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Effects of oxidized lipids and lipoproteins on cardiac function

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1. ABSTRACT

Oxidative modifications of lipids and lipoproteins have long been linked to the pathogenesis of cardiovascular diseases including atherosclerosis and coronary disease. Furthermore, overwhelming evidence indicate that oxidized lipids are also associated with myocardial dysfunction and cardiomyopathy. Oxidized lipid derivatives are generated by enzymatic and non-enzymatic reactions with unsaturated lipids in the cell and foods. In addition, blood LDL particles are prone to oxidation leading to the formation of oxidized LDL (oxLDL), which is often associated with obesity, diabetes and metabolic disease. Whether produced endogenously or delivered by the diet, oxidized lipid derivatives induce multiple metabolic and functional disturbances in the cell leading eventually to cell injury and death. As obesity is already associated with increased oxidative stress and excess lipid deposition in the heart, the cytotoxic effects of oxidized lipids in cardiomyocytes are more pronounced in obese subjects. The overall objective of this review is to provide a synthesis of recent findings about the effects oxidized lipids in the heart. First, the origin of oxidized lipids and lipoproteins is reported. Then, the effects of oxidized lipids in cardiomyocytes are reviewed and discussed. Finally, potential preventive interventions are highlighted and discussed.

2. INTRODUCTION

The prevalence of obesity has increased significantly during the last decades. This trend that has been reported in the United States of America and most countries of the world includes every age, sex, race and socioeconomic group (1) (2). The concern about the health risks associated with the rising obesity has become universal because obesity has been linked

to increased mortality resulting from acute and chronic co-morbidities including diabetes and cardiovascular diseases (3). Obesity is also associated with a state of oxidative stress and low grade chronic inflammation, conditions that are implicated in the development of multiple chronic diseases (4). Oxidative stress is initiated by excess production of reactive oxygen species (ROS) which are known to react with cellular lipids and generate highly reactive lipid oxidation derivatives. In addition, the concentration of oxidized low density lipoproteins (oxLDL) in blood and uptake by the cells are also increased by obesity and diabetes (5) (6), adding to risks of cardiovascular diseases (7) (8) (9) (10) (11).

Cardiomyopathy refers to a group of diseases of the heart muscle, or myocardium. The disease is characterized by weakens and/or dysfunction of heart muscle which is often reflected in cardiomyocyte contractile function. Obesity is among the factors that contribute to the development and progression of cardiomyopathy (12) (13) (14). The mechanisms for this causal relationship is that excess nutrients resulting from over-nutrition exceeds the storage capacity of adipose tissue leading to excess lipid deposition in cardiomyocytes, which in turn increases metabolic stress leading to cell dysfunction (13) (15). Interestingly, enlarged pericardial fat depot is also indicative of myocardial lipid content and increased risk of cardiomyopathy (12) (16) (17) (18), suggesting that excess fat deposition in and around the heart may contribute to the onset of this pathology through some signaling processes (19). The fact that circulating oxLDL level is increased by obesity and that oxLDL is detected in pericardial fat suggests that oxLDL is also another risk factor for the pathogenesis of cardiomyopathy (20) (21).

Oxidized lipids are derivatives of lipid oxidation initiated by enzymatic and non-enzymatic reactions with unsaturated lipids (22) (23) (24). The products of these reactions interact with cellular constituents and induce cell toxicity (25). Owing to the popularity of fried foods and the widespread of processed and fast-food industry, typical Western diet contains large quantities of oxidized fats (26) which could increase the level of oxidized lipoproteins in blood and therefore raises the risk of cardiovascular diseases. As obesity is associated with increased oxidative stress, the negative effects of dietary oxidized lipids are more pronounced in obese subjects. The toxicity of oxidized lipids and lipoproteins is demonstrated in a variety of cells and may compromise cell function and survival. An overwhelming number of studies demonstrate that the deposition of oxLDL in macrophages and endothelial cells is involved in the development and progression of vascular diseases (27) (28) (29). In addition, strong evidence indicate that oxidized lipids alter metabolic regulation and contractile function of cardiomyocytes leading to cardiac dysfunction and cardiomyopathy, independently of vascular alterations (11) (30) (31) (32). This review focuses primarily on the impact of oxidized lipids and lipoproteins on cardiomyocytes and cardiac dysfunction, and highlights the role of oxidized lipids in cardiomyopathy.

3. ORIGINS OF OXIDIZED LIPIDS

Oxidized lipids could be subdivided in two main groups according to their origins; oxysterols which are the oxygenated derivatives of cholesterol (23) and derivatives of unsaturated fatty acid oxidation which could be free or complexed with phospholipids and triglycerides (22) (33). In healthy conditions, the level of oxidized lipids in blood is low but could increase significantly with diseases or consumption of unhealthy diet. For instance, average blood oxysterol is about 1 mM in healthy subjects, but increases significantly with hyperlipidemia, obesity and diabetes, and could reach 20-30 mM in hypercholesterolemic subjects (23). Oxidized lipids could be generated endogenously in the cell and bloodstream, or ingested in the diet. The relevance of these sources is discussed below.

3.1. Dietary oxidized lipids

While dietary sources of oxysterols are limited to foods of animal origin since only these contain cholesterol in appreciable amounts, derivatives of oxidized unsaturated fatty acids are found in both animal- and plant-derived foods. Several products of cholesterol oxidation have been identified in food including 7beta-hydroperoxycholesterol, 7beta-hydroxycholesterol, 7-ketocholesterol, 25-hydroxycholesterol, cholesterol-5alpha,6alpha-epoxide (alpha-epoxide) and cholesterol-5beta,6beta-epoxide (beta-epoxide) (23) (34), but the total amount is variable and can reach up to 10% of total cholesterol (23) (35). In addition, a multitude of fatty acid oxidation derivatives has been reported in the diet, which complexity depends on the number of unsaturation in the carbon chain of the precursors (monoene, diene and triene fatty acids) and on the severity of oxidation (33). The biological activity and toxic effects vary among lipid oxidized species, depending on the location of oxygen substitution and subsequent chemical reactions. The most known sources of oxidized fatty acid derivatives are oil-fried foods. Polyunsaturated fatty acids (PUFAs) in the oil are labile and can undergo peroxidative damage when subjected to high temperatures in the presence of oxygen, resulting in the formation of lipid hydroperoxides. With further heating, hydroperoxides are oxidized to secondary oxidation products including hydroxides and aldehydes. Among the omega 6-PUFAs derivatives is 4-hydroxy-2-nonenal (4-HNE), a highly reactive and toxic derivative found in fried food that is also detected in oxLDL (36), which level could reach over 30 micro g/100 g in fast food fries could

(37). Substandard conditions of processing and/or preservations can also promote the oxidation of omega 3-PUFAs due to the large number of double bonds within fatty acid carbon chain. In these conditions, oxidative derivative 4-Hydroxy-2-hexenal (4-HHE) is detected in some fish oil supplements and in blood (38) (39) (40). In addition to being toxic, oxidation of fish oil supplements may interfere with its intended beneficial effects (41), such that a loss in omega-3 FA content to the detriment of peroxides diminishes the anti-inflammatory effect of these supplements.

Because of the popularity of processed and fried foods and the wide-spread of fast food industry, the consumption of oxidized fats in the diet has increased worldwide (42) (43) (44). Oxidation products of PUFAs are cytotoxic and have been implicated in the development of cardiovascular diseases and other chronic diseases as well (45) (46) (47). Previous studies indicate that dietary oxidized lipids are absorbed and incorporated into chylomicrons by the small intestine, and secreted into the lymph for delivery to the bloodstream (48) (49) (50). Although the mechanisms of absorption of oxidized lipids are not clear, some studies indicate that dietary oxidized lipids undergo further modifications in the gastrointestinal tract (51) (52) (53), and can react with proteins of the intestinal mucosa (40) which may interfere with the absorption process. Interestingly, oxidized lipids are more efficiently absorbed in diabetic subjects and animal models increasing the level of blood oxidized lipids and adding more risk to already high oxidative stress conditions (54) (55). Furthermore, dietary oxidized lipids taken by the liver are re-packaged in VLDL and secreted into the circulation, thereby increasing the oxidized lipids availability (56).

3.2. Oxidized lipoproteins

In blood, oxidized lipids have been detected in circulating lipoproteins, of which oxLDL is the mostly studied (7) (6) (10). Blood level of oxLDL is significantly increased in chronic metabolic diseases such as obesity (5) (6) (57) (58), diabetes (59) (60) and metabolic syndrome (61) (62) (63). In addition, oxidative modification of LDL is enhanced by hyperlipidemia, hyperglycemia and oxidative stress, conditions which are common in obese and diabetic subjects. Interestingly, the association of oxLDL with abdominal obesity, expressed as waist circumference, is stronger than the association with body mass index (BMI) expressed in kg/m^2 (7) (58) (64) (65) (66). While the production of oxLDL has been demonstrated experimentally *in vitro*, the mechanisms of formation of oxLDL *in vivo* is not fully elucidated. Some investigations speculate that lipids of LDL particles could be oxidized in blood in the vascular system when they are in contact with extracellular matrix where they are exposed to ROS and inflammatory mediators (67). Others propose that enzymes originating from neutrophils or macrophages, such as myeloperoxidase or lipoxygenases, are involved in LDL oxidation (7) (68). In addition, prenylcysteine oxidase 1, a pro-oxidation enzyme present in circulating lipoproteins, is also suspected to initiate LDL oxidation (69). In these conditions, cholesterol and PUFAs in LDL are susceptible to oxidation, leading to the formation of oxidized lipids, which in turn induce modifications of apolipoprotein B (apoB). Another possible route of apoB oxidation is through the interaction with dietary oxidized lipids incorporated in intestine-derived lipoproteins (70). It has been shown that aldehydes, the oxidative products of PUFAs, form covalent bonds with lysine amino groups of apoB100, a process that reduces the negative charge of LDL and increases its electrophoretic mobility (71). In addition to LDL, VLDL and HDL are also susceptible to oxidation and some studies detected significant amounts of oxidized VLDL (oxVLDL) and HDL (oxHDL) in blood (7) (56). Intravascular lipoprotein oxidation is also counter-regulated by the presence of anti-oxidation factors including anti-oxidation vitamins, such as vitamin E, and proteins such as paraoxonase and platelet-activating factor acetyl hydrolase present mainly in HDL (72). In general, oxidized lipoproteins, such as oxLDL and oxVLDL, comprise a multitude of oxidized lipids that includes oxysterols and derivatives unsaturated oxidized fatty acids which are demonstrated by several lipidomic studies (22) (73) (74) (75).

3.3. Intracellular oxidized lipids

In the cell, oxidized lipids can be formed by non-enzymatic pathways that involve interaction with ROS (24) (76) (77) or by enzymatic reactions catalyzed by cyclooxygenases, lipoxygenases and P450 monooxygenases (33). These reactions target PUFAs in phospholipids of the cell membrane as well as membranes of the cell organelles. The rate of production of oxidized lipids is further enhanced by the availability of free PUFA in the cell, and it has been demonstrated that phospholipase A2 (PLA2), an enzyme which releases fatty acids from phospholipids of the membrane, increases the formation oxidized lipids (22) (33). Among the products of enzymatic reactions of PUFAs are prostaglandins, leukotrienes, thromboxanes, and hydroxy- and epoxy-FAs. Some of these derivatives are required for normal physiology and function, but also could be damaging when present at abnormal levels (78). Interestingly, most of these derivatives are increased in the heart under ischemic condition (79), though it is not clear if they are active players in cardiac toxicity. The metabolism, and biological activity of these derivatives have been described in details elsewhere (78), and are not discussed in this review. The non-enzymatic peroxidation of PUFAs acts through oxygen radical-dependent reactions and generates more stables

and toxic derivatives including 4-HNE, 4-HHE, malonaldehyde (MDA), isoprostanes (IsoPs), epoxides and other end-products. Several studies indicate that products of lipid peroxidation are increased by obesity, diabetes and metabolic syndrome (80) (81) (82) (83), pathological conditions which are associated with concomitant increase of oxidative stress and ectopic lipid deposition in vital organs including the heart (83). In these conditions, the presence of excess lipids and free radicals jointly promotes lipid oxidation and enhances the contents of lipid oxidation products including hydroxides and isoprostanes in cardiomyocytes (83).

4. RECEPTORS OF OXIDIZED LIPOPROTEINS

Several membrane receptors are involved in the uptake of oxLDL among which Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) and cluster differentiating 36 (CD36) are the mostly studied.

4.1. Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1)

LOX-1 is a cell membrane receptor with an apparent molecular weight of 50 kDa discovered by Sawamura *et al.* (84). LOX-1 has the specificity to bind oxLDL (85) and oxidized phospholipids (oxPLs), but not native LDL (86). The role of LOX-1 as a receptor for oxLDL is first demonstrated in endothelial cells (84) and macrophages (87), and has been implicated in foam cell formation and atherosclerosis (88). Interestingly, LOX-1 is also expressed in cardiac cells including fibroblast (89) and cardiomyocytes (90), and there is a strong interest to examine the role of LOX-1 in these cells. In normal conditions, the expression of LOX-1 is low, but is upregulated in several pathological conditions, including obesity (91), hypertension (92), diabetes mellitus (93), oxidative stress (94) and hyperlipidemia (93). LOX-1 expression is also induced by several mediators including glucose (95), VLDL (93), inflammatory markers CRP (96) and angiotensin II (97). Since most of these pathological conditions (inflammation, hyperlipidemia, hypertension and diabetes) are directly or indirectly associated with obesity, their presence could have additive or synergistic effects on the regulation of LOX-1 expression. Recent investigations indicate that LOX-1 expression is also important in cardiomyocyte function (98) (99) (100). In this regard, LOX-1 deletion in mice reduces cardiac hypertrophy and remodeling induced by hypertension or myocardial ischemia, and prevents collagen deposition and fibrosis (98) (99). By contrast, activation of LOX-1 by oxLDL induces oxidative stress and instigates damages in isolated cardiomyocytes (100), effects that could implicated in cardiomyocyte dysfunction (101). Because LOX-1 is an important mediator of ROS generation it may induce inflammation and metabolic dysfunction under pathological conditions such as obesity and diabetes (102).

4.2. Cluster Differentiating 36 (CD36)

CD36 is a transmembrane glycoprotein from the class B scavenger receptor family (103). Several ligands bind to CD36 including long chain fatty acids (LCFAs) (103) (104), apoptotic cells, oxLDL and oxPLs (103) (105) (106). CD36 is identified in multiple cell types including cardiomyocytes, adipocytes and macrophages (83) (107). The expression of CD36 is markedly increased by obesity and diabetes, and is induced by FAs, glucose, inflammation mediators (83) (107) (108) and oxidized lipids (109). Binding of CD36 to ligands such as oxidized lipids activates several intracellular pathways related to lipid metabolism, inflammation and oxidative stress (83). Multiple studies have linked high expression of CD36 to cardiovascular diseases including cardiomyopathy and cardiac dysfunction (83) (110).

5. TOXIC EFFECTS OF OXIDIZED LIPIDS AND LIPOPROTEINS

Whether delivered in oxLDL or formed endogenously, oxidized lipids in the cell represent a large mixture of primary and secondary lipid derivatives including aldehydes, electrophilic lipids and other oxidized lipids (24) (111) (112) (113). The toxicity of oxidized lipids have been demonstrated in multiple cell types including macrophages and endothelial cells, which are known to be among the initiating factors of cardiovascular diseases. In addition to being involved in coronary vascular diseases, oxidized lipids are also linked to cardiomyocytes dysfunction. While the involvement of oxidized lipids in macrophages and endothelial cells have been discussed in several previous publications (102) (88) (114), this review summarizes existing knowledge regarding the effects of oxidized lipids in cardiomyocytes. Indeed, oxLDL and variety of oxidized lipid derivatives have been detected in the heart (115) (116) (117) and isolated cardiomyocytes (118) (74) (119) (120), and have been linked to cardiomyocyte hypertrophy and dysfunction (117) (118) (120). Furthermore, oxidized lipid derivatives, such as 4-HNE, 4-HHE, gamma-ketoaldehydes (gamma-KAs) and others, are capable of reacting with nucleophilic groups in proteins and generate adducts (121) (122). These adducts have been identified in cardiomyocytes (121) (122), and their levels are associated with the induction of inflammation, endoplasmic reticulum (ER) stress and apoptosis. Because oxidized lipids have higher polarity than their non-oxidized precursors they share the common property of moving faster between the cell compartments, and are able to interact with various components of the cell and affect multiple pathways. Taking this in consideration, the toxic

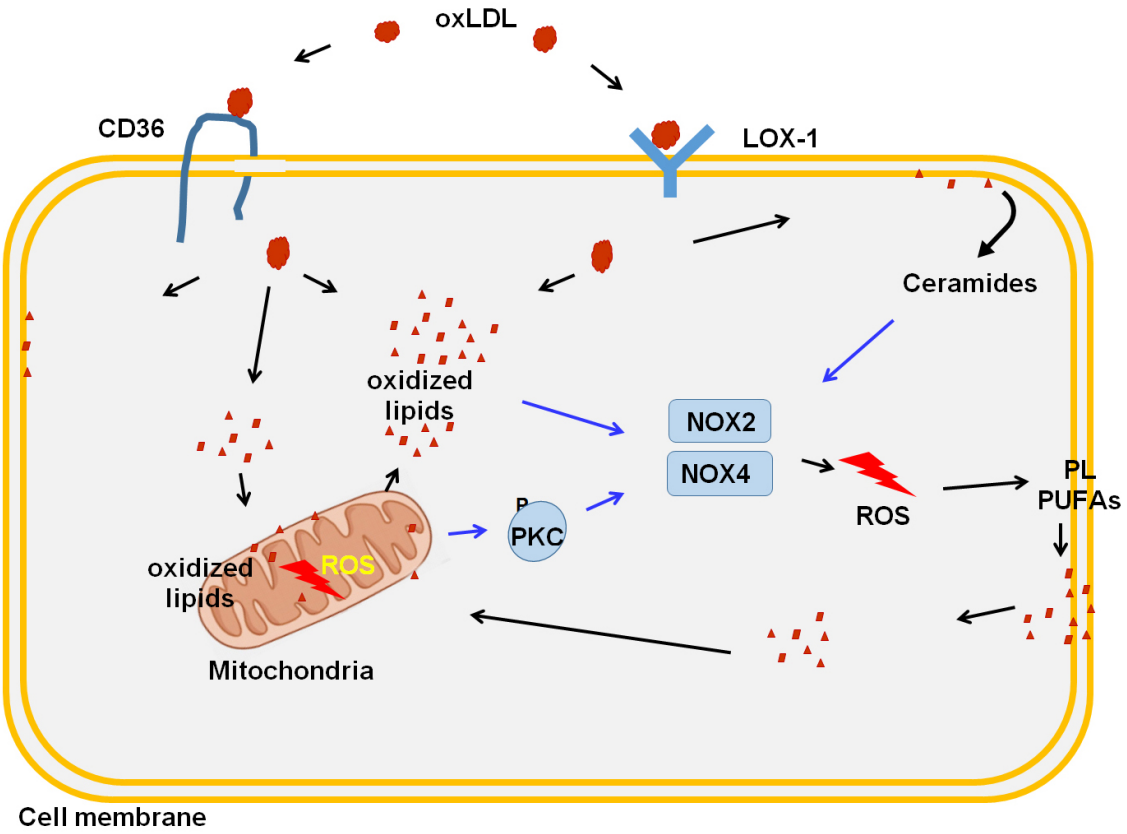

Figure 1. Proposed mechanisms by which oxidized lipids induces oxidative stress in cardiomyocyte. Membrane receptors CD36 band LOX-1 mediate the process of binding and endocytosis of oxLDL particles. In the cell, oxidized lipids liberated from oxLDL can act at different site to promote ROS production. In mitochondria, oxidized lipids increase ROS production which in turn react with PUFAs in cardiolipin and other complex lipids, and generate endogenously formed oxidized lipids. Oxidized lipids could also interact with the cell membrane phospholipids and activate ceramide production. Ceramides and ROS activate PKC, which in turn induces NOX2 and NOX4 leading to the production of ROS. In addition, NOX-derived ROS react with PUFAs in the cell membrane and increase the production of oxidized lipids. oxLDL, oxidized lipoproteins; CD36, cluster differentiating 36; LOX-1, Lectin-like oxidized low-density lipoprotein receptor-1; PL, phospholipids; ROS, reactive oxygen species; NOX, NADPH oxidase; PKC, protein kinase C; PUFAs, polyunsaturated fatty acids.

effects of oxidized lipids are linked to multiple cellular disturbances including oxidative stress, inflammation, apoptosis and insulin resistance.

5.1. Oxidative stress

Oxidative stress is defined as the damages and disturbances related to ROS overproduction. In physiological conditions, ROS are produced in the cell at low rate, and they play an important role in signaling and defense mechanisms. The life-time of ROS is relatively short, and their rate of production is regulated by powerful and divers antioxidant systems to minimize their damaging effects. ROS are, however, highly reactive and can inter-react with cell components including lipids, proteins and DNA leading to serious irreversible damages. In some medical conditions, the imbalance between pro-oxidants and ant-oxidant mechanisms in favor of the former result in oxidative stress that could lead the development of multiple diseases. Oxidized lipids induce ROS production and oxidative stress mainly through two mechanisms involving mitochondria and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (fig1).

5.1.1. ROS production in mitochondria

The mitochondria are considered the “power house” of cardiomyocytes which are in continuous need of energy. It is thus understandable that dysregulation of the function and integrity of mitochondria will have a major impact on the cell function and survival. Mitochondria are also an important site of ROS production mainly through aerobic oxidation of fatty acids (123). Several studies indicate that oxidized lipids exert multiple undesirable effects in mitochondria (124) (125). Oxidized lipids could act directly in mitochondria as demonstrated by the measurement of ROS production in cardiomyocytes with mitochondria-specific fluorescent dye showing strong increase immediately following exposure to oxLDL or 4-HNE (126) (127). This effect is also associated with dysregulation of mitochondrial electron transport chain, alteration of Ca^{2+} channel and cell contractile response

(126) (127). Similarly, oxLDL induces ROS production in other cell models exposed to oxLDL including mesangial cells (128), macrophages (129), vascular smooth muscle cells (130) and endothelial cells (131) (132). In addition, ROS generated in mitochondria are able to inter-react with mitochondrial lipids and increase the production of toxic lipid derivatives (133) (134) leading to membrane depolarization (31) (126). The presence of oxidized lipids in mitochondria, such as 4-HNE, is well demonstrated and is attribute to the oxidation of PUFAs of cardiolipin (134), protein that is present almost exclusively located in the inner mitochondrial membrane. Since cardiolipin plays a crucial role in maintaining the structure of mitochondria membrane, the presence of 4-HNE may affect the structural integrity and function of mitochondria (135). Because of their proximity, lipids of the mitochondrial respiratory chain complexes are exposed first-hand to mitochondrial ROS and oxidative modification. Moreover, the respiratory chain complexes contain iron-sulfur clusters, heme groups and copper, all of which can interact with ROS leading to a reduction of their enzymatic activity and dysfunction of the whole respiratory chain (135) (136) (137). This oxidative damages not only result in a reduction ATP generation (138) but can also increase ROS production by the oxidative phosphorylation (OXPHOS) subunits, contributing to the intensification of oxidative stress (137). All of these modifications indicate that oxidized lipids, in the cell or delivered by oxLDL, trigger a sequence of reactions in cardiomyocytes starting by the induction of ROS production which in turn reacts with mitochondrial lipids leading to further increase of oxidized lipids (fig1).

5.1.2. ROS production by nicotinamide adenine dinucleotide phosphate (NADPH) oxidases

In addition to mitochondria, ROS are derived from several enzymes including xanthine oxidase, uncoupled nitric oxide synthases and NADPH oxidase (NOX), with the latter being the mostly studied enzyme in relation to oxidized lipids. Alteration of NOX activity is often associated with heart disease including ventricular remodeling and heart failure (139). Among NOX isoforms, NOX2 and NOX4 are the main isoforms expressed in cardiomyocytes (39) (140), and they are involved in heart hypertrophy through increased production of ROS (140) (141) (142). Oxidized lipids regulate the expression and activity of NOX as indicated by the fact that knockdown of LOX-1, oxLDL receptor, in cardiomyocytes significantly reduces the expression of NOX2 and NOX4 (143). Moreover, oxidized lipid derivatives including oxLDL, oxysterols and oxPLs, induce NOX expression and increase ROS production (144) (145) (146) (147) (148). Furthermore, inhibition of NOX with specific inhibitor VAS2870 diminishes oxLDL-induction of ROS production (149). While cholesterol derivative 7-ketocholesterol (7-Kchol) induces the expression of NOX4 and enhances ROS production, silencing NOX expression with siRNA reduces 7-Kchol-induced ROS generation suggesting that NOX is an important mediator of the cytotoxic effects of oxysterols through the increase of ROS production (148). These findings highlight the role of NOX as a mediator of oxidized lipids in ROS production and cardiac remodeling (fig1).

5.2. Inflammation

Inflammation is another adverse effect of oxidized lipids, which is associated with the pathogenesis of cardiomyopathy. Several studies indicate that oxLDL increases the production of pro-inflammatory cytokines including interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-alpha) (90) (150). Oxidized lipids induce inflammation in cardiomyocytes through the activation of inflammatory pathways controlled by p38-mitogen-activated protein kinase (p38MAPK) and NF-kB (151) (152), a mechanism which is also reported in other type of cells (142). An early study by Cominacini *et al.* indicates that binding of oxLDL to LOX-1 receptor induces the activation of NF-kB through and increases ROS production (131). In line with this, a study by Yakoyama *et al.* shows that deletion of LOX-1 in mice reduces the expression of p38MAPK and NF-kB, blunts the production of TNF-alpha and interleukin-1 beta (IL-1beta), and abrogates the symptoms of cardiomyopathy (90). These findings are corroborated by the findings that cardiac LOX-1 transgenic mice increases the accumulation of oxLDL, and enhances oxidative stress and inflammation (153). Moreover, these studies demonstrate the existence of interconnections between inflammation and oxidative stress, both of which are increased by oxidized lipids. On the one side, activation of NOX and increased production of ROS activate p38MAPK and contribute significantly to cardiac inflammation (154). On the other side, exposure of cardiomyocytes to TNF-alpha induces strong increase of intracellular ROS and lipid peroxidation leading to cell injury (155) (156). This mechanism is in line with the investigations by Shah and colleagues in which they show that silencing NOX in cardiomyocytes reduces oxidative stress and prevents cardiac hypertrophy and remodeling (157). Although the effect of oxidized lipids on oxidative stress and inflammation are sometimes examined independently, it is clear that these effects are inter-connected and share common target elements and downstream signaling pathways.

Ceramides are also another possible mediator of oxidized lipid-induced inflammation. Activation of neutral sphingomyelinase (N-SMase), an enzyme that catalyze ceramide synthesis, is activated by oxLDL leading to increased production of ceramides and activation of inflammatory kinase p38MAPK (158) (159). In support of this, direct exposure of

cardiomyocytes to cell-permeable C2- and C6-ceramides activates the pathway of pro-inflammatory kinases c-Jun NH_2-terminal Kinase (JNKs) (160), a process that may involve protein kinase C (PKC) (161).

5.3. Apoptosis

There is a general agreement that oxidized lipids induce apoptosis (162), an effect that has been demonstrated for different types of oxidized lipids including oxysterols, oxPLs and PUFA-oxidized products. In general, there are at least two main apoptotic pathways; the death receptor mediated by TNF-receptor (TNFR) also called extrinsic pathway and the mitochondrial or intrinsic pathway. The pro-apoptotic effect of oxidized lipids is mostly linked to mitochondrial intrinsic pathways (35) (163). Several investigations demonstrate that oxysterols, such as 7beta-hydroxycholesterol and cholesterol-5beta,6beta-epoxide, and oxidative products of PUFAs such as NHE-1, initiate the intrinsic pathway by inducing a loss of mitochondrial membrane potential and increasing ROS production (31) (164) (165) (35) leading to the release of pro-apoptotic molecules cytochrome C and caspases (164) (166). Similarly, oxysterols are pro-apoptotic (167) (165). In this regard, several investigations indicate that exposure of cells to oxysterols, such as 7-kchol and 7 beta-hydroxycholesterol (7beta-OH), increases the release of cytochrome C from the mitochondria, where it is normally localized, into the cytosol where it could initiate the intrinsic pathway (34) (35) (165) (168).

Peroxidation of omega-6 PUFAs generates an array of primary lipid oxidation products and lipid electrophiles, among which 4-HNE is the most investigated aldehydic end-product. In cardiomyocytes, the mechanism of 4-HNE-induced apoptosis is linked to mitochondrial dysregulation and the release of cytochrome C and activation of caspase-3 (138) (169) (170) (171). The involvement of 4-HNE in cardiomyocyte apoptosis is also demonstrated *in vivo* in hypertensive rats in which inhibition of 4-HNE reduces TUNEL staining-positive apoptotic cells and blunts the expression of pro-apoptotic mediators BAX and caspase-3 (164) (172). The prevention of apoptosis by 4-HNE inhibition is associated with an improvement of mitochondrial permeability accompanied with a reduction of cardiac hypertrophy (172). In agreement with these results, 20-Hydroxyeicosatetraenoic acid (20-HETE), a hydroxylated derivative of arachidonic acid, sensitizes mitochondria to the calcium-induced loss of membrane potential which may lead to cell injury and apoptosis (79).

Other possible mediators of oxLDL-induced mitochondrial apoptosis are ceramides (163) (173) (174) (175). It has been reported that oxLDL enhances sphingomyelinase activity in mitochondrial outer membrane, increases ceramide production and activates caspases (174), suggesting that ceramide generation is indispensable for oxLDL-induced apoptosis (176). In addition, oxPLs which are abundant in oxLDL increase ceramide production (177), and induce damage in mitochondria triggering the intrinsic apoptotic cascade (166). The involvement of ceramides in stress-induced cardiac dysfunction and mitochondrial apoptosis is also demonstrated in myocardial ischemia or hypoxia (160) (178) (179) (180) (181). In addition, the accumulation of ceramide is also reported in *in vitro* in cardiomyocyte undergoing apoptosis (179) (182), and in other types of cells exposed to oxLDL (176) (183). Ceramides can also exert a negative inotropic effects in cardiac myocytes by the inhibition of intracellular Ca^{2+} mobilization, thereby adding stress on mitochondria that could further induce the intrinsic apoptosis pathways (184).

Increased ROS production can also mediate oxidized lipid-induced mitochondrial apoptosis (167). It is known that ROS can induce apoptosis through the modulation of the expression of pro-apoptosis mediators caspases and BAX (130) (162) (168), and anti-apoptotic protein B cell lymphoma-2 (Bcl-2) (162). In addition, oxLDL, oxysterols and oxPLs are all inducers of NOX activity and catalyzes the production of ROS, which in turn induces mitochondrial apoptosis pathways. In support of this hypothesis, overexpression of NOX4 in cardiomyocytes induces oxidative stress, mitochondrial insufficiency and apoptosis (185). Interestingly, while ceramides are known to stimulate NOX activity (186), inhibition of NOX with specific inhibitors abrogates ceramide-induced ROS production and prevents cell apoptosis (187). In addition, ceramides are able to activate PKC (161), which in turn induces NOX activity leading to increased ROS production and apoptosis (188). TNF-α also increases NOX subunit $p47^{phox}$ phosphorylation and translocation to cell membrane, a mechanism required for the assembly and activation NOX (189).

Finally, another important effect of oxidized lipids is related to their interaction with cytoplasmic cell membrane. Several studies report that oxidized lipids react with lipids in the cell membrane and induce important changes in lipid content and/or organization, effects that are demonstrated for oxysterols 7-ketocholesterol and 7beta-hydroxycholesterol (25) (190), and oxLDL (191). These interactions enhance the accumulation of polar lipids, destabilize the cell membrane and increase cell permeability leading to cell death (192). Interestingly, investigations by Leonarduzzi *et al.* demonstrate that while 7-ketocholesterol alone induces apoptosis, but adding a mixture of oxysterols diminishes the apoptotic effect of 7-ketocholesterol (193). These findings suggest that oxysterols are not equally apoptotic and they may interact with one

another probably through competition mechanisms. Likewise, ROS production and inflammatory markers are differentially induced by specific oxidized species (194) leading to the speculation that different pathways might be involved (35) (190) (195).

5.4. Insulin resistance

The link between oxidized lipids and insulin resistance has been reported in several studies, but there is a question whether oxidized lipids are the cause or the results of resistance. A large study involving 2,774 insulin resistant subjects shows a strong positive association between blood levels of oxLDL and insulin resistance index (HOMA-IR), independently of obesity (196). These finding led to the conclusion that the level of oxLDL in blood is a strong precursor of the risk of insulin resistance, independently of obesity. Other studies indicate that oxLDL blood levels are associated with BMI (197) and markers of insulin resistance and diabetes (7) (63) (65) (196) (198). Likewise, diets enriched with oxidized fatty acid derivatives (199) and high concentrations of oxysterols 7alpha-hydroxycholesterol and 7beta-hydroxycholesterol in blood (57) are associated with obesity and insulin resistance. In agreement with these observational studies, blocking oxLDL with specific antibody in obese primates improved insulin sensitivity (200).

Although the association between oxidized lipids and insulin resistance has been established, the mechanistic basis of the causal effect of this relationship is still in discussion. To address these questions, some investigations tested the effect of oxidized lipids on insulin signaling in cell culture (201). A recent study in cardiomyocytes indicate that low doses of oxLDL increases the activity of protein kinase C and reduces the expression of glucose transporter 4 (GLUT4) (201). Similarly, oxLDL disrupts insulin signaling in macrophages and myocytes, an effect which is linked to the induction of CD36 expression (202) (203). In agreement with this, 4-HNE, the peroxidation products of omega 6-PUFAs, reduces glucose uptake and insulin signaling in hepatocytes (204) (205), adipocytes (206) (207), isolated muscle (208) (209) and myocytes (208). This inhibitory effect of insulin signaling by oxidized lipids is attributed to the activation of inflammatory pathways regulated by JNK and NF-κB (207). Oxidized lipids could also impair insulin sensitivity indirectly through the induction of intracellular oxidative stress which in turn induces inflammation (210) (211). Alternatively, oxLDL could alter insulin signaling indirectly through intracellular lipo-toxic pathways and mediators, such as ceramides which are increased by oxidative stress and inflammation. Several studies indicate that oxLDL increases the activity of sphingomyelinase (SMase) and enhances intracellular content of ceramides (158) (183) (212) (213) (214) (215). Excess ceramide production is known to stimulate p38MAPK and NF-kB pathways leading to insulin resistance (158) (159) (183) (212). A summary of these regulatory mechanisms is presented in fig2.

5.5. Cardiac and cardiomyocyte dysfunction

There is strong evidence to indicate that oxidized lipids are implicated in cardiac remodeling and cardiomyopathy. First, the levels of oxidized lipids are elevated in myocardium of patients and animal models with cardiomyopathy and cardiac dysfunction (216) (217) (218). Furthermore, immuno-histochemical examinations of myocardium biopsies coupled with cardiac echography in patients with cardiomyopathy and/or cardiac failure show that 4-HNE protein adducts are increased in the heart (217) (219) (120), and are positively correlated with reduced cardiac performance (120). Likewise, the concentration of blood soluble LOX-1, an indicator of LOX-1 expression in tissues, is significantly increased in patients with cardiac hypertrophy and is positively associated with a reduction of heart performance as indicated with an abnormally low ejection fraction (220) (221). These findings led to the conclusion that LOX-1 expression could be used as an earlier indicator of heart dysfunction. These observational studies are corroborated with experiments in isolated cardiomyocytes and animal models with hypertrophied failing heart (90) (222). In addition, while silencing LOX-1 expression prevents the progression of experimentally-induced cardiomyopathy in mice (90), blocking LOX-1 with specific antibody inhibits oxLDL-induced cardiomyocyte hypertrophy (223). All together, these findings highlight the involvement of oxLDL and LOX-1 in cardiac dysfunction (90), suggesting a prominent role of LOX-1 in cardiomyocytes.

Some investigations attempted to elucidate the molecular mechanisms by which oxidized lipids induce cardiac remodeling and failure (127). In cardiomyocyte cultures, oxLDL induces cell damage and irregular electrical activity characterized by intense contractile and electrophysiological response including prolongation of action potential duration, depolarization of resting membrane potential, and modification of transmembrane ion currents (20) (31). All of these effects are dependent on the amount of lipid hydroperoxides in oxLDL (31). Other studies indicate that short perfusion of hearts with 4-HNE induces coronary vasodilation (224) and decreases systolic pressure (225), an indication of a decline in heart performance. In agreement with this, perfusion of cardiomyocytes with of 4-HNE solution induces changes in the cell electrophysiology and metabolism characterized by rapid depletion of ATP content and alteration of current through K^+ channels (226). Similarly, acute exposure of cardiomyocytes to 4-HNE

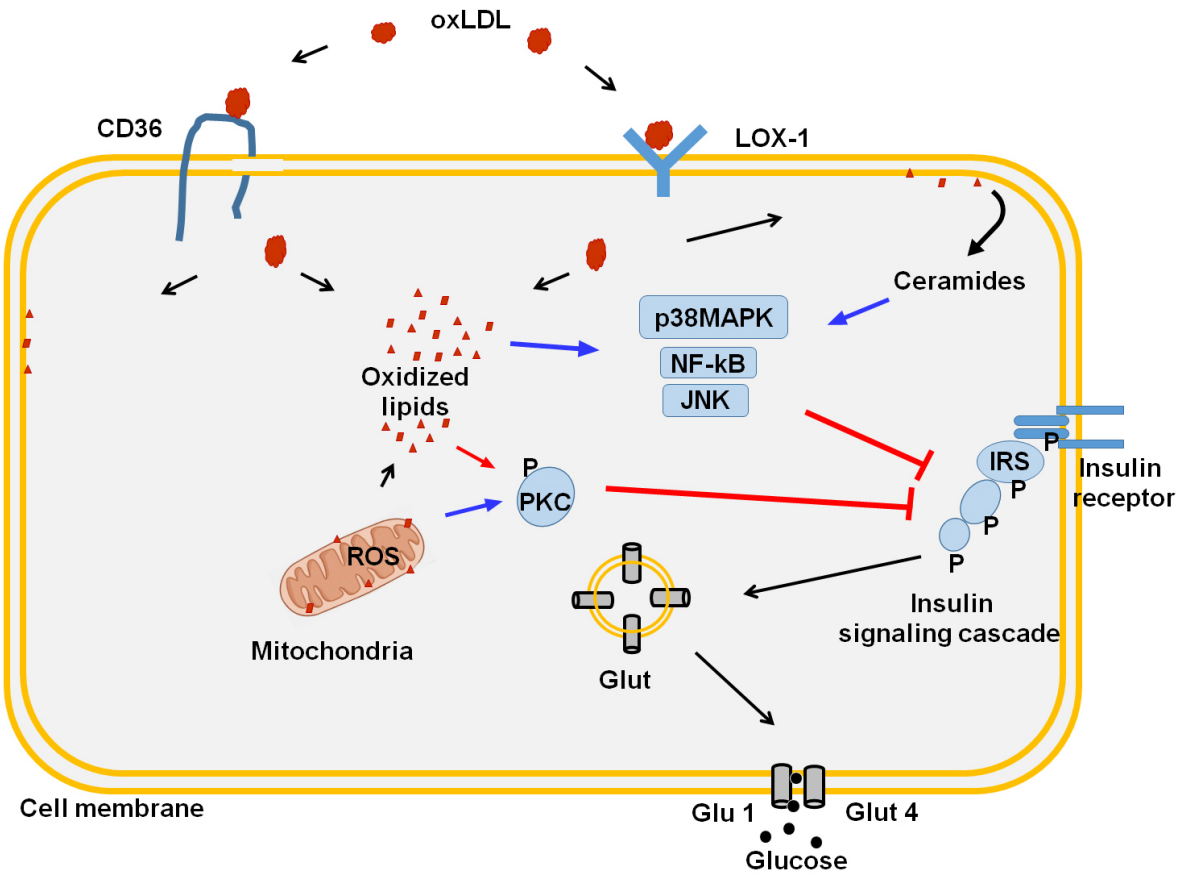

Figure 2. Proposed mechanisms depicting the effects of oxidized lipids on insulin signaling and glucose uptake in cardiomyocytes. In the cell, oxidized lipids, liberated from oxLDL or formed endogenously by ROS reactions, activate p38MAPK and NF-kB pathways leading to the production of inflammatory cytokines, which in turn inhibit the phosphorylation of proteins that mediate insulin signaling. In addition, oxidized lipids and ROS activate PKC which is known to block insulin signaling. Consequently, insulin signal is not transmitted to vesicles containing glucose transporters (Glut) to initiate their transduction to the plasma membrane. oxLDL, oxidized lipoproteins; CD36, cluster differentiating 36; LOX-1, Lectin-like oxidized low-density lipoprotein receptor-1; IRS-1, insulin receptor substrate 1; GLUT, glucose transporter; p38MAPK, p38mitogen-activated protein kinase; NF-κB, nuclear factor κB.

increases ROS production which in turn causes intracellular Ca^{2+} overload (127). All together, these findings demonstrate that oxidized lipid derivatives, alter cardiomyocyte contractile function and induce a depletion of energy source, effects that may explain the links between oxidized lipid cytotoxicity and cardiac dysfunction.

6. PREVENTIONS

In chronic metabolic disease such as obesity and diabetes, oxidative stress is often associated with inflammation and metabolic alterations. These disorders are strongly interconnected and mutually reinforced. Once this chronic vicious circle is established, therapeutic interventions designed to reverse this cycle of negative events is complicated. In this scheme of events, focusing on prevention is the most effective approach, at the first place. To reduce oxidized lipids and prevent oxidative stress, there are some preventive nutritional interventions that could be considered. As stated in the first parts of this review, oxidized lipids could be ingested in the diet or formed endogenously in the body. Therefore, effective preventions should target both sides; reduce the amount of oxidized lipids in the diet and limit endogenous production of oxidized lipids. The first and the most obvious prevention is to reduce the amount of consumption of oxidized lipids. In addition, adhering to a healthy balanced diet containing adequate amounts of anti-oxidants should limit weight gain and reduce oxidative stress, and consequently diminish endogenous production of oxidized lipids.

The most important factor that regulate endogenous production of oxidized lipids is oxidative stress, in other term excess production ROS. Enhancing anti-oxidant system in the body is an important and achievable action to reduce oxidative stress. In healthy conditions, ROS production is countered by an efficient anti-oxidant enzymes such as superoxide dismutase, catalase and glutathione

peroxidase. Dietary micro-nutrients such as vitamins and polyphenols are also important anti-oxidants.

6.1. Anti-oxidant vitamins

Vitamins E is considered the most prevalent natural anti-oxidant vitamin known to scavenge ROS. Several clinical trials have been conducted to determine the effects of vitamin E supplementation on blood oxLDL in healthy subjects (227) (228) or patients with diverse chronic diseases (229) (230). Although the doses and length of treatment vary among these studies, there is a large agreement that vitamin E reduces blood level of oxLDL and could be used efficiently as a preventive measure to protect LDL from oxidation (231) (232). Interestingly, vitamin E supplementation also downregulates the expression of oxLDL receptor CD36 (233), suggesting that this component is beneficial to prevent LDL oxidation as well as reduce cellular uptake. These mechanisms could explain the protective effect of vitamin E against oxLDL induction of inflammation and insulin resistance in fibroblast culture (234).

Given its reducing ability, vitamin C is also a potential water-soluble antioxidant that could act in the aqueous phase both intra- and extracellularly, and therefore could enhance the action of lipophilic antioxidants such as vitamin E. Some studies also tested the ability of vitamins A and beta carotene to reduce oxidative stress (235) (236). Although vitamin C and beta-carotene may have the ability to prevent LDL oxidation *in vitro* (235) (236) (237), controversy still exist as to the ability and efficacy of these components when used separately (238) (239). Some investigations, however, indicate that vitamin C and beta carotene could enhance the anti-oxidation capability of vitamin E, when used in combination (240) (241).

6.2. Polyphenols

Polyphenols are a large group of phenolic compounds with high anti-oxidant activity found in various vegetables and fruits (242). Polyphenols are categorized into flavonoids, such as flavonols, flavones, flavan-3-ols, anthocyanidins, flavanones and isoflavones, and non-flavonoids including major subclasses of stilbenes and phenolic acids, all of which exhibit anti-oxidation proprieties (243) (244). In cardiomyocytes, polyphenols extracted from various plants suppress NOX activity, reduce lipid oxidation, regulate mitochondria function (245) (246), and prevent cell hypertrophy and apoptosis (247) (248) (249). In support of these *in vitro* studies, investigations in animal models (248) (250) (251) and clinical trials (252) (253) (254) indicate that consumption of polyphenols reduce blood oxLDL, decrease ROS production, and prevent cardiomyopathy (248) (250).

7. CONCLUSIONS

The involvement of oxidized lipids and lipoproteins in cardiovascular diseases is well established and the effects of oxidized lipid in macrophages and endothelial cells have been reported by numerous studies. More recent investigations also indicate the existence of strong links between oxidized lipids and cardiac dysfunction. The expression and functionality of oxidized lipoprotein receptors, LOX-1 and CD36, and the presence of oxidized lipids have been demonstrated in cardiomyocytes. Furthermore, strong evidence indicate that oxidized lipids are detrimental to cardiac metabolism and function, and instigate multiple cellular disturbances in cardiomyocytes including oxidative stress, inflammation and insulin resistance. In addition, several *in vitro* investigations demonstrate that derivatives of lipids oxidation alter the structure and integrity of mitochondria leading to the initiation of intrinsic apoptosis pathway. Obesity, in addition to unhealthy diet rich in oxidized lipids, promotes oxidative stress and increases the availability of oxidized lipids. Therefore, adhering to a low calorie-antioxidant-rich balanced diet is possibly the most effective preventive way to avoid the detrimental effect of oxidized lipids.

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