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REVIEW

Antioxidant and Prooxidant Activities of α-Lipoic Acid and Dihydrolipoic Acid

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Reactive oxygen (ROS) and nitrogen oxide (RNOS) species are produced as by-products of oxidative metabolism. A major function for ROS and RNOS is immunological host defense. Recent evidence indicate that ROS and RNOS may also function as signaling molecules. However, high levels of ROS and RNOS have been considered to potentially damage cellular macromolecules and have been implicated in the pathogenesis and progression of various chronic diseases. α-Lipoic acid and dihydrolipoic acid exhibit direct free radical scavenging properties and as a redox couple, with a low redox potential of −0.32 V, is a strong reductant. Several studies provided evidence that α-lipoic acid supplementation decreases oxidative stress and restores reduced levels of other antioxidants *in vivo***. However, there is also evidence indicating that α-lipoic acid and dihydrolipoic acid may exert prooxidant properties** *in vitro***. α-Lipoic acid and dihydrolipoic acid were shown to promote the mitochondrial permeability transition in permeabilized hepatocytes and isolated rat liver mitochondria. Dihydrolipoic acid also stimulated superoxide anion production in rat liver mitochondria and submitochondrial particles. α-Lipoic acid was recently shown to stimulate glucose uptake into 3T3-L1 adipocytes by increasing intracellular oxidant levels and/or facilitating insulin receptor autophosphorylation presumably by oxidation of critical thiol groups present in the insulin receptor β-subunit. Whether α-lipoic acid and/or dihydrolipoic acid-induced oxidative protein modifications contribute to their versatile effects observed** *in vivo* **warrants further investigation.** © 2002 Elsevier Science (USA)

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α-Lipoic acid (LA) and its reduced form, dihydrolipoic acid (DHLA), have gained considerable attention due to their roles as biological thiol antioxidants. Several features have been described for LA, which make it an outstanding antioxidant (Packer *et al.*, 1995, 1997; Packer, 1998; Roy and Packer, 1998). LA readily crosses the blood–brain barrier and is a "metabolic antioxidant"; i.e., it is accepted by human cells as substrate and is reduced to DHLA. Therefore, unlike ascorbic acid, DHLA is not destroyed by quenching free radicals, but rather can be recycled from LA. Moreover, LA and DHLA are amphipathic molecules and may act as antioxidants both in hydrophilic and lipophilic environments. This review summarizes recent evidence about antioxidant and prooxidant properties of LA and DHLA.

Reactive Oxygen and Nitric Oxide Species, Their Function, and Oxidative Stress

Molecular oxygen is essential for the survival of all aerobic organisms. Partially reduced metabolites of molecular oxygen, such as superoxide anion and hydrogen peroxide, are formed during normal metabolism in mitochondria and peroxisomes and also by the activities of a variety of enzyme systems such as cytochrome P-450, plasma membrane associated oxidases, lipoxygenase, and xanthine oxidase. Hydrogen peroxide, though a weaker oxidizing agent than superoxide anion, functions as an intermediate in the production of more reactive and toxic oxygen metabolites, such as hypochlorous acid formed by the action of myeloperoxidase and hydroxyl radical formed via oxidation of transition metals. These partially reduced metabolites of molecular oxygen are referred to as reactive oxygen species (ROS) due to their higher reactivities relative to molecular oxygen. When produced in high concentrations, nitric oxide (NO) also functions as a source of highly toxic oxidants collectively called reactive nitrogen oxide species (RNOS), including peroxynitrite, nitroxyl, and nitrogen dioxide, which are formed via reaction of NO with superoxide anion or molecular oxygen (Thannickal and Fanburg, 2000; Espey *et al.*, 2000; Nordberg and Arner, 2001).

A major function for ROS and RNOS is immunological host

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defense in which these molecules are generated as toxic agents by macrophages and neutrophils to eliminate microbes and other foreign molecules (Bastian and Hibbs, 1994). The important roles of NO in neurotransmission and regulation of blood pressure have been also well established (Ignarro, 1991; Prast and Philippu, 2001). Recent evidence obtained from nonphagocytic cells suggests that several cytokines, growth factors, hormones, and neurotransmitters trigger rapid production of ROS and/or RNOS, which may function as signaling molecules in various signal transduction pathways (Lander, 1997; Finkel, 2000). However, high levels of ROS and RNOS have been considered to potentially damage cellular macromolecules, such as lipids, proteins, and DNA.

The harmful effects of ROS and RNOS can be counteracted by the antioxidant defense system, which consists of enzymatic scavengers such as superoxide dismutase, catalase, and glutathione peroxidase and nonenzymatic low-molecular-weight antioxidant compounds such as reduced glutathione (GSH) and thioredoxin (Thannickal and Fanburg, 2000; Nordberg and Arner, 2001). Thus, oxidative stress may be broadly defined as an imbalance between oxidant production and the antioxidant capacity of the cell to prevent oxidative injury. Although several reactions in biological systems contribute to the steady-state concentrations of superoxide anion and hydrogen peroxide, mitochondria seem to be quantitatively the most important cellular source (Cadenas and Davies, 2000). Overproduction of ROS and RNOS has been implicated in the pathogenesis and progression of chronic inflammatory disease, atherosclerosis, cancer, diabetes, and the aging process (Cross *et al.,* 1987; Halliwell *et al.,* 1992). Oxidative stress-induced inner mitochondrial membrane permeabilization has been also considered to play an important role in cell death in a variety of pathological states, such as ischemia/reperfusion and age-associated neurodegenerative disease (Kristian and Siesjo, 1998; Vieira and Kroemer, 1999).

Sources of α-Lipoic Acid

Naturally occurring *R*-enantiomer of LA is an essential cofactor in α-ketoacid dehydrogenase complexes and the glycine cleavage system, where it is covalently attached in an amide linkage to the ε-amino group of a lysine residue, and hence presents as lipoamide (Reed, 1974). Vegetables and animal tissues contain low amounts of *R*-LA detected in the form of lipoyllysine. The most abundant plant sources of *R*-LA are spinach, followed by broccoli and tomatoes, which contain 3.15 ± 1.11, 0.94 ± 0.25, and 0.56 ± 0.23 μg lipoyllysine/g dry wt, respectively. The highest concentration of lipoyllysine in animal tissues was found in kidney, heart, and liver containing 2.64 ± 1.23, 1.51 ± 0.75, and 0.86 ± 0.33 μg lipoyllysine/g dry wt, respectively (Lodge *et al.,* 1997).

Synthetic racemic LA, a 1:1 mixture of *R*- and *S*-enantiomers, has been long used as a therapeutic agent in the treatment of diabetic neuropathy (Packer *et al.,* 2001) and as a nutritional supplement in European countries and the United States. The pharmacokinetics of exogenously administered LA were investigated in healthy volunteers. The mean peak plasma concentration of LA following a single oral administration of 200 or 600 mg LA was 3.1 ± 1.5 or 13.8 ± 7.2 μM, respectively. The mean peak plasma concentration of LA after intravenous administration of 200 mg LA was found to be 40.3 ± 11.3 μM. The mean plasma half-life of LA was approximately 30 min for iv or oral administration (Teichert *et al.,* 1998). It is noteworthy that the plasma concentration of LA was reported to reach a level of 100 to 200 μM in diabetic patients following intravenous administration of 600 mg LA (Rosak *et al.,* 1996).

FIG. 1. Chemical structure of α-lipoic acid (A) and dihydrolipoic acid (B).

Chemistry, Uptake, and Metabolism of α-Lipoic Acid

α-Lipoic acid is a disulfide derivative of octanoic acid that forms an intramolecular disulfide bond in its oxidized form (Fig. 1). High electron density resulting from special position of the two sulfur atoms in the 1,2-dithiolane ring confers upon LA a high tendency for reduction of other redox-sensitive molecules according to environmental condition (Biewenga and Bast, 1995). Similar to other vicinal thiols, DHLA is more easily oxidized than monothiols, leading to high activity in –SH/S–S– interchange reactions. With a low redox potential of −0.32 V, the LA/DHLA redox couple is a strong reductant (Searls and Sanadi, 1960).

Exogenously supplied LA (1.65 g/kg diet, 5 weeks) was absorbed, transported to tissues, and reduced to DHLA in adult hairless mice (Podda *et al.,* 1994). Two enzyme systems were identified to reduce LA to DHLA (Pick *et al.,* 1995). The mitochondrial dihydrolipoamide dehydrogenase is capable of reducing LA to DHLA at the expense of NADH. This enzyme shows a marked preference for the natural, *R*-enantiomer of LA. Cytosolic glutathione reductase also catalyzes a slower reduction of LA, with a preference for the *S*-enantiomer at the expense of NADPH. Hence, various tissues may differ in their relative rates of NADH- or NADPH-dependent reduction of LA enantiomers according to their mitochondrial content (Haramaki *et al.,* 1997).

α-Lipoic acid was shown to be catabolized largely through β-oxidation of the valeric acid side chain in rats. Major metabolites identified were bisnorlipoic acid, tetranorlipoic acid, and β-hydroxy-bisnorlipoic acid (Harrison and McCormick, 1974). After single oral dose of 1 g LA to healthy humans, an intermediate metabolite in the course of β-oxidation, 3-ketolipoic acid, and dimethylated products following β-oxidation, such as 4,6-bismethylmercapto-hexanoic acid and 2,4-bismethylmercapto-butanoic acid, were identified (Locher *et al.,* 1995;

Biewenga *et al.,* 1997). In a comprehensive study, the excretion and biotransformation of LA were recently investigated following single oral dose of ^{14}C-labeled LA to mice (30 mg/kg), rats (30 mg/kg), and dogs (10 mg/kg) and unlabeled LA to humans (600 mg) (Schupke *et al.,* 2001). Mitochondrial β-oxidation was found to play major role in the metabolism of LA and a total of 12 metabolites were identified. The circulating metabolites were found to be subjected to reduction of the 1,2-dithiolane ring and subsequent S-methylation. This study also provided evidence suggesting that conjugation with glycine may occur as a separate metabolic pathway in competition with β-oxidation, predominantly in mice.

Antioxidant Activities of α-Lipoic Acid and Dihydrolipoic Acid

Direct radical scavenging properties. Using various model systems, LA and DHLA was found to be highly reactive against a variety of ROS *in vitro*. LA at concentrations of 0.05–1 mM scavenged hydroxyl radical, hypochlorous acid, and singlet oxygen. LA also formed stable complexes with Mn^{2+}, Cu^{2+}, and Zn^{2+} and chelated Fe^{2+}. DHLA (0.01–0.5 mM), however, was shown to scavenge hydroxyl radical, hypochlorous acid, peroxyl radical, and superoxide radicals and to chelate both Fe^{2+} and Fe^{3+} (Packer *et al.,* 1995; Matsugo *et al.,* 1996). Several studies have also explored the reactivity of LA and/or DHLA toward RNOS. Both LA and DHLA at concentrations of 0.01–0.5 mM efficiently protected against peroxynitrite-induced inactivation of α_1-antiproteinase and inhibited nitration of L-tyrosine by peroxynitrite (Whiteman *et al.,* 1996; Nakagawa *et al.,* 1999). Recently, LA and DHLA were shown to react with and to decompose peroxynitrite. However, the rate of direct reactions between LA or DHLA with peroxynitrite was not fast enough to be considered important under *in vivo* conditions (Trujillo and Radi, 2002). LA and LA-plus, a synthetic amide analog of LA with better cellular reduction and retention than DHLA, were also found to be ineffective in scavenging NO. However, LA or LA-plus at a concentration of 0.1 mM inhibited NO production from macrophages stimulated with lipopolysaccharide and interferon-γ (Guo *et al.,* 2001).

Redox interactions with other antioxidants. In addition to direct scavenging activity, LA/DHLA redox couple appears to be able to regenerate other antioxidants. Considering a redox potential of -0.32 V for the LA/DHLA redox couple compared to that of the GSH/GSSG couple (-0.24 V), DHLA is able to directly reduce GSSG to GSH (Jocelyn, 1967). Intraperitoneal (5–16 mg/kg body wt/day, 11 days) or intragastric (150 mg/kg body wt/day, 8 weeks) LA administration to rats also increased glutathione levels in various tissues *in vivo* (Busse *et al.,* 1992; Bustamante *et al.,* 1998; Khanna *et al.,* 1999). Mechanistic studies conducted in human cells in culture demonstrated that, following intracellular reduction of LA, DHLA was rapidly released into the extracellular space and reduced cystine to cysteine, whereupon cysteine was taken up by the neutral amino acids transporters and was used in glutathione synthesis. Enhancement of intracellular availability of cysteine, by bypassing the rate-limiting cystine transport system, was proposed to be the underlying mechanism of LA-induced elevation of GSH levels observed both *in vitro* and *in vivo* (Han *et al.,* 1997). In addition to increasing cellular GSH level, studies conducted *in vitro* demonstrated that DHLA (2 mM) reduced dehydroascorbate and the semidehydroascorbyl radical (Kagen *et al.,* 1992). There is also evidence indicating that intraperitoneal LA (0.2 mg/mouse/day, 3 days) or DHLA (0.8 mg/mouse/day, 3 days) administration may increase cerebral ubiquinol levels in mice (Gotz *et al.,* 1994). Hence, reduction of GSSG, dehydroascorbate, and/or ubiquinone by the LA/DHLA couple may contribute to vitamin E regeneration from its oxidized form in biological systems (Packer and Suzuki, 1993; Packer *et al.,* 1995).

In vivo antioxidant activity. Using various experimental models, several studies provided evidence that LA supplementation decreases oxidative stress and restores reduced levels of other antioxidants under various physiological and pathophysiological conditions *in vivo*. LA supplementation (600 mg/day, 2 months) to healthy humans decreased urinary F2-isoprostanes levels, a biomarker of lipid peroxidation, and increased the lag time of their LDL oxidation *ex vivo* (Marangon *et al.,* 1999). Physical exercise has been repeatedly shown to be associated with elevated oxidative stress (Witt *et al.,* 1992; Ji, 1995). Intragastric LA administration (150 mg/kg body wt/day, 8 weeks) to rats protected against exhaustive exercise-induced oxidative lipid damage in heart, liver, and skeletal muscle; increased total glutathione levels in liver and blood; and prevented exercise-induced decline in heart glutathione *S*-transferase activity (Khanna *et al.,* 1999).

Aging is believed to be due to the lifelong production of ROS and RNOS as by-products of oxidative metabolism that lead to the accumulation of DNA, lipid, and protein damage at multiple cellular and tissue level, which eventually induces the appearance of the age phenotype at the organismal level (Finkel and Holbrook, 2000). Dietary supplementation of LA [0.2% (wt/wt)] for 2 weeks markedly lowered age-associated increase in oxidant production by cardiac myocytes, restored the decline in myocardial ascorbic acid level, and reduced oxidative DNA damage in cardiac tissue of old rats (Suh *et al.,* 2001). Similarly, intraperitoneal administration of LA (100 mg/kg body wt/day, 2 weeks) attenuated cerebral lipid peroxidation and prevented age-associated decrease in the levels of ascorbic acid, vitamin E, and GSH in old rat brain (Arivazhagan and Panneerselvam, 2000). At the mitochondria level, in addition to GSH, ascorbic acid, and vitamin E, intraperitoneal LA administration (100 mg/kg body wt/day, 2 weeks) increased the reduced activities of enzymes such as isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and succinate dehydrogenase in mitochondria isolated from old rat liver or

kidney (Arivazhagan *et al.,* 2001). Dietary supplementation of LA [0.5% (wt/wt), 2 weeks] also completely reversed the age-related decline in hepatocellular GSH levels and the increased vulnerability of hepatocytes to *tert*-butylhydroperoxide (Hagen *et al.,* 2000).

It should be emphasized that several other studies have also provided evidence for protective effects of LA supplementation against oxidative tissue damage in various animal models of ischemia–reperfusion, hepatic disorders, and diabetes (Packer, 1994; Bustamante *et al.,* 1998; Packer *et al.,* 2001). Due to its versatile antioxidant activities, LA administration as a chemoprotector has been suggested to alleviate the severity of toxic side effects of anticancer drugs such as cisplatin, which is widely used against a variety of human neoplasms (Rybak *et al.,* 1999). The clinical use of cisplatin is limited by the onset of severe side effects, such as nephrotoxicity, ototoxicity, and peripheral neuropathy. Several lines of evidence suggest that cisplatin-induced toxicity is mediated by ROS as a consequence of antioxidant depletion in tissues such as cochlea and kidney (Rybak *et al.,* 1995). Intraperitoneal administration of a single dose of LA (25–100 mg/kg body wt) was shown to restore diminished activities of renal and cochlear superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase and to suppress elevated lipid peroxidation in cochlea and kidney of cisplatin-injected rats (Rybak *et al.,* 1999; Somani *et al.,* 2000).

Prooxidant Actions of α-Lipoic Acid and Dihydrolipoic Acid

Redox reactions with free and heme iron. Using various model systems LA and/or DHLA was reported to exert prooxidant properties *in vitro*. DHLA (0.5 mM) accelerated iron-dependent hydroxyl radical generation and lipid peroxidation in liposomes, probably by reducing Fe^{3+} to Fe^{2+}. This prooxidant action of DHLA was inhibited by LA. Under certain circumstances, DHLA at a concentration of 0.1 mM accelerated inactivation of α_1-antiproteinase exposed to ionizing radiation under N_2O/O_2 atmosphere while at a concentration of 1 mM it increased the loss of creatine kinase activity in human plasma exposed to gas-phase cigarette smoke (Scott *et al.,* 1994).

In a comprehensive study, the reactivity of LA and DHLA as well as other thiol compounds toward different redox states of myoglobin was evaluated. In contrast to the disulfides of glutathione and cysteine, LA efficiently interacted with the high oxidation state of myoglobin, ferrylmyoglobin, by either directly reducing the heme iron to form metmyoglobin or by reacting with the pyrrole ring to form sulfmyoglobin. Cysteine and GSH reduced ferrylmyoglobin to metmyoglobin or oxymyoglobin with the formation of thiyl radicals. Dihydrolipoic acid also reduced metmyoglobin to oxymyoglobin; however, no thiyl radical was detected (Romero *et al.,* 1992). DHLA thiyl radicals was recently observed by electron spin resonance (ESR) spectrometry through an oxidation process referred to as "thiol pumping," in which phenol was oxidized to phenoxyl radical in the presence of horseradish peroxidase and hydrogen peroxide and the phenoxyl radicals so formed were used to oxidize 5 mM DHLA. In this system, ESR signals for the DHLA thiyl radical and the disulfide radical anion were observed. The disulfide radical anion reacts rapidly with molecular oxygen to form superoxide anion, which was also trapped in this study (Mottley and Mason, 2001).

Effect on mitochondrial permeability transition. The mitochondrial permeability transition (MPT) is a nonselective permeabilization of the inner mitochondrial membrane, which may result in loss of matrix components, substantial swelling of the organelle, outer membrane rupture, cytochrome *c* release, and eventually cell death (Zoratti and Szabo, 1995). MPT is typically promoted by accumulation of Ca^{2+} ions, oxidation of mitochondrial NADPH, inorganic phosphate, and thiol oxidants, whereas it is prevented by thiol reductants and mitochondrial $NADP^+$ reduction (Zoratti and Szabo, 1995; Valle *et al.,* 1993; Fagian *et al.,* 1990). Accumulating evidence suggests that MPT might be indeed related to the redox state of mitochondria and that the link between mitochondrial Ca^{2+} accumulation and MPT might be oxidative stress (Kowaltowski *et al.,* 2001). LA and DHLA at concentrations of 0.010–0.1 mM were shown to promote MPT in permeabilized hepatocytes and isolated rat liver mitochondria (Saris *et al.,* 1998). Despite being a dithiol, DHLA more effectively stimulated MPT than LA, suggesting that mechanisms other than oxidation to LA might be involved. Presence of desferal, an iron chelator, in the incubation buffer had no effect on DHLA-induced MPT, indicating that reduction of contaminating buffer Fe^{3+} and, consequently, free radical generation by iron redox cycling is not involved in the action of DHLA on MPT (Morkunaite-Haimi and Saris, personal communication). However, DHLA-induced MPT was inhibited by the radical scavenger butylhydroxytoluene (2,6-di-*ter*-butyl-4-methylphenol), but it was slightly potentiated in the presence of ascorbic acid (Saris *et al.,* 1998). In comparison to the other mitochondrial electron transport chain substrates, MPT was most sensitively stimulated by DHLA when pyruvate was used as the substrate and showed similar characteristics to that observed in presence of the superoxide-generating system, xanthine *plus* xanthine oxidase (Morkunaite-Haimi *et al.,* 2000). Indeed, it was recently found that DHLA at concentrations of 0.01–0.1 mM stimulated superoxide anion production in rat liver mitochondria and submitochondrial particles, indicating that DHLA-induced MPT might be mediated by superoxide anion (Morkunaite-Haimi and Saris, personal communication).

There is evidence indicating that LA may directly oxidize vicinal thiol groups present in proteins. LA (0.05–0.5 mM) was shown to inactivate both purified and microsomal NADPH–cytochrome P450 reductase by oxidizing its thiol groups (Slepneva *et al.,* 1995). However, it is not known whether

LA-induced MPT is due to direct oxidation of critical thiol groups present in mitochondrial membrane proteins that are involved in the pore assembly. LA (1 mM) was previously shown to stimulate mitochondrial Ca^{2+} release, presumably by oxidizing some protein vicinal thiols. LA-stimulated Ca^{2+} release was inhibited by cyclosporine A, a major inhibitor of MPT. Furthermore, DHLA (1 mM) stimulated Ca^{2+} release to the same extent as LA, but only after a lag phase, which was suggested to indicate that oxidation to LA was required (Schweizer and Richter, 1996). It should be also emphasized that LA and α-lipoamide (0.2 mM) were recently reported to prevent oxidant-induced apoptosis in cultured J774 cells by stabilizing lysosomes against oxidative stress (Persson *et al.*, 2001). This protective effect was attributed to chelation of intralysosomal iron by LA or α-lipoamide, which might consequently prevent intralysosomal Fenton reactions and rupture of lysosomal membranes.

Effect on cellular glucose uptake. LA was shown to lower elevated glucose levels in animal models of diabetes and individuals with type 2 diabetes (Packer *et al.*, 2001). Mechanistic studies conducted in insulin-responsive cells in culture demonstrate that LA (2.5 mM) rapidly stimulates glucose uptake by activating the insulin-signaling pathway (Yaworsky *et al.*, 2000). Several lines of evidence suggest that the insulin-signaling pathway is sensitive to intracellular redox status and that oxidation of critical thiol groups present in the β-subunit of insulin receptor may increase its intrinsic tyrosine kinase activity and thereby activate insulin-signaling pathway (Schmid *et al.*, 1999). Short-term incubation of 3T3-L1 adipocytes with LA (0.25 mM) was recently shown to stimulate glucose uptake by increasing intracellular oxidant levels (Moini *et al.*, 2002). LA also increased tyrosine phosphorylation of immunoprecipitated insulin receptors, presumably by oxidation of critical thiol groups present in the insulin receptor β-subunit. However, long-term incubation of 3T3-L1 adipocytes with LA (0.25 mM) increased intracellular GSH levels and inhibited the rate of glucose uptake. These results provided evidence that LA modulates glucose uptake by changing the intracellular redox status and that the insulin receptor might be a potential cellular target for LA action (Moini *et al.*, 2002). However, it is not known whether LA modulates ROS-regulated signaling pathways *in vivo*. Recently, it was reported that levels of cortical ROS generation and cerebellar NO synthase are significantly depressed in 9-month-old mice relative to 3-month-old mice (Bondy *et al.*, 2002). Dietary supplementation of 9-month-old mice with melatonin (40 ppm, 6 months) restored both ROS and NO synthase levels while LA supplementation (1650 ppm, 6 months) restored NO synthase level to those found in 3-month-old mice. Dietary supplementation with ubiquinone (200 ppm, 6 months) or vitamin E (1000 ppm, 6 months), however, had no effect on either ROS generation or NO synthase level (Bondy *et al.*, 2002).

Concluding Remarks

Several lines of evidence indicate that LA exerts potent antioxidant activity *in vitro* and *in vivo*. Dietary supplementation with LA has been successfully utilized in a variety of model conditions associated with an imbalance of redox status, such as ischemia–reperfusion (Mitsui *et al.*, 1999), polyneuropathy (Packer *et al.*, 2001), diabetes (Packer *et al.*, 2001), AIDS (Patrick, 2000), hypertension (Vasdev *et al.*, 2000; Takaoka *et al.*, 2001), and hepatic disorders (Bustamante *et al.*, 1998). Recently, the fact that the versatile protective effects of LA and DHLA are entirely the result of their antioxidant activities has been questioned. It was speculated that LA might exert protective effects by catalyzing the formation of intramolecular disulfides in certain signaling proteins that function as detectors of oxidants and trigger heat-shock and phase II responses (McCarty, 2001). Some observations obtained from *in vitro* model systems support the idea that LA and/or DHLA may directly or indirectly cause oxidation of cellular proteins and thereby modulate biological processes. It should be noted that the physiological function of ROS and RNOS as modulators of cellular events, their chemical nature and intracellular levels, and their specific protein targets are not yet fully understood. Whether prooxidant effects of LA and/or DHLA are beneficial or harmful may depend on the biological system and will require careful evaluation. Nevertheless, the prooxidant effects of LA and DHLA warrant further investigation.

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