherosclerotic risk factors, such as hypertension, dyslipidaemia, peripheral artery disease, metabolic syndrome, diabetes, and obesity.

Among others, specific oxidative pathways involving both pro-oxidant and antioxidant enzymes seem to play a major role in the production of reactive oxidant species (ROS), such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, myeloperoxidase, superoxide dismutase, and glutathione peroxidase.

In this review, we will discuss: 1) the most relevant enzyme systems involved in the formation and detoxification of ROS, 2) the relationship between oxidative stress and cardiovascular risk, and 3) therapeutic implications to modulate oxidative stress.

Key words: oxidative stress, cardiovascular diseases, NADPH oxidase

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INTRODUCTION

The term oxidative stress was coined by Sies [1] to describe a disturbance in the balance between reactive oxygen species (ROS) production and antioxidant clearance. Currently, oxidative stress is defined as an event where a transient or permanent perturbation in the oxidative balance state generates physiological consequences within the cell, depending on the specific target and ROS concentrations. In physiological conditions, ROS concentrations fluctuate in a controlled manner and are modulated by enzymatic and non-enzymatic antioxidant systems [2]. If this homeostatic state fails, such as in the case of hypertension, dyslipidaemia, diabetes, and obesity and acute conditions such as sepsis and respiratory failure, ROS levels increase [3, 4]. Also, xenobiotic triggers can influence the antioxidant status, among them: radiation, drugs, habits like smoking, and environmental agents. All these triggers can induce the synthesis and increase the activity of pro-oxidant enzymatic systems that are identified to be significantly involved in the progression of the atherosclerotic disease [5]. In particular, nicotinamide adenine dinucleotide phosphate (NADPH) oxidases and myeloperoxidase (MPO) are among the best established enzymatic systems involved in atherosclerotic progression [3] and are involved in the formation of ROS such as isoprostanes and superoxide anion (O_2^-) [5].

Atherosclerosis is a chronic process of progressive hardening and narrowing of arteries that reduces the flow and delivery of blood and oxygen throughout the body, leading to plaque formation [5]. It starts in childhood and progresses throughout life, with several risk factors favouring its progression [5]. Atherosclerotic plaque progression is caused by molecular changes induced by cytokines and ROS, mainly due to the interaction between endothelial cells, low-density lipoprotein (LDL), and macrophages.

In particular, in the early stages of atherogenesis, LDLs are oxidised by ROS, giving formation to oxidised LDLs (oxLDLs), which are no longer cleared from sub-endothelial space and start to accumulate in the subendothelium [5]. Oxidised LDL activates the endothelium by inducing the production of adhesion molecules, which recruit monocytes and T-cells, which are considered a key stimulator for the immune system response [5]. Monocytes differentiate into macrophages that internalise LDL and, along with T-cells, release pro-inflammatory cytokines and ROS to keep oxidising LDLs [5]. This contributes to the formation of an atherosclerotic plaque by apoptosis and foam cell formation [5].

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Table 1. Overview of pro- and anti-oxidant systems

Pro-oxidants		Antioxidants		
Enzymatic systems	Pro-oxidant compounds	Antioxidant enzymes	Direct antioxidant	Indirect antioxidant
Myeloperoxidase (MPO)	Superoxide anion (O_2^-)	Superoxide dismutase (SOD)	Glutathione	Polyphenols
NADPH oxidase	Hydrogen peroxide (H_2O_2)	Catalase	Ascorbic acid	Hydrogen sulphide (H_2S)
Inducible nitric oxide synthase (iNOS)	Isoprostanes	Glutathione peroxidase (GPx)	Tocopherols	
Uncoupled endothelial nitric oxide synthase (eNOS)	Thromboxane	Nitric oxide synthase (NOS)		
Lipoxygenases (LOX)		Paraoxonase (PON)		

NADPH — nicotinamide adenine dinucleotide phosphate

Table 2. Nicotinamide adenine dinucleotide phosphate (NADPH) isoforms and localisation within vasculature

NADPH isoforms	Vasculature distribution	Product
NOX1	VSMCs, ECs	Superoxide anion
NOX2	VSMCs, macrophages, fibroblasts, platelets, ECs	Superoxide anion
NOX4	VSMCs, ECs, fibroblasts	Hydrogen peroxide
NOX5	VSMCs, ECs	Superoxide anion

ECs — endothelial cells; VSMCs — vascular smooth muscle cells

These events lead to the formation of the so-called "fatty streak" and ultimately to atherosclerotic plaque [3]. Plaque rupture will induce platelet aggregation, coagulation cascade activation, and thrombus development and, as a consequence, acute artery occlusion [3].

In this review, we will discuss 1) the most relevant enzyme systems involved in the formation and detoxification of ROS, 2) the relationship between oxidative stress and cardiovascular risk, and 3) therapeutic implications for the treatment of atherosclerosis and its complications.

OXIDATIVE STRESS AND ATHEROSCLEROSIS

An overview of pro- and anti-oxidant systems is provided in Table 1. Among the enzymatic systems involved in ROS formation, a relevant role is played by NADPH oxidase (NOX), MPO, lipoxygenases (LOX), and uncoupled endothelial nitric oxide synthase (eNOS).

NADPH oxidase (Table 2) is an enzymatic system composed of several subunits and different isoforms responsible for the formation of ROS, mostly O_2^- and hydrogen peroxide (H_2O_2), that in turn are responsible for the formation of active eicosanoids such as isoprostanes and thromboxanes [6].

The impact of the different NOX isoforms on the process of human atherosclerosis is still a matter of debate. While the role of NOX2 in atherosclerosis initiation and progression has been widely described, other NOX isoforms seem to differently affect the atherosclerotic process. Thus, NOX1 and NOX5 are known to essentially elicit O_2^- formation and to contribute to proliferation of human smooth muscle cells. NOX4 yields the formation of H_2O_2, which possesses vasodilating properties via eNOS activation.

Several studies have demonstrated that the modulation of NADPH oxidase activity is associated with reduced risk of atherosclerotic disease. Apocynin, a compound that reduces the NADPH oxidase subunit $p47^{phox}$ translocation to the membrane subunit NOX2, inhibited atherosclerotic plaque formation in animals [7, 8]. Moreover, apocynin dose-dependently lowered total monocyte plaque accumulation, platelet adhesion, and atherosclerotic progression [7]. These data were supported in mice treated with a specific antibody blocking NADPH oxidase activity. Thus, Quesada et al. [8] found a significant regression of atherosclerotic plaque in mice fed with a high-fat diet and given a specific NOX2 inhibitor (NOX2ds-tat).

Experimental knock-out models of NADPH oxidases fully elucidated the role of NOX2 activity in atherosclerotic progression. Judkins et al. [9] studied a double knockout model of accelerated atherosclerosis represented by $NOX2^{(-/y)}/ApoE^{(-/-)}$ mice, and found reduced early lesion development in NOX2$^{(-/y)}$/ApoE$^{(-/-)}$ animals compared to ApoE$^{(-/-)}$ mice. Similar results were also obtained in ApoE$^{(-/-)}$/p47$^{phox(-/-)}$ compared with ApoE$^{(-/-)}$ mice [10].

In human models over-expression of some NADPH oxidase subunits was associated with accelerated atherosclerotic lesion progression. Thus, the hyper-expression of p22^{phox}, the membrane-bound subunit of NADPH oxidase, was observed in the vessel wall of atherosclerotic coronary arteries using coronary sections from autopsied cases [11].

The human model of NADPH oxidase activity deletion, namely chronic granulomatous disease (CGD), permits confirmation of the central role of NADPH oxidase in the development of atherosclerotic diseases. CGD is prevalently characterised by NOX2 hereditary deficiency (X-linked) or, more rarely, by hereditary deficiency of p47^{phox} subunit [12, 13]. Patients with CGD disclosed lower cellular ROS formation in comparison to those with hereditary p47^{phox} deficiency [13]. The interplay between NADPH oxidase and atherosclerosis was further studied in women carrying NOX2 deficiency [14]. Thus, Sibley et al. [15] compared CGD patients with control subjects and showed a 22% lower internal carotid artery wall volume with a similar reduction detected in both the p47^{phox}- and gp91^{phox}-deficient subtypes [15].

In addition to NOX2, MPO is fully represented within vulnerable plaque, where it serves as an enzymatic source of oxidant species such as hypochlorous acid, chloramine, tyrosyl radicals, and nitrogen dioxide to generate atherogenic forms of both LDL and high-density lipoprotein (HDL) [16]. The ability of MPO-derived ROS to promote lipid peroxidation [17] is supported by an experimental study that showed that knockout mice for MPO had a reduction of F2-isoprostanes formation of about 85% [17].

Lipoxygenases play a significant role in the atherosclerotic process and are expressed in the vascular wall [18]. In particular, 5-LOX is expressed in human atherosclerotic plaques [19] and catalyses the transformation of free arachidonic acid into leukotriene B4 (LTB4), a potent chemo-attractant and leukocyte activator [20]. Experiments in mice demonstrated that LTB4 antagonism by binding its specific receptor on the cell surface decreases foam cell translocation into the plaque [21]. Experiments on knockout models support the relevance of 12/15-LOX in the atherosclerotic process. Thus, 12/15-LOX$^{(-/-)}$ mice on a high-fat diet presented reduced atherosclerosis, compared with control ApoE$^{(-/-)}$ mice [22].

The role of NOS is more controversial because it may act as a pro- and antioxidant system. Under normal conditions, eNOS exerts anti-atherogenic effects in the vascular wall [5], and in eNOS-deficient ApoE$^{(-/-)}$ mice an increased coronary atherosclerosis has been described [23].

On the contrary, eNOS becomes "uncoupled" in pathophysiological conditions, resulting in the production of the pro-oxidant species O_2^- by the transfer of electrons from NADPH through flavins to molecular oxygen and results in a pro-oxidant activity of eNOS [24].

Antioxidant enzyme systems

The vasculature is protected from excessive ROS formation by antioxidant enzyme systems, including superoxide dismutases (SODs), catalase, glutathione peroxidases (GPxs), and paraoxonases (PONs) [3]. These systems protect against atherogenesis by scavenging ROS, facilitating endothelium-dependent vasorelaxation, inhibiting inflammatory cell adhesion to endothelium, and altering vascular cellular responses, such as vascular smooth muscle cells (VSMCs) and endothelial cell apoptosis, VSMCs proliferation, hypertrophy, and migration.

The SOD family is represented by three isoforms, namely SOD1, SOD2, and SOD3, which are able to protect against atherogenesis by converting O_2^- into H_2O_2. Thus, overexpression of SOD1 delayed atherosclerotic lesion development in ApoE$^{(-/-)}$ mice [25], while SOD2 deficiency induced accelerated atherosclerosis in the same animal model [26]. SOD1 and SOD2 deficiency results in VSMCs hyperplasia and hypertrophy mediated by different kinases [27]. Also, SOD3 displayed a protective effect; it was shown to prevent LDL oxidation in in-vitro experiments on rabbit endothelial cells [28, 29].

Eight isoforms of GPx have been described so far, but not all of them are well characterised. GPx1 is contained in red blood cells while GPx3 is the only circulating isoform. GPx seems to play a central role in protecting arterial walls from atherosclerotic progression by detoxifying intracellular H_2O_2 [30]. An increased atherosclerosis was observed in GPx1$^{(-/-)}$ mice [31], and the overexpression of GPx4 decreased atherosclerosis and delayed lesion progression in a similar animal model [32].

Paraoxonase, which exists in three isoforms (PON1, PON2, and PON3), is another antioxidant enzyme with atheroprotective effect. PON1 was shown to prevent LDL and HDL oxidation in vitro. PON1-knockout mice had higher levels of oxidised phospholipids compared with wild-type [33]. Furthermore, PON1 deficiency increased aortic atherosclerosis in wild-type and ApoE$^{(-/-)}$ mice [33, 34]. Similar results were obtained in PON2-deficient ApoE$^{(-/-)}$ mice, which disclosed increased mitochondrial oxidative stress and exacerbated atherosclerosis when fed with both chow and Western diet [35]. Finally, significantly smaller atherosclerotic lesions in PON3 transgenic mice were found in PON3-transgenic mice fed with an atherogenic diet [36].

OXIDATIVE STRESS RELATED TO CARDIOVASCULAR RISK FACTORS

Several cardiovascular risk factors such as type 2 diabetes mellitus (T2DM), hypertension, atrial fibrillation (AF), peripheral artery disease (PAD), obesity, metabolic syndrome (MetS), dyslipidaemia, habit of smoking, and pollution are associated with an increased production of ROS (Fig. 1) [37].

Urinary 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-PGF$_{2\alpha}$), which is derived from the non-enzymatic oxidation of arachidonic acid, and serum levels of soluble NOX2-derived peptide

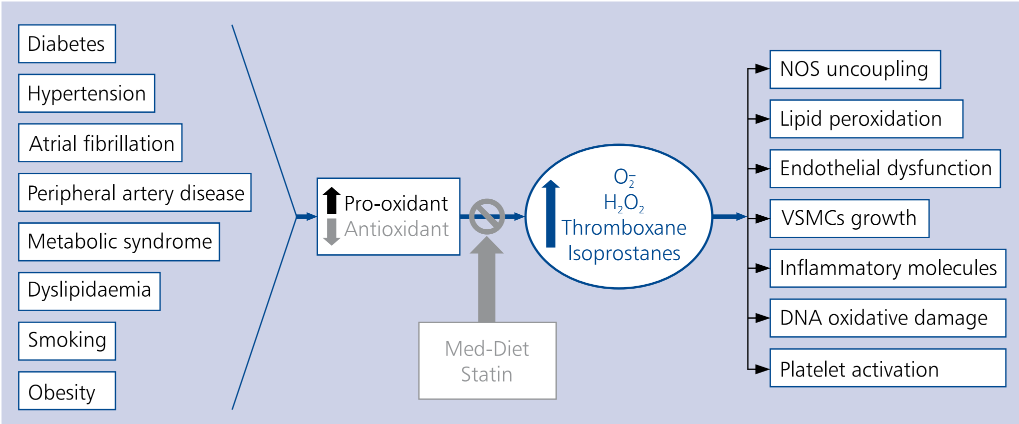

Figure 1. Relationship between cardiovascular risk factors and oxidative stress; Med-Diet — Mediterranean Diet; NOS — nitric oxide synthase; VSMCs — vascular smooth muscle cells

(sNOX2-dp), a peptide released upon NOX2 activation, are among the most studied biomarkers of oxidative stress [14, 38]. Urinary excretion of 8-iso-PGF$_{2\alpha}$ is a validated and accepted reliable biomarker of in vivo oxidative stress, which has been investigated in healthy subjects and patients with cardiovascular diseases (CVDs) [39]. Useful markers of oxidative stress are also MPO and oxLDL [39].

Increased values of urinary 8-iso-PGF$_{2\alpha}$ [40] and serum sNOX2-dp levels as well as higher levels of MPO and oxLDL have been detected in subjects with or at risk for CVD, such as those with T2DM, obesity, PAD, hypercholesterolaemia, MetS, and hypertension.

A large body of evidence indicates that patients with T2DM have an increased ROS production, together with enhanced lipid peroxidation and isoprostane formation [41, 42]. Of note, oxidative stress seems to be dependent upon glycaemic control. Thus, when analysing 62 non-insulin-dependent DM subjects, urinary 8-iso-PGF$_{2\alpha}$ excretion was seen to be significantly higher (419 ± 208 pg/mg creatinine) than in age-matched controls (208 ± 92 pg/mg creatinine; $p < 0.001$) [43]; the authors also observed that an improvement in metabolic control was associated with a reduction in 8-iso-PGF$_{2\alpha}$ levels (from 533 ± 276 to 365 ± 226 pg/mg creatinine; $p = 0.001$). Similarly, obese patients displayed increased urinary excretion of isoprostanes, as demonstrated by Davi et al. [44] when comparing 24 obese women to 24 nonobese. Although numerous studies have documented an increased oxidative stress in obese subjects, the relationship between the degree of obesity and systemic oxidative stress in humans is still an open question. No correlation at all, a positive correlation, or a link with obesity-related diseases have been described so far [45].

Isoprostanes and sNOX2-dp have also been investigated in patients affected by PAD. Compared to controls, PAD patients had increased sNOX2-dp (18.1 ± 17.6 vs. 34.4 ± 21.2 pg/mL respectively; $p < 0.001$) and isoprostanes (126.9 ± 122.9 vs. 199.1 ± 130.4 pg/mg creatinine, respectively; $p = 0.005$) [46]. Also, MPO was evaluated in PAD patients. In a large cohort of 1324 African-Americans and 1237 non-Hispanic white individuals, high MPO was significantly associated with lower ankle-brachial index and the presence of PAD [47]. It should be taken into consideration that, in addition to ROS formation, MPO contributes to oxidative stress by oxidising LDL and inactivating NO [48]. To confirm its central role in atherosclerotic progression the evidence was derived from a prospective study. Thus, in a cohort of 156 PAD patients, MPO was an independent predictor of vascular outcomes including myocardial infarction (MI) and stroke for a value ≥ 183.7 pM [49].

Also, MetS is associated with increased levels of isoprostanes and oxLDL. Higher F2-isoprostanes were found in MetS patients (n = 30, 808 pmol/mmol creatinine) as compared to 30 controls balanced for age and sex (664 pmol/mmol creatinine; $p = 0.05$) [50]. Oxidised LDL [51], a key element in the process of atherosclerosis, was evaluated in patients with MetS. Holvoet et al. [52] performed a large study on 3033 subjects: 1147 with and 1886 without MetS. The authors found elevated values of oxLDL in patients with MetS (1.45 ± 0.82 mg/dL) compared to those without (1.23 ± 0.67 mg/dL; $p < 0.001$). Hypercholesterolaemia is another risk factor with solid evidence as a trigger for ROS production. When comparing 40 hypercholesterolaemic adults to 40 matched controls, O_2^-, a direct product of NADPH oxidase activation, was found to be increased and correlated to markers of platelet activation such as CD40 ligand [53]. This phenomenon seems to be involved in the early stages of the atherosclerotic disease initiation because similar results were found in children. Thus, compared to normocholesterolaemic children, those with hypercholesterolaemia had higher MPO plasma levels that were associated with increased oxLDL [54]. Oxidative stress is also a critical component of hypertension, where NADPH oxidase represents the primary source of ROS. Mechanistically, angiotensin II, through angiotensin-1 receptor signalling, mediates the vascular up-regulation and activation of NADPH oxidase. In support of this is a study comparing the effect of the angiotensin II inhibitor irbesartan with diuretic therapy in hypertensive patients. Irbesartan-treated subjects presented lower level of O_2^- [55].

Among the xenobiotic triggers for oxidative stress, smoking and air pollution are critical risk factors associated with increased risk of cardiovascular event (CVE), and they are suggested to act by increasing ROS production. A recent report identified air pollution within the top 10 risk factors for all-cause disease, greater than that caused by risk factors such as sedentary lifestyle or high cholesterol [56]. Both smoking and pollution result in the combustion of complex carbon-rich materials, with notable similarities in the compositions of these fumes. The biological pathways linking pulmonary exposure to these fumes and CVD development remain the subject of ongoing research, although oxidative stress is considered the most probable candidate in mediating these pathways [45]. Exposure to air pollution is accompanied by an increase in circulating oxidative stress markers. Thus, when investigating oxidative stress markers in 113 workers exposed to metal-rich particulate matters and 61 non-exposed volunteers, plasma levels of soluble sNOX2-dp and 8-iso-PGF$_{2\alpha}$ were found to be significantly increased in the exposed group [57]. Regarding smoking habit, analysis of oxidative biomarkers in a crossover, single-blind study performed in 40 healthy subjects (20 smokers and 20 non-smokers, matched for age and sex) demonstrated that among smokers, the use of electronic cigarettes was associated with lower levels of sNOX2-dp and urinary isoprostanes compared to regular tobacco smokers [58].

THE RELATIONSHIP BETWEEN OXIDATIVE STRESS AND CARDIOVASCULAR RISK

Human studies in subjects affected by genetic modification of ROS-producing enzymes such as NADPH oxidase and MPO suggest that they play a key role in the development of CVE. Thus, the C242T polymorphism in the gene for the p22^{phox} subunit of NADPH oxidase was evaluated in 237 patients with coronary stenosis during a median follow-up of 7.8 years. The 242T allele was found to be a predictor of lower risk of recurrence of CVE in high-risk patients and was associated with reduced systemic oxidative stress [59].

Similarly, individuals with total or subtotal MPO deficiency or loss-of-function polymorphisms have presented a reduced rate of coronary heart disease (CHD) [60].

Several observational studies have supported the association between oxidative stress and CVE. Solid evidence was found when analysing several pro-oxidant molecules such as 8-iso-PGF$_{2\alpha}$ and oxLDL and enzymes such as MPO. Plasma or urinary excretion of 8-iso-PGF$_{2\alpha}$ has been extensively investigated in patients with acute or chronic coronary artery disease (CAD). Cipollone et al. [61] measured 8-iso-PGF$_{2\alpha}$ in urinary samples from patients with unstable angina (n = 32), stable angina (n = 32), and from 40 healthy subjects. Urinary excretion of F2-isoprostanes in patients with unstable angina were higher (339 ± 122 pg/mg creatinine) compared to patients with stable angina (236 ± 83 pg/mg creatinine; $p < 0.001$) and in control subjects (192 ± 71 pg/mg creatinine; $p < 0.001$). In a further study, plasma levels of F2-isoprostanes were also significantly elevated in patients with angiographically documented CAD (n = 54), compared to controls (n = 50; 9.0 ± 4.0 vs. 6.0 ± 3.0 µmol/mol; $p < 0.001$) [62]. Similarly, in a large case-control study, including 799 patients with CAD and 925 controls, Kim et al. [63] demonstrated that CAD patients had higher 8-epi-PGF$_{2\alpha}$ compared to controls (1332.9 ± 29.4 vs. 1123.6 ± 20.1 pg/mg creatinine, respectively; $p < 0.001$).

Of note, the levels of F2-isoprostanes were found to be associated with the number of affected vessels in a study in patients with angiographically proven CAD [64] and were confirmed also in a larger study in 241 subjects undergoing coronary angiography [65]. This evidence suggests a relationship between oxidative stress and the rate of coronary atherosclerosis.

Also, the interplay between oxLDL and CVE was largely investigated. A cross-sectional study performed in CHD patients [66] investigated the association between oxLDL and the severity of CAD. The study included 63 acute coronary syndrome (ACS) patients, 35 patients with angiographic stable angina, 28 heart transplant patients with post-transplant CAD, 79 heart transplant patients without CAD, and 65 control subjects. oxLDL levels were significantly higher in patients with CAD compared to those without CAD. Thus, plasma levels of oxLDL were 3.7-fold higher ($p < 0.001$) in patients with stable angina pectoris, 4.0-fold higher ($p < 0.001$) in patients with unstable angina pectoris, and 4.8-fold higher ($p < 0.001$) in patients with acute MI, compared to controls.

This data was corroborated by a second study on 135 patients with acute MI; in patients with MI (1.95 ± 1.42 ng/5 µg LDL protein), oxLDL levels were significantly higher than in patients with unstable (1.19 ± 0.74 ng/5 µg LDL protein; $p < 0.0005$) or stable angina (0.89 ± 0.48 ng/5 µg LDL protein; $p < 0.001$) or in 46 controls (0.58 ± 0.23 ng/5 µg LDL protein; $p < 0.001$) [67]. Taken together, these studies demonstrated that oxLDL levels correlated with the severity of ACS.

Several studies have investigated the relationship between MPO activity and CAD. In a first case-control study, leukocyte-MPO was significantly higher in 158 CAD patients (18.1 U/mg) in comparison to matched controls

(13.4 U/mg; $p < 0.001$) [68]. Similarly, in 384 patients presenting with ST-segment elevation MI, MPO was higher in patients experiencing death or non-fatal MI than in survivors (50.6 vs. 33.5 ng/mL; $p = 0.001$) [69]. MPO seems to play a role also in the development of heart failure. Thus, plasma MPO levels were elevated in patients with chronic systolic heart failure compared with healthy subjects (1158 ± 2965 vs. 204 ± 139 pM, respectively; $p < 0.001$). In this population, MPO levels increased in parallel with increasing functional New York Heart Association classes ($p < 0.001$) [70].

The predictive role of 8-iso-$PGF_{2\alpha}$ has been tested by LeLeiko et al. [71] in 108 patients presenting with ACS with a significant increase of cardiac event rate across tertiles of F2-isoprostanes. The predictive power of 8-iso-$PGF_{2\alpha}$ was also evaluated in patients affected by AF, in a large study that also tested the ability of sNOX2-dp to predict CVE. Analysing 1002 anticoagulated AF patients, a significant difference at baseline of median levels of urinary 8-iso-$PGF_{2\alpha}$ (160 vs. 100 pg/mg creatinine; $p < 0.001$) and sNOX2-dp (13 vs. 9 pg/mL; $p < 0.001$) between patients with and without CVE was found [72]. In particular, a significant increase in the cumulative incidence of CVE and cardiovascular deaths was observed across tertiles for 8-iso-$PGF_{2\alpha}$ and sNOX2-dp [72].

Also, MPO seems to be able to play a predictive role for CVE. A large study performed in 1090 patients with ACS with a six-month follow-up demonstrated that patients with elevated MPO levels (> 350 μg/L) had an increased risk of death and MI [73]. Another large study, which tested MPO activity in 604 consecutive patients presenting with chest pain at the emergency department, showed similar results [74]. Thus, the incidence of MI at 30 days increased with increasing quartiles of MPO levels, ranging from 13.9% in the first quartile (< 119.4 pM) to 38.4% in the fourth quartile (≥ 394.0 pM; $p < 0.001$ for trend). Similarly, above-median levels of MPO (> 55 ng/mL) predicted mortality (odds ratio 1.8; $p = 0.034$) in 512 acute MI patients [75]. Also, data from the TACTICS-TIMI 18 trial support a predictive role for MPO. Studying 1524 ACS patients treated with tirofiban and randomised to early invasive or conservative management, the authors found that patients with elevated baseline MPO (> 884 pM) were at higher risk of non-fatal MI or re-hospitalisation for ACS at 30 days [76]. As opposed to 8-iso-$PGF_{2\alpha}$, sNOX2-dp and MPO that demonstrated a univocal capacity to predict CVE oxLDL presented divergent results.

In a prospective study on 238 patients with documented CAD, Shimada et al. [77] found that oxLDL levels were predictive of CVE in a follow-up of 52 months. Thus, the levels of circulating oxLDL were significantly higher in patients with CVE than in patients without (20.3 vs. 17.6 U/mL; $p = 0.002$).

A large study joining two different populations: 18,140 men from the Health Professionals Follow-up Study and 32,826 women from the Nurses' Health Study, confirmed the link between oxLDL and CVE. Hence, stratification of subjects according oxLDL demonstrated that the highest quintile of oxLDL was significantly associated with an increased risk of CHD in a multivariate model [78]. Similar predictive power for CVE was found for oxLDL in the in the FRISC-II (Fragmin and fast Revascularisation during Instability in Coronary artery disease) trial, evaluating 433 patients with unstable CAD. OxLDL levels > 76 U/L were associated with a higher risk of recurrent MI at two years [79].

Conversely, data from the MONICA/KORA Ausburg study [80] demonstrated that oxLDL concentrations were not predicative for CVE. Although oxLDL levels were higher in 333 CHD cases compared with 1727 non-CHD subjects (103.3 vs. 87.8 U/L; $p = 0.001$), the predictive value of oxLDL became non-significant after adjustment for lipid profile.

Similarly, in the Framingham offspring study, which measured immunoglobuline G antibodies to oxLDL in 1192 men and 1427 women with an eight-year follow-up, no association with CVE at follow-up was found [81]. It is arguable that the different methodology used in clinical studies may account for these conflicting results, but the value of oxLDL in predicting CVE should be further investigated.

ANTIOXIDANT STATUS AND CARDIOVASCULAR DISEASE

Reduced levels of antioxidant systems were associated with an increased risk for CVE; low levels of plasma SOD3 are independently associated with a history of MI in patients with CAD [82]. Also, a study on GPx3, an enzyme that catabolises hydrogen peroxide into water, supports this evidence. Thus, serum activities of GPx3, SOD, and catalase were measured in a prospective study on 900 AF patients, demonstrating that reduced levels of GPx3 increased the risk of CVE [83].

Among antioxidant vitamins, vitamins C and E have been the most widely investigated in observational and interventional trials.

The WHO/MONICA (monitoring of trends and determinants in cardiovascular disease) project found an inverse correlation between CHD mortality and vitamin E plasma levels [84]. Blood vitamin E levels predicted CVEs such as MI and stroke in 1012 elderly people affected by AF during 27 months of follow-up [85]. Furthermore, Singh et al. [86] found that plasma levels of vitamins C and E were inversely related to CHD in a population of urban Indians.

However, a large meta-analysis of interventional trials with vitamins C and E showed harmful effects such as enhanced risk of all-cause mortality or haemorrhagic stroke [87] in patients treated with antioxidants, compared to controls. These unexpected results could be due to several factors including selection of patients, dose of antioxidant, and antioxidant status at baseline. Conversely, no association was observed between CHD and other vitamins (vitamins A and C).

THERAPEUTIC PERSPECTIVES AND CONCLUSIONS

Taken together, all data so far indicate the presence of an oxidative imbalance in several cardio-metabolic conditions, which is associated with an increased risk of ischaemic complications. However, no specific antioxidant treatment has been recommended so far to prevent atherosclerotic progression or CVEs, given the lack of randomised interventional trials.

Thus, a first approach in the management of patients with, or at risk of, atherosclerotic complications should be represented by lifestyle interventions and nutritional counselling.

In this last context, the role of a healthy dietary pattern such as the Mediterranean Diet (Med-Diet) has been investigated by several studies, giving promising results.

The Med-Diet is characterised by a high amount of foods rich in polyphenols such as fruit, vegetables, and extra-virgin olive oil (EVOO) and modest consumption of red wine. In the Lyon Diet Heart Study, the Med-Diet reduced cardiovascular complications by 50% in secondary prevention [88]. The PREDIMED trial [89], randomising 7447 people at high vascular risk to the Med-Diet supplemented with EVOO, mixed nuts, or control diet [89], demonstrated that Med-Diet reduced the risk of CVD complications by 30% over a follow-up of about five years in the two arms supplemented with EVOO or nuts [89]. Consistent with this finding, we recently demonstrated that adherence to the Med-Diet reduced the risk of CVE in patients affected by AF. Of note is that Med-Diet adherence was inversely correlated with sNOX2-dp and F2-isoprostanes [90]. Moreover, the Med-Diet is also associated with increased levels of the antioxidant GPx3, as was demonstrated in the same population of AF patients [91]. The inverse balance between antioxidants and pro-oxidants in patients at high adherence to the Med-Diet suggests that the Med-Diet can influence the CVE rate by modulating the oxidative imbalance.

Similarly to the alimentary strategies, the efficacy of drugs in preventing CVE by modulating oxidative stress are attractive but still far from conclusive. These strategies are based on the principle of targeting specific oxidant pathways potentially implicated in atherothrombosis. Among them, an intriguing attractive approach could be represented by the inhibition of NOX2, which is up-regulated in the atherosclerotic process and is predictive of CVE [72].

However, a matter of concern is the role played by NOX2 in the innate system. A complete suppression of NOX2 activity is associated with serious life-threatening infectious disease as depicted by the clinical history of patients with CGD [92]. It is interesting to note, however, that serious infection complications where not reported in the case of 50% reduction of NOX2 activity, as observed in NOX2 deficiency carriers [93]. Among the drugs modulating NOX2, statins represent a promising candidate because these drugs inhibit the activity of NOX2 subunit Ras-related C3 botulinum toxin substrate 1 [94]. Thus, in a randomised study in hypercholesteraemic patients, 40 mg atorvastatin ingestion was associated with immediate down-regulation of NOX2 [94]. Moreover, atorvastatin was also shown to reduce ROS formation during post-percutaneous coronary intervention reperfusion, imposing a protective effect on the myocardial cell during ischaemic reperfusion injury [95]. Similar protective effects were obtained using rosuvastatin, another powerful lipid-lowering molecule [96]. However, prospective studies are needed to investigate if the protective effects of statins against CVD are also attributable to NOX2 inhibition.

Another therapeutic option could be represented by apocynin, which would have less negative impact on the innate immune system, as indicated by the more favourable clinical history of patients with hereditary deficiency of $p47^{phox}$ [97]. Few studies have analysed the effects of apocynin in humans [98–100]. In each study, apocynin administration for a few hours was well tolerated, but chronic systemic administration of apocynin in humans has never been tested.

In conclusion, waiting for results from randomised interventional trials investigating new strategies to modulate oxidative stress, lifestyle modifications, and nutritional approach should represent the first-line interventions to lower systemic oxidative stress in patients at risk of or with established atherosclerosis. Specifically, the Med-Diet is the only proven dietary pattern to be associated with lower levels of oxidative biomarkers, and thus adherence to the Med-Diet should always be investigated in patients with cardio-metabolic diseases.

Conflict of interest: *none declared*

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