GLUT2-null mice are hyperglycemic, hypoinsulinemic, hyperglucagonemic, and glycosuric and die within the first 3 weeks of life. Their endocrine pancreas shows a loss of first phase glucose-stimulated insulin secretion (GSIS) and inverse α to β cell ratio. Here we show that reexpression by transgenesis of either GLUT1 or GLUT2 in the pancreatic β cells of these mice allowed mouse survival and breeding. The rescued mice had normal-fed glycemia but fasted hypoglycemia, glycosuria, and an elevated glucagon to insulin ratio. Glucose tolerance was, however, normal. In vitro, insulin secretion assessed following hyperglycemic clamps was normal. In vitro, islet perfusion studies revealed that first phase of insulin secretion was restored as well by GLUT1 or GLUT2, and this was accompanied by normalization of the glucose utilization rate. The ratio of pancreatic insulin to glucagon and volume densities of α to β cells were, however, not corrected. These data demonstrate that 1) reexpression of GLUT1 or GLUT2 in β cells is sufficient to rescue GLUT2-null mice from lethality, 2) GLUT1 as well as GLUT2 can restore normal GSIS, 3) restoration of GSIS does not correct the abnormal composition of the endocrine pancreas. Thus, normal GSIS does not depend on transporter affinity but on the rate of uptake at stimulatory glucose concentrations.

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Transgenic Reexpression of GLUT1 or GLUT2 in Pancreatic β Cells Rescues GLUT2-null Mice from Early Death and Restores Normal Glucose-stimulated Insulin Secretion*

Genetic inactivation of the glucose transporter GLUT2 by homologous recombination in embryonic stem cells allowed us to generate mice deficient in the activity of this transporter (1). These GLUT2-null mice were unable to survive past the third week of life and displayed a form of diabetes mellitus characterized by hyperglycemia, relative hypoinsulinemia, and elevated glucagon levels. Their glucose tolerance was severely impaired, and as assessed in islet perfusion experiments, their pancreatic β cells had completely lost the first phase while maintaining a second phase of insulin secretion. The cellular composition of the islets of Langerhans was also modified with an inversion of the α to β cell ratio due to an absolute increase in α cell number and absolute decrease in β cell numbers. As this change in cellular composition appeared only after birth, which is the time at which glucose sensitivity by pancreatic β cells is being established, we postulated that the altered cellular composition of the islets was due to a defect in the secretion by β cells of autocrine proliferation factors or of paracrine factors normally restricting α cell proliferation.

Glucose-stimulated insulin secretion (GSIS) is initiated by the uptake of glucose by the glucose transporter GLUT2. Glucose is then phosphorylated by glucokinase and further metabolized through the glycolytic pathway. Activation of the mitochondrial metabolism then leads to generation of coupling factors, ATP (2, 3) and glutamate (4), which trigger the distal steps in insulin granule exocytosis. The high $K_m$ (6 mM) hexokinase IV, referred to as glucokinase, is the rate-limiting step in glucose metabolism by β cells, and it therefore has a high control strength over the entire process of glucose utilization, glucose oxidation, and insulin secretion (5). The role of the high $K_m$ (–17 mM) GLUT2 in glucose-sensing by β cells has been much debated (6, 7). Based on measurements of glucose transport and glucose phosphorylation rates by rat β cells, it was demonstrated that the rate of uptake was largely in excess over the rate of phosphorylation (8). This led to the conclusion that GLUT2 played purely a permissive role in glucose sensing by allowing an unrestricted access of glucose to glucokinase. Consequently, it was suggested that GLUT2 could be replaced by another glucose transporter isoform, even with a lower $K_m$ for glucose, provided that the total transport capacity was sufficient. Furthermore, initial studies claimed that GLUT2 was not present in human β cells and that uptake could be accounted for solely by GLUT1 or GLUT3 (9), which have a $K_m$ for glucose of 1–3 mM. Numerous studies have, however, demonstrated that GLUT2 is also expressed by human β cells (10, 11). From the above data, it cannot therefore be claimed that GLUT2 is not required for normal GSIS. On the other hand, a number of studies suggested that GLUT2 could play a specific role in GSIS, in particular since GLUT2 but not GLUT1 reexpression in glucose-unresponsive insulinoma cells could restore some glucose-sensing capacity (12–14).

Here we directly compared the impact of GLUT1 or GLUT2 reexpression specifically in the pancreatic β cells of GLUT2-null mice on mouse survival, whole body glucose homeostasis, GSIS, and the cellular composition of the islets of Langerhans. We demonstrate that reexpression of either transporter in pancreatic β cells allowed mouse survival, growth, and breeding, normalization of whole body glucose homeostasis, and correction of GSIS but not restoration of the cellular composition of

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1 The abbreviations used are: GSIS, glucose-stimulated insulin secretion; kb, kilobase pairs; PCR, polymerase chain reaction; RIP, rat insulin promoter.

2 P. Duprand B. Thorens, unpublished information.
the endocrine pancreas. This therefore indicates that the absence of GLUT2 causes early death of GLUT2-null mice by impairing the normal secretory function of the pancreatic β cells, that the altered cellular composition of the pancreatic islets is not due to a defect in glucose sensing by β cells, and finally, that GSIS can function as well with the low or high Kₘ glucose transporters GLUT1 or GLUT2 present in the plasma membrane of β cells.

**MATERIALS AND METHODS**

**Transgene Vector and Generation of Transgenic Mice**—The transgene vector contains the rat insulin promoter I (15), the human GLUT1 cDNA (1.95 kb; Ref. 16), and the intron-exon sequences of simian virus 40 (Fig. 1). The transgene was isolated as a 3.1-kb BamHI fragment from the rat insulin promoter (RIP) RIP1-GLUT2 vector and injected into fertilized oocytes (C57Bl/6F1 mice). Genotyping of mice was done by Southern blot and/or PCR of tail DNA. For Southern blot, DNA was digested with EcoRI-HindIII. The transgene was identified by the presence of a 1.6-kb band when using the 1.6-kb probe EcoRI-HindIII (Fig. 1). When using PCR, transgenic mice were identified by a 750-base pair band with primers 1 (TCA GCC AAG GAC AAA GAA AGC AGC AGC) and 2 (CAG ACA GAA GGG GAC ACT CAT AGT). The RIP1-GLUT2 transgene construct was prepared using a 2.3-kb KpnI-Xbal fragment that contained the full-length rat GLUT2 cDNA (17) and which was downstream of the RIP1 promoter. The same vector described above. The transgene was isolated as a 3.4-kb NdeI-ClaI fragment from the vector and injected into fertilized C57Bl/6 oocytes. Genotyping of mice was done by Southern blot and/or PCR of tail DNA. For Southern blot, DNA was digested with EcoRI. The transgene was identified by the presence of a 2-kb band when using the 2-kb EcoRI probe (Fig. 1). For the PCR genotyping, transgenic mice were identified by a 525-base pair band with primers 1 (TGG CCC CTCG TGC TGA ACT GGT) and 2 (GGT GTC GTA TGC GGT GTG). To generate transgenic knockout mice, GLUT2⁻/⁻ mice were bred with transgenic mice. Resulting RIPG1LU1×GLUT2⁻/⁻ and RIPG1LU2×GLUT2⁻/⁻ mice were crossed to generate transgenic RIPG1LU1×GLUT2⁻/⁻ and RIPG1LU2×GLUT2⁻/⁻ mice. In the rest of the text, these mice are referred to as RIPG1×G1 or RIPG2×G2 with appropriate indication of homo- or heterozygosity in superscript next to the name of the respective gene. Genotyping for the GLUT2 alleles was performed by Southern blot as described (1) and/or PCR. For the PCR approach, the GLUT2 mutant allele was identified with a GLUT2 gene primer (TGG TGG ATT ACA TGG CAT GAT) and a primer located in the neomycin gene (GGG GTG GGG TGG TAG TAG ATA) yielding a 665-base pair band. The GLUT2 wild-type allele was identified by a 916-base pair fragment with a set of primers located at each side of the neomycin gene (GAG ATG ATT ATG TGA TTT) and (GAG GGG AGA AGG AGG TGA). RIPG2⁻/-×G2⁻/⁻ mice were genotyped for homozygosity of the transgene by PCR amplification using TaqMan analysis, which was performed by a commercial service (BioDytx, Fullsirdorf, Basel).

**Islet Isolation and in Vitro Culture**—For islet isolation, pancreas were removed from rats in a collageenase solution (collagene type IV; Worthington), 2 mg/ml in Hanks’ balanced salt solution containing 10 mM Hepes, pH 7.4. Tissue was minced and incubated for 30 min at 37 °C. After washing of the digested tissue, islets were hand-picked under a stereomicroscope and either used at once or cultured overnight in RPMI 1640 containing 10% fetal calf serum, 10 mM Hepes, 1 mM sodium pyruvate, and 50 μM β-mercaptoethanol (1).

**Northern and Western Blot Analysis**—Total RNA was extracted from various tissues by the acid guanidinium thiocyanate technique (18) and analyzed for the presence of GLUT1, GLUT2, and GLUT4 mRNA by Northern blot analysis as described (19). Total islet lysates were prepared in a solution containing 80 mM Tris-HCl, pH 6.8, 5 mM EDTA, 2 mM phenylmethylsulfonylfluoride, 5% SDS (20). Lysates were separated on 10% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose filters, and GLUT1 and GLUT2 were detected with specific antibodies as described (20) using secondary antibodies linked to horseradish peroxidase and revealed by chemiluminescence (Amersham Pharmacia Biotech, ECL kit). The GLUT1 antibody recognizes an epitope that is identical in the mouse, rat, and human transporter. For GLUT2, because of species-specific differences of the C-terminal peptide as epitope, different antibodies are used for rat and mouse GLUT2 (21).

**Immunofluorescence Microscopy and Morphometric Analysis**—Indirect immunofluorescence microscopy was performed on frozen sections of pancreas fixed with paraformaldehyde/lysine-periodate fixative as described (22) using GLUT1 (23), GLUT2 (21), glucagon, and insulin antibodies (Linco) and either fluorescein-conjugated rabbit anti-guinea pig or goat anti-rabbit immunoglobulin antibodies (Dako). Volume density measurements were carried out by point-counting method (24) on a total of 15 islets greater than 100 μm in diameter and from three pancreas for each group of mice.

**Glucose Utilization**—Freshly isolated islets in batches of 50 were incubated for 2 h at 37 °C in the presence of different glucose concentrations and tracer amounts of [3H]glucose (NEN Life Science Products). After stopping the reaction and lysing the cells with 1 N HCl, the [3H]H₂O produced was separated from the reaction supernatant by passage over Dowex 1 × 2 (Fluka, Switzerland) columns. 0.1 μCi of [3H]H₂O (America Radiolabeled Chemicals) was passed over similar columns to quantify the recovery efficiency. Measurement of eluted radioactivity was by scintillation counting.

**Insulin Secretion and Radioimmunoassay**—Insulin secretion from overnight cultured islets was performed by perfusion as described (1). Islets were first perfused at a rate of 1 ml/min for 40 min in the presence of 2.8 mM glucose in Hepes-buffered Krebs-Ringer solution containing 0.5% bovine serum albumin and 0.5 mM isobutylmethyl xanthine. Glucose concentration was then increased to 16.7 mM for 20 min and returned to 2.8 mM glucose for the last part of the perfusion experiments. One-mL fractions were collected and used for insulin determination, and islets were lysed in ethanol-acid for total insulin content measurement. Radioimmunoassays were performed with a kit from Linco (Saint-Louis, MO) using rat insulin as standard.

**Intraperitoneal Glucose Tolerance Test**—Food was removed 5 h before the test. Glucose (dissolved into a sodium chloride solution, 0.1% w/v), was injected into the intraperitoneal cavity. Blood glucose was monitored 30 min before the glucose injection and at the indicated times from tail vein blood and using a glucose meter (GlucoD cyt, Roche Molecular Biochemicals).

**Hyperinsulinemic Clamps**—Mice were anesthetized with pentobarbital (65 mg/kg) injected intraperitoneally. An indwelling catheter was implanted into the vena cava through the left femoral vein and slid under the back skin to exit at the back of the neck, as described previously (19, 25). The mice were allowed to recover for 2 days. The day of the experiment mice were fasted for 5 h before the start of the experiment. Awake mice were infused through the implanted catheter with a 33% glucose solution at rates adjusted to maintain the blood glucose concentration at 10 or 20 mM, as determined from tail vein blood. After 180 min of glucose clamp, blood was collected in the presence of 1 mM EDTA and 1 μg/ml aprotinin. The blood was immediately centrifuged, and the plasma was removed and kept at −70 °C until insulin determination. Plasma insulin was determined by radio immunoassay (Linco, St. Louis, Mo).

**RESULTS**

Transgenic mice were generated that express either the GLUT1 or GLUT2 cDNA under the control of the RIP (Fig. 1). The resulting RIPGLUT1 and RIPGLUT2 mice were crossed with GLUT2⁻/⁻ mice, and following further appropriate breeding, RIPGLUT1×GLUT2⁻/⁻ and RIPGLUT2×GLUT2⁻/⁻ mice were obtained. In the rest of the text, these mice are referred to as RIPG1×G1 or RIPG2×G2 with appropriate indication of homo- or heterozygosity in superscript next to the name of the respective gene. Transgenic expression of either transporter in β cells rescued the mice from early lethality and allowed the rescued mice to grow to the adult age and to breed normally. Fig. 2 shows that the growth curves of these mice were similar to that of control littermates with, nonetheless, body weight slightly below the normal value.

Western blot analysis of transgene expression in islet lysates showed that GLUT1 was expressed at a similarly high level in the three transgenic lines. Fig. 3A shows that in the line chosen for this study, this level was severalfold greater than that of GLUT1 in brain membranes, which is a very abundant source of this transporter. GLUT2 was, however, undetectable in control islets. For the GLUT2 transgene, the level of transport was chosen as it is possible to distinguish it from endogenous mouse GLUT2 due to the species specificity of the GLUT2 antibodies. Fig. 3A shows that GLUT2 expression in heterozygous RIPG2⁻/-×G2⁻/- islets was 3 and 10 percent that of the amount of GLUT2 expressed in rat islets in transgenic lines 1 and 2, respectively. To increase the level of GLUT2
in transgenic line 2, we crossed these mice together to obtain homozygous transgenic mice (RIPG2 \(^{1/1} \times \text{G2}^{2/2}\)), which expressed approximately twice as much GLUT2 as the heterozygous transgenic animals, as revealed by Western blot analysis (not shown). Immunofluorescence microscopy (Fig. 3B) shows transgene expression in the pancreatic \(\beta\) cells of RIPG1 \(^{3/3} \times \text{G2}^{2/2}\) and RIPG2 \(^{1/1} \times \text{G2}^{2/2}\) line 2, mice.

The blood parameters of the control and transgenic mice are presented in Table I. Blood glucose levels were not different in the fed state, but transgenic mice were slightly hypoglycemic in the fasted state. These mice were very glycosuric. The urinary glucose loss, due to absence of GLUT2 from the proximal convoluted tubule, may account for the fasted hypoglycemia. Insulin levels in the transgenic mice were significantly lower in both the fed and fasted states as compared with the control mice, and glucagon levels were significantly higher in the fed state in the transgenic mice. Although fed glucagon levels were similar in all types of mice, the glucagon to insulin ratio was always much greater in the rescued mice.

To determine whether the reexpression of GLUT1 or GLUT2 in pancreatic \(\beta\) cells restored glucose-stimulated insulin secretory response, we performed hyperglycemic clamps in control, RIPG1 \(^{3/3} \times \text{G2}^{2/2}\), and RIPG2 \(^{1/1} \times \text{G2}^{2/2}\) line 2 mice and determined the insulinemic levels at the end of the perfusion periods (180 min). Fig. 4B shows that both a 10 mM and 20 mM glucose clamp strongly stimulated insulin secretion in the three groups of mice. Although in RIPG2 \(^{3/3} \times \text{G2}^{2/2}\) mice the insulinemia was lower than in the other two groups of mice, the differences were not statistically significant. To more directly study the kinetics of glucose-induced insulin secretion we next performed islet perfusion experiments. These results are presented in Fig. 5. The kinetics of GSIS was completely restored by reexpression of GLUT1 or GLUT2; notably, the first phase of secretion was now present, and the magnitude of the secretion was similar between rescued and control mice. The secretory pattern of GLUT2 \(^{2/2}\) islets is presented in Fig. 5, left panel. In the case of GLUT2-rescued islets, these experiments were performed with islets from RIPG2 \(^{1/1} \times \text{G2}^{2/2}\) line 2 mice. With islets from the RIPG2 \(^{2/2} \times \text{G2}^{2/2}\) line 1 and RIPG2 \(^{1/1} \times \text{G2}^{2/2}\) line 2 mice, which reexpress GLUT2 at lower level, the pattern of secretion was very variable, with most of the time abnormal first phase of secretion. In contrast, with the islets from RIPG2 \(^{2/2} \times \text{G2}^{2/2}\) line 2 mice, the pattern of secretion was normal in all experiments (Fig. 5, right panel). In this last set of experiments, the control mice were not littermates but C57Bl/6 mice; this may account for the slightly different pattern of second phase secretion.

![FIG. 1. Schematic representation of the transgenes used for GLUT1 or GLUT2 reexpression in pancreatic \(\beta\) cells. GLUT1 cDNA, rat GLUT1 cDNA; GLUT2cDNA, rat GLUT2 cDNA.](image1)

![FIG. 2. Growth curve of the RIPG1\(^{1} \times \text{G2}^{2/2}\) and RIPG2\(^{1} \times \text{G2}^{2/2}\) mice and of their control littermates, \(i.e.\) mice with at least one allele of the wild-type GLUT2 gene. The rescued mice grew to the adult age, and the growth curves were similar to those of control mice with, however, some significant but small differences in body weight. Data are the mean ± S.E., \(n = 13–18\), mice for data in left panel, and \(n = 8–13\) mice for data in right panel. *, body weight significantly different from that of control mice at the indicated age, with \(p < 0.05\).](image2)
RIPG1 severe restriction over the glucose concentration range. A lets lysates. The glucose utilization rate of islets from RIPG1 GLUT1 and GLUT2 expression in the islets of RIPG1 

GLUT2 reexpression. The glucose utilization rate of islets from RIPG1 

GLUT2 and GLUT4 in mixed leg muscles, epidydymal fat, liver, kidney, and brain of RIPG1×G2−/− and RIPG1×G2−/− mice.

We described that in GLUT2−/− mice, there was an inversion of the α to β cell ratio that was due to an absolute increase in the α cell number and an absolute decrease in the β cell number. This inversion of the endocrine cell ratio appeared only after birth, a time when glucose sensing by β cells is being established. We therefore postulated that the loss of glucose sensing by β cells could lead to this change in α/β cell number, probably by impairing the synthesis/secretion of β cell autocrine proliferation factors or paracrine regulators of α cells proliferation. We therefore evaluated the insulin and glucagon content as well as the volume density of the α cell population in the different groups of mice. Table II shows that, in GLUT1− and GLUT2-rescued mice, the ratio of total pancreatic glucagon over insulin content was higher than in control mice. This was also observed when assessing the total pancreatic hormone mRNA content of rescued and control mice (Fig. 7A). These differences were correlated with an increase in the volume density of the α cells in RIPG1×G2−/− and RIPG2×G2−/− mice as compared with control mice (Fig. 7B).

Finally, to evaluate whether correction of glucose metabolism was due only to transgenic expression of glucose transporters in pancreatic β cells of rescued mice and not to induced changes in GLUTs expression in other tissues involved in glucose metabolism, we assessed the expression of GLUT1, GLUT2 and GLUT4 in mixed leg muscles, epidydymal fat, liver, kidney, and brain of RIPG1×G2−/− and RIPG1×G2−/−.
Fig. 8 shows that the expression of these transporters was similar in the tissues studied in RIPG1<sup>3</sup>G2<sup>1</sup> and RIPG1<sup>3</sup>G2<sup>2</sup> line 2 and control mice, except for GLUT2, which was not detectable in the liver and kidney of the RIPG1<sup>3</sup>G2<sup>2</sup>/− mice, as expected.

**DISCUSSION**

The salient findings of the present study are as follows: first, the cause of the early lethality of the GLUT2-null mice is the absence of GLUTs from pancreatic β-cells and the consequent impaired GSIS and not the absence of GLUT2 from the other tissues, where it is normally expressed; second, reexpression of the low Km GLUT1 or high Km GLUT2 can similarly restore normal GSIS and glucose utilization; third, the altered cellular composition of the pancreatic islets is not corrected even though normal glucose sensing is restored.

In addition to being the main glucose transporter species of pancreatic β-cells, GLUT2 is present in intestine, liver, kidney, and hypothalamic and brain stem centers. The present study demonstrates that reexpressing GLUT2 or GLUT1 in the pancreatic islets is sufficient to prevent lethality and to allow the mice to survive to the adult age and breed normally. This therefore indicates that the absence of this transporter from all the other tissues where it is normally expressed is not required for mouse survival. The absence of hepatic GLUT2 suppresses glucose uptake without preventing quantitatively normal glucose output, which occurs by a membrane traffic-based pathway (19, 26). The absence of GLUT2 from kidney proximal tubule is associated with a marked glycosuria, indicating that GLUT2 is essential for renal glucose reabsorption. The urinary glucose loss is probably the cause of the slight hypoglycemia observed in the rescued mice during fasting.

The plasma insulin and glucagon levels of the rescued mice were, however, very different from those of control mice. Strikingly, the fed and fasted insulin plasma levels were always much lower than in the controls. The level of circulating insulin could be related to the dramatically impaired kidney glucose reabsorption, which by itself would tend to strongly reduce the glycemic levels, as achieved for instance by phlorizin treatment of diabetic animals. At the same time the glucagon levels were relatively elevated, and the glucagon to insulin ratio was always severalfold greater than in controls. The reasons for the increased glucagonemia is not clear. It could be required for a constant stimulation of hepatic glucose production to compensate for the urinary glucose loss. We have however shown that the rates of hepatic glucose production were almost identical in fasted control and rescued mice (19), arguing against this explanation. Alternatively, elevated glucagonemia may result from the low insulinemia of these mice since insulin has been described to have a major role in tonically inhibiting glucagon production and secretion in insulinopenic rats (27).

Reexpression of GLUT1 or GLUT2 in the pancreatic β-cells restored a normal glucose tolerance following intraperitoneal glucose load and a normal insulinemia during hyperglycemic clamps. These observations suggested that GSIS was restored equally well by both transporter isoforms. Further analysis of GSIS in islet perifusion experiments indeed demonstrated that
the kinetics of insulin secretion, in particular the rapid onset of secretion, was completely normalized by either GLUT1 or GLUT2. This was also correlated by a restoration of the normal glucose dose-dependent increase in glucose utilization. These data therefore show that the different kinetic properties of these two transporters is not important for normal glucose signaling to secretion in β cells. In the transgenic mice used in the present study, GLUT1 was expressed at a very high level, severalfold that of brain membrane preparations, which must ensure a very high rate of transport. On the other hand, the GLUT2-rescued mice expressed rat GLUT2 to only a fraction of the level found in normal rat islets. Although we cannot directly compare the level of expression of rat GLUT2 in transgenic islets with the normal level of expression of mouse GLUT2.

![Fig. 5. GLUT1 or GLUT2 reexpression in pancreatic β cells correct the kinetics of glucose-stimulated insulin secretion. Left, islets from GLUT2−/−, RIPG1×G2−/−, and RIPG1×G2−/− (Ctrl) mice were perifused with the indicated concentrations of glucose in the presence of isobutylmethyl xanthine (IBMX). Reexpression of GLUT1 in GLUT2−/− islets led to a complete normalization of the secretory response to glucose. Right, islets from RIPG2−/−×G2−/− line 2 and control (Ctrl) mice were perifused with the indicated concentrations of glucose and in the presence of isobutylmethyl xanthine. Reexpression of GLUT2 led to normalization of the secretory response. Here the control mice were C57Bl/6 mice obtained from a commercial source and not littermates of the rescued mice, which could explain the slightly different secretory response. The data are the mean ± S.E. for n = 6 perifusions for the left panel and n = 3 in the right panel, except for GLUT2−/− mice, where a representative perifusion is shown. More extensive data with similar patterns have been published (1). The first phase of secretion is defined as the phase with rapid onset that is not present in the GLUT2−/− mice and which lasts for approximately the first 10 min of perifusion at 16.7 mM glucose. KRBH, Hepes-buffered Krebs-Ringer solution.](image)

**Fig. 6. Glucose usage by islets from RIPG1×G2−/−, RIPG2−/−×G2−/− and control mice.** Glucose usage was determined at different glucose concentrations by measuring the appearance of H2O from D-[3H]glucose. Glucose utilization is the same in the different rescued mice. The left panel shows also the glucose utilization of GLUT2−/− islets, which plateaus at 6 mM glucose. Data are mean ± S.E. for three separate measurements at each glucose concentrations in the left panel and for five separate measurements in the right panel. *, different from control and rescued islets with p < 0.01.

| Mice          | Pancreas Peptides | 5.0 mEq/100 ml IBMX | 6.7 mEq/100 ml IBMX | 7.8 mEq/100 ml IBMX | 8.9 mEq/100 ml IBMX |
|---------------|-------------------|---------------------|---------------------|---------------------|---------------------|
| RIPG1×G2−/−  | Insulin total     | 266 ± 20.6          | 126 ± 9.2           | 50 ± 3.2            | 10 ± 1.0            |
|               | Insulin Glucagon total | 14.2 ± 1.2         | 6.8 ± 0.4           | 2.7 ± 0.2           | 0.5 ± 0.1           |
| RIPG2−/−×G2−/− | Glucagon         | 96 ± 6.4            | 45 ± 3.0            | 18 ± 1.5            | 4 ± 0.5             |
| RIPG1×G2−/−  | Glucagon/Insulin  | 0.36                | 0.36                | 0.37                | 0.37                |

*Significantly different from RIPG1×G2−/− or RIPG2−/−×G2−/−, with p < 0.05.
GLUT2 in mouse islets because we used species-specific antibodies, our data showed that restoration of GSIS in transgenic islets depends on the level of GLUT2 expression. When the level was too low, as in the RIPG2\textsuperscript{−/−}G2\textsuperscript{−/−} line 1 mice, GSIS assessed by perifusion experiments was usually abnormally low with impaired first phase secretion. The situation was similar with the heterozygous RIPG2\textsuperscript{1/2}\textsuperscript{−/−}G2\textsuperscript{1/2}\textsuperscript{−/−} line 2 mice, which expressed 10% of the normal rat GLUT2 levels. The secretory response was, however, normal all the times in homozygous RIPG2\textsuperscript{1/2}\textsuperscript{1/2}\textsuperscript{−/−}G2\textsuperscript{1/2}\textsuperscript{1/2}\textsuperscript{−/−} line 2 mice. Thus, even though the lowest level of transgenic GLUT2 expression allowed mouse survival, a normal kinetics of GSIS required this level to be that obtained in the homozygous RIPG2\textsuperscript{+/+}\times G2\textsuperscript{−/−} line 2 mice. This last observation deserves some comments. Indeed, even though the presence of a low level of GLUT2 in pancreatic \(\beta\) cells is sufficient to allow mouse survival without restoring the normal kinetics of secretion, as assessed by perifusion experiments, one can ask whether the survival is because of restoration of some glucose sensitivity by pancreatic \(\beta\) cells, which cannot be clearly established in the perifusion experiments, or whether it is due to the presence of GLUT2 in islets, allowing other functions of these cells to be performed that are vital for the animal. Against this are our previous studies (1) that revealed that insulin injection in GLUT2\textsuperscript{−/−} mice allowed them...
Normal β-Cell Glucose Sensing with GLUT1 or GLUT2

Whether a similar need for GLUT2 exists in islets can be directly evaluated using the described GLUT1- and GLUT2-rescued mice. Finally these mice will also serve as an excellent model for the in vivo study of the role of GLUT2 in other tissues. Such studies have already been published regarding the role of GLUT2 in liver function (19, 26). They will be used to study the role of this transporter in intestinal transepithelial glucose transport and in glucose sensing by different glucosetive units present in the central nervous system or peripheral endocrine or neuronal cells.

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to survive. This therefore strongly suggests that even the low level of GLUT2 reexpression seen in RIPG2×G2−/− line1 mice rescues them from early death by restoring some GSIS that cannot be observed in perfusion experiments.

An interesting observation made previously with GLUT2-null mice was that the composition of the islets of Langerhans was inverted with more α cells and fewer β cells. This was hypothesized to result from impaired glucose sensing by β cells and a consequent impaired synthesis or secretion of factors stimulating β cell growth or inhibiting α cell proliferation. This hypothesis was based on the fact that the cellular composition was normal at the day of birth but was already abnormal 5 days later. This indicated that the defect appeared after the normal time of establishment of glucose sensitivity by β cells. On the other hand, analysis of cellular composition of the pancreatic islets of human diabetic patients also demonstrated an alteration in the α to β cell ratio similar to that observed in the GLUT2-null mice (28). As β cells from human diabetic patients are also characterized by a loss of glucose sensing, this strengthened the hypothesis that cellular alterations were secondary to loss of glucose sensing by β cells. Our present data, however, show that restoring normal GSIS with GLUT1 or GLUT2 did not lead to correction of this altered cellular composition, which was also reflected at the level of total pancreatic insulin and glucagon mRNA or peptide levels. Therefore, the altered α to β cell ratio cannot be explained by impaired β cell glucose sensing or by a defect intrinsic to the pancreatic islets since only β cells do express GLUT2. This defect may nevertheless be due to the absence of GLUT2 from other tissues. One possibility is that the absence of GLUT2 from central centers controlling the autonomous nervous system may lead to a constant tone of the sympathetic nervous system, which control glucagon secretion and possibly α cell proliferation. Finally, one likely possibility is that the absence of GLUT2 from the kidney, which prevents glucose reabsorption, reduces the level of insulin required to maintain normal glycemia. The low insulinemia in both fed and fasted rescued mice may therefore prevent the normal tonic inhibition of glucagon synthesis and secretion that appears to normally control these hormone levels. This has indeed been demonstrated by Dumonteil et al. (27), who shows that in streptozocin diabetic rats, the increase in circulating glucagon and proglucagon gene expression could be corrected by insulin but not by glucose.

Together the present results show that glucose sensing by pancreatic β cells is required for mouse survival and that this glucose sensing is dependent on the capacity of the glucose transport system to carry a sufficient glucose flux at stimulatory glucose concentrations but is not dependent on the pres- ence of a specific transporter isoform. Whether GLUT2 still plays a specific role in β cell physiology that cannot be compensated by GLUT1 is nevertheless not ruled out. Glucose controls many functions of β cells; in particular, it stimulates the transcription of several genes, such as those for insulin (29), L-pyruvate kinase (30), or macrophage migration inhibitory factor (31), and the translation of preexisting mRNAs, such as those for insulin (32) and the proconvertases PC1/3 and PC2 (33). A specific requirement for GLUT2, as compared with GLUT1, has indeed been reported in the control by glucose of L-pyruvate kinase gene expression in hepatocytes (34).