Mechanisms of Ageing and Development 151 (2015) 101–113

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

Oxidized LDL and NO synthesis—Biomarkers of endothelial dysfunction and ageing

Daniela Gradinaru[a,b,*], Claudia Borsa[a], Cristina Ionescu[a], Gabriel Ioan Prada[a,c]

[a] Ana Aslan National Institute of Gerontology and Geriatrics, 9 Caldarusani Street, Sector 1, P.O. Box 2-4, 011241 Bucharest, Romania
[b] Carol Davila University of Medicine and Pharmacy, Faculty of Pharmacy, Department of Biochemistry, 6 Taian Vuia Street, Sector 2, 020956 Bucharest, Romania
[c] Carol Davila University of Medicine and Pharmacy, Faculty of Medicine, Department of Geriatrics and Gerontology, 37 Dionisie Lupu Street, Sector 2, 020021 Bucharest, Romania

ARTICLE INFO

Article history:
Available online 21 March 2015

Keywords:
Oxidized LDL
Nitric oxide
Ageing
Biomarker
Endothelial dysfunction

ABSTRACT

Oxidized LDL (oxLDL) and nitric oxide (NO) exert contradictory actions within the vascular endothelium microenvironment influencing key events in atherogenesis. OxLDL and NO are so far regarded as representative parameters of oxidative stress and endothelial dysfunction, new targets in prevention, diagnosis and therapy of cardiovascular diseases, and also as candidate biomarkers in evaluating the human biological age. The aim of this review is to explore recent literature on molecular mechanisms and pathophysiological relationships between LDL oxidation, NO synthesis and vascular endothelium function/dysfunction in ageing, focusing on the following aspects: (1) the impact of metabolic status on both LDL oxidation and NO synthesis in relation with oxidative stress, (2) the use of oxidized LDL and NO activity as biomarkers in human studies reporting on cardiovascular outcomes, and (3) evidences supporting the importance of oxidized LDL and NO activity as relevant biomarkers in vascular ageing and age-related diseases.

Contents

Abbreviation: LDL, low-density lipoprotein; oxLDL, oxidized low-density lipoprotein; LOX-1, lectin-like oxidized LDL receptor-1; FFA, free fatty acids; ApoB-100, apolipoprotein B-100; H_2O_2, hydrogen peroxide; SOD, superoxide dismutase; ROS, reactive oxygen species; $O_2^{\bullet -}$, superoxide anion; $HO^{\bullet}$, hydroxyl radical; $LO^{\bullet}$, alkoxyl radical; $LOO^{\bullet}$, peroxyl radical; $ONOO^-$, peroxynitrite; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; NF-kB, nuclear factor κB; $p66^{Shc}$, 66-kDa isoform of Shc adaptor protein; BH_4, tetrahydrobiopterin; Dyslip, dyslipidemia; Hypergly, hyperglycemia; IR, insulin resistance.

* Corresponding author at: Carol Davila University of Medicine and Pharmacy, Faculty of Pharmacy, Department of Biochemistry, 6 Taian Vuia Street, sector 2, 020956, Bucharest, Romania. Tel.: +40 744339630; fax: +40 212231480.
E-mail address: daniela.gradinaru@umf.ro (D. Gradinaru).

http://dx.doi.org/10.1016/j.mad.2015.03.003

"longevity is a vascular question. A man is as old as his arteries"

William Osler, 1892

"It has been said that one is as old as one's arteries. In view of the supreme importance of endothelium in arterial function, I should like to modify. . . this statement by saying that

one is as old as one's endothelium."

Rudolf Altschul, 1954

1. Introduction

The vascular endothelium, with its broad spectrum of paracrine and autocrine functions, can be regarded as a multifunctional organ and "chief governor" of body homeostasis. Occupying a strategic location between the blood and tissues, the endothelial cells exist in a "high-risk position" and react progressively to aggressive factors, at first by modulation of the constitutive functions: permeability (*i.e.*, increased transcytosis of lipoproteins) and biosynthesis (*i.e.*, enhanced synthesis of the basement membrane and extracellular matrix) (Simionescu and Antohe, 2006; Sima et al., 2009). Even though the endothelial cells are resourceful cells that have the functional-structural attributes to adapt to the ever-changing surrounding milieu, to use innate mechanisms to confront and defend against insults, the ageing process induces a progressive failure of protective mechanisms, leading to vascular alterations (Dantas et al., 2012). It is becoming evident that ageing results in well-defined phenotypic changes and, as a consequence, a heightened susceptibility of the cardiovascular system to diseases, even in absence of traditional risk factors (*e.g.*, hypertension, hypercholesterolemia, diabetes, and smoking). Moreover, age-related alterations in cellular homeostatic mechanisms also impact the aged vasculature making it more liable to the damaging effects of the traditional pathophysiological conditions (Ungvari et al., 2010).

Endothelial dysfunction, a systemic pathological state defined as imbalance between vasodilating and vasoconstricting compounds produced by and acting on the endothelium, precedes development of atherosclerosis, leading to reduced vasodilation, pro-inflammatory and pro-thrombotic states (Deanfield et al., 2007).

In the last three decades, oxidation of low density lipoproteins (LDL) and nitric oxide (NO) synthesis have been discovered in parallel, studied extensively and considered as important mechanisms contributing to endothelial dysfunction, vascular ageing and disease. Oxidized LDL and NO exert contradictory actions within the vascular endothelium microenvironment influencing key events in endothelial dysfunction and atherogenesis such as: leukocyte adhesion, platelet aggregation and vascular smooth-muscle cell proliferation and migration. While oxidized LDL (oxLDL) – an oxidative stress biomarker has been identified as a non-traditional, pro-atherogenic emerging risk factor for coronary heart disease, NO is a free radical signal-transducing molecule that maintains the vasodilating tone, modulates *in vitro* lipid peroxidation reactions and alters pro-inflammatrory gene expression (Holvoet et al., 2008a; Borsa et al., 2012).

The LDL oxidation and NO activity are so far regarded as representative parameters of oxidative stress and endothelial dysfunction, new targets in prevention, diagnosis and therapy of cardiovascular diseases, and also as candidate biomarkers in evaluating the human biological age (Rodriguez-Manas et al., 2009; Verhoye and Langlois, 2009; Maiolino et al., 2013a,b; Zuliani et al., 2012; Paik et al., 2013; Bürkle et al., 2015; Moreno-Villanueva et al., 2015a,b; Capri et al., 2015; Baur et al., 2015).

The aim of this review is to explore recent literature on molecular mechanisms and pathophysiological relationships between LDL oxidation, NO synthesis and vascular endothelium function/dysfunction in ageing, focusing on the following aspects: (1) the impact of metabolic status on both LDL oxidation and NO synthesis in relation with vascular oxidative stress, (2) the use of oxidized LDL and NO activity as representative biomarkers in human studies reporting on cardiovascular outcomes, and (3) evidences supporting the importance of oxidized LDL and NO activity as relevant biomarkers in vascular ageing and age-related diseases.

2. Relationships between NO synthesis and LDL oxidation in endothelial dysfunction

In the endothelial microenvironment, concurrently, a variety of substances that influence endothelial function have been recognized, but among them NO and oxLDL are the best characterized key players sharing significant antagonistic roles, and being involved in all phases of atherogenesis (Borsa et al., 2012).

Nitric oxide, a non-eicosanoid component of endothelial-derived relaxation factor (EDRF) is the most important vasodilating molecule being continuously synthesized by the endothelial constitutive isoform of nitric oxide synthase (eNOS and NOS III) under the action of different neurohumoral mediators such as acetylcholine and circulating hormones (catecholamines, vasopressin and aldosterone), plasma constituents (thrombin, sphingosine 1-phosphate), platelet products (serotonin, adenosine diphosphate), and autacoids (histamine, bradykinin and prostaglandin E4) (Michel and Vanhoutte, 2010).

In addition to maintenance of normal organ blood flow, endothelium derived NO has the following pleiotropic vasoprotective, cardioprotective and anti-atherogenic effects, summarized in numerous review articles (Michel and Vanhoutte, 2010; Ungvari et al., 2010; Jin and Loscalzo, 2010; Bermúdez et al., 2008): (1) prevents abnormal constriction (vasospasm) of the coronary arteries, which favors intraluminal clot formation and inhibits platelets aggregation and adhesion to endothelium surface (anti-thrombotic effect); (2) inhibits the release and action of the vasoconstrictor and mitogenic peptide endothelin-1, decreases endothelial permeability and reduces vessel tone, reducing lipoproteins flux into the vessel wall, vascular smooth muscle cell proliferation and migration (cell growth inhibition and anti-atherogenic effect) (Verma et al., 2003); (3) inhibits the NF-κB activation and determines a disruption of pro-inflammatory cytokine – induced signaling pathways; NO reduces the endothelial expression of intracellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1(ELAM) (anti-inflammatory effect) (Rubio and Morales-Segura, 2004); (4) suppresses endothelial cell apoptosis and preserves the endothelial progenitor cell (EPC) function and regulation of tissue energy metabolism (anti-apoptotic, vasoprotective and cardioprotective effect) (Ungvari et al., 2010); (5) controls mitochondrial oxygen consumption and maintains cellular redox state. At physiological levels, NO is a highly reactive free radical that can also attenuate the metal/peroxide oxidative chemistry, as well as lipid peroxidation, and may limit oxidative injury to mammalian cells (antioxidative effect) (Wink et al., 2001; Müller et al., 2004).

Oxidative stress is one of the causative factors involved in ageing and pathogenesis of cardiovascular disease. While the chronological age is classified as major nonmodifiable risk factor for cardiovascular disease, the majority of modifiable atherosclerotic risk factors like hypertension, dyslipidemia, chronic hyperglycemia and cigarette smoking are real harmful stimuli that accelerate disease progression by augmenting the production of reactive oxygen species (ROS) (Nilsson, 2008).

The damaging effects of oxidative stress on cardiovascular system determine endothelial dysfunction through reduction in nitric

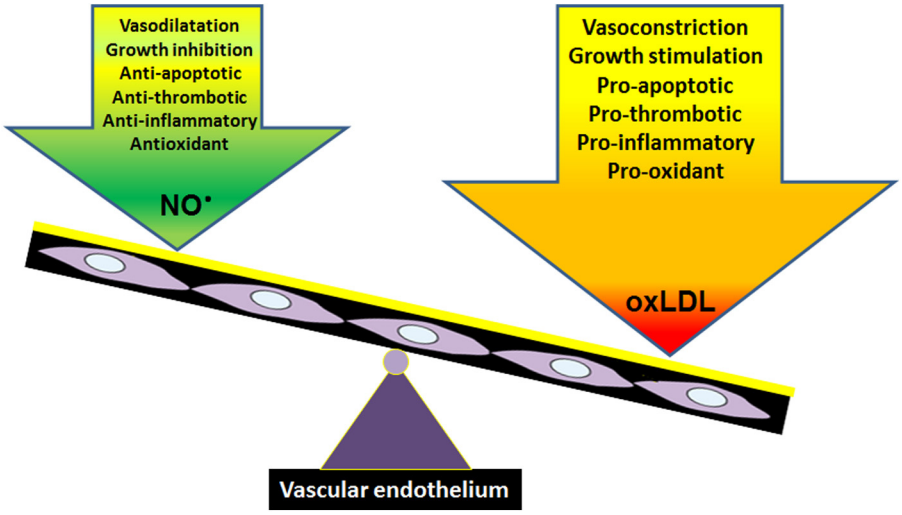

Fig. 1. Key significant anti-atherogenic effects of nitric oxide (NO) *vs.* pro-atherogenic actions of oxidized LDL (oxLDL) as biomarkers involved in the endothelial dysfunction.

oxide (NO) synthesis and bioavailability, inflammatory response, and lipid peroxidation. The endothelium is continuously exposed to various physiological molecules that may have a direct impact on nitric oxide actions (Chikani et al., 2004). Plasma lipoproteins, by virtue of their close interactions with endothelial cells in the vasculature and the susceptibility of their surface lipids to oxidative modification, are perfect biological "sensors" of oxidative stress in the arterial wall (Le, 2015). LDLs as main blood cholesterol carriers, containing relevant amount of polyunsaturated fatty acids (PUFAs) – major substrate for lipid peroxidation, are among various molecular targets the most affected by the oxidative stress associated with metabolic imbalance (hyperlipidemia, hyperglycemia, insulin resistance). Therefore, the oxidative modification hypothesis of atherosclerosis recognizes the crucial role of oxLDL as a byproduct of LDLs exposure to ROS (Steinberg and Witzum, 2010).

OxLDL promotes endothelial dysfunction and contributes to the atherosclerotic plaque formation, progression and destabilization, by several mechanisms described in numerous recent review articles (Maiolino et al., 2013b; Pirillo et al., 2013; Xu et al., 2013; Le, 2015): (1) chemotactic recruitment, activation, and proliferation of monocytes/macrophages in the arterial wall, through the induction of the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular-cell adhesion molecule-1 (VCAM-1), thus stimulating their binding to endothelial cells; (2) its identification and rapid uptake by macrophages, followed by foam cells formation; (3) stimulation of smooth muscle cells (SMCs) migration and proliferation in the tunica intima, following the increase of the expression of growth factors, such as platelet-derived growth factor (PDGF) and basic fibroblast growth factor (FGF) by endothelial cells and macrophages. Subsequently, oxLDL stimulate collagen production by SMCs and increase secretion of matrix metalloproteinases 1 and 9 (MPP-1 and MPP-9) inducing SMCs apoptosis; (4) cytotoxicity exerted mainly on the endothelial cells, which promote their apoptosis and the release in the subendothelial space of lipids and lysosomal enzymes; (5) stimulation of platelet adhesion and aggregation by decreasing endothelial production of nitric oxide, increasing prostacyclin production; (6) blockage of coronary artery relaxation (vasoconstriction) by downregulating eNOS expression, by inhibiting NO and increasing endothelin production. The above evidences explain the pro-inflammatory, pro-oxidant, pro-thrombotic, and vasoconstrictor actions of oxLDL, accounting for its global pro-atherogenic effect on vascular endothelium.

Consequently, NO and oxLDL are important biological mediators that promote protective *vs.* pathogenic effects in the vasculature, simultaneously and concurrently. By reducing NO synthesis and bioavailability, oxLDL breaks the balance of the vessel wall and results in defective endothelial-dependent vasodilation (Fig. 1).

Endothelial dysfunction refers to inability of the endothelium to regulate vascular homeostasis, and essentially describes "tipping" of the physiological balance in favor of vasoconstrictive, pro-inflammatory and pro-thrombotic effects that promote atherosclerosis. These endothelial function abnormalities strongly related to both biomarkers – NO and oxLDL, could be detected early in the development of CVD, often before symptoms are clinically evident (Roberts and Porter, 2013).

However, taking into account data derived from studies based on molecular and clinical approaches these two biomarkers do not seem to exert their antagonistic influence on vascular endothelium with the same significance. Impairment in NO, a common feature in patients with endothelial dysfunction, is considered to predict atherosclerosis and cardiovascular events (Ignarro and Napoli, 2004), whereas upregulation of oxLDL levels is a hallmark feature of atherosclerosis development (Steinberg and Witzum, 2010). Elevated levels of oxidized LDL, mainly formed within the arterial wall, are commonly related to the pro-atherogenic profile (Steinberg, 2009).

Numerous reviews summarized the mechanisms of oxidative stress in association with endothelial dysfunction, LDL oxidation and NO degradation, with ROS being the common mechanism by which different CVD risk factors trigger atherosclerosis (Cai and Harrison, 2000; El Assar et al., 2012; Pirillo et al., 2013; Le, 2015).

On the whole, the decline in NO bioavailability is caused by the cumulative effects of many factors and processes: the decreased expression of the endothelial NO synthase, a reduction of substrate or cofactors for eNOS, alterations of cellular signaling, eNOS inhibition by asymmetric dimethyl arginine, reduced NO production and accelerated NO degradation by hyperlipidemia, chronic
hyperglycemia, oxidative stress and obviously, LDL oxidation. Endothelial dysfunction occurs in response to vascular risk, hence metabolic status may influence both ageing-related vascular oxidative stress and inflammation (Borsa et al., 2012).

3. OxLDL and NO synthesis – oxidative stress and cardiovascular risk biomarkers

Given the central role of the endothelium in the development and clinical course of atherosclerosis, endothelial biomarkers testing may serve as useful tools in cardiovascular risk and outcomes assessments. Current evidence suggests that endothelial function is an integrative marker of the net effects of damage from traditional and emerging risk factors on the arterial wall and its intrinsic capacity for repair. These endothelial-dependent biomarkers are important in the atherosclerosis initiation/progression, the disease state of stable/unstable transitions, as well as clinical research outcome (Deanfield et al., 2007).

An important step forward in clinical studies was to explore the metabolic determinants of oxLDL and the relation between oxLDL and endothelial function measured as endothelium NO release and action. Hence, in this review we focused mainly on the interrelations of metabolic atherogenic risk factors with lipoxidative stress and endothelial dysfunction biomarkers. Numerous oxidative stress biomarkers show strong associations with the onset and progression of coronary artery disease (CAD) and predict cardiovascular events, being surrogate biomarkers and likely complementing diagnostic investigations (Meisinger et al., 2005; Tsimikas et al., 2012; Wang et al., 2013). Particularly, the LDL particles' susceptibility to oxidation is included among the "downstream markers" of oxidative stress (Borsa et al., 2012).

An indirect marker of *in vivo* oxidation of plasma LDL is the increase of the titer of autoantibodies against neo-epitopes in oxLDL (Tsimikas et al., 2007) but its relevance in coronary heart disease (CHD) remains controversial. By contrast, a direct, widely applied sensitive immunoassay quantifying the circulating levels of oxLDL uses the monoclonal antibody 4E6, directed against oxidized apolipoprotein B-100 moiety of LDL. However, all the methods using different antibodies to oxLDL most probably detect and measure the circulating minimally oxidized LDL which represents only a minor fraction of LDL ranging from 0.001%, in healthy controls, to approximately 5%, in patients with acute coronary events (Holvoet et al., 2008a). Moreover, circulating oxLDLs are strongly correlated to LDL-cholesterol and apoB100, making it difficult to unravel their independent contribution to cardiovascular risk (van der Zwan et al., 2009). For this reason, it has been suggested that the oxLDL/LDL-cholesterol ratio (*i.e.*, the relative amount of oxLDLs) could be the best indicator of the risk associated with oxLDLs levels.

Most cohort studies underline the associations between oxLDL and cardiovascular events or mortality, in particular those including a very high-risk population, specifically with age-related chronic metabolic diseases and their complications. For an overview on the association of oxLDL with cardiovascular events the reader is referred to a recent review by Maiolino et al. (2013b).

In middle-aged and elderly subjects, obesity and dyslipidemia are the strongest predictors of levels of oxLDL (Kopprasch et al., 2002; Holvoet et al., 2004). Recently, the association between dyslipidemia and oxidation of LDL has been demonstrated in elderly individuals, even in the pre-diabetic state (Gradinaru et al., 2013). In this regard, an increase in immunologically detected epitopes of oxLDL in subjects with abdominal obesity was reported by several authors (Couillard et al., 2005; Weinbrenner et al., 2006; Njajou et al., 2009; Babakr et al., 2014). Thus, higher levels of oxLDL were associated with increased incidence of metabolic syndrome (MetS) overall, as well as its components of abdominal obesity, hyperglycemia and hypertriglyceridemia in the population-based, prospective, observational study CARDIA (The Coronary Artery Risk Development in Young Adults) (Holvoet et al., 2008b). Moreover, oxLDL was suggestively associated with a greater prevalence of internal carotid intimal-medial thickness (IMT) and detectable coronary artery calcium (CAC) in a 997 participants (aged 45–84 years) of MESA Study (Multi-Ethnic Study of Atherosclerosis) without clinical CAD. However, this study pointed out that metabolic abnormalities and oxidative endothelial damage may lead to atherosclerotic disease through distinct mechanisms (Vaidya et al., 2011). Increased circulating oxLDL levels have been related to CVD in some studies, although not always independently after adjustment of classical lipid markers. In this sense, Wu et al. (2006) suggested in a prospective cohort study that oxLDL as an individual parameter, measured with antibody 4E6, was not an independent overall predictor of CHD, being less predictive than apoB and total cholesterol/HDL-cholesterol ratio. Also, in 2524 healthy middle-aged subjects (Asklepios study) oxLDL was affected by many biological and lifestyle factors, as well as subclinical atherosclerosis (Verhoye and Langlois, 2009). Autoantibodies to oxLDLs (anti-oxLDLAbs) were detectable in the serum of subjects with and without atherosclerosis, but it is unclear if they play a pathogenic or a protective role in atherogenesis or if they are simply a marker of atherosclerosis. In a prospective cohort study (748 patients) of the GENICA study who underwent coronary angiography and assessment of incident CV events at follow-up, high titer of anti-oxLDLAbs is a marker which predicts long term cardiovascular mortality in high risk patients (Maiolino et al., 2013a). Conversely, also in a recent study, Zuliani et al. (2012) using data for the InCHIANTI dataset (1025 older community dwelling individuals), a 9-year follow-up population-based study, no association emerged between higher oxLDLs levels (measured with antibody 4E6) and CVD/cardiac mortality, suggesting that in advanced age the prognostic information added by oxLDLs might be negligible. This study underlined also that LDL-cholesterol (LDL-C), triglycerides, and HDL-cholesterol (HDL-C) are the most important determinants of oxLDLs levels, indirectly suggesting an association between small dense LDLs and LDLs oxidation. Interestingly, a negative association between oxLDLs levels and age was found in this study population, perfectly mirroring the relationship between LDL-C and age. This finding suggests that after 65 years of age, although the oxidative stress might increase with ageing, circulating oxLDLs tend to decrease as a consequence of the progressive reduction of the substrate.

It is noteworthy that circulating levels of oxLDL reflect both the quantity and the "quality" of LDL particles, as cholesterol, phospholipids, polyunsaturated fatty acids and apolipoprotein B-100 are the LDL substrates for oxidation, being significantly influenced by the presence of endogenous lipophilic antioxidants: α- and γ-tocopherol, β-carotene, ubiquinol-10 (Esterbauer et al., 1992). Therefore, the estimation of *in vitro* LDL susceptibility to oxidation includes evaluation of specific products of the lipid peroxidation chain reaction, after the exposure of isolated LDL particles to a standard oxidative stress. The extent in formation of thiobarbituric reactive substances (TBARS), conjugated dienes, lipid hydroperoxides, and aldehydes, could indicate its oxidizability.

As indicator of NO activity, the measurement of endothelium-dependent vasodilation (flow-mediated dilation method, FMD) by inducing reactive hyperemia in the brachial artery, is the golden standard in clinical practice (Hirata et al., 2010). NO present in the circulation is resulting from endothelial and smooth muscle cells, thrombocytes, leukocytes and cardiomyocytes (Pacher et al., 2007). Systemic NO activity is the net result of a balance between its production and its inactivation by oxygen free radicals. NO released *in vivo* rapidly autooxidizes to yield nitrite (NO_2^-), which interacts with oxyhemoglobin yielding nitrate (NO_3^-). Because nitrite plus nitrate are relatively stable compounds in blood, their levels may be a biochemical index of systemic NO production being assessed as NO metabolic-pathway products, NO*x* ($NO_2^- + NO_3^-$) (Lundberg and Weitzberg, 2005).

Given the vast range of vasoprotective effects of NO, the term endothelial dysfunction generally refers to a reduced NO bioavailability, through decreased eNOS expression. Indeed, using these procedures, it has been demonstrated that patients with confirmed CVD, and importantly, even those who carry risk factors for future CVD events, had impaired endothelial-dependent vasodilation (Roberts and Porter, 2013; van der Zwan et al., 2009).

Among the twenty-five recent human studies reporting on associations between oxidized LDL, vascular endothelial function and different cardiovascular outcomes, fifteen reported the clinical assessment of the brachial artery flow-mediated dilation (FMD) as a measure of endothelium (NO)-dependent response, and ten studies reported the biochemical evaluation of plasma NO stable metabolites, NO*x* (nitrite + nitrate). Their application in the assessment of endothelial dysfunction in adult and elderly subjects, is summarized in the Tables 1 and 2.

With respect to the contradictory actions and interrelations of oxLDL and NO within the vascular endothelium, we recently proposed the use of a new marker of endothelial dysfunction, namely the ratio of oxLDL to NO*x* (oxLDL/NO*x*), which could be a more accurate estimation of the *in vivo* cumulative implications of oxLDL and NO in atherogenesis (Borsa et al., 2012; Gradinaru et al., 2012, 2013). Thus, in 170 elderly hyperlipidemic subjects we pointed out the strong positive associations of this ratio with the atherogenic index and the atherogenic risk markers: total cholesterol/HDL-C and oxLDL/HDL-C ratios. These significant relationships were in support to propose the ratio oxLDL/NO*x* as a potential marker of endothelial dysfunction. The future in depth studies, should take into consideration its association with clinical parameters of vascular endothelial functions, to further test this new candidate marker as biomarker of vascular ageing.

For these reasons, we used the susceptibility of LDL to oxidation and NO metabolic pathway products in MARK-AGE European Study to Establish Biomarkers of Human ageing, as biomarkers of oxidative stress and endothelial dysfunction in evaluation of biological age (Bürkle et al., 2015; Moreno-Villanueva et al., 2015a,b; Capri et al., 2015; Baur et al., 2015).

4. Oxidized LDL and NO synthesis in human vascular ageing

Ageing of the cardiovascular system has represented a real challenge for the human health, ever since we start to age at the first heartbeat (Thorin and Thorin-Trescases, 2009). Atherosclerosis is associated with premature biological ageing, as atherosclerotic plaques show evidence of cellular senescence characterized by reduced cell proliferation, irreversible growth arrest and apoptosis, elevated DNA damage, epigenetic modifications, and telomere shortening and dysfunction (Wang and Bennett, 2011). Diseases of the vascular system such as hypertension, chronic coronary disease and diabetes have long been considered to be age-related in terms of their onset and progression. In endothelial cells these changes result in a phenotype that is pro-inflammatory, pro-atherosclerotic, and pro-thrombotic. Endothelial cell (EC) senescence can be induced by a number of factors implicated in vascular pathologies, particularly by sustained cell replication and oxidative stress (Erusalimski, 2009).

Ageing is associated with an increase in arterial stiffness and an impairment of endothelial function – early important event leading to CVD (El Assar et al., 2012). Many studies attempting to elucidate the mechanisms behind this decline have used animal models (Wu et al., 2014). The key role of endothelium derived NO in protecting the cardiovascular system during ageing is underscored by the findings that eNOS knockout mice exhibit a premature cardiac ageing phenotype associated with early mortality (Li et al., 2004)

Among mechanisms that are proposed to contribute to age-dependent endothelial dysfunction, the most acknowledged evidenced the following aspects: (1) reduction of NO bioavailability, caused by diminished NO synthesis and/or by augmented NO scavenging due to oxidative stress; (2) increased oxidative stress in the endothelial microenvironment; (3) increased oxidation of LDL; (4) development of a low-grade pro-inflammatory environment; (5) increased activities of vasoconstrictor factors; (5) impaired endothelial cell function and maintenance repair systems by endothelial progenitor cells (EPC) (El Assar et al., 2012; Wadley et al., 2013; Ungvari et al., 2010).

Higher levels of oxidative stress and inflammation are major determinants of reduced vascular function in human ageing, being included in the vascular health triad concept (Wadley et al., 2013). Elevated circulating levels of high sensitivity CRP (hsCRP) and oxidized LDL were associated with CVD (Obradovic et al., 2015); and other inflammatory markers (*e.g.*, tumor necrosis factor-alpha [TNFα], sVCAM-1, sE-selectin, interleukin [IL]-6, IL-18, and MCP-1) were correlated with age, independently of other cardiovascular risk factors (Ungvari et al., 2010). In particular, elderly people often present a low-grade, chronic, systemic inflammation with age, termed "inflammaging" (Franceschi et al., 2007). Ageing and increased levels of systemic pro-inflammatory markers and oxLDL were associated with arterial stiffness (Donato et al., 2007; Kampus et al., 2007; Brinkley et al., 2009; Kim et al., 2013). Reduced brachial artery flow-mediated dilation (FMD) and elevated nitrotyrosine expression in brachial artery ECs were pointed out in older compared to young subjects (Kim et al., 2013). Additionally, in older men increased NAD(P)H oxidase-p47(phox) enzyme expression, together with nuclear factor-kappa B p65, a component of the redox-sensitive NF-κB, were pointed out (Donato et al., 2007). Of particular interest is the impact of oxidative stress on plasma LDL, but very low-density lipoprotein (VLDL), beta-VLDL and even HDL undergo oxidative modification that must be taken into consideration in the complex process of atherosclerosis (Parthasarathy et al., 2008). Age-related evaluations of arteries markers of functional and structural changes in healthy subjects (175 subjects, aged 40–70 years), showed a significant association between oxLDL and carotid intima-media thickness (IMT) (Kampus et al., 2007). In a cross-sectional study in 2295 elderly persons from the health, ageing, and body composition study, elevated plasma oxLDL levels were associated with higher arterial stiffness, independent of other traditional CVD risk factors (Brinkley et al., 2009). In a recent 3-year longitudinal study in 57 nonobese men (aged 34–55 years) changes in arterial stiffness measured as brachial-ankle pulse wave velocities (ba-PWV) were positively correlated with the changes in waist-hip ratio (WHR), oxLDLs, plasma malondialdehyde (MDA), hs-CRP, and IL-6 levels suggesting that changes in oxidative stress, pro-inflammation or abdominal obesity could play important roles in accelerating arterial stiffness (Kim et al., 2013). The effect of age on atherogenicity of LDL and inflammatory markers studied in 2944 healthy women (aged 30–79 years), underlined increases in plasma oxLDL levels after 50 years and higher levels of inflammatory markers: hCRP, TNF-α) and IL-6 after 60 years of age (Paik et al., 2013). Since total cholesterol levels decrease after 60 years of age, the oxLDL increases with age may be due to enhancement in LDL atherogenicity under preponderantly pro-oxidant and pro-inflammatory environment.

Circulating oxLDL is associated with the development of not only atherosclerosis but also numerous other degenerative and age-related diseases, such as rheumatoid arthritis, multiple sclerosis, osteoporosis, macular degeneration and Alzheimer's and Parkinson's diseases (Profumo et al., 2012; Besler and Comoğlu, 2003; Dildar et al., 2010; Robman et al., 2004; Mazière et al., 2010; Kankaanpää et al., 2009).

Table 1
Human studies reporting the relationship between oxidized LDL (oxLDL) and vascular endothelial function (VEF) assessed by clinical methods, in the investigation of different cardiovascular (CV) outcomes in adult and elderly subjects.

Population under study	Aim	OxLDL tests	VEF tests	Relevant findings	References
Healthy middle-aged and older adults, 127 men and women, aged 48–77 years	Relationship between dietary niacin intake, VEF and oxLDL	OxLDL-Ab-4E6 – ELISA	Brachial artery FMD	OxLDL was inversely related to niacin intake; FMD was positively related to dietary niacin intake	Kaplon et al. (2014)
Hypertensive patients ($N=88$), overweight (OW) and obese (OB)	Relationship between BMI, immune and VEF	Anti-oxLDL Abs	Brachial artery FMD	OB had lower levels of anti-oxLDL Abs and impaired FMD	Fonseca et al. (2013)
Randomized, double-blind, placebo-controlled study ($N=30$) of smoking cessation and γ-tocopherol (γ-T) supplementation	Effect of improvements in γ-T status on VEF and oxidative stress	OxLDL-Ab-4E6 – ELISA	Brachial artery FMD	OxLDL levels were unaffected by smoking cessation or γ-T-rich supplementation, whereas FMD was significantly improved	Mah et al. (2013)
Double control sandwich model intervention study, 21 hypercholesterolemic subjects	Effects of hazelnut consumption on CV risk markers	OxLDL-Ab-4E6 – ELISA	Brachial artery FMD	Antiatherogenic effect of hazelnut-enriched diets by improving endothelial function and oxLDL levels	Orem et al. (2013)
47 overweight and obese postmenopausal women completed a 4-month program of 1 h low-intensity physical activity (PA)	Impact of a lower-than-advised level of PA on small artery VEF and oxidative stress	OxLDL-Ab-4E6 – ELISA	Small artery reactive hyperemia index (saRHI)	PA improves antioxidant capacity, resting heart rate (RHR), and saRHI in postmenopausal women	Merino et al. (2013)
22 healthy sedentary subjects aged 50–77 years, treated with fenofibrate ($N=12$)	Influence of short-term treatment (7 days) on endothelial function	OxLDL-Ab-4E6 – ELISA	Brachial artery FMD	Fenofibrate improved FMD after 2 and 7 days, reduced oxLDL and increased eNOS expression in vascular EC	Walker et al. (2012)
Healthy, nonsmoking adults, 187 men and 127 women, aged 18–79 years	Relationship between, plasma norepinephrine (PNE) and VEF	OxLDL-Ab-4E6 – ELISA	Brachial artery FMD	VEF was inversely related to sympathetic activity	Kaplon et al. (2011)
Random population sample, 89 smokers and 261 non-smokers	Association between WBC telomere length, oxLDL and VEF	OxLDL-Ab-4E6 – ELISA	Distensibility of the carotid artery (CA)	Higher level of oxLDL is associated with shorter WBC telomeres and increased stiffness of the CA	Nawrot et al. (2010)
Hypertensive subjects ($N=94$), naive of antihypertensive medication	Effect of antihypertensive therapy on EVF	Anti-oxLDL Abs	Brachial artery FMD	Anti-oxLDL Abs titer increased and FMD was improved after antihypertensive therapy	Brandão et al. (2010)
Cross-sectional study, $N=70$ anuric hemodialysis patients	Relationship between malnutrition-inflammation score (MIS) and the VEF	OxLDL-Ab-4E6 – ELISA	Brachial artery FMD	MIS and oxLDL were independent significant predictors of FMD in a multivariate analysis	Demir et al. (2010)
Prospective study, 19 subjects with primary biliary cirrhosis (PBC)	Effect of low-dose atorvastatin treatment on dyslipidemia and VEF	OxLDL-Ab-4E6 – ELISA	Brachial artery FMD	Statin treatment improved CV risk markers and VEF	Stojakovic et al. (2010)
Randomized, controlled, parallel feeding trial, 50 adults with metabolic syndrome (MS) received diet supplemented with mixed nuts	Effect of nuts consumption on markers of oxidation and endothelial function	OxLDL- Ab-4E6 – ELISA LDL oxidation susceptibility	Peripheral artery tonometry (PAT)	No significant differences in oxLDL or endothelial function during the intervention	López-Uriarte et al. (2010)
Multicenter study, 25 patients with hereditary gp91(phox) deficiency, 25 healthy, 25 obese	Relationship between NADPH-oxidase and FMD	OxLDL-Ab-4E6 – ELISA	Brachial artery FMD	NOx significantly correlated with FMD; gp91(phox) is involved in the modulation of arterial tone	Violi et al. (2009)
Population-based cohort study: 624 men and women (age range 50–87 years)	Metabolic determinants of oxLDL and the relation between oxLDL and FMD	OxLDL-Ab-4E6 – ELISA LDL oxidation susceptibility	Brachial artery FMD	oxLDL/apoB100 ratio was negatively related to FMD after adjustment for age, sex, glucose tolerance status, and Framingham risk score	van der Zwan et al. (2009)
25 men with a previous hospital-diagnosed myocardial infarction	Relationship between oxLDL and C-reactive protein (CRP) with endothelial function, in CHD subjects	IgG and IgM antibodies (Ab) against oxLDL	FMD in isolated resistance arteries from subcutaneous fat biopsies	Maximum vessel dilatation was inversely related to IgG-Ab levels; correlation between FMD, CRP and plasma levels of IgG-Ab	Crisby et al. (2009)

Plasma oxLDL were assessed using the competitive ELISA method with monoclonal antibody 4E6 (Ab-4E6), or the anti-oxidized LDL antibodies (anti-oxLDL Abs). The endothelial function was assessed by flow-mediated dilation (FMD) of the brachial artery, FMD in isolated resistance arteries from subcutaneous fat biopsies, as small artery reactive hyperemia index (saRHI), as distensibility of the carotid artery (CA) or by peripheral artery tonometry (PAT).

Table 2
Human studies reporting the relationship between oxidized LDL (oxLDL) and vascular endothelial function (VEF) assessed as plasma NO metabolites, in the investigation of different cardiovascular (CV) outcomes in adult and elderly subjects.

Population under study	Aim	OxLDL tests	VEF tests	Relevant findings	References
35 subjects with normolipidemia and 30 subjects with hyperlipidemia	Relationship between VEF, asymmetric dimethylarginine (ADMA) and oxLDL	OxLDL-Ab-4E6 – ELISA	Plasma NO*x*	OxLDL values were significantly higher and NO*x* decreased in dyslipidemic subjects *vs.* normo-lipidemic subjects	Ercan et al. (2014)
273 subjects, aged 60–75 years, with pre-diabetes (*N* = 90, IFG), and type 2 diabetes mellitus (*N* = 95,T2DM), *vs.* control	Relationship between advanced oxidation and advanced glycation of proteins (AOPP and AGEs), oxLDL and NOx	LDL oxidation susceptibility	Plasma NO*x*	Higher levels of plasma AOPPs, AGEs, oxLDL, NOx, atherosclerosis risk markers, and insulin resistance were pointed out in IFG and T2DM	Gradinaru et al. (2013)
Cross-sectional study: 90 subjects aged 65–78 years, with pre-diabetes (*N* = 30, IFG), and type 2 diabetes mellitus (*N* = 35)	Relationship between vitamin D status, systemic oxidative stress and VEF	LDL oxidation susceptibility	Plasma NO*x*	Serum 25-hydroxyvitamin was inversely associated with oxidative stress and endothelial dysfunction, in subjects with hypovitaminosis D	Gradinaru et al. (2012)
170 elderly, aged 60–70 years: hypercholesterolemia (*N* = 125) *vs.* control (*N* = 45)	Metabolic determinants of oxLDL and NO*x* in hyperlipidemia	OxLDL-Ab-4E6 – ELISA	Plasma NO*x*	OxLDL/NO*x* ratio correlated significantly with traditional cardiovascular risk markers	Borsa et al. (2012)
84 patients with metabolic syndrome (MS) and 42 healthy controls	Relationship between plasma lipoprotein (a) (Lp[a]) levels and atherosclerotic burden	OxLDL-Ab-4E6 – ELISA Anti-oxLDLAbs	Plasma NO*x*	NO*x* levels were significantly lower in patients with high Lp(a) as compared with those with normal Lp(a)	Muñoz-Torrero et al. (2012)
24 patients with rheumatoid arthritis (RA), 25 with psoriatic arthritis (PsA), *vs.* control	Association oxLDL, NO*x*, with subclinical atherosclerosis	OxLDL-Ab-4E6 – ELISA	Plasma NO*x*	Ox-LDLs and NO*x* may be markers of accelerated atherosclerosis in RA and PsA	Profumo et al. (2012)
Double-blind, randomized, crossover dietary-intervention study, 24 women with high-normal blood pressure (BP) or stage 1 essential hypertension	Influence of a polyphenol-rich olive oil diet on BP, oxLDL and endothelial function	OxLDL-Ab-4E6 – ELISA	Plasma NO*x* brachial artery FMD	Polyphenol-rich olive oil diet led to a significant decrease in BP, oxLDL, and increase in plasma NOx and FMD	Moreno-Luna et al. (2012)
50 stable coronary artery disease (CAD) patients, 50 unstable CAD and 50 control	Relationships between myeloperoxidase (MPO), oxLDL and NO*x* levels	OxLDL-Ab-4E6 – ELISA	Plasma NO*x*	Plasma MPO levels were significantly positively correlated with oxLDL and negatively correlated with NO*x* levels	Samsamshariat et al. (2011)
Randomized crossover study, 20 participants	Effect of Mediterranean diet supplemented with coenzyme Q10 (Med+CoQ diet) on postprandial oxidative stress	OxLDL-Ab-4E6 – ELISA	Plasma NO*x* brachial artery FMD	Med and Med+CoQ diets produced a lower postprandial decrease in total nitrite, a higher postprandial increase in FMD, and a lower postprandial oxLDL	Yubero-Serrano et al. (2011)
62 patients diagnosed with essential arterial hypertension and 45 healthy controls	Diagnostic value of endothelial dysfunction and oxidative stress markers	IgG antibodies against oxLDL	Plasma NO*x*	Prostacyclin and oxLDL had the best diagnostic value	Kuklinska et al. (2009)

Plasma oxidized LDL were assessed using the competitive ELISA method with monoclonal antibody 4E6 (Ab-4E6), the anti-oxidized LDL antibodies (anti-oxLDL Abs), or the LDL susceptibility to *in vitro* oxidation. The endothelial function was evaluated as plasma NO metabolic pathway products, NO*x* (NO_2^- + NO_3^-).

Recent studies underscore the association between white blood cell (WBC) telomere length, as index of systemic ageing, oxidized LDL, and human vascular ageing expressed by the distensibility of the carotid artery. Higher levels of oxidized LDL were associated with shorter WBC telomeres and increased stiffness of the carotid artery (Nawrot and Staessen, 2008; Nawrot et al., 2010). Also, emerging evidence suggests that increasing NO bioavailability or eNOS expression activates telomerase and delays endothelial cell senescence (Hayashi et al., 2008). Interestingly, NO can play a dual role in atherosclerosis, some pathological vascular conditions being linked with excessive rather than reduced NO production. The inducible nitric oxide synthase isoform (iNOS), involved in immune response is synthesized by many cell types in response to pro-inflammatory cytokines (IL-6, TNF-α, and gamma-interferon) and produces quantities of NO far exceeding those produced by the eNOS isoform. These elevated levels of NO are associated with the general cytotoxic effects of NO, as NO is a free radical with an unpaired electron (Armitage et al., 2009). Therefore, regulation of nitric oxide synthases and bioavailability of their product become critical for the development and progression of vascular diseases, such as atherosclerosis (Napoli et al., 2006). High levels of NO produced from iNOS in endothelial cells and macrophages can induce injury to the endothelium. Peroxynitrite ($ONOO^-$), the harmful product of NO interaction with superoxide ($O_2^{\bullet -}$), is also produced in significant amounts in atherosclerotic lesions (Chatterjee et al., 2009). Thus, while the low concentrations of NO generated by eNOS protect against atherosclerosis by promoting vasodilatation, inhibiting leucocyte and platelet adhesion and/or aggregation and smooth muscle cell proliferation; higher concentrations of NO generated by iNOS promote atherosclerosis either directly or *via* the formation of NO adducts, such as peroxynitrite (Moncada and Higgs, 2006). By combining two experimental approaches in a large sample of subjects with a wide age range (18–91 years), Rodriguez-Manas et al. (2009) determined the existence of age-dependent endothelial dysfunction, both *in vitro* and *in vivo*, in subjects with no clinical cardiovascular diseases and no classical risk factors. In isolated mesenteric microvessels from these subjects, an age-dependent impairment of the endothelium dependent relaxations to bradykinin was observed. Moreover, aged microvessels showed superoxide anions ($O_2^{\bullet -}$) and peroxynitrite ($ONOO^-$) formation, as well as enhancement of NADPH oxidase and iNOS synthase expression. The expression of mRNA for eNOS did not change in mesenteric microvessels from aged subjects, whereas it was markedly enhanced for the iNOS isoform. Hence, a paradox appears whereby in ageing humans there is elevated NO production within the vasculature, associated with reduced NO bioavailability, due to free radical scavenging. The induction of the inflammatory iNOS isoform can be also related to the endothelial dysfunction associated with ageing and age-related diseases. Higher levels of plasma NO metabolic pathway products (NO*x*) were also pointed out in adults and elderly subjects with metabolic disorders: impaired fasting glucose (IFG) and type 2 diabetes mellitus (T2DM) (Ghasemi et al., 2011; Gradinaru et al., 2012, 2013), and also in stimulated blood cells (PBMNC) from T2DM subjects (Volpe et al., 2014). This suggests that in chronic hyperglycemia the role of eNOS changes from an anti-atherogenic effect to a pro-atherogenic effect and exacerbates *in vitro* inflammatory responses and iNOS expression.

In a prospective 4 years cohort study (204 subjects, aged over 55 years), Sverdlov et al. (2014) evaluated the effects of ageing on platelet aggregability and responsiveness to NO, in correlation with plasma asymmetric dimethylarginine (ADMA), an endogenous eNOS inhibitor. Thus, ageing was associated with marked deterioration of responsiveness of platelets to NO and increases in plasma ADMA concentrations to a proportional extent with adverse impact on CV outcomes.

5. OxLDL and NO – mechanisms in endothelial dysfunction and ageing

The NO-signaling system has a number of potential points of vulnerability—biochemical and cellular processes, which may result in impairment of the entire NO cascade. Enhanced ROS generation and overproduction of peroxynitrite in the presence of risk factors also facilitates activation of redox-dependent transcriptional factors such as NF-κB and increase iNOS (Sverdlov et al., 2014; Kolluru et al., 2012).

Numerous experimental studies have supported the harmful effects of hyperlipidemia, hyperglycemia and insulin resistance, at multiple steps in atherogenesis, including direct contributions to endothelial dysfunction, through several underlying mechanisms which involve together with NO and oxLDL, new causative factors: lectin-like oxidized LDL receptor-1 (LOX-1), $p66^{Shc}$ adaptor protein, NF-κB and tetrahydrobiopterin (BH_4) (Xu et al., 2013; Pirillo et al., 2013; El Assar et al., 2012; Nilsson, 2008; Ungvari et al., 2012; Wadley et al., 2013).

5.1. Lectin-like oxidized LDL receptor-1

Lectin-like oxidized LDL receptor-1 (LOX-1), the main oxLDL receptor in endothelial cells, macrophages and smooth muscle cells is implicated in pathogenesis of atherosclerosis. Soluble form of LOX-1 (sLOX-1) is associated with early stages of acute coronary syndrome (Hayashida et al., 2005), coronary plaque vulnerability (Zhao et al., 2011) and plaque rupture (Kobayashi et al., 2013), being considered a biochemical marker of atherosclerosis-related diseases (Pirillo and Catapano, 2013). Also, sLOX-1 is associated with metabolic disorders (obesity, T2DM, metabolic syndrome) (Tan et al., 2008). LOX-1 mediates the uptake of oxLDL by vascular cells being involved in endothelial dysfunction, monocyte adhesion, proliferation, migration, and apoptosis of SMC, foam cell formation, platelet activation, and plaque instability. These cellular events may be inhibited by anti-LOX-1 antibodies, and vascular LOX-1 expression and activity could be regulated by vasculoprotective drugs (Xu et al., 2013). LOX-1 mediates the oxLDL uptake in endothelial cells by activation of protein kinase C (PKC) β2 and c-Jun N-terminal kinases (JNK), and by phosphorylation of $p66^{Shc}$ adaptor proteins (Shi et al., 2011). Also, LOX-1 is implicated in NO-dependent endothelial impairment of coronary arterioles (Xu et al., 2007) by activation the signaling cascade involving NADPH oxidase or NF-kB–NADH oxidase – ROS (Cominacini et al., 2000; Xu et al., 2013). Thus, due to the important role in regulating oxLDL-NO – mediated vascular reactivity, LOX-1 could represent a potential therapeutic target in endothelial dysfunction and cardiovascular diseases.

5.2. $p66^{Shc}$ adaptor protein

The $p66^{Shc}$ adaptor protein has a dual role in vascular dysfunction being a mediator of oxidative stress-induced vascular dysfunction and a modulator of endothelial NO production (Shi et al., 2014; Yamamori et al., 2005; Franzeck et al., 2012). The $p66^{Shc}$ may mediate endothelial dysfunction by redox-enzyme action in mitochondrial ROS generation, translation of oxidative signals into apoptosis and increased ROS production by oxidized LDL. The mechanisms of oxLDL – dependent ROS generation include: phosphorylation of the $p66^{Shc}$ protein at ser36 through the lectin-like oxLDL receptor-1 (LOX-1), activation of protein kinase C beta-2, and c-Jun N terminal kinase (Shi et al., 2011). The deletion of ageing gene $p66^{Shc}$ increases endothelial nitric oxide synthase (eNOS) expression and nitric oxide (NO) bioavailability *via* protein kinase B and its overexpression inhibits eNOS-dependent NO production (Yamamori et al., 2005). Recent studies on cultured human

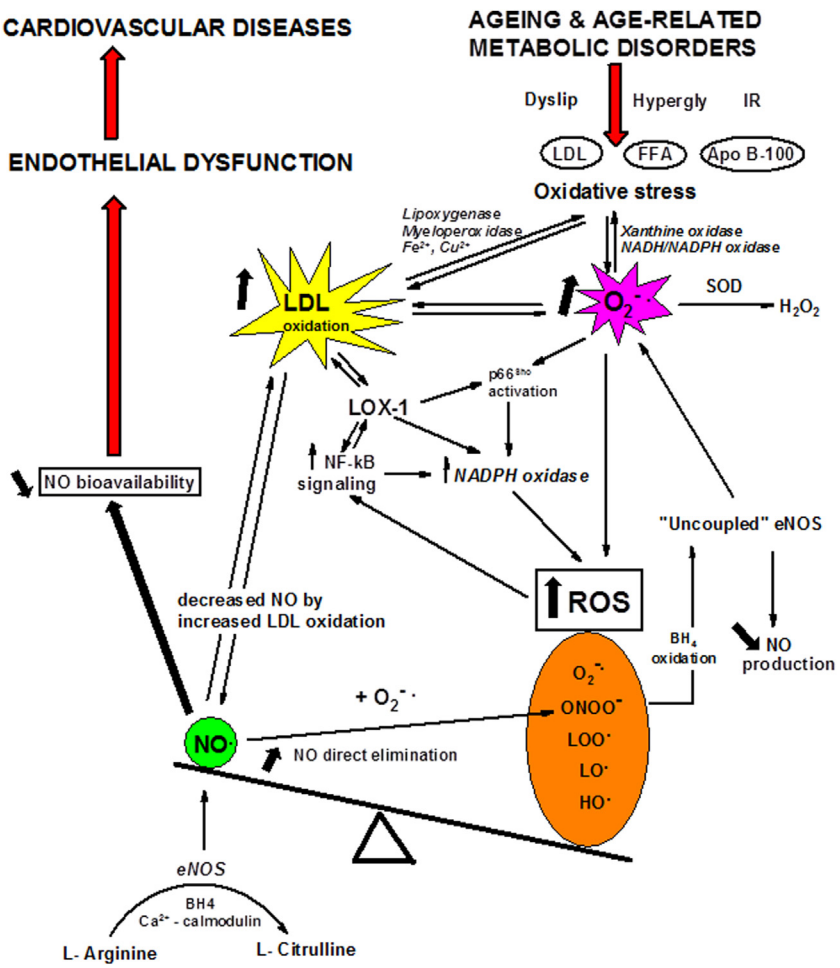

Fig. 2. Simplified scheme of the interrelations between oxidative stress, LDL oxidation and NO in ageing and age-related metabolic disorders leading to endothelial dysfunction and cardiovascular diseases.

endothelial cells underline the dual role of eNOS for $p66^{Shc}$ protein activation and ROS generation. The eNOS uncoupling is a crucial player in oxLDL-induced and $p66^{Shc}$-mediated intracellular ROS generation (Shi et al., 2014). Thus, eNOS uncoupling has been considered as a putative antioxidant therapeutical target in endothelial dysfunction and CVD.

5.3. Nuclear factor-kappa B

The transcription factor NF-κB, a key regulator of inflammation and oxidative stress provides an effective "transducer" for feeding forward activation of these processes. By stimulating inflammation and oxidative stress, NF-κB has a key role in mediating vascular endothelial dysfunction in humans (Xu et al., 2013; Ungvari et al., 2010). One of these roles includes the activation of the nuclear enzyme poly(ADP-ribose), polymerase (PARP-1), which leads to the production of inflammatory mediators such as iNOS, ICAM-1, and major histocompatibility complex class II. Lack of NO leads to vasodilator dysfunction and promotes endothelial apoptosis, whereas nitrative stress and increased H_2O_2 levels lead to poly(ADP-ribose) polymerase (PARP)-1 activation, which contributes to NF-κB -dependent gene transcription (Ungvari et al., 2010; Mangerich and Burkle, 2012). NF-κB, an integral factor in vascular health triad (Donato et al., 2009), is involved in transcriptional activation of pro-inflammatory and pro-oxidative genes leading to impaired vascular function. Activation of NF-kB correlates with reduced endothelium-dependent dilation (EDD) with advancing age (Pierce et al., 2009; Donato et al., 2007, 2009), contributing to vascular endothelial dysfunction *via* oxidative stress. Inactivation of NF-κB increased endothelial function and reduced NADPH oxidase activity (Pierce et al., 2009). The newly proposed model of vascular health, named vascular health triad (Wadley et al., 2013) integrate the associations and interactions of oxidative stress and inflammation with vascular dysfunction in ageing, primarily mediated by the transcriptional factor NF-κB and targeting downstream NO bioavailability. The NF-κB signaling inhibition might limit the vicious cycles of inflammation and oxidative stress. Thus, modulation of NF-κB signaling could be a potential therapeutic target in vascular ageing prevention.

5.4. Tetyrahydrobiopterin

Tetyrahydrobiopterin (BH_4), essential co-factor in eNOS regulation has been recently considered as biomarker of endothelial health (Tousoulis et al., 2013). Reduced synthesis or oxidative inactivation of BH_4 leads to reducing NO availability by uncoupled eNOS, which generates superoxide rather than NO. Increased levels of BH_4 enhance eNOS activity, promote vasodilation, and reduce oxidative stress in experimentally induced diabetes, ischemia/reperfusion, or hypertension (Faria et al., 2012; Shinozaki et al., 2000; Perkins et al., 2012). In human studies, BH_4 treatment improves endothelial dysfunction and decreases arterial stiffness in postmenopausal women, diabetes, hypercholesterolemia, and coronary disease (Moreau et al., 2012; Holowatz and Kenney, 2011; Heitzer et al., 2000). Recent studies in cultured human endothelial cells confirmed the BH_4 protective effect on eNOS coupling, as

BH4 treatment, prior to oxLDL stimulation, has prevented $p66^{Shc}$-mediated oxidative stress (Shi et al., 2014).

Based on the strong interrelationships pointed out in numerous experimental and clinical research (Xu et al., 2013; Ungvari et al., 2012; Wadley et al., 2013; Maiolino et al., 2013a,b; Borsa et al., 2012; Gradinaru et al., 2012, 2013), we summarized the mechanisms of oxidative stress-oxLDL-NO-induced endothelial dysfunction (Fig. 2). In ageing and age-related metabolic disorders (dyslipidemia, hyperglycemia, insulin resistance, metabolic syndrome) enhancement of oxidative stress, superoxide anion excessive generation and LDL oxidation are critically involved in reduced NO bioactivity and endothelial dysfunction by direct NO elimination. Superoxide radicals ($O_2^{\bullet -}$), are scavenged by nitric oxide to form peroxynitrite ($ONOO^-$), which can oxidize tetrahydrobiopterin (BH_4), the cofactor in NO production by eNOS enzyme, leading to eNOS uncoupling. Uncoupled eNOS will generate more $O_2^{\bullet -}$ and reduce NO production, activating the vicious cycle.

The binding of oxLDL to its specific receptor LOX-1 activates the NADPH oxidase on the cell membrane, which increases intracellular ROS formation. Increased ROS activates the redox-sensitive NF-κB signaling pathway, generating: increases of NF-κB binding to LOX-1 promoter and LOX-1 expression, and amplifies LOX-1-mediated oxLDL uptake. Thus, the oxLDL binding to LOX-1 affects NO bioactivity by two mechanisms: (1) increased ROS production, which reacts with intracellular NO generating cytotoxic $ONOO^-$, and down-regulates eNOS, decreasing NO bioavailability; (2) oxLDL, through LOX-1 receptor activates arginase II, competing with eNOS for L-arginine substrate, down-regulating NO formation, and contributing to vascular dysfunction. The eNOS uncoupling also lead to the p66Shc activation, an important mediator of oxidative stress-induced vascular dysfunction, which contributes to ROS overproduction from mitochondria and/or *via* NADPH oxidase. The oxidized LDL could also increase ROS production *via* phosphorylation of the p66Shc protein at ser36 through the LOX-1.

These complex mechanisms involved in oxLDL-NO interactions and endothelial dysfunction, atherosclerosis and CVD, could explain in part, the failure of antioxidant treatments in improving cardiovascular outcome in long-term clinical trials (Sesso et al., 2008; Parthasarathy et al., 2008).

Taken together, in dysmetabolic status and even ageing, the oxidative stress and LDL oxidation determine reduced NO bioavailability *via* combinatory effects of direct elimination and decreased production of NO. These NO reduced bioavailability compromises all the anti-atherogenic functions of the endothelium. Hypothesized mechanisms shown above could be a target for interventions to protect against endothelial dysfunction, atherogenesis and cardiovascular disease.

6. Conclusions

LDL oxidation and NO synthesis are direct contributors to atherogenesis and also important biomarkers indicating the overall status of the organism as a result of the progressive damage of the endothelium at cellular level under the action of pro-oxidant pathogenic factors and ageing. oxLDL and NO are therefore oxidative stress and endothelial dysfunction biomarkers that could be modulated in the course of ageing, age-related diseases and anti-ageing interventions. Vascular ageing, formerly considered an immutable and inexorable risk factor, is now viewed as a target process for intervention in order to achieve a healthier old age. Therapeutic approaches in the prevention and treatment of atherosclerosis based on improving NO bioactivity and reducing LDL oxidation, and their related molecular mechanisms targets, may become a challenge for future research.

It has been acknowledged that a discrepancy exists between the chronological age and the signs and "marks" of biological age, as evidenced by the clinical assessment of a patient. Therefore, establishing specific biomarkers of vascular endothelium function is important in the complex evaluation of the biological age. In this regard, oxLDL and NO*x* were included among the oxidative stress biomarkers studied within the MARK-AGE project, European Study to Establish Biomarkers of Human ageing.

Acknowledgements

The authors thank Prof. Alexander Burkle, Prof. Tilman Grune and Dr. Maria-Moreno Villanueva for fruitful discussions and support during the MARK-AGE study, very helpful in the preparation of this manuscript.

The authors gratefully acknowledge the European Commission for the financial support provided through the FP7 large-scale integrating project "European Study to Establish Biomarkers of Human ageing" (MARK-AGE; grant agreement no. 200880).

References

Armitage, M.E., Wingler, K., Schmidt, H.H., La, M., 2009. Translating the oxidative stress hypothesis into the clinic: NOX versus NOS. J. Mol. Med. (Berl.) 87 (11), 1071–1076.

Babakr, A.T., Elsheikh, O.M., Almarzouki, A.A., Assiri, A.M., Abdalla, B.E., Zaki, H.Y., Fatani, S.H., NourEldin, E.M., 2014. Relationship between oxidized low-density lipoprotein antibodies and obesity in different glycemic situations. Diabetes Metab. Syndr. Obes. 24 (7), 513–520.

Baur, J., Moreno-Villanueva, M., Kötter, T., Sindlinger, T., Bürkle, A., Berthold, M., Junk, M., 2015. MARK-AGE: extraction, cleaning and quality control of data. Mech. Aging Dev. Special Issue Biomark. Aging.

Bermúdez, V., Bermúdez, F., Acosta, G., Acosta, A., Añez, J., Andara, C., Leal, E., Cano, C., Manuel, V., Hernández, R., Israili, Z., 2008. Molecular mechanisms of endothelial dysfunction: from nitric oxide synthesis to ADMA inhibition. Am. J. Ther. 15 (4), 326–333.

Besler, H.T., Comoğlu, S., 2003. Lipoprotein oxidation, plasma total antioxidant capacity and homocysteine level in patients with multiple sclerosis. Nutr. Neurosci. 6 (2), 189–196.

Borsa, C., Ionescu, C., Gradinaru, D., 2012. Oxidized LDL and NO synthesis as biomarkers of atherogenesis – correlations with metabolic profile in elderly. In: Parthasarathy, S. (Ed.), Atherogenesis. InTech Open Access Publisher, Croatia, pp. 473–494.

Brandão, S.A., Izar, M.C., Fischer, S.M., Santos, A.O., Monteiro, C.M., Póvoa, R.M., Helfenstein, T., Carvalho, A.C., Monteiro, A.M., Ramos, E., Gidlund, M., Figueiredo Neto, A.M., Fonseca, F.A., 2010. Early increase in autoantibodies against human oxidized low-density lipoprotein in hypertensive patients after blood pressure control. Am. J. Hypertens. 23 (2), 208–214.

Bürkle, A., Moreno-Villanueva, M., Bernhard, J., Blasco, M., Zondag, G., Hoeijmakers, J., Toussaint, O., Grubeck-Löbenstein, B., Mocchegiani, E., Collino, S., Gonos, S., Sikora, E., Gradinaru, D., Dolle, M., Salmon, M., Kristensen, P., Griffiths, H., Libert, C., Grune, T., Breusing, N., Simm, A., Franceschi, C., Capri, M., Talbot, D., Caiafa, P., Friguet, B., Slagboom, E., Hervonnen, A., Hurme, M., Aspinall, R., 2015. MARK-AGE biomarkers of aging. Mech. Aging Dev. Special Issue Biomark. Aging.

Cai, H., Harrison, D.G., 2000. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ. Res. 87 (10), 840–844.

Capri, M., Moreno-Villanueva, M., Zoli, M., Scurti, M., Pini, E., Cevenini, E., Borelli, V., Schön, C., Fiegl, S., de Craen, A., Hervonnen, A., Bernhardtd, J., Sikora, E., Gonos, E., Toussaint, O., Grubeck-Loebenstein, B., Bürkle, A., Franceschi, C., 2015. MARK-AGE population: from the human model to practical and successful strategies. Mech. Aging Dev. Special Issue Biomark. Aging.

Chatterjee, A., Black, S.M., Catravas, J.D., 2009. Endothelial nitric oxide (NO) and its pathophysiologic regulation. Vasc. Pharmacol. 49 (4–6), 134–140.

Chikani, G., Zhu, W., Eric, J., 2004. Smart lipids: potential regulators of nitric oxide generation. Am. J. Physiol. Endocrinol. Metab. 287, E386–E389.

Cominacini, L., Pasini, A.F., Garbin, U., Davoli, A., Tosetti, M.L., Campagnola, M., Rigoni, A., Pastorino, A.M., Lo Cascio, V., Sawamura, T., 2000. Oxidized low-density lipoprotein (ox-LDL) binding to ox-LDL receptor-1 in endothelial cells induces the activation of nuclear factor-kappaB through an increased production of intracellular reactive oxygen species. J. Biol. Chem. 275 (17), 12633–12638.

Couillard, C., Ruel, G., Archer, W.R., Pomerleau, S., Bergeron, J., Couture, P., Lamarche, B., Bergeron, N., 2005. Circulating levels of oxidative stress markers and endothelial adhesion molecules in men with abdominal obesity. J. Clin. Endocrinol. Metab. 90 (12), 6454–6459.

Crisby, M., Kublickiene, K., Henareh, L., Agewall, S., 2009. Circulating levels of autoantibodies to oxidized low-density lipoprotein and C-reactive protein levels correlate with endothelial function in resistance arteries in men with coronary heart disease. Heart Vessels 24 (2), 90–95.

Dantas, A.P., Jiménez-Altayó, F., Vila, E., 2012. Vascular aging: facts and factors. Front. Physiol., http://dx.doi.org/10.3389/fphys.2012.00325, Article 325.

Deanfield, J.E., Halcox, J.P., Rabelink, T.J., 2007. Endothelial function and dysfunction: testing and clinical relevance. Circulation 115 (10), 1285–1295.
Demir, M., Kucuk, A., Sezer, M.T., Altuntas, A., Kaya, S., 2010. Malnutrition-inflammation score and endothelial dysfunction in hemodialysis patients. J. Ren. Nutr. 20 (6), 377–383.
Dildar, K., Sinem, F., Gökhan, E., Orhan, Y., Filiz, M., 2010. Serum nitrosative stress levels are increased in Alzheimer disease but not in vascular dementia. Alzheimer Dis. Assoc. Disord. 24 (2), 194–197.
Donato, A.J., Eskurza, I., Silver, A.E., Levy, A.S., Pierce, G.L., Gates, P.E., Seals, D.R., 2007. Direct evidence of endothelial oxidative stress with aging in humans: relation to impaired endothelium-dependent dilation and upregulation of nuclear factor-kappaB. Circ. Res. 100 (11), 1659–1666.
Donato, A.J., Pierce, G.L., Lesniewski, L.A., Seals, D.R., 2009. Role of nuclear factor-kappaB in age-related vascular endothelial dysfunction in humans. Aging Albany (N.Y.) 1 (8), 678–680.
El Assar, M., Angulo, J., Vallejo, S., Peiró, C., Sánchez-Ferrer, C.F., Rodríguez-Mañas, L., 2012. Mechanisms involved in the aging-induced vascular dysfunction. Front. Physiol. 3, 132.
Ercan, M., Firtina, S., Konukoglu, D., 2014. Comparison of plasma viscosity as a marker of endothelial dysfunction with nitric oxide and asymmetric dimethylarginine in subjects with dyslipidemia. Clin. Hemorheol. Microcirc. 57 (4), 315–323.
Erusalimski, J.D., 2009. Vascular endothelial senescence: from mechanisms to pathophysiology. J. Appl. Physiol. 106 (1), 326–332.
Esterbauer, H., Gebicki, J., Puhl, H., Jürgens, G., 1992. The role of lipid peroxidation and antioxidants in oxidative modification of LDL. Free Radical Biol. Med. 13 (4), 341–390.
Faria, A.M., Papadimitriou, A., Silva, K.C., Lopes de Faria, J.M., Lopes de Faria, J.B., 2012. Uncoupling endothelial nitric oxide synthase is ameliorated by green tea in experimental diabetes by re-establishing tetrahydrobiopterin levels. Diabetes 61, 1838–1847.
Fonseca, H.A., Fonseca, F.A., Monteiro, A.M., Bianco, H.T., Boschcov, P., Brandão, S.A., Juliano, L., Gidlund, M., Izar, M.C., 2013. Obesity modulates the immune response to oxidized LDL in hypertensive patients. Cell Biochem. Biophys. 67 (3), 1451–1460.
Franceschi, C., Capri, M., Monti, D., Giunta, S., Olivieri, F., Sevini, F., Panourgia, M.P., Invidia, L., Celani, L., Scurti, M., Cevenini, E., Castellani, G.C., Salvioli, S., 2007. Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans. Mech. Aging Dev. 128, 92–105.
Franzeck, F.C., Hof, D., Spescha, R.D., Hasun, M., Akhmedov, A., Steffel, J., Shi, Y., Cosentino, F., Tanner, F.C., von Eckardstein, A., Maier, W., Lüscher, T.F., Wyss, C.A., Camici, G.G., 2012. Expression of the aging gene p66Shc is increased in peripheral blood monocytes of patients with acute coronary syndrome but not with stable coronary artery disease. Atherosclerosis 220, 282–286.
Ghasemi, A., Zahedias, S., Azimzadeh, I., Azizi, F., 2011. Increased serum nitric oxide metabolites in dysglycaemia. Ann. Hum. Biol. 38, 577–582.
Gradinaru, D., Borsa, C., Ionescu, C., Margina, D., 2013. Advanced oxidative and glycoxidative protein damage markers in the elderly with type 2 diabetes. J. Proteomics 92, 313–322.
Gradinaru, D., Borsa, C., Ionescu, C., Margina, D., Prada, G.I., Jansen, E., 2012. Vitamin D status and oxidative stress markers in the elderly with impaired fasting glucose and type 2 diabetes mellitus. Aging Clin. Exp. Res. 24 (6), 595–602.
Hayashi, T., Yano, K., Matsui-Hirai, H., Yokoo, H., Hattori, Y., Iguchi, A., 2008. Nitric oxide and endothelial cellular senescence. Pharmacol. Ther. 120 (3), 333–339.
Hayashida, K., Kume, N., Murase, T., Minami, M., Nakagawa, D., Inada, T., Tanaka, M., Ueda, A., Kominami, G., Kambara, H., Kimura, T., Kita, T., 2005. Serum soluble lectin-like oxidized low-density lipoprotein receptor-1 levels are elevated in acute coronary syndrome a novel marker for early diagnosis. Circular 112 (6), 812–818.
Heitzer, T., Krohn, K., Albers, S., Meinertz, T., 2000. Tetrahydrobiopterin improves endothelium-dependent vasodilation by increasing nitric oxide activity in patients with type II diabetes mellitus. Diabetologia 43, 1435–1438.
Hirata, Y., Nagata, D., Suzuki, E., Nishimatsu, H., Suzuki, J., Nagai, R., 2010. Diagnosis and treatment of endothelial dysfunction in cardiovascular disease. Int. Heart J. 51 (1), 1–6.
Holowatz, L.A., Kenney, W.L., 2011. Acute localized administration of tetrahydrobiopterin and chronic systemic atorvastatin treatment restore cutaneous microvascular function in hypercholesterolaemic humans. J. Physiol. 589, 4787–4797.
Holvoet, P., De Keyzer, D., Jacobs Jr., D.R., 2008a. Oxidized LDL and the metabolic syndrome. Future Lipidol. 3 (6), 637–649.
Holvoet, P., Kritchevsky, S.B., Tracy, R.P., Mertens, A., Rubin, S.M., Butler, J., Goodpaster, B., Harris, T.B., 2004. The metabolic syndrome, circulating oxidized LDL, and risk of myocardial infarction in well-functioning elderly people in the health, aging, and body composition cohort. Diabetes 53 (4), 1068–1073.
Holvoet, P., Lee, D.H., Steffes, M., Gross, M., Jacobs Jr., D.R., 2008b. Association between circulating oxidized low-density lipoprotein and incidence of the metabolic syndrome. J. Am. Med. Assoc. 299 (19), 2287–2293.
Ignarro, L.J., Napoli, C., 2004. Novel features of nitric oxide, endothelial nitric oxide synthase, and atherosclerosis. Curr. Atheroscler. Rep. 6 (4), 281–287.
Jin, R.C., Loscalzo, J., 2010. Vascular nitric oxide: formation and function. J. Blood Med. 1, 147–162.
Kampus, P., Kals, J., Ristimäe, T., Muda, P., Ulst, K., Zilmer, K., Salonen, R.M., Tuomainen, T.P., Teesalu, R., Zilmer, M., 2007. Augmentation index and carotid intima-media thickness are differently related to age, C-reactive protein andoxidized low-density lipoprotein. J. Hypertens. 25 (4), 819–825.
Kankaanpää, J., Turunen, S.P., Moilanen, V., Hörkkö, S., Remes, A.M., 2009. Cerebrospinal fluid antibodies to oxidized LDL are increased in Alzheimer's disease. Neurobiol. Dis. 33 (3), 467–472.
Kaplon, R.E., Gano, L.B., Seals, D.R., 2014. Vascular endothelial function and oxidative stress are related to dietary niacin intake among healthy middle-aged and older adults. J. Appl. Physiol. (1985) 116 (2), 156–163.
Kaplon, R.E., Walker, A.E., Seals, D.R., 2011. Plasma norepinephrine is an independent predictor of vascular endothelial function with aging in healthy women. J. Appl. Physiol. (1985) 111 (5), 1416–1421.
Kim, J.Y., Kim, O.Y., Paik, J.K., Kwon, D.Y., Kim, H.J., Lee, J.H., 2013. Association of age-related changes in circulating intermediary lipid metabolites, inflammatory and oxidative stress markers, and arterial stiffness in middle-aged men. AGE 35, 1507–1519.
Kobayashi, N., Takano, M., Hata, N., Kume, N., Yamamoto, M., Yokoyama, S., Shinada, T., Tomita, K., Shirakabe, A., Otsuka, T., Seino, Y., Mizuno, K., 2013. Soluble lectin-like oxidized LDL receptor-1 (sLOX-1) as a valuable diagnostic marker for rupture of thin-cap fibroatheroma: verification by optical coherence tomography. Int. J. Cardiol. 168 (4), 3217–3223.
Kolluru, G.K., Bir, S.C., Kevil, C.G., 2012. Endothelial dysfunction and diabetes: effects on angiogenesis, vascular remodeling, and wound healing. Int. J. Vasc. Med. 2012, 918267.
Kopprasch, S., Pietzsch, J., Kuhlisch, E., Fuecker, K., Temelkova-Kurktschiev, T., Hanefeld, M., Kühne, H., Julius, U., Graessler, J., 2002. In vivo evidence for increased oxidation of circulating LDL in impaired glucose tolerance. Diabetes 51 (10), 3102–3106.
Kuklinska, A.M., Mroczko, B., Musial, W.J., Usowicz-Szarynska, M., Sawicki, R., Borowska, H., Knapp, M., Szmitkowski, M., 2009. Diagnostic biomarkers of essential arterial hypertension: the value of prostacyclin, nitric oxide, oxidized-LDL, and peroxide measurements. Int. Heart J. 50 (3), 341–351.
Le, N.A., 2015. Lipoprotein-associated oxidative stress: a new twist to the postprandial hypothesis. Int. J. Mol. Sci. 16 (1), 401–419.
Li, W., Mital, S., Ojaimi, C., Csiszar, A., Kaley, G., Hintze, TH, 2004. Premature death and age-related cardiac dysfunction in male eNOS-knockout mice. J. Mol. Cell. Cardiol. 37 (3), 671–680.
López-Uriarte, P., Nogués, R., Saez, G., Bulló, M., Romeu, M., Masana, L., Tormos, C., Casas-Agustench, P., Salas-Salvadó, J., 2010. Effect of nut consumption on oxidative stress and the endothelial function in metabolic syndrome. Clin. Nutr. 29 (3), 373–380.
Lundberg, J.O., Weitzberg, E., 2005. NO generation from nitrite and its role in vascular control. Arterioscl. Thromb. Vasc. Biol. 25 (5), 915–922.
Mah, E., Pei, R., Guo, Y., Ballard, K.D., Barker, T., Rogers, V.E., Parker, B.A., Taylor, A.W., Traber, M.G., Volek, J.S., Bruno, R.S., 2013. γ-Tocopherol-rich supplementation additively improves vascular endothelial function during smoking cessation. Free Radical Biol. Med. 65, 1291–1299.
Maiolino, G., Pedon, L., Cesari, M., Frigo, A.C., Barisa, M., Rossitto, G., Seccia, T.M., Zanchetta, M., Rossi, G.P., 2013a. Antibodies to malondialdehyde oxidized low-density lipoproteins predict long term cardiovascular mortality in high risk patients. Int. J. Cardiol. 168 (1), 484–489.
Maiolino, G., Rossitto, G., Caielli, P., Bisogni, V., Rossi, G.P., Calò, L.A., 2013b. The role of oxidized low-density lipoproteins in atherosclerosis: the myths and the facts. Mediators Inflamm., 714653.
Mangerich, A., Burkle, A., 2012. Pleiotropic cellular functions of PARP1 in longevity and aging: genome maintenance meets inflammation. Oxid. Med. Cell. Longev. 2012, 321653.
Mazière, C., Savitsky, V., Galmiche, A., Gomila, C., Massy, Z., Mazière, J.C., 2010. Oxidized low density lipoprotein inhibits phosphate signaling and phosphate-induced mineralization in osteoblasts. Involvement of oxidative stress. Biochim. Biophys. Acta 1802 (11), 1013–1019.
Meisinger, C., Baumert, J., Khuseyinova, N., Loewel, H., Koenig, W., 2005. Plasma oxidized low-density lipoprotein, a strong predictor for acute coronary heart disease events in apparently healthy, middle-aged men from the general population. Circulation 112, 651e7.
Merino, J., Ferré, R., Girona, J., Aguas, D., Cabré, A., Plana, N., Vinuesa, A., Ibarretxe, D., Basora, J., Buixadera, C., Masana, L., 2013. Even low physical activity levels improve vascular function in overweight and obese postmenopausal women. Menopause 20 (10), 1036–1042.
Michel, T., Vanhoutte, P.M., 2010. Cellular signaling and NO production. Pflugers Arch. 459 (6), 807–816.
Moncada, S., Higgs, E.A., 2006. The discovery of nitric oxide and its role in vascular biology. Br. J. Pharmacol. 147 (Suppl. 1), S193–S201.
Moreau, K.L., Meditz, A., Deane, K.D., Kohrt, W.M., 2012. Tetrahydrobiopterin improves endothelial function and decreases arterial stiffness in estrogen deficient postmenopausal women. Am. J. Physiol. Heart Circ. Physiol. 302, H1211–H1218.
Moreno-Luna, R., Muñoz-Hernandez, R., Miranda, M.L., Costa, A.F., Jimenez-Jimenez, L., Vallejo-Vaz, A.J., Muriana, F.J., Villar, J., Stiefel, P., 2012. Olive oil polyphenols decrease blood pressure and improve endothelial function in young women with mild hypertension. Am. J. Hypertens. 25 (12), 1299–1304.
Moreno-Villanueva, M., Capri, M., Breusing, N., Sevini, F., Ghezzo, S., Schön, C., de Craen, A.J., Hervonnen, A., Bernhardt, J., Grune, T., Franceschi, C., Bürkle, A., 2015a. MARK-AGE standard operating procedures (SOPs): a successful effort. Mech. Aging Dev. Special Issue Biomark. Aging.

Moreno-Villanueva, M., Kötter, T., Sindlinger, T., Baur, J., Bürkle, A., Berthold, M., 2015b. MARK-AGE database. Mech. Aging Dev. Special Issue Biomark. Aging.
Müller, S., König, I., Meyer, W., Kojda, G., 2004. Inhibition of vascular oxidative stress in hypercholesterolemia by eccentric isosorbide mononitrate. J. Am. Coll. Cardiol. 44 (3), 624–631.
Muñoz-Torrero, J.F., Rivas, D., Alonso, R., Crespo, L., Costo, A., Roman, M., Martín, C., Zamorano, J., 2012. Influence of lipoprotein (a) on inflammatory biomarkers in metabolic syndrome. South. Med. J. 105 (7), 339–343.
Napoli, C., de Nigris, F., Williams-Ignarro, S., Pignalosa, O., Sica, V., Ignarro, L.J., 2006. Nitric oxide and atherosclerosis: an update. Nitric Oxide 15 (4), 265–279.
Nawrot, T.S., Staessen, J.A., 2008. Genetic variation and environmental factors in biological and arterial aging. Verh. K. Acad. Geneeskd. Belg. 70 (5–6), 323–338.
Nawrot, T.S., Staessen, J.A., Holvoet, P., Struijker-Boudier, H.A., Schiffers, P., Van Bortel, L.M., Fagard, R.H., Gardner, J.P., Kimura, M., Aviv, A., 2010. Telomere length and its associations with oxidized-LDL: carotid artery distensibility and smoking. Front Biosci. (Elite Ed.) 2, 1164–1168.
Nilsson, P.M., 2008. Early vascular aging (EVA): consequences and prevention. Vasc. Health Risk Manage. 4 (3), 547–552.
Njajou, O.T., Kanaya, A.M., Holvoet, P., Connelly, S., Strotmeyer, E.S., Harris, T.B., Cummings, S.R., Hsueh, W.C., 2009. Association between oxidized LDL, obesity and type 2 diabetes in a population-based cohort, the health, aging and body composition study. Diabetes Metab. Res. Rev. 25 (8), 733–739.
Obradovic, M.M., Trpkovic, A., Bajic, V., Soskic, S., Jovanovic, A., Stanimirovic, J., Panic, M., Isenovic, E.R., 2015. Interrelatedness between C-reactive protein and oxidized low-density lipoprotein. Clin. Chem. Lab. Med. 53 (1), 29–34.
Orem, A., Yucesan, F.B., Orem, C., Akcan, B., Kural, B.V., Alasalvar, C., Shahidi, F., 2013. Hazelnut-enriched diet improves cardiovascular risk biomarkers beyond a lipid-lowering effect in hypercholesterolemic subjects. J. Clin. Lipidol. 7 (2), 123–131.
Pacher, P., Beckman, J.S., Liaudet, L., 2007. Nitric oxide and peroxynitrite in health and disease. Physiol. Rev. 87 (1), 315–424.
Paik, J.K., Chae, J.S., Kang, R., Kwon, N., Lee, S.H., Lee, J.H., 2013. Effect of age on atherogenicity of LDL and inflammatory markers in healthy women. Nutr. Metab. Cardiovasc. Dis. 23 (10), 967–972.
Parthasarathy, S., Litvinov, D., Selvarajan, K., Garelnabi, M., 2008. Lipid peroxidation and decomposition – conflicting roles in plaque vulnerability and stability. Biochim. Biophys. Acta 1781 (5), 221–231.
Perkins, K.A., Pershad, S., Chen, Q., McGraw, S., Adams, J.S., Zambrano, C., Krass, S., Emrich, J., Bell, B., Iyamu, M., Prince, C., Kay, H., Teng, J.C., Young, L.H., 2012. The effects of modulating eNOS activity and coupling in ischemia/reperfusion (I/R). Naunyn Schmiedebergs Arch. Pharmacol. 385, 27–38.
Pierce, G.L., Lesniewski, L.A., Lawson, B.R., Beske, S.D., Seals, D.R., 2009. Nuclear factor-kappaB activation contributes to vascular endothelial dysfunction via oxidative stress in overweight/obese middle-aged and older humans. Circulation 119 (9), 1284–1292.
Pirillo, A., Catapano, A.L., 2013. Soluble lectin-like oxidized low density lipoprotein receptor-1 as a biochemical marker for atherosclerosis-related diseases. Dis. Mark. 35 (5), 413–418.
Pirillo, A., Norata, G.D., Catapano, A.L., 2013. LOX-1, OxLDL, and atherosclerosis. Mediators Inflamm. 2013, 152786.
Profumo, E., Di Franco, M., Buttari, B., Masella, R., Filesi, C., Tosti, M.E., Scrivo, R., Scarno, A., Spadaro, A., Saso, L., Rigano, R., 2012. 2012. Biomarkers of subclinical atherosclerosis in patients with autoimmune disorders. Mediators Inflamm. 2012, 503942, http://dx.doi.org/10.1155/2012/503942.
Roberts, A.C., Porter, K.E., 2013. Cellular and molecular mechanisms of endothelial dysfunction in diabetes. Diabetes Vasc. Dis. Res. 10 (6), 472–482.
Robman, L., McNeil, J., Dimitrov, P., Dowrick, A., Tikellis, G., Nicolas, C., Cameron, J., Guymer, R., McGrath, B., McCarty, C., 2004. Methodology of the cardiovascular health and age-related maculopathy (CHARM) study. Ophthalmic Epidemiol. 11 (3), 161–179.
Rodriguez-Manas, L., El-Assar, M., Vallejo, S., Lopez-Doriga, P., Solis, J., Petidier, R., Montes, M., Nevado, J., Castro, M., Gomez-Guerrero, C., Peiro, C., Sanchez-Ferrer, C.F., 2009. Endothelial dysfunction in aged humans is related with oxidative stress and vascular inflammation. Aging Cell 8 (3), 226–238.
Rubio, A.R., Morales-Segura, M.A., 2004. Nitric oxide, an iceberg in cardiovascular physiology: far beyond vessel tone control. Arch. Med. Res. 35 (1), 1–11.
Samsamshariat, S.Z., Basati, G., Movahedian, A., Pourfarzam, M., Sarrafzadegan, N., 2011. Elevated plasma myeloperoxidase levels in relation to circulating inflammatory markers in coronary artery disease. Biomark. Med. 5 (3), 377–385.
Sesso, H.D., Buring, J.E., Christen, W.G., Kurth, T., Belanger, C., MacFadyen, J., Bubes, V., Manson, J.E., Glynn, R.J., Gaziano, J.M., 2008. Vitamins E and C in the prevention of cardiovascular disease in men: the physicians' health study II randomized controlled trial. JAMA 300 (18), 2123–2133.
Shi, Y., Cosentino, F., Camici, G.G., Akhmedov, A., Vanhoutte, P.M., Tanner, F.C., Lüscher, T.F., 2011. Oxidized low-density lipoprotein activates p66Shc via lectin-like oxidized low-density lipoprotein receptor-1, protein kinase C-beta, and c-Jun N terminal kinase in human endothelial cells. Arterioscler. Thromb. Vasc. Biol. 31, 2090–2097.
Shi, Y., Lutscher, T.F., Camici, G.G., 2014. Dual role of endothelial nitric oxide synthase in oxidized LDL-induced, p66Shc-mediated oxidative stress in cultured human endothelial cells. PLoS One 9 (9), e107787.
Shinozaki, K., Nishio, Y., Okamura, T., Yoshida, Y., Maegawa, H., Kojima, H., Masada, M., Toda, N., Kikkawa, R., Kashiwagi, A., 2000. Oral administration of tetrahydrobiopterin prevents endothelial dysfunction and vascular oxidative stress in the aortas of insulin-resistant rats. Circ. Res. 87, 566–573.
Sima, A., Stancu, C., Simionescu, M., 2009. Vascular endothelium in atherosclerosis. Cell Tissue Res. 335 (1), 191–203.
Simionescu, M., Antohe, F., 2006. Functional ultrastructure of the vascular endothelium: changes in various pathologies. Handb. Exp. Pharmacol. 176 (1), 41–69.
Steinberg, D., Witzum, J.L., 2010. Oxidized low-density lipoprotein and atherosclerosis. History of discovery. Arterioscler. Thromb. Vasc. Biol. 30 (12), 2311–2316.
Steinberg, D., 2009. The LDL modification hypothesis of atherogenesis: an update. J. Lipid. Res. 50, S376–S381.
Stojakovic, T., Claudel, T., Putz-Bankuti, C., Fauler, G., Scharnagl, H., Wagner, M., Sourij, H., Stauber, R.E., Winkler, K., März, W., Wascher, T.C., Trauner, M., 2010. Low-dose atorvastatin improves dyslipidemia and vascular function in patients with primary biliary cirrhosis after one year of treatment. Atherosclerosis 209 (1), 178–183.
Sverdlov, A.L., Ngo, D.T., Chan, W.P., Chirkov, Y.Y., Horowitz, J.D., 2014. Aging of the nitric oxide system: are we as old as our NO? J. Am. Heart Assoc. 3 (4), e000973.
Tan, K.C.B., Shiu, S.W.M., Wong, Y., Leng, L., Bucala, R., 2008. Soluble lectin-like oxidized low density lipoprotein receptor-1 in type 2 diabetes mellitus. J. Lipid Res. 49 (7), 1438–1444.
Thorin, E., Thorin-Trescases, N., 2009. Vascular endothelial aging, heartbeat after heartbeat. Cardiovasc. Res. 84 (1), 24–32.
Tousoulis, D., Papageorgiou, N., Androulakis, E., Siasos, G., Latsios, G., Tentolouris, K., Stefanadis, C., 2013. Diabetes mellitus-associated vascular impairment: novel circulating biomarkers and therapeutic approaches. J. Am. Coll. Cardiol. 62 (8), 667–676.
Tsimikas, S., Brilakis, E.S., Lennon, R.J., Miller, E.R., Witztum, J.L., McConnell, J.P., Kornman, K.S., Berger, P.B., 2007. Relationship of IgG and IgM autoantibodies to oxidized low density lipoprotein with coronary artery disease and cardiovascular events. J. Lipid Res. 48 (2), 425–433.
Tsimikas, S., Willeit, P., Willeit, J., Santer, P., Mayr, M., Xu, Q., Mayr, A., Witztum, J.L., Kiechl, S., 2012. Oxidation-specific biomarkers, prospective 15-year cardiovascular and stroke outcomes, and net reclassification of cardiovascular events. J. Am. Coll. Cardiol. 60 (21), 2218–2229.
Ungvari, Z., Kaley, G., de Cabo, R., Sonntag, W.E., Csiszar, A., 2010. Mechanisms of vascular aging: new perspectives. J. Gerontol. A Biol. Sci. Med. Sci. 65A (10), 1028–1041.
Vaidya, D., Szklo, M., Cushman, M., Holvoet, P., Polak, J., Bahrami, H., Jenny, N.S., Ouyang, P., 2011. Association of endothelial and oxidative stress with metabolic syndrome and subclinical atherosclerosis: multi-ethnic study of atherosclerosis. Eur. J. Clin. Nutr. 65 (7), 818–825.
van der Zwan, L.P., Teerlink, T., Dekker, J.M., Henry, R.M.A., Stehouwer, C.D.A., Jakobs, C., Heine, R.J., Scheffer, P.G., 2009. Circulating oxidized LDL: determinants and association with brachial flow-mediated dilation. J. Lipid Res. 50 (2), 342–349.
Verhoye, E., Langlois, M.R., 2009. Circulating oxidized low-density lipoprotein: a biomarker of atherosclerosis and cardiovascular risk? Clin. Chem. Lab. Med. 47 (2), 128–137.
Verma, S., Buchanan, M.R., Anderson, T.J., 2003. Endothelial function testing as a biomarker of vascular disease. Circulation 108, 2054–2059.
Violi, F., Sanguigni, V., Carnevale, R., Plebani, A., Rossi, P., Finocchi, A., Pignata, C., De Mattia, D., Martire, B., Pietrogrande, M.C., Martino, S., Gambineri, E., Soresina, A.R., Pignatelli, P., Martino, F., Basili, S., Loffredo, L., 2009. Hereditary deficiency of gp91(phox) is associated with enhanced arterial dilatation: results of a multicenter study. Circulation 120 (16), 1616–1622.
Volpe, C.M., Abreu, L.F., Gomes, P.S., Gonzaga, R.M., Veloso, C.A., Nogueira-Machado, J.A., 2014. The production of nitric oxide, IL-6, and TNF-alpha in palmitate-stimulated PBMNCs is enhanced through hyperglycemia in diabetes. Oxid. Med. Cell. Longev. 2014, 479587.
Wadley, A.J., Jet, J.C.S., van Zanten, V., Aldred, S., 2013. The interactions of oxidative stress and inflammation with vascular dysfunction in aging: the vascular health triad. AGE 35, 705–718.
Walker, A.E., Kaplon, R.E., Lucking, S.M., Russell-Nowlan, M.J., Eckel, R.H., Seals, D.R., 2012. Fenofibrate improves vascular endothelial function by reducing oxidative stress while increasing endothelialnitric oxide synthase in healthy normolipidemic older adults. Hypertension 60 (6), 1517–1523.
Wang, J.C., Bennett, M., 2011. Aging and atherosclerosis Mechanisms, functional consequences, and potential therapeutics for cellular senescence. Circ. Res. 111, 245–259.
Wang, Y., Chun, O.K., Song, W.O., 2013. Plasma and dietary antioxidant status as cardiovascular disease risk factors: a review of human studies. Nutrients 5 (8), 2969–3004.
Weinbrenner, T., Schröder, H., Escurriol, V., Fito, M., Elosua, R., Vila, J., Marrugat, J., Covas, M.I., 2006. Circulating oxidized LDL is associated with increased waist circumference independent of body mass index in men and women. Am. J. Clin. Nutr. 83 (1), 30–35.
Wink, D.A., Miranda, K.M., Espey, M.G., Pluta, R.M., Hewett, S.J., Colton, C., Vitek, M., Feelisch, M., Grisham, M.B., 2001. Mechanisms of the antioxidant effects of nitric oxide. Antioxid. Redox Signal. 3 (2), 203–213.
Wu, J., Xia, S., Kalionis, B., Wan, W., Sun, T., 2014. The role of oxidative stress and inflammation in cardiovascular aging. Biomed. Res. Int. 2014, 615312.
Wu, T., Willett, W.C., Rifai, N., Shai, I., Manson, J.E., Rimm, E.B., 2006. Is plasma oxidized low-density lipoprotein, measured with the widely used antibody

4E6, an independent predictor of coronary heart disease among U.S. men and women? J. Am. Coll. Cardiol. 48 (5), 973–979.
Xu, S., Ogura, S., Chen, J., Little, P.J., Moss, J., Liu, P., 2013. LOX-1 in atherosclerosis: biological functions and pharmacological modifiers. Cell. Mol. Life Sci. 70 (16), 2859–2872.
Xu, X., Gao, X., Potter, B.J., Cao, J.M., Zhang, C., 2007. Anti-LOX-1 rescues endothelial function in coronary arterioles in atherosclerotic. ApoE knockout mice. Arterioscler. Thromb. Vasc. Biol. 27 (4), 871–877.
Yamamori, T., White, A.R., Mattagajasingh, I., Khanday, F.A., Haile, A., Qi, B., Jeon, B.H., Bugayenko, A., Kasuno, K., Berkowitz, D.E., Irani, K., 2005. p66shc regulates endothelial NO production and endothelium-dependent vasorelaxation: implications for age-associated vascular dysfunction. J. Mol. Cell. Cardiol. 39 (6), 992–995.
Yubero-Serrano, E.M., Delgado-Casado, N., Delgado-Lista, J., Perez-Martinez, P., Tasset-Cuevas, I., Santos-Gonzalez, M., Caballero, J., Garcia-Rios, A., Marin, C., Gutierrez-Mariscal, F.M., Fuentes, F., Villalba, J.M., Tunez, I., Perez-Jimenez, F., Lopez-Miranda, J., 2011. Postprandial antioxidant effect of the Mediterranean diet supplemented with coenzyme Q10 in elderly men and women. Age (Dordr.) 33 (4), 579–590.
Zhao, Z.W., Zhu, X.L., Luo, Y.K., Lin, C.G., Chen, L.L., 2011. Circulating soluble lectin-like oxidized low-density lipoprotein receptor-1 levels are associated with angiographic coronary lesion complexity in patients with coronary artery disease. Clin. Cardiol. 34 (3), 172–177.
Zuliani, G., Morieri, M.L., Volpato, S., Vigna, G.B., Tch, C.B., Maggio, M., Cherubini, A., Bandinelli, S., Guralnik, J.M., Ferrucci, L., 2012. Determinants and clinical significance of plasma oxidized LDLs in older individuals. A 9 years follow-up study. Atherosclerosis 226 (1), 201–720.