Lactate induces insulin resistance in skeletal muscle by suppressing glycolysis and impairing insulin signaling

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Abstract

Lactate induces insulin resistance in skeletal muscle by suppressing glycolysis and impairing insulin signaling. Am J Physiol Endocrinol Metab 283:E233–E240, 2002. First published April 23, 2002; 10.1152/ajpendo.00557.2001.—Elevation of plasma lactate levels induces peripheral insulin resistance, but the underlying mechanisms are unclear. We examined whether lactate infusion in rats suppresses glycolysis preceding insulin resistance and whether lactate-induced insulin resistance is accompanied by altered insulin signaling and/or insulin-stimulated glucose transport in skeletal muscle. Hyperinsulinemic euglycemic clamps were conducted for 6 h in conscious, overnight-fasted rats with or without lactate infusion (120 μmol·kg⁻¹·min⁻¹) during the final 3.5 h. Lactate infusion increased plasma lactate levels about fourfold. The elevation of plasma lactate had rapid effects to suppress insulin-stimulated glycolysis, which clearly preceded its effect to decrease insulin-stimulated glucose uptake. Both submaximal and maximal insulin-stimulated glucose transport decreased 25–30% (P < 0.05) in soleus but not in epitrochlearis muscles of lactate-infused rats. Lactate infusion did not alter insulin’s ability to phosphorylate the insulin receptor, the insulin receptor substrate (IRS)-1, or IRS-2 but decreased insulin’s ability to stimulate IRS-1- and IRS-2-associated phosphatidylinositol 3-kinase activities and Akt/protein kinase B activity by 47, 75, and 55%, respectively (P < 0.05 for all). In conclusion, elevation of plasma lactate suppressed glycolysis before its effect on insulin-stimulated glucose uptake, consistent with our hypothesis. A major characteristic of type 2 diabetes and obesity (9, 17, 23). The mechanisms by which this insulin resistance develops are unclear. Skeletal muscle is the major tissue responsible for insulin action on peripheral glucose uptake (2). In muscle, insulin resistance is associated with impaired insulin action on both glucose transport and intracellular glucose metabolism (3, 13, 14). Researchers have debated as to which of these defects is the primary defect in the development of insulin resistance or type 2 diabetes (7, 30, 31, 34). We (19, 20) have put forth the hypothesis that impairment (or suppression) of intracellular glucose metabolism could precede and cause impairment of insulin’s action on glucose transport (or insulin resistance) in skeletal muscle. We demonstrated that suppression of insulin-stimulated glycolysis preceded insulin resistance during high-fat feeding in rats (19). Also, suppression of either glycolysis or glycogen synthesis in skeletal muscle during physiological insulin stimulation was shown to decrease insulin-stimulated glucose uptake (20). We also reported that suppression of glycogen synthesis preceded the development of insulin resistance during growth hormone infusion (18). Although these data strongly support our hypothesis, the cause-effect relationship remains to be established. We have undertaken the present study to produce additional evidence that suppression of intracellular glucose metabolism can lead to insulin resistance.

Lactate was shown to decrease glycolysis, and thus its own production, in isolated rat skeletal muscle (6, 27). In humans, lactate infusion inhibited endogenous lactate production (32). Because the major site of lactate production in vivo is skeletal muscle (8), these data suggest that lactate inhibits glycolysis in skeletal muscle in vivo. In rats, acute (i.e., 2.5-h) or chronic (24-h) lactate infusion has been shown to induce insulin resistance (24, 36). It is possible that lactate induces insulin resistance in skeletal muscle by suppressing glycolysis, consistent with our hypothesis. A major goal of the present study was to test whether lactate suppresses glycolysis in vivo in conscious rats.

INSULIN RESISTANCE, defined as an impairment in insulin’s ability to stimulate peripheral glucose uptake, is a major characteristic of type 2 diabetes and obesity (9, 17, 23). The mechanisms by which this insulin resistance develops are unclear. Skeletal muscle is the major tissue responsible for insulin action on peripheral glucose uptake (2). In muscle, insulin resistance is associated with impaired insulin action on both glucose transport and intracellular glucose metabolism (3, 13, 14). Researchers have debated as to which of these defects is the primary defect in the development of insulin resistance or type 2 diabetes (7, 30, 31, 34). We (19, 20) have put forth the hypothesis that impairment (or suppression) of intracellular glucose metabolism could precede and cause impairment of insulin’s action on glucose transport (or insulin resistance) in skeletal muscle. We demonstrated that suppression of insulin-stimulated glycolysis preceded insulin resistance during high-fat feeding in rats (19). Also, suppression of either glycolysis or glycogen synthesis in skeletal muscle during physiological insulin stimulation was shown to decrease insulin-stimulated glucose uptake (20). We also reported that suppression of glycogen synthesis preceded the development of insulin resistance during growth hormone infusion (18). Although these data strongly support our hypothesis, the cause-effect relationship remains to be established. We have undertaken the present study to produce additional evidence that suppression of intracellular glucose metabolism can lead to insulin resistance.

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and whether this effect precedes changes in insulin action on glucose uptake. Suppression of intracellular glucose metabolism may decrease glucose uptake by causing accumulation of intracellular glucose and thus decreasing the transmembrane glucose gradient (35). Whereas intracellular glucose does not accumulate in muscle under normal or type 2 diabetic conditions (7), whether it could accumulate under other metabolic states is not known. On the other hand, skeletal muscle insulin resistance is often associated with impaired insulin signaling and decreased insulin-stimulated glucose transport. A second goal of the present study was to test whether lactate-induced insulin resistance is accompanied by impaired insulin signaling and/or decreased insulin stimulation of glucose transport.

**METHODS**

**Animals and Catheterization**

Male Wistar rats weighing 275–300 g were obtained from Simonsen (Gilroy, CA) and studied 5 days after arrival. Animals were housed under controlled temperature (22 ± 2°C) and lighting (12-h light: 0600–1800, 12-h dark: 1800–0600) with free access to water and standard rat chow. At least 4 days before the experiment, animals were placed in individual cages with tail restraint, as previously described (19, 20), which was required to protect tail blood vessel catheters during experiments. Animals were free to move about and were allowed unrestricted access to food and water. Two tail vein infusion catheters were placed on the day before the experiment, and one tail artery blood-sampling catheter was placed ≥4 h before the experiment (i.e., −0700) (19, 20). All procedures were approved by the Institutional Animal Care and Use Committee at the University of Southern California.

**Animal Experiments**

Experiments were conducted after an overnight fast; food was removed at 1700 on the day before the experiment. Three studies were carried out. In study 1, we determined the effects of lactate infusion on insulin-stimulated whole body glucose fluxes (i.e., glucose uptake and glycolysis). Animals received an infusion of porcine insulin (Novo Nordisk, Princeton, NJ) at a constant rate of 30 pmol·kg⁻¹·min⁻¹ for 6 h. During the insulin infusion, plasma glucose was clamped at basal levels by exogenous glucose infusion (hyperinsulinemic euglycemic clamp). After the initial 150-min clamp, the clamp was continued with a constant infusion of lactate [120 μmol·kg⁻¹·min⁻¹; L(+)-lactate sodium salt diluted in phosphate buffer, 0.06 M NaH₂PO₄, and 0.0134 M Na₂HPO₄, pH 4.5 (24, 36)] during the remaining 210 min. Whole body glucose fluxes were estimated using [³H]methylglucose, as previously described (16).

**Measurement of Insulin Signaling Events**

**Preparation of rat tissue lysates.** Fifty milligrams of muscle were homogenized using a polytron at half-maximum speed for 1 min on ice in 500 μl of buffer A (in mM: 20 Tris (pH 7.5), 5 EDTA, 10 Na₂HPO₄, 100 NaF, and 2 Na₃VO₄) containing 1% NP-40, 1 mM phenylmethylsulfonyl fluoride, 10 mg/ml aprotenin, and 10 mg/ml leupeptin. Tissue lysates were solubilized by continuous stirring for 1 h at 4°C and centrifuged for 10 min at 14,000 g. The supernatants were stored at −80°C until analysis.

**Tyrosine phosphorylation of the insulin receptor and insulin receptor substrates 1 and 2.** Muscle lysates (500 μg protein) were subjected to immunoprecipitation overnight at 4°C with 1 μl of insulin receptor (IR) polyclonal antibody (1:500 dilution; gift from Dr. Steven Shoelson, Joslin Diabetes Center) or 5 μl of IR substrate (IRS)-1 or IRS-2 polyclonal antibody (1:100 dilution; gift from Dr. Morris White, Joslin Diabetes Center) coupled to protein A-Sepharose. The immunoprecipitates were washed, and phosphorylation was visualized as described (22) using anti-phosphotyrosine monoclonal antibody at 1:200 dilution (Upy 99, Santa Cruz, CA).

**Determination of phosphatidylinositol 3-kinase activity.** Tissue lysates (500 μg protein) were subjected to immunoprecipitation with 5 μl of IRS-1 polyclonal antibody or 5 μl of IRS-2 polyclonal antibody (1:100 dilution) coupled to protein A-Sepharose (Sigma, St. Louis, MO). The immunocomplex was washed, and phosphatidylinositol (PI) 3-kinase activity was determined, as previously described (21, 22).

**Determination of Akt activity.** Tissue lysates (500 μg protein) were subjected to immunoprecipitation for 4 h at 4°C with 2 μg of a polyclonal Akt antibody that recognizes both Akt1 and Akt2 (Upstate Biotechnology, Lake Placid, NY) coupled to protein G-Sepharose beads (Pharmacia Biotech, Piscataway, NJ) for 4 h. Immune pellets were washed, and Akt activity was determined as previously described (21, 22).

**Determination of glycogen synthase kinase-3 activity.** Muscle lysates (500 μg protein) were subjected to immunoprecipitation for 4 h at 4°C with glycogen synthase kinase (GSK)-3β antibody (1:100 dilution; gift from Dr. Hagit Eldar-Finkelman, Tel-Aviv University, Tel-Aviv, Israel) coupled to protein
A-Sepharose beads. Immune pellets were washed, and GSK-3 activity was determined as previously described (12).

**Muscle GLUT4 Content**

Muscle homogenates (30 µg protein) were subjected to 8% SDS-PAGE and transferred to Hybond-P membranes (Amer- sham Pharmacia Biotech, Piscataway, NJ). The membranes were incubated with an anti-GLUT4 polyclonal antibody (Biogenesis, Brentwood, NH) and exposed to a secondary antibody (horseradish peroxidase-conjugated anti-rabbit IgG from Amersham). Immunodetection was performed using an enhanced chemiluminescence kit from Pierce Chemical (Rockford, IL).

**Other Assays**

Plasma glucose was analyzed by a glucose oxidase method on a Beckman Glucose Analyzer II (Beckman, Fullerton, CA). Plasma lactate was measured by a lactate oxidase method on a YSI lactate analyzer (Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin was measured by radioimmunoassay with a kit from Linco Research (St. Charles, MO). Plasma concentrations of [3H]glucose and 3H2O were determined as previously described (18–20).

**Calculations**

To estimate non-steady-state rates of whole body glucose fluxes (study 1), plasma glucose, [3H]glucose specific activity, and 3H2O data were smoothed using the OOPSEG algorithm (4). The rates of total glucose appearance and glucose uptake were then calculated using the non-steady-state Steele's equation (33). Endogenous glucose production (EGP) was calculated by subtracting the glucose infusion rate from the rate of total glucose appearance. Whole body glycolysis was calculated from the rate of increase in plasma 3H2O concentration, which was calculated at each sampling time as the slope of the smoothed 3H2O curve divided by the specific activity of plasma [3H]glucose, as previously described by us (20).

**Statistical Analysis**

Data are expressed as means ± SE. The significance of the differences in mean values among different treatment groups was evaluated using a one-way ANOVA, followed by ad hoc analysis with the Tukey test. The significance of the effects of treatment within the groups was evaluated using the paired t-test. P < 0.05 was considered statistically significant.

**RESULTS**

**Plasma Insulin, Glucose, and Lactate Levels During the Insulin and/or Lactate Infusion**

Insulin infusion raised plasma insulin to ~500 pM, and this level was maintained throughout the clamp and was not altered by lactate infusion (data not shown). During the insulin infusion, plasma glucose was clamped at the basal levels (~5.8 mM; Fig. 1). Plasma lactate levels were threefold higher during the hyperinsulinemic euglycemic clamps compared with the saline-infused group. Lactate infusion, initiated at 150 min, further increased plasma lactate levels from ~1.1 to ~4.2 mM. Glucose infusion rate required to clamp plasma glucose increased rapidly during the first hour and reached a steady state within 2 h of clamp initiation. Lactate infusion did not alter glucose infusion rate during the first 30–60 min but gradually decreased it thereafter, resulting in a 34% decrease at the end of the 3.5-h infusion (P < 0.01).

**Effects of Lactate Infusion on Insulin-Stimulated Glucose Fluxes (Study 1)**

Figure 2 shows the time courses of changes in specific activities of plasma [3H]glucose and 3H2O with lactate infusion (150–360 min) during the hyperinsulinemic glucose clamp. Plasma glucose specific activity reached a steady state (with a constant 3H-glucose infusion) before the lactate infusion (Fig. 2A). During the first hour of lactate infusion, little change was observed with the specific activity, indicating minimal changes in insulin-stimulated glucose uptake. However, glucose uptake increased slowly thereafter, reflecting a slow decline of insulin-stimulated glucose uptake. The rate of increase in plasma 3H2O was con-
stant before the lactate infusion, indicating a constant rate of glycolysis (Fig. 2B). Lactate infusion had rapid effects to slow the increase in plasma $^{3}$H$_2$O, indicating that lactate rapidly suppressed insulin-stimulated glycolysis. The calculated rates of insulin-stimulated glucose fluxes (Fig. 2C) clearly demonstrate that lactate had a rapid effect to suppress glycolysis, preceding decreases in insulin-stimulated glucose uptake. EGP did not change during the first 2 h of lactate infusion but increased slightly during the final hour of lactate infusion (4.3 ± 0.6 vs. 2.1 ± 0.6 mg·kg$^{-1}$·min$^{-1}$; $P < 0.05$). This change in EGP accounted for ~20% of the change in glucose infusion rate with lactate infusion (Fig. 1C).

**Effects of Lactate Infusion on Insulin-Stimulated Glucose Transport Activities (Study 2)**

We next examined whether lactate-induced insulin resistance (i.e., decrease in insulin-stimulated glucose uptake) was accompanied by any changes in insulin’s ability to stimulate glucose transport. Glucose transport activity was assessed in vitro in isolated epitrochlearis and soleus muscles after a 10-min incubation with insulin after the hyperinsulinemic clamps with or without lactate infusion. Neither submaximal nor maximal insulin-stimulated glucose transport activity was altered in epitrochlearis muscles (Fig. 3A). In contrast, both submaximal and maximal insulin-stimulated glucose transport activities were significantly decreased in soleus muscles of lactate-infused animals ($P < 0.05$; Fig. 3B). Thus the lactate infusion decreased insulin’s ability to stimulate glucose transport activity in the slow-twitch oxidative (type I) soleus muscle, although this effect was not seen in the fast-twitch glycolytic (type IIb) epitrochlearis muscles.

**Effects of Lactate Infusion on Muscle GLUT4 Content and Insulin Signaling (Study 3)**

A previous study (24) reported that lactate infusion in rats for 24 h, at a rate similar to that in the present study, decreased muscle GLUT4 protein content by 40%. We examined whether insulin resistance induced with the 3.5-h lactate infusion (Fig. 1) was associated with decreased GLUT4 protein content in soleus and

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**Fig. 2.** Specific activities of plasma $[^{3}$H$]$glucose (A) and $[^{3}$H$]$H$_2$O (B) and calculated glucose fluxes (C) before and during lactate infusion in hyperinsulinemic euglycemic clamps. Values are means ± SE for 7 experiments (study 1). In B, error bars are too small and completely masked by symbols. Inset, close-up of initial changes and error bars.

**Fig. 3.** Insulin-stimulated glucose transport activities in epitrochlearis (A) and soleus (B) muscles, assessed in vitro with 3-O-$[^{3}$H$]$methylglucose. Open bars, control rats (i.e., no lactate infusion); closed bars, lactate-infused rats. Broken lines, glucose transport activities in basal (i.e., no insulin stimulation) muscles. Values are means ± SE for 8 or 10 experiments (study 2). *$P < 0.05$ vs. control.
gastrocnemius muscles. GLUT4 content in gastrocnemius muscle showed a tendency to increase with insulin infusion (i.e., the 6-h hyperinsulinemic clamp; \( P > 0.05 \); Fig. 4) but was not decreased by the 3.5-h lactate infusion. GLUT4 content in soleus muscle was altered by neither insulin nor lactate (\( P > 0.05 \)).

Insulin infusion resulted in two- to fourfold stimulation of tyrosine phosphorylation of the insulin receptor, IRS-1, and IRS-2 above basal levels (i.e., those in saline-infused rats; Fig. 5). Lactate infusion during the final 3.5 h of the hyperinsulinemic clamps did not alter insulin-stimulated phosphorylation of the insulin receptor or IRS molecules. Insulin increased IRS-1- and IRS-2-associated PI 3-kinase activities 2.5-fold above basal levels. In lactate-infused rats, insulin-stimulated IRS-1- and IRS-2-associated PI 3-kinase activities were decreased by 27 and 53%, respectively (\( P < 0.05 \) for all). Thus insulin stimulation (the increments above basal levels) of IRS-1- and IRS-2-associated PI 3-kinase activities were suppressed 47 and 75%, respectively. Insulin increased Akt activity 2.5-fold, and this action of insulin was 55% inhibited by lactate infusion (\( P < 0.05 \)). These data demonstrate that lactate infusion impairs IRS-1 and IRS-2-associated PI 3-kinase activities, critical events for insulin stimulation of downstream Akt activity and glucose transport.

Fig. 4. GLUT4 content in gastrocnemius (open bars) and soleus (solid bars) muscles after 360-min saline infusion (saline) or hyperinsulinemic euglycemic clamp without (insulin) or with lactate (insulin + lactate) infusion. Values are means ± SE for 6 experiments (study 3).

Fig. 5. Insulin receptor (A), insulin receptor substrate (IRS)-1 (B), and IRS-2 (C) phosphorylation, IRS-1- (D) and IRS-2 (E)-associated phosphatidylinositol (PI) 3-kinase activities, and Akt activity (F) in gastrocnemius muscles after 360-min saline infusion (saline) or hyperinsulinemic euglycemic clamp without (insulin) or with lactate (insulin + lactate) infusion. Values are means ± SE for 6 experiments (study 3). *\( P < 0.05 \) vs. saline; #\( P < 0.05 \) vs. insulin.
infusion. Values are means ± SE for 6 experiments (study 3). *P < 0.05 vs. saline.

GSK-3 activity was decreased by insulin as expected (P < 0.05; Fig. 6); GSK-3 is phosphorylated and inactivated by Akt. However, it is very interesting to find that lactate infusion did not alter GSK-3 activity despite significant alteration of Akt activity, demonstrating a dissociation of changes in Akt and GSK-3 activities.

DISCUSSION

The present study demonstrates that an elevation of plasma lactate level suppressed insulin-stimulated glycolysis in vivo in conscious rats and that this effect preceded the effect of lactate to decrease insulin-stimulated glucose uptake. This finding is consistent with our hypothesis that metabolic impairment could precede and cause insulin resistance (i.e., decrease in insulin-stimulated glucose uptake) in skeletal muscle and suggests that lactate may induce insulin resistance in skeletal muscle by first suppressing glycolytic flux. In addition, the present study demonstrates that lactate-induced insulin resistance was accompanied by decreases in insulin-stimulated glucose transport activities (soleus muscles) and specific alterations of insulin signaling for glucose transport in skeletal muscle. These data suggest that the sequence of events leading to lactate-induced insulin resistance may first involve metabolic impairment followed by altered insulin signaling and ultimately impaired glucose transport.

Because skeletal muscle is the major site of insulin-stimulated glucose utilization (2), it is likely that the effect of lactate (~4.2 mM) to decrease insulin-stimulated glycolysis occurred mainly in skeletal muscle. Our data are consistent with those of previous studies in isolated skeletal and cardiac muscles (6, 10, 11, 27). Pearce and Connett (27) showed that 8 mM lactate resulted in profound decreases in insulin-stimulated glucose oxidation and lactate production in isolated soleus muscles. Clark et al. (6) also reported that lactate inhibited insulin-stimulated glycolysis in epitrochlearis muscles and that the rate of glycolysis was inversely proportional to the rate of lactate oxidation. Inhibition of glycolysis by lactate has been extensively studied in cardiac muscle (10, 11); the lactate effect appears to involve inhibitions of 6-phosphofructo-1-kinase (PFK-1) and pyruvate dehydrogenase (PDH), two rate-limiting enzymes of glycolysis, similar to the situation of fatty acid inhibition of glycolysis (19). In cardiac muscles, lactate increases the level of citrate (which is an inhibitor of PFK-1), decreases the level of fructose-2,6-bisphosphate (which is a well-known stimulator of PFK-1), and increases the ratio of NADH to NAD (which would inhibit PDH) (10, 11). Whether these changes also occur in skeletal muscle is less clear. In the present study, lactate increased citrate levels in soleus muscle by 70% (data not shown).

Did the suppression of glycolysis by lactate cause subsequent development of insulin resistance? So far, we have demonstrated that suppression of either glycolysis or glycygen synthesis precedes development of impaired glucose uptake during high-fat feeding in rats (19) or during acute infusion of Intralipid (20), amylin (20), growth hormone (18), or lactate (present study). Although these data strongly support our hypothesis, they do not prove a causal relationship. However, recent human studies provided more direct evidence supporting our hypothesis; Ristow et al. (28, 29) reported that deficiency of PFK-1, a rate-limiting enzyme in glycolysis, causes insulin resistance in humans. Thus available data support our hypothesis, and we propose that lactate-induced insulin resistance results from initial suppression of glycolysis.

If suppression of glucose metabolism causes impaired insulin-stimulated glucose uptake, what could be the mechanisms? We (20) previously provided evidence that the classic mechanism involving glucose 6-phosphate (G-6-P) inhibition of hexokinase cannot account for the decrease in insulin-stimulated glucose uptake during metabolic suppression with Intralipid or amylin infusion. Therefore, we hypothesized that metabolic impairment (and consequent increase in G-6-P or other metabolites) somehow leads to impairment of insulin’s ability to stimulate the glucose transport system (20). G-6-P has been shown to interact with protein kinases and phosphatases to regulate the activities of glycogen synthase and phosphorylase (5, 37). Therefore, it may be conceivable that increased G-6-P level activates certain protein kinases or phosphatases to alter insulin signaling and impair insulin’s ability to stimulate glucose transport. However, it may also be possible that increased G-6-P level increases substrate flux through a minor glucose metabolic pathway (e.g., pentose phosphate pathway) to induce insulin resistance.

It is interesting to note that lactate decreased insulin-stimulated glucose transport activity in soleus but not in epitrochlearis muscles. Soleus is composed of red fibers (slow-twitch oxidative, 89%; fast-twitch oxida-
tive glycolytic, 11%) (1), whereas epitrochlearis is mainly (60–65%) composed of white fibers (i.e., fast-twitch glycolytic fibers) (26). Our data are consistent with the finding of Lombardi et al. (24) and Vettor et al. (36) that lactate infusion, at a rate similar to ours, decreased insulin-stimulated glucose uptake in red but not in white fiber muscles in vivo. Taken together, these data suggest that the effect of lactate to decrease insulin-stimulated glucose uptake in red fiber muscles was mediated by impairment of insulin’s ability to stimulate glucose transport. Lombardi, Vettor, and colleagues (24, 36) attributed muscle fiber-specific effects of lactate to differences in lactate transport capacity between the muscle fibers, which has been shown to be greater in red than in white fiber muscles (25). In the present work, we did not study the effect of lactate on glycosylation in individual muscles. It would be important to examine whether the lack of lactate effect on insulin-stimulated glucose transport in epitrochlearis muscle is associated with a lack of lactate effect on insulin-stimulated glycosylation. We found specific alterations of insulin signaling in gastrocnemius muscle (see the next paragraph), which is a mixture of red (55%) and white (45%) fibers (1). It is possible that significant changes in insulin signaling occurred only in red but not in white fibers of gastrocnemius muscles. If so, the present data might underestimate the changes in insulin signaling that occurred in red gastrocnemius muscles.

The present study demonstrates that lactate altered specific steps of insulin signaling; although insulin-stimulated phosphorylation of the insulin receptor, IRS-1, and IRS-2 was not affected, insulin-stimulated IRS-1- and IRS-2-associated PI 3-kinase activities were substantially decreased in lactate-infused rats, suggesting that the interactions of IRS-1 and IRS-2 with PI 3-kinase may be altered. Stimulation of PI 3-kinase is a crucial event for stimulation of glucose transport activity. An impairment of insulin stimulation of IRS-associated PI 3-kinase activities would impair downstream signaling processes, as confirmed with Akt activation, and could reduce insulin’s ability to translocate GLUT4 to the plasma membrane. Therefore, the present study suggests that insulin stimulation of glucose transport activity is impaired by lactate because of its specific effect to alter insulin signaling for GLUT4 translocation without changing GLUT4 protein content. The effects of lactate on insulin signaling (i.e., blunted insulin stimulation of IRS-associated PI 3-kinase in the absence of changes in IRS phosphorylation) were similar to those observed with glucosamine in our previous study (22) but were different from those observed with free fatty acids (15). In the latter study, a blunting of insulin-stimulated IRS-1 tyrosine phosphorylation was observed. The exact mechanisms by which lactate alters IRS-associated PI 3-kinase activity remain to be studied and could involve decreased binding of p85 (the regulatory subunit of PI 3-kinase) to IRs.

In conclusion, the present study demonstrates that lactate suppresses insulin-stimulated glycosylation in vivo and that this effect precedes the effect of lactate to decrease insulin-stimulated glucose uptake. This finding suggests that lactate may induce insulin resistance in skeletal muscle by first suppressing glycosylation. Lactate-induced insulin resistance was accompanied by decreases in insulin-stimulated glucose transport activities and specific alterations of insulin signaling for stimulation of glucose transport without change in GLUT4 protein content. Thus the present study provides additional evidence to support our hypothesis that impairment (or suppression) of intracellular glucose metabolism can precede and possibly cause impairment of insulin action to stimulate glucose transport (or glucose uptake) in skeletal muscle.

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