Effect of Insulin and Insulin-Like Growth Factor-I on Glucose Transport and Its Transporters in Soleus Muscle of Lean and Obese Mice

Y. Le Marchand-Brustel, S.J. Heydrick, D. Jullien, N. Gautier, and E. Van Obberghen

The mechanisms underlying insulin and insulin-like growth factor-I (IGF-I) action on glucose transport share similar processes leading to Glut 4 translocation after respective receptor activation. Among these steps are phosphorylation of insulin receptor substrate-1 (IRS-1) and activation of phosphatidylinositol-3-kinase (PI3-kinase). This enzyme could be involved in stimulated glucose transport in muscle, since its inhibitor, wortmannin, blocks the hormonal effect in muscle. PI3-kinase is activated by insulin and IGF-I in a rapid and transient manner in incubated soleus muscles. When PI3-kinase activation was studied in muscle of obese insulin-resistant mice, there was a marked alteration in the response to insulin both in vivo and in vitro. PI3-kinase activation by IGF-I was also altered in obese mice, although to a lesser degree.

INSULIN and insulin-like growth factor-I (IGF-I) are structurally related polypeptides that elicit a similar pattern of biological effects after binding to their respective cell surface receptors. Like their ligands, insulin receptors and IGF-I receptors are highly homologous,[1,2] consisting of heterotetrameric structures, with two α-subunits responsible for ligand binding and two β-subunits with intrinsic tyrosine kinase activity. It has long been assumed that insulin's effects are mostly metabolic, whereas those of IGF-I are growth-promoting. This is clearly an oversimplification, since IGF-I is able to exert hypoglycemic effects reflecting its stimulatory action on glucose uptake in muscles (for review, see Froesch[3]). Thus, IGF-I has recently received attention as a potential therapeutic tool in decreasing blood glucose in patients with insulin resistance.[4]

In the studies summarized here, we used the isolated mouse soleus muscle to compare insulin and IGF-I effects on glucose uptake. We particularly studied early steps in the mechanism of action of the peptides, such as stimulation of phosphatidylinositol-3-kinase (PI3-kinase). We then extended our studies to a model of insulin resistance, the mouse made obese by gold thioglucose injection. In these obese hyperglycemic mice, skeletal muscle glucose transport is insulin-resistant as a result of defects at the receptor level and in postreceptor steps. We have looked at whether those muscles were also defective in IGF-I action. We will first briefly summarize the present knowledge of the steps involved in insulin and IGF-I actions and then present some of the results obtained in muscle of lean and obese mice.

MATERIALS AND METHODS

Animals

Soleus muscles were isolated from 7- to 9-week-old male Swiss-Albino mice (Iffa-credo, Lyon, France). They were maintained at a constant temperature of 22°C on a 12-hour light cycle until the time of study and were fed ad libitum. Obesity was induced by gold thioglucose injection at 3 weeks of age as previously described,[5] and mice were used at 6 to 8 months of age.

Muscle Incubation

As previously described,[5] immediately after isolation, preincubations were performed for 15 minutes in 1 mL Krebs-Ringer bicarbonate buffer (pH 7.3) containing 1% bovine serum albumin and 2 mmol/L pyruvate. Muscles were then incubated for 5 minutes (unless otherwise indicated) with IGF-I (human recombinant IGF-I; a gift of Lilly Laboratories, Indianapolis, IN) or insulin in the same buffer. All incubations were performed at 37°C under a 95% O_2:5% CO_2 atmosphere.

Determination of PI3-kinase Activity in Immunoprecipitates

Muscles were homogenized by brief sonication in 0.5 mL lysis buffer (140 mmol/L NaCl, 10 mmol/L Tris hydrochloride, pH 8.1, 1 mmol/L $CaCl_2$, 10% glycerol, 1% Nonidet P-40, 10 μg/mL aprotinin, 50 μmol/L leupeptin, 200 μmol/L sodium vanadate, and 1 mmol/L phenylmethylsulfonyl fluoride). Solubilization was achieved by continuous stirring at 4°C. After centrifugation (12,000 × *g* for 8 minutes), the supernatant was used for protein determination and immunoprecipitation with antibodies to phosphotyrosine or insulin receptor substrate-1 (IRS-1) as described previously.[6] The antibodies were preadsorbed for 90 minutes at 4°C to protein G–sepharose (5 μL serum for 20 μL hydrated beads per sample). The beads were washed thrice with HEPES NaCl–1% Triton. The homogenate (400 μL) was added on the beads and agitated for 60 minutes at 4°C. The immune pellets were washed thrice with phosphate-buffered saline/1% Nonidet P-40/200 μmol/L sodium vanadate, twice with 0.5 mol/L LiCl/10 mmol/L HEPES (pH 7.4)/200 μmol/L sodium vanadate, and twice with 10 mmol/L Tris hydrochloride, pH 7.4/100 mmol/L NaCl/1 mmol/L EDTA/200 μmol/L sodium vanadate. PI3-kinase assay was performed directly on the immunoprecipitates. The substrate, phosphatidylinositol (10 μg per sample), was dried and sonicated in 10 mmol/L HEPES and 1 mmol/L EGTA. Five microliters of this mixture was added to the beads, and the reaction was initiated by addition of 25 μL of a reaction mixture consisting of 40 mmol/L HEPES (pH 7.5), 20 mmol/L $MgCl_2$, and 50 μmol/L [γ^{32}P]adenosine triphosphate (40 μCi). The reaction was stopped after 20 minutes by addition of 40 μL 4N HCl. The phospholipids were extracted in 160 μL chloroform:methanol (1:1 vol/vol) and spotted on a thin-layer chromatography plate precoated with potassium oxalate 1%. Following autoradiography, the band corresponding to PI3-kinase was excised, and its radioactivity was quantified by liquid scintillation counting.

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Measurement of 2-Deoxyglucose Uptake

A solution containing [^{3}H]deoxyglucose ([DOG] 0.1 mmol/L, 0.5 μCi/mL) and [^{14}C]sucrose (0.1 μCi/mL) was added in the medium for 5 minutes. The sucrose was used as a marker of extracellular space. The amount of [^{3}H]DOG and [^{14}C]sucrose taken up by the muscle was measured by scintillation counting. Protein concentration was determined by Bio-Rad assay (Fullerton, CA).

RESULTS AND DISCUSSION

Overview of the Mechanism of Insulin and IGF-I Action

The IGF-I and insulin receptor cDNA sequences are similar, with approximately 60% overall sequence homology. Despite homology of the ligands and the receptors, insulin and IGF-I bind to their noncognate receptor with a lower affinity. For example, both in intact mouse soleus muscle[7] or in partially purified receptors prepared from rat soleus muscles,[8] IGF-I is approximately 100 to 150 times less potent than insulin in displacing labeled insulin from its receptor, whereas the ability of insulin to displace IGF-I from its receptors is negligible. In both cases, the transmembrane receptor β-subunit possesses a tyrosine kinase domain, with an adenosine triphosphate binding site and regulatory sites of tyrosine autophosphorylation. For both receptors, ligand binding stimulates the β-subunit tyrosine kinase, leading to autophosphorylation, receptor conformational changes, and tyrosine phosphorylation of intracellular substrates.[9] As shown in Fig 1, these receptors phosphorylate a major endogenous substrate, namely IRS-1,[10] and the *src*-homology/collagen protein (shc).[11] IRS-1, which presents an apparent molecular weight of 165 to 180k on sodium dodecyl sulfate–polyacrylamide gel electrophoresis, contains at least 20 potential tyrosine phosphorylation sites, some of which are substrates for the insulin and IGF-I receptor kinase and then serve as docking sites for SH_2-containing proteins.[12] Thus, tyrosine phosphorylation of IRS-1 acts as a "molecular switch" to engage SH_2-containing proteins during insulin and IGF-I stimulation. These proteins include Grb2 (involved in *ras* regulation), SH-PTP2 (a protein tyrosine phosphatase), Nck, and p85α and p85β (the two isoforms of the PI3-kinase regulatory subunit). The *src*-homology/collagen protein also binds to Grb2 and activates the *ras* pathway. Apart from its tyrosine phosphorylation sites, IRS-1 contains several serine/threonine residues that are phosphorylated in response to insulin.[13] Although the exact role of these Ser/Thr phosphorylations is not known, they could be of importance in the regulation of insulin or IGF-I signaling.[14,15]

PI3-kinase catalyzes the phosphorylation of inositides on the 3-position of the *myo*-inositol ring. This enzyme consists of a 110-kd catalytic subunit (p110) and an 85-kd regulatory subunit (p85) that contains two SH_2 domains, allowing its binding to tyrosine-phosphorylated IRS-1. Both insulin and

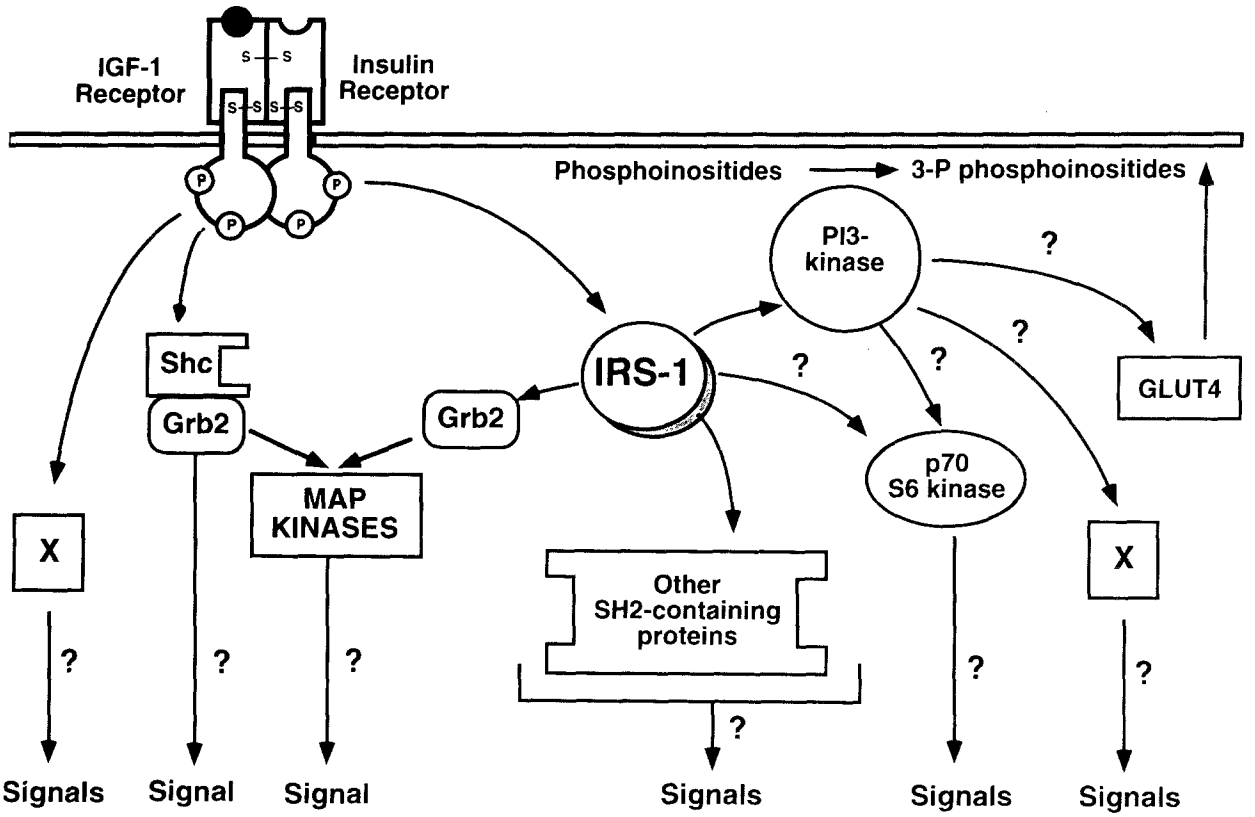

Fig 1. Schematic representation of the mechanism of insulin or IGF-I action. Insulin and IGF-I bind to their own receptors and phosphorylate IRS-1 and shc. Different proteins containing SH_2 domains, such as PI3-kinase, bind to tyrosine-phosphorylated IRS-1. The increase in glucose uptake results from translocation of Glut 4–containing vesicles from an intracellular location to plasma membranes, a step in which PI3-kinase or other molecules could be involved. Adapted from Myers et al.[12]

IGF-I activate PI3-kinase through its association of p85 with tyrosine-phosphorylated IRS-1. PI3-kinase activation had first been linked to growth factor stimulation of mitogenesis. More recently, it has been shown to be involved in insulin's metabolic effects, since its blockade by wortmannin and LY294002, two inhibitors of PI3-kinase, abolishes insulin's effect on glucose transport in adipose tissue and muscle[16,17] (see below). Lastly, insulin stimulates rapid glucose uptake into muscle and fat cells by recruiting to the cell membrane intracellular vesicles containing the glucose transporter Glut 4.[18] More recently, IGF-I has also been shown to induce the same translocation process in muscle.[8] However, the exact molecular mechanism of the translocation process is still unknown.

Effect of IGF-I and Insulin on Glucose Uptake in Soleus Muscles: Potential Role of PI3-kinase Activation in Stimulated Glucose Transport

Using isolated soleus muscles, we have shown that the maximal effect of insulin and IGF-I was similar on maximally stimulated 2-DOG uptake, an index of glucose transport and phosphorylation (Fig 2). However, the potency of IGF-I in stimulating glucose uptake was only 5% to 8% that of insulin.[7,8,19] No additive effects were observed when the two peptides were combined at maximally effective concentrations. To determine whether PI3-kinase activity was important in insulin and IGF-I action on glucose transport in muscle, wortmannin, described as an inhibitor of the enzyme, was used. As shown in Table 1, insulin's effect on glucose transport was blocked by addition of the inhibitor. In a similar fashion, wortmannin also blocked the effect of IGF-I (data not shown). These results obtained in muscle are in accordance with results observed in freshly isolated adipocytes or in 3T3-L1 adipocytes, in which wortmannin and LY294002, a second inhibitor of PI3-kinase, blocked insulin-induced glucose transport. Thus, PI3-kinase is likely to be important in insulin- and IGF-I–induced glucose transport.

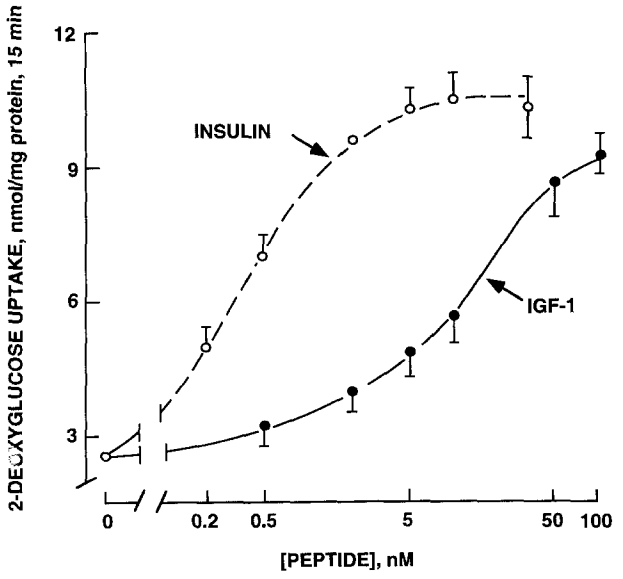

Fig 2. Insulin and IGF-I effects on glucose uptake in soleus muscles. Values are the mean ± SEM for 6 muscles. Reprinted with permission from *Endocrinology* 105:723-730, 1979.[7] ©The Endocrine Society.

Table 1. Effect of Wortmannin on Insulin-Stimulated Glucose Transport in Muscles

Incubation Condition	DOG Uptake (nmol/mg protein)	
	Basal	Insulin-Stimulated
Control	0.36 ± 0.04	1.03 ± 0.08
Wortmannin 1 μmol/L	0.33 ± 0.04	0.63 ± 0.08
Wortmannin 5 μmol/L	0.26 ± 0.03	0.32 ± 0.04

NOTE. Values are the mean ± SEM of 4 to 8 muscles.

Effect of Insulin and IGF-I on PI3-kinase Activation in Soleus Muscle of Lean and Obese Mice

To study the effect of insulin on the activation of PI3-kinase, isolated soleus muscles were incubated with insulin for various lengths of time, muscles were homogenized, and PI3-kinase activity was then determined in immunoprecipitates obtained with antibodies to phosphotyrosine. As shown in Fig 3, with a maximally effective dose of insulin (50 nmol/L), anti–P-Tyr-PI3-kinase was maximally stimulated within 3 to 5 minutes. This initial rapid activation was followed by a partial deactivation. With a more physiological insulin concentration, 2 nmol/L, stimulation of anti–P-Tyr-PI3-kinase was less important and occurred more slowly, peaking at 30 minutes. IGF-I stimulation was

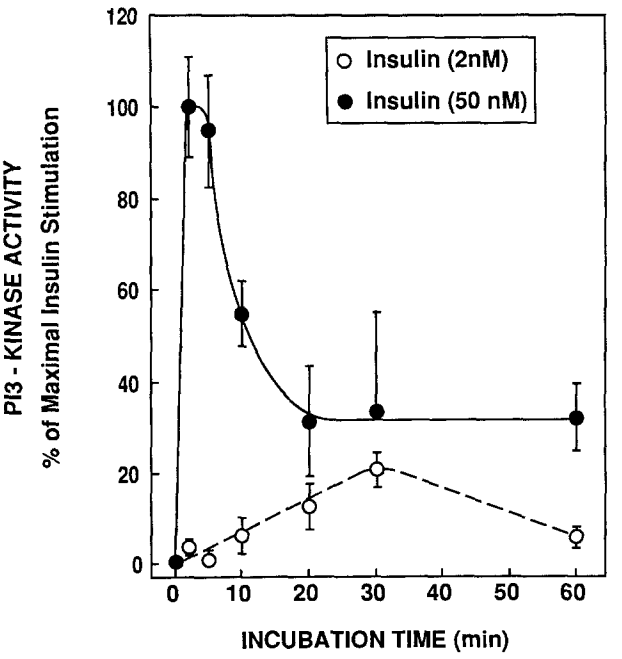

Fig 3. Time course of insulin stimulation of PI3-kinase activity in soleus muscles incubated in the presence of 2 or 50 nmol/L insulin for various periods of time as indicated. PI3-kinase activity was assayed in antiphosphotyrosine immunoprecipitates. Results are presented as a percentage of 50-nmol/L insulin stimulation of PI3-kinase activity at 2 minutes (ie, insulin minus basal). Results are the mean ± SEM from determinations in 4 to 10 muscles.

also transient (data not shown). Although the mechanism of the deactivation process is not known, it could be proposed that IRS-1 serine/threonine phosphorylation is involved. Compatible with this view, we have recently shown that following treatment of cells with okadaic acid, a serine/threonine phosphatase inhibitor, IRS-1 becomes hyperphosphorylated on Ser/Thr and less able to be tyrosine-phosphorylated and to bind PI3-kinase.[15]

We then studied PI3-kinase activation in muscle of obese insulin-resistant mice, both in vivo and in vitro. The model used is the mouse made obese and insulin-resistant following gold thioglucose injection. This substance destroys part of the hypothalamus and induces hyperphagia. Mice become obese and insulin resistance develops, particularly in skeletal muscle.[5,20] When DOG uptake is measured in soleus muscle from these mice, there is a diminished response to insulin, resulting from defects at both the receptor level and postreceptor steps. Indeed, we have shown that tyrosine kinase activity of insulin receptors prepared from obese insulin-resistant mice was defective.[21] However, there are also alterations at postreceptor levels, since the stimulation of glucose uptake is abnormal in response to other stimuli such as anoxia.[22] When IGF-I–stimulated glucose uptake was measured in muscle of gold thioglucose-obese mice, the maximal response to IGF-I was altered in a manner similar to the response to insulin.[7] This was not due to a decrease in the number of IGF-I receptors, since labeled IGF-I binding was similar in muscles of obese and lean mice.[7] Similarly, IGF-I receptor binding and tyrosine kinase activity were found to be normal in skeletal muscles of genetically obese mice[23] and of obese insulin-resistant patients in whom a decrease in insulin receptor number and kinase activity was found.[24] However, although the number and function of IGF-I receptors are not altered in obesity and diabetes, a similar alteration of IGF-I– and insulin-stimulated glucose uptake is observed in both obese rodents[7,25] and humans.[26]

The key step in both insulin and IGF-I action on glucose transport consists of the translocation of Glut 4 glucose transporters from an intracellular pool to the plasma membrane.[8,27] A decreased glucose transport in response to insulin and IGF-I could be the consequence of a decreased expression of the transporter. This is not the case, since the total Glut 4 transporter number is not altered in muscle from obese or diabetic subjects.[28-31] This result indicates that the defective glucose transport observed in the insulin-resistant state linked to obesity results from a defect in the translocation process rather than in Glut 4 expression.[32]

Therefore, we studied the ability of insulin to increase PI3-kinase activity in obese mice. As shown in Fig 4, PI3-kinase activity increased to a much lower extent in obese mice than in lean controls when insulin was administered in vivo or when soleus muscles were incubated with insulin in vitro. Parallel results were obtained when PI3-kinase activity was measured in pellets obtained with antibodies to phosphotyrosine (Fig 4) or to IRS-1 (data not shown).[6,33] This result is not surprising, since PI3-kinase activation by insulin occurs as a result of the interaction of the p85 subunit with tyrosine-phosphorylated IRS-1. This defect is partly the consequence of the decreased tyrosine kinase activity of the insulin receptor, but is also the consequence of alterations independent of the receptor. Indeed, in reconstitution experiments, the ability of normal insulin receptors to activate PI3-kinase was lower in obese mice than in lean controls.[6] This indicates that there is a defective step at the level of either IRS-1 or PI3-kinase. Since levels of these proteins are unchanged in obese mice compared with lean, a possibility is that IRS-1 is less able to be tyrosine-phosphorylated by insulin receptors, perhaps as the consequence of an increased serine/threonine phosphorylation. When IGF-I was used to activate PI3-kinase, the defects in obese mice were comparable to those observed with insulin but less pronounced (Y. Le Marchand-Brustel, et al, unpublished results, January 1995). These results

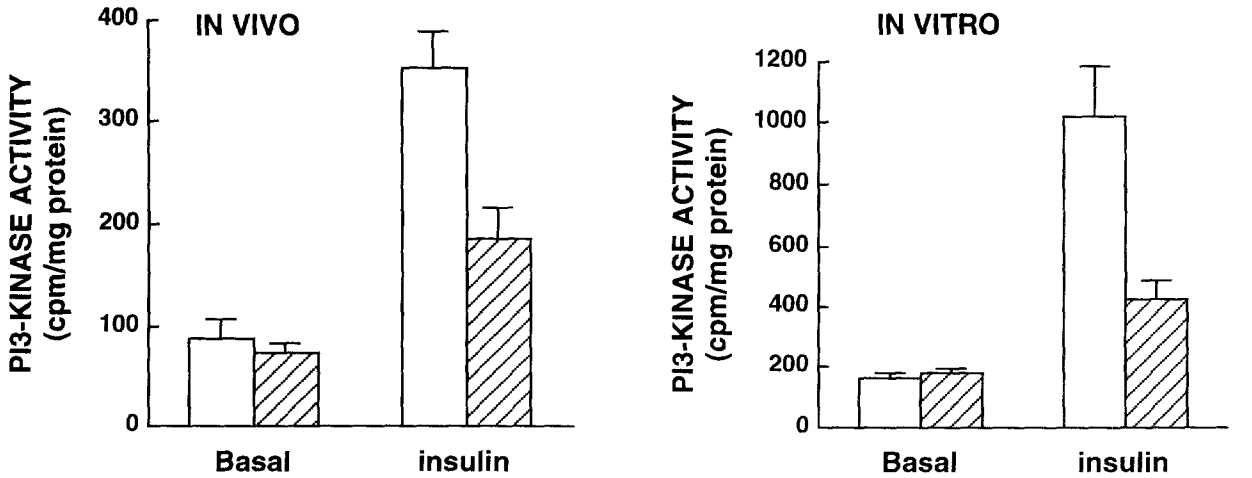

Fig 4. Effects of obesity on insulin-stimulated PI3-kinase activity in soleus muscles in vivo or in vitro. For in vivo experiments, anesthetized mice (lean [□] or gold thioglucose–obese [▨]) were injected with isotonic NaCl solution (140 mmol/L) or insulin (0.5 U/kg) 5 minutes before soleus muscles were removed. Soleus muscles were incubated in vitro for 2 minutes without or with insulin (50 nmol/L). PI3-kinase activity was then determined in antiphosphotyrosine immunoprecipitates. Results are expressed as cpm of ^{32}P incorporated in PIP/mg muscle protein. Values are the mean ± SEM of 4 to 8 muscles.

indicate than PI3-kinase in response to insulin is defective as the consequence of both receptor and postreceptor defects, whereas in response to IGF-I, only postreceptor defects are responsible for decreased activation of PI3-kinase.

In conclusion, our results indicate that both insulin and IGF-I are able to stimulate glucose transport and to activate PI3-kinase. The exact role of PI3-kinase in Glut 4 translocation is still not known, but different observations argue for an implication. First, inhibitors of PI3-kinase block insulin-induced glucose transport, although their specificity can be questioned, since inhibition of mitogen-activated protein kinase by wortmannin has been described.[17] Second, the PI3-kinase p110 presents homology with Vps 34, a protein that appears to be critical for vesical trafficking in yeast.[34,35] Third, the observation that insulin rapidly activates PI3-kinase in its main target tissues, adipose tissue and muscle,[6,36,37] is suggestive of an important role for the enzyme in insulin action. However, PI3-kinase is also activated in liver, where glucose transport is not regulated by insulin.[38] Finally, glucose transport and PI3-kinase activation are affected in obese insulin-resistant mice, in response to both insulin and IGF-I.

Our studies summarized here clearly illustrate that the effects of insulin and IGF-I on the recently identified signaling molecule, PI3-kinase, are indistinguishable, at least by the available technology. Drug discovery and design relating to insulin resistance should aim to find agents targeting the postreceptor defects, or to search for substances acting on the IGF-I receptor, since responses to IGF-I are altered less than responses to insulin.

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