Stimulation of Glucose and Amino Acid Transport and Activation of the Insulin Signaling Pathways by Insulin Lispro in L6 Skeletal Muscle Cells

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ABSTRACT

The monomeric insulin analogue insulin lispro (Lys B28, Pro B29) is a rapid-acting insulin with a shorter duration of activity than human regular insulin. This compound has the advantage of reducing early postprandial hyperglycemia and the accompanying late hypoglycemia, thereby improving overall blood glucose control. To date, all published studies of the functional properties of insulin lispro have been conducted in whole animals. This study aimed to characterize the cellular actions of insulin lispro and the signals it elicits in an insulin-sensitive muscle cell line, L6 cells. Comparing the cellular actions of insulin lispro with those of human regular insulin, a number of observations were made. (1) Insulin lispro stimulated glucose and amino acid transport into L6 myotubes with a dose dependency and time course virtually identical to those of human regular insulin. (2) Insulin lispro was as effective as human regular insulin in stimulating time-dependent phosphorylation of insulin receptor substrate 1 (IRS-1), p70 ribosomal S6 kinase, and two isoforms of mitogen-activated protein kinase (ERK1 and ERK2). (3) Insulin lispro’s ability to induce the association of IRS-1 with the p85 subunit of phosphatidylinositol 3-kinase was similar to that of human regular insulin. (4) As with human regular insulin, 100 nmol of the fungal metabolite wortmannin completely inhibited insulin lispro stimulation of glucose uptake. We concluded that the cellular actions of insulin lispro are similar to those of human regular insulin with respect to glucose and amino acid uptake and that the biochemical signals elicited are also comparable. Key words: lispro, glucose transport, amino acid, p70 S6 kinase, wortmannin.
INTRODUCTION

The monomeric insulin analogue insulin lispro (Lys B28, Pro B29) is identical to human insulin with one exception: the lysine and proline residues at positions 28 and 29 on the B-chain are interchanged. This amino acid substitution confers on insulin lispro the unique physiochemical characteristic of weak self-association at low concentrations. The monomeric state allows insulin lispro to be absorbed faster from the site of administration, reaching higher serum concentration at a more rapid rate with a shorter duration of activity than currently available insulin formulations. Therefore, insulin lispro has the advantage of decreasing glucose excursions during and after meals with less risk of late hypoglycemia—a disadvantage of current therapeutic short-acting insulin—thereby improving overall blood glucose control.

Compared with human regular insulin, insulin lispro is equipotent in binding to the human placental insulin receptor. Previously published functional studies on the action of insulin lispro have been performed in whole animals. To fully understand insulin lispro’s metabolic actions and the signals it elicits, it is important to compare its short- and long-term actions with those of human regular insulin in a system in which separate variables can be studied independently. This is not achievable in human and animal studies because several circulating factors will be altered on insulin administration (insulin, glucose, fatty acids, counter regulatory hormones). Muscle cells in culture, on the other hand, offer a system in which separate variables can be studied independently.

The acute and chronic effects of insulin on L6 muscle cells (cell line derived from the leg muscle of 1-day-old rats) have been described previously. These cells have a glucose transport system that is kinetically, pharmacologically, and biochemically similar to that of rat skeletal muscle and is stimulated rapidly by insulin. Insulin also stimulates the uptake of short-chain neutral amino acids across the plasma membrane by system A, an integral membrane-bound protein that catalyzes Na:amino acid cotransport in L6 muscle cells. In skeletal muscle, insulin stimulates system A by a rapid mechanism that increases the maximal velocity of transport independently of ongoing protein synthesis.

The complete mechanism by which insulin influences metabolic and mitogenic processes is unknown. However, many of the intermediate participants in the insulin signaling pathways have been identified, and the changes that these proteins undergo in response to insulin are well documented. Upon insulin binding, the insulin-receptor—intrinsic tyrosine kinase is activated by autophosphorylation on tyrosine residues, and this is followed by the rapid multisite phosphorylation of insulin receptor substrate 1 (IRS-1). The next step in the insulin signal transduction cascade is the binding of many signaling molecules via their Src homology 2 (SH2) domains to the phosphotyrosine residues of IRS-1 and the consequent activation of their catalytic or regulatory activities.

The purpose of the present study was to compare the effectiveness of insulin lispro and human regular insulin in the stimulation of glucose and amino acid transport; in the phosphorylation of IRS-1, p70 ribosomal S6 kinase, and two isoforms of mitogen-activated protein kinase (MAPK), ERK 1 and ERK 2; and in the association of IRS-1 with phosphatidylinositol (PI) 3-kinase. Finally, we used the fungal metabolite wortmannin (WM).
to assess the effect of inhibition of PI 3-kinase on insulin lispro action.

MATERIALS AND METHODS

Materials

Insulin lispro and human regular insulin were provided by Eli Lilly Canada (Toronto, Ontario, Canada). α-Minimal essential medium (α-MEM) was obtained from Ontario Cancer Institute (Toronto, Ontario, Canada). Fetal bovine serum (FBS) and antibiotic/antimycotic solution were obtained from GIBCO/BRL (Burlington, Ontario, Canada). 2-Deoxy-3H-D-glucose (2DG) and 2-(14C-methylamino) isobutyric acid (MeAIB) were purchased from Dupont (Boston, Massachusetts). Unlabeled 2-deoxy-D-glucose, cytochalasin B (CB), WM, and unlabeled MeAIB were obtained from Sigma Chemicals (St. Louis, Missouri). Monoclonal antiphosphotyrosine, polyclonal anti-S6 kinase antibodies, and polyclonal antibody against the 85-kd subunit of PI 3-kinase (p85) for immunoblotting and polyclonal anti-IRS-1 antibody for immunoprecipitation were purchased from Upstate Biotechnology, Inc. (Lake Placid, New York). Anti-phospho-mitogen-activated protein kinase (MAPK) antibody was obtained from New England Biolab (Beverly, Massachusetts). Protein A sepharose was purchased from Pharmacia (Uppsala, Sweden). All electrophoresis and immunoblotting reagents were purchased from Bio-Rad (Mississauga, Ontario, Canada).

Cell Culture and Incubations

Cells from a spontaneously fusing subclone of the original L6 muscle cells were grown and differentiated into myotubes as previously described. For transport studies, cells were treated with trypsin, seeded in 12-well plates (2.5-cm-diameter well), and maintained in growth medium as described above, except for supplementation with 2% FBS. Cells were maintained under the same conditions in 6-well plates for preparation of whole-cell lysates and in 10-cm-diameter dishes for immunoprecipitations.

2-Deoxy-3H-D-Glucose Uptake

L6 myotubes were deprived of serum for 5 hours (for dose-response and short-time-course studies) with α-MEM, 0.1% FBS (volume/volume solvent), and 25 mmol glucose (serum-deprivation media) before experimental manipulations. 2DG uptake measurements were carried out as described previously.

Briefly, following all stimulations and incubations with inhibitors, cell monolayers were washed twice with HEPES-buffered saline (HBS) (140 mmol NaCl, 20 mmol HEPES-Na, 2.5 mmol MgSO4, 1 mmol CaCl2, 5 mmol KCl [pH 7.4]), and any remaining liquid was aspirated. Cells were then incubated for 5 minutes in HBS containing 10 μmol unlabeled 2DG and 10 μmol 2DG (1 μCi/mL) in the absence of insulin. The reaction was terminated by washing three times with ice-cold 0.9% NaCl. Nonspecific uptake was determined in the presence of 10 μmol CB. Cell-associated radioactivity was determined by lysing the cells with 0.05 N NaOH, followed by liquid scintillation counting. Total cellular protein was determined using the Bradford method. For the long time course, conditions were manipulated so that cells were maintained for the last 24 hours in serum-deprivation media.
2-(14C-Methylamino)isobutyric Acid Uptake

Cells were serum-deprived for 5 hours as described above for 2DG uptake. During the last hour they were also depleted of amino acids by incubation in HBS supplemented with 25 mmol glucose. MeAIB uptake was measured for 15 minutes essentially as described by Hundal et al.20 Briefly, following all stimulations, cell monolayers were washed twice with HRS, and all remaining liquid was aspirated. Cells were then incubated for 15 minutes in HBS containing 20 μmol MeAIB (2 μCi/mL) and 10 μmol unlabeled MeAIB. The reaction was terminated and radioactivity associated with the cells determined as described above for 2DG uptake. Non-specific uptake was determined in the presence of a saturating concentration of unlabeled MeAIB (10 mmol).

Detection of Insulin Receptor Substrate 1 Phosphorylation and Association of the 85-kd Subunit of Phosphatidylinositol 3-Kinase

L6 myotubes were treated with 100 nmol insulin lispro or human regular insulin for either 5 or 30 minutes. Cells were then washed twice with ice-cold phosphate-buffered saline containing 100 μmol sodium orthovanadate and once with buffer A (137 mmol NaCl, 20 mmol Tris [pH 7.5], 1 mmol MgCl₂, 1 mmol CaCl₂, and 100 μmol sodium orthovanadate). Cells were then lysed with 1 mL of buffer A containing 10% glycerol, 1% NP-40, protease inhibitors (2 mmol phenylmethylsulfonyl fluoride, 10 μmol E-64, 1 μmol pepstatin A, 1 μmol leupeptin), and 100 μmol sodium orthovanadate. Lysates were passed five times through a 25-gauge syringe and then incubated for 15 minutes at 4 °C under constant rotation. Debris and unbroken cells were removed by centrifugation. Supernatants were incubated overnight with 1 μg of anti-IRS-1 antibody followed by a 1-hour incubation with 20 μL protein A sepharose beads (Pharmacia, Uppsala, Sweden) (100 mg/mL). Immune complexes were collected by centrifugation for 1 minute, and pellets were then washed four times with 100 mmol phosphate-buffered saline–containing sodium orthovanadate and 0.1% NP-40. Pellets were finally resuspended in 30 μL 2X Laemmli sample buffer and boiled for 5 minutes. Proteins were resolved by 7.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and then electrotransferred onto polyvinylidene difluoride (PVDF) membranes. Membranes were cut and probed for either tyrosine-phosphorylated IRS-1 or p85. To detect tyrosine-phosphorylated IRS-1, the upper part of the blot was probed with antiphosphotyrosine antibody (monoclonal, 1:5000 dilution), and protein was detected by the enhanced chemiluminescence method using sheep anti-mouse immunoglobulin conjugated to horseradish peroxidase (HRP) as the secondary antibody. The lower part of the blot was probed with anti-p85 antibody (polyclonal, 1:1000 dilution), and protein was detected using anti-rabbit immunoglobulin G (IgG) conjugated to HRP.

Detection of Mitogen-Activated Protein Kinase and p70 Ribosomal S6 Kinase Phosphorylation

L6 myotubes were treated with 100 nmol insulin lispro or human regular insulin for the indicated time periods. Cells were lysed in a solution containing 10% glycerol, 4%
SDS, 115 mmol Tris/HCl (pH 6.8), 10 mmol dithiothreitol, 0.25 mg/mL bromphenol blue, protease inhibitors (100 μmol phenylmethylsulfonyl fluoride, 10 μmol E-64, 1 μmol pepstatin, 1 μmol leupeptin), and phosphatase inhibitors (40 mmol NaF, 7.5 mmol sodium pyrophosphate, 1.5 mmol sodium orthovanadate). Lysates were passed five times through a 25-gauge syringe to shear the DNA and boiled for 3 minutes. To detect MAPK phosphorylation, 30 μg of total cellular protein were resolved by 10% SDS-PAGE, electrotransferred onto PVDF membranes, and then immunoblotted with phosphospecific MAPK antibody* (polyclonal, 1:1000 dilution). Protein was detected by the enhanced chemiluminescence method using goat anti-rabbit IgG conjugated to HRP (1:5000 dilution). For p70 S6 kinase phosphorylation, 15 μg of total cellular protein were resolved by 7.5% SDS-PAGE, and immunoblotted with anti-S6 kinase antibody (polyclonal, 1 μg/mL) followed by HRP-conjugated goat anti-rabbit IgG.

Statistical Analysis

Statistical analysis was performed using either an unpaired Student's t test or analysis of variance (ANOVA) (Fisher, multiple comparisons), as indicated in the results.

RESULTS AND DISCUSSION

Insulin Lispro Dose-Dependent Stimulation of Glucose Uptake

Insulin lispro elicited a dose-dependent stimulation of 2DG uptake into L6 myotubes. This was assessed by the uptake of the nonmetabolizable analogue 2DG. As shown in Figure 1, the response of L6
myotubes to insulin lispro closely paralleled the response to human regular insulin over the concentration range 1 nmol to 1 μmol, peaking at 200 nmol. Similar results were obtained with porcine insulin both in this study (data not shown) and previous studies. There was no significant difference (P > 0.05, unpaired t test) in glucose uptake stimulated by insulin lispro or by human regular insulin at any of the concentrations studied here.

**Insulin Lispro Time-Dependent Stimulation of Glucose Uptake**

L6 myotubes were exposed to 200 nmol of either insulin lispro or human regular insulin for various time periods (Figure 2A). Within 5 minutes, both insulin lispro and human regular insulin stimulated 2DG uptake into L6 myotubes by twofold, and this level was maintained for at least 1 hour. There was no significant difference (P > 0.05, unpaired t test) between the actions of the two insulin molecules during any of the short time periods investigated.

Over the course of 24 hours, insulin lispro stimulated 2DG uptake by 2.9-fold from a basal level of 5.9 ± 0.3 pmol/min per mg protein to a stimulated level of 17.1 ± 1.3 pmol/min per mg protein (Figure 2B). The response of L6 myotubes to insulin lispro was very similar to that elicited by human regular insulin, which stimulated 2DG uptake from a basal level of 6.3 ± 0.9 to 17.4 ± 1.2 pmol/min per mg protein, corresponding to a 2.8-fold increase. There was no significant difference (P > 0.05, unpaired t test) between these values, indicating that insulin lispro has a course of action, as assessed over a 24-hour period, that does not deviate significantly from that of human regular insulin. Taken together, these time courses demonstrate that insulin lispro and human regular insulin stimulate glucose uptake in a biphasic manner consisting of an acute response and a more prolonged phase initiated at some point after 8 hours.

**Inhibition of Insulin Lispro Stimulation of Glucose Uptake by Wortmannin**

WM is a selective inhibitor of PI 3-kinase. It potently inhibits the enzyme by binding irreversibly to the 110-kd catalytic subunit. PI 3-kinase is necessary for the maintenance of basal as well as insulin-stimulated glucose uptake, because inhibition of PI 3-kinase with WM results in decreased basal glucose transport, decreased insulin-stimulated glucose transport, and impairment of the translocation of glucose transporters to the plasma membrane in L6 skeletal muscle cells, rat skeletal muscle, and 3T3-L1 cells. Insulin lispro as well as human regular insulin stimulated glucose uptake into L6 cells by approximately twofold within 30 minutes (Figure 2A). Treatment of L6 myotubes with 100 nmol WM for 45 minutes (a 15-minute pretreatment followed by a 30-minute treatment in the absence or presence of 200 nmol insulin) resulted in complete inhibition of insulin lispro–stimulated 2DG uptake (control: 4.6 ± 0.8 pmol/min per mg protein; insulin lispro plus WM: 4.8 ± 0.5 pmol/min per mg protein) and near inhibition of human regular insulin–stimulated 2DG uptake (control: 4.6 ± 0.8 pmol/min per mg protein; human regular insulin plus WM: 6.4 ± 0.9 pmol/min per mg protein) (Figure 3). A higher value was obtained for human regular insulin in the presence of WM because of one experiment in which WM only partially inhibited 2DG uptake. The reason for this was unclear. However, the
Figure 2. Time course of insulin lispro stimulation of 2-deoxyglucose (2DG) uptake. L6 myotubes (serum starved) were treated with 200 nmol of either insulin lispro (InsLP) or human regular insulin (HumR) for short time periods (A) or for longer time periods (B). 2DG uptake was subsequently determined over a 5-minute period. Results represent the mean ± SE of at least three independent experiments, each consisting of five replicates of each condition. Where error bars are absent, the SE was too small to appear on this scale.
responses to human regular insulin and insulin lispro in the presence of 100 nmol WM were not statistically different (P > 0.05, ANOVA). These results indicate that insulin lispro, like human regular insulin, utilizes a PI 3-kinase-dependent pathway to stimulate the transport of glucose.

**Insulin Lispro Dose-Dependent Stimulation of Amino Acid Uptake**

The nonmetabolizable amino acid MeAIB was used to assess the effect of insulin lispro on amino acid uptake in comparison with human regular insulin. Short-chain–neutral amino acids are translocated across the plasma membrane of skeletal muscle by the insulin-responsive transporter system A.25 To determine whether insulin lispro can mimic the actions of human regular insulin on system A–mediated amino acid transport, L6 myotubes were depleted of free amino acids and stimulated for 30 minutes with different concentrations of either insulin lispro or human regular insulin. Then the activity of system A was assessed as the uptake of the nonmetabolizable amino acid MeAIB. As illustrated in Figure 4A, increasing the concentration of insulin lispro or human regular insulin led to a parallel increase in MeAIB uptake. MeAIB transport into L6 myotubes was stimulated by 200 nmol insulin lispro from a basal value...
Figure 4. Insulin lispro stimulation of methylaminoisobutyric acid (MeAIB) uptake. L6 myotubes (serum starved) were incubated either (A) for 30 minutes in the presence of the indicated concentrations of insulin lispro (InsLP) or human regular insulin (HumR) or (B) with 200 nmol of either InsLP or HumR for the indicated times. MeAIB uptake was subsequently determined over a 15-minute period. Results represent the mean ± SE of three (A) or five (B) independent experiments. Each condition was replicated five times within each experiment. Where error bars are absent, the SE was too small to appear on this scale.
of 13.9 ± 0.3 pmol/min per mg protein to 19.9 ± 2.2 pmol/min per mg protein and by 200 nmol human regular insulin from a basal value of 13.3 ± 1.3 pmol/min per mg protein to 19.2 ± 1.7 pmol/min per mg protein. There was no significant difference ($P > 0.05$, unpaired $t$ test) between the insulin lispro- and human regular insulin–stimulated MeAIB uptake at any of the concentrations investigated. These results suggest that the two insulins may regulate system A by similar mechanisms.

**Time-Dependent Stimulation of Amino Acid Transport by Insulin Lispro**

Insulin lispro stimulation of amino acid transport into L6 myotubes was also time dependent. Amino acid–depleted L6 myotubes were incubated separately with 200 nmol of each of the agonists for either 30 or 60 minutes before assay of MeAIB uptake. The time dependence of MeAIB transport in response to insulin is illustrated in Figure 4B. At 30 minutes there was a 39% increase in MeAIB uptake above the basal value, and by 1 hour this stimulation had increased to 53% above the basal value. For human regular insulin, the corresponding figures were 35% and 50%, respectively. The stimulation of MeAIB transport into L6 myotubes by insulin lispro was not significantly different from that of human regular insulin at any of the time points investigated ($P > 0.05$, unpaired $t$ test).

**Effect of Insulin Lispro on Tyrosine Phosphorylation of Insulin Receptor Substrate 1 and the Association of the 85-kd Subunit of Phosphatidylinositol 3-Kinase with Insulin Receptor Substrate 1**

Following the activation of the insulin receptor tyrosine kinase and tyrosine phosphorylation of IRS-1, many signaling molecules bind via their SH2 domains to the phosphotyrosine residues of IRS-1. These include the adaptor protein Grb2, Nck, Syp, and, notably, PI 3-kinase, which is involved in mediating the rapid metabolic effects of insulin. The p85 subunit of PI 3-kinase contains two SH2 domains that facilitate its interaction with IRS-1.

To determine the effect that insulin lispro might have on tyrosine phosphorylation of IRS-1 and its association with p85, IRS-1 was immunoprecipitated from L6 myotubes treated with either insulin lispro or human regular insulin, resolved by 7.5% SDS-PAGE, and then subjected to immunoblotting with antiphosphotyrosine antibody. Insulin lispro elicited the tyrosine phosphorylation of IRS-1, with the maximal phosphorylation observed at 5 minutes and reduced at 30 minutes (Figure 5A). This phenomenon was analogous to that observed with human regular insulin. Hence, qualitatively, the time course of tyrosine phosphorylation of IRS-1 stimulated by insulin lispro was similar to that stimulated by human regular insulin.

Insulin lispro also stimulated the association of p85 with IRS-1 (Figure 5A). This was determined by immunoblotting the lower portion of the IRS-1 immunoprecipitate blot with anti-p85 antibody to evaluate the amount of p85 that coimmunoprecipitated with IRS-1. As seen in Figure 5A, the amount of p85 that coimmunoprecipitated with IRS-1 from cells treated with insulin lispro was similar to the amount that coimmunoprecipitated from cells treated with human regular insulin. The association of p85 with IRS-1 over time occurred in parallel with the tyrosine phosphorylation of IRS-1. Maximal association of p85 with IRS-1 was observed at 5 minutes, and this association...
Effect of Insulin Lispro on the Phosphorylation of Mitogen-Activated Protein Kinases ERK1 and ERK2

The mitogenic activity of insulin requires the activation of the MAPK family of serine/threonine protein kinases. In response to insulin and other growth factors, the MAPKs are phosphorylated on tyrosine as well as threonine residues and they in turn function as serine/threonine kinases. To determine the effect of insulin lispro on this pathway, the phosphorylation of two isoforms of MAPK (ERK1 and ERK2) was investigated at two time periods, 5 and 20 minutes. Cell lysates from cells treated with either insulin lispro or human regular insulin were resolved by 10% SDS-PAGE and immunoblotted with phospho-MAPK-specific antibody.
specific antibody. Insulin lispro stimulated the phosphorylation of both ERK1 and ERK2 (Figure 5B). At 5 minutes, the same degree of phosphorylation of ERK2 was induced by insulin lispro and human regular insulin. The phosphorylation state of ERK1 was not influenced by either insulin lispro or human regular insulin at this time point. At 20 minutes the phosphorylation of both ERK1 and ERK2 was stimulated by the two agonists. When the intensity of the corresponding bands was compared, it was apparent that at the 20-minute time point, insulin lispro signaled to the same extent as did human regular insulin. Qualitatively, the effect of insulin lispro on the phosphorylation of ERK1 and ERK2 was similar to that of human regular insulin at both time points investigated.

**Effect of Insulin Lispro on the SDS-PAGE Migration of p70 Ribosomal S6 Kinase**

p70 S6 Kinase is a 70-kd polypeptide that is activated in response to insulin and other growth factors and plays a crucial role in cell-cycle control, gene transcription, protein translation, and insulin-dependent biosynthesis of glucose transporter 1 protein. Activation of p70 S6 kinase is associated with multiple phosphorylation of the enzyme on serine and threonine residues, which can be monitored as the slower electrophoretic migration of a family of bands on SDS gels. This polypeptide has at least four serine/threonine phosphorylation sites, and the observed shift in electrophoretic mobility has been interpreted as an indication of the level of phosphorylation. To investigate how insulin lispro affects the p70 S6 kinase signaling pathway, we compared the retardation in electrophoretic mobility (as an indication of the level of phosphorylation) induced by insulin lispro with that induced by human regular insulin. L6 myotubes were treated with either insulin lispro or human regular insulin for 5, 10, 15, or 20 minutes. The mobility shift of p70 S6 kinase is illustrated in Figure 6A and quantified in Figure 6B. A shift from the basal position (lane 5) is evident in lanes 4 to 1 (representing the time course of human regular insulin) and from lanes 6 to 10 (representing the time course of insulin lispro). Qualitatively, the retardation in electrophoretic mobility of p70 S6 kinase induced by insulin lispro is similar to that for human regular insulin. This is confirmed in Figure 6B, which quantifies the time course of this retardation in electrophoretic mobility. To quantify the mobility shift, the basal position was assigned a value of zero, and then the mobility shift upward from the bottom of the basal band was determined using Adobe Photoshop software. From this summary of four independent experiments, it is apparent that both agonists stimulated a time-dependent decrease in the mobility of p70 S6 kinase on SDS gels. Statistical analysis confirmed that there was no significant difference ($P > 0.05$, unpaired $t$ test) between the mobility shift stimulated by insulin lispro and that stimulated by human regular insulin at any of the four time periods investigated.

**CONCLUSIONS**

Clinical trials using insulin lispro have concluded that insulin lispro provides much better glycemic control than does human regular insulin. Other recently published reports have indicated that in-
Figure 6. Time course of insulin lispro stimulation of phosphorylation of p70 ribosomal S6 kinase. L6 myotubes (serum starved) were treated with 100 nmol of insulin lispro (InsLP) or human regular insulin (HumR) for the indicated time periods, after which the cells were lysed and prepared. (A) Fifteen µg of total protein were resolved by 7.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotted with polyclonal anti-p70 S6 kinase antibodies. (B) The basal mobility was assigned a value of zero, and the mobility shift of p70 S6 kinase from stimulated L6 myotubes was measured relative to the basal value. Results are the mean ± SE of four independent experiments. B = basal.
sulin lispro can improve glycemic control in patients receiving continuous subcutaneous insulin infusion without increasing the risk of hypoglycemia,\textsuperscript{35} that it is effective in the treatment of insulin resistance resulting from human insulin antibodies,\textsuperscript{36} and that it can be successfully used in the treatment of patients with insulin resistance.\textsuperscript{37} In addition, it has been reported that there is no difference in the immunogenicity of insulin lispro or human regular insulin in patients with non-insulin-dependent diabetes mellitus or insulin-dependent diabetes mellitus. In the present study we demonstrated that insulin lispro, like human regular insulin, was effective in stimulating metabolic processes such as glucose and amino acid transport and in activating different arms of the insulin signaling pathway, namely, the PI 3-kinase/p70 S6 kinase and ERK signaling cascades. These results suggest that altering the structure of the human insulin molecule to create insulin lispro does not affect its cellular functions.

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