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Forum Review

Utilization of the Insulin-Signaling Network in the Metabolic Actions of α-Lipoic Acid—Reduction or Oxidation?

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ABSTRACT

α-Lipoic acid is a naturally occurring cofactor of mitochondrial dehydrogenase complexes and a potent antioxidant. It can interchange between a reduced form and an oxidized form, thereby displaying reducing (antioxidant) and prooxidant properties, respectively. It is suggested that α-lipoic acid through its prooxidant properties acutely stimulates the insulin-signaling cascade, thereby increasing glucose uptake in muscle and fat cells. On the other hand, α-lipoic acid appears to protect the insulin-signaling cascade from oxidative stress-induced insulin resistance through its reducing capacities. In addition, α-lipoic acid seems to inhibit hepatic gluconeogenesis by interfering with fatty acid oxidation, as well as to increase peripheral glucose utilization by activating pyruvate dehydrogenase resulting in increased glucose oxidation. These different properties render α-lipoic acid a potentially attractive therapeutic agent for the treatment of insulin resistance. Moreover, given the potential role of oxidative stress in the pathogenesis of secondary complications in diabetes, α-lipoic acid might be beneficial in the prevention/treatment of these complications as was recently shown for diabetic neuropathy. *Antioxid. Redox Signal.* 7, 1032–1039.

INTRODUCTION

α-Lipoic acid (1,2-dithiolane-3-pentanoic acid; LA), which is synthesized by plants and animals, has diverse biological functions. It is a cofactor of mitochondrial dehydrogenase complexes such as pyruvate dehydrogenase (PHD), α-ketoglutarate dehydrogenase, and branched chain α-ketoacid dehydrogenase. The PDH complex catalyzes the oxidative decarboxylation of pyruvate, linking glycolysis to the tricarboxylic acid cycle. Therefore, LA may have a potentially modulatory effect on carbohydrate metabolism. In addition, by virtue of its two SH groups, LA can interchange between a reduced form [dihydrolipoic acid (DHLA)] and an oxidized form (Fig. 1), thereby displaying reducing (antioxidant) and prooxidant properties, respectively. There are three distinct antioxidant actions of LA: capacity to regenerate endogenous antioxidants, such as glutathione, vitamin C, and vitamin E, due to its low redox potential; direct scavenging of reactive oxygen species (ROS); and metal-chelating activity resulting in reduced ROS production through a metal-catalyzed chemical reaction (26, 44).

LA was shown to have antidiabetic properties as follows (see Table 1): It improved glucose metabolism in patients with type 2 diabetes (22, 36). In animal models, LA restored insulin-stimulated glucose uptake into insulin-resistant skeletal muscle of obese Zucker rats (21, 47, 58). Treatment of streptozotocin-diabetic rats with LA caused a significant reduction in plasma glucose levels and enhanced insulin-stimulated glucose uptake into muscle (29). The exact mode of how LA improves glucose metabolism is not entirely clear, but reduced oxidative stress, improved glucose oxidation, and inhibition of hepatic gluconeogenesis may play a role (30, 36, 52). The present review will discuss potential modes of action, and will specifically focus on the possibility that the insulin-signaling network is utilized by LA by virtue of its unique redox properties.

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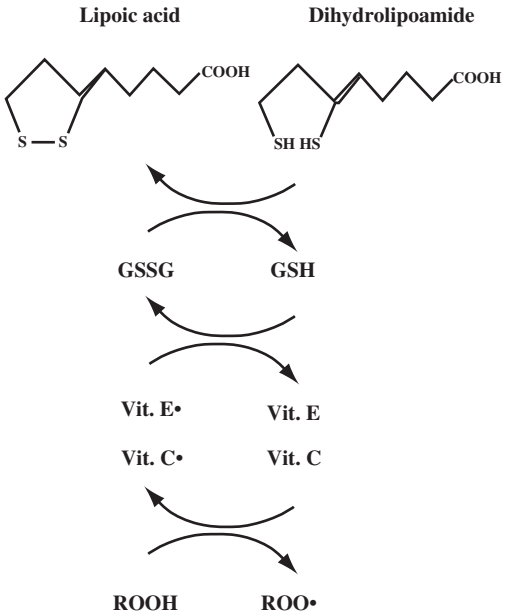

FIG. 1. Interaction between vitamin E, vitamin C, glutathione, and LA redox cycles. Adapted from a recent review (45).

METABOLISM, OCCURRENCE, AND ANTIOXIDANT PROPERTIES OF LA

LA (or thioctic acid) is a short-chain fatty acid (C8) synthesized in the liver from octanoic acid and a sulfur source (24, 62). Its catabolism also takes place in the liver: LA is metabolized by mitochondrial β-oxidation (55). There are two different enantiomers of LA: the *R*(+) isoform and the *S*(+) isoform. *R*(+)-LA is the naturally occurring stereoisoform of LA. However, synthetic LA occurs as a racemic mixture of *R*(+) and *S*(+) isoforms, which seem to have different potency, *e.g.*, it was previously shown that *R*(+)-LA is more potent than *S*(+)-LA in its ability to stimulate glucose uptake in L6 myotubes (13), as well as to increase insulin-mediated glucose uptake in obese Zucker rats (31). In fact, the *S*(+) isoform was even found to inhibit insulin action (33). On the other hand, the *S*(+) enantiomer exerts a slightly increased affinity for glutathione reductase (48). This enzyme has a key role in glutathione recycling and therefore maintenance of cellular reduced glutathione (GSH) concentrations. Thus, the two stereoisoform of LA differ in the potency in which they exert the various biological activities of this compound.

Common antioxidants are either water-soluble or lipid membrane-soluble agents. In contrast, LA has both hydrophilic and hydrophobic properties. This amphiphilic character of LA is unique among antioxidants. LA can therefore elicit its antioxidant action in the cytosol as well as in the plasma membrane (aquous and lipid media of the cell), and in serum and lipoproteins (aquous and lipid media of the blood) (26). LA's antioxidant properties consist of the following: its capacity to directly scavenge ROS; its metal-chelating activity, resulting in reduced ROS production; and its ability to regenerate endogenous antioxidants, such as glutathione, vitamin C, and vitamin E (26, 44). These interactions between different antioxidants create a network in which DHLA takes a central position due to its capacity to reduce other antioxidants (Fig. 1). Especially the restoration of glutathione, which is linked to many physiological processes, is of great importance. Interestingly, LA not only reduces glutathione, but also increases its synthesis. Besides increasing the GSH/oxidized glutathione (GSSG) ratio, LA also enhances the biosynthesis of GSH (45). Thus, LA plays an important part in the control of cellular redox status.

LA ACTIVATES THE INSULIN-SIGNALING CASCADE: POTENTIAL ROLE FOR OXIDATION

Insulin-signaling cascade

Insulin stimulates glucose uptake into fat and muscle cells through the recruitment of glucose transporter 4 (GLUT4) from intracellular membrane compartments to the plasma membrane (GLUT4 translocation) (9, 32, 59). Upon binding of insulin to its receptor, the latter is phosphorylated on several tyrosine residues via autophosphorylation. The insulin

TABLE 1. POTENTIAL EFFECTS OF LA

Effect	*Mode of action*	*Subjects/model*	*Reference*
Insulinomimetic properties	Prooxidant	Cultured adipocytes and muscle cells	13, 34, 42, 70
Improvement of insulin resistance	Antioxidant	Diabetic patients	19, 22
		Zucker (*fa*/*fa*) rats	16, 21, 58
		Cultured adipocytes and muscle cells	39, 52
Inhibition of hepatic gluconeogenesis	Inhibition of fatty acid oxidation (?)	Healthy and STZ-treated rats	30
Improvement of glucose oxidation	Increase in PDH activity	Diabetic patients (type 2)	36
Diabetic neuropathy	Antioxidant (?)	Diabetic patients (type 1 and 2)	49, 72, 73
Diabetic embryopathy	Antioxidant (?)	STZ-treated rats	67

STZ, streptozotocin.

receptor (IR) substrate (IRS) family of proteins specifically interacts with the phosphorylated insulin receptor (25, 68), facilitating the phosphorylation of IRS on several tyrosine residues by the IR kinase (65). In turn, the phosphorylated tyrosine residues on IRS themselves become docking sites for proteins with Src homology 2 (SH2) domains. Among these proteins, phosphatidylinositol 3-kinase (PI 3-kinase) is mediating most, if not all, of the metabolic effects of insulin for glucose metabolism, including GLUT4 translocation (61). Activation of PI 3-kinases leads to phosphorylation of inositol phospholipids on the D3 position of the inositol ring, thereby generating PI (3,4,5)trisphosphate and PI (3,4)bisphosphate.

Downstream of PI 3-kinase, the serine/threonine kinase Akt (also known as protein kinase B) is activated and has been implicated in insulin-induced glucose transport (7, 23, 28, 64). Other possible downstream effectors of PI 3-kinase involved in GLUT4 translocation are the atypical protein kinase C (PKC) isoforms λ and ζ (3, 4, 37). The precise insulin signaling steps downstream of Akt and atypical PKCs are largely elusive. Interestingly, the Rab GTPase-activating protein AS160 was recently found to be phosphorylated by Akt in response to insulin and to regulate GLUT4 translocation (53) (Fig. 2).

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 (its capacity to transport glucose), in addition to increasing its abundance at the plasma membrane (27). This activation step may involve stimulation of p38 mitogen-activated protein kinase (p38 MAPK). Insulin activates p38 MAPK. Moreover, preincubation with inhibitors of p38 MAPK reduced insulin-stimulated glucose uptake, but did not affect GLUT4 translocation in muscle cells and adipocytes (35, 56, 60). It was therefore hypothesized that insulin increases the intrinsic activity of translocated GLUT4 via a p38 MAPK-dependent pathway (Fig. 2).

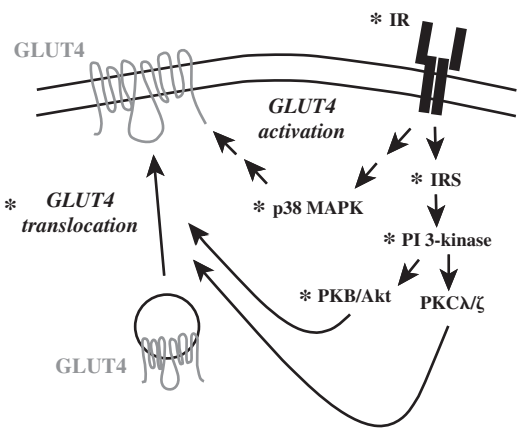

FIG. 2. Suggested model for insulin-stimulated glucose uptake. Separate pathways lead to GLUT4 translocation and GLUT4 activation, respectively. For details, see text. *Activated by LA.

LA activates the insulin-signaling cascade

LA was found to stimulate glucose uptake in fat cells (3T3-L1 adipocytes) and muscle cells (L6) in culture. This increase in glucose uptake was accompanied by rapid translocation of the glucose transporters GLUT4 from an internal membrane fraction to the plasma membrane (13, 70). Similar to insulin, treatment with LA resulted in increased tyrosine phosphorylation of the IR and IRS-1 (42, 70). In addition, LA stimulated IRS-1-associated PI 3-kinase as well as Akt activity in 3T3-L1 adipocytes and L6 myotubes (34, 70). Pretreatment with the PI 3-kinase inhibitor wortmannin completely abolished LA-stimulated glucose uptake (13, 42, 70) and GLUT4 translocation (34), as was previously shown for insulin-mediated glucose uptake. In addition, LA led to rapid activation of p38 MAPK, and pretreatment with SB203580, a selective inhibitor of p38 MAPK, reduced LA-stimulated glucose uptake, but did not affect LA-mediated GLUT4 translocation (34). Thus, LA appears to engage the insulin-signaling pathway and thereby to increase glucose uptake into muscle and fat cells. Therefore, LA is often referred to as an insulinomimetic agent (Fig. 2).

Where does LA intersect with the insulin-signaling pathway?

In vitro, the IR and to a higher extent IRS-1 are tyrosine-phosphorylated after stimulation with LA (42, 70). Thus, LA must intersect with early events of the insulin-signaling cascade. It was recently reported that *in vitro* incubation of immunoprecipitated IR with LA leads to increased tyrosine phosphorylation of the IR (42). Therefore, LA appears to directly interact with the IR tyrosine kinase, resulting in autophosphorylation of the receptor. Oxidation of critical cysteine residues in the β subunit of the IR was postulated to increase its tyrosine kinase activity (54). Hence, given its prooxidant properties, LA may oxidize these cysteine groups, thereby activating IR tyrosine kinase.

Similarly, the activity of cellular protein tyrosine phosphatases (PTPases) requires a reduced form of the thiol side chain of the catalytic cysteine residue for phosphotyrosine hydrolysis (5) and, thus, oxidation of this active-site cysteine residue results in inactivation of the enzyme (11, 38). As reversible protein-tyrosine phosphorylation of IR and IRS is balanced by PTPases such as PTP1B, decreased catalytic activity of the latter will enhance propagation of the insulin signal. Indeed, it was recently postulated that insulin stimulation elicits local generation of hydrogen peroxide (H_2O_2), causing oxidative inhibition of PTP1B, which in turn enhances the early and distal insulin-signaling cascade resulting in GLUT4 translocation and glucose uptake (40, 41). By analogy, it may be speculated that LA directly oxidizes PTP1B on its catalytic cysteine residue, thereby leading to increased tyrosine phosphorylation of the IR and IRS. Interestingly, LA was recently shown to acutely increase ROS production in rat soleus muscle (12). Moreover, LA was found to inhibit PTPase activity and decrease thiol reactivity of PTP1B (8). Thus, LA may directly or indirectly—via increased production of oxidants—inactivate PTP1B and thereby lead to activation of IR.

The question remains whether these *in vitro* observations can explain the effects occurring *in vivo*. Plasma concentrations of LA observed *in vivo* never reach those that are required to activate the insulin-signaling cascade (70 μ*M* versus at least 500 μ*M*). Thus, this discrepancy appears to minimize the operational significance of LA's insulinomimetic activity *in vivo*. On the other hand, this might simply indicate that the LA concentration in plasma does not reflect the accumulated concentration of LA in its target tissues or that the effectiveness and metabolism of LA may be variable in different tissues. Regardless of whether the *in vitro* insulin mimicking effects of LA are operational *in vivo*, they provide "proof of concept" that pharmacological insulin mimicking effect can be achieved by modulating redox state.

In summary, 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. The resulting insulin mimicking effect may arise from either activation of positive components or inhibition of negative regulators through oxidation. But not only does LA activate the insulin-signaling cascade, moreover its reduced form, DHLA, seems to protect the insulin-signaling pathway from oxidative stress-induced changes.

LA RESCUES OXIDATIVE STRESS-INDUCED INSULIN RESISTANCE: POTENTIAL ROLE FOR REDUCTION

Insulin resistance is a hallmark of type 2 diabetes. In addition, there is good evidence for its occurrence in type 1 diabetes (1, 10, 71). Insulin resistance is defined by a decreased ability of insulin to promote the uptake of glucose into peripheral tissues (*i.e.,* skeletal muscle and adipose tissue) and to inhibit hepatic glucose output (Fig. 3). However, the causes and cellular mechanisms responsible for insulin resistance are not yet fully understood.

Whereas acute local production of ROS resulting in oxidation of signaling moieties may positively regulate insulin signaling, there is evidence that increased oxidative stress occurs in diabetic patients and may contribute causatively to peripheral insulin resistance (14, 43, 46, 50). Thus, depending on the biological context (cellular localization, the specific ROS or reactive nitrogen species produced, duration of exposure, which cellular components interact with the ROS), ROS may be involved both in normal insulin signal transduction processes and in the induction of insulin resistance.

The notion that oxidative stress may be causatively linked to insulin resistance was further supported by work performed in cultured adipocytes showing that exposure to H_2O_2 reduced insulin-stimulated GLUT4 translocation and glucose uptake (15, 51, 52). Whereas one group reported decreased insulin-stimulated tyrosine phosphorylation of IR and IRS after exposure to H_2O_2 (15), the other group found no changes (52). However, in the latter study, insulin-stimulated Akt phosphorylation was significantly reduced. Moreover, the changes were associated with a decrease in GSH levels. Pretreatment with LA restored GSH levels and reversed the effect on glucose uptake, GLUT4 translocation, and Akt phosphorylation (52). Very similar results were reported for muscle cells in culture (39). Importantly, the concentrations used in these experiments reflect the concentrations of LA measured in human plasma. It was therefore hypothesized that LA may protect against oxidative stress-induced insulin resistance by its capacity to maintain intracellular redox state (52). Interestingly, patients with type 2 diabetes showed improved insulin-mediated glucose disposal when LA was administered (19, 20). In addition, LA administration increased insulin sensitivity in diabetic patients (22, 36).

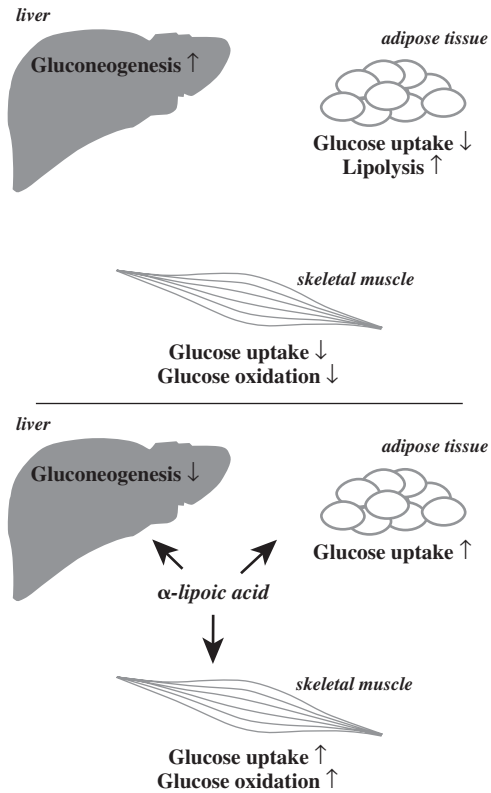

FIG. 3. Potential beneficial effects of LA on insulin resistance and hyperglycemia. (Upper panel) Changes found in insulin resistance. Under treatment with LA, these changes are partly reversed, resulting in improved glucose homeostasis **(lower panel).** For more details, see text.

Thus, in contrast to its insulinomimetic properties, LA's protective effect on oxidative stress-induced insulin resistance seems to depend on its reducing properties and therefore on its reduced form DHLA. Besides its potential as an insulin-sensitizing compound, LA may also have an important impact in the treatment/prevention of secondary complications in diabetic patients because oxidative stress appears to be a key factor in their development (6, 45).

LA MODIFIES OTHER TARGETS INVOLVED IN GLUCOSE HOMEOSTASIS

Besides its insulinomimetic actions, LA potentially improves glucose metabolism by a mechanism independent of its oxidizing and reducing actions. It was reported that ad-

ministration of LA to normal and diabetic rats causes an inhibition of hepatic gluconeogenesis, thereby inducing hypoglycemia (30). This effect of LA appears to be mediated by interfering with hepatic fatty acid oxidation. As a consequence, less acetyl-CoA is produced. Acetyl-CoA is the essential activator of the key gluconeogenetic enzyme pyruvate carboxylase (66), and reduced acetyl-CoA levels therefore result in impaired gluconeogenesis. Given the important contribution of nonopposed hepatic gluconeogenesis to (fasting) hyperglycemia in diabetic patients, this effect further underscores the potential therapeutic value of LA in the treatment of diabetic patients, although the doses required were higher than those required for the antioxidant, chronic treatment.

Another potential target of LA treatment is the PDH complex. There is evidence that activity of the PDH complex is impaired in diabetic patients, resulting in decreased glucose oxidation (17). Indeed, lactate and pyruvate levels are increased in patients with type 2 diabetes (2, 36), and treatment of diabetic patients with LA decreased pyruvate and lactate levels (36). Similarly, dichloracetate, an activator of PDH, lowers pyruvate, lactate, and glucose levels in diabetic rats (57). Activation of PDH therefore not only results in increased peripheral glucose utilization, but also interrupts the Cori cycle and reduces the availability of three-carbon precursors for gluconeogenesis. Of importance, pyruvate dehydrogenase kinase 4 (PDK4), which phosphorylates and thereby inactivates PDH, was found to be up-regulated in diabetes (18, 69). Therefore, LA may restore glucose homeostasis in diabetic patients by improving PDH activity levels. Indeed, it was reported in a very recent article that R(+)-LA increased pyruvate metabolism and activation state of the PDH complex in cultured rat hepatocytes (63).

CONCLUSION

The antihypoglycemic effect of LA in diabetes appears to be the result of interactions on different levels (Table 1). LA appears to involve glucose uptake into skeletal muscle, as well as hepatic glucose production. These properties therefore render LA a potentially attractive therapeutic agent for the treatment of insulin resistance and diabetes. Moreover, given the role of oxidative stress in the pathogenesis of secondary complications in diabetes, LA might be beneficial in the prevention/treatment of these complications as was recently shown for diabetic neuropathy (49, 73), and embryonic malformations (67). Clearly, more clinical studies are mandatory to examine the proposed beneficial effects of LA in diabetic patients.

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ABBREVIATIONS

DHLA, dihydrolipoic acid; GLUT4, glucose transporter 4; GSH and GSSG, reduced and oxidized glutathione, respectively; H_2O_2, hydrogen peroxide; IR, insulin receptor; IRS, insulin receptor substrate; LA, α-lipoic acid; p38 MAPK, p38 mitogen-activated protein kinase; PDH, pyruvate dehydrogenase; PI 3-kinase, phosphatidylinositol 3-kinase; PKC, protein kinase C; PTPase, protein tyrosine phosphatase; PTP1B, protein tyrosine phosphatase 1B; ROS, reactive oxygen species.

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