

STIMULATION BY α -LIPOIC ACID OF GLUCOSE TRANSPORT ACTIVITY IN SKELETAL MUSCLE OF LEAN AND OBESE ZUCKER RATS

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Summary

α -Lipoic acid (ALA), a potent biological antioxidant, improves insulin action of skeletal muscle glucose transport and metabolism in both human and animal models of insulin resistance. In order to obtain further insight into the potential intracellular mechanisms for the action of ALA on insulin-stimulated glucose transport in skeletal muscle, we investigated the effects of direct incubation with ALA (2 mM) on 2-deoxyglucose (2-DG) uptake by epitrochlearis muscle from either insulin-sensitive lean (*Fa/-*) or insulin-resistant obese (*fa/fa*) Zucker rats. ALA stimulated 2-DG uptake in muscle of lean animals by 76%, whereas ALA stimulated 2-DG uptake by only 48% in muscle from obese animals. The stimulation of 2-DG uptake due to ALA was enhanced 30-55% in the presence of insulin. In contrast, ALA action on 2-DG uptake was not additive with the effects of electrically-stimulated muscle contractions in either insulin-sensitive or insulin-resistant muscle. Wortmannin (1 μ M), an inhibitor of phosphatidylinositol-3-kinase, completely inhibited insulin action on 2-DG uptake, but inhibited ALA action by only 25%. Collectively, these results indicate that although a portion of ALA action on glucose transport in mammalian skeletal muscle is mediated via the insulin signal transduction pathway, the majority of the direct effect of ALA on skeletal muscle glucose transport is insulin-independent.

Key Words: insulin action, epitrochlearis muscle glucose transport, wortmannin

α -Lipoic acid (ALA; thioctic acid) occurs endogenously in tissues and functions as a co-factor for enzymatic reactions involved in the oxidative decarboxylation of α -keto acids (e.g. pyruvate dehydrogenase, α -ketoglutarate dehydrogenase) (1). ALA is a potent biological antioxidant (2) and, when administered exogenously, significantly improves

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insulin-stimulated whole body and skeletal muscle glucose disposal in insulin-resistant animal models (3) and humans (4, 5). Several studies using cultured muscle cells (6, 7), isolated diaphragm (8), and perfused heart preparations (9) have demonstrated that ALA can directly activate glucose transport. In mammalian skeletal muscle, glucose transport can be activated by two independent mechanisms, one insulin-dependent and the other insulin-independent (10). The activation of phosphatidylinositol 3-kinase (PI3-kinase) is an essential step in the intracellular pathway for insulin-dependent stimulation of glucose transport (11). To date, no study has addressed the effect of ALA on glucose transport in an intact mammalian locomotor skeletal muscle, the primary tissue of glucose disposal (12), nor has the possible involvement of PI3-kinase in ALA action in this tissue been investigated. Therefore, the purpose of the present study was 1) to assess the effect of ALA on glucose transport activity in the isolated epitrochlearis muscle of lean and obese Zucker rats and 2) to gain insight into the possible biochemical basis for this effect.

Methods

Animals. Female obese Zucker (*fa/fa*) rats (280-300 g) and lean littermates (*Fa/-*) (150-170 g) were purchased at 7-8 weeks of age from Harlan (Indianapolis, IN). Animals were housed 2 per cage and maintained on chow (Purina, St. Louis, MO) and water ad libitum. All procedures were approved by the University of Arizona Animal Use and Care Committee. Lean animals were restricted to 4 g of chow after 5 p.m. the evening before the experiment, while obese animals received 6 g of chow at this time. At 9 a.m. on the day of the experiment, animals were deeply anesthetized with pentobarbital sodium (50 mg/kg body weight i.p.). Both epitrochlearis muscles were then surgically removed and prepared for *in vitro* incubation as described below.

Glucose transport activity. Epitrochlearis muscles were initially incubated for 60 min in 3 ml of oxygenated Krebs-Henseleit buffer (KHB; ref. 13) containing 8 mmol/l glucose, 32 mmol/l mannitol, and 0.1% bovine serum albumin (BSA, radioimmunoassay grade; Sigma Chemical, St. Louis, MO). The flasks were shaken in a Dubnoff incubator at 37°C, and the gas phase in each flask was 95% O₂:5% CO₂. In the first series of experiments, the KHB contained either no additions, a maximally effective concentration of ALA (2 mM racemic mixture; ASTA Medica, Frankfurt, Germany), a maximally effective concentration of insulin (2 mU/ml; Eli Lilly, Indianapolis, IN), or ALA and insulin in combination. In the second series of experiments, the buffer contained either no additions or ALA, and maximally-effective electrical stimulation of muscle contractions proceeded as previously described (14). In a third series of experiments, pairs of muscles were treated either with no additions, insulin, contractions, or ALA. One muscle from each pair was contemporaneously treated with 1 μM wortmannin (Sigma Chemical), an effective inhibitor of PI3-kinase activation (15) and a selective inhibitor of insulin-stimulated glucose transport in muscle (16, 17). The wortmannin was initially dissolved in DMSO as a 1 mM stock before addition to the medium. When added, wortmannin was present during the entire 60 min pre-incubation period. It should be noted that the wortmannin was not present during the 10 min electrical stimulation period.

Following these initial treatments, all muscles were rinsed for 10 min at 37°C in 3 ml of oxygenated KHB containing 40 mmol/l mannitol, 0.1% BSA, and any compounds present previously. The muscles were then transferred to flasks containing 2 ml of oxygenated KHB, 0.1% BSA, 1 mmol/l 2-deoxy [1,2-³H]glucose (2-DG) (300 mCi/mol) and 39 mmol/l [U-¹⁴C]mannitol (0.8 mCi/mol) (ICN Radiochemicals, Irvine, CA), and any previous additions. After this final 20 min incubation at 37°C, muscles were trimmed of fat, extraneous muscle tissue, and connective tissue, frozen between aluminum blocks cooled to the temperature of liquid N₂, weighed, and dissolved in 0.5 ml of 0.5 N NaOH. Glucose transport activity was then determined as described by Henriksen and Ritter (18). This

method for assessing glucose transport activity in epitrochlearis muscles of this size has been thoroughly studied and validated (19).

Results and Discussion

Dose-response effect of ALA on muscle glucose transport. At 0.5 mM, ALA elicited a significant ($p < 0.05$) stimulation of 2-DG uptake in the epitrochlearis muscle of both lean and obese animals (Fig. 1). In both lean and obese groups, a maximal response of 2-DG uptake was brought about by 2 mM ALA, as 5 mM ALA did not cause a further increase in 2-DG uptake. Moreover, the increase in 2-DG uptake due to 2 mM ALA was 76% in muscle from lean Zucker rats, whereas the increase due to this concentration of ALA in muscle from obese rats was only 48% ($p < 0.05$ vs. lean). These data represent

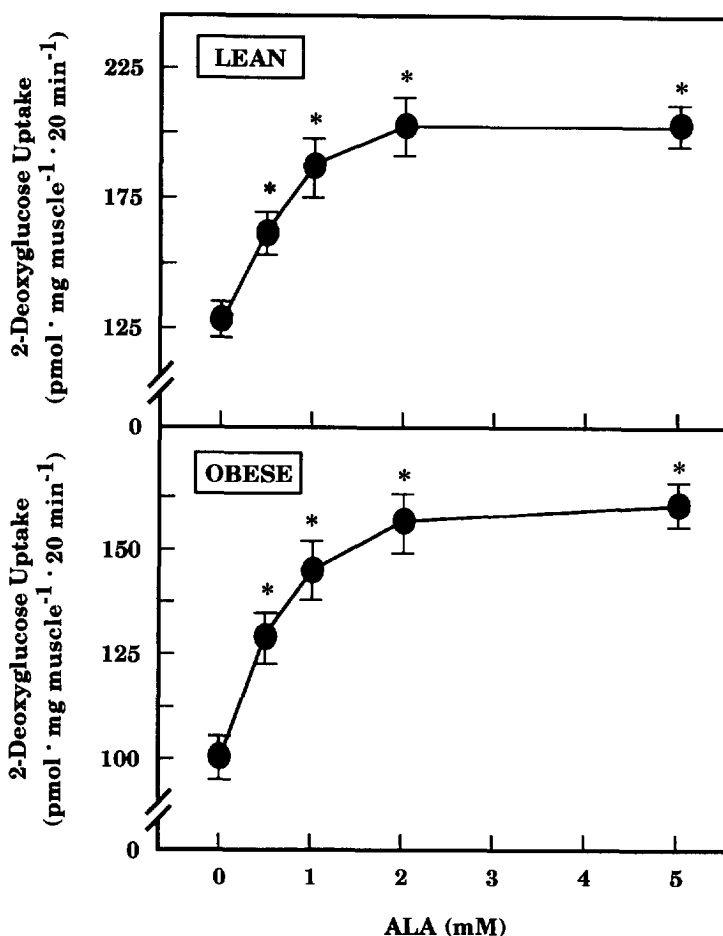


Fig. 1.

Dose-response effect of direct incubation with α -lipoic acid on in vitro skeletal muscle glucose transport activity. ALA-stimulated 2-DG uptake was assessed in muscle from lean (upper panel) and obese (lower panel) Zucker rats. Data are means \pm SE for 5-6 muscles per group. * $p < 0.05$ vs. group incubated in the absence of ALA.

the first demonstration that ALA itself can activate glucose transport in an intact mammalian locomotor skeletal muscle. The resistance of glucose transport stimulation by ALA in muscle of the obese animals is similar to that seen for stimulation by contractions (20; Fig. 3), but is less than that observed for stimulation by insulin (3, 20, 21; Fig. 2).

Interactions between ALA and insulin. Insulin (2 mU/ml) stimulated 2-DG uptake in muscle from both lean and obese Zucker rats; however, this maximal rate of insulin-stimulated 2-DG uptake in the obese group was only 50% ($p < 0.05$) of the rate observed in the lean group (Fig. 2, left panels). In both lean and obese muscle, the combination of ALA and insulin was more than additive. In lean muscle, the effect of ALA on stimulation

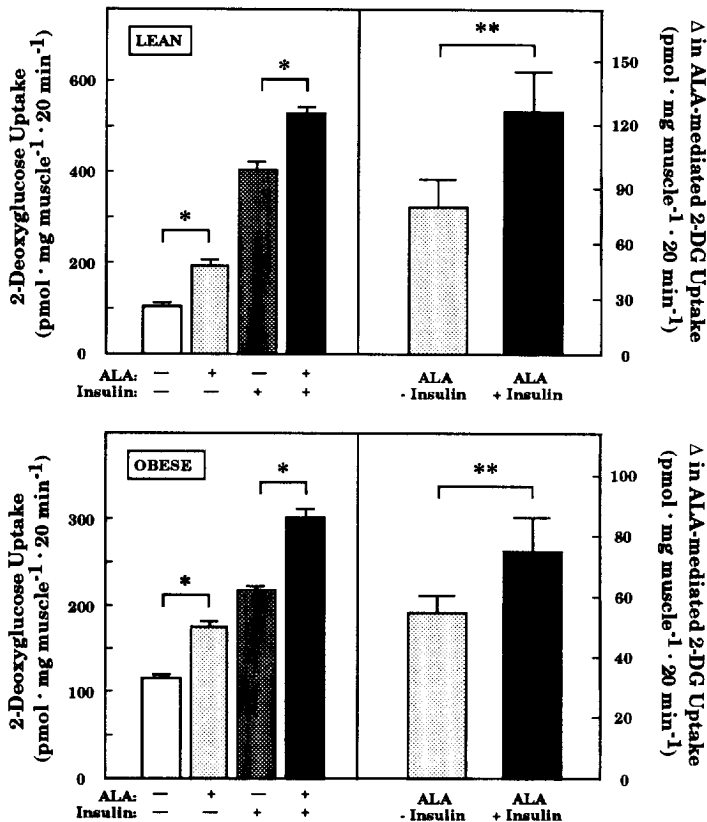


Fig. 2.

Effects of direct incubation with α -lipoic acid in the absence or presence of insulin on in vitro skeletal muscle glucose transport activity. 2-DG uptake was assessed in muscle from lean (upper panel) and obese (lower panel) Zucker rats under the following conditions: with no additions (open bar), in the presence of 2 mM ALA (lightly shaded bar), in the presence of 2 mU/ml insulin (darkly shaded bar), and in the presence of both ALA and insulin (black bar). The right panels display the increase due to ALA above either basal (lightly shaded bar) or above the insulin-stimulated 2-DG uptake (black bar). Data are means \pm SE for 5-6 muscles per group. * $p < 0.05$ vs. corresponding group without ALA (i.e., significant ALA effect). ** $p < 0.05$ vs. corresponding group without insulin (i.e., significant interaction between ALA and insulin).

of 2-DG uptake was enhanced by 55% (Fig. 2, upper right panel), while in obese muscle the effect of ALA was enhanced by 33% in the presence of insulin (Fig. 2, lower right panel) (both $p < 0.05$). This indicates that in the presence of insulin, the ability of ALA to activate glucose transport in muscle is increased, and may underlie at least part of the *in vivo* effect of the compound to enhance insulin action (3).

Interactions between ALA and contractions. The addition of ALA to muscles stimulated to contract did not increase any further the contraction-induced rate of 2-DG uptake, in preparations from either lean or obese animals (Fig. 3). This indicates that there was no interaction between ALA and muscle contractions on stimulation of glucose transport activity. Moreover, this lack of additivity provides evidence that at least part of the intracellular pathway for activation of glucose transport is shared by ALA and contractile activity. At the present time, the exact intracellular elements involved in this particular pathway are unknown.

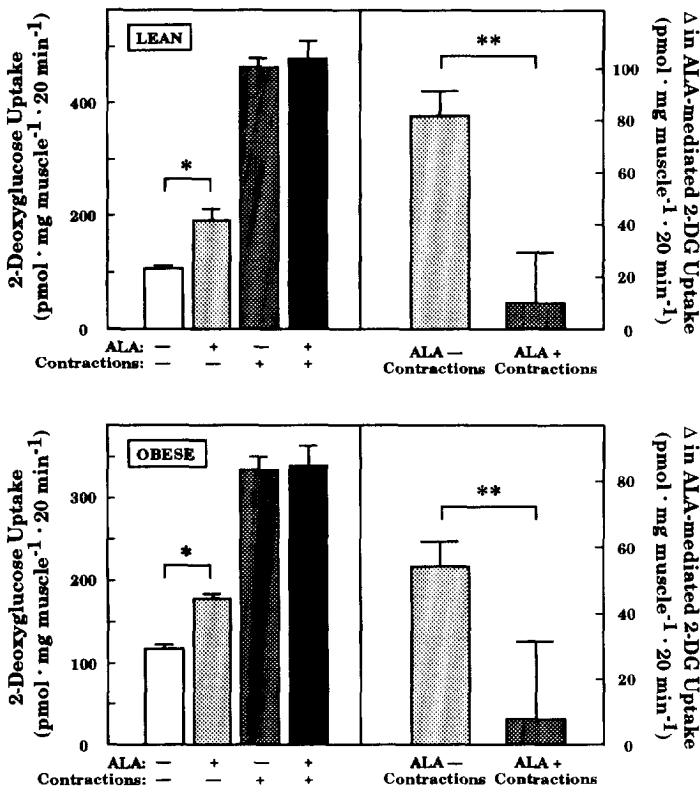


Fig. 3.

Effects of direct incubation with α -lipoic acid with or without muscle contractions on skeletal muscle glucose transport activity. 2-DG uptake was assessed in muscle from lean and obese Zucker rats under the following conditions: with no additions (open bar), in the presence of 2 mM ALA (lightly shaded bar), following 10 tetanic contractions (darkly shaded bar), and following stimulation with both ALA and muscle contractions (black bar). The right panels display the increase due to ALA above either basal (lightly shaded bar) or the contraction-stimulated 2-DG uptake (black bar). Data are means \pm SE for 5-6 muscles. * $p < 0.05$ vs. corresponding group without ALA (i.e., significant ALA effect). ** $p < 0.05$ vs. corresponding group without contractions (i.e., no additivity between ALA and contractions).

Effects of PI3-kinase inhibition on stimulation of glucose transport by insulin, contractions, or ALA. In order to elucidate the potential involvement of intracellular factors involved in the pathway of glucose transport activation utilized by ALA, wortmannin was used. Wortmannin is an effective inhibitor of the activation of PI3-kinase (15), and can selectively inhibit the insulin-dependent pathway for activation of glucose transport in isolated skeletal muscle, while having no effect on the contraction/hypoxia-dependent pathway (16, 17). The effect of 1 μ M wortmannin on stimulation of glucose transport activity by insulin, contractions, or ALA in epitrochlearis muscles of lean and obese Zucker rats is shown in Fig. 4. Wortmannin at this concentration had no significant effect on basal 2-DG uptake, nor did this PI3-kinase inhibitor reduce the rate of contraction-stimulated 2-DG uptake, in agreement with the results of Lee et al. (16). In contrast, wortmannin completely inhibited the stimulation of 2-DG uptake by insulin in muscle from both lean and obese animals. At the same concentration, wortmannin was only able to inhibit ~25% of the stimulation of 2-DG uptake by 2 mM ALA.

There are two major interpretations that can be made from the above results. First, the vast majority (~75%) of the intracellular pathway for activation of glucose transport by

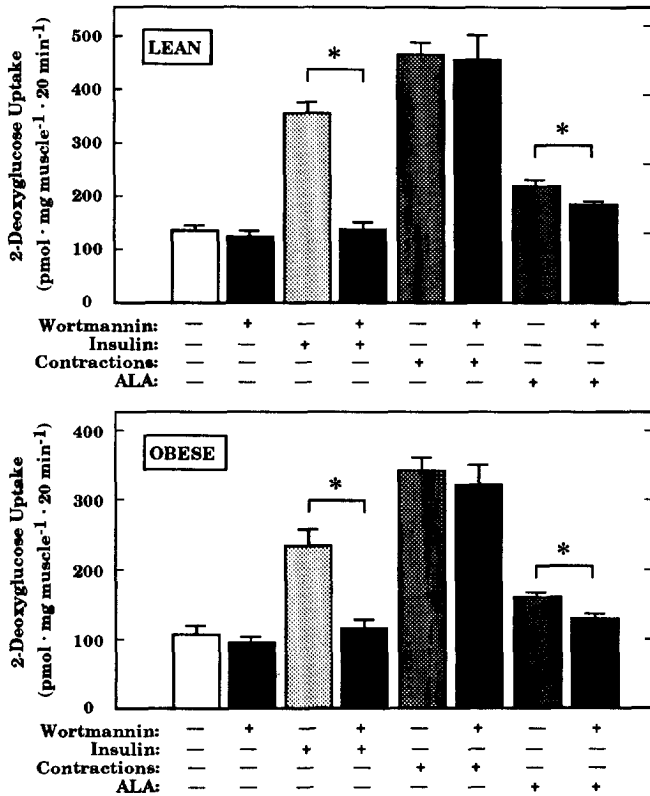


Fig. 4.

Effect of wortmannin on glucose transport activity stimulated by insulin, contractions, or α -lipoic acid. Paired muscles from lean and obese Zucker rats were stimulated with either insulin (2 mU/ml), muscle contractions (10 tetanic contractions), or 2 mM ALA in the absence or presence of 1 μ M wortmannin, and 2-DG uptake was assessed. Data are means \pm SE for 5-8 muscles. * p <0.05 vs. corresponding group without wortmannin (i.e., significant wortmannin effect).

ALA is PI3-kinase-independent and is common to the pathway for activation of glucose transport by muscle contractions. Second, a small portion (~25%) of the activation of glucose transport by ALA does recruit a pathway utilizing PI3-kinase, possibly common to that used by insulin stimulation. One could speculate that the interaction between ALA and insulin on muscle glucose transport (Fig. 1) could involve enhanced activation of PI3-kinase. This, of course, requires further investigation.

We have previously reported that acute *in vivo* administration of ALA to obese Zucker rats enhances insulin-stimulated glucose transport activity in the epitrochlearis muscle, as assessed *in vitro* (3). The present data suggest that at least a portion of that improvement could be due to the interaction of ALA with the elements of the insulin-dependent pathway of glucose transport activation, including PI3-kinase, in skeletal muscle. Other factors known to be affected by ALA administration *in vivo*, such as the reduction in plasma free fatty acids (3), could also be important in this *in vivo* effect of ALA.

Tsakiridis et al. (7) have demonstrated in cultured L₆ myocytes that ALA can activate both glucose transport activity and PI3-kinase activity. In addition, this group has shown that the activation of glucose transport activity by ALA can be completely inhibited by wortmannin (7), indicating that in this muscle cell line the effects of ALA are likely being mediated by activation of the insulin signaling pathway. This differs quantitatively from our present findings, as we found substantial involvement of insulin-independent mechanisms for ALA action. This highlights the potential differences in metabolic responses to various stimuli and possibly in intracellular signaling components between muscle cell lines and intact mammalian skeletal muscle preparations, and emphasizes that one should exercise caution in making direct comparisons between the two models of investigation.

It is of interest that Wojtaszewski et al. (22) have recently published evidence that wortmannin at 1 μM can completely inhibit insulin-stimulated glucose uptake and can also partially (~30%) inhibit contraction-stimulated glucose uptake in fast-twitch glycolytic skeletal muscle, using the hindlimb perfusion technique. Higher concentrations of wortmannin (>3 μM) were needed for complete inhibition of the contraction-dependent pathway for stimulation of glucose transport in perfused muscle. The observation that wortmannin can significantly inhibit contraction-stimulated glucose transport contradicts the present findings (Fig. 4) and those of Lee et al. (16) and Yeh et al. (17) using the isolated epitrochlearis, also a predominantly fast-twitch glycolytic muscle (23). Although there is no obvious explanation for this discrepancy, Wojtaszewski et al. (22) have speculated that there may exist in skeletal muscle a sub-fraction of PI3-kinase involved in regulation of muscle contraction that is less sensitive to the effects of wortmannin.

In summary, we have demonstrated that ALA, a potent biological antioxidant, activates glucose transport in skeletal muscle from lean and obese Zucker rats by both insulin-dependent and insulin-independent mechanisms. There is also evidence that an interaction between ALA and insulin takes place that results in a small but significant enhancement of the effect of ALA on glucose transport activity. The intracellular mechanisms underlying these effects remain to be investigated.

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