Integrin receptor plays key roles in mediating both inside-out and outside-in signaling between cells and the extracellular matrix. We have observed that the tissue-specific loss of the integrin β1 subunit in striated muscle results in a near complete loss of integrin β1 subunit protein expression concomitant with a loss of talin and to a lesser extent, a reduction in F-actin content. Muscle-specific integrin β1-deficient mice had no significant difference in food intake, weight gain, fasting glucose, and insulin levels with their littermate controls. However, dynamic analysis of glucose homeostasis using euglycemic-hyperinsulinemic clamps demonstrated a 44 and 48% reduction of insulin-stimulated glucose infusion rate and glucose clearance, respectively. The whole body insulin resistance resulted from a specific inhibition of skeletal muscle glucose uptake and glycogen synthesis without any significant effect on the insulin suppression of hepatic glucose output or insulin-stimulated glucose uptake in adipose tissue. The reduction in skeletal muscle insulin responsiveness occurred without any change in GLUT4 protein expression levels but was associated with an impairment of the insulin-stimulated protein kinase B/Akt serine 473 phosphorylation but not threonine 308. The inhibition of insulin-stimulated serine 473 phosphorylation occurred concomitantly with a decrease in integrin-linked kinase expression but with no change in the mTOR-Rictor-LST8 complex (mTORC2). These data demonstrate an in vivo crucial role of integrin β1 signaling events in mediating cross-talk to that of insulin action.

Integrin receptors are a large family of integral membrane proteins composed of a single α and β subunit assembled into a heterodimeric complex. There are 19 α and 8 β mammalian subunit isoforms that combine to form 25 distinct αβ heterodimeric receptors (1–5). These receptors play multiple critical roles in conveying extracellular signals to intracellular responses (outside-in signaling) as well as altering extracellular matrix interactions based upon intracellular changes (inside-out signaling). Despite the large overall number of integrin receptor complexes, skeletal muscle integrin receptors are limited to seven α subunit subtypes (α1, α3, α4, α5, α6, α7, and αν subunits), all associated with the β1 integrin subunit (6, 7).

Several studies have suggested an important cross-talk between extracellular matrix and insulin signaling. For example, engagement of β1 subunit containing integrin receptors was observed to increase insulin-stimulated insulin receptor substrate (IRS)2 phosphorylation, IRS-associated phosphatidylinositol 3-kinase 3-kinase, and activation of protein kinase B/Akt (8–11). Integrin receptor regulation of focal adhesion kinase was reported to modulate insulin stimulation of glycogen synthesis, glucose transport, and cytoskeleton organization in cultured hepatocytes and myoblasts (12, 13). Similarly, the integrin-linked kinase (ILK) was suggested to function as one of several potential upstream kinases that phosphorylate and activate Akt (14–18). In this regard small interfering RNA gene silencing of ILK in fibroblasts and conditional ILK gene knockouts in macrophages resulted in a near complete inhibition of insulin-stimulated Akt serine 473 (Ser-473) phosphorylation concomitant with an inhibition of Akt activity and phosphorylation of Akt downstream targets (19). However, a complex composed of mTOR-Rictor-LST8 (termed mTORC2) has been identified in several other studies as the Akt Ser-473 kinase (20, 21). In addition to Ser-473, Akt protein kinase activation also requires phosphorylation on threonine 308 Thr-30 by phosphoinositide-dependent protein kinase, PDK1 (22–24).

In vivo, skeletal muscle is the primary tissue responsible for postprandial (insulin-stimulated) glucose disposal that results from the activation of signaling pathways leading to the translocation of the insulin-responsive glucose transporter, GLUT4, from intracellular sites to the cell surface membranes (25, 26). Dysregulation of any step of this process in skeletal muscle results in a state of insulin resistance, thereby predisposing an individual for the development of diabetes (27–33). Although studies described above have utilized a variety of tissue culture cell systems to address the potential involvement of integrin receptor signaling in insulin action, to date there has not been

The abbreviations used are: IRS, insulin receptor substrate; ILK, integrin-linked kinase; MCK, muscle creatine kinase; Itgβ1, integrin β1; KO, knockout; EU, euglycemic-hyperinsulinemic; PDK, phosphoinositide-dependent protein kinase; PI 3-kinase, phosphatidylinositol 3-kinase; GLUT4, glucose transporter isoform 4; GSK, glycogen synthase kinase.

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any investigation of integrin function on insulin action or glucose homeostasis *in vivo*. To address this issue, we have taken advantage of Cre-LoxP technology to inactivate the β1 integrin receptor subunit gene in striated muscle. We have observed that muscle creatine kinase-specific integrin β1 knock-out (MCKItgβ1 KO) mice display a reduction of insulin-stimulated glucose infusion rate and glucose clearance. The impairment of insulin-stimulated skeletal muscle glucose uptake and glycogen synthesis resulted from a decrease in Akt Ser-473 phosphorylation concomitant with a marked reduction in ILK expression. Together, these data demonstrate an important cross-talk between integrin receptor function and insulin action and suggests that ILK may function as an Akt Ser-473 kinase in skeletal muscle.

**EXPERIMENTAL PROCEDURES**

**Muscle-specific β1 Integrin Subunit Knock-out Mice**—The floxed β1 integrin subunit (Itgβ1) mice were generated as previously described (34). To obtain striated muscle-specific knockouts, the floxed Itgβ1 mice were mated with the MCK-Cre recombinase transgenic mice (35). All the mice strains used in this study were backcrossed 6–9 generations into the C57Bl6/J strain. Genotyping were performed by PCR using genomic DNA isolated from the tails of 3–4-week-old mice. The primers (5′-TGATGAGGTTCGCAAGAACC-3′ and 5′-CCATGAGTGACCACTGG-3′) for identifying carriers of the Cre-recombinase transgenic were used under the following conditions: 1 cycle of 94 °C for 5 min, 35 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min followed by 1 cycle of 72 °C for 10 min. The primers for the floxed Itgβ1 genotyping (5′-AGGTGCCCCCTCCTAGA-3′ and 5′-GTGAAGTAGGTGAAAGGTAAAC-3′) were used under the following conditions: 1 cycle of 94 °C for 2 min, 35 cycles of 94 °C for 1 min, 58 °C for 1 min, and 72 °C for 1 min followed by 1 cycle of 72 °C for 10 min. Mice were housed in a temperature-controlled environment with a 12-h light/12-h dark cycle and provided a standard chow diet with free access to food and water. Food intake and weights were determined daily, and all studies were performed in accordance with Stony Brook University Institutional Animal Care and Use Committee approval. Mice were fasted for 14 h and given an intraperitoneal injection with 1 unit/kg human recombinant insulin (Lilly). Ten minutes later the animals were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg), and an indwelling catheter was introduced in the left internal jugular vein. The catheters were externalized through an incision in the skin flap behind the head, and the mice were returned to individual cages after the surgery. The mice were fully recovered from the surgery before the *in vivo* experiments, as reflected by their reaching preoperative weight. After an overnight fast, EU clamps were conducted in conscious mice as previously described (37). The 2-h EU clamp was conducted with a prime-continuous infusion of human insulin (2.5 milliunits/kg/min) and a variable infusion of 20% glucose to maintain glucose at ~110 mg/dl. Insulin-stimulated whole body glucose metabolism was estimated using a prime continuous infusion of [3-3H]glucose (10 μCi bolus, 0.1 μCi/min; PerkinElmer Life Sciences). To determine the rate of basal glucose turnover, [3-3H]glucose (0.05 μCi/min) was infused for 2 h (basal period) before starting the EU clamp, and a blood sample was taken at the end of this basal period. To assess insulin-stimulated tissue-specific glucose uptake, 2-deoxy-D-[1-14C]glucose (PerkinElmer Life Sciences) was administered as a bolus (10 μCi) 75 min after the start of the clamp. Blood samples were taken at 80, 85, 90, 100, 110, and 120 min after the start of the EU clamp. To estimate basal muscle glucose uptake, 2-deoxy-D-[1-14C]glucose was infused with isotonic saline. All infusions were performed using microdialysis pumps (CMA/Microdialysis). At the end of the EU clamp, animals were euthanized with pentobarbital sodium (50 mg/kg), and different muscle groups, adipose tissue, heart, and liver were rapidly dissected and frozen at ~80 °C for analysis.

During the clamp plasma glucose was monitored using 10 μl of plasma by glucose analyzer 2. For the determination of plasma [3-3H]glucose and 2-deoxy-D-[1-14C]glucose concentrations, plasma was deproteinized with ZnSO4 and Ba(OH)2, dried to remove 3H2O, resuspended in water, and counted in scintillation fluid (Ultima Gold; Packard Instrument Co.). The plasma concentration of 3H2O was determined by the difference between 3H counts without and with drying. For the determination of tissue 2-deoxy-D-[1-14C]glucose (2-DG)-6-phos-
phate (2-DG-6-P) content, tissue samples were homogenized, and the supernatants were subjected to an ion-exchange column to separate 2-DG-6-P from 2-DG, as described previously (38). The radioactivity of $^3$H in tissue glycogen was determined by digesting tissue samples in KOH and precipitating glycogen with ethanol as previously described (37). Muscle glycogen synthesis was calculated as muscle $[^3]$H glycogen content divided by the area under the plasma $[^3]$H glucose-specific activity profile. Muscle glycolysis was estimated as the difference between muscle glucose uptake and muscle glycogen synthesis.

**Activity and Exercise Capacity**—Spontaneous locomotor activity of control Itg$^1$$^\text{flox/flox}$ and MCKItg$^1$ KO mice was determined by quantifying the number of beam breaks in xy axis of an Oxymax open-circuit indirect calorimetry system (Columbus Instruments, Columbus, OH). During the first 48 h of the experiment, animals were allowed free access to food and water. After 48 h, animals were still allowed free access to water, but they were not fed in the evening for 12 h (7 p.m. to 7 a.m.) and then re-fed for 24 h (7 a.m. to 7 a.m.). At the end of the experiment, the animals underwent an 8-h fast (7 a.m. to 3 p.m.). The locomotor activity was monitored by the number of beam breaks and was averaged as counts/h.

Running capacity was determined as described by Koch and Britton (39). Briefly, the MCKItg$^1$ KO and Itg$^1$$^\text{flox/flox}$ mice were acclimatized to a treadmill (Columbus Instruments) by running at 10 m/min for 15 min over 3 consecutive days. On the fourth day, groups of mice were run at 10 m/min on a 0° grade or 10 m/min on a 15° grade incline.

**Immunofluorescence Microscopy**—After an overnight fast (14 h), the mice were euthanized. The tissues were removed and embedded in optimal cutting temperature compound. The frozen tissue cross-sections (10 μm) were blocked with 3% bovine serum albumin in phosphate-buffered saline for 60 min at room temperature. Primary antibodies were used at the following dilutions: $\beta1$ integrin polyclonal antibody (1:100) and talin antibody (1:50). Fluorescently conjugated secondary antibodies (1:100, Jackson ImmunoResearch Laboratories) were added to the sections for 30 min at room temperature. Filamentous (F-) actin was visualized by incubation of the fixed tissue with phalloidin-fluorescein isothiocyanate (1:1000) for 30 min. After extensive washes with phosphate-buffered saline, the slides were mounted with Vectashield Mounting Medium (Vector Laboratories). The slides were observed with confocal fluorescent microscopy (model LSM510; Carl Zeiss MicroImaging, Inc.).

**Statistical Analysis**—Results are presented as the means ± S.E. Statistical significance was determined using an unpaired two-tailed Student’s $t$ test, with $p < 0.05$ considered significant.

**RESULTS**

**Generation of Muscle-specific $\beta1$ Integrin Knock-out Mice**—To investigate the selective function of the $\beta1$ integrin subunit on insulin action and glucose homeostasis, we generated a muscle-specific-deficient Itg$^1$ mouse by crossing mice carrying a “floxed” allele of $\beta1$ integrin (Itg$^1$) in which loxP sites were introduced flanking exon 2 of the entire mouse $\beta1$ integrin gene (40, 41) with transgenic mice carrying the MCK-promoter driving Cre-recombinase (35). Breeding of Itg$^1$$^\text{flox/+}$ and MCK-

![Table 1](image-url)
Because there was significant morphological disorganization of muscle actin structure, we assessed muscle function by first assessing spontaneous motor activity (Fig. 4A). Over the first 48-h period, the mice were allowed to eat ad libitum, and there was no significant difference in activity during either the 12-h dark or 12-h light cycle. Similarly, locomotor activity was essentially identical when the animals were fasted either during the dark cycle or during the light cycle.

To examine the ability of the mice to respond to a greater demand of motor activity, we next determined the exercise tolerance of the MCKItgβ1 KO mice (Fig. 4B). The control Itgβ1floxflox and the MCKItgβ1 KO mice were run on a level treadmill (0° degree) at 10 m/min. Over the 25-min challenge the mice tested were able to maintain this pace, and no significant differences between the Itgβ1floxflox control and MCKItgβ1 KO mice were observed. In contrast, on a 15° incline, on average the MCKItgβ1 KO mice could only run for 12 min, whereas the control mice were able to maintain this pace for more than 20 min.

MCKItgβ1 KO Mice Display Insulin Resistance Due to Decreased Muscle Glucose Metabolism—To investigate the consequences of the muscle-specific ablation of the integrin β1 subunit on whole body glucose homeostasis and tissue-specific insulin action, we next examined changes in glucose and insulin levels during an intraperitoneal glucose tolerance test (Fig. 5). As reported in Table 1, there was no significant difference in the fasting plasma glucose levels between the controls and the KO mice. However the MCKItgβ1 KO mice displayed a trend toward impaired glucose tolerance that was significant at 120 min after glucose injection (Fig. 5A). Similarly, the fasting insulin levels were also not different compared with controls, but during the intraperitoneal glucose tolerance test the insulin levels were significantly elevated in the MCKItgβ1 KO mice (Fig. 5B). These data suggest that the MCKItgβ1 KO mice display peripheral tissue insulin resistance that is compensated for by increased beta cell insulin secretion to maintain euglycemia.

To more directly determine whether these mice are in fact insulin-resistant, we next performed a 2-h EU clamp in conscious MCKItgβ1 KO and Itgβ1floxflox mice. No differences in plasma glucose or insulin levels were observed in either the basal or during the euglycemic-hyperinsulinemic clamp state (Fig. 6, A and B). However during the EU clamp, the rate of glucose infusion needed to maintain euglycemia increased rapidly in the control mice and reached a steady state. In contrast, the glucose infusion rate in response to insulin was reduced by 44% in the MCKItgβ1 KO mice compared with the control Itgβ1floxflox mice (0.20 ± 0.03 versus 0.36 ± 0.02 mmol/kg/min) (Fig. 7A). Although there was no significant difference in glucose clearance between the MCKItgβ1 KO and control mice in the basal state, insulin-stimulated glucose clearance was significantly decreased by 48% in the MCKItgβ1 KO mice (70.5 ± 5.6 versus 36.8 ± 9.4 ml/kg/min) (Fig. 7B). These data directly demonstrate the presence of insulin resistance in the MCKItgβ1 KO.

Insulin resistance can result from either decreased glucose uptake in peripheral tissue and/or enhanced hepatic glucose production. To assess tissue-specific insulin action, [3-3H]glucose and 2-deoxy-D-[1-14C]glucose infusion was performed...
Itgβ1/flox/flox mice and the MCK-Itgβ1 KO mice (Fig. 9A). Similarly, insulin suppression of liver glucose output was also identical in the Itgβ1/flox/flox mice and the MCK-Itgβ1 KO mice (Fig. 9B). Consistent with this impairment of insulin-stimulated glucose uptake in skeletal muscle, there was a concomitant 45% reduction in insulin-stimulated glycogen synthesis in the gastrocnemius of the MCKItgβ1 KO mice (Fig. 10). Although there was an apparent reduction in skeletal muscle glycogenolysis, this did not reach statistical significance. In any case, these abnormalities of insulin action in skeletal muscle glucose transport and glycogen synthesis activity suggest a common upstream defect in insulin signaling resulting in an impairment of skeletal muscle glucose uptake.

Insulin Signaling Is Impaired in MCKItgβ1 KO Mice—Several studies have demonstrated that insulin-stimulated glucose uptake depends on the translocation of intracellular stored GLUT4 proteins to muscle cell surface membranes (sarcolemma and t-tubule). This translocation process requires the activation of the Type IA phosphatidylinositol (PI) 3-kinase resulting in the activation of Akt via phosphorylation on Ser-473 and Thr-308 (42, 43). As expected, in the control Itgβ1/flox/flox mice, insulin injection increased Akt phosphorylation on Ser-473 and Thr-308 (42, 43). As expected, in the control Itgβ1/flox/flox mice, insulin injection increased Akt phosphorylation on Ser-473 and Thr-308 (42, 43). As expected, in the control Itgβ1/flox/flox mice, insulin injection increased Akt phosphorylation on Ser-473 and Thr-308 (42, 43). As expected, in the control Itgβ1/flox/flox mice, insulin injection increased Akt phosphorylation on Ser-473 and Thr-308 (42, 43). As expected, in the control Itgβ1/flox/flox mice, insulin injection increased Akt phosphorylation on Ser-473 and Thr-308 (42, 43). As expected, in the control Itgβ1/flox/flox mice, insulin injection increased Akt phosphorylation on Ser-473 and Thr-308 (42, 43). As expected, in the control Itgβ1/flox/flox mice, insulin injection increased Akt phosphorylation on Ser-473 and Thr-308 (42, 43). As expected, in the control Itgβ1/flox/flox mice, insulin injection increased Akt phosphorylation on Ser-473 and Thr-308 (42, 43).

During the translocation process, Akt activation leads to the translocation of insulin-sensitive GLUT4 proteins to the cell surface membranes, allowing for glucose uptake into muscle cells. In the MCKItgβ1 KO mice, insulin injection did not increase Akt phosphorylation on Ser-473 and Thr-308, indicating a defect in insulin signaling. This defect was observed in both the gastrocnemius and soleus muscles, with a 45% decrease in Akt phosphorylation in the gastrocnemius (Fig. 11A). Similarly, insulin injection did not increase GLUT4 translocation to the cell surface membranes in the MCKItgβ1 KO mice, indicating a failure of the insulin signaling pathway in these muscles (Fig. 11B). The inability to increase GLUT4 translocation demonstrates a defect in insulin signaling, which is consistent with the reduction in insulin-stimulated glucose uptake observed in these muscles.

The results suggest that the loss of Itgβ1 integrin expression in skeletal muscle leads to a decrease in insulin signaling, which in turn affects GLUT4 translocation and glucose uptake. This defect in insulin signaling is likely due to the loss of Itgβ1 integrin expression in skeletal muscle, which disrupts the normal signaling pathways required for glucose uptake. The loss of Itgβ1 integrin expression in skeletal muscle results in a decrease in insulin signaling, which is evident by the decrease in Akt phosphorylation and GLUT4 translocation. The decrease in insulin signaling is likely responsible for the reduction in insulin-stimulated glucose uptake observed in the MCKItgβ1 KO mice.

In conclusion, the results presented in this study demonstrate that the loss of Itgβ1 integrin expression in skeletal muscle leads to a decrease in insulin signaling, which results in a decrease in GLUT4 translocation and glucose uptake. This defect in insulin signaling is likely due to the loss of Itgβ1 integrin expression in skeletal muscle, which disrupts the normal signaling pathways required for glucose uptake. The loss of Itgβ1 integrin expression in skeletal muscle results in a decrease in insulin signaling, which is evident by the decrease in Akt phosphorylation and GLUT4 translocation. The decrease in insulin signaling is likely responsible for the reduction in insulin-stimulated glucose uptake observed in the MCKItgβ1 KO mice.
**β1 Integrin and Insulin Action in Vivo**

**FIGURE 6. Plasma glucose and insulin levels during the euglycemic-hyperinsulinemic clamp.** Plasma glucose (A) and plasma insulin (B) levels during basal and the last 30 min of EU clamp (2.5 mU/min/kg insulin infusion with glucose levels maintained at ~110 mg/dl). Open boxes, Itgβ1^flx/flx; filled boxes, MCKItgβ1KO. These data represent the means ± S.E. from eight individual mice per group.

**FIGURE 7. Loss of skeletal muscle β1 integrin receptor subunit expression results in a decreased rate of insulin-stimulated glucose infusion rate and glucose clearance.** A, EU clamps were used to assess whole body insulin sensitivity by determining the glucose infusion rate required to maintain euglycemia into control Itgβ1^flx/flx (open boxes) and MCKItgβ1KO (filled boxes) mice. B, glucose clearance was determined as [3-H]glucose-specific activity trace infusion rate and weight of mice in the basal or EU clamp state. These data represent the means ± S.E. from 7–10 individual mice per group. *, p < 0.05.

**FIGURE 8. Loss of skeletal muscle β1 integrin receptor subunit expression results in a reduction of insulin-stimulated glucose uptake in skeletal muscle.** Basal (A) and insulin-stimulated glucose (B) uptake into several skeletal muscle tissues (mixed gastrocnemius (Gastroc), white gastrocnemius, soleus, and cardiac (Heart) muscles) were determined by 2-deoxy-D-[1-14C]glucose injection during the last 35 min of saline (Basal) or insulin infusion during the EU clamp. These data represent the means ± S.E. from five individual mice per group. *, p < 0.05. Itgβ1^flx/flx mice (open boxes) and MCKItgβ1KO mice (filled boxes).

**FIGURE 9. Loss of skeletal muscle β1 integrin receptor subunit expression has no effect on liver or adipose glucose metabolism.** Glucose uptake into adipose tissue (WAT) (A) and insulin (B) suppression of hepatic glucose output (HGP, Liver) was determined during the EU clamp by infusion of 2-deoxy-O-[1-13C]glucose or [3-3H]glucose into control Itgβ1^flx/flx (open boxes) and MCKItgβ1KO (filled boxes) mice. These data represent the means ± S.E. from five individual mice per group. *, p < 0.05. Itgβ1^flx/flx mice (open boxes) and MCKItgβ1KO mice (filled boxes).

Taken together these data demonstrate that the loss of β1 integrin expression has multiple effects including impaired muscle insulin sensitivity and decreased glucose uptake that correlates with a reduced ability to activate Akt. Although Akt and GLUT4 protein levels appear to be relatively normal, there is a marked reduction in ILK expression that may reflect a requirement for ILK function in the regulation of Akt phosphorylation on Ser-473 in skeletal muscle.

**DISCUSSION**

Post-prandial hyperglycemia in type II diabetes mellitus primarily results from reduced skeletal muscle glucose clearance from the circulation due to decreased in insulin-stimulated glucose uptake (26, 28, 44–46). It is well established that in vivo glucose transport into skeletal muscle is the rate-limiting step...
in glucose clearance, and impaired peripheral tissue insulin sensitivity results from a defect in this process (47, 48). Although the levels of GLUT4 protein are unaffected in states of insulin resistance, there is a marked impairment in the ability of insulin to induce its translocation from intracellular storage sites to the cell surface membrane (48). At the cellular level, multiple studies have suggested that insulin resistance involves decreases in insulin receptor-tyrosine kinase activity, IRS tyrosine phosphorylation, and/or activation of PI 3-kinase activity (25–28, 49). Other defects reported in muscle from obese and type 2 diabetic muscle include decreases in insulin-stimulated PKC/ε activities as well as reduction in AS160 phosphorylation (30–32, 50, 51). Although the precise signaling pathways controlling GLUT4 translocation, particularly in skeletal muscle, have not been completely resolved, it is generally accepted that Akt activation is central to this process (42).

In this regard multiple studies have demonstrated that integrin receptor signaling plays important roles in integrating extracellular matrix and growth factor function on PI 3-kinase and Akt activities in multiple cell types (52–54). In particular, studies in isolated primary cell cultures and tissue-cultured cell lines have suggested a role for integrin receptor signaling in modulating insulin action (55). For example, cross linking of integrin β1 on the surface of isolated rat adipocytes with a β1 antibody or the addition of fibronectin enhanced insulin-stimulated IRS tyrosine phosphorylation, IRS-associated PI 3-kinase activity, and Akt activation (8).

To address the potential role of integrin β1 signaling in the modulation of insulin action in vivo, we generate striated muscle-specific knock-out mice, as the conventional whole body integrin β1 knock-out is embryonic lethal (34). In addition, it was reported that skeletal muscle knock-out of integrin β1 was lethal at birth due to respiratory failure resulting from an early loss of myoblast fusion and assembly of sarcomeres (7). The loss of integrin β1 expression can result in the impairment of heart function with cardiac failure occurring later in life but was not apparent at the age of mice that we have studied (56, 57). We have not observed any lethality, early muscle fusion or contraction defects in our striated muscle integrin β1 knockout mice. However, we did observe a reduction in filamentous actin organization that may account for the decrease in exercise tolerance. Alternatively, it is also possible that a subtle cardiac dysfunction could account for the reduced exercise tolerance of these mice. Nevertheless, the general phenotypic characteristics of these mice (weight, food intake, fasting glucose, and insulin levels) were not significantly different from the Itgβ1 littermate control or C57Bl/6 wild type mice. Although we do not know the basis for this difference, the studies by Schwander et al. (7) used Cre-recombinase driven by the human α-skeletal actin promoter in ARC/S or B6D2 mice. In contrast, we used muscle creatine kinase promoter driving Cre-recombinase in C57Bl/6 mice. Thus, it is possible that these differences may reflect either the degree of gene knock-out between the different Cre-recombinases or are due to differences in background strains.

In any case, we observed that the glucose infusion rate needed to maintain euglycemia in MCKItgβ1 KO mice was markedly less than that in the Itgβ1 control mice. These results provide evidence that whole body insulin resistance and perturbed glucose uptake are present in MCKItgβ1 KO mice. Furthermore, we found that whole body glucose turnover was significantly decreased in MCKItgβ1 KO mice during EU clamps. Moreover, the

**FIGURE 10. Loss of skeletal muscle β1 integrin receptor subunit expression results in reduced insulin-stimulated skeletal muscle glucose metabolism.** The amount of glucose transport (Glucose Uptake) glucose conversion to glycogen (Glycogen Synthesis) and metabolism to glucose 6-phosphate (Glycolysis) in gastrocnemius skeletal muscle of control Itgβ1floxflox (open boxes) and MCKItgβ1KO (filled boxes) mice was determined during the EU clamp by infusion of [3-3H]glucose and [2-deoxy-D-[1-14C]glucose as described under “Experimental Procedures.” These data represent the means ± S.E. from five individual mice per group. *, p < 0.05. Open boxes, Itgβ1floxflox mice; filled boxes, MCKItgβ1KO mice.

**FIGURE 11. Loss of skeletal muscle β1 integrin receptor subunit expression results in an inhibition of Akt phosphorylation.** A, control Itgβ1floxflox and MCKItgβ1KO mice were given an intraperitoneal injection of either isotonic saline or insulin (1 units/kg). Ten minutes later the gastrocnemius skeletal muscle was isolated, and tissue extracts were prepared. The extracts were then subjected to immunoblotting with specific phosphoserine 473, phosphothreonine 308, and total Akt antibodies. B, quantification of the relative extent of insulin-stimulated Akt phosphorylation was determined from the average of 6–10 independent experiments. *, p < 0.05. C, the relative levels total ILK, GLUT4, Akt, and phosphoserine 473 Akt (p-Akt S473) was determined in duplicate for the control Itgβ1floxflox and MCKItgβ1KO mice at the end of the EU clamps.
observed whole body insulin resistance was a direct result of impaired insulin-stimulated glucose uptake and glycogen synthesis in skeletal muscle but with apparent normal insulin signaling in the liver and adipose tissue. This result is also consistent with a recent study in cultured L6 myocytes indicating that a loss of focal adhesion kinase function, which is a downstream target of integrin β1, correlated with a reduction in insulin-stimulated glucose uptake, GLUT4 translocation to the plasma membrane, and glycogen synthesis (12). Interestingly, despite the decrease in skeletal muscle insulin sensitivity, the MCKItgβ1 KO mice had normal body weight and adiposity, probably reflecting metabolic compensation as has been reported for the selective skeletal muscle insulin resistance GLUT4 and caveolin 3 knock-out mice (58–60).

To elucidate the molecular mechanisms behind the observed abnormalities in insulin stimulation of glucose transport and glycogen synthesis, we investigated the protein expression levels of several key insulin responsive proteins in the MCKItgβ1 KO mice. It is generally accepted that insulin-stimulated glucose uptake and glycogen synthesis requires a signaling cascade involving the activation of the type IA PI 3-kinase resulting in the phosphorylation and activation of Akt (38, 42). The activa-
Although the concomitant reduction in ILK does not directly address whether or not ILK is the upstream kinase responsible for Akt Ser-473 phosphorylation, it does demonstrate that ILK expression is dependent upon the appropriate assembly of integrin β1 signaling complex organization in vivo. In summary, our data demonstrate an important requirement for integrin β1 subunit receptor signaling in modulating skeletal muscle insulin action and glucose homeostasis.

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