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Diabetes has emerged as a major threat to worldwide health. The exact mechanisms underlying the disease are unknown; however, there is growing evidence that the excess generation of reactive oxygen species (ROS) associated with hyperglycemia, causes oxidative stress in a variety of tissues. In this context, various natural compounds with pleiotropic actions like lipoic acid (LA) are of interest, especially in metabolic diseases such as diabetes. LA, either as a dietary supplement or a therapeutic agent, modulates redox potential because of its ability to match the redox status between different subcellular compartments as well as extracellularly. Both the oxidized (disulphide) and reduced (di-thiol: dihydro-lipoic acid, DHLA) forms of LA show antioxidant properties. LA exerts antioxidant effects in biological systems through ROS quenching but also via an action on transition metal chelation. Dietary supplementation with LA has been successfully employed in a variety of *in vivo* models of disease associated with an imbalance of redox status: diabetes and cardiovascular diseases. The complex and intimate association between increased oxidative stress and increased inflammation in related disorders such as diabetes, makes it difficult to establish the temporal sequence of the relationship.

Key words: Lipoic-acid – antioxidant – metabolism- nutrition - diabetes

Abbreviations:

AMPK, AMP-activated protein kinase
ARE, antioxidant response element
DHLA, dihydro-lipoic acid
eNOS, endothelial nitric oxide synthase
EPA, eicosapentaenoic acid
EpRE/ARE, electrophile/antioxidant response element
ER, endoplasmic reticulum
FAS, fatty acid synthase
FOXO, Forkhead Box O
G6Pase, glucose-6-phosphatase
GLUT4, glucose transporter type 4
GPx, glutathione peroxidase
GSH, reduced glutathione
GSSG, oxidized glutathione
GST, glutathione transferase
HDL, high-density lipoprotein
4-HNE, 4-hydroxy-2-enal
HSF, heat shock factor
Hsp, heat shock protein
HUVEC, human umbilical vein endothelial cells
ICAM1, intercellular adhesion molecule-1
IGF, insulin-like growth factor
IL-6, Interleukin-6
IR, insulin receptor
I/R, ischemia/reperfusion
IRS, insulin receptor substrate
IκB, inhibitor of NF-κB
IκKβ, IκB kinase β
JNK, c-jun N-terminal kinase
LA, α-lipoic acid
LDA, Lipid derived aldehydes
LOOH, lipid hydroperoxides
NADPH, nicotinamide adenine dinucleotide phosphate
NF-κB, nuclear factor-κB
NO_2-FAS, nitro-fatty acid derivates
Nrf2, nuclear factor E2-related factor 2
NTR, NADPH-dependent thioredoxin reductase
4-ONE, 4-oxo-trans-2-nonenal
ox-LDL, oxidized low density-lipoprotein
p38 MAPK, p38 mitogen-activated protein kinase
PI3K, phosphatidylinositol 3-kinase
PUFA, polyunsaturated fatty acid;
RNS, reactive nitrogen species
ROS, reactive oxygen species
TRx, thioredoxin
TNF-α, tumor necrosis factor-α
VCAM-1, vascular cell adhesion molecule-1
VSMC, vascular smooth muscle cell

1. Introduction

The direct and indirect antioxidant properties of alpha-lipoic acid (LA) have been proposed to exert potential therapeutic effects for the treatment of diabetes and its associated complications. Diabetes is increasing at an alarming rate worldwide. The majority of diabetes-related deaths arise from vascular complications such as myocardial infarction, cerebrovascular disease, and peripheral vascular disease. The exact mechanisms underlying the disease are unknown; however, there is growing evidence that the excess generation of reactive oxygen species (ROS) associated with hyperglycemia, causes oxidative stress in a variety of tissues. Diseases such as hypertension, atherosclerosis, hyperlipidemia and diabetes are associated with endothelial dysfunction, probably mediated via NADPH oxidase-driven generation of ROS [1, 2]. It is clearly established that ROS are generated as products of oxygen metabolism in aerobic organisms [3]. ROS are produced as intermediates in reduction-oxidation (redox) reactions transforming O_2 to H_2O. The major superoxide sources of the ROS generated in endothelial cells include nicotinamide dinucleotide phosphate (NADPH) oxidases, xanthine oxidase, the mitochondria, and, under certain conditions, endothelial NO synthases [4]. Exposure to ROS from a variety of sources has led organisms to develop defense mechanisms via antioxidant agents. Several classes of antioxidant and pro-antioxidant agents may be considered, but it is important to clarify some points concerning the specificity of each antioxidant agent. An antioxidant can be defined as “any substance that, when present in very low concentrations compared to that of an oxidizable substrate, significantly delays or inhibits the oxidation of that substrate" [5]. Recently, new data concerning redox-dependent components, led to a refinement in the definition of oxidative stress as an imbalance in pro-oxidants and antioxidants with the associated disruption of redox circuitry and macromolecular damage [6]. Even with the existence of enzymatic and non-enzymatic antioxidants, it is now recognized that the age-related accumulation of oxidatively damaged macromolecules is associated with an increase in rates of ROS generation and a reduction in antioxidant factors [7, 8]. Despite obvious theoretical advances and supportive laboratory work [9-11], clinical studies have failed to confirm the benefits of dietary supplements of natural compounds with antioxidant properties in the prevention of cardiovascular diseases [12-14]. Clinical trials that investigated the impact of antioxidant vitamins on the progression of diabetes-related and vascular complications generated negative or inconclusive results. In this context, some natural compounds with pleiotropic actions like LA, which primary biological effects are associated with its antioxidant properties, are of interest, especially in metabolic diseases such as diabetes.

2. Chemistry and metabolism of alpha-lipoic acid

LA is also called thioctic acid and is chemically named 1,2-dithiolane-3-pentanoic acid ($C_8H_{14}O_2S_2$). Both the oxidized (disulphide) and reduced (di-thiol: dihydro-lipoic acid, DHLA) forms of LA show antioxidant properties (Fig. 1). LA exists as two different enantiomers: the biologically active (R)-isomer and the (S)-isomer. Commercial LA is usually a racemic mixture of the R- and S-form. In one study, volunteers were given 600 mg of R,S-LA, and plasma concentrations of R-LA were 40-50% higher than S-LA [15].

LA rapidly crosses the cell monolayer in a pH-dependent manner. LA transport can be inhibited by compounds such as benzoic acid and medium-chain fatty acids, suggesting that the monocarboxylate transporter was the likely carrier responsible for intestinal absorption of LA [16]. Some studies identified LA as a substrate for the Na^+-dependent multivitamin transporter contributing to its gastrointestinal uptake [17]. Experimental evidence obtained in recent years indicated that the sodium-dependent multivitamin transporter (SMVT; a product of the SLC5A6 gene) is an important transmembrane protein responsible for translocation of vitamins and other essential cofactors such as LA and biotin [18].

It has also been suggested that food intake reduces the bioavailability of LA. Therefore, it is recommended that LA should be taken 30 min before or 2 h after eating [19]. The half-life of LA in plasma is 30 min. LA either from dietary sources or as nutritional supplement is readily absorbed, metabolized and excreted. Endogenous plasma levels of LA and DHLA are respectively 1-25×10^{-9} g.ml^{-1} and 30-140 ×10^{-9} g.ml^{-1}. When LA is administered in the diet, it accumulates in several tissues and a substantial part is converted to DHLA via a lipoamide dehydrogenase. In the reduction reaction, mitochondrial NADH-dependent dihydro-lipoamide dehydrogenase exhibits a marked preference for R (+)-LA whereas cytosolic NADH-dependent glutathione reductase shows greater activity toward the S (-)-LA stereoisomer; the mechanisms of LA reduction are highly tissue-specific. NADH contributes to 90% of the reduction of LA in the heart, 63% in the kidney and 50% in the liver.

Vegetable and animal tissues contain low amounts of R-(+)-LA detected in the form of lipoyllysine (attachment of LA to specific lysine residues) [20]. The most abundant vegetable sources of R-LA are spinach, broccoli and tomatoes, which contain 3.2, 0.9 and 0.6×10^{-3} g lipoyllysine/g dry weight, respectively. In animal tissues, the highest concentration of lipoyllysine is found in kidney, heart and liver, containing 2.6, 1.5 and 0.9×10^{-3} g lipoyllysine/g dry weight, respectively. Recently, levels of lipoyllysine in proteins from rat tissues were directly measured for the first time by reversed-phase high-performance liquid chromatography with fluorescence detection [21]. The amounts in each tissue were different from previous reports [22]. The mean amounts per gram wet weight were as follows: kidney - 3.67 μg, heart - 2.09 μg, liver - 1.97 μg, brain - 0.59 μg, lung - 0.30 μg, pancreas - 0.38 μg and spleen - 0.20μg. Concentrations seem to be dependent on the animal species [23].

LA is both water and lipid-soluble and is widely distributed in cellular membranes, cytosol and extra-cellular spaces. LA readily crosses the blood-brain barrier. Cellular transport of LA occurs probably via several systems, such as the medium-chain fatty acid transporter, an Na+-dependent vitamin transport system, and an H+-linked monocarboxylate transporter for intestinal uptake [24]. The cellular reduction of LA to DHLA is accomplished by NAD(P)H-driven enzymes such as thioredoxin reductases in particular, or NADPH-dependent thioredoxin reductase (NTR). Glutathione (GSH) is a key player in this antioxidative system, with a significant function in ROS scavenging and as a redox buffer to keep the cellular redox state in balance. Data accumulated in recent years indicate that in cells such as erythrocytes, GSH is a key player in this antioxidative system, with a significant function in ROS scavenging. LA and DHLA readily cross the erythrocyte membrane. Thioredoxin reductase is the major enzyme responsible for LA reduction in erythrocytes. Red cells reduced the S-isomer of LA about 40−50% more efficiently than the R-isomer when both were present at low concentrations [25]. Erythrocytes take-up and reduce LA to DHLA; subsequently, DHLA is released to the extracellular milieu, thus reflecting the activity of disulfide reductases.

In a biological system, the reduction of GSSG and dihydro-ascorbate by DHLA contributes to vitamin E regeneration from its oxidized form. This process is associated with the conversion of peroxyl radical ($LOO^{\bullet}$) to lipid hydroperoxides (LOOH). $LOO^{\bullet}$ is generated from lipid radical ($L^{\bullet}$) from an unsaturated lipid (LH) if O_2 is available [26].

The chemical reactivity of LA is mainly conferred by its dithiolane ring. The oxidized (LA) and reduced (DHLA) forms create a potent redox couple. It has been reported that LA/DHLA has a redox potential of -320 mV while the redox potential of GSH/GSSG is -240 mV. This difference suggests that DHLA offers more protection from oxidative damage than does GSH. Therefore, the LA/DHLA couple has been called "universal antioxidant". In fact, the LA/DHLA redox couple appears to be able to regenerate several antioxidants (see following section) and, unlike ascorbic acid, DHLA is not destroyed while quenching free radicals but can be recycled from LA (Fig. 2).

3. Alpha-lipoic acid as a biological antioxidant

3.1 Is LA a direct and/or an indirect antioxidant?

The assessment of oxidative stress, defined as the association between an increased production of oxygen-derived species and an exhaustion of the stores of antioxidants, requires a multimodal approach. Oxidative damage itself can be much better estimated by quantifying the oxidative byproducts of the lipids and proteins associated with an evaluation of the remaining stores of the functional antioxidants, or the activity of antioxidant enzymes, than by the total antioxidant stores [3, 27, 28].

Many criteria must be considered when evaluating the antioxidant potential of a compound: 1)- specificity of free radical quenching, 2)- metal chelating activity, 3)- interaction with other antioxidants, 4) - concentration in the intracellular compartment and extracellular fluid, 5) - induction of proteins implicated in antioxidant protection. In light of these criteria, one can postulate that a compound may be a direct and/or an indirect antioxidant.

3.2 LA: a direct antioxidant

Several classes of antioxidant agents may be considered, but it is important to clarify some points concerning the specificity of each antioxidant agent. An antioxidant can be defined as any substance that when present in very low concentrations compared to that of an oxidizable substrate, significantly delays or inhibits the oxidation of that substrate. Early chemical studies indicated that LA and DHLA scavenge hydroxyl radicals, hypochlorous acid and singlet oxygen [29]. In our laboratory, we also observed that DHLA was able to reduce the superoxide-driven oxidation of a sensitive spin probe, in a manner comparable to that of superoxide dismutase [30]. Recently, LA and DHLA were shown to react with peroxynitrite: ($ONOO^-$) a highly reactive oxidant species resulting from the rapid reaction of nitric oxide ($^{\bullet}NO$) with superoxide anion ($O_2^{\bullet-}$), which is thought to be the main mediator of all of the cytotoxic effects of nitric oxide. However, according to Trujillo & Radi [31] the direct

reaction between LA or DHLA with peroxynitrite was not fast enough to be considered important under *in vivo* conditions.

In vivo, several studies showed that dietary supplementation with LA induced a decrease in oxidative stress, while restoring diminished levels of the other antioxidants. LA supplementation (600 mg/day *per os* for two months) to healthy humans decreased urinary levels of F2-isoprostane, a biomarker of lipid peroxidation, and increased the lag time of LDL oxidation [32]. The mechanisms by which ROS act as mediators in aging have been investigated in detail. In this context, several lines of evidence suggest that LA is able to decrease cerebral lipid peroxidation and to prevent age-associated decreases in endogenous antioxidant levels in old brain tissue [33, 34].

3.3 Alpha-lipoic acid as an INDIRECT biological antioxidant

Antioxidant via metal chelation

In addition to being direct reactive oxygen species scavengers, both LA and DHLA chelate redox-active metals in vitro and in vivo. Several studies have also explored the reactivity of LA and DHLA towards metals. In addition to its ability to directly quench ROS in biological systems, LA also exerts antioxidant effects by acting on transition metal chelation. In fact, LA is a potent chelator of divalent metal ions *in vitro*, and forms stable complexes with Mn^{2+}, Cu^{2+}, Fe^{2+} and Zn^{2+}. It has been demonstrated that LA had a profound dose-dependent inhibitory effect upon Cu^{2+}-catalyzed ascorbic acid oxidation [35]. LA also inhibited Cu^{2+}-catalyzed liposomal peroxidation. It is suggested that prior intracellular reduction of LA to DHLA is not an obligatory mechanism for an antioxidant effect of the drug, which may also operate via Cu^{2+}-chelation. The R-enantiomer and racemic mixture of the drug seemed more effective than the S-enantiomer in metal chelation. Suh *et al.*, 2005 [36] reported the protective effects of R- LA on cortical iron content in aged rats, thereby lowering age-related oxidative stress. DHLA-mediated chelation of iron and copper in the brain had a positive effect in the pathobiology of Alzheimer's disease by lowering free radical damage [37]. It has also been demonstrated that DHLA prevented Cu^{2+}-mediated oxidation of LDL in vitro [38]. In this field, investigations have been conducted on the effects of LA and DHLA on iron- or copper-catalyzed oxidation of ascorbate, a sensitive assay for the redox activity of these metal ions [39]. DHLA, but not LA, significantly inhibited ascorbate oxidation mediated by Fe^{3+}-citrate, suggesting that reduced thiols are required for iron binding. DHLA also strongly inhibited $Cu^{2+}(histidine)_2$-mediated ascorbate oxidation in a concentration-dependent manner, with complete inhibition at a DHLA:Cu^{2+} molar ratio of 3:1. In contrast, no inhibition of copper-catalyzed ascorbate oxidation was observed with LA. It is suggested that DHLA chelates and inactivates redox-active transition metal ions in small-molecular, biological complexes without affecting iron- or copper-dependent enzyme activities.

Antioxidant via electrophilic mechanisms (Fig. 3)

Exposure of cells to high levels of oxygen results in the generation of reactive oxygen species, which react with membrane phospholipids to generate lipid electrophiles associated with oxidative stress. Membrane phospholipids undergo enzymatic and non-enzymatic oxidation of their polyunsaturated fatty acid (PUFA) side chains to generate a variety of oxidized phospholipid products, including

hydroperoxides and cyclic peroxides [40]. The peroxidation of membrane lipids containing ω-3 and/or ω-6 polyunsaturated fatty acids results in the formation of several classes of reactive compounds such as malondialdehyde (MDA), acrolein, and 4-hydroxy-2-nonenal (HNE) [41]. The endogenous generation of reactive aldehydes has been studied for decades and is known to contribute to numerous disease pathologies by altering genomic, cell signaling, and metabolic processes [42]. Reactive aldehydes are a class of highly reactive organic chemical compounds obtained by the oxidation of primary alcohols, characterized by the common group R-CHO consisting of a carbonyl center bonded to hydrogen and an R group [43]. These compounds arise predominantly as a consequence of oxidative stress within the cellular microenvironment, where prooxidant forces overcome natural antioxidant capacities.

The first line of defense that cells utilize to eliminate reactive aldehydes is conjugation with glutathione (GSH). HNE is able to spontaneously form Michael adducts with GSH to form GSH conjugates but the mechanisms controlling the detoxification of lipid electrophiles depend on cell type. Diffusible oxidation products of membrane phospholipids are an important source of bioactive compounds that chemically modify cellular proteins, DNA and macromolecules. In proteins, cysteine residues are the major nucleophilic targets of HNE and its oxidation product: 4-oxononenal (ONE) [44].

LA-induced heme-oxygenase-1 (HO-1) expression and cellular protection (Fig. 3)

HO-1 belongs to a family of cytoprotective and detoxification genes that possess AREs in their regulatory regions. As we mentioned above, the Nrf family of transcription factors can bind ARE. HO-1 induction also exerts anti-inflammatory effects in vascular cells, including the inhibition of adhesion molecules and pro-inflammatory cytokine secretion; HO-1 overexpression inhibits vascular cell adhesion molecule-1 expression in human endothelial cells [45-47]. It has been demonstrated *in vitro* that LA induced HO-1 expression via Nrf2 in human monocytes [48]. The consequences of HO-1 induction by LA in monocytes are potentially important for vascular disease. Recently, the effect of LA pretreatment on Nrf2-responsive gene expression of HepG2 cells exposed to As^{3+} has been analyzed. LA pretreatment can down-modulate the response mediated by Nrf2 and provide protection to As^{3+} exposed HepG2 cells [49]. Interestingly, LA induced a small increase in HO-1 , this finding was consistent with several reports linking HO-1 cytoprotection with low (less than 5-fold) induction of the enzyme, while high levels of HMOX1 expression (greater than 15-fold) were associated with significant oxygen cytotoxicity [50]. HO-1 is a cytoprotective molecule, which has potent anti-inflammatory cardioprotective properties, [51] but additionally, its essential metabolite, carbon monoxide (CO), appears toxic when CO binds to hemoglobin (Hb).

Paradoxically, LA may act as a pro-oxidant

Paradoxically, LA may act as a pro-oxidant to cause a mild cellular insult that induces nuclear localization of Nrf2. It has been reported that administration of R-LA in a cell culture model increased GSH only after 24 h. This result suggests a Nrf2-dependent mechanism rather than a direct antioxidant or GSH-recycling one [52]. LA, acting as a pro-oxidant, may increase Nrf2-dependent

transcriptional activity by forming lipoyl-cysteinyl mixed disulfides on Keap1, the protein that sequesters Nrf2 and bridges it to ubiquitin ligases [53, 54].

4. Effects of LA, DHLA on cellular glucose uptake

Mechanistic studies on the effects of LA on the redox status of insulin responsive cells revealed that LA stimulated glucose uptake by affecting components of the insulin signaling pathway. LA stimulated glucose uptake upon translocation and regulation of the intrinsic activity of GLUT4, an effect that might be mediated by p38 MAPK [55]. Evidence suggests that the insulin-signaling pathway is sensitive to the redox status. The effect of LA and DHLA was examined in the context of insulin abnormalities and insulin resistance. It has been reported that LA increases glucose uptake in insulin-sensitive [56] and insulin-resistant muscle tissues [57]. It has also been demonstrated [58] that the administration LA to obese animals increased insulin-stimulated glucose uptake in the whole body. In addition to these effects, it has been shown recently that, in isolated working rat hearts, LA stimulates glucose oxidation without affecting glycolysis, lactate oxidation or palmitate oxidation [59]. In differentiated 3T3-L1 adipocytes R-α-LA and oxidized isoforms are effective in stimulating glucose transport by a mechanism entailing changes in the intracellular redox status, LA also facilitated the auto-phosphorylation of the insulin receptor by a mechanism that may involve oxidation of the cysteine residues in the α- and β-subunits [24, 52].

Studies on muscle cell lines indicate that exposure to LA stimulates glucose uptake by redistribution of GLUT1 and GLUT4 to the plasma membrane, and tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) [55]. In type II diabetes mellitus, there is evidence that LA ameliorates insulin resistance and impaired glucose metabolism in the periphery [60, 61].

5. Effects of LA and DHLA on mitochondria metabolism

Mitochondria are constantly undergoing fission and fusion to adapt to changing conditions of the cell, and mitochondrial dysfunction has been reported in various cardiovascular pathologies. Mitochondria are also important regulators of cell death. The switch to a cell death program can be mediated by the opening of the mitochondrial permeability transition pore (mPTP) in the inner mitochondrial membrane [62]. This large pore allows components with a molecular mass up to 1.5 kDa to diffuse through the inner membrane, which induces rupture of the outer membrane and the release of pro-apoptotic factors. Pores are influenced by pro-oxidants that stimulate mPTP opening by oxidizing pyridine nucleotides; thiols [63] and antioxidant compounds with reduced SH groups [64].

Paradoxically, LA and DHLA at concentrations between 0.01 – 0.1 mM promote mPTP opening. Despite being a dithiol, DHLA was more effective than LA, suggesting another mechanism than oxidation of LA. DHLA-induced mPTP opening was inhibited in the presence of some antioxidant agents, and the involvement of ROS in the mechanism for mPTP stimulation is supported by this inhibition [65, 66]. This finding contrasts with the strong antioxidant capacity of LA or DHLA in cells and tissues. In bovine heart sub-mitochondrial particles, the stimulation of $O_2^{\bullet-}$ production by DHLA was studied using Electronic Paramagnetic Resonance [67]. The DHLA-induced increase in the signal

was reduced by two radical scavengers: BHT and TEMPO. Superoxide generation was observed in mitochondria in the presence of two different substrates: succinate or pyruvate. These effects are not yet fully understood, but the mechanisms may be attributed to ubisemiquinone-dependent oxidation of DHLA to thiyl radicals that will be able to generate superoxide by auto-oxidation.

Mitochondria are a major intracellular source of pro-oxidants and are reversibly responsive to changes in redox status [4]. There is now evidence that the mitochondrial Krebs cycle enzyme α-ketoglutarate dehydrogenase (KGDH) is a component of the mitochondrial antioxidant system and a key sensor of redox status. It induces critical changes in mitochondrial and cellular metabolism to prevent oxidative damage [68]. KGDH is uniquely sensitive to oxidative stress, capable of undergoing fully reversible free radical mediated inhibition. LA is an essential cofactor for the E2 component of α-ketoacid dehydrogenase complexes, exclusively located in mitochondria, e.g., pyruvate dehydrogenase (PDH), KGDH, and branched chain α-ketoacid dehydrogenase complexes. The former catalyzes the oxidative carboxylation of pyruvate and plays a fundamental role in carbohydrate metabolism and bioenergetics, for which PDH bridges anaerobic and aerobic energy metabolism. In conclusion, it appears that R-α-LA is a cofactor for four enzyme complexes exclusively located in mitochondria, and is essential for energy production and the regulation of carbohydrate and protein metabolism. In conclusion, LA modulates mitochondrial processes involved in energy homeostasis; this effect has recently been studied in fatty liver [69].

6. LA and DHLA as treatments for cardiovascular diseases

6.1 Effects on endothelial vascular cells:

Endothelial dysfunction including impairment of endothelium-dependent vasodilator function and increased endothelial activation contributes to the pathophysiology of cardiovascular diseases such as hypertension, atherosclerosis and diabetic vasculopathy. The microvascular complications of diabetes, including nephropathy, retinopathy and neuropathy, are common manifestations of diabetes. The changes in the endothelial phenotype in these conditions occur in response to diverse stimuli including inflammatory cytokines, hyperlipidemia and hyperglycemia. The increased production of ROS is involved in the genesis of these alterations in the endothelial phenotype, and endothelial dysfunction primarily reflects decreased availability of NO. Vascular endothelium is a potentially important target for new therapies. The impact of LA treatment on endothelial function has been studied in vitro and in vivo.

The therapeutic doses of LA in humans range from 200 to 1,800 mg /day. As we previously reported, the half-life of racemic LA in plasma is 30 min and the endogenous plasma levels of LA are between 15-20 µM. Given its potential antioxidant properties, the possible health benefits of LA supplementation have been tested in cardiovascular diseases. One common factor contributing to the development of atherosclerosis, diabetes mellitus and hypertension is the overproduction of ROS associated with chronic inflammation. Most of the risk markers for cardiovascular disease include a pro-inflammatory component which modifies the release of active molecules from endothelial cells. Jones *et al.* [70] studied the uptake, reduction and antioxidant effects of LA in cultured human

endothelial cells. The LA/DHLA redox couple enhances both the antioxidant defenses and the function of these cells, and thus preserves nitric oxide-dependent vascular relaxation in humans [71]. Since oxidative stress can cause endothelial dysfunction, the interactions of LA, and its efficacy have been tested in the prevention of oxidant damage in EA-hy926 cells, a cell line that is derived from the fusion of human umbilical vein endothelial cells (HUVEC) with human lung carcinoma cells [72]. These cells retain endothelial cell features including oxidative modifications of human LDL and calcium-dependent eNOS activation. Extracellular oxidant stress generated by incubating these cells with LDL and copper caused a time-dependent increase in lipid peroxidation of both cells and LDL, preceded by the disappearance of α-tocopherol in LDL. LA (40-80 μM) blunted these effects.

Extensive evidence suggests that ROS-induced vascular dysfunction is one of the main features of diabetes; this state of ROS accumulation is strongly associated with impaired endothelium-dependent NO metabolism. Recent studies suggest that improved endothelial function due to LA is at least partially attributed to recoupling of eNOS and increased NO bioavailability [73]. The synthesis of NO is blocked by the inhibition of the NOS active site with guanidino-substituted analogues of L-arginine such as asymmetric dimethylarginine (ADMA) a naturally occurring amino acid found in the plasma and various tissues [74]. Plasma levels of ADMA in humans and rats in most studies range from 0.3 to 0.5 mmol/L [75]. Endothelial dysfunction is increased by various cardiovascular risk factors, metabolic diseases, and systemic or local inflammation. One mechanism that has been implicated in the development of endothelial dysfunction is the presence of elevated levels of ADMA. In this field, our studies suggest that the elevation in blood pressure and glucose induced by a fructose diet is accompanied by early overexpression of iNOS and an increase in oxidative stress. Our findings suggest that the elevated levels of ADMA observed could in part be secondary to the early development of oxidative stress [76]. An increased concentration of ADMA predicts cardiovascular events in different populations including diabetic patients [77, 78]. In cultured endothelial cells, it has been demonstrated that LA decreases ADMA levels by increasing DDAH II mRNA expression and DDAH activity [79]. It has been suggested that DDAH activity is impaired by oxidative stress. Indeed, agents with antioxidant properties such as probucol increase DDAH activity [80]. In light of these findings, one can postulate that an LA-induced increase in DDAH activity is partly mediated by its antioxidant properties. Recently, a great deal of attention has been given to the antioxidant mechanisms of LA in different tissues. Consequently, LA/DHLA redox couple affects biological processes including the activity of enzymes and receptors and the regulation of gene transcription [81-85].

6.2 Prevention and treatment of diabetes

LA and Diabetic retinopathy

Diabetic retinopathy (DR) is one of the most common complications of diabetes and is a leading cause of blindness in people of working age in industrialized countries. Approximately 25% of type 1 diabetic patients may have signs of retinopathy [86]; the DR prevalence is very important in type-2 diabetic patients (39% in a study performed in 1993) [87]. DR is characterized as a microvascular complication of diabetes. Vascular alterations in the early stage of the disease include alterations in blood flow, death of retinal pericytes and subtle increases in vascular permeability [88]. The mechanisms by which diabetes causes microvascular complications and disease progression in the

retina are not fully understood. However, studies have shown that DR has features of chronic inflammation. Inflammation is the body's defense against pathogens and is also a critical step in wound healing. This process involves multiple mediators such as pro-inflammatory cytokines, chemokines and adhesion molecules that initiate the interaction between leukocytes and the endothelium. The accumulation of neutrophils correlates with the upregulation of ICAM-1 immunoreactivity in the vessels and is associated with capillary closure [89]. Increases in IL-6, ICAM-1 and VCAM-1 have been shown to be related to the progression of DR. While normal inflammation is beneficial, excessive or uncontrolled inflammation can cause tissue injury [90, 91]. Risk factors of DR include hyperglycemia, hypertension and dyslipidemia. These factors have been associated with inflammation by different mechanisms, including oxidative stress, dysregulation of NOS and the formation of advanced glycation end-products (AGEs) [92]. Overall, these changes lead to a net increase in ROS, and diabetes-induced sustained oxidative stress is a major cause of retinal inflammation. In our laboratory, we demonstrated that the scavenging capacities of plasma were decreased in diabetic patients. A significant decrease in plasma vitamin C and an increase in ascorbyl free radical /vitamin C ratios were noted in type 2 diabetic patients. Our results suggest a possible use of AFR/vitamin C ratios as indicators of oxidative stress as we reported in diabetic patients [93]. During these last years, we demonstrated that there are numerous sources of ROS in the retina and the level of oxidative damage will depend upon the efficiency of the antioxidant system. Retinal complications may be encountered during the development of hypertension as a response to oxidative stress [2]. The lifelong accumulation of chronic oxidative damage will lead to dysfunction in retinal cells and increase their susceptibility to exogenous and endogenous insult such as ischemia [2, 94, 95].

A large clinical study (RETIPON) has recently been reported. The aim was to evaluate the effect of LA for the prevention of diabetic macular edema [96]. Patients were randomized (235 patients with type II diabetes mellitus) to the treatment group with 600 mg LA per day or the placebo group. Every 6 months, stereo fundus photographs, HbA1c levels, and an ophthalmological examination were documented. The primary endpoint was the occurrence of clinically significant macular edema (CSME) within a follow-up period of 2 years. A daily dosage of 600 mg LA did not prevent the occurrence of CSME in diabetic patients.

Does LA treatment prevent or delay the onset of diabetic complications?

Many trials in diabetic subjects and animal models of diabetes have attempted to determine whether LA treatments can prevent or delay the onset of diabetic complications. As detailed in recent reviews [97-99], LA has been shown to possess a number of beneficial effects both in the prevention and in the treatment of diabetes in several experimental conditions. However, a general problem with all animal studies is the selection of the optimal dose of LA and the species-specific metabolism of antioxidant vitamins. The results in patients are contradictory [29]. In contrast, LA presents beneficial effects in the treatment of symptomatic diabetic neuropathy, which is a major health problem. Obtaining glucose levels close to normoglycemia is the primary approach for the prevention of diabetic neuropathy, but these glycemic objectives are not achievable in a number of patients. Seven controlled randomized clinical trials of LA in patients with diabetic neuropathy have been completed (alpha-lipoic acid: in Diabetic neuropathy: Dekan, Oral Pilot: Orpil, Symptomatic diabetic neuropathy:

Sydney, Neurological Assessment of thioctic acid in neuropathy: Nathan II). These trials used different study designs, durations, doses and patient populations. A comprehensive analysis of these trials was undertaken and the meta-analysis (n = 1,258) gave a precise evaluation of the efficacy and safety of 600 mg LA I.V. for 3 weeks in diabetic patients suffering from symptomatic poly-neuropathy [100].

The protection of human LDL against glycation by LA was also investigated; this effect is of clinical significance because diabetic patients have a higher frequency of atherosclerosis than non-diabetic patients [101]. In addition, it has been reported that non-covalent hydrophobic binding of LA to albumin was involved in its protective effects.

Finally, most currently available therapies are symptomatic (focusing on pain relief) rather than disease-modifying. With the exception of good glycemic control, there is currently no effective treatment to slow the progression of or reverse diabetic poly-neuropathy. The recognition of the difficulty in reversing established DN has focused efforts on slowing its progression [102]**.**

6.3 Prevention and treatment of hypertension

The protective effects of LA against hypertension have also been studied in experimental conditions. Since there is strong evidence that excess dietary NaCl is associated with the development of hypertension, Vasdev *et al.* [103] determined whether giving salt-sensitive rats a dietary supplement of 500 mg/kg of LA could lower blood pressure. The results showed that the dietary supplement of LA attenuated the increase in systolic arterial pressure. LA was also effective in preventing an increase in tissue aldehyde conjugates and cytosolic Ca^{2+} in salt-induced hypertensive rats [104]. The antihypertensive effects of LA were associated with an attenuation of oxidative stress in the aortic artery and with the preservation of glutathione peroxidase activity in the plasma of rats receiving 10% D-glucose in their drinking water [105]. In these experimental conditions, dietary supplements of LA prevented the rise in systolic blood pressure and the development of insulin resistance, as reflected by a higher homeostasis model assessment (HOMA). Recent studies have demonstrated that oxidative stress may be the unifying factor for the damaging effect of hypertension and hyperglycemia, the cardiovascular protection of LA appearing to be associated with its antioxidant properties [85, 106]. The findings of these studies extend our understanding of the role of antioxidants in cardiovascular diseases [84, 107].

7. Conclusions: perspectives in protection of cardiovascular functions

To date, there has been no convincing demonstration that nutritional or pharmacological agents can prevent the occurrence or the progression of chemotherapy-induced cardiomyopathy. The formation of reactive oxygen species and the dysregulation of calcium homeostasis are both implicated in the development of anthracycline-related toxicity [108-110] where iron chelators do not provide optimal protection. The use of LA in the treatment of neuropathy suggests that this agent could be helpful in the protection of patients undergoing chemotherapy. In fact, a small case series suggested that LA

may ameliorate chemotherapy-induced neuropathy [111] and that, at least, LA may be a reasonable complement to other empiric therapies for the treatment of these neurological side-effects [112, 113].

In conclusion, a great deal of attention has been given to the bio-thiol antioxidants LA and its metabolite DHLA since they effectively protect cells against ROS-induced damage. Consequently, the LA/DHLA redox couple affects important biological processes including the regulation of gene transcription and the activity of enzymes and receptors. In our opinion, the lack of clear beneficial effects in some clinical trials to date does not disprove the clinical usefulness of LA in different populations, including diabetic patients. However, long-term and thorough clinical studies are needed to confirm the therapeutic potential of LA. Indeed, LA offers the hope of effective and safe treatment that may allow preventing the damaging outcomes of diabetes.

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Conflict of interest:

The authors declare that they have no personal, financial or other relationships with other people or organizations within 3 years of beginning the work submitted that could inappropriately influence, or be perceived to influence, the work submitted.

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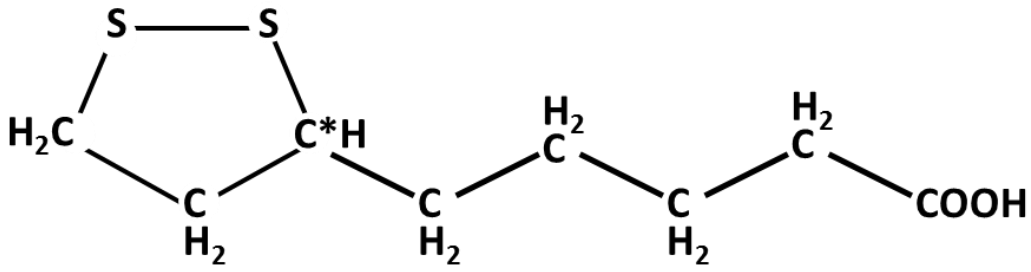
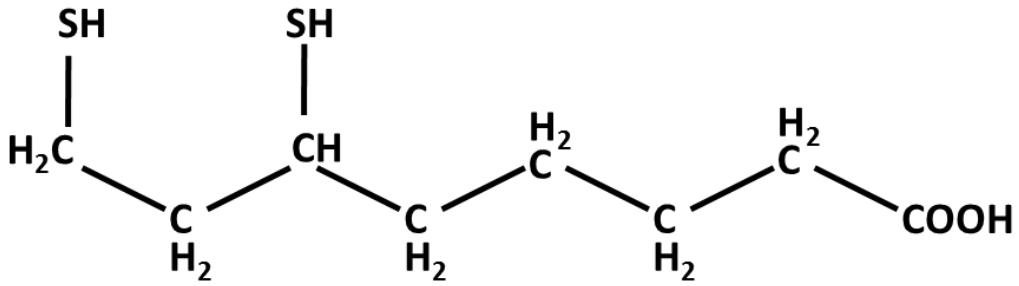
Figure 1:

Chemical structure of alpha-lipoic acid (A) and dihydrolipoic acid (B).

* the structure contains a chiral center



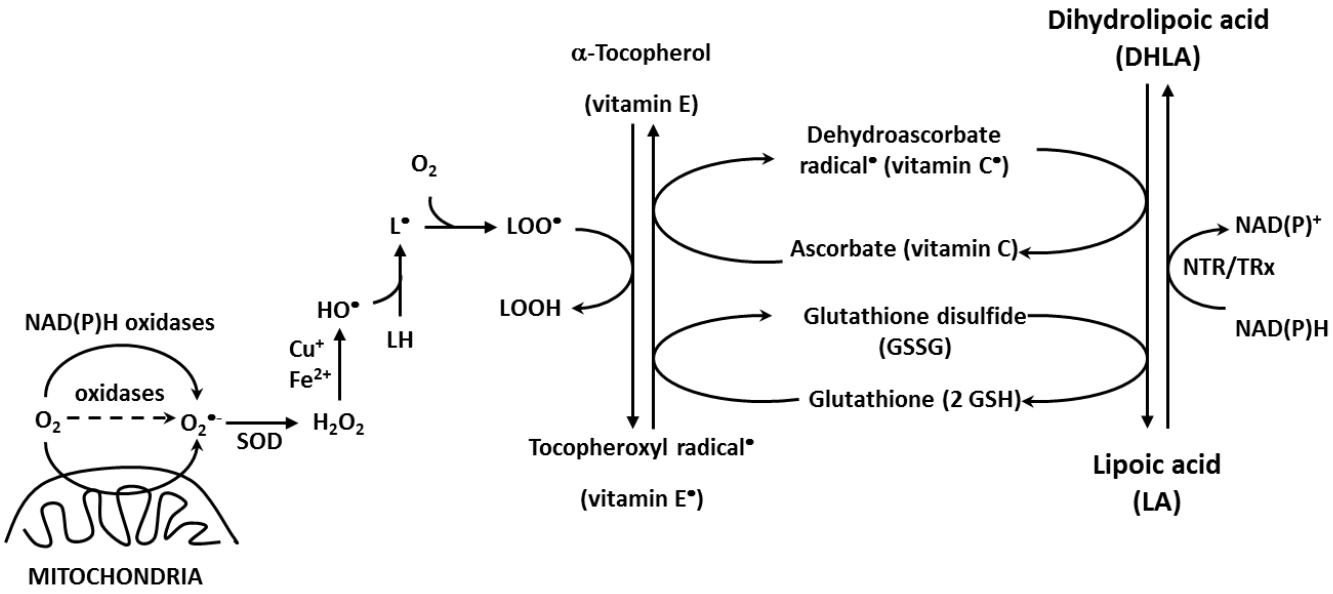
A



B

Figure 2: The pathways of the antioxidants: glutathione: GSH, α-lipoic acid (LA) and dihydrolipoic acid (DHLA). LA and DHLA increase the efficiency of te vitamin C cycle and activate the vitamin E cycle.

thioredoxin (TRx); NADPH-dependent thioredoxin reductase (NTR); unsaturated lipid (LH); lipid hydroperoxides (LOOH); peroxyl radical ($LOO^{\bullet}$)



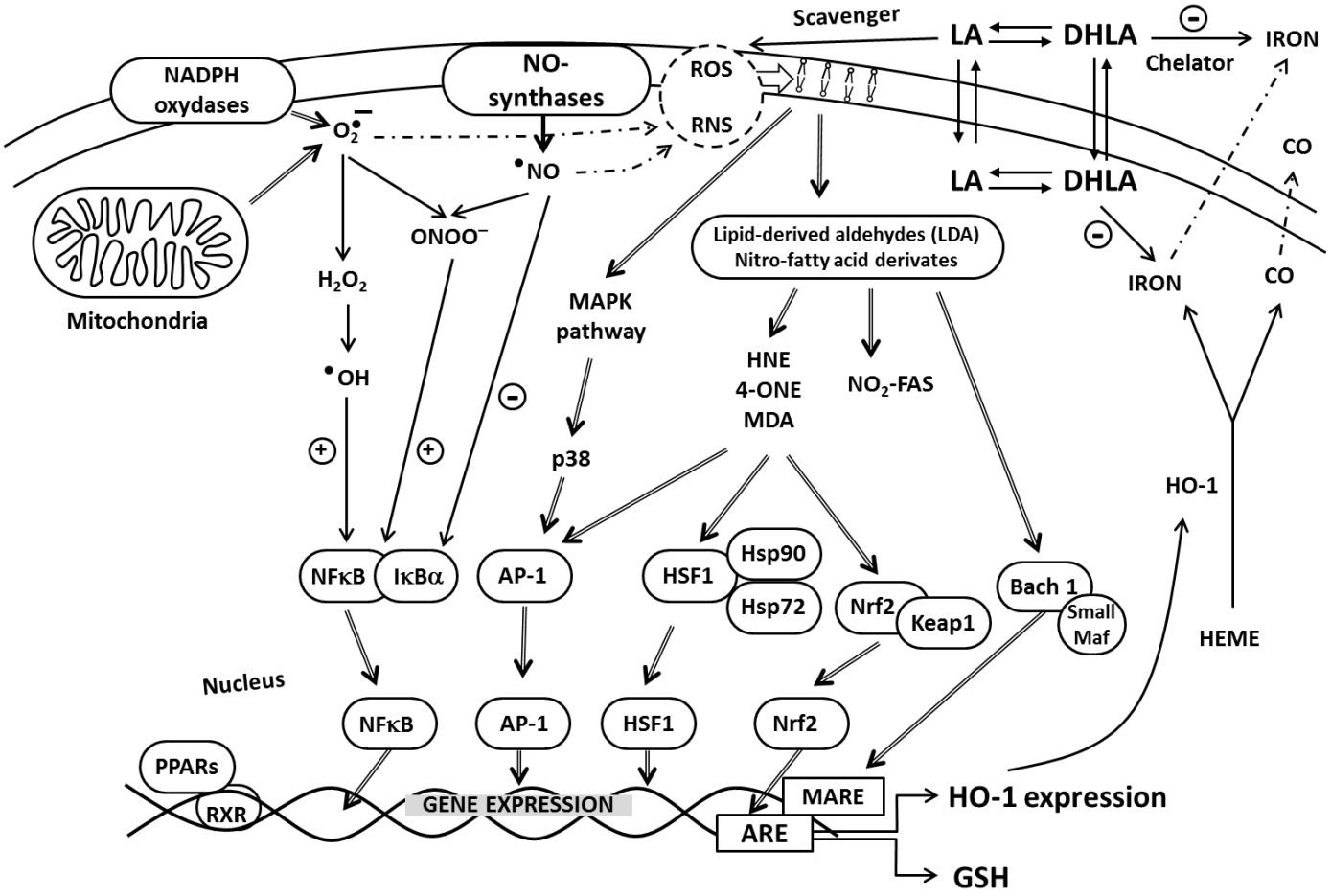


Figure 3: General scheme for the induction of gene expression through the signaling pathways and mechanisms of cellular actions of lipoic acid (LA) and dihydro-lipoic acid (DHLA).

Induction of these genes which include antioxidative (reduced glutathione (GSH) and heme oxygenase-1 (OH-1)) genes in adaptative responses that enhance the resistance of cells to environmental stresses mediated by Reactive Oxygen Species (ROS) and Reactive Nitrosative Species (RNS) and electrophilic compounds (Lipid Derived Aldehydes: LDA, Nitro-Fatty acid derivates: NO2-FAS). ROS, RNS and endogenous and exogenous electrophiles/ activators (see text for more details) can alter the AP-1, HSF1, NF-κB/IkB, Nrf2–Keap1 and Bach-1 signaling complexes. Subsequent nuclear translocation exits and the induction of antioxidant response element (ARE)-driven genes results in the upregulation of HO-1 and GSH.