

# Effects of a unique conjugate of $\alpha$ -lipoic acid and $\gamma$ -linolenic acid on insulin action in obese Zucker rats

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**Peth, J. Anthony, Tyson R. Kinnick, Erik B. Youngblood, Hans J. Tritschler, and Erik J. Henriksen.** Effects of a unique conjugate of  $\alpha$ -lipoic acid and  $\gamma$ -linolenic acid on insulin action in obese Zucker rats. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.* 278: R453–R459, 2000.—The purpose of this study was to assess the individual and interactive effects of the antioxidant  $\alpha$ -lipoic acid (LPA) and the n-6 essential fatty acid  $\gamma$ -linolenic acid (GLA) on insulin action in insulin-resistant obese Zucker rats. LPA, GLA, and a unique conjugate consisting of equimolar parts of LPA and GLA (LPA-GLA) were administered for 14 days at 10, 30, or 50 mg·kg body wt<sup>-1</sup>·day<sup>-1</sup>. Whereas LPA was without effect at 10 mg/kg, at 30 and 50 mg/kg it elicited 23% reductions ( $P < 0.05$ ) in the glucose-insulin index (the product of glucose and insulin areas under the curve during an oral glucose tolerance test and an index of peripheral insulin action) that were associated with significant increases in insulin-mediated (2 mU/ml) glucose transport activity in isolated epitrochlearis (63–65%) and soleus (33–41%) muscles. GLA at 10 and 30 mg/kg caused 21–25% reductions in the glucose-insulin index and 23–35% improvements in insulin-mediated glucose transport in epitrochlearis muscle. The beneficial effects of GLA disappeared at 50 mg/kg. At 10 and 30 mg/kg, the LPA-GLA conjugate elicited 29 and 38% reductions in the glucose-insulin index. These LPA-GLA-induced improvements in whole body insulin action were accompanied by 28–63 and 38–57% increases in insulin-mediated glucose transport in epitrochlearis and soleus muscles and resulted from the additive effects of LPA and GLA. At 50 mg/kg, the metabolic improvements due to LPA-GLA were substantially reduced. In summary, these results indicate that the conjugate of the antioxidant LPA and the n-6 essential fatty acid GLA elicits significant dose-dependent improvements in whole body and skeletal muscle insulin action on glucose disposal in insulin-resistant obese Zucker rats. Moreover, these actions of LPA-GLA are due to the additive effects of its individual components.

insulin resistance; antioxidant; essential fatty acid; muscle glucose transport

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RESISTANCE TO INSULIN-MEDIATED glucose transport in skeletal muscle is associated with glucose intolerance, hyperinsulinemia, hypertension, increased plasma triglycerides, and decreased high-density lipoprotein cho-

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lesterol (6, 22). Collectively, this clustering of cardiovascular disease risk factors has been referred to as the "insulin resistance syndrome" (6) or "syndrome X" (22) and can lead to conditions such as atherosclerosis and type 2 diabetes (6, 22).

The metabolic antioxidant  $\alpha$ -lipoic acid (LPA) has been shown recently in various clinical trials to improve insulin-mediated glucose metabolism in type 2 diabetic subjects (12–14, 17). Improvements in insulin-stimulated glucose transport in insulin-resistant rat skeletal muscle (9, 15, 24) and cardiac muscle (25) have also been demonstrated following both acute and chronic treatments with LPA. LPA also has been shown to have a beneficial role in the prevention and treatment of neurodegenerative disorders (21), including the improvement of symptoms of diabetic polyneuropathy (20), a condition associated with oxidative damage (19, 20). An n-6 essential fatty acid and a prostaglandin precursor  $\gamma$ -linolenic acid (GLA) is also known to improve various indexes of diabetic polyneuropathy (3, 4, 16). Recently, combined treatment with LPA and GLA, either as individual components (10) or as a conjugate (4), has been demonstrated to have a synergistic effect in improving neurovascular function in diabetic rats. However, the effect of combined treatment with LPA and GLA on glucose metabolism in insulin-resistant skeletal muscle has not been investigated.

The present study was designed to investigate the effects of chronic treatments with LPA, GLA, and a unique conjugate consisting of equimolar parts of LPA and GLA (LPA-GLA) on oral glucose tolerance and insulin-stimulated skeletal muscle glucose transport in the obese Zucker rat, a well-established model of insulin resistance, glucose intolerance, hyperinsulinemia, and dyslipidemia. Because free fatty acids are known to negatively modulate insulin-stimulated glucose transport activity in skeletal muscle (2), the plasma levels of this variable were also assessed.

## MATERIALS AND METHODS

**Animals.** Female obese Zucker rats (*fa/fa*) were purchased at 7–8 wk of age from Harlan (Indianapolis, IN). Animals were housed two to three per cage and maintained on chow (Purina, St. Louis, MO) and water ad libitum. All procedures used were approved by the University of Arizona Animal Use and Care Committee.

**Chronic in vivo treatment.** At 8–9 wk of age, obese animals were assigned to either a vehicle-treated control group or to intervention groups receiving a racemic mixture of LPA

(ASTA Medica, Frankfurt, Germany), GLA (Scotia Pharmaceuticals, Carlisle, UK), or the LPA-GLA conjugate (Scotia Pharmaceuticals). LPA-treated animals and matched controls received either vehicle (8.3 ml/kg body wt of 120 mM Tris buffer, pH 7.4) or 10, 30, or 50 mg/kg LPA by intraperitoneal injection. GLA- and LPA-GLA-treated animals and matched controls received either vehicle (corn oil) or the respective compounds at 10, 30, or 50 mg/kg by gavage. Because the mode of administration of vehicle did not affect any of the variables measured, these values were pooled in the presentation of the final results. All animals were treated for 14 consecutive days.

**Oral glucose tolerance tests.** Oral glucose tolerance tests (OGTTs) were performed on all animals after 11 days of treatment. Rats were restricted of food (4 g of chow given at 5 PM, which was immediately consumed) the evening before the experiment. Starting at 8 AM, all rats were administered 1 g/kg glucose feeding by gavage. Blood samples (<0.5 ml) were obtained from a cut at the tip of the tail at 15, 30, and 60 min following the glucose feeding. Whole blood samples were mixed with EDTA (18 mg/ml final concentration) and centrifuged at 13,000 *g* to separate the plasma. Plasma samples were analyzed spectrophotometrically for glucose (Sigma, St. Louis, MO) and free fatty acids (WAKO, Richmond, VA) and by radioimmunoassay for insulin (Linco Research, St. Charles, MO). Immediately following completion of the OGTT, all rats received a 2-ml injection of sterile 0.9% saline subcutaneously to compensate for plasma loss and were allowed access to food and water ad libitum. Treatments resumed for a further 3 days.

**Assessment of *in vitro* muscle glucose transport.** At 8 AM, ~24 h after the final treatment and having been restricted to 4 g of chow during the previous 13 h, animals were deeply anesthetized with pentobarbital sodium (50 mg/kg ip). Right soleus muscles and both right and left epitrochlearis muscles were surgically removed and prepared for *in vitro* incubation. Soleus muscles were prepared into strips weighing ~30 mg. Muscles were incubated initially for 60 min at 37°C in 3 ml of oxygenated Krebs-Henseleit buffer (KHB) containing 8 mM glucose, 32 mM mannitol, and 0.1% bovine serum albumin (BSA). One muscle preparation from each animal was incubated in medium containing no insulin, whereas the other muscle preparation was incubated in medium containing a maximally effective concentration of insulin (2 mU/ml). Thereafter, muscles were rinsed for 10 min at 37°C in 3 ml of

oxygenated KHB containing 40 mM mannitol, 0.1% BSA, and, if present previously, insulin. The muscles were then transferred to flasks containing 2 ml of oxygenated KHB, 0.1% BSA, 1 mM 2-deoxy-[1,2-<sup>3</sup>H]glucose (2-DG; 300 mCi/mol; Sigma) and 39 mM [U-<sup>14</sup>C]mannitol (0.8 mCi/mol) (ICN Radiochemicals, Irvine, CA), and insulin, if present previously. After this final 20-min incubation at 37°C, muscles were trimmed of fat, extraneous muscle tissue, and connective tissue, frozen in liquid N<sub>2</sub>, weighed, and dissolved in 0.5 ml of 0.5 N NaOH. Glucose transport activity was then assessed as described previously (8).

**Muscle GLUT-4 protein levels.** The level of GLUT-4 glucose transporter protein in whole homogenates of epitrochlearis and soleus muscles was determined using the method described previously (8).

**Statistical analysis.** All data are presented as means  $\pm$  SE. The significance of differences between groups was assessed via a factorial analysis of variance with a post hoc Fishers protected least-significant difference test (Statview, version 5.0, Cary, NC).  $P < 0.05$  were considered significant.

## RESULTS

**Plasma glucose, insulin, and free fatty acids.** After treatment with LPA, GLA, or LPA-GLA, final body weights, incubated epitrochlearis and soleus muscle wet weights, and plasma glucose values were not significantly different from the vehicle-treated group (Table 1). Treatment with LPA resulted in an 18% decrease ( $P < 0.05$ ) in plasma insulin at the 30 mg/kg dose and an 18% decline in plasma free fatty acids at the 30 and 50 mg/kg doses, relative to vehicle-treated controls. Significant changes in plasma insulin and free fatty acid levels were not observed following treatment with GLA. However, an 11% increase in plasma insulin was observed in the 50 mg/kg GLA group. Treatment with LPA-GLA resulted in reductions of 24 and 28% ( $P < 0.05$ ) in plasma insulin at the 10 and 30 mg/kg levels, respectively. Plasma free fatty acid levels were 16, 27, and 15% lower ( $P < 0.05$ ) in the 10, 30, and 50 mg/kg LPA-GLA groups, respectively, compared with the vehicle-treated control value.

Table 1. Effect of chronic LPA, GLA, or LPA-GLA treatments on plasma glucose, insulin, and free fatty acids in obese Zucker rat

Group	Final Body Wt, g	Epitrochlearis Wt, mg	Plasma Glucose, mg/dl	Plasma Insulin, $\mu$ U/ml	Free Fatty Acids, $\mu$ eq/ml
<b>LPA</b>					
Obese vehicle treated	310 $\pm$ 4	34.9 $\pm$ 0.5	135 $\pm$ 3	176 $\pm$ 5	1.67 $\pm$ 0.06
Obese chronic 10 mg/kg LPA treated	305 $\pm$ 6	38.7 $\pm$ 2.1	128 $\pm$ 5	173 $\pm$ 7	1.54 $\pm$ 0.10
Obese chronic 30 mg/kg LPA treated	314 $\pm$ 7	37.3 $\pm$ 0.8	134 $\pm$ 2	145 $\pm$ 14*	1.37 $\pm$ 0.07*
Obese chronic 50 mg/kg LPA treated	304 $\pm$ 7	37.1 $\pm$ 1.0	132 $\pm$ 3	154 $\pm$ 20	1.38 $\pm$ 0.10*
<b>GLA</b>					
Obese vehicle treated	298 $\pm$ 4	34.0 $\pm$ 0.7	136 $\pm$ 2	160 $\pm$ 5	1.67 $\pm$ 0.06
Obese chronic 10 mg/kg GLA treated	300 $\pm$ 3	34.7 $\pm$ 1.0	131 $\pm$ 3	144 $\pm$ 12	1.51 $\pm$ 0.06
Obese chronic 30 mg/kg GLA treated	301 $\pm$ 5	36.4 $\pm$ 1.0	128 $\pm$ 2	149 $\pm$ 8	1.60 $\pm$ 0.03
Obese chronic 50 mg/kg GLA treated	297 $\pm$ 4	37.2 $\pm$ 1.3	132 $\pm$ 3	178 $\pm$ 18	1.64 $\pm$ 0.08
<b>LPA-GLA Conjugate</b>					
Obese vehicle treated	303 $\pm$ 2	36.2 $\pm$ 1.4	137 $\pm$ 3	147 $\pm$ 5	1.73 $\pm$ 0.03
Obese chronic 10 mg/kg LPA-GLA treated	296 $\pm$ 3	36.4 $\pm$ 0.6	132 $\pm$ 3	111 $\pm$ 7*	1.45 $\pm$ 0.08*
Obese chronic 30 mg/kg LPA-GLA treated	293 $\pm$ 5	35.1 $\pm$ 0.6	133 $\pm$ 2	106 $\pm$ 10*	1.26 $\pm$ 0.06*
Obese chronic 50 mg/kg LPA-GLA treated	297 $\pm$ 2	34.8 $\pm$ 1.1	133 $\pm$ 3	138 $\pm$ 13	1.47 $\pm$ 0.08*

Values are means  $\pm$  SE for 6–9 animals/group. LPA,  $\alpha$ -lipoic acid; GLA,  $\gamma$ -linolenic acid. \*  $P < 0.05$  vs. obese vehicle treated.

**OGTT responses.** Chronic treatment with LPA at 10 mg/kg had no significant effect on glucose disposal during the OGTT (Fig. 1, *top left*). However, 30 mg/kg LPA-treated animals had significantly lower (16 and 12%,  $P < 0.05$ ) 30- and 60-min glucose values compared with controls. Similarly, plasma glucose was 15 and 12% lower than vehicle-treated controls at 30 and 60 min in the 50 mg/kg LPA-treated group. In addition, the total areas under the curve (AUC) for the glucose responses were significantly lower in both 30 and 50 mg/kg LPA-treated groups (Fig. 2, *top left*). Chronic 10 mg/kg LPA treatment resulted in no changes in plasma insulin levels during the OGTT (Fig. 1, *bottom left*). However, plasma insulin responses in the 30 mg/kg LPA-treated group were significantly lower than vehicle-treated control animals at 15 and 30 min and in the 50 mg/kg LPA-treated group at 15 min. In addition, the insulin AUC was significantly lower than control in the 30 and 50 mg/kg LPA-treated groups (Fig. 2, *middle left*). The glucose-insulin index (an indirect index of peripheral insulin action on glucose disposal calculated as the product of the glucose and insulin AUCs) was significantly decreased (indicating decreased insulin resistance) in the 30 and 50 mg/kg LPA-treated groups compared with control and the 10 mg/kg values (Fig. 2, *bottom left*).

In the 30 mg/kg GLA-treated group, glucose levels at 15, 30, and 60 min were significantly lower than controls (18, 15, and 15%, respectively; Fig. 1, *top center*), as was the glucose AUC (15% lower; Fig. 2, *top center*). However, no significant differences in plasma glucose values or glucose AUC were observed in either the 10 or 50 mg/kg GLA-treated animals compared with controls. Significantly decreased plasma insulin levels were observed at 30 min in the 10 mg/kg GLA-treated group (19% lower) and at 60 min in the 10 and 30 mg/kg GLA-treated groups (18 and 15% lower, respectively) relative to controls (Fig. 1, *bottom center*). Additionally, the insulin AUC was significantly lower in the 10 and 30 mg/kg GLA-treated groups (Fig. 2, *middle center*). However, no effects on the plasma

insulin response or the insulin AUC were observed in the 50 mg/kg GLA-treated group. The glucose-insulin index was significantly lower in the 10 and 30 mg/kg GLA-treated groups compared with control (Fig. 2, *bottom center*).

The LPA-GLA conjugate-treated animals displayed significantly lower plasma glucose levels than control animals after an oral glucose load in both the 10 mg/kg group (19 and 17% lower at 15 and 30 min, respectively) and the 30 mg/kg group (18 and 22% lower at 15 and 30 min, respectively; Fig. 1, *top right*). The glucose AUC was significantly lower (17%) in the 30 mg/kg LPA-GLA-treated animals (Fig. 2, *top right*). No significant differences in plasma glucose values or glucose AUC were observed in 50 mg/kg LPA-GLA-treated animals compared with controls, and the glucose AUC in the 50 mg/kg LPA-GLA-treated animals was significantly greater than that in the 30 mg/kg group. LPA-GLA treatment resulted in 23 and 28% lower plasma insulin values than controls in the 30 mg/kg group at 30 and 60 min after an oral glucose load (Fig. 1, *bottom right*). In addition, the insulin AUC was significantly lower than controls in the 10 and 30 mg/kg LPA-GLA-treated groups (21 and 25% lower, respectively; Fig. 2, *middle right*). However, no differences from controls in plasma insulin response or insulin AUC were observed in the 50 mg/kg LPA-GLA-treated group. The glucose-insulin index was significantly decreased in both the 10 and 30 mg/kg LPA-GLA-treated groups compared with control but increased significantly at the 50 mg/kg dose (Fig. 2, *bottom right*).

**Skeletal muscle glucose transport.** Basal rates of muscle 2-DG uptake were not different from that of the vehicle-treated group (data not shown), with the exception of the 30 and 50 mg/kg GLA-treated groups, in which basal 2-DG uptake was reduced in the epitrochlearis by 15% ( $106 \pm 4$  pmol $\cdot$ mg $^{-1}\cdot$ 20 min $^{-1}$  vs.  $90 \pm 5$  and  $90 \pm 9$ ) and in the soleus by 14–17% ( $245 \pm 6$  pmol $\cdot$ mg $^{-1}\cdot$ 20 min $^{-1}$  vs.  $204 \pm 19$  and  $211 \pm 4$ ). Insulin-mediated (i.e., increase above basal) 2-DG uptake in the epitrochlearis and soleus muscles from all

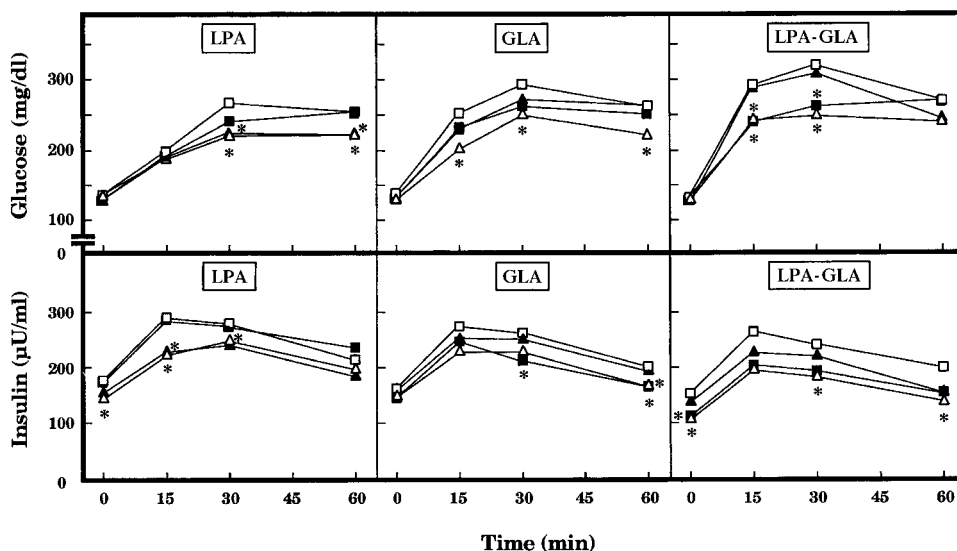
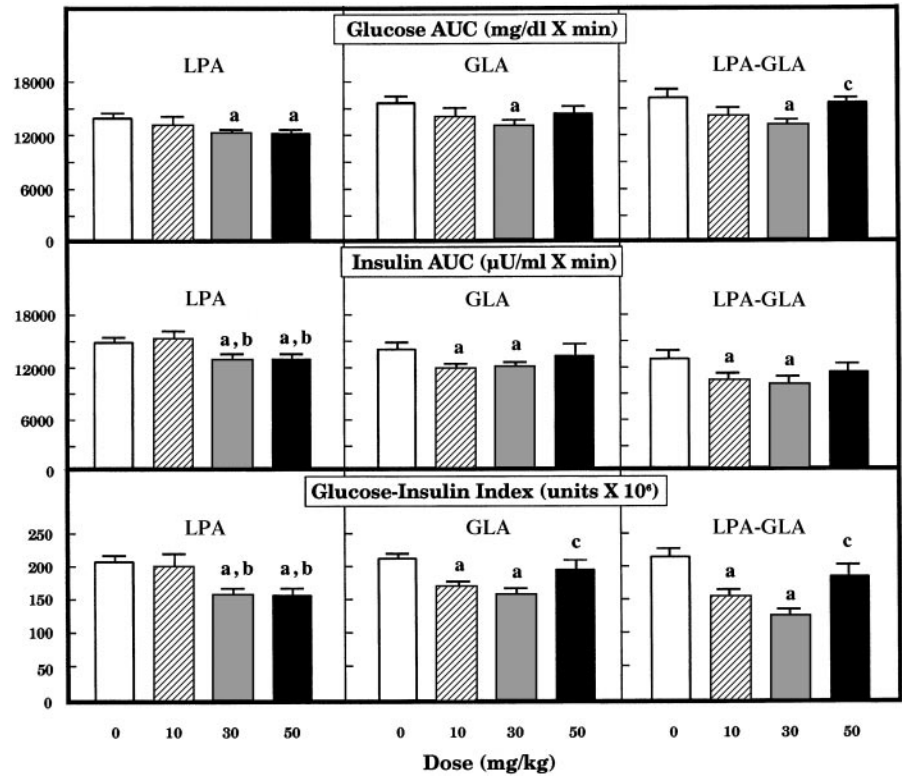


Fig. 1. Glucose and insulin responses during oral glucose tolerance test in obese Zucker rats following chronic treatment with either vehicle,  $\alpha$ -lipoic acid (LPA),  $\gamma$ -linolenic acid (GLA), or LPA-GLA conjugate. Animals were treated for 11 days with either vehicle ( $\square$ ) or experimental compounds at 10 mg/kg ( $\blacksquare$ ), 30 mg/kg ( $\triangle$ ), or 50 mg/kg ( $\blacktriangle$ ). Glucose and insulin values during a 1 g/kg oral glucose tolerance test were then determined. Data are means  $\pm$  SE for 6–9 animals/group. \* $P < 0.05$  vs. vehicle-treated group at same time point.

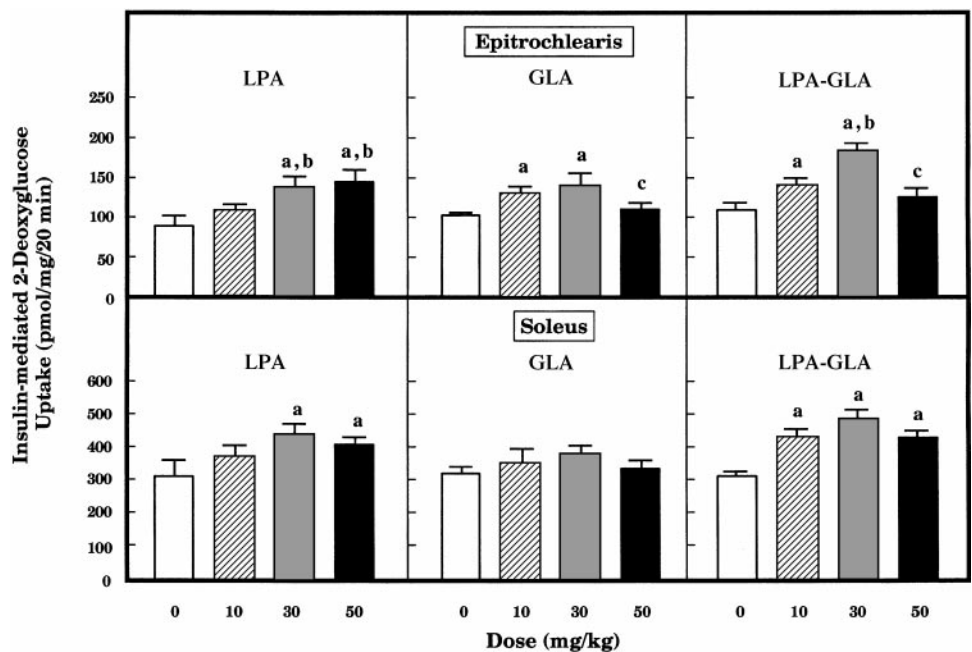
Fig. 2. Effects of chronic treatment of obese Zucker rats with LPA, GLA, or LPA-GLA conjugate on total areas under the curves (AUC) for glucose and insulin during oral glucose tolerance test and glucose-insulin index. Values are means  $\pm$  SE for animals treated with vehicle (open bars) or experimental compound at 10 mg/kg (hatched bars), 30 mg/kg (shaded bars), or 50 mg/kg (solid bars). Data for AUCs were taken from Fig. 1. Glucose-insulin index is calculated as product of glucose AUC and insulin AUC for each animal. <sup>a</sup> $P < 0.05$  vs. vehicle-treated group. <sup>b</sup> $P < 0.05$  vs. 10 mg/kg-treated group. <sup>c</sup> $P < 0.05$  vs. 30 mg/kg-treated group.



vehicle-, LPA-, GLA-, and LPA-GLA-treated animals are shown in Fig. 3. Insulin-mediated 2-DG uptake was significantly greater than controls in both the epitrochlearis and soleus muscles from 30 and 50 mg/kg LPA-treated groups (Fig. 3, *top left* and *bottom left*). Treatment with GLA resulted in significantly greater increases in 2-DG uptake than controls in the epitrochlearis in the 10 and 30 mg/kg groups (Fig. 3, *top center*). However, no significant differences from controls were observed in the soleus muscles from

GLA-treated animals at any dose (Fig. 3, *bottom center*). Chronic LPA-GLA conjugate treatment resulted in significantly greater insulin-mediated increases in 2-DG uptake than controls in the epitrochlearis in 10 and 30 mg/kg groups, but this effect was significantly reduced at the 50 mg/kg dose (Fig. 3, *top right*). Treatment with the LPA-GLA conjugate caused significantly elevated 2-DG uptake in the soleus in the 10, 30, and 50 mg/kg groups (Fig. 3, *bottom right*).

Fig. 3. Effect of chronic treatment of obese Zucker rats with LPA, GLA, or LPA-GLA conjugate on insulin-mediated skeletal muscle glucose transport activity. Net increase in 2-deoxyglucose uptake due to insulin was assessed in isolated epitrochlearis and soleus muscles from animals treated with vehicle (open bars) or experimental compound at 10 mg/kg (hatched bars), 30 mg/kg (shaded bars), or 50 mg/kg (solid bars). Values are means  $\pm$  SE for 6–9 animals/group. <sup>a</sup> $P < 0.05$  vs. vehicle-treated group. <sup>b</sup> $P < 0.05$  vs. 10 mg/kg-treated group. <sup>c</sup> $P < 0.05$  vs. 30 mg/kg-treated group.



These improvements in insulin-mediated 2-DG uptake were not associated with any significant increases in skeletal muscle GLUT-4 protein levels (data not shown).

*Assessment of additivity of LPA and GLA effects.* A comparison of the actual effects of the LPA-GLA conjugate and the theoretical values calculated by summing the individual effects of each component is presented in Fig. 4. At doses of 10 and 30 mg/kg, there was a close agreement between the experimental values and the calculated theoretical additive value for the glucose-insulin index (Fig. 4, *top*), for insulin-mediated 2-DG uptake in the epitrochlearis (Fig. 4, *center*), and for insulin-mediated 2-DG uptake in the soleus (Fig. 4, *bottom*). There was no additivity of the LPA and GLA effects at the highest dose administered, 50 mg/kg (data not shown).

## DISCUSSION

In the present study, we have shown for the first time that a unique conjugate of the antioxidant LPA and the n-6 essential fatty acid GLA can significantly improve glucose tolerance (Figs. 1 and 2), lessen the exaggerated insulin response following a glucose load (Figs. 1 and 2), reduce fasting plasma insulin and free fatty acid levels (Table 1), and enhance insulin action on skeletal muscle glucose transport activity (Fig. 3) in an animal model of insulin resistance, hyperinsulinemia, and dyslipidemia, the obese Zucker rat. The beneficial effects of the chronic LPA-GLA conjugate treatments on these variables were dose dependent, with pronounced effects at 10 and 30 mg/kg doses. However, nearly all of the aforementioned beneficial effects of the LPA-GLA conjugate were substantially reduced at the highest dose administered, 50 mg/kg.

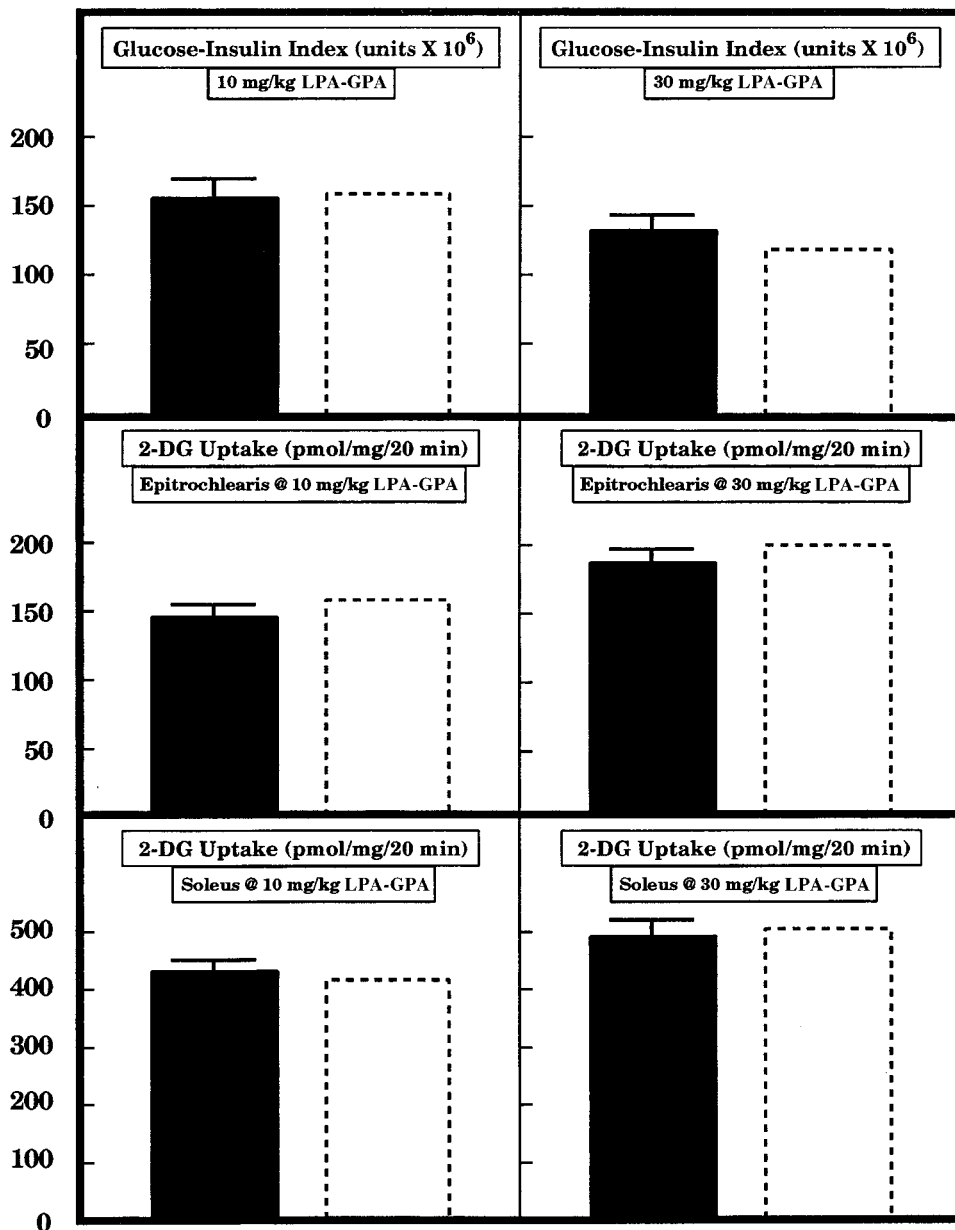


Fig. 4. Comparison of experimental values (solid bars) for glucose-insulin index and insulin-mediated glucose transport activity in epitrochlearis and soleus muscles following chronic treatment of obese Zucker rats at 10 or 30 mg/kg LPA-GLA and theoretical values (open bars with dashed borders) based on additivity of individual effects of LPA and GLA. Experimental values were taken from Figs. 2 and 3. Theoretical value at each dose was calculated by adding differences between experimental value at that dose and corresponding control values in LPA and GLA groups and then adding this sum to control value in LPA-GLA group. 2-DG, 2-deoxy-[1,2-<sup>3</sup>H]glucose.

The effects of this LPA-GLA conjugate appeared in most cases to be due to the additive effects of the individual components of the conjugate, as noted for insulin-mediated skeletal muscle glucose transport activity (Fig. 4). At the 10 mg/kg dose, LPA had minimal effects on the parameters investigated, in agreement with our previous findings (15, 24). The effect of the LPA-GLA conjugate at this dose was almost entirely due to the individual effects of the GLA. At the 30 mg/kg dose, both LPA and GLA had significant effects on whole body and skeletal muscle glucose disposal, and these individual effects generally accounted for the effect of the LPA-GLA conjugate. However, it is noteworthy that at the 10 mg/kg dose, neither LPA nor GLA individually induced a reduction in plasma insulin or free fatty acids in the unfed obese Zucker rat, yet the LPA-GLA conjugate elicited a significant lowering of both variables (Table 1). This suggests an interaction between LPA and GLA on these particular variables. Whereas LPA still elicited a significant modulation of insulin action at the whole body (Figs. 1 and 2) and skeletal muscle levels (Fig. 3) at the highest dose administered (50 mg/kg), the GLA effect at this dose was no longer significant. Indeed, the substantially reduced effect of the LPA-GLA conjugate at this dose, mentioned previously, was likely caused by this reduction in GLA action. These findings imply that whereas GLA can elicit metabolic improvements at lower doses, these effects are lost at higher doses, perhaps due to its lipid composition having deleterious effects on skeletal muscle glucose transport activity, as is the case with free fatty acids (2).

The present findings on the metabolic adaptations in skeletal muscle brought about by the LPA-GLA conjugate are an important adjunct to the previous neurovascular investigations using this compound. It was recently reported that treatment with LPA and GLA in combination resulted in a synergistic interaction for improvements in neurovascular function in a diabetic rat model of polyneuropathy (4, 10). On the basis of these findings, we hypothesized that the LPA-GLA conjugate would also display such a synergistic interaction on insulin action in the obese Zucker rat. However, it is clear that the interaction between LPA and GLA on whole body and skeletal muscle insulin action was only additive and was restricted to lower doses of the conjugate.

The increase in insulin action on skeletal muscle glucose transport due to the LPA treatments were of physiological relevance, as significant correlations between the glucose-insulin index and the insulin-mediated increase in 2-DG uptake were found for both the epitrochlearis ( $r = -0.601$ ,  $P < 0.05$ ) and the soleus ( $r = -0.581$ ,  $P < 0.05$ ). Although the exact cellular mechanism(s) through which LPA functions to improve skeletal muscle glucose transport is not well understood, several possibilities exist. It has been demonstrated that LPA treatment of  $L_6$  myocytes stimulates glucose uptake and is associated with enhanced phosphatidylinositol-3 kinase (PI-3 kinase) activity and intracellular redistribution of GLUT-4, similar to that

caused by insulin (7). Henriksen et al. (9) found that a portion (~25%) of LPA action on glucose transport in mammalian skeletal muscle is mediated via the insulin signal transduction pathway, with the majority of the direct effect being independent of PI-3 kinase activity.

Interestingly, LPA at 30 and 50 mg/kg resulted in a significant reduction in plasma free fatty acids (Table 1), an effect not seen following treatment with GLA alone. More striking was the observation that free fatty acids were significantly reduced at the lowest dosage of the LPA-GLA conjugate. Because free fatty acids can negatively influence insulin action on skeletal muscle glucose transport (2), it is possible that the reduction in this variable was mechanistically linked to the LPA- and LPA-GLA conjugate-induced improvements in muscle glucose transport. This was certainly not the cause for the improvement in insulin action due to GLA.

Treatment with the n-6 essential fatty acid GLA has been demonstrated to improve impaired nerve function in diabetic humans (16) and in animal models of diabetes (3, 4). A relationship between essential fatty acids and insulin resistance has been established. Das (5) measured lower than normal plasma levels of certain essential fatty acids in individuals with diabetes, hypertension, and coronary heart disease, conditions associated with insulin resistance (6, 22). It was observed that the decline in essential fatty acids in these conditions may be an important contributing component in the pathobiology of insulin resistance (5). Also, administration of the essential fatty acid conjugated linoleic acid to Zucker diabetic fatty rats has been shown recently to improve peripheral insulin sensitivity, possibly via activation of the peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ ) (11).

In the present study, GLA at 10 and 30 mg/kg improved whole body insulin action on glucose disposal and in vitro glucose transport in the insulin-resistant epitrochlearis muscles (but not the soleus) of the obese Zucker rat (Figs. 1–3). The mechanism(s) for these effects is currently not known. It is possible that the effects of GLA, a prostaglandin precursor, are mediated via prostaglandins themselves. Prostaglandins of the E series have been shown to increase insulin-sensitive glucose metabolism in the rat soleus (18). Furthermore, Takahashi et al. (26) demonstrated that treatment with evening primrose oil, which is rich in GLA, reduced fasting plasma glucose and increased levels of prostaglandin  $E_1$  in type 2 diabetic subjects. Another possibility is that the effect of GLA to improve insulin action in insulin-resistant skeletal muscle is through the activation of PPAR- $\gamma$ , similar to the action of conjugated linoleic acid (11). The much lesser effect of GLA to improve insulin action in soleus muscle, which consists of primarily type I fibers (1), compared with the epitrochlearis (Fig. 3), which is made up of mainly type IIb fibers (23), may be a result of the different fiber type compositions of these muscles. It is clear that further investigations are necessary to elucidate the cellular and molecular actions of LPA and GLA on the glucose transport system in insulin-resistant skeletal muscle.

### Perspectives

We have demonstrated in the present study that the chronic in vivo administration of a unique conjugate of the antioxidant LPA and the n-6 essential fatty acid GLA to the insulin-resistant, hyperinsulinemic, and dyslipidemic obese Zucker rat significantly improved whole body insulin action on glucose disposal, likely because of enhanced insulin-stimulated glucose transport activity in skeletal muscle. These metabolic effects of the LPA-GLA conjugate were due to the additive effects of the individual components of the molecule and were observed only at the two lowest doses administered. This conjugate therefore represents an important potential intervention in the diabetic state, because it can elicit improvements not only in insulin action on skeletal muscle glucose metabolism but has very favorable effects on neurovascular dysfunction, a common complication in diabetes. Future investigations should focus on identifying the underlying cellular and molecular mechanisms responsible for the individual and combined beneficial effects of LPA and GLA on insulin action in conditions of insulin resistance.

We thank Melanie B. Schmit for excellent technical assistance.

This work was supported in part by a grant from ASTA Medica (Frankfurt, Germany).

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Received 14 June 1999; accepted in final form 21 September 1999.

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