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Abstract: Diabetes are chronic metabolic diseases with a high prevalence worldwide. These pathologies and insulin resistance are associated with the development of cardiovascular and nervous diseases. The development of these disorders reflects complex pathological processes in which the oxidative stress caused by reactive oxygen species (ROS) and reactive nitrogen species (RNS) plays a pivotal role. It is widely accepted that diabetes impairs endothelial nitric oxide synthase (eNOS) activity and increases the production of ROS, thus resulting in diminished NO bioavailability and increased oxidative stress. Alpha-lipoic acid (LA) possesses beneficial effects both in the prevention and in the treatment of diabetes. LA is a potent antioxidant with insulin-mimetic and anti-inflammatory activity. LA in the diet is quickly absorbed, transported to the intracellular compartments, and reduced to dihydrolipoic acid (DHLA) under the action of enzymes. LA, which plays an essential role in mitochondrial bioenergetic reactions, has drawn considerable attention as an antioxidant for use in managing diabetic complications such as retinopathy, neuropathy and other vascular diseases.

Keywords: lipoic acid, antioxidant, diabetes, metabolism, prevention, treatment.

Résumé: Les diabètes sont des désordres métaboliques chroniques avec une prévalence élevée à travers le monde. Ces pathologies et la résistance à l'insuline sont associées au développement d'atteintes cardiovasculaires et nerveuses. Le développement de ces atteintes reflète des processus pathologiques complexes mettant en jeu un stress oxydatif joue un rôle majeur en impliquant les espèces radicalaires oxygénées (ERO) et nitro-oxygénées (ERN). Il est généralement admis que les diabètes modifient l'activité des oxyde nitrique (NO) synthases endothéliales (eNOS), conduisant à une diminution de la biodisponibilité du NO et une augmentation du stress oxydatif. L'acide alpha-lipoïque (AL) possède des effets bénéfiques dans la prévention et le traitement des diabètes. AL est un antioxydant puissant pourvu d'activités insulino-mimétiques et anti-inflammatoires. AL présent dans la nourriture est rapidement absorbé, transporté dans les compartiments intracellulaires et réduit en acide dihydrolipoïque (ADHL) sous l'effet d'enzymes. AL qui joue un rôle essentiel dans les réactions bioénergétiques mitochondriales, suscite beaucoup d'attention comme antioxydant dans la prise en charge des complications liées au diabète telles que les rétinopathies, les neuropathies et les autres atteintes vasculaires.

Mots-clés : acide lipoïque, antioxydant, diabètes, métabolisme, prévention, traitement

Introduction

Diabetes is increasing at an alarming rate worldwide. The prevalence of type 2 diabetes is increasing, and diabetes is a strong adverse prognostic factor among patients with cardiovascular (CV) disease. 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 (Vergely et al. 1998; Vergely et al. 2001). Clinical studies have failed to confirm the benefits of dietary supplements of natural compounds with antioxidant properties in the prevention of cardiovascular diseases. Clinical trials that investigated the impact of antioxidant vitamins on the progression of diabetes-related and vascular complications generated negative or inconclusive results (2002; Hercberg et al. 2004). In this context, some natural compounds with pleiotropic actions like lipoic acid (LA) are of interest, especially in metabolic diseases such as diabetes. It appears that LA or its reduced form, dihydrolipoic acid (DHLA) possess many biochemical functions acting as biological antioxidants, as metal chelators, able to regenerate endogenous antioxidants such as vitamins C and E, and modulator of the signaling transduction of several pathways (Rochette et al. 2013b). This article is an up-to-date review of current thinking regarding LA and its use in providing antioxidant drug therapy for diabetic diseases.

Chemistry and metabolism of lipoic acid

LA is also called thioctic acid and is chemically named 1,2-dithiolane-3-pentanoic acid ($C_8H_{14}O_2S_2$): with an oxidized (disulfide, LA) and a reduced (di-thiol: dihydro-lipoic acid, DHLA) forms of LA. LA exists as two different enantiomers: the biologically active (R)-isomer and the (S)-isomer. Vegetable and animal tissues contain low amounts of R-(+)-LA detected in the form of lipoyllysine (attachment of LA to specific lysine residues). (Figure 1) 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, which contain 2.6, 1.5, and 0.9 × 10−3 g lipoyllysine/g dry weight, respectively.

LA has both hydrophilic and hydrophobic properties. Being both water and fat-soluble means that ALA is widely distributed in plants and animals in both cellular membranes and in the cytosol. Therefore, it can elicit its actions in both the cytosol and plasma membrane. LA readily crosses the blood-brain barrier. Cellular transport of LA occurs probably via several systems, such as the medium-chain fatty acid transporter, a Na^+-dependent vitamin transport system, and an H^+-linked monocarboxylate transporter for intestinal uptake. The cellular reduction of LA to DHLA is accomplished by NAD(P)H-driven enzymes, thioredoxin reductase, lipoamide dehydrogenase, and glutathione reductase. Erythrocytes take up and reduce LA by glucose metabolism; subsequently, DHLA is released to the extracellular space,

thus reflecting the activity of disulfide reductases. Red cells reduced the S-isomer of LA about 40–50% more efficiently than the R-isomer when both were present at low concentrations (May et al. 2007). The intracellular redox status is usually determined by the glutathione/glutathione disulfide (GSH / GSSG), thioredoxin reduced/thioredoxin oxidized, and cysteine/cysteine couples and their ability to reversibly modulate cysteine- and methionine moieties in proteins. In these mechanisms, the mitochondria functions are important **(Figure 2).** When LA is administered in the diet, it accumulates in several tissues and a substantial part is converted to DHLA via a lipoamide dehydrogenase. The mechanisms of LA reduction are highly tissue specific (Packer et al. 2001).

Concerning the metabolism of LA and DHLA, it is demonstrated that the rapid gastrointestinal transport of LA into the blood plasma is followed by an equally rapid clearance, reflecting both uptake into tissues, (e.g. the liver, brain, heart, and skeletal muscle) as well as glomerular filtration and renal excretion (Schupke et al. 2001).

Interplay between LA metabolism and redox mechanisms

The redox environment of a linked set of redox couples as found in biological fluids, organelles, cells, or tissuesis defined as the summation of the products of the reduction potential and reducing capacity of the linked redox couples.

The relationships between the effects of LA and the thiol redox state have been established. The chemical reactivity of LA is mainly conferred by its dithiolane ring **(Figure 1).** 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/oxidized glutathione 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 the “universal antioxidant”. A true antioxidant is defined as ‘‘any substance that, when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate’. Common antioxidants are either water-soluble or lipid membrane-soluble agents. As we reported, in contrast, LA has both hydrophilic and hydrophobic properties. This amphiphilic character of LA is unique among antioxidants. In fact, the LA/DHLA redox couple appears to be able to regenerate several antioxidants and, unlike ascorbic acid, DHLA is not destroyed while quenching free radicals but can be recycled from LA. In an oxidative space, paradoxically, LA and DHLA may act as pro-oxidants. (see next paragraphs)

There is also evidence from both in vitro and more physiological studies that LA increases or maintains cellular GSH levels by acting as a tran-scriptional inducer of genes governing GSH synthesis**.** Treatment with LA increases hepatic nuclear Nrf2 levels and induces Nrf2-mediated gene transcription in vivo (Suh et al. 2004). Nrf2 is a key transcription factor that

mediates the expression of antioxidant and detoxification genes regulated by the antioxidant response element (ARE). Nuclear Nrf2 levels determine the expression of these genes, including those for GSH synthesis. Nrf2, in association with small Maf and Jun protein family, forms an upstream transcriptional complex. This heterodimer state of Nrf2 binds to the ARE sequence of DNA and regulates ARE-driven genes that encode for detoxification enzymes as well as antioxidant proteins to augment the cellular first line defense system against oxidative stress (Anuranjani and Bala 2014). Synthesis of GSH occurs via a two-step ATP-requiring enzymatic process. The first step is catalyzed by glutamate-cysteine ligase (GCL). The second step is catalyzed by GSH synthase. A variety of activators such as LA release and translocate Nrf2 into the nucleus. Furthermore, LA elevates the CGL activity and increases GSH synthesis by reducing the ratio of cystine to cysteine; cysteine being the rate-limiting substrate for this reaction **(Figure 3)** (Lu 2013).

Further to the effects of LA on Nrf-2-mediated antioxidant gene expression, LA has also been shown to inhibit the activation of NF-KB. The NF-κB protein complex is located in the cytoplasm in an inactive form by its binding to a inhibitor of NF-κB (IκB) protein. After stimulation by a variety of stimuli, signal responsive IKK α and β (TNF-α-inducible IκB kinase complex named IKK1 and IKK2) are activated, resulting in the phosphorylation of IκB and its proteasomal degradation. IκB degradation liberates NF-κB, allowing it to translocate to the nucleus and induce gene expression. LA inhibits IκB degradation and NF-κB-dependent gene expression by inhibition of IKK2, suggesting that LA inhibits NF-κB activation independent of its antioxidant function. LA inhibits NF-κB activation at the level of or upstream of, IKK-α and IKK-β. MAPKs are important group of protein kinases in the signaling pathway that can transduce signals from the cell surface to changes in gene expression (Ying et al. 2011).

The clinical properties of LA are related to the molecular actions of LA on the insulin signaling pathway. 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 glucose transporters, an effect that might be mediated by p38 mitogen-activated protein kinase (p38MAPKs) (Figure 4) (Konrad et al. 2001). There is considerable evidence that a defect in glucose transport is responsible for the acquired insulin resistance of glucose uptake observed in diabetes. This metabolic impairment could conceivably be explained by a variety of defects in GLUT4 regulation including alterations in GLUT4 expression and translocation, defects in the insulin signaling pathway and alterations in the temporal and spatial pattern of signaling molecules (Karim et al.). It has been demonstrated that LA induced a rapid redistribution of GLUT1 and GLUT4, leading to stimulation of glucose uptake in adipocytes, and that it stimulated tyrosine and serine/threonine kinases (Yaworsky et al. 2000).

Biological functions of alpha-lipoic acid as a biological antioxidant

The chemical reduction and oxidation (redox) properties of LA suggest that it may have potent antioxidant potential. A significant number of studies now show that LA and its reduced form: DHLA directly scavenge ROS and reactive nitrogen species (RNS) species and protect cells against a host of insults where oxidative stress is part of the underlying etiology**.**

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 (Lorgis et al. 2010; Rochette et al. 2011). 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.

Lipoic acid: a DIRECT antioxidant

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(Packer et al. 1995). 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. Recently, LA and DHLA were shown to react with peroxynitrite: (ONOO-) a highly reactive oxidant species resulting from the rapid reaction of nitric oxide (▪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, [30] the direct reaction between LA or DHLA with peroxynitrite was not fast enough to be considered important under in vivo conditions (Trujillo and Radi 2002).

In vivo, several studies showed that dietary supplementation with LA induced a decrease in oxidative stress, while restoring diminished levels of the other antioxidants (Marangon et al. 1999). LA and DHLA may be able to regenerate endogenous antioxidants such as vitamins C and E, and has the beneficial property of neutralizing free radicals without itself becoming one in the process (Figure 3). Therefore, they can elicit their antioxidant actions in both the cytosol and plasma membrane in contrast to vitamin C (which is lipophobic) and vitamin E (which is lipophilic). Another important action of LA and DHLA, is the ability of reducing the oxidized forms of other antioxidants such as GSH; GSH being one of the most important low

molecular weight cellular antioxidants, buffering the thiol redox state (Petersen Shay et al. 2008).

Alpha-lipoic acid as an INDIRECT biological antioxidant

Antioxidant via metal chelation LA/DHLA are considered as chelator compounds because they are able to chelate divalent transient metal ions both in vivo and in vitro but by different mechanisms of action. Moreover, the actions of ALA/DHLA as chelating compounds do not cause metal depletion. Because of the presence of two thiol groups, LA and DHLA both have metal chelating properties. 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+}. 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. It has been demonstrated that LA had a profound dose-dependent inhibitory effect upon Cu^{2+}-catalyzed ascorbic acid oxidation (Ou et al. 1995). The R-enantiomer and racemic mixture of the drug seemed more effective than the S-enantiomer in metal chelation (Suh et al. 2005).

Antioxidant via electrophilic mechanisms

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 (Rochette et al. 2013c). 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 (Niki 2009). The peroxidation of membrane lipids results in the formation of several classes of reactive compounds such as malondialdehyde (MDA), acrolein, and 4-hydroxy-2-nonenal (HNE) (Rochette et al. 2013a). The endogenous generation of reactive aldehydes contribute to numerous disease pathologies by altering genomic, cell signaling, and metabolic processes (Fritz and Petersen). Reactive aldehydes 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 GSH.

Mitochondria are a major intracellular source of pro-oxidants and electrophilic stress and are reversibly responsive to changes in redox status. 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 (McLain et al.). 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 (Valdecantos et al.).

As a required cofactor for the reductive elimination of hydroperoxides by glutathione peroxidase, glutathione is rapidly oxidized resulting in the formation of a disulfide between two glutathione molecules. Glutathione reductase then catalyzes the conversion of oxidized glutathione (GSSG) to GSH maintaining a reductive environment. Protein glutathionylation is readily reversible through the action of glutaredoxin, an enzyme that regenerates reduced sulfhydryls on protein cysteine residues with the concomitant production of GSSG. Glutathionylation of KGDH protected LA from modification by the electrophilic lipid peroxidation product 4-hydroxy-2-nonenal (Applegate et al. 2008).

Lipoic acid -induced heme-oxygenase-1 (HO-1) expression and cellular protection

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 (Chan et al. 2011). It has been demonstrated in vitro that LA induced HO-1 expression via Nrf2 in human monocytes (Ogborne et al. 2005). The consequences of HO-1 induction by LA in monocytes are potentially important for vascular disease. HO-1 is a cytoprotective molecule, which has potent anti-inflammatory cardioprotective properties (Rochette et al. 2013a).

Lipoic acid as a pro-oxidant

Paradoxically, LA is able to act as a **pro-oxidant**. Pro-oxidant effect of ALA is also described in experimental studies, but it is generally observed at higher concentrations than the usual plasmatic concentration observed after oral or intravenous infusion of ALA found in human studies. 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 (Moini et al. 2002a; Moini et al. 2002b). 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 (Dinkova-Kostova and Talalay 2008; Zhang et al. 2004). In fact, the pro-oxidant action of ALA/DHLA is not fully understood but could be related to different direct or indirect reactions and is related with the pleiotropic actions of ALA or DHLA in the different organs and systems.

Effects of LA, DHLA on cellular glucose uptake

Evidence suggests that the insulin-signaling pathway is sensitive to 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 (Khanna et al. 1999) and insulin-resistant muscle tissues. Studies on muscle cell lines indicate that exposure to LA stimulates glucose uptake by the redistribution of glucose transporters to the plasma membrane, and tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) (Konrad et al. 2001).

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. 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 (Khanna et al. 1999) and insulin-resistant muscle tissues (Streeper et al. 1997). In type II diabetes mellitus, there is evidence that LA ameliorates insulin resistance and impaired glucose metabolism in the periphery (Lee et al. 2005).

LA may intercept with the insulin-signaling pathway by directly or indirectly (through the induction of intracellular ROS) oxidizing components of the insulin-signaling cascade. There is evidence that the insulin-mediated increase in cell-surface GLUT4 does not suffice to fully account for the increase in glucose uptake in response to insulin: insulin may also increase the intrinsic activity of GLUT4, in addition to increasing its abundance at the plasma membrane. It was hypothesized that insulin increases the intrinsic activity of translocated GLUT4 via a p38 MAPK-dependent pathway (Figure 4). (Konrad et al. 2001). LA was found to stimulate glucose uptake and the increase in glucose uptake was accompanied by rapid translocation of the glucose transporters GLUT4 from an internal membrane fraction to the plasma membrane. Similar to insulin, treatment with LA resulted in increased tyrosine phosphorylation of the insulin receptor (IR) and substrate (IRS-1). LA is often referred to as an insulinomimetic agent (Moini et al. 2002b). In summary, Extensive evidence suggests that LA has potential therapeutic value in lowering glucose levels in diabetic conditions and that the intracellular redox status plays a role in the modulation of insulin action and insulin resistance.

Effects of lipoic acid on mitochondria metabolism

Mitochondria are important regulators of cell functions. 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. Paradoxically, LA and DHLA at concentrations between 0.01 and 0.1 mM promote mPTP opening. There is now evidence that the mitochondrial Krebs cycle enzyme, alpha-ketoglutarate dehydrogenase (KGDH), is a component of the mitochondrial antioxidant system and a key sensor of redox status. A variety of co-factors such as LA are utilized by KGDH to catalyze the multistep reaction. These findings may help to explain some pathological conditions and diseases associated with aging (McLain et al.).

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 (Gustafsson and Gottlieb 2008; Javadov and Kuznetsov 2013). 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 and antioxidant compounds with reduced SH groups (Chernyak and Bernardi 1996; Zoratti and Szabo 1995).

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(Moini et al. 2002a). 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 The DHLA-induced increase in the signal was reduced by two radical scavengers: BHT and TEMPO (Morkunaite-Haimi et al. 2003).

Clinical applications of lipoic acid: treatments for prevention and treatment of diabetes

In the cardiovascular area, dietary supplementation with LA has been successfully tested in a variety of in vivo model conditions and clinical situations associated with an imbalance of redox status: ischemia-reperfusion, heart failure, hypertension, and diabetes. 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. There is an abundance of evidence suggesting that insulin resistance plays a significant role in the physiopathology of the vasculature (Diabetes, oxidative stress and therapeutic strategies (Rochette et al. 2014).

Patients with diabetes exhibit peripheral arteriole lesions that are associated with reduced blood flow. Endothelial dysfunction, vascular smooth muscle cell dysfunction, inflammation and hypercoagubility are the key factors in diabetic–peripheral arterial disease (Marso and Hiatt 2006). Microangiopathy is mainly characterized by the impaired ability of capillaries to vasodilate in response to injury. Part of this impaired hyperaemic response may be due to the rigidity of the thickened basement membrane. Other factors play a significant role, especially endothelial dysfunction, which appears to be partially linked to metabolic diseases (Richard et al. 2012).Protective effects of LA against cardiometabolic diseases have also been studied in experimental conditions. Since there is strong evidence that excess dietary NaCl is associated with the development of hypertension , it has been determined whether giving salt-sensitive rats a dietary supplement of 500 mg/kg of LA could lower blood pressure (Abbott et al. 2004). The results showed that 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 (Vasdev et al. 2005). 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 (El Midaoui and de Champlain 2002). In these experimental conditions, dietary supplements of LA for 3 weeks prevented the rise of systolic blood pressure and the development of insulin resistance, as reflected by a higher homeostasis model assessment (HOMA). The antihypertensive and the hypoglycemic effects of LA appeared to be associated with its anti-oxidative properties.

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 (Grassi et al. 2009). LA presents beneficial effects in the treatment of symptomatic diabetic neuropathy. 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 (Ziegler et al. 2011). 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 (Ziegler 2004).

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 after 5 years of diabetes, increasing to 60% after 10 years (Fong et al. 2004). 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 (Klein and Klein 1997). 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 (Haritoglou et al. 2011). 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.

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 (Golbidi et al. 2011; Singh and Jialal 2008), 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 (Koufaki 2014). 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.

Summary and conclusion remarks

LA, a natural thiol, or its reduced form, DHLA possess many biochemical functions acting as biological antioxidants, as metal chelators, able to regenerate endogenous antioxidants such as vitamins C and E, and modulator of the signaling transduction of several pathways. The LA/DHLA couple has been called the “universal antioxidant”. Furthermore, many studies have reported that LA can regulate the transcription of genes associated with anti-oxidant and anti-inflammatory pathways. 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 and clinical trials. In the future, a combination of currently used pharmaceutics, together with natural anti-oxidative compounds, such as LA could be taken into account in the design of both in the prevention and in the treatment of diabetes and other metabolic diseases.

Conflict of interest

The authors confirm that this article content has no conflicts of interest.

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Legend to Figures

Figure 1.
Chemical structures of lipoic acid (LA), dihydrolipoic acid (DHLA) and lipoyllysine (LA attached to biologic lysine residues).
* the structure contains a chiral center.

Figure 2. The main transformations of LA/DHLA redox couple.
Mitochondrial synthesis of LA. Cellular uptake of exogenous LA and its extracellular release; 1)mitochondrial reduction of R-LA to R-DHLA in presence of dihydro-lipoamide dehydrogenase and 2) cytosolic reduction of S/R-LA to S/R-DHLA in presence of glutathione reductase or thioredoxin reductase. The link between LA/DHLA system and cellular cysteine uptake and GSH synthesis.

Figure3 : LA and DHLA regenerate endogenous antioxidants such as vitamins C, E and GSH.
The pathways of the antioxidants: glutathione: GSH, LA, and dihydrolipoic acid (DHLA). LA and DHLA increase the efficiency of the vitamin C cycle and activate the vitamin E cycle. thioredoxin; NADPH-dependent thioredoxin reductase; unsaturated lipid (LH); lipid hydroperoxides; peroxyl radical (LOO°).

Figure 4: Antioxidant mechanisms of LA/DHLA and modulation of intracellular thiol redox status: activation of insulin-signaling pathway and Nrf2-ARE signaling pathway.
Intracellular thiol redox changes induce insulin receptor (IR) phosphorylation with activation of some kinases : IRS1/PI3-kinase/Akt that enhance GLUT4 translocation or p38 MAPK that influence GLUT 4 abundance in cells membranes and increase its intrinsic activity. The increase in GLUT 4 translocation and activation enhances intracellular glucose uptake and reduces glycemia. (IRS1 - insulin receptor substrate, IP3-kinase - phosphatidylinositol 3-kinase, p38 MAPK - mitogen-activated protein kinase.)
Oxidation of some cysteine residues from Keap1, the structure that binds and inactivates Nrf2, is followed by release and activation of Nrf2. Subsequently it localizes in nucleus where it binds ARE-gene, enhancing the transcription gene for γ-glutamyl cysteine ligase (GCH), that increases GCH activity and GSH synthesis. (ARE - Antioxidant Response Element, GCH is the rate enzyme-limiting in GSH synthesis). Figure 4: Antioxidant mechanisms of LA/DHLA and modulation of intracellular thiol redox status: activation of insulin-signaling pathway Nrf2-ARE signaling pathway.
Intracellular thiol redox changes induce insulin receptor (IR) phosphorylation with activation of some kinases : IRS1/PI3-kinase/Akt that enhance GLUT4 translocation or p38 MAPK that influence GLUT 4 abundance in cells membranes and increase its intrinsic activity. The increase in GLUT 4 translocation and activation enhances intracellular glucose uptake and

reduces glycemia. (IRS1 - insulin receptor substrate, IP3-kinase - phosphatidylinositol 3-kinase, p38 MAPK - mitogen-activated protein kinase.)

Oxidation of some cysteine residues from Keap1, the structure that binds and inactivates Nrf2, is followed by release and activation of Nrf2. Subsequently it localizes in nucleus where it binds ARE-gene, enhancing the transcription gene for γ-glutamyl cysteine ligase (GCH), that increases GCH activity and GSH synthesis. (ARE - Antioxidant Response Element, GCH is the rate enzyme-limiting in GSH synthesis).

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