ATP-sensitive K⁺ channel-mediated glucose uptake is independent of IRS-1/phosphatidylinositol 3-kinase signaling

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Minami, Kohtaró, Mizuo Morita, Atsunori Saraya, Hideki Yano, Yasuo Terauchi, Takashi Miki, Takayuki Kuriyama, Takashi Kadowaki, and Susumu Seino. ATP-sensitive K⁺ channel-mediated glucose uptake is independent of IRS-1/phosphatidylinositol 3-kinase signaling. Am J Physiol Endocrinol Metab 285: E1289–E1296, 2003. First published August 21, 2003; 10.1152/ajpendo.00278.2003.—We previously found that disruption of Kir6.2-containing ATP-sensitive K⁺ (KATP) channels increases glucose uptake in skeletal muscle, but the mechanism is not clear. In the present study, we generated knockout mice lacking both Kir6.2 and insulin receptor substrate-1 (IRS-1). Because IRS-1 is the major substrate of insulin receptor kinase, we expected disruption of the IRS-1 gene to reduce glucose uptake in Kir6.2 knockout mice. However, the double-knockout mice do not develop insulin resistance or glucose intolerance. An insulin tolerance test reveals the glucose-lowering effect of exogenous insulin in double-knockout mice and in Kir6.2 knockout mice to be similarly enhanced compared with wild-type mice. The basal 2-deoxyglucose uptake rate in skeletal muscle of double-knockout mice is increased similarly to the rate in Kir6.2 knockout mice. Accordingly, disruption of the IRS-1 gene affects neither systemic insulin sensitivity nor glucose uptake in skeletal muscles of Kir6.2-deficient mice. In addition, no significant changes were observed in phosphatidylinositol 3-kinase (PI3K) activity and its downstream signal in skeletal muscle due to lack of the Kir6.2 gene. Disruption of Kir6.2-containing KATP channels clearly protects against IRS-1-associated insulin resistance by increasing glucose uptake in skeletal muscles by a mechanism separate from the IRS-1/PI3K pathway.

insulin receptor substrate-1; insulin secretion; insulin sensitivity; pancreatic β-cell; skeletal muscle

ATP-sensitive K⁺ (KATP) channels regulate various cellular functions such as secretion and neural excitability by coupling cell metabolism to membrane potential (2, 5, 9, 21, 25, 26, 28). The KATP channel is a heterooctamer composed of four pore-forming subunits, Kir6.1 or Kir6.2, and four sulfonylurea receptor subunits, SUR1, SUR2A, or SUR2B (8, 13). Differing combinations of Kir6.1 or Kir6.2 and SUR1, SUR2A, or SUR2B constitute KATP channels with distinct nucleotide and pharmacological sensitivities (24). For example, the pancreatic β-cell KATP channel comprises Kir6.2 and SUR1 (12, 23), and the cardiac and skeletal muscle KATP channels comprise Kir6.2 and SUR2A (1, 4). In pancreatic β-cells, glucose is transported through the glucose transporter GLUT2, and the subsequent metabolism of glucose produces ATP. The increase in the ATP/ADP ratio closes the KATP channels, depolarizing the β-cell membrane and opening the voltage-dependent calcium channels to allow calcium influx. The rise in intracellular calcium concentration triggers insulin granule exocytosis. Because there is almost no insulin secretion in response to glucose in mice lacking Kir6.2 (18), the pancreatic β-cell KATP channel clearly plays a critical role in glucose-induced insulin secretion. Although Kir6.2 knockout mice lack glucose-induced insulin secretion, they exhibit only slight glucose intolerance and do not develop overt diabetes (18). Furthermore, an insulin tolerance test shows enhanced insulin action in Kir6.2 knockout mice (18). We (17) found recently that glucose uptake in skeletal muscles of Kir6.2 knockout mice is increased both in vivo and in vitro. Chutkow et al. (7) also reported that disruption of KATP channels enhances glucose uptake in skeletal muscle.

In the present study, we crossed Kir6.2 knockout mice with insulin receptor substrate-1 (IRS-1) knockout mice to clarify the mechanism of KATP channel-mediated glucose uptake. IRS-1 knockout mice exhibit insulin resistance in skeletal muscles (3, 30, 35). However, double-knockout mice with disruption of both the Kir6.2 and IRS-1 genes do not develop insulin resistance or overt diabetes. Disruption of Kir6.2 does not increase phosphatidylinositol 3-kinase (PI3K) activity or Akt/PKB phosphorylation, indicating that KATP channel-mediated enhancement of glucose uptake is not mediated by IRS-1/PI3K signaling.

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MATERIALS AND METHODS

Generation of double-knockout mice. Homozygous mutant mice for the Kir6.2 gene (Kir6.2−/−) (15) and for the IRS-1 gene (IRS-1−/−) were generated as described previously (30). To generate double-knockout mice, Kir6.2−/− were crossbred with IRS-1−/−. To homogenize the genetic background, the F1 generation was used for multiple breedings to obtain the F2 generation, which comprised nine different genotypes on a similar mixed genetic background: Kir6.2+/+ IRS-1+/+, Kir6.2−/+ IRS-1−/−, Kir6.2−/− IRS-1−/−, Kir6.2−/− IRS-1+/+, Kir6.2−/− IRS-1+/+, and Kir6.2−/− IRS-1−/−. Among the nine genotypes generated, mice with the following four genotypes were used for the present studies: Kir6.2−/− IRS-1−/− (wild-type, WT), Kir6.2−/− IRS-1−/−, Kir6.2−/− IRS-1+/+, and Kir6.2−/− IRS-1−/−. The genotypes were confirmed by PCR, as previously described (15, 30). The mice were fed normal chow and maintained using standard husbandry procedures. Animal care and procedures were approved by the Animal Care Committee of Chiba University School of Medicine.

Oral glucose tolerance test and insulin tolerance test. An oral glucose tolerance test (OGTT) was performed on male mice fasted for 16 h at 12 wk of age. Oral glucose (5 g/kg) was administered intraperitoneally in conscious adult mice fed ad libitum. Blood samples were taken before and 2, 5, and 10 min after the glucose injection. Plasma samples were analyzed for glucose content. The body weight of each mouse after an overnight fast (16 h) was measured at 12–20 wk of age. Serum insulin levels were determined with an ELISA kit (Morinaga, Yokohama, Japan). The insulin response was quantified by determining the area under the curve (AUC) from 0 to 10 min following an oral glucose load (200 mg/kg body wt).

RESULTS

General features of the double-knockout mice. Double-knockout mice (Kir6.2−/− IRS-1−/−) were generated by breeding Kir6.2−/− and IRS-1−/−. Each genotype of mice was identified using the PCR method. WT, Kir6.2+/− IRS-1+/+, Kir6.2+/− IRS-1−/−, and Kir6.2−/− IRS-1−/− were born at the expected Mendelian frequency. Although the growth of Kir6.2−/− IRS-1−/− was similar to that of WT, Kir6.2−/− IRS-1−/− showed growth retardation, as previously reported (18, 30). The body weight of Kir6.2−/− IRS-1−/− was −70% of WT. Kir6.2−/− IRS-1−/− also showed growth retardation similar to that of Kir6.2−/− IRS-1−/− (Table 1).

Blood glucose and insulin levels in ad libitum-fed and fasted mice. To evaluate the effects of double disruption of both the Kir6.2 and IRS-1 genes on glucose metabolism in vivo, we measured blood glucose and serum insulin levels fed ad libitum and after 16 h of fasting (Table 1). Fed glucose levels were similar among the four groups of mice. As in a previous study (3), fed insulin levels of Kir6.2+/− IRS-1−/− were increased significantly compared with WT (P = 0.0075). In addition, Kir6.2+/− IRS-1−/− showed significantly higher fed insulin levels than Kir6.2+/− IRS-1+/+ (P < 0.0001). In the fasted state, Kir6.2+/− IRS-1−/− had blood glucose levels comparable to those of WT (P = 0.3741), whereas Kir6.2+/− IRS-1−/− and Kir6.2−/− IRS-1−/− had lower blood glucose levels (P = 0.0003 for Kir6.2+/− IRS-1+/+ vs. WT and P = 0.0002 for Kir6.2−/− IRS-1−/− vs. WT). Although the fasting insulin levels of Kir6.2+/− IRS-1−/− and WT were similar (P = 0.0681),
the levels of Kir6.2−/−IRS-1−/− were significantly increased compared with those of Kir6.2−/−IRS-1+/+ (P = 0.0150). These results indicate that lack of IRS-1 induces hyperinsulinemia, especially in the fed state, without affecting blood glucose levels, whereas disruption of Kir6.2 decreases fasting blood glucose levels without affecting serum insulin levels. Overt diabetes did not develop in mice with disruption of both Kir6.2 and IRS-1.

**Blood glucose and serum insulin levels during OGTT.** We then compared OGTT (1.5 g/kg) in the four groups of mice (Fig. 1) to clarify the effects of double disruption of the Kir6.2 and IRS-1 genes on acute glucose metabolism. As reported previously (18, 30), Kir6.2+/−IRS-1−/− have normal glucose tolerance, whereas Kir6.2−/−IRS-1+/+ have impaired glucose tolerance (Fig. 1A). The AUC of blood glucose is largest in Kir6.2−/−IRS-1+/+ among the four groups (Fig. 1B).

Surprisingly, the impaired glucose tolerance observed in Kir6.2−/−IRS-1+/+ was not further impaired by disruption of IRS-1 (in Kir6.2−/−IRS-1−/−), and the glucose disappearance rate from peak at 30 min was enhanced compared with Kir6.2−/−IRS-1+/+ (Fig. 1A).

Table 1. Body weight and blood glucose and serum insulin levels in the test animals

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<tr>
<td>Body weight, g</td>
<td>28.3 ± 0.3(10)</td>
<td>29.3 ± 0.7(10)</td>
<td>19.3 ± 0.4(9)†‡</td>
<td>21.3 ± 0.6(12)‡</td>
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<td>Fed ad libitum</td>
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<tr>
<td>Blood glucose, mmol/l</td>
<td>9.85 ± 0.29(17)</td>
<td>9.87 ± 0.37(43)</td>
<td>10.23 ± 0.35(27)</td>
<td>10.13 ± 0.51(30)</td>
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<td>Serum insulin, pmol/l</td>
<td>158 ± 29(8)</td>
<td>149 ± 12(21)</td>
<td>353 ± 28(10)‡‡</td>
<td>505 ± 62(16)‡‡</td>
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<tr>
<td>Fasted (16 h)</td>
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<tr>
<td>Blood glucose, mmol/l</td>
<td>6.42 ± 0.42(14)</td>
<td>4.18 ± 0.32(15)†</td>
<td>5.57 ± 0.30(15)†</td>
<td>4.04 ± 0.30(14)‡§</td>
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<tr>
<td>Serum insulin, pmol/l</td>
<td>37.1 ± 7.7(8)</td>
<td>29.7 ± 4.9(8)‡</td>
<td>56.8 ± 6.9(8)*</td>
<td>57.6 ± 10.1(7)‡*</td>
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Data are means ± SE; no. in parentheses, no. of mice. Body weight was measured at 16 wk of age, other parameters at 12–20 wk of age. *P < 0.05; †P < 0.01 compared with wild type; ‡P < 0.01 compared with Kir6.2+/−IRS-1+; §P < 0.05 compared with Kir6.2+/−IRS-1−.
In accord with previous data (18, 30), the drop in blood glucose induced insulin secretion is enhanced in Kir6.2+/−/IRS-1+/− but diminished in Kir6.2+/−/IRS-1−/− (Fig. 1D). Kir6.2+/−/IRS-1−/− also had a lower ΔI/ΔG than WT but showed a higher index than Kir6.2+/−/IRS-1+/− during 0–60 min (P = 0.0486). These observations show that the acute insulin response is impaired by lack of the Kir6.2 gene, whereas disruption of the IRS-1 gene partially restores glucose-induced insulin secretion even in the absence of the Kir6.2 gene.

Assessment of insulin sensitivity in vivo. We then assessed insulin sensitivity by ITT (0.5 U/kg ip). In accord with previous data (18, 30), the drop in blood glucose level after insulin injection was less in Kir6.2+/−/IRS-1−/− than in WT, whereas it was significantly greater in Kir6.2+/−/IRS-1+/− (Fig. 2). The blood glucose level decrease in Kir6.2−/−/IRS−/− and in WT but similar to that in Kir6.2+/−/IRS−/− (Fig. 2). These results show that disruption of the Kir6.2 gene leads to enhancement of insulin sensitivity even in the absence of the IRS-1 gene.

2-[3H]DG uptake in skeletal muscles and fat tissues. We previously found that 2-[3H]DG uptake in skeletal muscles of Kir6.2 knockout mice is enhanced (17). Here, we measured 2-[3H]DG uptake to determine whether disruption of Kir6.2 increases glucose uptake in the absence of IRS-1. In skeletal muscles [gastrocnemius, soleus, and extensor digitorum longus (EDL)], the rate constant of net uptake of 2-[3H]DG was similar in Kir6.2+/−/IRS−/− and WT (Fig. 3A). However, in Kir6.2−/−/IRS−/−, the 2-[3H]DG uptake rates in gastrocnemius and EDL were significantly higher than in WT (Fig. 3A). Moreover, 2-[3H]DG uptake in Kir6.2−/−/IRS−/− was enhanced in gastrocnemius compared with Kir6.2+/−/IRS−/−, demonstrating that disruption of the Kir6.2 gene enhances glucose uptake even in the absence of the IRS-1 gene. In contrast, the rate constant of net uptake of 2-[3H]DG in white adipose tissues (epididymal and retroperitoneal fat) was higher in Kir6.2−/−/IRS−/− than in WT (Fig. 3B). 2-[3H]DG uptake in retroperitoneal fat also was increased in Kir6.2−/−/IRS−/− compared with WT (Fig. 3B).

Expression of GLUT1 and GLUT4 in skeletal muscle. To test the possibility that change in expression of glucose transport proteins is involved in Kir6.2-mediated glucose uptake, we performed immunoblotting of GLUT1 and GLUT4 proteins. No significant difference in levels of GLUT4 protein was detected in skeletal muscles among all four groups of mice (Fig. 4). GLUT1 protein was not detected by immunoblotting in any of the groups (data not shown). Therefore, it is unlikely that enhanced glucose uptake in Kir6.2−/−/IRS−/− and Kir6.2−/−/IRS−/− is due to upregulation of GLUT1 or GLUT4 expression.

PI3K activity in skeletal muscle. Because the metabolic action of insulin is mediated by activation of PI3K (22), we compared skeletal muscle PI3K activity with and without insulin injection. In the presence of exogenous insulin, no significant difference in PI3K activity was observed among four groups of mice (Fig. 5A; P values are indicated in the figure). However, the fold increase in PI3K activity after insulin injection was significantly lower in Kir6.2+/-/IRS-1−/− than in WT.
PI3K activity were similar in Kir6.2−/−IRS-1−/− and Kir6.2−/−IRS-1−/− (P = 0.7675), suggesting that disruption of the Kir6.2 gene does not affect the PI3K signal. The fold increases in PI3K activity in Kir6.2−/−IRS-1+/+ and Kir6.2−/−IRS-1−/− were comparable (P = 0.9607), indicating that lack of the IRS-1 gene does not affect insulin action when the Kir6.2 gene is disrupted.

**Phosphorylation of Akt/PKB in skeletal muscle.** Akt/PKB is one of the signaling molecules downstream of PI3K that regulates glucose uptake in skeletal muscle. It is activated by phosphorylation at Thr308 and Ser473 (34). The abundance of Akt/PKB proteins in all four groups of mice is similar (Fig. 6A). In addition, similar insulin-induced phosphorylation (at both Thr307 and Ser473) of Akt/PKB is detected among the four groups of mice (Fig. 6, B and C), demonstrating that disruption of the IRS-1 or the Kir6.2 gene does not affect Akt/PKB phosphorylation.

**AMPK in skeletal muscle.** Because AMPK is known to regulate insulin-independent glucose uptake in skeletal muscle (16), we investigated protein expression and phosphorylation of AMPK. Protein expression of AMPK in skeletal muscle of Kir6.2−/−IRS-1−/− was decreased significantly compared with WT (P = 0.0452; Fig. 7A). In Kir6.2−/−IRS-1+/+ and Kir6.2−/−IRS-1−/−, AMPK protein levels showed no significant change.

(Fig. 5B), indicating that insulin action is diminished by the lack of IRS-1. The difference in the fold increase between Kir6.2−/−IRS-1+/+ and WT was not significant (P = 0.0869). In addition, the fold increases in

![Fig. 4. Immunoblot analyses of GLUT4. Equal amounts of tissue lysates (40 μg) were separated by 7.5% SDS-PAGE, probed with anti-GLUT4 antibody, and visualized with enhanced chemiluminescence. Bands were quantified with a densitometer, and each bar represents mean ± SE (n). Representative photograph is shown. Difference between WT and each group was not statistically significant.](image-url)

![Fig. 5. Phosphatidylinositol 3-kinase (PI3K) activity in the presence or absence of insulin injection (A) and fold increase of its activity after insulin injection (B) in skeletal muscle. After treatment with or without insulin for 5 min, gastrocnemius lysates were immunoprecipitated with anti-phosphotyrosine antibody and then subjected to PI3K assay as described in MATERIALS AND METHODS. A: results are expressed as percent WT without insulin, and each bar represents mean ± SE (n). Difference between WT and each group was not statistically significant. Photograph shows representative results. B: results are expressed as ratio of PI3K activity with and without insulin, and each bar represents mean ± SE (n). *P < 0.05. NS, not significant.](image-url)
P/11005 0.1542 for Kir6.2/H11002/11002 IRS-1/H11001/11001 vs. WT and P/11005 0.1184 for Kir6.2/H11002/11002 IRS-1/H11002/11002 vs. WT). Phosphorylation of AMPK (an activated form) is shown in Fig. 7B. Although Kir6.2/H11001/11001 IRS-1/H11002/11002 have levels of phosphorylated AMPK similar to those of WT (P = 0.3065), both Kir6.2/H11002/11002 IRS-1/H11001/11001 + + + and Kir6.2/H11002/11002 IRS-1/H11002/11002 −/− had levels significantly lower than in WT (P = 0.0183 for Kir6.2/H11002/11002 IRS-1/H11001/11001 + + + vs. WT and P = 0.0101 for Kir6.2/H11002/11002 IRS-1/H11002/11002 −/− vs. WT). These results show that lack of the IRS-1 gene reduces protein expression of AMPK in skeletal muscle and that lack of the Kir6.2 gene reduces the phosphorylated form of AMPK.

Fig. 6. Immunoblotting of Akt/PKB (A), Thr308 phosphorylated (p-)Akt/PKB (B), and Ser473 p-Akt/PKB (C). The gastrocnemius lysates (40 μg) were subjected to SDS-PAGE and transferred to Immobilon P membranes. The membranes were probed with anti-Akt/PKB or anti-p-Akt/PKB (Thr308 or Ser473) as described in MATERIALS AND METHODS. Photographs show representative results. A: Akt/PKB protein expression levels of all 4 groups of mice are similar (n = 3). B and C: results are expressed as percent WT with insulin, and each bar represents mean ± SE (n). Difference between WT and each group is not statistically significant.

Fig. 7. Immunoblotting of cAMP-activated protein kinase (AMPK; A) and phosphorylated AMPK (B). Gastrocnemius lysates (40 μg) were subjected to SDS-PAGE and transferred to Immobilon P membranes. Membranes were probed with anti-AMPK or anti-phospho-AMPK (Thr172) as described in MATERIALS AND METHODS. Photographs show representative results. Results are expressed as percent WT, and each bar represents mean ± SE (n). *P < 0.05. NS, not significant.

DISCUSSION

Double-knockout mice with disruption of both the Kir6.2 and IRS-1 genes show no overt diabetes and no insulin resistance. We previously found that Kir6.2-deficient mice exhibit enhanced insulin sensitivity (18) and that Kir6.2-containing KATP channels participate in glucose uptake in skeletal muscles (17), but the mechanism was unclear. Because IRS-1 is a major substrate of insulin receptor kinase and mediates downstream insulin signals, lack of the IRS-1 gene causes insulin resistance (30). Accordingly, a KATP channel-associated increase in insulin action or glucose uptake would be mediated by the IRS-1 signal, so disruption of the IRS-1 gene should reduce both insulin sensitivity and glucose metabolism in Kir6.2 knockout mice. Lack of the Kir6.2 gene should not affect insulin action or glucose metabolism when the IRS-1 gene is disrupted. However, the present study demonstrates that disruption of the Kir6.2 gene enhances insulin sensitivity and glucose metabolism in the absence of the IRS-1 gene and that disruption of the IRS-1 gene does not affect insulin sensitivity or glucose metabolism in mice lacking the Kir6.2 gene (Figs. 2 and 3). These results indicate that KATP channel-associated increases in insulin action and glucose uptake are not mediated by the IRS-1 signal.

IRS-1-mediated glucose uptake requires activation of PI3K followed by activation of downstream signals such as atypical PKCs and Akt/PKB (6, 10, 14, 27, 31, 33). Accordingly, disruption of the IRS-1 gene causes reduction of insulin-stimulated activation of PI3K (Refs. 3 and 30 and Fig. 5B). We also found that lack of the Kir6.2 gene does not increase PI3K activity in either the presence or the absence of the IRS-1 gene (Fig. 5B), indicating that a Kir6.2-associated increase in glucose uptake is independent of PI3K activity.
Furthermore, the phosphorylation of Akt/PKB and the amounts of the protein are not affected by the lack of the Kir6.2 gene, indicating that the Akt/PKB signal is not involved in Kir6.2-associated increases in glucose uptake.

Glucose uptake in skeletal muscles also is stimulated by an insulin-independent, AMPK-dependent mechanism (19, 20). Upon phosphorylation, AMPK stimulates glucose uptake additively to insulin action. Accordingly, if the AMPK signal is enhanced, glucose uptake should be increased. However, this was not the case in Kir6.2 knockout mice, as the phosphorylated form of AMPK was not increased but decreased in mice lacking the Kir6.2 gene (Fig. 7B). Although the pathways involved in K\textsubscript{ATP} channel-mediated glucose uptake remain to be elucidated, the present results establish that they are independent of AMPK and the IRS-1/Pi3K signal.

The acute insulin response was evaluated by OGTT (Fig. 1C). Although disruption of the Kir6.2 gene leads to impaired glucose-induced insulin secretion, lack of the IRS-1 gene causes hypersecretion. Double-knockout mice have a poor insulin response at 30 min but a significant response at 60 min, which may account for the acceleration of the disappearance rate of blood glucose after 30 min compared with Kir6.2 knockout mice. Thus disruption of IRS-1 gene partially restores glucose-induced insulin secretion, even in the absence of Kir6.2 gene. Islet insulin content of double-knockout mice was decreased by 84% of that of WT mice (data not shown), similarly to that of IRS-1 knockout mice (15). Kir6.2 knockout islets showed insulin content similar to that of WT islets (data not shown). Glucose-induced insulin secretion in Kir6.2 knockout islets is severely impaired (18). In addition, IRS-1 knockout mice exhibit \( \beta \)-cell hyperplasia, whereas insulin secretion from the isolated islets is diminished (15, 32). Thus it is likely that the increased insulin secretion in the double-knockout mice is due to \( \beta \)-cell hyperplasia rather than to enhanced insulin secretion from individual \( \beta \)-cells.

Despite impaired acute insulin secretion, fed insulin levels are increased in double-knockout mice similarly to those of IRS-1 knockout mice. IRS-1 knockout mice have \( \beta \)-cell hyperplasia (15, 32), which may account for the hyperinsulinemia. In general, \( \beta \)-cell hyperplasia and the resulting hyperinsulinemia are thought to be a compensatory response to insulin resistance. However, the double-knockout mice in the present study have no insulin resistance, as assessed by ITT (Fig. 2). Therefore, the hyperinsulinemia observed in these mice is not due to insulin resistance, and the lack of IRS-1 can lead to hyperinsulinemia independently of the onset of insulin resistance, at least in the absence of Kir6.2.

In conclusion, analysis of mice with disruption of both the Kir6.2 and IRS-1 genes reveals that K\textsubscript{ATP} channel-associated enhancement of glucose uptake in skeletal muscles is independent of the IRS-1/Pi3K-signaling pathway. In addition, these double-knockout mice provide a useful model to study the interaction of \( \beta \)-cell function and peripheral glucose metabolism.

DISCLOSURES

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REFERENCES


