Effects of exercise training and antioxidant R-ALA on glucose transport in insulin-sensitive rat skeletal muscle

VITOON SAENGSRISUWAN, FELIPE R. PEREZ, TYSON R. KINNICK, AND ERIK J. HENRIKSEN

Muscle Metabolism Laboratory, Department of Physiology,
University of Arizona, College of Medicine, Tucson, Arizona 85721-0093

Received 14 June 2001; accepted in final form 14 September 2001

Saengsirisuwan, Vitoon, Felipe R. Perez, Tyson R. Kinnick, and Erik J. Henriksen. Effects of exercise training and antioxidant R-ALA on glucose transport in insulin-sensitive rat skeletal muscle. J Appl Physiol 92: 50–58, 2002; 10.1152/japplphysiol.000617.2001.—We have recently demonstrated (Saengsirisuwan V, Kinnick TR, Schmit MB, and Henriksen EJ, J Appl Physiol 91: 145–153, 2001) that exercise training (ET) and the antioxidant R(+)-α-lipoic acid (R-ALA) interact in an additive fashion to improve insulin action in insulin-resistant obese Zucker (fa/fa) rats. The purpose of the present study was to assess the interactions of ET and R-ALA on insulin action and oxidative stress in a model of normal insulin sensitivity, the lean Zucker (fa/−) rat. For 6 wk, animals either remained sedentary, received R-ALA (30 mg·kg body wt−1·day−1), performed ET (treadmill running), or underwent both R-ALA treatment and ET. ET alone or in combination with R-ALA significantly increased (P < 0.05) peak oxygen consumption (28–31%) and maximum run time (52–63%). During an oral glucose tolerance test, ET alone or in combination with R-ALA resulted in a significant lowering of the glucose response (17–36%) at 15 min relative to R-ALA alone and of the insulin response (19–36%) at 15 min compared with sedentary controls. Insulin-mediated glucose transport activity was increased by ET alone in isolated epimysial (30%) and soleus (50%) muscles, and this was associated with increased GLUT-4 protein levels. Insulin action was not improved by R-ALA alone, and ET-associated improvements in these variables were not further enhanced with combined ET and R-ALA. Although ET and R-ALA caused reductions in soleus protein carbonyls (an index of oxidative stress), these alterations were not significantly correlated with insulin-mediated soleus glucose transport. These results indicate that the beneficial interactive effects of ET and R-ALA on skeletal muscle insulin action observed previously in insulin-resistant obese Zucker rats are not apparent in insulin-sensitive lean Zucker rats. 

It is well established that endurance exercise training leads to an enhancement of insulin-mediated glucose metabolism (see reviews in Refs. 11, 15, 16). In normal rodent models, moderate- or high-intensity exercise training can improve glucose tolerance (2, 20), whole body insulin sensitivity (21, 22), and insulin action on skeletal muscle glucose transport activity in rodent models (13, 30, 34). The increased insulin action on skeletal muscle glucose transport after exercise training is associated with increased GLUT-4 protein expression (7, 13, 25, 30, 31, 34) as well as with adaptive responses of enzymes involved in glucose phosphorylation and oxidation (15, 16).

α-Lipoic acid (ALA) is a naturally occurring cofactor for several mitochondrial enzyme complexes that catalyze the oxidative decarboxylation of α-keto acids, and, when administered exogenously, ALA can act as a potent water-soluble antioxidant (26). It has previously been shown that ALA can modulate glucose metabolism in insulin-sensitive cells and tissues (see Ref. 12 for a recent review). When administered in vitro, ALA increases glucose utilization in the rat diaphragm (10) and enhances glucose uptake by rat myocardium (33, 37), L6 myocytes (6, 24), and locomotor skeletal muscles from both insulin-sensitive and insulin-resistant rats (14). In addition, we have demonstrated that parenteral administration of ALA to the obese Zucker (fa/fa) rat, an animal model of obesity-associated insulin resistance, significantly improves glucose tolerance and insulin action on skeletal muscle glucose transport (19, 27, 31, 36) with a substantially lesser acute effect on insulin-sensitive rats (19).

We have recently demonstrated in the obese Zucker rat a significant interaction between exercise training and chronic ALA administration on maximal run time to exhaustion and on insulin-stimulated glucose transport activity in skeletal muscle (31). However, the potential interactions between these two interventions have not yet been investigated in an animal model of normal insulin sensitivity. In this context, the purpose of the present investigation was to test the hypothesis that exercise training and chronic treatment with the R(+)-enantiomer of ALA (R-ALA), in combination, could improve insulin-stimulated glucose transport in skeletal muscle of lean Zucker (fa/−) rats to a greater extent than either intervention used individually. Additionally, we wished to further investigate the poten-
tial relationship between insulin-stimulated glucose transport and oxidative stress (as reflected in protein carbonyl level) in normal skeletal muscle. Lean Zucker rats underwent 6 wk of exercise training and 6 wk of parenteral administration of R-ALA, individually and in combination. Subsequently, peak aerobic capacity (peak O2 consumption; \(V_{\text{O2 peak}}\)), maximal run time to exhaustion, oral glucose tolerance, insulin-stimulated muscle glucose transport, muscle GLUT-4 protein level, tissue protein carbonyl level (a marker of oxidative stress) (5, 28), and the activities of enzymes involved in glucose phosphorylation (total hexokinase activity) and glucose oxidation (citrate synthase activity) were determined. The investigation of these potential interactions in normal muscle is important in determining whether the beneficial metabolic interactions between these interventions, which we have established in the insulin-resistant obese Zucker rat, are applicable to conditions of normal insulin action.

**METHODS**

**Animals and treatments.** Female lean Zucker (fa/–) rats (Harlan, Indianapolis, IN) were received at 5–6 wk of age and weighed 130–140 g. Animals were housed in a temperature-controlled room (20–22°C) at the Central Animal Facility of the University of Arizona. A reversed 12:12-h light-dark cycle (lights on 1900–0700) was maintained so that training occurred during the dark cycle when the rats are most active. Animals had free access to water and chow (Harlan Teklad Rodent Diet, Madison, WI). This chow does not contain any lipoic acid but does contain 90.2 IU/kg of vitamin E, an antioxidant. However, the amount of vitamin E consumed from the diet would be very small (~1 IU per rat per day), and it is unlikely that this dietary source of antioxidants affected the results of this study. All procedures were approved by the University of Arizona Animal Use and Care Committee.

Lean animals were treated exactly as in our previous study with obese Zucker rats (31). Lean Zucker rats were randomly assigned to one of four groups: 1) a group that remained sedentary and was vehicle treated, 2) an R-ALA-treated group, 3) an exercise-trained group, or 4) a combined R-ALA-treated and exercise-trained group. Animals in the R-ALA-treated groups received 30 mg/kg body wt of purified R-ALA (ASTA Medica, Frankfurt, Germany) dissolved in 120 mM Tris buffer (pH 7.4) by intraperitoneal injection (a maximally effective dose in obese Zucker rats; Ref. 36) every evening for 6 wk, whereas sedentary control animals received 8.3 ml/kg body wt of 120 mM Tris buffer (pH 7.4). Animals in the exercise-trained groups ran in the morning on a 10-lane motor-driven rodent treadmill for 6 wk at 4% grade. During the first 3 wk of training, animals ran 7 days/wk, and the training protocol was quickly increased to 60 min/day, continuously rotating through the following 15-min cycles: 24 min/min for 10 min, 26 min/min for 3 min, and 28 min/min for 2 min. Over the final 3 wk of training, animals ran 75 min/day, 5 days/wk by using these same 15-min cycles. The combined treatment animals performed the treadmill-training protocol exactly as described above, while also receiving daily treatments with R-ALA.

**Oral glucose tolerance tests.** After 6 wk of treatment, an oral glucose tolerance test (OGTT) was performed on each animal. At 6 PM of the evening before the test, rats were restricted to 4 g of chow. Between 8 and 9 AM on the day of the OGTT, ~15 h after the last R-ALA treatment and/or 24 h after the last exercise bout, rats were administered a 1 g/kg body wt glucose load by gavage. Blood was drawn from a cut at the tip of the tail at 0, 15, 30, 60, and 90 min after the glucose feeding, thoroughly mixed with EDTA (18 mM final concentration), and centrifuged at 13,000 g to separate the plasma. Plasma was stored at −80°C and subsequently assayed for glucose (Sigma Chemical, St. Louis, MO), insulin (Linco Research, St. Charles, MO), and free fatty acids (Wako, Richmond, VA). Immediately after completion of the OGTT, each animal was given 2 ml of sterile 0.9% saline subcutaneously to compensate for plasma loss, and animals in the exercise-training groups were run for 30 min.

\(V_{\text{O2 peak}}\). \(V_{\text{O2 peak}}\) was assessed in each animal during a treadmill test 48 h after the OGTT by using the method of Bedford et al. (1). Sedentary animals were familiarized with treadmill running by running for periods of 5–10 min three times per week in the 2 wk leading to the measurement of \(V_{\text{O2 peak}}\). No exercise was performed on the day before \(V_{\text{O2 peak}}\) tests. However, R-ALA was given to the R-ALA and the combined exercise-trained and R-ALA-treated groups on this day.

Animals ran on a motorized treadmill in an airtight Plexiglas chamber. Grade and speed of the treadmill were increased every 3 min from a basal level of 0% grade and 13.4 m/min through the following stages: 16.1 m/min at 5%, 21.4 m/min at 10%, 26.8 m/min at 10%, 32.2 m/min at 12%, 32.2 m/min at 15%, 32.2 m/min at 18%, and 32.2 m/min at 21%. The test was terminated when the rats were unable to keep pace with the treadmill belt. \(O_2\) (Ametek S-3A1, Applied Electrochemistry, Pittsburgh, PA) and \(CO_2\) (Ametek CD-3A) were measured in expired gases every 3 min for the determination of \(O_2\) uptake (ml \(O_2\)-kg body wt \(-1\)·min \(-1\)). Exercise training and R-ALA treatments were resumed the day after \(V_{\text{O2 peak}}\) assessment.

**Glucose transport activity in skeletal muscle.** Approximately 72 h after the \(V_{\text{O2 peak}}\) test, 24 h after the final exercise bout, and 15 h after the final R-ALA treatment, animals were weighed and deeply anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt). Determination of muscle glucose transport activity was initiated at 8 AM after an overnight food restriction as described in Oral glucose tolerance tests. One soleus and both epitrochlearis muscles were dissected and prepared for in vitro incubation. Whereas the epitrochlearis muscles were incubated intact, the soleus muscle was prepared in two strips (~25 mg) and incubated. Muscles were incubated in the unmounted state. Each muscle was incubated for 1 h at 37°C in 3 ml of oxygenated (95% \(O_2\)-5% \(CO_2\)) Krebs-Henseleit buffer (KHB) supplemented with 8 mM glucose, 32 mM mannitol, and 0.1% BSA (radioimmunoassay grade, Sigma Chemical). One epitrochlearis muscle and one soleus strip were incubated in the absence of insulin, and the contralateral epitrochlearis muscle and second soleus strip were incubated in the presence of a maximally effective concentration of insulin (2 mU/ml; Humulin R, Eli Lilly, Indianapolis, IN). After this initial incubation period, the muscles were rinsed for 10 min at 37°C in 3 ml of oxygenated KHB containing 40 mM mannitol, 0.1% BSA, and insulin, if previously present. Thereafter, the muscles were transferred to 2 ml of KHB, containing 1 mM 2-[1,2,3\(^{3}H\)]deoxyglucose (2-DG; 300 mCi/mmol; Sigma Chemical), 39 mM [U-14\(^{C}\)]mannitol (0.8 mCi/mmol; ICN Radiochemicals, Irvine, CA), 0.1% BSA, and insulin, if previously present. At the end of this final 20-min incubation period at 37°C, the muscles were removed, trimmed of excess fat and connective tissue, quickly frozen, and weighed. Epitrochlearis muscles were divided into two pieces, which were individually reweighed. One piece from each epitrochlearis muscle and the entire soleus strip were...
dissolved in 0.5 ml of 0.5 N NaOH. After the muscles were completely solubilized, 5 ml of scintillation cocktail were added, and the specific intracellular accumulation of 2-DG was determined as described previously (13) by using mannitol to correct for the extracellular accumulation of 2-DG. Glucose transport activity was measured as the intracellular accumulation of 2-DG (in pmol · mg muscle wt⁻¹ · 20 min⁻¹).

**Biochemical assays.** The remaining two pieces of epitrochlearis were pooled, reweighed, and homogenized in 40 volumes of ice-cold 20 mM HEPES (pH 7.4) containing 1 mM EDTA and 250 mM sucrose. These homogenates were used for determination of total protein content by using the bicinchoninic acid method (Sigma Chemical), GLUT-4 protein level (31), total hexokinase activity (38), and citrate synthase activity (35). In addition, the contralateral soleus and plantaris muscles, liver, and heart were removed, trimmed of fat and connective tissue, quickly frozen in liquid nitrogen, and used for subsequent determination of these same variables as well as for the measurement of protein carbonyl levels by using the method of Reznick and Packer (28). Briefly, pieces of frozen tissue (50–90 mg) were gently homogenized in 1.5 ml of a 50 mM phosphate buffer (pH 7.4) containing 0.1% digitonin, 1 mM EDTA, and protease inhibitors (40 μg/ml phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin, 7 μg/ml pepstatin, and 5 μg/ml aprotinin). If needed, nucleic acids were removed with 1% streptomycin sulfate, and extracted soluble proteins were then reacted with 10 mM 2,4-dinitrophenylhydrazine (DNPH) in 2.5 M HCl for 1 h at room temperature. Proteins were precipitated with 10% TCA, and protein pellets were washed with ethanol/ethyl acetate (1:1) (vol/vol) to remove free DNPH and lipid contaminants. Final precipitates were dissolved in 6 M guanidine HCl and incubated at 37°C for 10 min. The carbonyl contents of these samples were then assayed by using a spectrophotometric assay at 370 nm and an absorption coefficient of 22,000 M⁻¹·cm⁻¹ (28). Protein content of the final samples was quantified by reading the absorbance at 280 nm with the use of a BSA standard curve. These protein contents were typically in the range of 0.3–0.5 mg. In our hands, this assay had a coefficient of variance of 11%.

**Statistical analysis.** All values are expressed as means ± SE. The significance of differences among the four experimental groups was assessed by a factorial ANOVA with a post hoc Fisher’s protected least-significant difference test, and relationships between two variables were assessed by linear regression analysis (StatView version 5.0, SAS Institute, Cary, NC). A level of P < 0.05 was set for statistical significance.

---

**Table 1. Effects of exercise training and chronic R-ALA treatment on body weight, VO₂peak, maximum run time to fatigue, and plasma glucose, insulin, and free fatty acids**

<table>
<thead>
<tr>
<th>Group</th>
<th>Initial Body Wt, g</th>
<th>Final Body Wt, g</th>
<th>Change in Body Wt/day, g/day</th>
<th>VO₂peak, ml·kg⁻¹·min⁻¹</th>
<th>Maximum Run Time, min</th>
<th>Plasma Glucose, mg/dl</th>
<th>Plasma Insulin, μU/ml</th>
<th>Plasma Free Fatty Acids, mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedentary</td>
<td>155 ± 2</td>
<td>220 ± 5</td>
<td>1.55 ± 0.10</td>
<td>63.3 ± 1.2</td>
<td>12.2 ± 0.4</td>
<td>113 ± 4</td>
<td>24 ± 2</td>
<td>0.57 ± 0.04</td>
</tr>
<tr>
<td>R-ALA-treated</td>
<td>150 ± 4†</td>
<td>201 ± 5‡</td>
<td>1.20 ± 0.06‡</td>
<td>65.5 ± 1.3</td>
<td>10.4 ± 3</td>
<td>119 ± 6</td>
<td>18 ± 1§</td>
<td>0.35 ± 0.03§</td>
</tr>
<tr>
<td>Exercise-trained</td>
<td>159 ± 2</td>
<td>217 ± 3</td>
<td>1.39 ± 0.07</td>
<td>83.1 ± 1.7†</td>
<td>18.4 ± 0.7‡</td>
<td>119 ± 6</td>
<td>18 ± 1§</td>
<td>0.43 ± 0.06</td>
</tr>
<tr>
<td>Combined</td>
<td>149 ± 2‡</td>
<td>202 ± 2‡</td>
<td>1.27 ± 0.05§</td>
<td>81.3 ± 1.4†</td>
<td>19.8 ± 0.9†</td>
<td>113 ± 6</td>
<td>22 ± 1</td>
<td>0.57 ± 0.06</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6–9 animals/group and were measured after an overnight food restriction (4 g of chow). R-ALA, R-(-)α-lipoic acid; VO₂peak, peak oxygen consumption. *P < 0.05 vs. sedentary control group. †P < 0.05 vs. R-ALA-treated group. ‡P < 0.05 vs. exercise-trained group. §P < 0.05 vs. all other groups.

---

**RESULTS**

**Body weights, muscle weights, and VO₂peak.** The R-ALA-treated and the combined treatment groups had slightly lower (8–9%, P < 0.05) final body weights compared with either the sedentary or the exercise-trained groups due to significantly lower average rates of body weight gain over the experimental period (18–23%, Table 1). Wet weights of the whole soleus, plan-
tari, heart, and heart wet weight-to-body weight ratio were not different among the various groups (data not shown).

Animals in both the exercise-training and the combination groups had significantly higher peak aerobic capacities compared with the sedentary control (31 and 29%, respectively) or the R-ALA-treated group (27 and 24%, respectively) (Table 1). In addition, exercise training alone or in combination with R-ALA treatment caused significantly longer maximum run times than those of the sedentary control (52 and 63%, respectively) or the R-ALA-treated group (54 and 65%, respectively) (Table 1).

Plasma glucose, insulin, and free fatty acids. There were no differences in plasma glucose among the various groups after the overnight food restriction (Table 1). R-ALA treatment had no effect on plasma levels of insulin, whereas exercise training induced significant decreases in plasma insulin (23–33%) and free fatty acids (62–136%) compared with all other groups. In contrast, R-ALA treatment resulted in the highest level of circulating free fatty acids, an effect that was prevented by exercise training of R-ALA-treated animals.

OGTT responses. Glucose and insulin responses during the OGTT in the experimental groups are displayed in Fig. 1. Compared with the sedentary control group, R-ALA treatment alone had no effect on plasma glucose or insulin at any time point during the test. At the 15-min time point, exercise training alone or in combination with R-ALA treatment significantly lowered the glucose response (17 and 36%, respectively) compared with the R-ALA treatment and induced significant reduction of the insulin response (36 and 19%, respectively) relative to the sedentary control.

Whereas there were no significant differences among groups for the glucose area under the curve (AUC), exercise training alone was associated with a significantly lower insulin AUC compared with the sedentary group (Fig. 2). The glucose-insulin index, defined as the product of the glucose and insulin AUCs, is an indirect index of in vivo peripheral insulin action (4). R-ALA treatment in the lean animals led to a trend toward a higher glucose-insulin index compared with sedentary control (Fig. 2). This response toward a worsening of whole body insulin sensitivity was prevented by exercise training of the R-ALA-treated animals.

Muscle glucose transport. To examine whether the interventions altered the skeletal muscle glucose transport system, basal and insulin-stimulated 2-DG uptake in isolated epitrochlearis and soleus muscles was determined (Fig. 2). Basal 2-DG uptake in either muscle was not different among experimental groups. In the epitrochlearis, the rate of insulin-stimulated 2-DG uptake (Fig. 3A) was enhanced by exercise training alone (16%) and by exercise training in combination with R-ALA treatment (17%) compared with the sedentary control group. In the soleus muscle (Fig. 3B), exercise training alone significantly increased the insulin-stimulated rate of 2-DG uptake (28% vs. sedentary and 29% vs. R-ALA). These significant increases relative to the sedentary and R-ALA groups were maintained in the combined treatment group.

GLUT-4 protein and enzyme responses. Total protein concentrations for a given muscle type did not differ significantly among the various groups (data not shown). Total GLUT-4 protein level (Fig. 4) and the activities of total hexokinase (Fig. 5) and citrate synthase (Fig. 6) enzymes were determined in the epitrochlearis, soleus, plantaris, and heart. No increases

![Fig. 2. Areas under the curve (AUCs) for glucose (mg·dl⁻¹·min⁻¹; A) and insulin (mU·ml⁻¹·min⁻¹; B) during an oral glucose tolerance test and the glucose-insulin index (mg·dl⁻¹·min⁻¹ × mU·ml⁻¹·min⁻¹ × 10⁶; C) in lean Zucker rats after the 6-wk interventions. Data for AUCs were taken from Fig. 1. Values are means ± SE of 6–9 animals/group. Sed, sedentary control group; ALA, R-ALA-treated group; Exer, exercise-trained group; Combo, combined treatment group. *P < 0.05 vs. R-ALA-treated group.](http://jap.physiology.org/
in GLUT-4 protein level were observed in either muscle type after chronic treatment with R-ALA. Exercise training, alone or in combination with R-ALA treatment, caused significant increases in the GLUT-4 protein level in the epitrochlearis (21 and 18%, respectively, vs. sedentary control and 15 and 12%, respectively, vs. R-ALA-treated animals), soleus (21 and 31%, respectively, vs. sedentary control and 15 and 25%, respectively, vs. R-ALA-treated animals), plantaris (14 and 16%, respectively, vs. sedentary control and 16 and 18%, respectively vs. R-ALA-treated animals), and heart (22 and 19%, respectively, vs. sedentary control).

Total hexokinase activity was increased by R-ALA treatment alone or exercise training alone (69 and 75%, respectively) in the epitrochlearis with no further significant increase when these two interventions were combined. This parameter was markedly enhanced by 186% in the soleus muscle from the combination treatment group. In the plantaris, exercise training alone brought about significant increases in hexokinase activity compared with the sedentary or the R-ALA-treated groups (53 and 85%, respectively), whereas no significant alterations between groups were observed in the heart. Citrate synthase activity in epitrochlearis was increased (31–48%) after either R-ALA treatment alone, exercise training alone, or in the combination treatment group. Exercise training, alone or in combination with R-ALA treatment, resulted in significant increases (59%) in this variable in the soleus muscle. The combination treatment enhanced citrate synthase activity in the plantaris by 37% compared with the sedentary or the R-ALA-treated groups, whereas only exercise training alone increased citrate synthase ac-

![Graph](image-url)
Activity in the heart (29%) relative to the sedentary control group.

**Protein carbonyls.** The effect of the antioxidant R-ALA and exercise training interventions on tissue protein carbonyls, a marker of oxidative stress (5, 28), was examined. R-ALA treatment, alone or in combination with exercise training, resulted in significant decreases (91–108%) in protein carbonyl levels in the liver. Protein carbonyl levels in the soleus after exercise training alone or in combination with R-ALA treatment were significantly lower (49–59%) compared with the sedentary group. In the plantaris muscle, exercise training alone or R-ALA treatment alone lowered protein carbonyl levels by 47% and 74%, respectively, relative to the sedentary group. No changes in this parameter were observed in the heart muscle after either intervention. The correlation between protein carbonyl level and insulin-mediated 2-DG uptake in the soleus muscle from the various experimental groups was assessed. No significant correlation was observed ($P = 0.1025$). In addition, no significant correlation was observed in the soleus between protein carbonyl level and citrate synthase activity ($P = 0.9212$) (data not shown).

**DISCUSSION**

Our laboratory has recently reported (31) that, in the markedly insulin-resistant, hyperinsulinemic, and...
dyslipidemic obese Zucker rat, endurance exercise training and the antioxidant R-ALA interact in an additive fashion to improve skeletal muscle glucose transport. In contrast to these findings, we have demonstrated in the present investigation that the combination of endurance exercise training and R-ALA treatment in the insulin-sensitive lean Zucker rat does not result in a further improvement of insulin-stimulated glucose transport in skeletal muscle compared with the effects of exercise training alone (Fig. 3). Moreover, we have shown that chronic treatment of normal rats with R-ALA alone does not improve insulin action on whole body glucose disposal (Figs. 1 and 2) and skeletal muscle glucose transport (Fig. 3), unlike the beneficial modulation of glucose metabolism in insulin-resistant rodents (19, 27, 31, 36) and humans (17, 18, 23) associated with chronic administration of ALA. It appears, therefore, that the ability of ALA to enhance insulin action on glucose metabolism in skeletal muscle is restricted to conditions of insulin resistance.

The level of carbonyl formation in proteins is an indicator of oxidative damage in tissues (5) and reflects the degree of long-term oxidative stress (28). We have demonstrated in the present investigation that the levels of protein carbonyls in the soleus, plantaris, myocardium, and liver of the insulin-sensitive lean Zucker rats (Fig. 7) were 31–60% less ($P < 0.05$) than those levels measured in the same tissues of insulin-resistant obese Zucker rats (31). In this previous investigation (31), our laboratory showed that reductions in soleus muscle protein carbonyl levels after endurance exercise training or chronic administration of R-ALA were significantly correlated with improvements in insulin-mediated glucose transport activity, supporting a role of oxidative stress in the etiology of muscle insulin resistance. However, this relationship between oxidative stress and insulin action is obviously not a

![Figure 7](image)

**Fig. 7.** Effects of chronic treatment with R-ALA, Exer, or Combo on protein carbonyl levels in the liver, soleus, plantaris, and heart. Values are means ± SE of 6–9 animals/group. a $P < 0.05$ vs. Sed. b $P < 0.05$ vs. Exer.

![Figure 8](image)

**Fig. 8.** Simple linear regression analysis of the relationship between insulin-mediated glucose transport activity and protein carbonyl level in the soleus muscle of lean Zucker rats subjected to the various interventions. Insulin-mediated glucose transport is defined as the net increase in 2-deoxyglucose uptake above basal due to insulin (using data from Fig. 3). Regression equation: $y = -0.004x + 2.302, r = -0.321, P = 0.1025$. 

J Appl Physiol • VOL 92 • JANUARY 2002 • www.jap.org
simple one, as reductions in the level of protein carbonyls in the soleus muscle of the exercise-trained or R-ALA-treated lean Zucker rats were not significantly correlated with any significant enhancement of insulin-mediated glucose transport activity (Fig. 8). Taken together, these data support the hypothesis that reductions of already elevated protein carbonyl levels (such as those in tissues of the obese Zucker rat), elicited by either exercise training or R-ALA interventions, can be associated with enhancements of insulin action on skeletal muscle glucose transport. However, further decreases in these protein carbonyl levels below a given threshold value (e.g., the levels in muscle from the lean Zucker rat) do not result in an enhancement of insulin action.

An important observation in the present study is that chronic treatment of lean animals with R-ALA was associated with a significantly reduced rate of body weight gain (Table 1). As lean mass was apparently not affected (muscle wet weights were not different between sedentary and R-ALA-treated animals), the difference in body mass was likely due to a difference in fat mass. It has previously been noted that, in older rats, chronic treatment with R-ALA leads to increases in ambulatory activity and hepatocellular oxygen consumption (8), and we have also found increases in metabolic enzyme activities (hexokinase and citrate synthase; Figs. 5 and 6) in skeletal muscle of the R-ALA-treated animals. The possibility exists that chronic R-ALA treatment can increase the expression of specific genes involved in metabolism, allowing for an increase in metabolic and ambulatory activity and ultimately leading to a reduced body weight gain.

The alterations in plasma FFAs that resulted from the interventions in the lean animals are noteworthy (Table 1), as FFAs are known to negatively modify whole body and skeletal muscle glucose disposal (3). Whereas chronic R-ALA treatment of dyslipidemic, obese Zucker rats elicits decreases in plasma FFAs (31, 36), chronic treatment of lean animals with R-ALA brought about an unexpected and significant increase in plasma FFAs (Table 1), an effect that was significantly reduced by concomitant exercise training. This elevation in plasma FFAs after ALA treatment has previously been reported in normal chickens (9) and previously been reported in normal chickens (9) and may result from the ability of ALA to bind to albumin and displace fatty acids (32). Moreover, the elevated FFAs may help to explain the slight worsening of whole body insulin sensitivity after R-ALA treatment (Fig. 2). In support of this concept, in the group of lean animals receiving R-ALA treatment and exercise training in combination, the reduction in plasma FFAs relative to the R-ALA-treated group was accompanied by a relative enhancement of whole body insulin sensitivity.

We have again confirmed numerous previous investigations demonstrating that endurance exercise training enhances insulin-stimulated glucose transport activity in skeletal muscle (reviewed in Refs. 15, 16). These improvements in insulin action were associated with increased total GLUT-4 protein level (Fig. 4) and with increased activities of enzymes involved in glucose phosphorylation (hexokinase; Fig. 5) and glucose oxidation (citrate synthase; Fig. 6), in agreement with previous studies (15, 16).

In summary, we have provided new evidence that, in contrast to the insulin-resistant obese Zucker rat (31), chronic administration of the water-soluble antioxidant R-ALA to the insulin-sensitive lean Zucker rat does not enhance insulin-stimulated glucose transport activity in skeletal muscle. Moreover, again in contrast to our findings with the obese Zucker rat (31), we could find no evidence that the combination treatment of lean Zucker rats with exercise training and R-ALA could beneficially modify either maximal running performance or skeletal muscle glucose transport activity relative to endurance exercise training alone. Taken together, these results indicate that the positive interaction between endurance exercise training and antioxidant treatment with R-ALA for skeletal muscle insulin action is restricted to conditions of insulin resistance and is not seen in insulin-sensitive muscle.

We thank ASTA Medica (Frankfurt, Germany) for the gift of the R-ALA.

This work was supported in part by Grant-in-Aid 9951103Z from the Desert/Mountain Affiliate of the American Heart Association.

REFERENCES


