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Exercise training and the antioxidant α-lipoic acid in the treatment of insulin resistance and type 2 diabetes☆

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Abstract

One hallmark of the insulin-resistant state of prediabetes and overt type 2 diabetes is an impaired ability of insulin to activate glucose transport in skeletal muscle, due to defects in IRS-1-dependent signaling. An emerging body of evidence indicates that one potential factor in the multifactorial etiology of skeletal muscle insulin resistance is oxidative stress, an imbalance between the cellular exposure to an oxidant stress and the cellular antioxidant defenses. Exposure of skeletal muscle to an oxidant stress leads to impaired insulin signaling and subsequently to reduced glucose transport activity. Numerous studies have demonstrated that treatment of insulin-resistant animals and type 2 diabetic humans with antioxidants, including α-lipoic acid (ALA), is associated with improvements in skeletal muscle glucose transport activity and whole-body glucose tolerance. An additional intervention that is effective in ameliorating the skeletal muscle insulin resistance of prediabetes and type 2 diabetes is endurance exercise training. Recent investigations have demonstrated that the combination of exercise training and antioxidant treatment using ALA in an animal model of obesity-associated insulin resistance provides a unique interactive effect resulting in a greater improvement in insulin action on skeletal muscle glucose transport than either intervention individually. Moreover, this interactive effect of exercise training and ALA is due in part to improvements in IRS-1-dependent insulin signaling. These studies highlight the effectiveness of combining endurance exercise training and antioxidants in beneficially modulating the molecular defects in insulin action observed in insulin-resistant skeletal muscle.

Keywords: Lipoic acid; Skeletal muscle glucose transport; Diabetes; Obese Zucker rats

Contents

Abbreviations: IR, insulin receptor; IRS, IR substrates; PI3-kinase, phosphatidylinositol-3-kinase; PDK, 3-phosphoinositide-dependent kinases; AMP kinase, 5′-adenosine-monophosphate-activated protein kinase; p38 MAPK, p38 mitogen-activated protein kinase; ALA, α-lipoic acid; CLA, conjugated linoleic acid.

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Introduction

The long-term maintenance of plasma glucose concentrations under a variety of nutritional conditions and energetic demands is one of the most important and closely regulated processes in mammalian species. Whole-body glucose homeostasis is the product of input from three primary tissues: the liver, skeletal muscle, and β-cells of the pancreas. The liver functions as the primary source of endogenous glucose production in the body under conditions of increased peripheral glucose demand through the breakdown of glycogen stores (glycogenolysis) and the synthesis of new glucose (gluconeogenesis) from a variety of precursor molecules. The liver can also take up glucose from the circulation and store the glucose carbons as glycogen (glycogenesis). However, the most important site of glucose uptake is skeletal muscle, which makes up ~40% of body mass in mammalian species. It is widely accepted that, following an oral or intravenous glucose load or in response to an acute exercise bout, skeletal muscle is a major site of glucose disposal [1], though evidence to the contrary has been published [2]. The most important hormone responsible for the stimulation of skeletal muscle glucose transport and metabolism is insulin, which is secreted by the pancreatic β-cells in response to appropriate stimuli. In addition, the muscle contractions associated with exercise have a powerful effect on the glucose transport process [3].

Important advances in our understanding of the molecular regulation by insulin and contractions of the glucose transport system in muscle have been made over the past two decades. In addition, it is clear that defects in this molecular regulation of glucose transport by insulin are a primary characteristic of insulin-resistant states, including prediabetes and overt type 2 diabetes [4]. An accumulating body of evidence indicates that one potential factor contributing to the multifactorial etiology of insulin resistance is oxidative stress, and that exercise training and antioxidant therapies can be effective interventions in treating insulin resistance associated with this oxidative stress [5,6]. This review will briefly summarize our current understanding of the molecular defects associated with skeletal muscle insulin resistance, especially in the context of a state of oxidative stress. Moreover, this review will briefly cover current information on how exercise training and antioxidant therapy, both individually and in combination, can improve insulin action in insulin-resistant states.

Etiology of muscle insulin resistance and type 2 diabetes

Regulation of glucose transport activity in skeletal muscle

Glucose transport activity in skeletal muscle is acutely regulated by insulin through the activation of a series of intracellular proteins (for reviews, see Refs. [4,7]). In brief, insulin binding enhances the tyrosine kinase activity of the insulin receptor (IR) β-subunits, and this activated IR then phosphorylates IR substrates (IRS; primarily IRS-1 in skeletal muscle) at conserved pYXXM sequences on tyrosine residues. Tyrosine-phosphorylated IRS-1 can interact with the SH2 domains of the p85 regulatory subunit of phosphatidylinositol-3-kinase (PI3-kinase), thereby activating the p110 catalytic subunit of this enzyme. PI3-kinase catalyzes the production of phosphoinositide moieties, which can subsequently activate 3-phosphoinositide-dependent kinases (PDK). A downstream target of PDK is Akt, a serine/threonine kinase. An important role of Akt in the insulin-dependent regulation of glucose transport is supported by several studies [8–10], although contradictory findings have been made [11]. Ultimately, the activation of these steps results in the translocation of the glucose transporter protein isoform GLUT-4 to the sarcolemmal membrane, where glucose transport takes place via a facilitative diffusion process.

Muscle contractions can also stimulate glucose transport via insulin-independent mechanisms [12,13] that culminate with activation of GLUT-4 translocation [14,15]. In comparison to the body of knowledge on insulin signaling elements, relatively little is currently understood regarding the intracellular factors involved in the stimulation of GLUT-4 translocation and glucose transport by muscle contractions. Recent evidence supports a role of the activation of 5′-adenosine-monophosphate-activated protein kinase (AMP kinase), an enzyme stimulated by a decrease in cellular energy charge ([16–18] also see Ref. [19] for a review), although evidence questioning the essential role of AMP kinase in the regulation of glucose transport has been reported [20]. In addition, increases in intracellular calcium play an important role in the acute regulation of glucose transport during contractions [3], possibly by activation of a calcium- and calmodulin-dependent protein kinase [18].

Insulin resistance of muscle glucose disposal

In the context of glucose homeostasis, insulin resistance refers to the reduced ability of insulin to stimulate glucose

transport and metabolism in skeletal muscle or fat (another insulin-sensitive tissue) or to decrease glucose production by the liver. Insulin resistance of skeletal muscle glucose transport represents a major defect in the normal maintenance of euglycemia [4] and is often accompanied by a variety of metabolic and cardiovascular abnormalities, including hypertension, obesity, dyslipidemia, type 2 diabetes, and atherosclerosis [21–23]. This clustering of atherogenic risk factors in the same individual has been called "syndrome X" [21,23] or the "insulin resistance syndrome" [22], and the increased cardiovascular mortality associated with this condition has been directly attributed to the insulin resistance and the compensatory hyperinsulinemia [22,24,25].

Insulin resistance of skeletal muscle glucose transport is a key defect leading to the development of glucose intolerance in prediabetes and to the conversion from prediabetes to overt type 2 diabetes. The prediabetic state is a condition characterized by fasting plasma glucose levels between 100 and 125 mg/dl that do not reach the diagnostic criteria ($\geq$126 mg/dl) for diabetes [26], but only at the expense of hyperinsulinemia overcoming the insulin resistance that is already manifest. This insulin-resistant, prediabetic state affects $\sim$ 40 million people in the United States, and these individuals are at great risk for the conversion to overt type 2 diabetes [27,28]. It is estimated that upward of 18 million American currently have overt type 2 diabetes, and the number continues to increase dramatically [26]. Both of these insulin-resistant states-prediabetes and type 2 diabetes-contribute substantially to the excess morbidity and mortality in this country.

The obese Zucker (*fa*/*fa*) rat displays a missense mutation in the leptin receptor gene, leading to hyperphagia and obesity, and has long been used as an animal model of the prediabetic state, as it displays severe skeletal muscle insulin resistance, hyperinsulinemia, glucose intolerance, dyslipidemia, and central adiposity (see Ref. [29]). Skeletal muscle from obese Zucker rats displays significant defects in insulin-stimulated GLUT-4 protein translocation [30,31] and glucose transport activity [31–33] that are associated with impaired protein expression and functionality of critical elements of the insulin signaling cascade. These defects include a reduced protein expression of IRS-1 [34–36] and impaired insulin-stimulated IRS-1 tyrosine phosphorylation and PI3-kinase activation [34,36]. Similar defects in insulin signaling functionality are observed in skeletal muscle from human subjects with prediabetes and overt type 2 diabetes. For example, insulin stimulation of tyrosine phosphorylation of IR and IRS-1 and of the activities of PI3-kinase and Akt are impaired [37–40] and the activation of GLUT-4 protein translocation to the sarcolemma by insulin is reduced [41,42] in insulin-resistant human skeletal muscle.

Increasing evidence indicates that the protein expression and functionality of IR and IRS-1 can be negatively regulated by serine and threonine phosphorylation [43–50]. Serine phosphorylation of IR is associated with decreased tyrosine phosphorylation of IRS-1 and a subsequently diminished activation of PI3-kinase [43,45], while serine phosphorylation of IRS-1 causes enhanced degradation of this protein in cell lines [47,50]. Recent evidence also supports a role of enhanced serine phosphorylation of IRS-1 in the multifactorial development of insulin resistance (reviewed in Ref. [47]).

Role of oxidative stress in the etiology of insulin resistance

Oxidative stress-the imbalance between the cellular production of oxidants and the antioxidant defenses within cells-can play an important role in the multifactorial etiology of skeletal muscle insulin resistance (reviewed in Refs. [5,6,51,52]). For example, plasma levels of hydroperoxides, one marker of oxidative stress, are higher in subjects with type 2 diabetic compared to nondiabetic controls and are inversely correlated with the degree of metabolic control [53]. More definitive evidence linking oxidative stress and insulin resistance comes from cell culture and isolated muscle incubation studies. Rudich et al. [54,55] have demonstrated in 3T3-L1 adipocytes and L_6 myocytes that prolonged exposure to a low-grade oxidant stress (H_2O_2) markedly decreases insulin-stimulated glucose metabolism. This decreased insulin responsiveness is associated with increased GLUT-1 mRNA and protein levels and decreased GLUT-4 mRNA and protein levels [54,56], and with impaired PI3-kinase-dependent insulin signaling [55,57]. Moreover, Blair et al. [58] showed that exposure of L6 myocytes to a low level of H_2O_2 activates the p38 mitogen-activated protein kinase (p38 MAPK). Thus, these findings are consistent with the hypothesis that oxidative stress can directly and negatively impact insulin action on glucose transport, perhaps through a p38 MAPK-dependent mechanisms.

As noted above, serine phosphorylation of IRS-1 leads to increased degradation and decreased functionality of this protein [47,49]. A critical recent observation is that exposure of hepatoma cells to an oxidant stress (H_2O_2) induces increases in phosphorylation at Ser^{307} and Ser^{632} of IRS-1 [59], and that this oxidant exposure is associated with degradation of the IRS-1 protein [50].

We have also recently addressed the direct effect of oxidant stress on the regulation of insulin action in isolated rat skeletal muscle (V. Saengsirisuwan, J.S. Kim, J.A. Sloniger, and E.J. Henriksen, unpublished data). Isolated soleus muscles from insulin-sensitive lean Zucker rats exposed acutely to a low-grade oxidant stress (70–90 μM H_2O_2) display 30–50% decreases in the ability of insulin to stimulate glucose transport and glycogen synthase, the rate-limiting enzyme for glycogen synthesis. Moreover, this oxidant stress impairs insulin action on tyrosine phosphorylation of IR and IRS-1, activation of PI3-kinase, and serine

phosphorylation of Akt and glycogen synthase kinase-3, a target of Akt action. The oxidant stress also caused activation of p38 MAPK, a serine/threonine kinase thought to be associated with impaired insulin signaling in skeletal muscle [60,61]. Collectively, these results indicate that oxidative stress can directly and negatively impact insulin signaling and glucose transport in intact rat skeletal muscle, consistent with previous findings in muscle and nonmuscle cell lines.

It is well documented that chronic hyperglycemia, a hallmark of the diabetic state, is associated with enhanced formation of reactive oxygen species, advanced glycation end-products, and lipid peroxidation products, and is also frequently accompanied by decreased antioxidant defenses (reviewed in Refs. [51,52]). The glucotoxicity induced by 5–24 h of hyperglycemia causes insulin resistance of skeletal muscle glucose transport and metabolism [62]. While the mechanisms underlying this glucose-induced insulin-resistant state are not clearly defined and are certainly multifactorial, one mechanism may involve an increase in muscle oxidative stress. Short-term hyperglycemia (6 h at 15 mM glucose) in nondiabetic rats causes a marked skeletal muscle insulin resistance and an increase in muscle protein carbonyls, a marker of oxidative stress, both of which are prevented by co-infusion of the antioxidant *N*-acetylcysteine [63].

As noted above, the obese Zucker rat is a well-accepted rodent model of obesity-associated insulin resistance and has specific defects in the insulin signaling cascade in skeletal muscle. In addition, the obese Zucker rat displays characteristics of oxidative stress in muscle and liver [36,64]. Muscle of the obese Zucker rat contains elevated triglyceride levels [36] and the animal has marked dyslipidemia, making it highly susceptible to enhanced lipid peroxidation [64]. Moreover, a direct consequence of these metabolic abnormalities is the increased levels of protein carbonyls in skeletal muscle, cardiac muscle, and liver [64], reflecting a chronic state of oxidative stress in these tissues [65]. These elevated protein carbonyl levels in skeletal muscle of the obese Zucker rat are highly correlated with impairment of insulin action on glucose transport activity [64].

Interventions for improvement of insulin action in insulin-resistant states

Because of the critical role of skeletal muscle insulin resistance in the context of the "insulin resistance syndrome" and in the development of type 2 diabetes, interventions that can enhance insulin action in skeletal muscle are important components of treatment strategies in these insulin-resistant states. Effective nonpharmacological interventions include exercise training, especially endurance activities involving running, biking, or swimming, and specific dietary modifications leading to a reduction of visceral fat stores. In addition, a variety of pharmaceutical and nutriceutical interventions, including antioxidants, exist which target specific systemic and molecular defects associated with insulin resistance. Because the development of an insulin-resistant state is commonly due to a number of different defects, it is unlikely that a single type of intervention will be sufficient to fully correct the insulin resistance, and increasing attention is being giving to combination therapies in the treatment of these insulin-resistant states. The effectiveness of exercise training and antioxidant therapy, individually and in combination, in overcoming skeletal muscle insulin resistance will be discussed below.

Role of endurance exercise training

Endurance exercise training leads to improvements in glucose tolerance and insulin action on skeletal muscle glucose metabolism in insulin-resistant subjects with impaired glucose tolerance or type 2 diabetes [66,67] or in animal models of insulin resistance [29]. In type 2 diabetic humans, one potential mechanism for this training-induced enhancement of insulin action is an upregulation of skeletal muscle GLUT-4 protein expression [67]. While exercise training of animals with normal insulin sensitivity leads to enhanced insulin signaling, including IR and IRS-1 tyrosine phosphorylation and IRS-1-associated PI3-kinase activity [68], it is currently unclear whether exercise training also ameliorates the insulin signaling defects that characterize insulin-resistant skeletal muscle.

Studies using the insulin-resistant obese Zucker rat have provided insight into the potential molecular mechanisms associated with improvements in whole-body and skeletal muscle insulin action caused by endurance exercise training (reviewed in Ref. [29]). Moderate- to high-intensity exercise training increases glucose tolerance and glucose disposal in the obese Zucker rat, primarily due to adaptations in the glucose transport activity [36,64,69–71] and translocation of GLUT-4 protein [72,73]. Our research group has recently demonstrated favorable metabolic adaptations in skeletal muscle of the insulin-resistant obese Zucker rat in response to treadmill training, including significant enhancements of insulin-stimulated glucose transport, GLUT-4 protein expression, and activities of enzymes involved in glucose metabolism [36,64]. Moreover, we and others have shown that endurance exercise training by these prediabetic rodents induces upregulation of the protein expression of important proximal elements of the insulin signaling pathway, including IR, IRS-1, and PI3-kinase [35,36]. However, there is also evidence in the literature indicating that these training-induced improvements in insulin action in the obese Zucker rat can be elicited without concomitant alterations in these insulin signaling factors [74].

A recent investigation has addressed the effect of exercise training on skeletal muscle insulin signaling in insulin-resistant human subjects with type 2 diabetes [75].

After 8 weeks of aerobic exercise training, insulin action on whole-body glucose disposal was increased in the insulin-resistant subjects. However, the exercise training did not improve insulin action on IRS-1-associated PI3-kinase activity, and only Akt protein expression was increased in muscle of the exercise-trained type 2 diabetic subjects [75].

Role of antioxidant treatment

There are numerous pharmaceutical and nutriceutical interventions that improve insulin resistance of skeletal muscle glucose transport, including thiazolidinedione, sulfonylureas, and biguanides [7], and specific antihypertensive agents, such as angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists [76]. One class of nutriceutical compounds that has received considerable attention in the context of diabetes and diabetic complications is antioxidants [5,6,77]. α-Lipoic acid (ALA) is a water-soluble antioxidant [78] that has important effects in the treatment of insulin resistance [5,6]. The positive metabolic actions of this antioxidant have been demonstrated in a variety of experimental models. Evidence from cell culture studies, primarily using 3T3-L1 adipocytes and L6 myocytes, indicates that ALA can protect cells from the deleterious effects of oxidant stress [50,57,79,80] (reviewed in Ref. [52]). ALA can directly modulate glucose metabolism in both insulin-sensitive [81–83] and insulin-resistant [84–86] muscle tissues, with the *R*-(+)-enantiomer (R-ALA) displaying a greater effect than the *S*-enantiomer [82,86]. Interestingly, short-term exposure of L6 myocytes to ALA causes a transient increase in p38 MAPK phosphorylation [87], a finding we have confirmed in isolated rat skeletal muscle (V. Saengsirisuwan and E.J. Henriksen, unpublished data).

Furthermore, we have demonstrated that chronic in vivo treatment with ALA elicits improvements in whole-body glucose tolerance and insulin sensitivity, as well as in insulin action on skeletal muscle glucose transport, in insulin-resistant obese Zucker rats [36,64,84,86]. Chronic administration of ALA to rats made hypertensive and insulin resistant as a result of high glucose feeding results in reductions in systolic blood pressure, an increase in whole-body insulin sensitivity, and a reduction in markers of oxidative stress [88]. It has also been demonstrated recently that chronic administration of ALA to diabetes-prone Otsuka Long-Evans Tokushima Fatty (OLETF) rats prevents the age-dependent development of hyperglycemia, hyperinsulinemia, dyslipidemia, and plasma markers of oxidative stress [89].

The precise cellular mechanisms responsible for the stimulatory effect of ALA on insulin action remain unclear. Initial studies using insulin-sensitive adipocyte and muscle cell lines indicated that in vitro ALA exposure acutely activates critical elements of the insulin signaling pathways, including tyrosine phosphorylation of IR and IRS-1, activation of PI3-kinase, and serine phosphorylation of Akt [87,90]. The increased insulin action following ALA treatment of obese rats is associated with a reduction of hyperinsulinemia and dyslipidemia [39,67,90,93] and with enhancements of IRS-1 protein expression and insulin-stimulated IRS-1 associated with the p85 subunit of PI3-kinase, a surrogate measure of PI3-kinase activation [36]. Moreover, this antioxidant treatment is effective in ameliorating the oxidative stress of the obese Zucker rat, as chronic ALA treatment decreases muscle and liver protein carbonyl levels [64]. Chronic ALA treatment also reduces muscle triglyceride levels in both obese Zucker rats [36] and OLETF rats [89].

Studies involving ALA treatment of human type 2 diabetic subjects are less available. However, whole-body insulin sensitivity of glucose disposal in insulin-resistant type 2 diabetic subjects is improved by ~30% after a singe intravenous infusion of ALA [91] or following chronic intravenous or oral administration of this antioxidant [92,93].

Other nutriceutical compounds may elicit their effects as a result of their antioxidant properties. Conjugated linoleic acid (CLA), a dienoic derivative of linoleic acid with strong antioxidant characteristics [94], has been used as an intervention against cancer and heart disease [95] and diminishes visceral fat in obese Zucker rats [96] and adult humans [97]. In the context of insulin resistance, treatment with the trans10, cis12 isomer of CLA has been shown to enhance glucose tolerance and insulin-stimulated skeletal muscle glucose transport activity and reduce fasting plasma glucose, insulin, and free fatty acids in prediabetic obese Zucker rats [96,98] and type 2 diabetic Zucker diabetic fatty (ZDF) rats [99,100]. In the obese Zucker rat, these CLA-induced metabolic improvements were associated with reductions in skeletal muscle protein carbonyl and lipid levels [96]. Interestingly, we have shown a significant interaction between CLA and R-ALA for enhancement of insulin action on skeletal muscle glucose transport in the obese Zucker rat, at least in part due to reductions in oxidative stress and lipid storage in skeletal muscle [98].

Interactions between exercise training and antioxidant treatment

We have recently demonstrated in insulin-resistant obese Zucker rats that the combination of treatment with the antioxidant R-ALA and endurance exercise training is associated with greater improvements in insulin action on skeletal muscle glucose transport than either intervention individually [36,64]. As noted above, we have shown in the obese Zucker rat [36,64] that individual treatments with R-ALA and endurance exercise training elicit significant reductions in muscle and liver levels of protein carbonyls, a marker of tissue oxidative stress [65], and in intramuscular triglycerides, which is inversely related to insulin action

[101–103]. However, we observed no further decrease in muscle protein carbonyls and triglycerides when the antioxidant treatment and exercise training were combined [36,64]. Furthermore, while muscle GLUT-4 protein expression in the obese Zucker rat is upregulated by exercise training, no further enhancement of this adaptive response is caused by the addition of R-ALA treatment [36,64]. Therefore, it is unlikely that these factors are responsible for the metabolic interactions between exercise training and antioxidant intervention.

However, recent results from our group indicate that the interactive effect of antioxidant treatment and exercise training to improve insulin action on skeletal muscle glucose transport activity in the insulin-resistant obese Zucker rat may be related to alterations in specific elements of the insulin signaling pathway. We observed an essentially additive upregulation of the protein expression of IRS-1 and the association of tyrosine-phosphorylated IRS-1 with the p85 subunit of PI3-kinase in skeletal muscle of obese Zucker rats that received chronic R-ALA treatment during the endurance exercise training period [36].

Summary and perspectives

The interaction between exercise training and antioxidant treatment with R-ALA on the glucose transport system in insulin-resistant skeletal of the obese Zucker rat [36,64] is summarized schematically in Fig. 1. In this figure, the number of down arrows reflects the magnitude of defects in

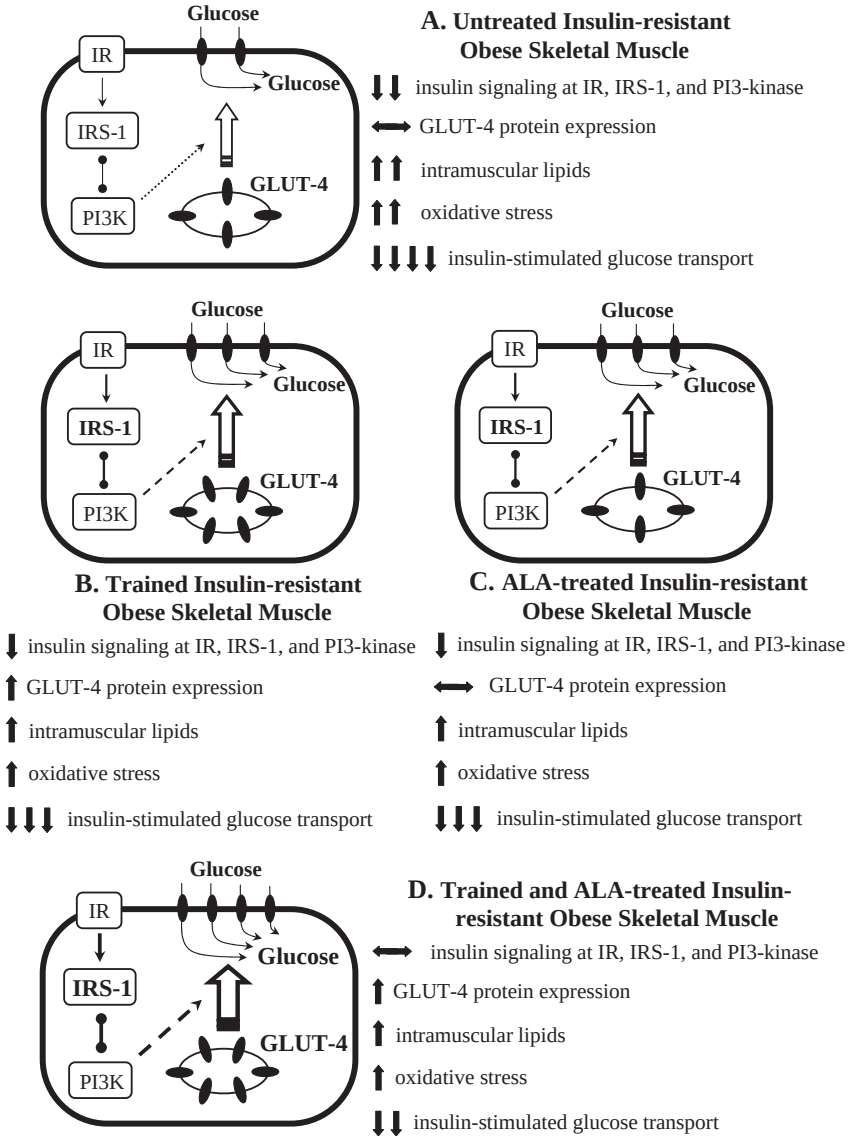

Fig. 1. Summary of the interactions between endurance exercise training and the antioxidant *R*-(+)-lipoic acid on insulin signaling and glucose transport activity in insulin-resistant skeletal muscle of the obese Zucker rat. In this figure, the down arrows indicate the relative degree of insulin resistance, with the number of down arrows reflecting the magnitude of the defects in these insulin-regulatable processes in muscle of obese Zucker rats compared to the insulin-sensitive lean Zucker rats. IR, insulin receptor; IRS-1, insulin receptor substrate 1; PI3K, phosphatidylinositol-3-kinase.

these insulin-regulatable processes in muscle of obese Zucker rats compared to lean Zucker rats. Relative to skeletal muscle from the insulin-sensitive lean Zucker rat, this insulin-resistant obese skeletal muscle is characterized by defects in the protein expression and functionality of critical elements of the insulin signaling pathway, including IR, IRS-1, PI3-kinase. Despite a normal protein expression of GLUT-4, there is a diminished GLUT-4 protein translocation and glucose transport activity in response to insulin. This muscle from the obese Zucker rat also displays elevated intramuscular lipids and evidence of oxidative stress, such as increased protein carbonyls (Fig. 1A). Exercise training by itself induces an increase in insulin action on glucose transport associated with increased IRS-1 functionality, due to both increased IR tyrosine phosphorylation and IRS-1 biosynthesis, and causes GLUT-4 biosynthesis. This exercise training intervention also decreases muscle lipids and reduces tissue oxidative stress (Fig. 1B), and the cumulative effect is that there is less insulin resistance in the exercise-trained obese Zucker rat compared to the sedentary obese Zucker rat. Likewise, R-ALA treatment individually brings about an enhancement of insulin-stimulated glucose transport in muscle by increasing IRS-1 biosynthesis and functionality, but is not associated with an increase in GLUT-4 biosynthesis. In addition, this R-ALA treatment reduces the elevated levels of muscle lipids and protein carbonyls to approximately the same extent as exercise training (Fig. 1C). There is still insulin resistance, but to a lesser degree, after exercise training or R-ALA treatment individually.

The interaction between exercise training and R-ALA on the glucose transport system likely results from an additive effect of the two interventions on IRS-1 biosynthesis and functionality, leading to the greatest effect of insulin to stimulate PI3-kinase. Activation of IR, IRS-1, and PI3-kinase is essentially normalized by the combination therapy. The downstream signals originating from activated PI3-kinase would subsequently cause a greater amount of GLUT-4 from the increased GLUT-4 pool (induced by the exercise training) to translocate and fuse into the sarclemmal membrane, thereby facilitating the greatest enhancement of glucose transport activity in these muscles. However, insulin-stimulated glucose transport activity, while greatly enhanced relative to the untreated obese muscle, is still not normalized, possibly because intramuscular triglycerides and protein carbonyls remain somewhat elevated relative to lean muscle (Fig. 1D).

This compelling concept of interactive metabolic effects of exercise training and R-ALA in the treatment of insulin resistance clearly requires further experimental testing to evaluate its validity. Moreover, it would be important to determine if other antioxidants, such as vitamin E, glutathione, and *N*-acetylcysteine, might also display these beneficial interactions with exercise training in the treatment of skeletal muscle insulin resistance. Most importantly, it will ultimately be critical to evaluate, in well-designed clinical trials, whether these interesting findings in an animal model of obesity-associated insulin resistance can be reproduced in human subjects with prediabetes or type 2 diabetes.

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