Membrane Lipid/Protein Oxidative Nitration in Atherosclerosis

Subjects: Biochemistry & Molecular Biology

Contributor: Mohamed Aly Abdelhafez

The role of oxidation and nitration of endothelial plasma memberane and lipoproteins in pathogenesis of atherosclerosis. The atherogenic role of nitro-tyrosine, and the protective role of nitrated fatty acids. The factors influencing endothelial lipoprotein lipase activity.

Oxyphosholipids oxy-LDL oxidative Nitration cell membrane nitrolipids 3-tyrosine protein nitrofatty acids

1. Introduction

The vascular smooth muscle cells (VSMCs) and endothelium is exposed to mediators that influence their biological functions. Being in direct contact with the peripheral tissue cells, the activity of these cells is susceptible to oxidation and nitration stress depending on local metabolic events. The vascular smooth muscle cells (VSMCs) is in need of nitric oxide generated by vascular endothelial cells (VECs) to satisfy efficiently nutrient or metabolites. The local microenvironment is exposed to both reactive oxygen species (ROS) and nitric oxide (NO). Plasma membranes of these cells are composed of phospholipids bilayer containing both unsaturated fatty acids (besides choline, inositol, serine, ethanolamine, and/or sphingosine), and cholesterol with intervening proteins as receptors, channels, enzymes, or else.

In addition, circulating platelets secrete eicosanoids along with those synthesized by VECs influenced by specific synthases and leukocytic myeloperoxidase. All these cells are ROS generators.

At vascular beds in peripheral tissues, both ROS and NO are generated. Nitric oxide is a precursor of reactive nitrogen species (RNS). ROS as well as RNS strongly encourage inflammasome formation. In this review we will discuss the role of membrane alterations secondary to oxidation and nitration in atherogenesis and discuss that the concept of altered lipoproteins components are not the only players in pathogenesis of atherosclerosis.

2. Lipid Oxidation and Nitration

Lipid oxidation and lipid nitration are processes affect the cell membranes as a result of oxidative attack on the unsaturated acyl chains of lipids by *reactive oxygen and nitrogen species* [1].

The free radical species ·nitric oxide (·NO) is a smooth muscle relaxant and inhibitor of platelet/leukocyte activation that is essential for maintenance of vascular homeostasis. Reaction of ··NO with superoxide anion (O_2^{*-}), yields peroxynitrite ($ONOO^-$), accounts for a major part of the accelerated ·NO disposal [2].

Oxidation of lipids is accomplished by ROS released by transitional metal-dependent Fenton oxidation, enzyme-catalyzed oxidation by lipoxygenase and myeloperoxidase (MPO), reaction with hypochlorous acid (HOCl) or via superoxide anion (O_2^{*-}) and H_2O_2-generating oxidases (as flavine-containing oxidases). In addition, oxidation by ·NO-derived reactive species (eg.· nitrogen dioxide (·NO_2), nitryl chloride (NO_2Cl), and peroxynitrite [3]. Lipid oxidation by these enzymes involves formation of enzyme-bound radical intermediates, including lipid alkyl (L·) and peroxyl (LOO·) radical species. Peroxidation of lipids may propagate in a succession of reactions. During the reaction, free peroxyl (LOO·) and/or alkyl (LO·) radicals react with ·NO. Two molecules of ·NO are consumed to form peroxynitrite (LOONO) that may decompose to secondary radical species, LO· NO2·. This decreases bioavailable ·NO and terminating succession of lipid peroxidation propagation process [4].

Peroxynitrite mediates oxidation of fatty acids whether free or in form of neutral lipids, phospholipids and/or LDL lipids; resulting in formation of conjugated diene, malondialdehyde, lipid peroxide, lipid hydroxide, F2-isoprostane, and oxysterol products [5][6].

Enzymes such as lipoxygenase, prostaglandin endoperoxide H synthase, and cytochrome P450 that oxidize lipids to bioactive eicosanoids play critical signaling roles in the regulation of vascular cell function and inflammatory responses and are ubiquitously expressed by all vascular cells under physiological and inflammatory conditions [7]. In the cellular environment, the hydroxyl radicals initiate lipid peroxidation in a chain reaction that depends on the presence of polyunsaturated fatty acids in the membrane phospholipids and on the oxygen concentration, resulting in the formation of peroxidized lipids. Peroxidized lipids tend to hydrolyze into oxidatively truncated oxidized phospholipids with shorter acyl chains as aldehydes and ketones which play key role in membrane permeability, binding with ligands, and ferroptosis execution [8]. They may influence the biological functions of membrane proteins either chemically through oxidation, conjugation with the truncated lipid products, or alteration of covalent attachment(s) with adjacent molecules. Cell membrane lipids and proteins properties are thus altered resulting in cell dysfunction or even cell death.

Reactive oxygen and nitrogen species induce membrane lipid or protein oxidation in the absence of efficient oxidative defense mechanisms [9]. Oxidation of membrane phospholipids, containing the polyunsaturated fatty acid, results in the accumulation of an end product, 2-(ω-carboxyethyl) pyrrole (CEP) that induce proinflammatory cytokines expression [10]. This process is mediated by toll-like receptor 2 (TLR2) in endothelial cells independently of vascular endothelial growth factor signaling [11]. CEP adducts also promote platelet activation and thrombosis [12] resulting in ischemic consequences.

Oxidized lipids and lipid-protein adducts are immunogenic producing oxidation-specific epitopes. Macrophage pattern recognition receptors (as CD36 and SR-A) recognize oxidation-specific epitopes as oxidized phospholipids (OxPL) and malondialdehyde (MDA)-modified structures. This mechanism is intimately working in the process of

apoptotic cell removal [13]. Antiphospholipid antibodies recognize the plasma membranes of apoptotic cells, but not viable cells [14]. Apoptotic cells not only serve as targets for antibodies but also provide autoantigens that trigger autoimmune responses [15].

3. Role of Oxidized Low Density Lipoproteins (oxLDL])

Native low-density lipoprotein (LDL) has no pro-atherogenic effects [16]. Cholesterol accumulation in atherosclerotic lesions is not due to cellular uptake of native LDL through the LDL receptor, but rather due to the uptake of an oxidation-modified form that are ligands for scavenger receptors present in monocytes/macrophages and smooth muscle cells (SMC) membranes [17]. In contrast to native LDL, oxidized LDL (oxLDL) uptake by scavenger receptors in macrophages or SMC is not under a negative feedback regulation. Therefore, this process results in uncontrolled influx and intracellular accumulation of cholesterol and its oxidation products which critical in the formation of foam cells. It is a major component of the atheroma plaque [18].

Circulating LDL is not susceptible for oxidation owing to the co-existence of antioxidants (*i.e.* tocopherol, ascorbate, uric acid) [19]. As LDL influxes towards the subendothelial space, that has low antioxidant concentrations, most oxidation reactions occur besides development of inflammatory vascular reactions. In this context, activated cells produce reactive oxygen and nitrogen species that convert native LDL into oxLDL. The reactive oxygen species are superoxide ($O_2^{\bullet -}$), hydroxyl ($^{\bullet}OH$) are generated by NADPH oxidase, xanthine oxidase, myeloperoxidase and/or lipooxygenase. Production of $^{\bullet}NO$ radicals is mediated by nitric oxide synthase (NOS). In addition, the oxidant and nitrating agent peroxynitrite, is synthesized from nitric oxide ($^{\bullet}NO$) and superoxide anion ($O_2^{\bullet -}$). Hydrogen peroxide (H_2O_2) is also generated by vascular cells and macrophages and is an additional oxidative factor [20]. oxLDL alters the endothelial production and bioavailability of nitric oxide ($^{\bullet}NO$), stimulates endothelial cells apoptosis and has direct chemotactic effects on monocytes. Polyunsaturated fatty acids in phospholipids undergo peroxidation. The oxidized phospholipid (OxPL) backbone may be fragmented into shorter peroxylipids as malondialdehyde (MDA). Moreover, oxLDL induces VSMCs expression and generation of growth, adhesion, and chemotactic factors. These reactions promote development of an inflammatory focus in the arterial intima [21][22].

The scavenger receptors LOX-1, SR-A, SR-B1, and CD36 recognize lipoproteins modified by oxidation, glycation, alkylation, and nitration to be endocytosed prior to their degradation. The reticuloendothelial macrophages, phagocytes, Kupffer cells, and dendritic cells are primarily responsible for the scavenger receptor-mediated removal of oxLDLs. Other cells may share this process as vascular smooth muscle cells (VSMCs), keratinocytes, endothelial cells, and neuronal cells [23].

Oxidatively modified apolipoprotein B, in turn, may disturb LDLR-mediated cellular uptake of LDL [24].

Scavenger receptors do not recognize the so-called “minimally oxidized LDL” (mmLDL), probably because of their weak antigenicity [25]. mmLDL predominantly contains hydroxides, hydroperoxides, endoperoxides and other trunkated lipid peroxidation products of phospholipids [26][27].

Furthermore, formation of lipid-protein adducts making the LDL particle more electronegative [28]. The electronegative LDL (LDL(−)) fraction accounts for approximately 4% (ranging from 0.5 to 9.8%) of all LDL, and it is characterized by the enrichment of TG, hydroperoxides, MDA, oxysterols lipid but is poor in α-tocopherol [29]. LDL(−) induce cytokine expressions and apoptosis of endothelial cells endothelial cells [30].

4. Oxidized Phospholipids and Lp(a) Role in Atherogenesis

Oxidized phospholipids (OxPL) are principle players of the atherosclerotic oxidized lipoproteins. They are principle components of Lp(a) that is a potent risk factor for atherothrombotic disease. OxPL plays an axial role in atherogenicity of Lp(a). The OxPL modification may explain why Lp(a) is such a potent risk factor for cardiovascular disease despite being present at low concentrations compared to LDL, and they account for the ability of elevated Lp(a) as an etiological factor in atherothrombotic disorder [31].

Lp(a) plasma concentrations versus OxPL-apoB are comparable in risk prediction. Both are positively associated with peripheral atherosclerotic cardiovascular disease progression [32].

5. Tissue Lipid Uptake

Lipid uptake bytissue cells necessitate transfer from the capillaries across the endothelial cell (EC) barrier. This process requires lipoprotein lipase (LPL), its binding protein glycosylphosphatidylinositol-anchored HDL binding protein 1 (GPIHBP1), cluster of differentiation 36 (CD36), and other mediators [33].

During fasting, the stored lipids undergo lipolysis by hormone-sensitive lipase, with release of free fatty acids (FFAs). In post-absorptive sate, FFAs are released from very low density lipoproteins (VLDL) and chylomicrons bny endothelial LPL. FFAs are taken up by peripheral cells facilitated by CD36 as a high-affinity receptor. Locally, LPL actions depend on rate of synthesis, release of its activators and/or inhibitors as well as EC expression of by glycosylphosphatidyl inositol anchored HDL binding protein (GPIHBP1). Transfer of FFAs released from triglyceride-rich lipoproteins seems partially to require EC CD36 [34].

CD36 enhances FA uptake and FA oxidation [35]. Human CD36 polymorphisms associate with defects of lipid handling and metabolic disorders (eg, hyperlipidemia, metabolic syndrome, type 2 diabetes) [36].

Lipoprotein lipase (LPL) is synthesized in metabolically-active tissues like heart, skeletal muscle, and adipose tissue. It is linked to the basolateral sides of endothelial cells (ECs) by GPIHBP, to get access to the capillary lumen, which is the site of LPL's catalytic action on triglycerides-rich lipoproteins [37].

There is gene expression distinction in capillary endothelial cells (ECs) and most large vessel ECs to correlate with the corresponding functions. Microvascular ECs, that are marked by CD36; are enriched in genes coding for GPIHBP1, LPL, and peroxisome proliferator activated receptor γ (PPARγ) to enhance triglyceride lipolysis and fatty acid uptake [38]. Heterogeneity of ECs gene expression is observed, even, within individual vessels. In the aorta, a

subset of ECs was found to express the gene signature typical of microvascular ECs, including CD36, LPL, and GPIHBP1. These ECs show a distinctive distribution, being more abundant in the greater curvature area of the aortic arch, a non-atherosclerosis prone region [39] and have higher expression of SR-B [40]. These findings suggest that although lipolysis of triglyceride-rich lipoproteins is thought to only occur in capillaries, some lipolysis and fatty acid uptake likely occur in the greater curvature areas of the aorta, while more LDL endocytosis occurs in other areas [41].

Apolipoprotein C3 (APOC3), produced by the liver and to a small extent by the intestine, inhibits LPL and is a key regulator of plasma triglyceride metabolism. APOC3 associates with ApoB-containing lipoproteins. APOC3 inhibits LPL activity and also hepatic uptake of remnants of triglyceride-rich lipoproteins. Its overexpression increases triglyceride levels [42].

APOC2 (apolipoprotein C2) is required for LPL activation. APOC2 mimetic peptides directly activate LPL and inhibit APOC3. Loss-of-function mutations in APOC2 in humans increase plasma triglyceride levels and can provoke acute pancreatitis [43].

Capillary ECs from tissues which most actively uptake circulating FAs have high levels of CD36 expression. CD36 facilitates FA transfer and that might be regulated by interaction with membrane integrins or integrin ligands [44].

CD36 facilitated FA transport and keeps Src phosphorylation of the insulin receptor to maintain its activity. Since CD36 regulation of insulin receptor is inhibited by saturated FAs, this may account for FA-induced muscle insulin resistance. Overall, these findings indicate that EC CD36 acts as a regulator of tissue FA delivery and its metabolic effects integrate both its transport and signaling functions [45]. CD 36 at the surface membrane of EC stimulates uptake of long-chain fatty acids influenced by peroxisome proliferator activated receptor γ (PPAR γ), PPAR γ coactivator1 alpha (PGC1α), Angiopoietin-2 , FA transporter protein , and vascular endothelia growth factor [41] . EC CD36 deficiency in ECs downregulates expression of PPAR target genes. This results in impaired LPL activity [46].

6. LDL Receptors (LDLR) and VLDL Receptors (VLDLR)

Capillary EC uptake of circulating lipids may occur via caveolae transcytosism containing LDL to cross the endothelial barrier [47]. LDLR is clustered into coated pits. The initiation, growth, and maturation of coated pits and vesicles is a tightly regulated process dependent on the plasma membrane content of phosphatidylinositol-4,5-bisphosphate $(PI4,5)P_2$ [48]. Several factors may influence plasma membrane $PI4,5P_2$ level including phosphatidylinositol transfer protein, membrane clatherine vesicle, phospholipase- C linked to plasma membrane, oxysterol-binding protein, endoplasmic reticulum as well as Golgi apparatus. These factors control the activity of LDLR function in uptake of extracellular LDL [49]. LDLR recycling is activated once the ligand and receptor dissociate. It undergoes conformational changes to protect it against degradation and allowing recycling.

LDLR mainly recognizes apolipoprotein (Apo) B-100, whereas VLDLR specifically recognizes ApoE that is a component of rich-triglyceride lipoproteins, viz. chylomicrons, VLDL, and intermediate-density lipoprotein (IDL).

VLDLR has also been reported to promote lipid uptake by increasing TG hydrolysis by lipoprotein lipase and receptor-mediated endocytosis [50].

Like LDL, VLDL endocytosis is mediated by caveolae formation [51]. VLDLR expression is induced by PPARγ [52].

The highest levels of the VLDLR occur in muscle, heart, adipose tissue, and brain, all of which utilize lipoprotein-derived free fatty acids as an energy fuel. VLDLR is not ordinarily expressed in hepatocytes. However, VLDLR is highly expressed in the capillary endothelium. It plays an important role in the delivery of TGs to adipocytes, myocytes, or other cells in peripheral tissues [53]. It is also expressed in EC of small arterioles, and coronary arteries [54].

There is abundant evidence for the atherogenic properties of TG-rich lipoproteins [55]. VLDLR expression is insulin dependent [56]. Unlike LDLR, VLDLR expression is not regulated by cellular cholesterol content [57]. While apoE is a ligand for both VLDLR and LDLR binding of lipoproteins, VLDLR differs from LDLR in that it does not bind apoB as a ligand [58].

7. Protein Tyrosine Nitration

Protein tyrosine nitration in biological systems is linked with free radical reactions, implying the intermediacy of •Tyr and subsequent reactions with either nitric oxide (•NO) or nitrogen dioxide ($^{\bullet}NO_2$) radicals. The nitration of protein tyrosine residues to 3-nitrotyrosine disturbs nitric oxide (•NO) signaling and metabolism towards pro-oxidant processes, which is defined as “nitroxidative stress”. Protein 3-nitrotyrosine has been established as a biomarker of “nitro-oxidative stress”[59][60].

Tyrosine nitration is an oxidative process that may occur inside the plasma membrane of cells during the lipid peroxidation reactions. Lipid peroxyl radicals (LOO•) can oxidize tyrosine to •Tyr. The lipid alkoxyl radical (LO•), and lipid peroxy radicle (LOO•) formed during lipid peroxidation may also participate in tyrosine oxidation [61].

Many biochemical consequences of protein tyrosine nitration develop changes in activity, whether acquiring of immunogenic responses, interference in tyrosine kinase-dependent pathways, alteration of protein assembly and configuration, facilitation of protein degradation, and/or participation in the creation of proteasome-resistant protein aggregates [62]. Nitration of protein and peptide tyrosyl residues was associated with a decrease in their ability to be degraded in proteasomes [63]. 3-Nitrotyrosine (3-NT-Tyr) is encountered as a worse cardiovascular risk factor; it is greater in vascular beds with more advanced atherosclerotic processes [64].

The overproduction of 3-NT-Tyr induced by a high glucose level was shown to be associated with the downregulation of peroxisome proliferator-activated receptor β (PPARβ), whose activity to protect against endothelial dysfunction [65].

The blood levels of 3-NT-Tyr are significantly higher in subjects with metabolic derangement states as metabolic syndrome. Lifestyle modifications (erobic exercise and the Mediterranean diet) lead to significant decreases in 3-NT-Tyr levels. Increased 3-NT-Tyr level is encountered with insulin resistance (IR) states [64] and chronic kidney disease [66]. 3-Nitrotyrosine generally tends to increase with the presence of cardiovascular risk factors such as age, obesity, smoking, consumption of highly processed foods, as well as metabolic syndrome [67][68].

Nitrated lipoproteins are characterized by the presence of 3-NT-Tyr in the polypeptide chains of apolipoprotein A-I (apoA-I) and apolipoprotein B (apoB), resulting in the formation of nitrated apoA-I (NT-apoA-I) and nitrated apoB (NT-apoB), respectively [64]. Oxidation/nitration of the apolipoproteinB-100 (apoB-100) and/or the lipid components of the LDL confers the particle its pro-atherogenic features. In addition to promoting foam cell formation, oxLDL alters the endothelial production and bioavailability of •NO, stimulates endothelial cells apoptosis and has direct chemotactic effect on monocytes. Moreover, it induces vascular cell expression and production of growth and adhesion factors; overall promoting the formation of an inflammatory focus in the arterial intima [69].

The highest nitration level in the apoA-I in HDL particles within atherosclerotic lesions is over 100-fold more than in normal coronary arteries [70]. The nitration of HDL molecules is associated with a decreased activity of paraoxonase-1 and caspase-3 [71] and also influences the transport of cholesterol via ATP-binding cassette transporterA1 (ABCA1); it inhibits cholesterol reverse-transport. Therefore, there is a reduction in the antioxidant and antiapoptotic properties of HDL particles and weaker antiatherogenic properties than native HDL, in addition to impaired reverse transport of cholesterol [72].

8. Nitric Oxide inhibition of LDL oxidation does not protect against atherogenesis

- NO-derived metabolites may exert oxidative modifications in LDL through peroxynirite, nitriogen dioxide ($^{\bullet}NO_2$) and/or the NO_2^-- [73],yet •NO itself inhibits lipid oxidation-dependent processes. It is highly reactive with lipid-derived radicals such as alkoxyl ($LO^{\bullet}$) or peroxyl ($LOO^{\bullet}$), yielding a variety of nitrogenated non-radical products. •NO acts as an effective chain-breaking by terminating lipid radical-mediated chain propagation reactions.

So, it protects membrane lipids and lipoproteins from oxidative modifications and redirecting the cytotoxic reactions mediated by $O_2^{\bullet-}$and peroxynitrite towards other oxidative pathways [74].

Peroxynitrite-reacted LDL results in extensive tyrosine nitration and accumulation of lipid peroxides, which alter configuration of apoB-100 folding and prevent the normal binding of LDL to its receptor. This accounts for prolonged longevity of LDL, promoting atherogenecity.

9. Potential Mechanisms of Action of NO_2-Fas in Pathogenesis of Atherosclerosis

Radical species •NO in a biological medium encounters $O_2^{\bullet-}$ to form peroxinitrite ($ONOO^-$). Upon $ONOO^-$ protonation the resulting peroxynitrous acid either directly reacts and oxidizes biomolecules (particularly cysteine

and selenocysteine) or decomposes via homolytic fission into the oxidizing hydroxyl radicals (•OH) and nitrating nitrogen dioxide (•NO_2) radicals [74]. Peroxynitrite is unique as a lipid oxidant, because it mediates peroxidation of unsaturated fatty acids in the absence of transition metal catalysts as iron [74].

The nitration of unsaturated fatty acids by the radical nitrogen dioxide (•NO_2) generates bioactive lipids adducts with amino acids, predominantly cysteine. The generated nitrated factor(s) is a component of some transcriptional regulatory proteins and enzymes involved in metabolism, cell signaling and/or redox homeostasis. All have its impact on their biological functions [75].

Nitrated fatty acids (NO_2-Fas) reduce foam cell formation by inhibiting nuclear factor kappa B (NF-κB), toll-like receptor 4 (TLR4), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and inhibiting STAT-1 phosphorylation. This results in inhibition of vascular smooth muscles (VSCMs) proliferation and reduces atherogenesis [76]. Nitrated oleic acid (NO_2-OA) and nitrated linoleic acid (NO_2-LA) can covalently bind to the p65 subunit of NF-κB, to repress NF-κB-dependent gene expression; inhibiting the secretion of the pro-inflammatory cytokines interleukin (IL)-6, tumor necrosis factor α (TNFα), MCP-1, and vascular cell VCAM-1 in macrophages. So, it inhibits the adherence of monocytes to endothelial cells [77][78].

Nitro-oleic acid (NO2-OA) preserves endothelial *NO production and function via enhanced endothelial nitric oxide synthase (eNOS) and heme oxygenase [79]. On the other hand, NO_2-LA is a potent inducer of *heme oxygenase 1 (HO-1)* gene expression, a central defensive enzyme in tissue anti-inflammatory responses to vascular injury [80]. This contributes for the inhibition of atherosgenesis. Conjugated linoleic acid (cLA) represents the preferential substrate for fatty acid nitration. cLA is more susceptible to nitration [81]. NO_2-cLA modulated hypoxia responses by increasing the expression of angiopoietin-like 4 (ANGPTL4) in endothelial cells [82].

NO_2-FAs induced PPAR-γ-dependent macrophage CD36 expression. NO_2-OA exerted an anti-atherosclerotic effect by reducing the TG content of macrophages [83]. PPARγ expression is up-regulated in intimal vascular smooth muscle cells (VSMC) and macrophages in early human atheromas despite a strong correlation with its insulin sensitizing action, the vascular protection observed with PPARγ and its ligands is independent from improvements in metabolic control [84]. PPARγ activation in vascular cells inhibits the production of cytokines such as TNFα, and monocyte chemoattractant protein-1 [85]. NO_2-OA and NO_2-LA suppress TNFα-stimulated release of inflammatory cytokines, such as IL-6, IL-8, and IL-12, from endothelial cells, and blocked TNFα-induced expression of intercellular cell adhesion molecule-1 (ICAM-1) [86].

Therefore, NO_2-FAs participate by multiple signaling events to promote the overall atherosclerosis protection.

10. Conclusion

Lipoproteins have to pass through the vascular endothelial barrier to get contact with the sub-intimal space. They, then have to interact with the cell membranes. This environment contains mediators as well as ROS and RNS. Changes in epitopes may alter protein antigenicity, configuration, and/or degradation. Fatty acids may undergo

nitration besides oxidation and fragmentation. Fragmented peroxylipids may adduct to the proteins forming pathogenic products. Phospholipid bilayer undergoes oxidation and appears to participate in the pathological process. Niro-fatty acids may have protective role against atherosclerosis in experimental studies. Multiplicity of factors interact with the components of the plasma membranes, may alter their functions in a manner pathogenic to atherosclerosis. More investigations are still necessitated to clarify the role of oxidative nitrogenous products under circumstances of oxidative nitration.

References

1. Ayala, A., Muñoz, M.F. and Argüelles, S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative medicine and cellular longevity. 2014 (1), p.360438.
2. Wang, H.D., Hope, S., Du, Y., Quinn, M.T., Cayatte, A., Pagano, P.J. and Cohen, R.A. Paracrine role of adventitial superoxide anion in mediating spontaneous tone of the isolated rat aorta in angiotensin II-induced hypertension. Hypertension. 1999.33(5).1225-1232.
3. O'Donnell, V.B. and Freeman, B.A.,. Interactions between nitric oxide and lipid oxidation pathways: implications for vascular disease. Circulation research 2000.88(1).12-21.
4. Ischiropoulos, H.,. Protein tyrosine nitration. Redox Biochemistry and Chemistry, 2024. p.100030.
5. Moore, K.P., Darley-Usmar, V., Morrow, J. and Roberts, L.J., Formation of F2-isoprostanes during oxidation of human low-density lipoprotein and plasma by peroxynitrite. Circulation research 1995. 77(2). 335-341.
6. Patel, R.P., Diczfalusy, U., Dzeletovic, S., Wilson, M.T. and Darley-Usmar, V.M., Formation of oxysterols during oxidation of low density lipoprotein by peroxynitrite, myoglobin, and copper. Journal of lipid research 1996. 37(11), 2361-2371.
7. Winterbourn, C.C., Toxicity of iron and hydrogen peroxide: the Fenton reaction. Toxicology letters 1995.82.969-974.
8. Chan, H.S., Coxon, D.T., Peers, K.E. and Price, K.R., Oxidative reactions of unsaturated lipids. Food Chemistry 1982. 9(1-2). 21-34.
9. Kim, Y.W., Yakubenko, V.P., West, X.Z., Gugiu, G.B., Renganathan, K., Biswas, S., Gao, D., Crabb, J.W., Salomon, R.G., Podrez, E.A. and Byzova, T.V., Receptor-mediated mechanism controlling tissue levels of bioactive lipid oxidation products. Circulation research 2015. 117(4).321-332.
10. West, X.Z., Malinin, N.L., Merkulova, A.A., Tischenko, M., Kerr, B.A., Borden, E.C., Podrez, E.A., Salomon, R.G. and Byzova, T.V., Oxidative stress induces angiogenesis by activating TLR2 with

novel endogenous ligands. Nature 2010. 467(7318) .972-976.

11. Cruz-Guilloty, F., Saeed, A.M., Duffort, S., Cano, M., Ebrahimi, K.B., Ballmick, A., Tan, Y., Wang, H., Laird, J.M., Salomon, R.G. and Handa, J.T., T cells and macrophages responding to oxidative damage cooperate in pathogenesis of a mouse model of age-related macular degeneration. PloS one 2014.9(2), p.e88201.
12. Chang, M.K., Binder, C.J., Miller, Y.I., Subbanagounder, G., Silverman, G.J., Berliner, J.A. and Witztum, J.L., Apoptotic cells with oxidation-specific epitopes are immunogenic and proinflammatory. The Journal of experimental medicine 2004 200(11).1359-1370.
13. Levine, J.S., Koh, J.S., Subang, R. and Rauch, J., Apoptotic cells as immunogen and antigen in the antiphospholipid syndrome. Experimental and molecular pathology 1999. 66(1),.82-98.
14. Chang, M.K., Binder, C.J., Miller, Y.I., Subbanagounder, G., Silverman, G.J., Berliner, J.A. and Witztum, J.L., Apoptotic cells with oxidation-specific epitopes are immunogenic and proinflammatory. The Journal of experimental medicine 2004. 200(11),359-1370.
15. Steinberg, D. Low density lipoprotein oxidation and its pathobiological significance. Journal of Biological Chemistry 1997. 272(34),.20963-20966.
16. Parthasarathy, S., Raghavamenon, A., Garelnabi, M.O. and Santanam, N., Oxidized low-density lipoprotein. Free Radicals and Antioxidant Protocols 2010. .403-417.
17. Takahashi S, Sakai J, Fujino T, Hattori H, Zenimaru Y, Suzuki J, Miyamori I, Yamamoto TT. The very low-density lipoprotein (VLDL) receptor: characterization and functions as a peripheral lipoprotein receptor. J Atheroscler Thromb. 2004,11(4):200-8. doi: 10.5551/jat.11.200.
18. Levitan, I., Volkov, S. and Subbaiah, P.V. Oxidized LDL: diversity, patterns of recognition, and pathophysiology. Antioxidants & redox signaling 2010. 13(1).39-75.
19. Prolo, C., Álvarez, M.N., Ríos, N., Peluffo, G., Radi, R. and Romero, N. Nitric oxide diffusion to red blood cells limits extracellular, but not intraphagosomal, peroxynitrite formation by macrophages. Free Radical Biology and Medicine 2015. 87.346-355.
20. Kotamraju, S., Hogg, N., Joseph, J., Keefer, L.K. and Kalyanaraman, B. Inhibition of oxidized low-density lipoprotein-induced apoptosis in endothelial cells by nitric oxide: peroxyl radical scavenging as an antiapoptotic mechanism. Journal of Biological Chemistry 2001. 276(20).17316-17323.
21. Chang, M.K., Binder, C.J., Miller, Y.I., Subbanagounder, G., Silverman, G.J., Berliner, J.A. and Witztum, J.L. Apoptotic cells with oxidation-specific epitopes are immunogenic and proinflammatory. The Journal of experimental medicine 2004. 200(11).1359-1370.
22. Zingg, J.M., Ricciarelli, R. and Azzi, A. Scavenger receptors and modified lipoproteins: fatal attractions?. IUBMB life 2000. 49(5).397-403.

23. Ahotupa, M. Lipid Oxidation Products and the Risk of Cardiovascular Diseases: Role of Lipoprotein Transport. Antioxidants 2024. 13(5).512.
24. Choi, S.H., Harkewicz, R., Lee, J.H., Boullier, A., Almazan, F., Li, A.C., Witztum, J.L., Bae, Y.S. and Miller, Y.I. Lipoprotein accumulation in macrophages via toll-like receptor-4–dependent fluid phase uptake. Circulation research 2009. 104(12).1355-1363.
25. Boullier, A., Friedman, P., Harkewicz, R., Hartvigsen, K., Green, S.R., Almazan, F., Dennis, E.A., Steinberg, D., Witztum, J.L. and Quehenberger, O. Phosphocholine as a pattern recognition ligand for CD361. Journal of lipid research 2005. 46(5).969-976.
26. Di Pietro, N., Formoso, G. and Pandolfi, A. Physiology and pathophysiology of oxLDL uptake by vascular wall cells in atherosclerosis. Vascular pharmacology 2016. 84.1-7.
27. Afonso, C.B. and Spickett, C.M. Lipoproteins as targets and markers of lipoxidation. Redox Biology 2019. 2.101066.
28. JL, S.Q. Electronegative low-density lipoprotein. Curr Opin Lipidol, 2004. 15.329-335.
29. Benítez, S., Camacho, M., Bancells, C., Vila, L., Sánchez-Quesada, J.L. and Ordóñez-Llanos, J. Wide proinflammatory effect of electronegative low-density lipoprotein on human endothelial cells assayed by a protein array. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids 2006. 1761(9).1014-1021.
30. Koschinsky, M.L. and Boffa, M.B. Oxidized phospholipid modification of lipoprotein (a): epidemiology, biochemistry and pathophysiology. Atherosclerosis 2022. 349.92-100.
31. Gilliland, T.C., Liu, Y., Mohebi, R., Miksenas, H., Haidermota, S., Wong, M., Hu, X., Cristino, J.R., Browne, A., Plutzky, J. and Tsimikas, S., Lipoprotein (a), oxidized phospholipids, and coronary artery disease severity and outcomes. Journal of the American College of Cardiology 2023. 81(18).1780-1792.
32. Beigneux, A.P., Davies, B.S., Gin, P., Weinstein, M.M., Farber, E., Qiao, X., Peale, F., Bunting, S., Walzem, R.L., Wong, J.S. and Blaner, W.S. Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 plays a critical role in the lipolytic processing of chylomicrons. Cell metabolism 2007. 5(4).279-291.
33. Goldberg, I.J., Eckel, R.H. and Abumrad, N.A. Regulation of fatty acid uptake into tissues: lipoprotein lipase-and CD36-mediated pathways. Journal of lipid research 2009..S86-S90.
34. Hajri, T., Han, X.X., Bonen, A. and Abumrad, N.A. Defective fatty acid uptake modulates insulin responsiveness and metabolic responses to diet in CD36-null mice. The Journal of clinical investigation 2002. 109(10).1381-1389.
35. Love-Gregory, L., Sherva, R., Sun, L., Wasson, J., Schappe, T., Doria, A., Rao, D.C., Hunt, S.C., Klein, S., Neuman, R.J. and Permutt, M.A. Variants in the CD36 gene associate with the

metabolic syndrome and high-density lipoprotein cholesterol. Human molecular genetics 2008. 17(11).1695-1704.

36. Bharadwaj, K.G., Hiyama, Y., Hu, Y., Huggins, L.A., Ramakrishnan, R., Abumrad, N.A., Shulman, G.I., Blaner, W.S. and Goldberg, I.J. Chylomicron-and VLDL-derived lipids enter the heart through different pathways: in vivo evidence for receptor-and non-receptor-mediated fatty acid uptake. Journal of Biological Chemistry 2010. 285(49).37976-37986.
37. Kanda, T., Brown, J.D., Orasanu, G., Vogel, S., Gonzalez, F.J., Sartoretto, J., Michel, T. and Plutzky, J. PPARγ in the endothelium regulates metabolic responses to high-fat diet in mice. The Journal of clinical investigation 2008. 119(1) 119(1):110-24. doi: 10.1172/JCI36233.
38. Kleinfeld, A.M. Lipid phase fatty acid flip-flop, is it fast enough for cellular transport?. The Journal of membrane biology 2000. 175.79-86.
39. Wang, Y., McNutt, M.C., Banfi, S., Levin, M.G., Holland, W.L., Gusarova, V., Gromada, J., Cohen, J.C. and Hobbs, H.H. Hepatic ANGPTL3 regulates adipose tissue energy homeostasis. Proceedings of the National Academy of Sciences 2015. 112(37).11630-11635.
40. Abumrad, N.A., Cabodevilla, A.G., Samovski, D., Pietka, T., Basu, D. and Goldberg, I.J. Endothelial cell receptors in tissue lipid uptake and metabolism. Circulation research 2021. 128(3).433-450.
41. Jong, M.C., Rensen, P.C., Dahlmans, V.E., van der Boom, H., van Berkel, T.J. and Havekes, L.M. Apolipoprotein C-III deficiency accelerates triglyceride hydrolysis by lipoprotein lipase in wild-type and apoE knockout mice. Journal of lipid research 2001. 42(10).1578-1585.
42. Ueda, M., Dunbar, R.L., Wolska, A., Sikora, T.U., Escobar, M.D.R., Seliktar, N., deGoma, E., DerOhannessian, S., Morrell, L., McIntyre, A.D. and Burke, F. A novel APOC2 missense mutation causing apolipoprotein C-II deficiency with severe triglyceridemia and pancreatitis. The Journal of Clinical Endocrinology & Metabolism 2017.102(5).1454-1457.
43. Nolan, D.J., Ginsberg, M., Israely, E., Palikuqi, B., Poulos, M.G., James, D., Ding, B.S., Schachterle, W., Liu, Y., Rosenwaks, Z. and Butler, J.M. Molecular signatures of tissue-specific microvascular endothelial cell heterogeneity in organ maintenance and regeneration. Developmental cell 2013. 26(2).204-219.
44. Samovski, D., Dhule, P., Pietka, T., Jacome-Sosa, M., Penrose, E., Son, N.H., Flynn, C.R., Shoghi, K.I., Hyrc, K.L., Goldberg, I.J. and Gamazon, E.R. Regulation of insulin receptor pathway and glucose metabolism by CD36 signaling. Diabetes 2018. 67(7).1272-1284.
45. Bergman, M., Manco, M., Sesti, G., Dankner, R., Pareek, M., Jagannathan, R., Chetrit, A., Abdul-Ghani, M., Buysschaert, M., Olsen, M.H. and Nilsson, P.M. Petition to replace current OGTT criteria for diagnosing prediabetes with the 1-hour post-load plasma glucose≥ 155 mg/dl (8.6 mmol/L). Diabetes research and clinical practice 2018. 146.18-33.

46. Frank, P.G., Pavlides, S. and Lisanti, M.P. Caveolae and transcytosis in endothelial cells: role in atherosclerosis. Cell and tissue research 2009. 335(1).41-47.
47. Antonescu, C.N., Aguet, F., Danuser, G. and Schmid, S.L. Phosphatidylinositol-(4, 5)-bisphosphate regulates clathrin-coated pit initiation, stabilization, and size. Molecular biology of the cell 2011. 22(14).2588-2600.
48. Islam, M.M., Hlushchenko, I. and Pfisterer, S.G. Low-density lipoprotein internalization, degradation and receptor recycling along membrane contact sites. Frontiers in Cell and Developmental Biology 2022. 10 .826379.
49. Oshio, Y., Hattori, Y., Kamata, H., Ozaki-Masuzawa, Y., Seki, A., Tsuruta, Y. and Takenaka, A. Very low-density lipoprotein receptor increases in a liver-specific manner due to protein deficiency but does not affect fatty liver in mice. Scientific reports 2021. 11(1). 8003.
50. Deng, L., Vrieling, F., Stienstra, R., Hooiveld, G.J., Feitsma, A.L. and Kersten, S. Macrophages take up VLDL-sized emulsion particles through caveolae-mediated endocytosis and excrete part of the internalized triglycerides as fatty acids. PLoS Biology 2022. 20(8).e3001516.
51. Tao, H. and Hajri, T. Very low density lipoprotein receptor promotes adipocyte differentiation and mediates the proadipogenic effect of peroxisome proliferator-activated receptor gamma agonists. Biochemical pharmacology 2011. 82(12) .1950-1962.
52. Tiebel, O., Oka, K., Robinson, K., Sullivan, M., Martinez, J., Nakamuta, M., Ishimura-Oka, K. and Chan, L. Mouse very low-density lipoprotein receptor (VLDLR): gene structure, tissue-specific expression and dietary and developmental regulation. Atherosclerosis 1999. 145(2).239-251.]
53. Wyne, K.L., Pathak, R.K., Seabra, M.C. and Hobbs, H.H. Expression of the VLDL receptor in endothelial cells. Arteriosclerosis, thrombosis, and vascular biology 1996. 16(3).407-415.
54. Ginsberg, H.N., Packard, C.J., Chapman, M.J., Borén, J., Aguilar-Salinas, C.A., Averna, M., Ference, B.A., Gaudet, D., Hegele, R.A., Kersten, S. and Lewis, G.F. Triglyceride-rich lipoproteins and their remnants: metabolic insights, role in atherosclerotic cardiovascular disease, and emerging therapeutic strategies—a consensus statement from the European Atherosclerosis Society. European heart journal 2021. 42(47).4791-4806.
55. Iwasaki, T., Takahashi, S., Takahashi, M., Zenimaru, Y., Kujiraoka, T., Ishihara, M., Nagano, M., Suzuki, J., Miyamori, I., Naiki, H. and Sakai, J. Deficiency of the very low-density lipoprotein (VLDL) receptors in streptozotocin-induced diabetic rats: insulin dependency of the VLDL receptor. Endocrinology 2005. 146(8).3286-3294.
56. Krauss, R.M., Lu, J.T., Higgins, J.J., Clary, C.M. and Tabibiazar, R. VLDL receptor gene therapy for reducing atherogenic lipoproteins. Molecular Metabolism 2023. 69. 101685.
57. Ali, O. and Szabó, A. Review of eukaryote cellular membrane lipid Composition, with special attention to the fatty acids. International Journal of Molecular Sciences 2023. 24(21).15693.

58. Radi, R. Protein tyrosine nitration: biochemical mechanisms and structural basis of functional effects. Accounts of chemical research 2013. 46(2).550-559.
59. Bartesaghi, S., Wenzel, J., Trujillo, M., López, M., Joseph, J., Kalyanaraman, B. and Radi, R. Lipid peroxyl radicals mediate tyrosine dimerization and nitration in membranes. Chemical research in toxicology 2010. 23(4).821-835.
60. Petruk, A.A., Bartesaghi, S., Trujillo, M., Estrin, D.A., Murgida, D., Kalyanaraman, B., Marti, M.A. and Radi, R. Molecular basis of intramolecular electron transfer in proteins during radical-mediated oxidations: Computer simulation studies in model tyrosine–cysteine peptides in solution. Archives of biochemistry and biophysics 2012. 525(1).82-91.
61. Vernon, G., Baranova, A. and Younossi, Z.M. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Alimentary pharmacology & therapeutics 2011. 34(3).274-285.
62. Koutoulogenis, G.S. and Kokotos, G. Nitro fatty acids (NO2-FAs): An emerging class of bioactive fatty acids. Molecules 2021. 26(24).7536.
63. Khoo, N.K., Fazzari, M., Chartoumpekis, D.V., Li, L., Guimaraes, D.A., Arteel, G.E., Shiva, S. and Freeman, B.A. Electrophilic nitro-oleic acid reverses obesity-induced hepatic steatosis. Redox Biology 2019. 22. 101132.
64. Piroddi, M., Palmese, A., Pilolli, F., Amoresano, A., Pucci, P., Ronco, C. and Galli, F. Plasma nitroproteome of kidney disease patients. Amino acids 2011. 40 .653-667.
65. Zhao, Y., Chang, Z., Zhao, G., Lu, H., Xiong, W., Liang, W., Wang, H., Villacorta, L., Garcia-Barrio, M.T., Zhu, T. and Guo, Y. Suppression of vascular macrophage activation by nitro-oleic acid and its implication for abdominal aortic aneurysm therapy. Cardiovascular drugs and therapy 2021. 35.939-951.
66. Mastrogiovanni, M., Trostchansky, A. and Rubbo, H. Fatty acid nitration in human low-density lipoprotein. Archives of biochemistry and biophysics 2020.
67. Kotamraju, S., Hogg, N., Joseph, J., Keefer, L.K. and Kalyanaraman, B. Inhibition of oxidized low-density lipoprotein-induced apoptosis in endothelial cells by nitric oxide: peroxyl radical scavenging as an antiapoptotic mechanism. Journal of Biological Chemistry 2001. 276.17316-17323.
68. Fioravanti, S., Pellacani, L., Tardella, P.A. and Vergari, M.C. Facile and highly stereoselective one-pot synthesis of either (E)-or (Z)-nitro alkenes. Organic Letters 2008. 10(7).1449-1451.
69. Woodcock, S.R., Bonacci, G., Gelhaus, S.L. and Schopfer, F.J. Nitrated fatty acids: synthesis and measurement. Free Radical Biology and Medicine 2013. 59 .14-26.

70. Zanoni, G., Valli, M., Bendjeddou, L., Porta, A., Bruno, P. and Vidari, G. Improved synthesis of (E)-12-nitrooctadec-12-enoic acid, a potent PPARγ activator. Development of a “Buffer-free” enzymatic method for hydrolysis of methyl esters. The Journal of Organic Chemistry 2010. 75(23).8311-8314.
71. Hsiai, T.K., Hwang, J., Barr, M.L., Correa, A., Hamilton, R., Alavi, M., Rouhanizadeh, M., Cadenas, E. and Hazen, S.L. Hemodynamics influences vascular peroxynitrite formation: Implication for low-density lipoprotein apo-B-100 nitration. Free Radical Biology and Medicine 2007. 42(4).519-529.
72. Trostchansky, A. and Rubbo, H. Lipid nitration and formation of lipid-protein adducts: biological insights. Amino Acids,2007 32.517-522.
73. Radi, R.,. Oxygen radicals, nitric oxide, and peroxynitrite: Redox pathways in molecular medicine. Proceedings of the National Academy of Sciences 2018. 115(23).5839-5848.
74. Radi, R., Beckman, J.S., Bush, K.M. and Freeman, B.A. Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. Archives of biochemistry and biophysics 1991. 288(2).481-487.
75. Delmastro-Greenwood, M., Freeman, B.A. and Wendell, S.G. Redox-dependent anti-inflammatory signaling actions of unsaturated fatty acids. Annual review of physiology 2014. 76(1).79-105.
76. Ni, H., Tan, X., Du, J. and Wang, Y. Nitro-fatty acids: mechanisms of action, roles in metabolic diseases, and therapeutics. Current Medicine 2024. 3(1). 3.
77. Cui, T., Schopfer, F.J., Zhang, J., Chen, K., Ichikawa, T., Paul, R.B., Batthyany, C., Chacko, B.K., Feng, X., Patel, R.P. and Agarwal, A. Nitrated fatty acids: endogenous anti-inflammatory signaling mediators. Journal of Biological Chemistry 2006. 281(47).35686-35698.
78. Hwang, J., Lee, K.E., Lim, J.Y. and Park, S.I. Nitrated fatty acids prevent TNFα-stimulated inflammatory and atherogenic responses in endothelial cells. Biochemical and biophysical research communications 2009.387(4).633-640.
79. Khoo, N.K., Rudolph, V., Cole, M.P., Golin-Bisello, F., Schopfer, F.J., Woodcock, S.R., Batthyany, C. and Freeman, B.A. Activation of vascular endothelial nitric oxide synthase and heme oxygenase-1 expression by electrophilic nitro-fatty acids. Free Radical Biology and Medicine 2010. 48(2).230-239.
80. Zhang, Q., Liu, J., Duan, H., Li, R., Peng, W. and Wu, C. Activation of Nrf2/HO-1 signaling: An important molecular mechanism of herbal medicine in the treatment of atherosclerosis via the protection of vascular endothelial cells from oxidative stress. Journal of advanced research 2021. 3.43-63.
81. Bonacci, G., Baker, P.R., Salvatore, S.R., Shores, D., Khoo, N.K., Koenitzer, J.R., Vitturi, D.A., Woodcock, S.R., Golin-Bisello, F., Cole, M.P. and Watkins, S. Conjugated linoleic acid is a

preferential substrate for fatty acid nitration. Journal of Biological Chemistry 2012. 287(53).44071-44082.

82. Lu, H., Sun, J., Liang, W., Zhang, J., Rom, O., Garcia-Barrio, M.T., Li, S., Villacorta, L., Schopfer, F.J., Freeman, B.A. and Chen, Y.E. Novel gene regulatory networks identified in response to nitro-conjugated linoleic acid in human endothelial cells. Physiological genomics 2019. 51(6).224-233.
83. Rosenblat, M., Rom, O., Volkova, N. and Aviram, M. Nitro-oleic acid reduces J774A. 1 macrophage oxidative status and triglyceride mass: involvement of paraoxonase2 and triglyceride metabolizing enzymes. Lipids 2016. 51.941-953.
84. Villacorta, L., Schopfer, F.J., Zhang, J., Freeman, B.A. and Chen, Y.E. PPARγ and its ligands: therapeutic implications in cardiovascular disease. Clinical science 2009. 116(3).205-218.
85. Joner, M., Farb, A., Cheng, Q.I., Finn, A.V., Acampado, E., Burke, A.P., Skorija, K., Creighton, W., Kolodgie, F.D., Gold, H.K. and Virmani, R. Pioglitazone inhibits in-stent restenosis in atherosclerotic rabbits by targeting transforming growth factor-β and MCP-1. Arteriosclerosis, thrombosis, and vascular biology 2007. 27(1).182-189.
86. Hwang, J., Lee, K.E., Lim, J.Y. and Park, S.I.,. Nitrated fatty acids prevent TNFα-stimulated inflammatory and atherogenic responses in endothelial cells. Biochemical and biophysical research communications. 2009, 387(4).633-640.