

α -Lipoic Acid Overview

2.1 Summary of the Benefits of α -Lipoic Acid

- α -Lipoic acid is well absorbed via the stomach and gut, and can easily be supplemented orally. It does not accumulate in tissues to a significant degree, and does not exhibit any clinically significant toxicity when taken in the amounts appropriate for preventative or therapeutic purposes (< 1500 mg/day).
- α -Lipoic acid is a natural antioxidant that effectively neutralizes a variety of free radicals, including oxygen radicals and ionized metals.
- α -Lipoic acid increases the tissue level of glutathione, which is the major intracellular antioxidant. Glutathione is reduced in a variety of diseases and with aging. Glutathione levels are also reduced during moderate to strenuous exercise.
- α -Lipoic acid interacts synergistically with other antioxidants. It regenerates both Vitamin C and Vitamin E, and it helps to maintain the proper ratio of reduced to oxidized Coenzyme Q₁₀ in the mitochondria.
- α -Lipoic acid functions as a cofactor (coenzyme) in enzyme complexes responsible for glucose utilization and energy metabolism. α -Lipoic acid is effective as an adjunct in the treatment of diabetes. It has been used widely for over 20 years in Germany to treat a wide array of pathologies, including those related to glucose intolerance.
- α -Lipoic acid protects the mitochondria. Mitochondria decay is associated with aging.
- α -Lipoic acid holds promise as a safe and effective approach for the treatment of other pathologies associated with lowered antioxidant status and/or increased levels of oxidative stress.

2.2 Scientific Rationale for the Development of Controlled Release α -Lipoic Acid

2.2.1 α -Lipoic Acid Background

α -Lipoic acid (LA; occasionally referred to as thioctic acid) is an eight-carbon disulfide compound containing a single chiral center (Figure 1). LA is reduced *in vivo* to its dithiol form, dihydrolipoic acid (DHLA). LA is synthesized in organisms ranging from bacteria to man. In humans, it is synthesized in liver and other tissues, where it functions as a natural cofactor in multi-enzyme dehydrogenase complexes, such as pyruvate dehydrogenase (PDH) and α -ketoglutarate dehydrogenase (8). Pyruvate dehydrogenase is localized in mitochondria where it catalyzes the oxidative decarboxylation of pyruvate to acetyl-CoA, a critical step in oxidative glucose metabolism (23). Thus, LA plays an essential role in mitochondrial-specific pathways that generate energy from glucose.

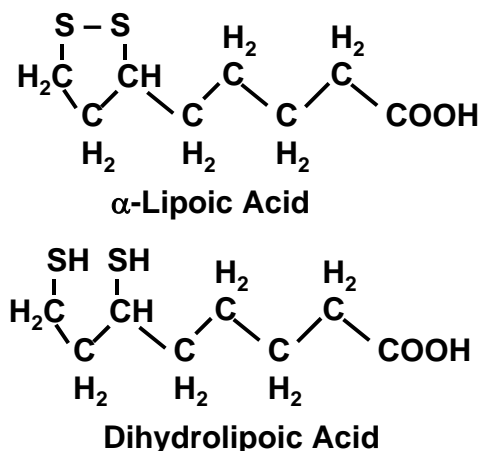


Figure 1. Chemical structures of α -lipoic acid and dihydrolipoic acid. α -Lipoic acid is an eight carbon disulfide compound containing a single chiral carbon. It is readily reduced *in vivo* to its dithiol form, dihydrolipoic acid. The R-enantiomer is the naturally occurring form of α -lipoic, while synthetic α -lipoic acid is a racemic mixture containing both the R- and S-enantiomers.

LA and its reduced form, dihydrolipoic acid (DHLA; Figure 1) are also potent antioxidants (10). Four distinct antioxidant actions of LA and DHLA have been observed: 1) reactive oxygen species (free radical) scavenging activity, 2) capacity to regenerate endogenous antioxidants such as glutathione, vitamin C and vitamin E, 3) metal chelating activity, and 4) repair of oxidized proteins (8,10). LA and DHLA function as a redox couple to regenerate endogenous antioxidants through a cooperative set of reactions. LA is soluble in both lipid and aqueous environments. Because LA is lipid soluble, it is highly effective at reducing free radicals, including lipid peroxides, in cellular membranes. Because LA is also water soluble, it is able to gain access to the cytosol, where it effectively scavenges free radicals at their mitochondrial source (8).

2.2.2 Free Radicals and Oxidative Stress

Free radicals are highly reactive molecular by-products produced in all cells as a result of normal metabolism, exercise, aging, and disease (Figure 2) (24-26). Due to their essential role in cellular metabolism, mitochondria are the chief sources of free radical production (26,27). Excessive production of free radicals or their inadequate neutralization by antioxidants leads to a condition known as oxidative stress. Free radicals interact with and damage proteins, lipids, and DNA leading to loss of function.

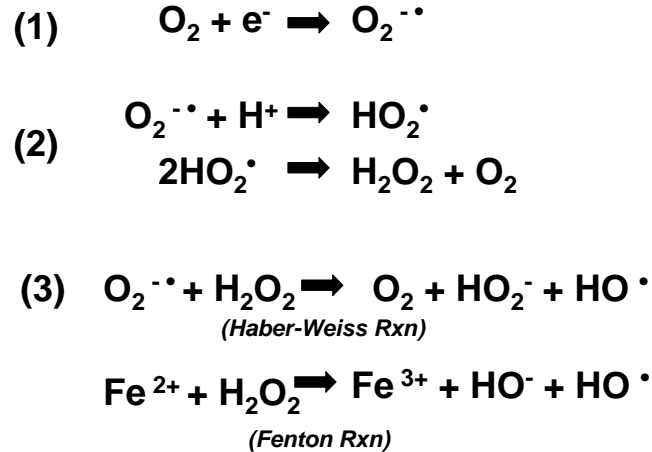


Figure 2. Selected examples of oxygen free radicals. (1) Superoxide anion is formed when molecular oxygen acquires an additional electron, (2) hydrogen peroxide can be generated by several metabolic reactions, e.g. from superoxide, and (3) hydroxyl radicals can be formed from either the superoxide anion or from hydrogen peroxide.

It is clear that increased oxidative stress is associated with a variety of pathological conditions including diabetes, atherosclerosis and cardiovascular disease, and neurodegenerative diseases (25,28-33). In particular, diabetes mellitus is strongly associated with increased oxidative stress (34-37), which could be a consequence of either increased production of free radicals (36,38-42), or reduced antioxidant defenses (43-46).

There is considerable evidence to indicate that oxidative stress plays an important role in the etiology of diabetic complications (47-50). Many of the biochemical pathways (e.g. protein glycation, polyol pathway, glucose autoxidation) associated with hyperglycemia can result in increased free radical production. Oxidative stress is not only associated with complications of diabetes, but has been also linked to insulin resistance (51-53). *In vitro*, oxidative stress causes insulin resistance at multiple levels (1). An additional potential target of oxidative stress is likely to be the β -cell (Figure 3).

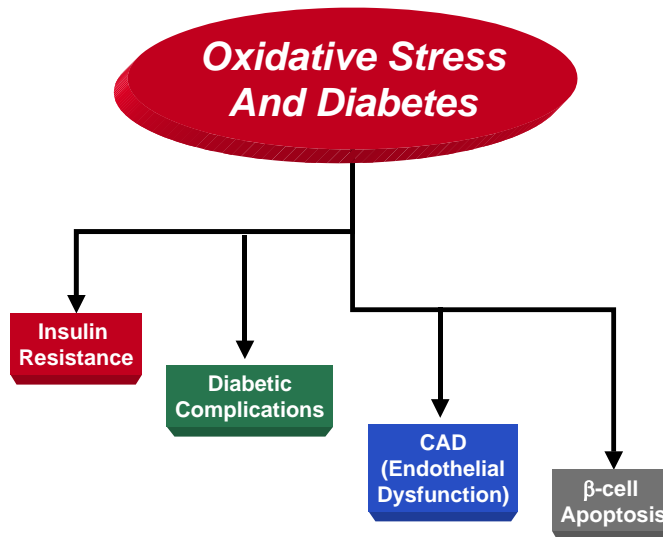


Figure 3. Potential areas of impact of oxidative stress in diabetes and the pre-diabetic state. Free radical generation leading to oxidative stress has the capacity to impact diabetes at multiple levels. The role of oxidative stress leading to microvascular complications has the most experimental support. Oxidative stress also plays a significant role in the development of macrovascular complications (coronary artery disease; CAD), including promoting atherosclerosis and the inhibition of nitric oxide mediated vasodilation (32). More recent evidence suggests an association of oxidative stress with both impaired insulin action (*in vitro and in vivo*) and the deterioration of β -cell function (1).

2.2.3 α -Lipoic Acid and Diabetes Mellitus

LA has been prescribed in Germany for over thirty years for the treatment of diabetic neuropathy (54,55). It is currently in clinical development for the same indication in the US. A comprehensive review of the clinical studies of LA in the treatment of diabetic neuropathy has recently been published by Ziegler *et al.* (6). More recently, several studies have shown that LA, when administered parenterally, improves insulin sensitivity in type 2 diabetics (1).

It is clear that IV administration of LA provides a metabolic benefit in type 2 diabetics by increasing insulin-stimulated glucose disposal (MCR) and insulin sensitivity (Figure 4). In contrast to IV LA administration, the improvement in insulin sensitivity following oral administration of LA is only minimal, ~20%. This is evident despite the higher doses employed (up to 1800 mg), and the longer treatment time (30 days oral vs.10 days IV) (7). Although the improvement in insulin sensitivity following oral administration of LA reached statistical significance in both studies, the magnitude borders on the limit of reproducibility for each of the two methods (*i.e.* euglycemic clamp and minimal model) (56,57), and should be regarded as a minimal effect, at best.

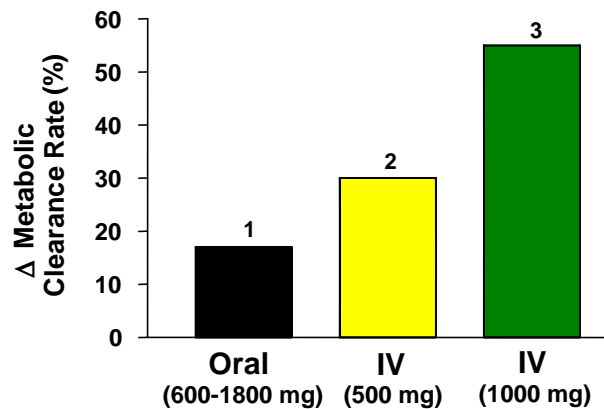


Figure 4. Effects of α -lipoic acid on insulin-stimulated glucose metabolism in individuals with type 2 diabetes. Intravenous (IV) administration of α -lipoic acid is able to significantly increase insulin sensitivity (as judged by % change (Δ) in metabolic clearance rate) in Type 2 diabetics, while oral administration exerts a minimal effect (see text for details). (1) Data replotted from (7), (2) Data replotted from (16), (3) Data replotted from (15).

2.2.4 Limited Efficacy of Current Oral Form of α -Lipoic Acid Due to Pharmacokinetic Profile

One possible explanation for the limited effect of oral LA on insulin sensitivity and lack of efficacy with regard to glucose control might be provided by the abbreviated time therapeutic plasma levels are maintained. This plasma profile is a function of the short half-life of LA, along with its extensive pre-systemic elimination, which is thought to be primarily hepatic (58). Human pharmacokinetic studies have found that LA possesses an extremely short plasma half-life of about 30 minutes after both oral and IV administration (2,58). Thus, following oral LA administration, a maximum plasma level is quickly reached, but falls just as quickly to a level insufficient to impact insulin sensitivity or glucose control. It is interesting to speculate that the superior ability of IV LA to improve insulin sensitivity might be due to the fact that IV administration achieves a higher plasma level of LA, and maintains it for a longer duration (2,58).

In this context, the question is raised as to whether maintaining a therapeutically effective level of LA in plasma for an appropriate length of time (*i.e.* mimicking the IV LA situation) would increase insulin sensitivity and eventually result in a beneficial impact on glucose control in individuals with type 2 diabetes. Until the introduction of Glucotize, there have been no commercially available oral solid-dosage forms of LA that could be used to pursue this idea. The clinical studies discussed above have used enteric-coated tablets, which protect LA from gastric acidity, but do not control the rate of LA release in plasma. This results in a 'spike' of LA. To test this hypothesis, MRI set out to develop and evaluate a novel and proprietary controlled release formulation of LA.

2.3 α -Lipoic Acid and Cardiovascular Disease

The endothelium lines the vasculature throughout the body where it was generally considered to function passively as a barrier, and also for transport. Over the last decade, it has become increasingly clear that the endothelium plays a broader role, and possesses a diverse assortment of physiological functions. Some of these include the regulation of vascular tone and blood fluidity, the modulation of monocyte adhesion, and lipid peroxidation. Endothelium-dependent regulation of vasomotor tone is mediated via the nitric oxide (NO) system. NO is a gas that is continuously produced in the endothelium from arginine in a reaction catalyzed by the enzyme nitric oxide synthase. The production of NO is also stimulated in response to growth factors and other agents. NO produced in the endothelium rapidly diffuses into the surrounding smooth muscle cells, where it binds to the enzyme guanylate cyclase resulting in the generation of cGMP. This second messenger causes smooth muscle relaxing which ultimately results in vasodilation.

There is an extensive body of literature indicating that the regulation of endothelial-mediated vasodilation is impaired in those who have established coronary artery disease, and in those who are at risk. Major risk factors include family history of CVD, elevated lipids (cholesterol and triglycerides), smoking, hypertension, diabetes, insulin resistance, and aging. Thus, impairment of endothelial-mediated vasodilation is accepted as a surrogate marker for the eventual development of CVD. Pharmacotherapy that improves the vasodilator function of the endothelium would potentially offer significant clinical benefits.

Impaired regulation of endothelial-mediated vasodilation can result from decreased synthesis or bioavailability of NO, or decreased NO action. The cause(s) of impaired regulation is unknown but a growing body of evidence indicates that increased oxidative stress plays a significant role (32). To be an

effective vasodilator, NO must diffuse from the endothelium to the underlying smooth muscle. There is increasing evidence that free radicals, molecules that stimulate the production of free radicals (e.g. advanced glycation end-products), and oxidized LDL all serve to neutralize or inactivate NO (59-62).

The negative impact of oxidative stress is also supported by a number of studies showing that antioxidants along with agents that elevate endogenous glutathione significantly improve endothelial-mediated vasodilation (63,64). In patients with coronary artery disease, oral administration of either a single dose (2 g) or multiple doses (500 mg/day for 30 days) of ascorbate significantly improved (approximately 2.4%-7.7%) brachial artery flow mediated dilation (65,66). These effects were significantly correlated with an increase in plasma ascorbate concentrations. The negative impact of oxidative stress is further supported by a study using L-2-oxo-4-thiazolidine carboxylate (OTC), a compound that elevates intracellular glutathione levels. In patients with coronary artery disease, administration of a single oral dose (4.5 g) of OTC significantly improved (4.4%) brachial artery flow mediated dilation (64).

As indicated earlier, α -lipoic acid and its reduced form, dihydrolipoic acid, are powerful antioxidants. α -Lipoic acid increases glutathione in tissue and regenerates endogenous levels of vitamins C and E. These properties along with its demonstrated safety and potency qualify it as a prime candidate to evaluate for its ability to improve endothelial dysfunction. In a preliminary study using normal individuals, oral administration of a single dose of Glucotize (600 mg) significantly improved brachial artery flow mediated dilation (unpublished data). These results provide further support for the involvement of oxidative stress in the impaired regulation of endothelial-mediated vasodilation, and identify Glucotize as a novel candidate therapy for the improvement of this condition.

2.4 α -Lipoic Acid and Aging

Aging is an inevitable biological event that is associated with a progressive decline in mitochondrial function (24,26,67). Work from several laboratories has revealed that mitochondrial membrane potential, respiratory control ratios, cellular oxygen consumption, cardiolipin content, and membrane fluidity decline with age, while proton leakage and oxidant production rise. The etiology of mitochondrial deterioration is primarily linked to increased oxidant formation resulting from aerobic metabolism, coupled with a general decline in endogenous antioxidant defenses. Increased oxidant formation imparts damage to mitochondrial DNA, protein, and lipids ultimately leading to impaired (or loss of) function. Increased oxidant formation is not restricted to aging, but is also evident in many age-associated pathologies such as diabetes, cardiovascular disease, neurodegenerative disease, cancer, and immune dysfunction (Figure 3). Therapeutic agents that reduce oxidative stress have the potential to improve mitochondrial function, combat disease, improve the quality of life, and delay the aging process.

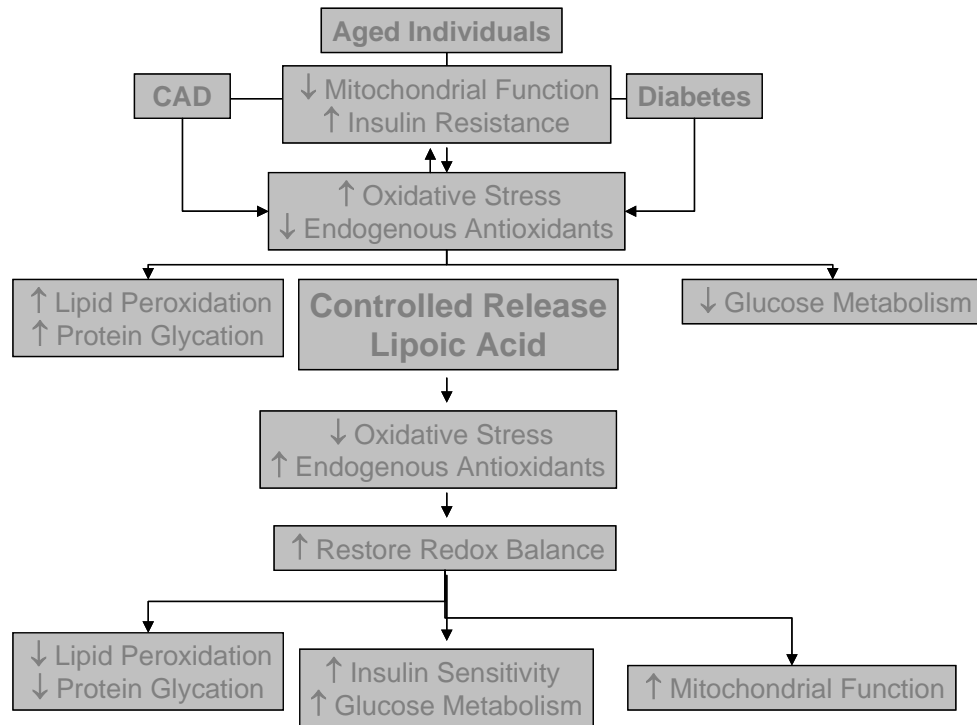


Figure 3. Association of increased oxidative stress and mitochondrial dysfunction with aging, diabetes, and coronary artery disease (CAD).

It has recently become apparent that the ability of α -lipoic acid to improve energy metabolism and decrease oxidative stress is not limited to disease states. α -Lipoic acid can partially reverse the decline in mitochondrial function and increase in oxidative stress associated with aging. Lykkesfeldt and colleagues have reported that dietary supplementation (*ad libitum* feeding) of aged rats for two weeks with α -lipoic acid (0.5%, w/w) reversed the age-associated impairment of hepatocyte ascorbate content and biosynthesis (68) More recently, this group has shown that α -lipoic acid treatment reversed the age-associated decline in hepatocyte mitochondrial O_2 consumption and increased mitochondrial membrane potential in rats (69). α -Lipoic acid treatment also reversed the age-associated decline in glutathione content, and attenuated the age-associated rise in lipid peroxidation. The improvement in mitochondrial metabolism and

antioxidant status of aged rats mediated by α -lipoic acid supplementation was reflected by a significant improvement in their motor activity. Although the plasma level of α -lipoic acid was not reported in these studies, it is likely that the normal feeding cycle of rodents resulted in a higher steady state plasma of α -lipoic acid compared to what can be achieved by oral administration of 'non-controlled release' dosage forms. Taken together, these data support the belief that α -lipoic acid supplementation provides multiple beneficial effects to the decline in mitochondrial function, energy metabolism, and oxidative stress associated with disease and aging, and that appropriate plasma levels need to be achieved to ensure maximum therapeutic benefit.

2.5 References

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