α-Lipoic Acid Prevents the Development of Glucose-Induced Insulin Resistance in 3T3-L1 Adipocytes and Accelerates the Decline in Immunoreactive Insulin During Cell Incubation

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Oxidative stress has been implicated in glucose toxicity. We tested the hypothesis that certain antioxidants may prevent insulin-resistant glucose transport that develops in adipocytes after sustained exposure to high glucose, provided insulin is present. The antioxidant α-lipoic acid has been proposed as an insulin sensitizer. 3T3-L1 adipocytes were preincubated 18 hours in media containing insulin (0.6 nmol/L) with low (5 mmol/L) or high (25 mmol/L) glucose with or without α-lipoate, dihydrolipoate (each 0.1 to 0.5 mmol/L), or *N*-acetylcysteine (1 to 5 mmol/L). After extensive re-equilibration in insulin and antioxidant-free media, basal and maximally insulin-stimulated (100 nmol/L) glucose transport was measured. Insulin was quantified by radioimmunoassay. Preincubation with α-lipoate and dihydrolipoate but not *N*-acetylcysteine increased subsequent basal glucose transport; the effect was much smaller than that of acute maximal insulin stimulation. Preincubation in high glucose without antioxidants inhibited acutely insulin-stimulated glucose transport by 40% to 50% compared with low glucose. This down- regulation was partially or completely prevented by each antioxidant. In cell-free media, the 2 reductants, dihydrolipoate and *N*-acetylcysteine, rapidly decreased immunoreactive insulin, but α-lipoate was ineffective. However, during incubation with adipocytes, α-lipoate, and dihydrolipoate promoted the decline in immunoreactive insulin nearly equally. Because insulin and high glucose are synergistic in inducing insulin resistance in this model, the reduction in immunoreactive insulin probably contributed to the protective effect of the antioxidants. 3T3-L1 adipocytes efficiently metabolize α-lipoate to dihydrolipoate, which may be released into the medium. The stimulation of glucose transport by α-lipoic acid may represent redox effects in subcellular compartments that are accessible to dihydrolipoate.
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SUSTAINED HYPERGLYCEMIA induces insulin resistance in humans and in experimental animals and impairs the insulin secretory response of β cells to acute hyperglycemia. These observations gave rise to the concept of glucose toxicity.[1] Glucose-induced insulin resistance reflects in great part impaired insulin-stimulated glucose transport into skeletal muscle and adipocytes. It accounts for the insulin resistance associated with poorly controlled type 1 diabetes and contributes to insulin resistance in type 2 diabetes.[1,2] The mechanisms that cause glucose toxicity are incompletely understood. Activation of protein kinase C,[3-5] enhanced glucose flux via the hexosamine biosynthetic pathway,[6-8] and increased production of reactive oxygen species (ROS)[9-11] have been implicated. The synergistic effects of glucose and insulin in down-regulating glucose transport and inducing insulin resistance were first described in isolated rat adipocytes in primary culture.[12]

3T3-L1 adipocytes are derived from a mouse fibroblast cell line,[13] which differentiates into adipocytes in response to defined hormonal manipulations.[14,15] This model has been widely used to study the mechanism of insulin's stimulation of glucose transport and GLUT4 trafficking. As in primary adipocytes, prolonged (8 to 18 hours) exposure of 3T3-L1 adipocytes to high glucose, as compared with low glucose, down-regulates basal and to a greater extent insulin-stimulated glucose transport, provided that low-dose insulin is present during preincubation.[15]

α-Lipoic acid (LA) is considered a naturally occurring antioxidant. It is an obligatory cofactor of α-ketoacid dehydrogenase complexes, ie, pyruvate, α-ketoglutarate, and branched-chain α-ketoacid dehydrogenases, which are located on the inner mitochondrial membrane. LA is rapidly transported into cells, where it can be converted to its reduced form, dihydrolipoic acid (DHLA), which is a potent reducing agent. Both LA and DHLA quench certain ROS, chelate some transition metals, and exhibit hydrophobic binding to proteins such as albumin.[16] Based on its antioxidant properties and the concept that diabetes is associated with increased oxidative stress,[9,10,17-20] the effects of LA on glucose metabolism and insulin sensitivity have been investigated.

Incubation of 3T3-L1 adipocytes or L6 myotubes with high concentrations (2.5 mmol/L) of LA or DHLA for 1 to 4 hours stimulated glucose transport nearly as well as insulin. LA and DHLA were equally effective. LA promoted the translocation of GLUT4 and GLUT1 to the cell membrane, and, like the effect of insulin, LA stimulation of glucose transport was prevented by pretreatment with wortmannin, a PI-3 kinase inhibitor.[21,22] In insulin-resistant (fa/fa) Zucker rats, parenteral treatment with LA enhanced the insulin response of glucose transport and metabolism in muscles, whereas no treatment effect was observed in muscles of lean (fa/−) insulin-sensitive

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rats. Concomitantly, plasma insulin and nonesterified fatty acid (NEFA) concentrations decreased in LA-treated fa/fa rats.[23] In 3T3-L1 adipocytes, pre-exposure to micromolar concentrations of H_20_2 results in marked impairment of insulin-stimulated glucose transport and GLUT4 translocation and disruption of the insulin-induced cellular redistribution of IRS-1 and PI-3 kinase.[24] Pretreatment of these cells with 200 μmol/L LA for 16 hours provided nearly complete protection from oxidative stress-induced insulin resistance.[17]

To test the hypothesis that oxidative stress may contribute to glucose-induced insulin resistance in 3T3-L1 adipocytes we tested the effects of including LA, DHLA, or *N*-acetylcysteine (NAC) during preincubation on the subsequent acute insulin response of glucose transport. NAC is a cell-permeable antioxidant that is structurally different from LA. It is widely used experimentally to protect cells from oxidative stress.[11,25-27] Because in our model the development of glucose-induced insulin resistance requires the presence of insulin during preincubation with high glucose,[15] the effect of the antioxidants on insulin concentrations was also assessed.

MATERIALS AND METHODS

±-α-Lipoic acid (DL-6,8-thioctic acid), ± dihydrolipoic acid (DL-6,8-thioctic acid, reduced form), and *N*-acetyl-L-cysteine were purchased from Sigma (St Louis, MO). Crystalline, human recombinant insulin was a gift from Lilly Research Laboratories (Indianapolis, IN). Tissue culture supplies were purchased from Gibco (Rockville, MD), with the exception of fetal calf serum (FCS) and calf serum, which were purchased from Biofluids (Rockville, MD). 2-[1,2-^{3}H]-deoxy-D-glucose (2-DOG) and [^{14}C]sucrose were purchased from American Radiolabelled Chemicals (St Louis, MO). Other reagents were from Sigma or Fisher Scientific (Swanee, GA).

Cell Culture and General Methods

3T3-L1 fibroblasts were grown and differentiated into adipocytes as previously described.[14,15] Differentiated cells were maintained in Dulbecco modified Eagle medium (DMEM) containing 25 mmol/L glucose and 10% FCS until they were used in experiments 10 to 14 days after the start of differentiation, when 90% to 95% of cells exhibited the adipocyte phenotype. For experiments, cells were placed for 18 hours at 37°C into DMEM containing 1% FCS, 0.6 nmol/L insulin, and either 5 or 25 mmol/L glucose, with or without the addition of antioxidants (LA, DHLA, or NAC) at the concentrations indicated.

After preincubation, adipocytes were washed 3 times with phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) at 37°C and incubated for 2 hours in serum, insulin, and antioxidant-free DMEM containing the same sugar concentrations as during preincubation, with 0.5% BSA, 25 mmol/L HEPES at 37°C in a 5% CO_2 atmosphere. Cells were rapidly washed and equilibrated for 10 minutes at 37°C with 0.1% BSA in glucose-free Krebs Ringer bicarbonate buffer (KRBH), as described.[15]

Glucose Transport Measurements

Cells prepared as described above were incubated for 15 minutes at 37°C in glucose free KRBH without or with a maximally stimulating acute insulin dose (100 nmol/L). Glucose transport was initiated by adding 2-DOG (0.05 mmol/L, 0.5 μCi/mL). [^{14}C]Sucrose (0.05 mmol/L, 0.05 μCi/mL) was added as an extracellular space marker. 2-DOG transport was measured over 5 minutes and normalized to the protein concentration in the cell extract, as described. The latter was measured spectrophotometrically using Coomassie protein assay reagent purchased from Pierce (Rockford, IL) against BSA standards.

Insulin Radioimmunoassay

The concentration of insulin in the media was determined by radioimmunoassay using kits purchased form Linco Research Inc (St Charles, MO) as recommended by the manufacturer. All media were diluted 1:10 before assay. Samples of media were removed for analysis within 5 minutes after preparation of the complete medium or after 18 hours of incubation at 37°C without or with cells. Aliquots were stored at −70°C until they were assayed within 72 hours. To assure that components of the medium did not interfere with the radioimmunoassay, standards were assayed in buffer with or without the complete media (except for insulin addition), at the same concentration as used in the assay. The standard curves prepared under the 2 conditions were identical. The final dilution of the media in the assay mixtures during overnight equilibration with anti-insulin antibody was 1:30.

Statistical Analyses

Means ± SEM are shown. The significance of differences between means was evaluated by 2-tailed, unpaired Student *t* test using Statview 4.5 statistical software (Statsoft, Tulsa, OK) and by 2-way analysis of variance (ANOVA) and Tukey's test for unbalanced design using Statistica software (Abacus Concepts, Berkeley, CA). $P < .05$ was considered significant.

RESULTS

The effects of including DHLA or LA during 18 hours of preincubation in media containing 0.6 nmol/L insulin in the presence of 5 or 25 mmol/L glucose on subsequent basal and insulin-stimulated glucose transport are illustrated in Fig 1. To facilitate data analysis and graphic representation, 2-DOG transport (nmol/mg/5 min) measurements under different conditions were expressed as a percentage of the mean acutely insulin-stimulated transport observed after preincubation in low glucose with 0.6 nmol/L insulin (without addition of LA or DHLA) assayed in the same experiment. Two-way ANOVA revealed that both glucose concentration (high *v* low glucose during preincubation) or the addition of LA or DHLA during preincubation markedly affected subsequent glucose transport ($P < .0001$ for each condition). As previously reported,[15] preincubation in high glucose markedly reduced basal and insulin-stimulated glucose transport compared with preincubation in low glucose ($P < .001$) and decreased insulin stimulation of transport above basal values (Δinsulin). The inclusion of DHLA or LA during preincubation tended to increase basal and insulin-stimulated transport under both conditions without a clear dose effect. When DHLA- or LA-treated samples preincubated in low glucose were compared with their respective untreated controls by Student *t* test, basal glucose transport was significantly increased by 0.1 mmol/L DHLA and by 0.1 and 0.5 mmol/L LA ($P < .001$, $P < .02$, and $P < .05$, respectively). The effect of 0.5 mmol/L DHLA was not significant. Stimulation of basal glucose transport by either agent was less than 2-fold. Acute stimulation with insulin (100 nmol/L) increased glucose transport more than 4-fold over basal values in untreated samples preincubated in low glucose. Maximal insulin-stimulated transport was increased further (by 25%) in cells treated with 0.1 mmol/L DHLA or 0.1 mmol/L LA ($P < .001$, $P < .01$, respectively), but not in the cells treated with 0.5 mmol/L DHLA or LA. In cells preincubated in high glucose, the depressed basal glucose transport was increased by each of the 4 treatments, the increase was significant in cells treated

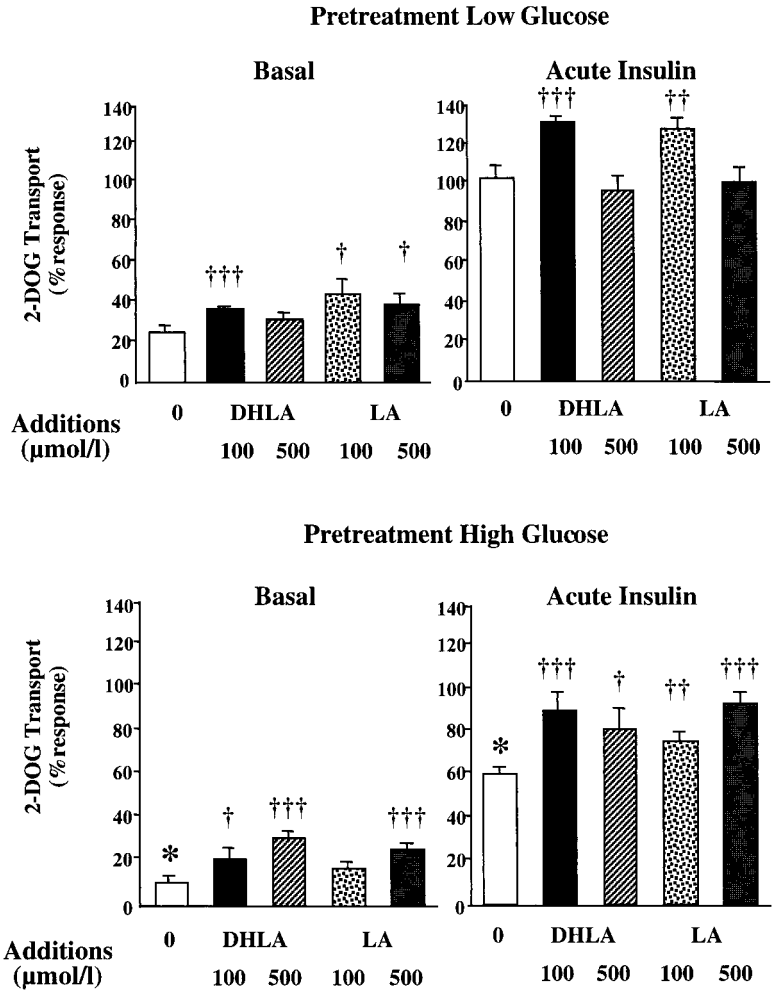

Fig 1. Effects of preincubation with LA or DHLA in media containing low or high glucose on basal and insulin-stimulated glucose transport. 3T3-L1 adipocytes were preincubated for 18 hours in DMEM containing 1% FCS, 0.6 nmol/L insulin, 5 or 25 mmol/L glucose, without or with LA or DHLA (100 or 500 μmol/L). Cells were then re-equilibrated for 2 hours in DMEM without insulin, FCS, or antioxidants and placed into glucose-free KRBH for 10 minutes then into KRBH without or with insulin (100 nmol/L) for 15 minutes before measurement of 2-DOG transport for 5 minutes as described.[15] 2-DOG transport measurements were normalized to the acutely insulin-stimulated transport of cells preincubated in low glucose (without LA or DHLA) in the same experiments. Under these conditions, mean 2-DOG transport was 1.19 ± 0.08 nmol/5 min (n = 26, from 13 experiments) with a range of 0.65 to 2.22 nmol/5 min in different experiments. Means ± SEM are shown; n = 20 to 26 for the control groups (without antioxidants), and 6 to 12 for the experimental groups, from 3 to 6 separate experiments. *$P < .001$ compared with control preincubated in low glucose. †$P < .05$, ††$P < .01$, †††$P < .001$, treatment effect v corresponding no-addition control by Student *t* test.

with 0.5 mmol/L DHLA ($P < .01$), 0.1 mmol/L LA ($P < .05$), and 0.5 mmol/L LA ($P < .001$), but not with 0.1 mmol/L DHLA. The depressed insulin-stimulated glucose transport of cells preincubated in high glucose was restored toward normal by all 4 treatment modalities. It was increased significantly by 0.1 mmol/L DHLA ($P < .001$), 0.5 mmol/L DHLA ($P < .05$), 0.1 mmol/L LA ($P < .01$), and 0.5 mmol/L DHLA ($P < .001$). Furthermore, the insulin-stimulated glucose transport of cells preincubated with high glucose and either dose of DHLA or LA was no longer significantly different from that of cells preincubated in low glucose. However, post hoc analysis by Tukey's test of the 2-way ANOVA did not reach statistical significance for any of the LA or DHLA treatment subgroups described above.

We then tested whether NAC would also protect 3T3-L1 adipocytes from glucose-induced insulin resistance (Fig 2). The experimental design was identical to that used for LA (Fig 1), except that NAC was used at 10-fold higher concentrations (1 and 5 mmol/L) than LA. Inclusion of NAC during preincubation in high glucose (in the presence of 0.6 nmol/L insulin) increased acutely insulin-stimulated glucose transport in a dose-dependent manner ($P < .02$ and $P < .001$ for 1 mmol/L and 5 mmol/L NAC, respectively) over controls preincubated

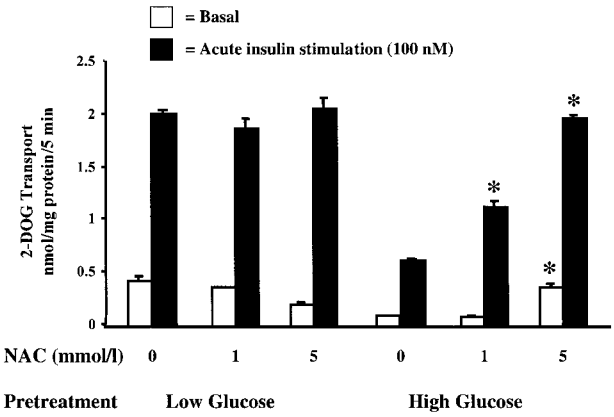

Fig 2. Effects of preincubation with NAC in media containing low or high glucose on basal and insulin-stimulated glucose transport. The experimental design is identical to that in Fig 1, except that NAC (1 or 5 mmol/L) was the antioxidant tested. *$P < .05$, **$P < .02$, ***$P < .001$, NAC effect *v* corresponding control without NAC. Data are means ± SD of 2 observations from 1 experiment that is representative of a total of 3 similar experiments.

in high glucose. NAC (5 mmol/L) also increased basal glucose transport in cells preincubated in high glucose ($P < .05$). NAC did not affect basal or insulin-stimulated glucose transport significantly in cells preincubated in low glucose and insulin. Inclusion of 5 mmol/L NAC during incubation with high glucose completely prevented the glucose-induced down-regulation of insulin- stimulated glucose transport.

The data in Figs 1 and 2 indicate that inclusion of antioxidants during preincubation in high glucose partially or completely prevented the development of glucose-induced insulin resistance, supporting a role for oxidative stress in glucose transport down-regulation in these cells. However, because insulin and high glucose are both required for the development of insulin resistance in our model, the protective effect could also be achieved if the antioxidants promoted insulin degradation. Therefore, we examined the effects of LA, DHLA, and NAC on the concentrations of immunoreactive insulin in the media. The media were sampled within 5 minutes of addition of antioxidants and again after 18 hours of incubation at 37°C without cells. Both DHLA and NAC caused a rapid, dose-dependent decrease in the concentration of immunoreactive insulin in the absence of cells (Fig 3). At the doses used in our experiments, 100 μmol/L and 500 μmol/L DHLA decreased immunoreactive insulin by ≈20% and ≈70%, respectively, within 5 minutes, and the decline was further enhanced after 18 hours at 37°C when only ≈10% of the original immunoreactive insulin remained in media supplemented with 0.5 mmol/L DHLA. The insulin concentration in the media (0.6 ± 0.1 nmol/L) was unchanged after 18 hours of incubation without antioxidants. The addition of LA (0.05 to 0.5 mmol/L) to insulin-containing media did not affect the insulin concentration during 18 hours of incubation without cells (Fig 3). Comparing the dose-response curves of DHLA and NAC (Fig 3), DHLA was 5- to 10-fold more potent than NAC in promoting the decline of immunoreactive insulin; 1 mmol/L NAC decreased immunoreactive insulin by ≈50% within 5 minutes, and 5 mmol/L NAC depleted it by ≈90% in 5 minutes and ≈95% in 18 hours.

Because preincubation with DHLA or LA protected cells similarly against glucose-induced down-regulation of glucose transport, yet only DHLA promoted the decrease in immunoreactive insulin in a cell-free system, we assayed media insulin after incubating the cells with or without 100 μmol/L or 500 μmol/L DHLA or LA (Fig 4). As previously reported,[14,15] 3T3-L1 adipocytes degrade insulin, and the immunoreactive insulin concentration decreased to 38% ± 2% (n = 9) of the initial concentration after 18 hours of incubation with cells in unsupplemented media, ie, it decreased from 0.6 to ≈0.2 nmol/L. Inclusion of 0.1 mmol/L DHLA or LA in the medium decreased immunoreactive insulin to approximately one half (≈0.1 nmol/L) of that remaining in the unsupplemented samples ($P < .001$), and the 2 agents were equally effective. At 0.5 mmol/L, DHLA reduced the insulin concentration in the medium to less than 5% of that originally present while 0.5 mmol/L LA reduced it to 12.6% ± 2.6% (≈75 pmol/L). The discrepancy between the effects of LA in the presence and absence of cells may reflect the fact that LA is internalized by cells and reduced, and DHLA may be subsequently released into the media.[16]

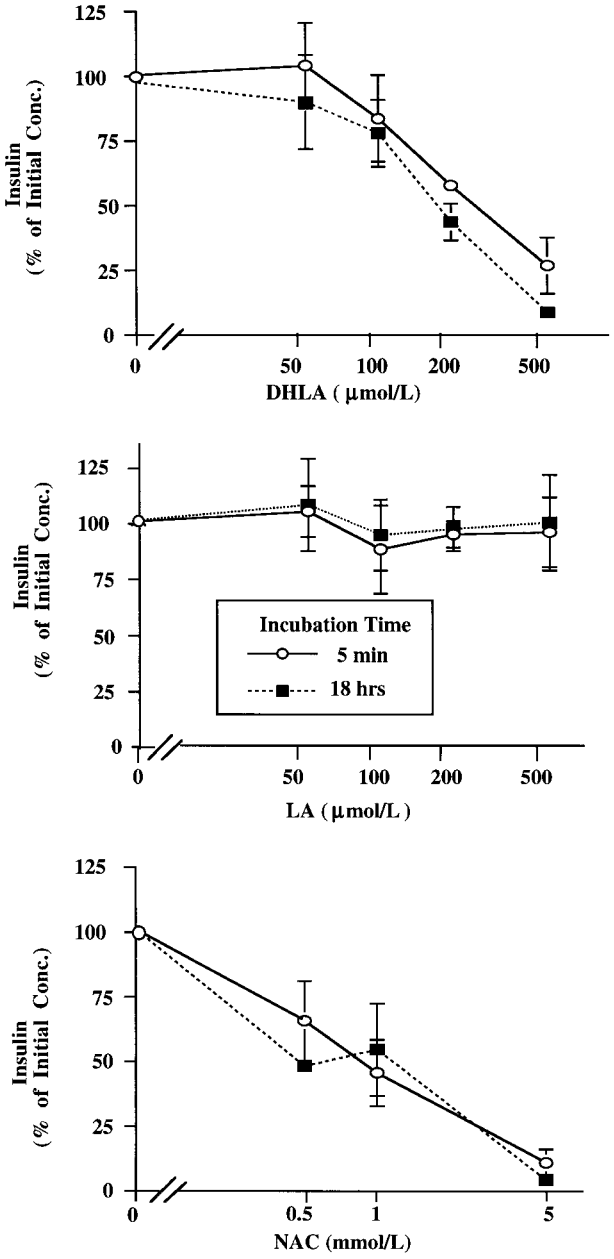

Fig 3. Effect of antioxidants on immunoreactive insulin in the absence of cells. Preincubation media were prepared as described in the legend to Fig 1, without or with the addition of antioxidants (LA, DHLA, or NAC) at the concentrations indicated on the abscissas in logarithmic scale. Insulin was quantitated by radioimmunoassay (Linco Research Kit). Samples were removed for analysis within 5 minutes of addition of the antioxidant or after 18 hours of incubation at 37°C without cells. Data are expressed as % of the immunoreactive insulin (0.6 ± 0.1 nmol/L, n = 11) present in the media without antioxidant addition. All media samples were diluted 1:10 before radioimmunoassay. Data are means ± SE of 4 to 6 determinations from 2 to 3 experiments.

DISCUSSION

The insulin molecule has 3 disulfide (S-S) bonds, which are highly conserved among species. Two S-S bonds link the α and

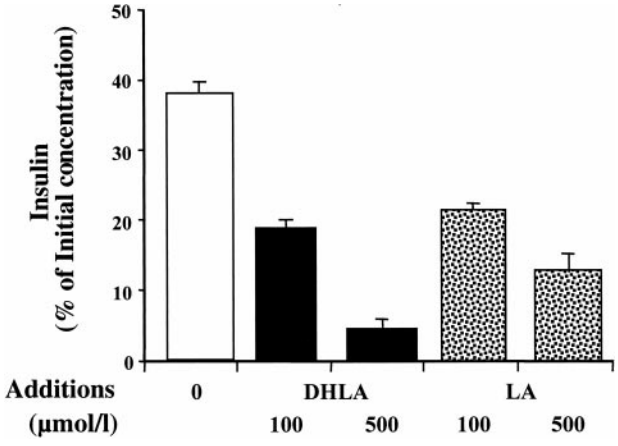

Fig 4. Effect of LA and DHLA on immunoreactive insulin during incubation with 3T3-L1 adipocytes. Cells were incubated, as described in Fig 1, for 18 hours in media containing 0.6 nmol/L insulin without or with 100 or 500 μmol/L LA or DHLA. At the end of incubation, the insulin concentration in the media was determined by radioimmunoassay as described in the legend to Fig 3. Data are normalized to the insulin concentration before incubation in media without antioxidants. Means ± SE of 5 to 6 determination from 3 experiments are shown.

β chain, and a third intrachain S-S bond links Cys-6 and Cys-11 of the α chain. DHLA is a strong reducing agent. The redox potential of the DHLA/LA couple is −0.32 V compared with the reduced glutathione (GSH)/oxidized glutathione (GSSG) couple, which has a redox potential of −0.24 V. Thus, DHLA will reduce GSSG to GSH, but GSH is incapable of reducing LA to DHLA.[16]

GSH is the most abundant thiol in mammalian cells and is considered the primary agent in the redox regulation of thiol proteins.[16] Because both NAC and DHLA are reducing agents, the most likely explanation for the rapid decrease in immunoreactive insulin within 5 minutes of exposure to these agents in a cell-free medium is that the reduction of 1 or more S-S bonds induces conformational changes that prevent recognition by the anti-insulin antibody. Reduction of both interchain S-S bonds results in the disassociation of the 2 chains. In in vitro studies, the dissociation of the insulin chains was used as an indicator to monitor the reduction of LA enantiomers to DHLA by purified pyruvate or α-ketoglutarate dehydrogenase complexes.[28] Garant et al[29] recently incubated cells overexpressing the insulin receptor (IR) with GSH or NAC. They documented rapid and reversible reduction of IR α-subunit disulfide bonds upon brief exposure to either agent. Although the oligomeric IR structure was unaltered, IR signaling was impaired.[29] It seems likely that in the present study, the decrease in immunoreactive insulin was paralleled by impaired bioactivity.

In contrast to DHLA and NAC, LA did not affect the concentration of immunoreactive insulin in the absence of cells. However, in the presence of 3T3-L1 adipocytes, LA markedly accelerated the decrease in immunoreactive insulin, and DHLA and LA were nearly equally effective. Jurkat T lymphocytes incubated with LA (50 to 500 μmol/L) rapidly reduced LA to DHLA. Intracellular DHLA concentrations increased and correlated with increasing intracellular GSH. Total glutathione (GSH + GSSG) increased more than 40% and was accounted for exclusively by increased GSH.[30] DHLA appeared to be released by the cells into the medium.[16] PC-12 cells incubated with NAC also showed marked increases in GSH as well as striking biologic effects. The latter were independent of the increase in GSH and reflected NAC's activity as a reducing agent. Furthermore, L-NAC and D-NAC were equally effective in increasing GSH, suggesting that NAC promoted the reduction of extracellular cystine to cysteine, the precursor of GSH.[25] Although direct proof is lacking, our data are consistent with previous observations in Jurkat cells, suggesting that LA was taken up by the cells, metabolically reduced to DHLA, and released into the media.[16,30] In 3T3-L1 adipocytes, the development of glucose-induced insulin resistance is insulin dependent.[14,15] We have reconfirmed this observation in the course of the present study (data not shown). Therefore, the decrease in immunoreactive insulin during preincubation with LA, DHLA, or NAC probably contributed to the protective effect of these agents against glucose-induced insulin resistance. It may represent the major effect in cells preincubated in high glucose + insulin + NAC, in view of the apparent correlation between the NAC dose–dependent decrease in immunoreactive insulin in the medium (Fig 3) and the restoration of acute insulin-stimulated glucose transport in cells preincubated in high glucose (Fig 2). Furthermore, in contrast to LA, NAC did not enhance basal or insulin-stimulated glucose transport in cells preincubated in low glucose + insulin.

In previous studies,[21,22] cultured L-6 myotubes or 3T3-L1 adipocytes were treated for 1 to 4 hours with LA or DHLA before measurement of glucose transport. At concentrations of 1 to 2.5 mmol/L, both agents stimulated glucose transport similarly and promoted the translocation of GLUT1 and GLUT4 to the plasma membrane.[21,22] Addition of insulin in the presence of LA caused no further stimulation of glucose transport.[21] However, pretreatment of Zucker rats in vivo with LA sensitized their muscles to acute insulin stimulation of glucose transport in vitro,[23] and R(+) LA was much more effective than S(−) LA.[31] Furthermore, preincubation of 3T3-L1 adipocytes with R(+) LA for 1 hour, followed by short-term insulin stimulation, enhanced glucose transport.[22] Brief incubation with R(+) LA in the absence of insulin stimulated glucose transport by engaging the insulin signaling pathway, causing enhanced tyrosine phosphorylation of the IR and IRS-1, increased association of PI-3 kinase with IRS-1, and stimulation of Akt activity.[32] The stimulatory effects of preincubation with LA or DHLA in the presence of low glucose reported here may reflect the above mechanisms. The effects were smaller than those described above, but we used racemic LA, lower doses, longer incubation, and a 145-minute washout period before measuring glucose transport. The persistence of the LA/DHLA stimulation of glucose transport after washout suggests that the effect of these drugs is more persistent than that of 0.2 nmol/L insulin, a high physiologic insulin concentration. The kinetics of the R(+) and S(−) enantiomers toward enzymes, eg, pyruvate dehydrogenase E2 subunit, lipoamide dehydrogenase, and glutathione reductase, are different.[16,17,33] Although in several systems R(+) LA was more effective than S(−) LA in increasing glucose transport,[21,22,31] under some conditions S(−) LA

appeared more potent at low doses.[17] Some of the variability in the responses observed in Fig 1 in this study may reflect the lack of parallelism of the dose responses to the R(+) and S(−) components of the racemic mixture. Future experiments using the more potent R(+) enantiomer may help answer the question whether R(+) LA protects against glucose-induced insulin resistance at doses that do not affect insulin.

The racemic mixture of LA has been used in vivo to attenuate insulin resistance in Zucker (fa/fa) rats[23] and in patients with type 2 diabetes.[34] In clinical trials, LA treatment improved diabetic neuropathy,[35] prevented hyperglycemia-induced increments of circulating pyruvate and lactate in patients with diabetes,[36] and appeared to enhance their insulin sensitivity.[34] In view of the short half-life of LA in the circulation,[37] it is unlikely that at the doses used in vivo LA treatment significantly decreased circulating insulin levels. However, if LA-treated cells release DHLA, which in turn reduces disulfide bonds of proteins, it may reduce insulin in the microenvironment of certain cells and/or modify ligands and/or receptors involved in insulin resistance. For example, tumor necrosis factor α, which may play a role in insulin resistance,[38] is a cytokine with a conserved intrachain S-S bond.[39]

NAC is extensively used in biologic research as a cell-permeable reductant and antioxidant. Blockade of a cellular response by NAC treatment, at doses similar to or higher than those used here, often implicates the participation of intracellular ROS in signal transduction.[26,27] Our data re-emphasize the need for cautious data interpretation; the possibility that a reductant may alter the extracellular milieu, ligands, and/or receptors needs to be considered particularly in studies of the role of ROS in signal transduction.

There is relatively little information concerning the metabolic fate of LA in different intact cells.[16,30] The fact that LA was nearly as effective as DHLA in decreasing immunoreactive insulin in the presence (but not in the absence) of cells supports the concept that 3T3-L1 adipocytes efficiently metabolize exogenously administered LA to DHLA and suggests that LA serves as substrate of the dehydrogenase complexes in intact cells. The release of DHLA or other reduced thiols by LA-treated cells may explain LA-induced increases in intracellular GSH[30,40] by promoting the reduction of cystine to cysteine in the microenvironment. The latter is preferentially transported into cells and promotes GSH synthesis.[25] DHLA associates with both the aqueous and membrane domain of cells[16,41]; thus DHLA may exert redox effects in cellular compartments that are not accessible to other reducing agents, eg, NAC or dithiothreitol.[32] The redox effects of intracellularly generated DHLA may mediate the insulin-like effects of LA on the signaling cascade and glucose transport in vitro,[32] and its action as an insulin sensitizer in insulin-resistant muscle in vivo.[23,31]

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