Age-Related Impairment in Insulin Release
The Essential Role of β2-Adrenergic Receptor

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In this study, we investigated the significance of β2-adrenergic receptor (β2AR) in age-related impaired insulin secretion and glucose homeostasis. We characterized the metabolic phenotype of β2AR-null C57Bl/6N mice (β2AR−/−) by performing in vivo and ex vivo experiments. In vitro assays in cultured INS-1E β-cells were carried out in order to clarify the mechanism by which β2AR deficiency affects glucose metabolism. Adult β2AR−/− mice featured glucose intolerance, and pancreatic islets isolated from these animals displayed impaired glucose-induced insulin release, accompanied by reduced expression of peroxisome proliferator–activated receptor (PPAR)γ, pancreatic ductal homeobox-1 (PDX-1), and GLUT2. Adenovirus-mediated gene transfer of human β2AR rescued these defects. Consistent effects were evoked in vitro both upon β2AR knockdown and pharmacologic treatment. Interestingly, with aging, wild-type (β2AR+/+) littersmates developed impaired insulin secretion and glucose tolerance. Moreover, islets from 20-month-old β2AR−/− mice exhibited reduced density of β2AR compared with those from younger animals, paralleled by decreased levels of PPARγ, PDX-1, and GLUT2. Over-expression of β2AR in aged mice rescued glucose intolerance and insulin release both in vivo and ex vivo, restoring PPARγ/PDX-1/GLUT2 levels. Our data indicate that reduced β2AR expression contributes to the age-related decline of glucose tolerance in mice. Diabetes 61:692–701, 2012

Impairment of glucose metabolism with age represents a major determinant of type 2 diabetes epidemics within the elderly population. The molecular mechanisms underlying these changes have not been fully elucidated and are likely attributable to multiple causes (1,2). Aging per se is associated with a continuous decrease in basal insulin release (3). The size of this effect is sufficient to increase the likelihood of developing abnormalities in glucose tolerance and even overt diabetes (2,4). The consequence of aging on glucose tolerance occurs in different species, having been identified in rats (5,6) as well as in humans (4,7,8). However, why insulin secretion deteriorates with aging remains a moot point.

The noradrenergic system provides fine-tuning to the endocrine pancreas activity through the function of α- and β-adrenergic receptors (ARs) (9,10). The reciprocal regulation exerted by insulin and the adrenergic system has been well documented through a large number of studies (11–13). More recent evidence shows that mice with simultaneous deletion of the three known genes encoding the βARs (β1, β2, and β3) present a phenotype characterized by impaired glucose tolerance (14). Studies with β2AR agonists further suggest that the β2AR may play an important role in regulating insulin secretion (15). In addition, different human polymorphisms in the β2AR gene have been associated with higher fasting insulin levels (16). Nevertheless, the impact of the β2AR subtype on glucose tolerance and insulin secretion is still unclear.

Similar to glucose tolerance, βAR function and responsiveness deteriorate with aging (17–20), but the precise mechanisms involved are unknown. However, current evidence indicates that aging may downregulate βAR signaling, β2AR in particular, by decreasing the expression of molecular components of the adrenergic signaling machinery (21–24). We have therefore hypothesized that age-dependent alterations in βAR function impair glucose-regulated insulin release by the pancreatic β-cells and may contribute to deterioration of glucose tolerance. To test this hypothesis, we explored the consequences of β2AR knockout on insulin secretion in mice and investigated the significance of the age-related changes in β2AR function with regard to glucose tolerance.

RESEARCH DESIGN AND METHODS
In vivo studies. We studied male mice with a homozygous deletion of the β2AR gene (β2AR−/−) and backcrossed >12 generations onto C57Bl/6N background. Founders were provided by Brian Koblika (Stanford University, Stanford, CA) (25). Wild-type littersmates (β2AR+/+) were used as controls. The animals were housed in a temperature-controlled (22°C) room with a 12-h light/dark cycle in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication no. 85-23, revised 1996), and experiments were approved by the ethics committee of the Federico II University. Mice were killed by cervical dislocation. Pancreata were excised and collected rapidly after mice were killed. Samples were weighted, fixed by immersion in 4% paraformaldehyde for histology, homogenized for determination of total insulin content, or snap-frozen in liquid nitrogen and stored at −80°C for subsequent analyses. For determination of insulin or glucagon content, pancreatic tissue was homogenized in acid ethanol and extracted at 4°C overnight. The acidic extracts were dried by vacuum, reconstituted, and subjected to insulin and glucagon measurements.

Glucose tolerance test and assessment of insulin secretion. Glucose tolerance test (GTT) was performed as previously described (9,26). Briefly, mice were fasted overnight and then injected with glucose (2 g/kg i.p.). Blood glucose was measured by tail bleeding (Glucose Analyzer II; Beckman Coulter, Brea, CA) at indicated time points. The assessment of insulin secretion before
and during glucose challenge was performed as previously described (9,27). Blood from the mandibular vein of overnight-fasted mice was collected at the indicated time for serum insulin assessment. The evaluation of glucagon secretion was performed by collecting blood from the mandibular vein of random-fed mice before and after injection of insulin (0.75 IU/kg i.p.). Serum insulin and glucagon secretion was performed by collecting blood from the mandibular vein of random-fed mice at the indicated time for serum insulin assessment. The evaluation of glucagon secretion was performed as previously described (9,27). Blots were probed with mouse monoclonal antibodies against adenylyl cyclase type VI (AC-VI) (Abcam, Cambridge, MA), pancreatic and duodenal homeobox (PDX)-1, GLUT2, peroxisome proliferator–activated receptor (PPARγ), G-protein–coupled receptor (GRK2), G protein αs (Gαs), clathrin heavy chain, and actin (Santa Cruz Biotechnology). Experiments were performed in triplicate to ensure reproducibility. Membrane extracts were obtained as previously described (29,38). Data are presented as arbitrary units using actin as internal control (clathrin heavy chain for membrane extracts) as indicated.

**Measurement of cAMP production in vitro and ex vivo.** Intracellular content of cAMP was determined using a cAMP ELISA-sciillation proximity assay (GE Healthcare, Piscataway, NJ) according to the manufacturer’s instructions. Briefly, we used 20 size-matched islets (for the ex vivo assays) and 4,000 cells/well INS-1 (for the in vitro assays). Islets and β-cells were washed once and preincubated at 37°C in HEPES-buffered Krebs-Ringer solution containing 1 mM/L glucose and 0.5 mM/L isobutylmethylxanthine (a phosphodiesterase inhibitor) for 1 h and incubated for another 15 min in the same buffer with or without 100 mM/L forskolin (MP Biomedicals, Solon, OH), 3 mM/L NaF (Thermo Fisher Scientific, Pittsburgh, PA), or 1 mM/L isoproterenol (Tocris Bioscience, Ellisville, MO). The reaction was stopped by addition of 50 mM/L HCl and neutralized with NaOH. The cAMP levels were normalized to the protein concentration.

**Statistical analysis.** All data are presented as means ± SE. Statistical differences were determined by one-way or two-way ANOVA as appropriate, and Bonferroni post hoc testing was performed when applicable. A P value <0.05 was considered significant. Statistical analysis was performed using GraphPad Prism (version 5.01; GraphPad Software Inc., San Diego, CA).

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**RESULTS**

**Metabolic phenotype of β2AR+/– mouse.** To investigate in vivo the relevance of the β2AR gene in the regulation of insulin secretion, we compared the metabolic phenotype of adult (6 months old) β2AR+/– and β2AR+/+ mice. Blood glucose was significantly higher in the null mice compared with that in their wild-type littermates both upon fasting and under random feeding conditions (Table 1). In addition, their fasting serum insulin levels were significantly reduced (Table 1). Upon glucose loading (GTT), the β2AR+/– mice displayed a marked reduction in glucose tolerance (Fig. LA and B). In β2AR+/– mice, we observed a threefold increase in insulin secretion 3 min after intraperitoneal glucose injection, presumably corresponding with the peak of first-phase insulin release. This was followed by a decrease at 10
FIG. 1. Metabolic profile of $\beta_2AR^{-/-}$ mice. Six-month-old $\beta_2AR^{-/-}$ mice and their wild-type littermates ($\beta_2AR^{+/+}$) were fasted for 16 h and subjected to intraperitoneal glucose loading (2 g/kg body weight). Blood glucose (A and B) and serum insulin (C and D) were monitored for 120 min after glucose administration ($n = 14–18$ animals per group). $\beta_2AR^{-/-}$ mice displayed glucose intolerance (A) and impaired insulin secretion (C). We calculated the AUC from glucose (B) and insulin excursion (D) curves. Peak insulin–to–peak glucose ratio (E) represents $\beta$-cell function, as better described in RESEARCH DESIGN AND METHODS. Bars represent means ± SE. *$P < 0.05$ vs. $\beta_2AR^{+/+}$, Bonferroni post hoc test. AUC, area under the curve.
min and then a gradual increase over 30 min that may indicate a second-phase response (27,41).

In β₂AR⁻/⁻ mice, the early phase of insulin secretory response to glucose was reduced by more than twofold. The late response was also significantly impaired in the β₂AR⁻/⁻ compared with β₂AR⁺/+ mice (Fig. 1C and D). The peak insulin-to-peak glucose ratio was also decreased (Fig. 1E), further indicating impaired insulin response to hyperglycemia in the null mice.

To investigate whether the alterations in glucose tolerance identified in the β₂AR⁻/⁻ mice were contributed by deranged glucagon release, we further measured blood glucose and plasma glucagon levels 30 min after insulin administration. Indeed, insulin administration determines a fall in blood glucose and a counterregulatory rise in plasma glucagon (2,30). However, β₂AR⁻/⁻ and β₂AR⁺/+ mice exhibited comparable glucose and glucagon responses to insulin administration (Supplementary Fig. 1A and B). Pancreatic islet histology also did not show any significant difference in these mice (Fig. 2A), similar to total insulin and glucagon pancreatic content (Fig. 2B and C).

We then posed the further question of whether the reduced glucose insulin secretion observed in the β₂AR⁻/⁻ mice in vivo may represent the direct consequence of the β₂AR⁻/⁻ lack in the β-cells or whether it is indirectly mediated by other regulatory factors. To answer this question, we analyzed glucose effect on islets isolated from the null mice. As shown in Fig. 2D, these islets responded poorly to increased glucose concentration in the culture medium compared with the islets from their wild-type littermates but were fully responsive to KCl depolarization.

Islets and β-cell profiling after β₂AR deletion. To gain further insight into the mechanism leading to impaired insulin secretion in mice lacking β₂AR, we profiled the expression of different genes relevant to β-cell regulation by real-time RT-PCR of islet mRNA. As shown in Fig. 3A and B, mRNA levels of both PDX-1 and GLUT2, two major genes involved in β-cell function, were decreased in islets from β₂AR⁻/⁻ mice by 75 and 60%, respectively. Also, mRNA levels of the PDX-1/GLUT2 upstream regulator PPARγ were decreased by 54% compared with islets from wild-type mice (Fig. 3C). Reliable results were obtained in
the INS-1Esh
PPAR
the downregulation of both PDX-1 and GLUT2 occurring in
3 completely prevented the effect of ICI (Supplementary Fig.
of INS-1E
b
696 DIABETES, VOL. 61, MARCH 2012 diabetes.diabetesjournals.org
immunoblotting experiments (Fig. 3D and E). PDX-1 and
GLUT2 mRNAs were also reduced to a similar extent in
total pancreatic tissue from the β2AR–/– mice (data not
shown).
We then sought to demonstrate whether these abnor-
malities in gene expression were directly caused by β2AR
silencing. To pursue this objective, we silenced with a
specific shRNA (Supplementary Fig. 2) the β2AR gene in
the glucose-responsive INS-1E β-cell line (INS-1Eβ2AR)
(Fig. 4A and B). As shown in Fig. 4C, this specific knock-
down impaired glucose-induced insulin secretion by 58% in
these cells. A similarly sized effect was achieved by treat-
ment with the specific β2AR antagonist ICI, while the β2AR
agonist fenoterol showed an opposite action (Supplemen-
tary Fig. 3A). Consistent with our ex vivo results, the INS-
1Eβ2AR displayed a reduction in PDX-1, GLUT2, and
PPARγ mRNA (Fig. 4D–F) and protein levels (Fig. 4G and
H). Interestingly, transient transfection of a PPARγ cDNA in
INS-1Eβ2AR β-cells increased glucose-induced insulin se-
cretion compared with the wild-type INS-1E control β-cells
(Fig. 4C). In addition, overexpression of PPARγ prevented
the downregulation of both PDX-1 and GLUT2 occurring in
the INS-1Eβ2AR β-cells (Fig. 4D–H). Consistently, treatment
of INS-1E β-cells with ICI decreased PDX-1 and GLUT2
mRNA and protein levels, while PPARγ overexpression
completely prevented the effect of ICI (Supplementary Fig.
3B–D), suggesting that β2AR controls insulin secretion
through a PPARγ/PDX-1–mediated mechanism.
To better define the β2AR downstream mechanism lead-
ing to PPARγ activation, we assessed the cAMP levels in
these cells, observing an impaired production of cAMP in
INS-1Eβ2AR β-cells both in basal condition and after stim-
ulation with the β2AR agonist isoproterenol (Supplementary
Fig. 4A). Accordingly, to rule out possible involvement of
other components of β2AR signaling machinery, we assessed
the protein level of AC-VI, GRK2, and Gαs, and we found
no significant difference (Supplementary Fig. 4B and C).
Parallel results were obtained in ex vivo experiments,
performed to investigate the possible age-related alter-
ations in the β2AR transduction pathway, comparing
pancreatic islets isolated from adult (6 months old) and
old (20 months old) β2AR+/– mice (Supplementary Fig. 4D
and E).
β2AR overexpression rescued the age-related impairment
in insulin release. Based on radioligand binding and real-
time RT-PCR analysis, the expression of both β2AR protein
and mRNA was significantly decreased in islets from aged
(20 months old) β2AR+/– mice compared with those isolated
from adult (6 months old) mice (Fig. 5A and B). PDX-1,
GLUT2, and PPARγ expression (both in terms of mRNA
and protein level) was also reduced, and insulin release
in response to glucose, though not that evoked by KCl de-
polarization, was impaired in islets from the aged mice (Fig.
5C–H), suggesting that the reduced β2AR density constrains
islet glucose response in these animals. To prove this hy-
thesis, we used an adenoviral construct driving over-
expression of human β2AR in mouse islets. Interestingly,
infection of islets isolated from wild-type old mice with this
construct induced a twofold increase in β2AR expression
(Fig. 5A and B) and returned glucose-induced insulin secre-
tion to levels comparable with those of islets from 6-month-
old mice (Fig. 5C) accompanied by restored expression of
PDX-1, GLUT2, and PPARγ (Fig. 5D–H).
In the in vivo setup, 20-month-old β2AR+/– mice exhibited
a significant reduction in fasting serum insulin levels
(Table 2) accompanied by impaired glucose tolerance and
insulin response upon GTT (Fig. 6A–E). We have therefore designed a gene therapy protocol aimed to prove that these abnormalities can be corrected by restoring β2AR density. Accordingly, we infected the pancreas of aged mice by Adβ2AR injection. This injection effectively rescued β2AR expression in the pancreatic tissue, returning it to levels comparable with those of 6-month-old mice (Supplementary Fig. 5A and B), and restored the expression of PDX-1, GLUT2, and PPARγ (Supplementary Fig. 5C–E). Injections in the distal pancreas did not induce β2AR expression in other tissues, such as the liver (Supplementary Fig. 6A and B) or the skeletal muscle (Supplementary Fig. 6C and D).

These effects were paralleled by significant improvement in glucose tolerance and insulin secretion during GTT (Fig. 6A–E). Fasting insulin levels also increased, reaching values similar to those measured in 6-month-old mice (Table 2), further underlining the relevance of β2AR function in enabling adequate pancreatic β-cell response to hyperglycemia.

**DISCUSSION**

In the present work, we provide evidence that β2AR gene deletion in mice causes reduction of glucose-stimulated insulin release by pancreatic β-cells. This phenotype is reminiscent of that observed in mice with targeted β-cell disruption of the Gaα1a gene (30). In these mice, however, the impairment of Gaα1a prevented response to multiple Gαs-related receptors, causing a severe phenotype, with gross abnormalities in pancreatic islets. Interestingly, in islets from β2AR−/− mice, PPARγ expression was reduced by 50%, leading to repression of the PPARγ downstream molecules PDX-1 and GLUT2, two key effectors of β-cell function (26,42,43). This downregulation resulted in a clear impairment in insulin release, though islet architecture and insulin content were not affected by the β2AR gene deletion.

Rosen et al. (44) showed that islets from mice with targeted elimination of PPARγ in β-cells were approximately twice as large as those from control mice. Thus, we can speculate that in our model the 50% reduction in...
PPARγ levels is sufficient to restrain β-cell function without altering islet mass.

The mechanistic significance of β2AR gene knockout was further sustained by in vitro studies in the INS-1E pancreatic β-cells, showing that the silencing of the β2AR as well as the pharmacological treatment with a specific β2AR antagonist impaired glucose response and down-regulated PPARγ expression, reducing both PDX-1 and GLUT2 levels. No alteration of αARs was observed instead (data not shown). In addition, treatment with the β2AR agonist fenoterol activated PPARγ/PDX-1/GLUT2 signaling, indicating that, at least in part, β2AR controls insulin secretion through this pathway. Indeed, in this study we show that exogenous PPARγ expression in INS-1E β-cells silenced for β2AR led to recovery of PDX-1/GLUT2 levels and glucose-stimulated insulin secretion. This finding is supported by recent evidence that directly relates β2AR to PPARγ (42,45–47), a key element in the process of insulin secretion in β-cells.

FIG. 5. β2AR ex vivo infection rescued age-dependent impairment of β-cell function. Density (A) and mRNA levels (B) of β2AR were evaluated on cell membranes of islets isolated from β2AR−/− mice. Insulin release (C) was determined upon exposure to the indicated concentration of glucose or KCl as described in RESEARCH DESIGN AND METHODS. mRNA levels of PDX-1 (D), GLUT2 (E), and PPARγ (F) were determined by real-time RT-PCR using the pooled total RNAs from five mice/group with cyclophilin as internal standard. Each bar represents means ± SE of five independent experiments in each of which reactions were performed in triplicate. Islets isolated from β2AR−/− mice were solubilized and aliquots of the lysates were blotted with PDX-1, GLUT2, and PPARγ antibodies. Actin was used as loading control. The autoradiographs shown (G) are representative of three independent experiments, which are quantified in H. *P < 0.05 vs. β2AR−/− 6 months, Bonferroni post hoc test.

### TABLE 2
Metabolic effects of β2AR overexpression in aged (20 months old) β2AR−/− mice

<table>
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<th></th>
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<th>6 months old</th>
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<tr>
<td></td>
<td>Untreated</td>
<td>AdEmpty</td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>38.4 ± 1.7*</td>
<td>38.1 ± 2.4*</td>
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<tr>
<td>Food intake (g/day)</td>
<td>4.0 ± 1.1*</td>
<td>4.2 ± 1.8*</td>
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<tr>
<td>Water intake (mL/day)</td>
<td>6.8 ± 1.2*</td>
<td>6.9 ± 1.7*</td>
</tr>
<tr>
<td>Random-fed blood glucose (mg/dL)</td>
<td>176.5 ± 8.6</td>
<td>178.4 ± 12.7</td>
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<tr>
<td>Fasting blood glucose (mg/dL)</td>
<td>84.2 ± 10.4</td>
<td>83.6 ± 11.9</td>
</tr>
<tr>
<td>Fasting serum insulin (ng/mL)</td>
<td>0.32 ± 0.04*</td>
<td>0.33 ± 0.05*</td>
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</table>

Data are means ± SE unless otherwise indicated. *P < 0.05 vs. adult (6 months old) β2AR−/− mice.
FIG. 6. Adenoviral vector-mediated β₂AR gene transfer in the mouse pancreas rescued age-related reduction in glucose tolerance. Blood glucose levels (A) and serum insulin (C) after 120 min of glucose administration (n = 14–18 animals per group). We calculated the AUC from glucose (B) and insulin excursion (D) curves. Twenty-month-old β₂AR⁻⁻ mice showed glucose intolerance (A and B), impaired insulin secretion (C and D), and also an impairment in β-cell function, evaluated measuring the peak insulin–to–peak glucose ratio (E). All of these parameters were restored after Adβ₂AR in vivo infection. *P < 0.05 vs. β₂AR⁺⁺ at 6 months (mos) of age, Bonferroni post hoc test. (See also Supplementary Figs. 5 and 6.)
secretion that has also recently been investigated in aging (43,48). Our results are consistent with these observations, sustaining also the hypothesis that cAMP levels could act as a connecting link through which β2AR signaling leads to activation of PPARγ (49,50). Moreover, the cAMP assays, performed both in INS-1E11β2AR pancreatic β-cells and in islets isolated from aged mice, showed an impairment in basal conditions and after stimulation with isoproterenol, while the responses to NaF and forskolin were not affected. Also, Goαs and AC-VI protein levels were not significantly different among the explored settings. This combination of events is usually observed in models of β2AR gene deletion or impaired β2AR signaling (18,34).

Whether and to what extent β2AR gene knockout in liver and peripheral tissues affects glucose homeostasis in the β2AR/−/− mice remain to be conclusively addressed. Indeed, variations at the β2AR locus have also been reported to associate with insulin resistance in type 2 diabetic patients (16). However, as shown in this work, the impaired glucose tolerance of β2AR/−/− mice is likely contributed by the defective β-cell function, as indicated by the major effect of β2AR lack on glucose-evoked insulin secretion.

In humans, glucose tolerance declines with age, resulting in a high prevalence of type 2 diabetes and impaired glucose tolerance in the elderly population (2,8). Now, at the individual level, glucose tolerance declines remains unclear, but it is likely determined by multiple factors including diminished insulin secretion (3,7). In rat models and in humans, a progressive decline in β-cell activity with age has been documented (4,6). In the present work, we show that the same occurs in the C57Bl/6N mouse and is paralleled by the development of abnormal glucose tolerance. Similar to previous findings in several human tissues (18–21,24), our results show that these changes are accompanied by reduced β2AR levels in mouse pancreatic islets. The decreased β2AR density in islets from aged mice recapitulates the mechanisms leading to the insulin secretory defect occurring in β2AR-null mice, indicating that it may contribute to the age-related impairment in glucose tolerance. Indeed, both in vivo and ex vivo experiments of β2AR gene transfer revealed that recovery of normal β2AR levels rescued insulin release and glucose tolerance in aged mice. Thus, in the mouse model progressive decline of islet β2AR density appears to contribute to the reduction in glucose tolerance that accompanies aging. Whether the same also occurs in humans needs to be clarified and is currently under investigation in our laboratory.

In conclusion, we have shown that β2AR physiologically regulates pancreatic β-cell insulin secretion by modulating PPARγ/PDX-1/GLUT2 function. Reduced β2AR expression contributes to the age-dependent deterioration of glucose tolerance.

ACKNOWLEDGMENTS

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No potential conflicts of interest relevant to this article were reported.

G.S. conceived the project, performed experiments, analyzed data, and wrote the manuscript. A.L. performed experiments, analyzed data, and wrote the manuscript. D.S. performed experiments and contributed to discussion. A.A. performed experiments. C.D.G. performed experiments. P.F. analyzed data and contributed to discussion. F.B. analyzed data and wrote the manuscript. B.T. designed research and supervised the project. C.M. analyzed data and wrote the manuscript. G.I. designed research, analyzed data, and wrote the manuscript. G.S. and G.I. are the guarantors of this work and, as such, had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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## Supplementary Table 1. shRNA sequences

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<th>shRNA sequence</th>
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<tr>
<td>shRNA scramble</td>
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<td>shRNA (\beta_2)AR</td>
<td>CCACAAGCAAGCCAUCCAGUUA</td>
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<td>shRNA (\beta_2)AR (x)</td>
<td>GGGAGGAAACUGUAACACAAACGACU</td>
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<tr>
<td>shRNA (\beta_2)AR (y)</td>
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## Supplementary Table 2. Real Time RT-PCR primer sequences (5’- 3’)

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<th>Reverse</th>
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</tr>
<tr>
<td>(\beta_2)AR (mouse)</td>
<td>GAGTGTGCAGGACGCAACCAC</td>
<td>CTGTCTGGTTCCCAGTGGGGCC</td>
</tr>
<tr>
<td>PDX-1 (rat and mouse)</td>
<td>AAAACCGTCCATGAAAGTGG</td>
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<td>GLUT2 (rat and mouse)</td>
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<td>PPAR(\gamma) (mouse)</td>
<td>ACGGGGTCTCTGGTTGAGGG</td>
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<td>PPAR(\gamma) (rat)</td>
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<td>TCACCGACTGAGACTTTTCT</td>
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<td>Cyclophilin (rat and mouse)</td>
<td>GCAGACAAAGTTCCAAGACAG</td>
<td>CACCCTGGCAGATGAATCC</td>
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Supplementary Figure 1. Blood glucose and plasma glucagon assessed after intraperitoneal insulin administration. Blood glucose (A) and plasma glucagon (B), evaluated in basal (random fed) conditions and 30 minutes after intraperitoneal administration of insulin (0.75 IU/Kg), were not significantly different among the two studied groups of adult (6-month-old) mice. Each bar represents the mean±SE of eight independent experiments. *: p<0.05 vs basal condition; Bonferroni post hoc test.
**Supplementary Figure 2.** β2AR silencing in pancreatic INS-1E β-cells.

To silence the expression of β2AR in INS-1E β-cells, we designed (sequences are shown in Supplementary Table 1) three shRNA, namely sh-β2AR, sh-β2AR(x), sh-β2AR(y). We decide to use the first one, which showed the best effectiveness, inducing a 73.7% decrease in the membrane density of β2AR, also better than the pooled mix of the three sh-β2AR. Each bar represents mean±SE from five independent experiments in each of whom reactions were performed in triplicate. *:p<0.05 vs control; Bonferroni post hoc test).
Supplementary Figure 3. Pharmacological modulation of β2AR.
INS-1E pancreatic β-cells were incubated (1 hour) with the β2AR selective agonist fenoterol (1 µMol/l) or antagonist ICI 118.551 (ICI, 0.1 µMol/l). This latter inhibited the insulin secretory response to 16.7 mMol/l glucose, that was completely rescued by overexpression of PPARγ (A). Neither fenoterol nor ICI determined significant changes in KCl-induced insulin release. Each bar represents the mean±SE from five independent experiments in each of whom reactions were performed in triplicate. (*:p<0.05 vs control, i.e. untreated INS-1E β-cells; Bonferroni post hoc test; basal is glucose 2.8 mMol/l). Fenoterol treatment caused a marked rise in mRNA level of PDX-1 (by 76%), GLUT2 (by 53%) and PPARγ (by 80.5%). On the contrary, ICI determined a significant decrease in mRNA level of the same genes: PDX-1 (by 37%), GLUT2 (by 48%) and PPARγ (by 53%), compared to untreated INS-1E β-cells (B). Bars represent the mRNA levels in treated cells and are relative to those in control cells; data are expressed as mean±SE of triplicate reactions for total RNA from each group in four independent experiments. Reliable results were obtained from western blot analysis (C-D); representative images (C) of triplicate experiments are shown; actin was used as loading control; bar graph in panel D show densitometry. *:p<0.05 vs control, i.e. untreated INS-1E cells; Bonferroni post hoc test; □: control, i.e. untreated INS-1E cells; ■: fenoterol; □: ICI 118.551; □: ICI 118.551 + PPARγ).
Supplementary Figure 4. Assessment of β2AR downstream. 
In vitro (A-C) and ex vivo (D-F) experiments were performed to investigate cAMP production and β2AR downstream. cAMP levels (A, D) were measured in basal condition and after stimulation with isoproterenol (1 μMol/l), NaF (3 mMol/l) and forskolin 100 μMol/l, as described in RESEARCH DESIGN AND METHODS. Each bar represents the mean±SE of four independent experiments in each of whom reactions were performed in triplicate. We found an impaired cAMP production in basal conditions and after stimulation with isoproterenol both in INS-1E_{shβ2AR} β-cells (A, *:p<0.05 vs unstimulated INS-1E β-cells; #:p<0.05 vs control) and in islets of Langerhans isolated from 20-month-old β2AR^{+/+} mice (D, *:p<0.05 vs unstimulated islets; #:p<0.05 vs β2AR^{+/+} 6 mos). We assessed by Western blot (B,E) protein levels of adenylate cyclase type VI (AC-VI), GRK2 and Gαs on membrane extracts, using chlatrin heavy chain (chlatrin HC) as loading control. Representative images of three independent experiments are shown. We quantified blots by densitometry in INS-1E pancreatic β-cells (B, : sh-scramble; : sh-β2AR; *:p<0.05 vs sh-scramble) and in isolated islets (E, : 6 mos, : 20 mos; *:p<0.05 vs β2AR^{+/+} 6 mos). Control indicates INS-1E β-cells not treated with shRNA; AU indicates arbitrary units; mos is months of age.
**Supplementary Figure 5.** Efficacy of $\beta_2$AR *in vivo* infection. Assessment of $\beta_2$AR expression on cell membranes of pancreatic tissue from $\beta_2$AR$^{+/+}$ mice after $\beta_2$AR overexpression *in vivo*. $\beta_2$AR density (A) was evaluated by radioligand assay. mRNA levels of $\beta_2$AR (B), PDX-1 (C), GLUT2 (D) and PPAR$\gamma$ (E) were determined by Real-time RT-PCR using the pooled total RNAs from four mice/group, using cyclophilin as internal standard. AdEmpty: Pancreata infected with a control adenovirus; Ad$\beta_2$AR: Pancreata infected with an adenovirus encoding for the human $\beta_2$AR gene. Each bar represents the mean±SE of four independent experiments in each of whom reactions were performed in triplicate; ■: 6 mos, □: 20 mos, ■■: 20 mos Adempty, ■■■: 20 mos Ad$\beta_2$AR; *:p<0.05 vs $\beta_2$AR$^{+/+}$ 6 mos; Bonferroni *post hoc* test; mos is months of age.
Supplementary Figure 6. Effect of Adβ2AR pancreatic injection on liver and muscle. The localized injections in the distal pancreas do not induce β2AR expression in other tissues. Density (A, C) and mRNA levels (B, D) of β2AR were evaluated on cell membranes of liver and quadriceps muscle (C, D) from β2AR<sup>+/+</sup> mice. mRNA levels were determined by Real-time RT-PCR using the pooled total RNAs from four mice/group with cyclophilin as internal standard. Each bar represents the mean±SE of five independent experiments in each of whom reactions were performed in triplicate. □: 20 mos, □□: 20 mos Adempty, □■: 20 mos Adβ2AR; AdEmpty: Mice infected with a control adenovirus; Adβ2AR: Mice infected with an adenovirus encoding for the human β2AR gene; mos is months of age.