Lipoic Acid Reduces Glycemia and Increases Muscle GLUT4 Content in Streptozotocin-Diabetic Rats

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Alpha lipoic acid (lipoate [LA]), a cofactor of α-ketodehydrogenase, exhibits unique antioxidant properties. Recent studies suggest a direct effect of LA on glucose metabolism in both human and experimental diabetes. This study examines the possbility that LA positively affects glucose homeostasis in streptozotocin (STZ)-induced diabetic rats by altering skeletal muscle glucose utilization. Blood glucose concentration in STZ-diabetic rats following 10 days of intraperitoneal (IP) injection of LA 30 mg/kg was reduced compared with that in vehicle-treated diabetic rats (495 ± 131 *v* 641 ± 125 mg/dL in fed state, $P = .003$, and 189 ± 48 *v* 341 ± 36 mg/dL after 12-hour fast, $P = .001$). No effect of LA on plasma insulin was observed. Gastrocnemius muscle crude membrane GLUT4 protein was elevated both in control and in diabetic rats treated with LA by 1.5- and 2.8-fold, respectively, without significant changes in GLUT4 mRNA levels. Gastrocnemius lactic acid was increased in diabetic rats (19.9 ± 5.5 *v* 10.4 ± 2.8 μmol/g muscle, $P < .05$ *v* nondiabetic rats), and was normal in LA-treated diabetic rats (9.1 ± 5.0 μmol/g muscle). Insulin-stimulated 2-deoxyglucose (2 DG) uptake into isolated soleus muscle was reduced in diabetic rats compared with the control group (474 ± 15 *v* 568 ± 52 pmol/mg muscle · 30 min, respectively, $P = .05$). LA treatment prevented this reduction, resulting in insulin-stimulated glucose uptake comparable to that of nondiabetic animals. These results suggest that daily LA treatment may reduce blood glucose concentrations in STZ-diabetic rats by enhancing muscle GLUT4 protein content and by increasing muscle glucose utilization.

ALPHA LIPOIC ACID ([LA] thioctic acid, 6,8-dithiooctanoic acid) is an essential cofactor of the α- oxoacid dehydrogenase complexes such as pyruvate dehydrogenase and α-ketoglutarate dehydrogenase, two major mitochondrial enzymes in glucose metabolism and the Krebs cycle.[1] In addition, the unique antioxidant properties of LA are well documented. It is an efficient hydroxyl and HOCl radical scavenger,[2] which inhibits iron-dependent OH · generation and peroxidation of brain phospholipids,[3] protects human erythrocytes from 2,2′-azobis (2-amidinopropane) dihydrochloride–induced hemolysis in vitro,[4] and inhibits cataract formation in rats treated with the oxidant buthionine sulfoxide.[5]

Presently, its main clinical application is in the treatment of diabetic polyneuropathy in Germany.[6-7] The rationale for this use is based on accumulating evidence supporting the involvement of enhanced oxidative stress in the pathogenesis of diabetes and its complications.[8-9] In addition, diverse effects of LA on glucose metabolism were described for in vitro and in vivo animal models and human studies. LA was found to induce an increase in glucose transport in muscle cells in culture (L6 myotubes) by stimulating translocation of the glucose transporters GLUT4 and GLUT1 from internal membrane pools to the plasma membrane.[10] Isolated muscles of obese Zucker rats (a model of obesity and insulin resistance) demonstrate increased insulin-stimulated glucose transport activity, glycogen synthesis, and glucose oxidation following 10 days of LA treatment.[11-13] The relevance of these observations was further evaluated in clinical trials using the euglycemic-hyperinsulinemic clamp technique. In type II diabetic patients, both short- and long-term treatments with LA induce an increase in insulin-stimulated glucose disposal, by 50% and 30%, respectively.[14]

Since skeletal muscle is the primary site of insulin-mediated glucose disposal,[15] increased glucose transport into skeletal muscle could account for these observations. Cellular uptake of glucose in mammalian cells is mediated by a family of glucose transporter proteins, GLUT4 being the major isoform present in skeletal muscle. GLUT4 is characterized by its localization in internal vesicles and is transported to the plasma membrane in response to insulin and several other stimuli.[16-17] In the streptozotocin-(STZ)-induced diabetes model, muscle GLUT4 content is reduced depending on diabetes duration and on muscle fiber type, yet the GLUT4 translocation mechanism upon insulin stimulation seems to remain intact.[18]

The findings of this study show that prolonged LA treatment in STZ-diabetic rats reduces blood glucose, elevates GLUT4 protein in gastrocnemius muscle, and normalizes muscle lactate concentration. These changes are associated with a reversal of the diabetes-induced impairment in insulin-dependent 2-deoxyglucose (2 DG) uptake into isolated soleus muscle.

MATERIALS AND METHODS

LA (racemic mixture) was provided by Asta Medica (Frankfurt, Germany). Recombinant human insulin (Novo Nordisk, Copenhagen, Denmark) was used for all experiments. All other chemicals were purchased from Sigma (St Louis, MO) unless otherwise stated. Anti-GLUT4 antibody was purchased from East Acres Biological, (Southbridge, MA) anti–α1 Na/K adenosine triphosphatase (ATPase) antibody from Upstate Biotechnology (New York), and anti–rabbit immunoglobulin G (IgG) from Amersham Life Science (Amersham, UK).

Animals

Male Sprague-Dawley rats were purchased from Harlan Laboratories (Jerusalem, Israel). They were kept on a 12-hour light-dark cycle at 23°C and housed in groups of four per cage, except for five animals in

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each experimental group that were housed in individual cages for measurement of food consumption. Standard rat chow and tap water were provided ad libitum. Diabetes was induced at the age of 6 to 8 weeks by a single intraperitoneal (IP) injection of a freshly prepared solution of STZ (65 mg/kg body weight) in 100 mmol/L citrate buffer (pH 4.5). Diabetes was defined as nonfasting tail-blood glucose concentration greater than 400 mg/dL on two separate occasions (Glucometer Elite; Bayer Diagnostics, Tarrytown, NY). Seven days following induction of diabetes, diabetic and control animals received IP injections of either vehicle (120-mmol/L Tris buffer, pH 7.4, 1 mL) or LA 30 mg/kg body weight dissolved in the same buffer, for 10 consecutive days. LA did not increase mortality rates in control or diabetic rats. Twelve hours after final treatment, animals were weighed and anesthetized (phenobarbital 80 mg/kg IP) and the soleus and gastrocnemius muscles were rapidly dissected out. Soleus muscle was immediately used for measurement of 2DG uptake. Gastrocnemius muscles were trimmed of fat and connective tissue, immediately frozen in liquid nitrogen, and transferred to −70°C, where they were kept until used for membrane preparations and metabolite determinations. Blood was collected by cardiac puncture, after which animals were killed by phenobarbital overdose.

Crude Membrane Preparation From Gastrocnemius Muscle and Western Blotting Procedure

Membranes were prepared using a previously described protocol.[19] Briefly, all procedures were performed at 4°C. Gastrocnemius muscles (0.5 g) were homogenized using a Polytron homogenizer (Kinematica, Littau, Switzerland) at the lowest setting for five 5-second strokes in 5 mL 250-mmol/L sucrose, 20-mmol/L $NaHCO_3$, 5 mmol/L NaN_3, 0.1 mmol/L phenylmethylsulfonyl fluoride, 1 μmol/L pepstatin A, 1 μmol/L leupeptin, and 10 μmol/L *trans*-epoxysuccinyl-L-leucylamido-(4-guanidino) butane (E64). The homogenates were centrifuged at 1,200 × *g* for 10 minutes to remove cell debris and nuclei. The supernatant was then centrifuged at 9,000 × *g* for 10 minutes to sediment mitochondria and lysosomes. The resultant supernatant (found to contain > 80% of total GLUT4) was then centrifuged at 190,000 × *g* for 60 minutes (L7-55 CA; Beckman Instruments, Palo Alto, CA) to obtain the crude membranes, as described previously.[19]

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis were performed as previously described[20] using either a 1:500 dilution of anti-GLUT4 antibody or anti–α1 Na/K ATPase antibody. Blots were incubated with either horseradish peroxidase–conjugated protein A (for GLUT4) or anti–rabbit IgG horseradish peroxidase (for Na/K ATPase). Luminescence was detected by ECL (Amersham). Density values of the different bands were obtained after scanning the blots by videodensitometry using the UVP-GDF 5000 system (UVP, San Gabriel, CA), and thereafter were quantified using Image Quant Version 3.3 (Molecular Dynamics, Sunnyvale, CA).

Total RNA Isolation From Gastrocnemius Muscle and Northern Blotting Procedure

Total cellular RNA was extracted from gastrocnemius muscle using the acid guanidium isothiocyanate-phenol-chloroform method as previously described.[21] For Northern blot analysis, 30 μg total RNA per lane was separated under denaturing conditions on 1% (wt/vol) agarose gels containing 8% (vol/vol) formaldehyde with a trace amount of ethidium bromide. The amount of ethidium bromide–stained 18S and 28S ribosomal RNA was used to confirm equal RNA loading on each lane. The RNA was transferred onto Hybond N^+ nylon membranes (Amersham). GLUT4 and β-actin mRNA levels were detected using random prime–labeled full-length cDNA under stringent hybridization conditions. Autoradiographic and densitometric analyses were performed using the bioimaging analyzer BAS 1000 (Fuji, Tokyo, Japan).

Determination of Lactic Acid Concentrations in Gastrocnemius Muscle

Gastrocnemius muscle tissue (30 to 100 mg) was freeze-dried. The muscle powder was extracted with 0.5 mol/L perchloric acid and neutralized with sodium carbonate. The neutralized extract was analyzed for lactate by the enzymatic method described by Katz et al.[22]

Measurement of Glucose Transport Activity in Isolated Soleus Muscle

Soleus muscles were surgically excised, divided from tendon to tendon into longitudinal strips (<24 mg per strip) connected at one end, and immediately incubated in either the presence or absence of insulin. One soleus muscle from each animal was incubated in the absence of insulin, and the contralateral muscle from each animal was incubated in medium containing a maximally effective concentration of insulin (2 mU/mL). Glucose transport activity was evaluated using the glucose analog 2DG as described previously.[23-24] 2DG uptake in the absence or presence of insulin followed linear kinetics for 60 minutes in the presence of 1 mmol/L 2DG. Nonspecific glucose uptake assessed by adding 25 mmol/L cytochalasin B to the medium accounted for less than 10% of total 2DG uptake. Specific inhibition of glucose transporters provides evidence that 2DG uptake was glucose transporter–mediated.

Other Assays

Protein content was measured using the Bio-Rad (Munchen, Germany) protein assay.[25] Insulin concentration was measured using a rat insulin radioimmunoassay kit (Linco Research, St Louis, MO). Blood glucose levels were determined either by the Elite glucometer or by the glucose oxidase method.[26]

Statistical Analysis

All data are reported as the mean ± SE. The significance of differences between variables was determined using Student's *t* test.

Table 1. Effect of LA Treatment on Fasting and Nonfasting Blood Glucose and Insulin Levels in Control and STZ-Diabetic Rats

	Blood Glucose (mg/dL)		Blood Insulin (ng/mL)
Group	Fed	Fasted	Fed
Control			
Vehicle-treated	159 ± 42 (13)	91 ± 8 (8)	2.2 ± 0.5 (8)
LA-treated	146 ± 28 (14)	80 ± 6 (8)	2.0 ± 0.5 (8)
Diabetic			
Vehicle-treated	641 ± 125 (16)	341 ± 36 (8)	1.1 ± 0.2 (8)*
LA-treated	495 ± 131 (19)†	189 ± 48 (8)‡	1.0 ± 0.3 (8)*

NOTE. Control and STZ-diabetic rats (7 days following induction of diabetes) received daily IP injection of either vehicle (120-mmol/L Tris buffer, pH 7.4, 1 mL) or LA (30 mg/kg body weight dissolved in 120 mmol/L Tris buffer) for 10 consecutive days. Following the final treatment, animals were either food-deprived or provided food ad libitum for 12 hours, after which animals were anesthesized and killed and blood samples were obtained. Blood glucose levels were determined using glucose oxidase assay, and insulin concentrations were determined using a radioimmunoassay kit. Results are the mean ± SE, and the number of animals for each result is presented in parentheses.

*$P < .002$ *v* nondiabetic animals.

†$P = .003$ *v* vehicle-treated diabetic animals.

‡$P = .001$ *v* vehicle-treated diabetic animals.

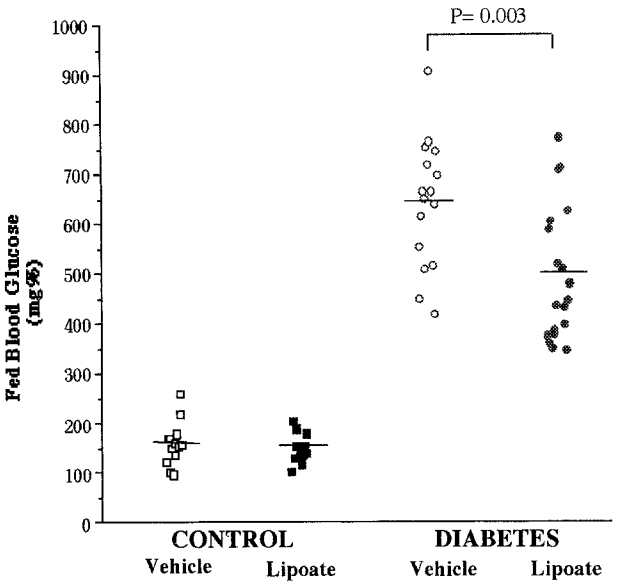


Fig 1. Nonfasting (fed-state) blood glucose level in control and STZ-diabetic rats treated with LA or vehicle. Blood samples were obtained as described in Table 1. Each point represents blood glucose of an individual nonfasted animal as determined by glucose oxidase assay. Horizontal lines represent mean values for each group, and significance was calculated with Student's *t* test.

RESULTS

LA Treatment Improves Fasting and Fed-State Glycemia in Diabetic Rats

Ten consecutive days of LA treatment (30 mg/kg body weight IP) had no significant effect on body weight or food consumption in both control and diabetic rats (data not shown). Fasting and fed-state blood glucose concentrations and fed-state circulating insulin levels from both control and diabetic rats treated for 10 days with LA or vehicle alone are presented in Table 1. Both fasting and nonfasting blood glucose concentrations were reduced in diabetic rats treated with LA compared with vehicle-treated rats (189 ± 48 *v* 341 ± 36 mg/dL, $P = .001$, and 495 ± 131 *v* 641 ± 125 mg/dL, $P = .003$, for fasting and fed state, respectively). Individual values for fed-state blood glucose concentrations in the four groups are presented in Fig 1. LA significantly reduced fed-state hyperglycemia by 23%, on average. Fed-state serum insulin concentration was 50% lower in STZ-diabetic rats, but was unaffected by LA treatment in both control and diabetic rats (Table 1).

LA Increases Gastrocnemius GLUT4 Protein But Not mRNA Content

To investigate the involvement of skeletal muscle glucose transporters in the hypoglycemic effect of LA, the amount of insulin-sensitive glucose transporter GLUT4 was determined in crude gastrocnemius muscle membranes. In a representative Western blot, GLUT4 appears as a double band corresponding to a molecular weight of 45 to 47 kd (Fig 2A). LA treatment significantly increased GLUT4 content in both diabetic and control rats. The amount of muscle immunoreactive GLUT4 (eight rats per group) was evaluated by laser-scanning densitometry and expressed in arbitrary units (Table 2). In membranes from diabetic rats, an average 32% reduction in muscle GLUT4 protein content was observed compared with control levels. LA-treated animals showed a significant increase in crude membrane GLUT4 content compared with vehicle-treated rats (1.5-fold and 2.8-fold for nondiabetic and diabetic animals, respectively). The α1 Na/K ATPase protein content was similar between the different experimental groups (Fig 2B and Table 2), suggesting that LA treatment did not alter the recovery of membrane proteins during the preparation procedure. LA-induced elevation of muscle GLUT4 transporters therefore appears to reflect a selective effect of prolonged LA treatment. This could result from either an elevated protein synthesis or a reduced protein degradation rate. To address this question, gastrocnemius GLUT4 mRNA expression was assessed by Northern blot analysis (Fig 3A). The amount of β-actin mRNA was evaluated in each lane (Fig 3B). Neither GLUT4 per se nor the ratio of GLUT4 mRNA to β-actin mRNA were significantly different in the four groups, indicating that under our experimental conditions neither STZ-induced diabetes nor LA treatment influenced GLUT4 mRNA levels (Fig 3C).

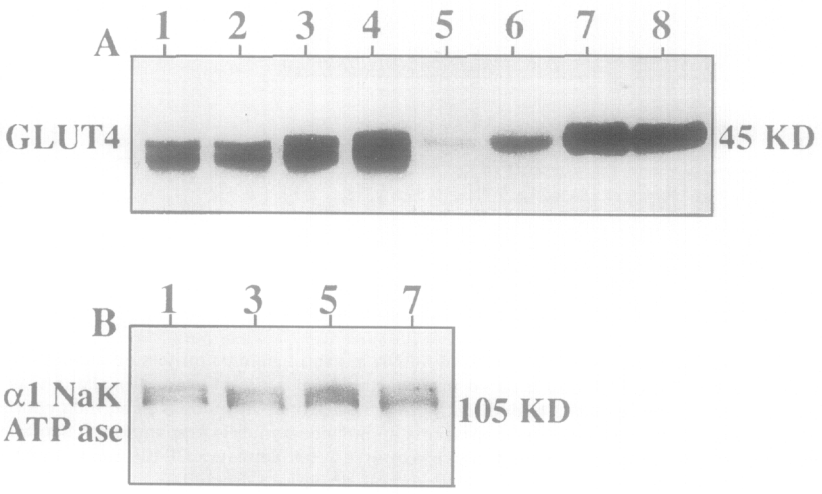


Fig 2. Western blot analysis of GLUT4 and α1 Na/K ATPase proteins in crude membrane prepared from gastrocnemius muscle of control and diabetic rats treated for 10 days with vehicle or LA. Total membrane proteins were purified from gastrocnemius muscle obtained from control and diabetic rats treated IP with either buffer or LA (30 mg/kg body weight/d) for 10 days. Membrane proteins (20 μg protein/lane) were separated by SDS-PAGE on 10% polyacrylamide gels, transferred to nitrocellulose membranes, and immunoassayed using anti-GLUT4 (A) or anti-α1 Na/K ATPase (B) antibodies. Blot represents 4 separate experiments yielding similar results and corresponds to 8 individual muscle preparations in each group. Lanes 1 and 2, nondiabetic vehicle-treated; 3 and 4, nondiabetic LA-treated; 5 and 6, diabetic vehicle-treated; 7 and 8, diabetic LA-treated.

Table 2. Effect of LA Treatment on the Relative Amount of GLUT4 and α1 Na/K ATPase Protein in Total Membranes of Gastrocnemius Muscle From Control and STZ-Diabetic Rats

Group	GLUT4	α1 Na/K ATPase
Control		
Vehicle-treated	1.00 ± 0.05	1.00 ± 0.10
LA-treated	1.53 ± 0.16*	1.15 ± 0.02
Diabetic		
Vehicle-treated	0.68 ± 0.07†	0.90 ± 0.10
LA-treated	1.93 ± 0.34‡§	1.08 ± 0.04

NOTE. Videodensitometry analysis was performed for autoradiographs from Western blots of crude gastrocnemius muscle membranes probed with anti-GLUT4 or anti–α1 Na/K ATPase antibodies. Gastrocnemius membranes were prepared from rats treated as described in Fig 2. Since GLUT4 appeared as a double band, both 45- and 47-kd bands were analyzed by videodensitometry. Data are expressed in arbitrary units relative to vehicle-treated nondiabetic rats. Results are the mean ± SE for 8 rats in each group.

*$P = .01$ *v* vehicle-treated nondiabetic animals.

†$P = .025$ *v* vehicle-treated nondiabetic animals.

‡$P = .017$ *v* vehicle-treated nondiabetic animals.

§$P = .003$ *v* vehicle-treated diabetic animals.

LA Treatment Prevents Diabetes-Induced Elevation in Gastrocnemius Lactic Acid Concentration

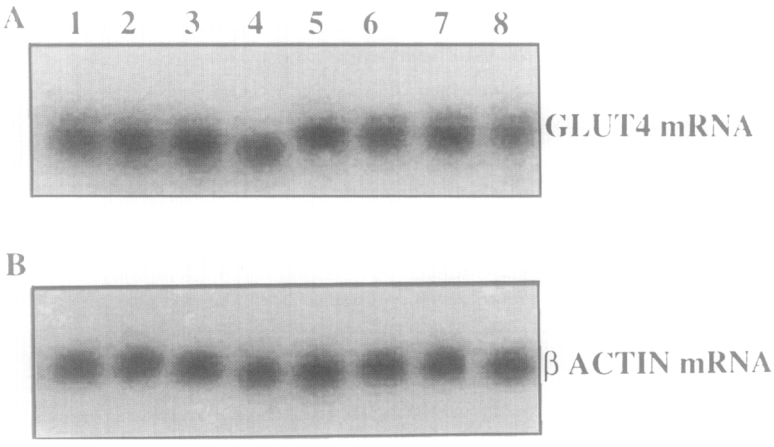
To assess whether LA-induced alterations in muscle GLUT4 content are associated with changes in intracellular carbohydrate or protein metabolism, the LA effect on gastrocnemius lactic acid concentration was evaluated. Muscle lactic acid concentration may be increased in diabetes through several mechanisms, including increased glycolysis, reduced pyruvate dehydrogenase activity, and/or enhanced proteolysis. Diabetic vehicle-treated rats were indeed found to exhibit a twofold increase in gastrocnemius lactic acid concentration compared with controls (19.9 ± 5.5 *v* 10.4 ± 2.8 μmol/g muscle, $P < .05$). Lactic acid concentration in gastrocnemius of nondiabetic rats was unaffected by LA treatment (10.4 ± 2.8 and 9.5 ± 2.0 μmol/g muscle for vehicle- and LA-treated rats, respectively). In contrast, LA treatment of diabetic rats reduced muscle lactic acid concentration to the control level, suggesting an improvement of muscle glucose utilization in diabetic rats due to LA treatment.

LA Prevents Diabetes-Induced Reduction in Insulin-Stimulated 2DG Uptake by Isolated Soleus

To directly evaluate whether LA effects are associated with increased glucose uptake activity, 2DG uptake was measured using isolated soleus muscle. Although this muscle differs from gastrocnemius with respect to muscle fiber-type composition, a similar pattern of GLUT4 regulation has been described in both muscles in various conditions, including STZ-diabetes.[27] Since gastrocnemius muscle is not suitable for in vitro transport measurements, soleus muscle was used for this purpose. Basal 2DG uptake was similar in control and diabetic animals treated or untreated with LA (Table 3). In contrast, a significant reduction in insulin-stimulated glucose transport activity was observed in STZ-diabetic rats 17 days after induction of diabetes (474 ± 15 *v* 568 ± 52 pmol 2DG/mg muscle · 30 min, $P = .05$, in diabetic and vehicle-treated nondiabetic rats, respectively). However, in diabetic rats under LA treatment, insulin-stimulated glucose uptake was similar to that of control animals (593 ± 25 *v* 568 ± 52 pmol 2DG/mg muscle · 30 min in LA-treated diabetic and vehicle-treated nondiabetic rats, respectively). Although glucose transporter levels and glucose transport activity were measured in two different muscles, taken together, these results suggest that LA treatment of diabetic animals increases insulin-dependent glucose uptake by elevating skeletal muscle GLUT4 content.

DISCUSSION

The present study demonstrates that LA treatment reduces blood glucose levels in STZ-diabetic rats and increases the muscle GLUT4 content. In the absence of changes in insulin levels and food consumption, a reduced blood glucose level can result from inhibition of hepatic glucose production and/or



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	mRNA (arbitrary units)	
	GLUT 4	GLUT 4 / β actin
Control vehicle treated	1.00±0.07	1.00±0.11
Control lipoic acid treated	1.09±0.06	1.00±0.10
Diabetes vehicle treated	0.89±0.05	0.90±0.15
Diabetes lipoic acid treated	1.07±0.09	0.87±0.05

Fig 3. Northern blot analysis of GLUT4 mRNA in gastrocnemius muscle obtained from control and diabetic rats treated for 10 days with vehicle or LA. Total RNA was isolated from gastrocnemius muscle and 30 μg/lane was fractionated on a 1% agarose gel, transferred to nitrocellulose membrane, and hybridized first with GLUT4 cDNA probe (A) and then with β-actin cDNA probe (B). Shown is a representative autoradiograph in which each lane represents an individual muscle preparation. A summary of the GLUT4 mRNA and GLUT4/β-actin mRNA densitometric analysis (n = 10 per group) is expressed as the mean ± SE (C). Lanes 1 and 2, nondiabetic vehicle-treated; 3 and 4, nondiabetic LA-treated; 5 and 6, diabetic vehicle-treated; 7 and 8, diabetic LA-treated.

Table 3. Effect of LA Treatment on 2DG Uptake by Isolated Soleus Muscle in the Absence and Presence of Insulin

Group	2DG Uptake (pmol/mg muscle · 30 min) Basal	Insulin	-Fold Increase
Control			
Vehicle-treated (n = 11)	200 ± 22	568 ± 52	3.24 ± 0.49
LA-treated (n = 10)	248 ± 23	593 ± 33	2.57 ± 0.27
Diabetic			
Vehicle-treated (n = 16)	241 ± 17	474 ± 15†	2.01 ± 0.14§
LA-treated (n = 20)	217 ± 25	593 ± 25*	3.55 ± 0.43‡

NOTE. Control and STZ-diabetic rats received 10 days of IP LA injections. 2DG uptake by isolated soleus muscle was assayed in the absence and presence of insulin. The ratio between 2DG uptake in the presence and absence of insulin was calculated for each individual animal (-fold increase). Results are expressed as the mean ± SE.

*$P = .001$ *v* vehicle-treated diabetic animals.

†$P = .05$ *v* vehicle-treated nondiabetic animals.

‡$P = .004$ *v* vehicle-treated diabetic animals.

§$P = .01$ *v* vehicle-treated nondiabetic animals.

augmentation of glucose utilization by peripheral tissues (mainly muscle and fat). This study shows that 10 days' LA treatment (30 mg/kg IP) did not cause a reduction in blood glucose concentration in fasted nondiabetic rats (Table 1), eliminating the possibility that under such conditions LA suppressed hepatic glucose production in these animals. In diabetic rats, our results support the notion that LA indeed enhances muscle glucose metabolism, as previously described in both Zucker rats[13] and type II diabetic patients.[14] The possibility of a concomitant inhibition of hepatic glucose production in diabetic rats cannot be ruled out by this study.

Glucose transport is the rate-limiting step in muscle glucose utilization, and hence a possible target for LA action. Alterations in GLUT4 expression and function have been documented in a variety of insulin-resistant states, including the hypoinsulinemic STZ-diabetes model.[28] We found a 32% reduction in membrane GLUT4 protein content in gastrocnemius muscle from diabetic rats compared with nondiabetic controls (Table 2). Ten days' LA treatment significantly elevated gastrocnemius GLUT4 content in both control and diabetic rats. Evidence for the physiological consequences of increased muscle GLUT4 protein in diabetic muscle includes the reduction in glycemia, normalization of insulin-stimulated glucose transport activity in isolated soleus muscle, and reduction in muscle lactic acid concentration. Reduced muscle lactate may represent increased glucose metabolism by the muscle, as well as reduced proteolysis, both of which may be associated with improved energetic status. In contrast to diabetic animals, the LA-induced increase in gastrocnemius GLUT4 content did not correlate with any of the aforementioned physiological effects in nondiabetic animals. This is consistent with some reports of muscle GLUT4 overexpression not associated with altered glycemia or insulin-dependent 2DG uptake by the muscle,[29] which could be explained by altered subcellular localization of the transporters or their intrinsic activity. The fact that muscle GLUT4 increased in control animals despite a lack of effect on glycemia suggests that the GLUT4 elevation represented a direct effect of LA treatment and was not secondary to its hypoglycemic action.

The increase in muscle GLUT4 protein content was not associated with similar alterations in GLUT4 mRNA (Fig 3). A reduction in muscle GLUT4 mRNA is well established in the literature for prolonged diabetes and diabetes induced by high-dose STZ.[28] However, in our STZ-diabetes model (short diabetes duration and STZ 65 mg/kg), reduced muscle GLUT4 mRNA is not a consistent finding.[16,30] In most conditions, alterations in GLUT4 protein and mRNA content are correlated,[18,28] although a differential regulation mode has been described.[31-33] This suggests a posttranscriptional mechanism, such as an increased translation rate for existing mRNA or a reduced protein degradation. Available data concerning the regulation of GLUT4 protein degradation mechanisms in muscle are scarce. A specific protease that recognizes vicinal sulfhydryl groups has been implicated in the regulation of GLUT4 degradation.[34] The increased oxidant stress observed in the STZ-diabetes model[35] may result in changes in SH/S-S groups and lead to increased susceptibility to proteolytic processes.[36] In mammalian tissues, LA can be converted to dihydrolipoate, which is capable of preventing generation of disulfide bonds.[3-5] Therefore, LA treatment may interfere with GLUT4 degradation processes, leading to increased protein stability.

Although the applicability of our results to humans has yet to be established, increasing muscle glucose utilization may be a significant finding relevant for both insulin-dependent and non–insulin-dependent diabetes mellitus.

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