Critical roles for the TSC-mTOR pathway in β-cell function

Hiroyuki Mori,1,2 Ken Inoki,1,2 Darren Opland,3,4 Heike Münzberg,3 Eneida C. Villanueva,2,3 Miro Faouzi,3 Tsuno Ikenoue,1 David J. Kwiatkowski,5 Ormond A. MacDougald,2,3 Martin G. Myers, Jr.,3,4 and Kun-Liang Guan1,6,7,8

1Life Sciences Institute, 2Department of Molecular and Integrative Physiology, 3Department of Medicine, 4Program in Neuroscience, 5Department of Biological Chemistry, and 7Institute of Gerontology, University of Michigan, Ann Arbor, Michigan; 3Division of Translational Medicine, Department of Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts; and 4Department of Pharmacology and Moores Cancer Center, University of California San Diego, La Jolla, CA

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TSC1 is a tumor suppressor that associates with TSC2 to inactivate Rheb, thereby inhibiting signaling by the mammalian target of rapamycin (mTOR) complex 1 (mTORC1). mTORC1 stimulates cell growth by promoting anabolic cellular processes, such as translation, in response to growth factors and nutrient signals. To test roles for TSC1 and mTORC1 in β-cell function, we utilized Rip2/Cre to generate mice lacking Tsc1 in pancreatic β-cells (Rip-Tsc1cko mice). Although obesity developed due to hypothalamic Tsc1 excision in older Rip-Tsc1cko animals, young animals displayed a prominent gain-of-function β-cell phenotype prior to the onset of obesity. The young Rip-Tsc1cko animals displayed improved glycemic control due to mTOR-mediated enhancement of β-cell size, mass, and insulin production but not determinants of β-cell number (proliferation and apoptosis), consistent with an important anabolic role for mTOR in β-cell function. Furthermore, mTOR mediated these effects in the face of impaired Akt signaling in β-cells. Thus, mTOR promulgates a dominant signal to promote β-cell/fislet size and insulin production, and this pathway is crucial for β-cell function and glycemic control.

Tuberous sclerosis complex; mammalian target of rapamycin; pancreatic β-cell; conditional knockout mice; rat insulin promoter 2
We examined that constitutive activation of mTOR in β-cells leads to embryonic lethality (16, 19, 27), we generated mice conditionally lacking Tsc1 in pancreatic β-cells using Rip2/Cre [Rip-Tsc1-knockout (KO) mice]. Although older Rip-Tsc1KO animals displayed hyperphagia and obesity due to the activation of mTOR in Rip/Cre-expressing hypothalamic neurons (26), we examined β-cell function in younger mice prior to the onset of obesity and insulin resistance. These studies demonstrated that constitutive activation of mTOR in β-cells by Tsc1KO causes β-cell hypertrophy, increased insulin synthesis and release, and consequent hyperinsulinemia. These results suggest that the cellular TSC-mTOR pathway regulates insulin production as well as the function of appetite-suppressing neural circuits.

**MATERIALS AND METHODS**

**Animals and animal care.** Tsc1lox/lox mice, with exons 17 and 18 of Tsc1 flanked by loxP sites by homologous recombination, have been described (25, 38). We generated β-cell and hypothalamic-specific Tsc1-knockout mice (Rip-Tsc1KO) by breeding Tsc1lox/lox mice with mice that express the Cre recombinase gene under the control of the rat insulin 2 gene promoter (The Jackson Laboratory). Mice were maintained on the mixed genetic background (C57Bl/6 × 129Sv × BALB/c). We performed experiments using Rip-Cre/Tsc1lox/lox (Rip-Tsc1KO) mice and littermates Tsc1lox/lox as control. Mice were housed on a 12:12-h light-dark cycle in the Unit for Laboratory Animal Medicine at the University of Michigan with free access to water and standard mouse chow. Animal experiments were conducted following protocols approved by the University Committee on the Use and Care of Animals.

**In vivo physiological studies.** Blood glucose levels were determined using an automated blood glucose reader (Accu-Check; Roche). Serum insulin and leptin levels were measured by ELISA (Crystal Chem, Downers Grove, IL). Glucose tolerance tests were performed on mice that were fasted overnight (16 h). Blood was collected immediately before as well as 15, 30, 60, and 120 min after the intraperitoneal injection of glucose (2 g/kg body wt). For insulin tolerance tests, mice were fasted for 3 h and injected with 0.75 U/kg body wt of human insulin (Novolin R; Novo Nordisk). For glucose-stimulated insulin secretion experiments, mice were deprived of food for 16 h and then anesthetized with pentobarbital sodium. Blood samples were taken from tail vein at 0, 1, 2, 5, 10, and 15 min after the intravenous glucose injection (2 g/kg body wt). Insulin levels were measured as described above. Serum glucose levels were measured by ELISA (Linco Research). Serum glucagon levels were measured by Hormone Assay & Analytical Services Core, MMPC, Vanderbilt University [National Institutes of Health (NIH) Grant no. DK-59637].

**Rapamycin treatment studies.** Rapamycin (LC Laboratories) was initially dissolved in 100% ethanol, stored at −20°C, and further diluted in an aqueous solution of 5.2% Tween-80 and 5.2% PEG 400 (final ethanol concentration 2%) immediately before use (41). Two-week-old Rip-Tsc1KO and control mice were injected with rapamycin intraperitoneally (1 mg/kg body wt every other day) for 5 or 6 wk. Both pancreas and brain tissues for Western blot analysis, RTPCR, and immunohistochemistry (IHC) were harvested after 12 h of the last treatment of rapamycin.

**Insulin content measurement.** For measurement of insulin content of whole pancreas, excised pancreata were frozen in liquid nitrogen and disrupted. Then the pancreas powder was suspended in cold acid-ethanol, and insulin was extracted overnight at 4°C. The supernatants were diluted and subjected to ELISA as described above. Pancreas insulin content was determined using a rat insulin ELISA kit following acid-ethanol extraction.

**RESULTS**

**IHC.** Pancreatic tissue was harvested following transcardiac perfusion with 4% paraformaldehyde and postfixed in 4% paraformaldehyde. Pancreatic sections were stained with antibodies against insulin and glucagon (DakoCytomation), phosphorylated S6 (p-S6; Ser235/236, Cell Signaling Technology), p-S6 (Ser235/236 Alexa Fluor 488 conjugate for double staining of pancreas), and β-catenin (Cell Signaling Technology). For analysis of pancreatic morphometry, we scored six animals/genotype and nine sections/animal spaced 100 μm apart using NI Vision Assistant (version 7.1.0; National Instruments). We also used ApopTag (Millipore) for terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay, Ki-67 antibody (Leica), and ProLong Gold antifade with 4′,6-diamidino-2-phenylindole (intronvite) for proliferation assay.

**Statistical analyses.** All data are presented as means ± SE and were analyzed by Student’s t-test or analyses of variance. The differences were considered to be significant if P < 0.05.
mice and littermate Tsc1lox/lox (control) mice. Western blotting confirmed markedly reduced Tsc1 expression in isolated islets from Rip-Tsc1cKO mice (Fig. 1A). Tsc2 content was also decreased in Rip-Tsc1cKO mice, consistent with the stabilization of TSC2 by TSC1 (Fig. 1A). We further determined the activation status of mTOR in islets by immunoblotting for p-S6, which serves as a convenient downstream readout for mTOR, since activated mTOR stimulates the phosphorylation of S6 by S6K. Loss of Tsc1 was associated with increased S6 phosphorylation (p-S6) in Tsc1cKO islets (Fig. 1A), indicating that mTOR was activated in the mutant islets. Immunostaining for p-S6 showed that, within the pancreas, mTOR was activated only in the β-cells of Rip-Tsc1cKO mice and not the neighboring cells (Fig. 1, B and C), consistent with a cell-autonomous effect of Tsc1 on mTOR. These data also revealed large islets, suggesting the potential expansion of islet mass in the Rip-Tsc1cKO mice. Tsc1 protein levels in brain, heart, kidney, liver, and muscle were indistinguishable between Rip-Tsc1cKO and control mice (data not shown). In addition to Tsc1 deletion in islets, we demonstrated previously that Rip-Tsc1cKO mice demonstrate Tsc1 excision and subsequently increase p-S6 immunoreactivity in the hypothalamus (Fig. 1D) (26).

Normal insulin responsiveness in 4-wk-old Rip-Tsc1cKO mice. The hypothalamic deletion of Tsc1 in Rip-Tsc1cKO mice leads to obesity and insulin resistance in older animals (26). Since obesity and insulin resistance would confound our examination of β-cell function in these animals, we initially examined insulin levels and parameters of glycemic control with age in Rip-Tsc1cKO mice to determine an appropriate young age prior to the onset of obesity at which to examine β-cell function (Fig. 2). We focused on animals 4 wk of age prior to the onset of obesity (Fig. 2A) and compared their phenotype to that of older animals. Serum insulin concentrations in Rip-Tsc1cKO mice were elevated relative to controls at all ages examined, although this was most dramatic in the older (8- and 24-wk-old) animals, consistent with the dramatic obesity and insulin resistance in these older animals (Fig. 2B). Indeed, whereas older Rip-Tsc1cKO mice displayed normal or increased blood glucose levels despite dramatically increased circulating insulin levels (consistent with insulin resistance), 4-wk-old Rip-Tsc1cKO mice demonstrated decreased blood glucose relative to controls (Fig. 2C). These data are consistent with the hypersecretion of insulin in young Rip-Tsc1cKO relative to the wild-type mice (4-wk-old; Fig. 2D), indicating a normal insulin response in the Rip-Tsc1cKO animals. To directly examine insulin sensitivity in these animals, insulin tolerance tests were performed. Consensurate with their obesity, by 8 wk of age, Rip-Tsc1cKO mice exhibited an impaired hypoglycemic response to exogenous insulin. Consistently, insulin-induced Akt phosphorylation in both liver and muscle were significantly compromised in Rip-Tsc1cKO mice at 12 wk of age along with the expression of IRS-1 protein in muscle (Supplemental Fig. S1; Supplemental Material for this article is available at the AJP-Endocrinology and Metabolism website). The finding that pair-feeding Rip-Tsc1cKO and control mice normalized the insulin responsiveness of Rip-Tsc1cKO animals (Fig. 2E) suggests that the insulin resistance of the older Rip-Tsc1cKO mice results from their increased adiposity rather than some other alteration (such as hypothalamic function).

In contrast to the insulin resistance in older animals, blood glucose levels fell at a similar rate and to a similar extent in Rip-Tsc1cKO compared with control mice at 4 wk of age (Fig. 2D, left). Thus, consistent with the reduction in blood glucose levels appropriate for the hyperinsulinemia displayed by 4-wk-old Rip-Tsc1cKO mice, these young animals exhibit normal insulin sensitivity, suggesting that the elevated insulin levels in young Rip-Tsc1cKO mice reflect a primary enhancement in

A

<table>
<thead>
<tr>
<th>Tsc1</th>
<th>Tsc2</th>
<th>p-S6 (Ser240/244)</th>
<th>S6</th>
<th>α-Tubulin</th>
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<td>Rip-cKO</td>
<td>Ct.</td>
<td>Islets</td>
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B

IHC: p-S6 (Ser235/236)

Low mag.

High mag.

Rip-cKO

control

C

Islet

Insulin

p-S6 Ser235/236

merge

D

Hypothalamus

IHC: p-S6

3V ME

3V

3rd ventricle

Rip-cKO

control

Fig. 1. Tuberous sclerosis complex 1 (Tsc1) knockout (KO) by Rip-Cre activates mammalian target of rapamycin (mTOR) complex 1 in islets. A: elevated S6 phosphorylation in Tsc1-knockout islets. Immunoblot analysis of Tsc1, Tsc2, phospho-S6 (p-S6), S6, and α-tubulin are indicated. Islets were isolated and extracts prepared from independent animals. B: S6 phosphorylation in Tsc1cKO β-cells. Immunostaining of the pancreatic sections from 4-wk-old Rip-Tsc1cKO and control mice were performed with p-S6 (Ser235/236) antibody. The scale bar represents 500 (low mag., low magnification) and 100 μm (high mag., high magnification). C: S6 phosphorylation was observed in Tsc1cKO β-cells. Double labeling of the pancreatic sections from 4-wk-old Rip-Tsc1cKO and control mice were performed with p-S6 (Ser235/236) antibody and insulin antibody. The scale bar represents 100 μm. D: Rip-Tsc1cKO increases S6 phosphorylation in hypothalamus. Representative p-S6 (Ser235/236) staining of hypothalamus from ad libitum-fed Rip-Tsc1cKO and control mice at 4-wk ages. Ct., control; Rip-cKO, Rip-Tsc1cKO, which is Tsc1 tissue-specific knockout using RipCre; IHC, immunohistochemistry; ME, median eminence; 3V, 3rd ventricle.
insulin production and that 4 wk represents an appropriate age to examine islet function without confounding effect of hypothalamic obesity.

*Increased β-cell size in 4-wk-old Rip-Tsc1cKO mice.* Given the apparent enlargement of islets observed in the p-S6 staining (Fig. 1B), we examined the morphology of the islets in Rip-Tsc1cKO mice more carefully (Fig. 3). As shown in Fig. 3A, the average islet area was increased significantly in Rip-Tsc1cKO pancreas at 4 wk of age compared with that of control littersmates. Consistently, the distribution of islet size was skewed toward larger islets in Rip-Tsc1cKO pancreas (Fig. 3B), and protein content per islet (not normalized for size) was increased almost twofold in islets isolated from Rip-Tsc1cKO mice compared with those from control mice (Fig. 3C). To determine whether the increase in islet mass reflected increased cell size (rather than cell number), the size of individual β-cells was also measured. We used insulin staining to mark the β-cells and β-catenin staining to highlight cell boundaries. We found that β-cells in Rip-Tsc1cKO were significantly larger than those in control mice (Fig. 3D). In contrast, Rip-Tsc1cKO had no effect on β-cell number (Fig. 3E). Furthermore, we did not observe significant differences in the proportion of Ki-67-positive nuclei or TUNEL-positive nuclei in the islets between control and Rip-Tsc1cKO mice at 4 wk of age (Fig. 3, F and G). Therefore, ablation of Tsc1 and subsequent mTOR activation primarily promotes increased cell growth/size rather than proliferation or number of β-cells. Consistent with this idea, there was no evidence of invasiveness of islet tissue.

*Increased mTOR activity in β-cells from Rip-Tsc1cKO mice promotes insulin production.* To test whether the phenotype of the pancreatic β-cells in Rip-Tsc1cKO animals is mediated by mTOR activation, we treated 2-wk-old Rip-Tsc1cKO and control mice with rapamycin, a specific mTOR inhibitor, at 1 mg/kg every other day for 2 wk (until 4 wk of age). Rapamycin treatment abrogated the increased mTOR activity in the mutant pancreas β-cells, as determined by the p-S6 immunostaining (Fig. 4A). In contrast, treatment with this low dose of rapamycin failed to normalize p-S6 immunoreactivity in the hypothalamus of Rip-Tsc1cKO animals (Fig. 4B) and did not restore feeding regulation or adiposity in these animals (Supplemental Fig. S2) (26). Thus, the effects of treating Rip-Tsc1cKO animals with 1 mg/kg rapamycin were not attributable to normalization of hypothalamic mTOR signaling but rather to the inhibition of hyperactive mTOR in the β-cells.

Serum insulin levels were higher in Rip-Tsc1cKO mice compared with control mice in both fasting and fed states (Fig. 4C). Rapamycin treatment significantly decreased the serum insulin levels in Rip-Tsc1cKO mice, making them similar to control mice (Fig. 4C).

We performed intraperitoneal glucose tolerance tests in the 4-wk-old rapamycin-treated and control mice. As shown in Fig. 4D, the injected glucose was cleared more rapidly in Rip-Tsc1cKO mice compared with controls. In both genotypes, the rapamycin-treated mice showed impairments in their ability to clear blood glucose. Insulin secretion in response to glucose was also dramatically increased in Rip-Tsc1cKO mice compared with that in control mice (Fig. 4E). This observation is consistent with the higher insulin level in the Rip-Tsc1cKO mice (Figs. 2A and 4C). Rapamycin treatment diminished the insulin secretion in response to glucose injection in both genotypes (Fig. 4E). A sharp increase of the serum insulin levels by glucose injection in Rip-Tsc1cKO mice suggests that
glucose-sensing and insulin secretion mechanisms in mutant \( β \)-cells are preserved.

**Normoglycemia in Rip-Tsc1cKO mice at 4 wk of age.** Surprisingly, despite a large increase in insulin levels in Rip-Tsc1cKO mice, the blood glucose levels were only modestly decreased relative to controls (Figs. 2B and 5A). Since insulin sensitivity in 4-wk-old animals was similar between genotypes, we examined circulating levels of the counterregulatory hormones glucagon and growth hormone (Fig. 5, B and C). Interestingly, both glucagon and growth hormone were increased significantly in Rip-Tsc1cKO mice compared with those of control mice, indicating that to maintain euglycemia, Rip-Tsc1cKO mice produce more glucagon and growth hormone to counterbalance the glucose-lowering effect of the elevated insulin. Consistent with this idea, rapamycin treatment normalized both glucagon and growth hormone levels concomitant with the reduction in insulin levels. These results suggest that Tsc1 deletion mediated by Rip/Cre results in increased \( β \)-cell size and islet mass, and higher insulin content and secretion by \( β \)-cells due to activation of mTORC1 signaling.

**Increased insulin production in Rip-Tsc1cKO mice.** Next, insulin content in pancreas was measured to determine the ability of Tsc1cKO \( β \)-cells to synthesize insulin. We found that Tsc1cKO pancreas had approximately twice as much insulin as controls (Fig. 6A). Rapamycin treatment significantly decreased the insulin content in Rip-Tsc1cKO mice and had a similar, albeit milder, effect in control mice. Importantly, both Rip-Tsc1cKO and control mice showed similar pancreatic insulin content under rapamycin treatment. These results demonstrate that mTORC1 plays an important role in normal insulin synthesis in \( β \)-cells, and the constitutive mTORC1 activation in Rip-Tsc1cKO mice is responsible for the high insulin levels in both the Tsc1KO \( β \)-cells and serum.

We also studied insulin release from size-matched isolated islets from Rip-Tsc1cKO and control mice (Fig. 6B). In response to high glucose, there was no significant difference in insulin secretion in Rip-Tsc1cKO islets compared with that of control islets. Given the overall increase in \( β \)-cell and islet size, these data suggest a primary effect of mTORC1 activation on \( β \)-cell mass and insulin content, rather than insulin secretion, to mediate increased circulating insulin in the Rip-Tsc1cKO animals.

Immunoblotting for crucial signaling mediators of mTOR and \( β \)-cell function from control and Rip-Tsc1cKO mice revealed that phosphorylation of 4E-BP1, S6k, and S6 was increased in Rip-Tsc1cKO islets (Fig. 6C). As expected, the phosphorylation of S6k in both Rip-Tsc1cKO and control islets was inhibited by rapamycin (data not shown). We also observed that Akt phosphorylation (Ser\(^{473} \) and Thr\(^{308} \)) was decreased dramatically in the Rip-Tsc1cKO islets, consistent with the negative feedback effect of high S6k in the Tsc1-knockout cells (Fig. 6D). Indeed, although we detected no increase in the phosphorylation of IRS-1 on Ser\(^{307} \) or decrease in IRS-1 or IRS-2 expression, the electrophoretic
mobility of IRS-2 was reduced in Rip-Tsc1cKO samples, consistent with increased serine phosphorylation (2), and this change was reversed by rapamycin treatment. The increased serine/threonine phosphorylation of IRS-2 in Rip-Tsc1cKO islets may blunt IRS-2-dependent signaling to Akt and other downstream effectors. Akt signaling, which depends upon its phosphorylation, promotes ß-cell size as well as proliferation and survival. Thus, the mTOR activation following Tsc1 ablation increases ß-cell mass and insulin production to augment glycemic control even in the face of blunted Akt signaling.

**DISCUSSION**

We have generated Rip-Tsc1cKO mice, in which Rip2/Cre deletes the Tsc1 gene in both pancreatic ß-cells and the
Fig. 5. Normoglycemia in Rip-cKO mice at 4-wk of age. A: blood glucose levels. Overnight fasting (16 h) or ad libitum-fed blood glucose levels were measured in 4-wk-old male mice. Control or Rip-cKO mice were treated with or without rapamycin as indicated (1 mg/kg body wt; n = 16–21). B: serum glucagon levels of fed-state 4-wk-old mice of each group with or without rapamycin (n = 5–8). C: serum growth hormone levels of fed-state 4-wk-old mice of each group with or without rapamycin (n = 11–16). Graphs show means ± SE. *P < 0.05; **P < 0.01.

hypothalamus, and studied islet and β-cell function in these animals. Although it is well known that mutation of Tsc1 causes tumors in multiple organs, there was no evidence of tumors in the pancreas or brain during the observation periods of this study (6 mo). Although we noted the development of prolactin-producing pituitary tumors in Rip-Tsc1cKO mice at advanced ages, this is unrelated to the phenotypes described in this report because the tumor occurred in only a few animals and at a later stage, and the prolactin levels were normal in young Rip-Tsc1cKO animals (data not shown).

Previous studies have established important roles of insulin signaling in maintaining β-cell size, cell number, and insulin secretion (5, 9, 17, 18, 21). Mutations of several genes in the insulin pathway, such as Irs (5, 17, 21), Pten (28, 36), and Pdk1 (9), affect multiple pathways, including mTOR. In contrast, Tsc1 mutation specifically activates mTORC1. The Irs2 Rip-Cre (Rip-Irs2cKO) mice showed insulin deficiency with a failure of β-cell growth (5, 17, 21), whereas Pten Rip-Cre (Rip-PtencKO) mice displayed increased islet mass and β-cell numbers. The use of rapamycin to inhibit mTOR has suggested crucial roles for mTOR in β-cell function. Not only does rapamycin inhibit β-cell proliferation in vitro, it also blocks the effects of Akt activation on β-cell mass and proliferation (2, 20, 22). Here, we utilize genetic ablation of the mTOR inhibitor Tsc1 to examine mTOR function in β-cells.

We observed significant increases in islet mass, β-cell size, and insulin content in Rip-Tsc1cKO mice; these effects were counteracted by rapamycin treatment, indicating the critical role of mTORC1 activation in β-cell mass. Although both islet and β-cell size were increased in the Rip-Tsc1cKO mice, the number of β-cells per islet was not increased, suggesting that mTOR activation increases cell growth but not β-cell number. Indeed, the similar number of Ki-67- and TUNEL-reactive cells in islets from Rip-Tsc1cKO and control mice is consistent with unaltered proliferation and apoptosis with mTOR activation in Rip-Tsc1cKO islets. By contrast, others have shown that inhibition of mTOR decreases β-cell proliferation and numbers, including in response to Akt activation (2, 3, 22, 46). Thus, although mTOR signaling appears to be required for cell proliferation, it is not sufficient; this finding is consistent with the results of others and with the low oncogenic potential of the tumors in TSC (10, 35). Importantly, however, our data reveal that increased mTOR signaling suffices to promote increased β-cell size and pancreatic islet mass even in the face of reduced baseline Akt signaling, consistent with the previous findings of others suggesting that mTOR mediates important aspects of the response to the insulin-like signaling pathway and Akt.

The enlarged β-cell size and increased pancreatic insulin content in Rip-Tsc1cKO mice likely results from increased overall protein synthesis, including insulin synthesis, that is driven by high mTOR activity. Although our data do not reveal an increase in per-islet insulin secretion in Rip-Tsc1cKO islets that are size-matched to controls (suggesting that mTOR may minimally effect the insulin secretory machinery), the overall increased pancreatic islet mass and insulin content result in the greater release of insulin in Rip-Tsc1cKO mice compared with controls. Although Rip-Cre has been reported to blunt insulin secretion even in the absence of conditional alleles, this is unlikely to be a factor in the Rip-Tsc1cKO mice because they exhibit increased first-phase insulin secretion in vivo. Also, although rapamycin treatment may promote the increased sensitivity on target tissues to insulin, the decreased glucose tolerance of rapamycin-treated animals, coupled with the decreased secretion of insulin, suggests a primary effect on β-cell function in this case. Thus, although the TSC-mTOR pathway does not mediate β-cell proliferation as does the PI3K pathway, mTOR plays a crucial role in the regulation of β-cell mass and islet function by controlling β-cell size and insulin production.

Our observations of β-cell hypertrophy and hyperinsulinemia are consistent with Tsc2 deletion studies in pancreatic β-cells (31, 35). However, Tsc2 knockout by Rip/Cre did not affect body weight, and the knockout mice maintain normal insulin sensitivity at advanced age. In contrast, we found that Rip-Tsc1cKO mice show obesity and insulin resistance. Furthermore, we observed that Tsc1 knockout increased only β-cell size and not cell proliferation/apoptosis/numbers, whereas Rachdi et al. (31) reported that Tsc2 knockout also increased β-cell proliferation. These differences could be due to a functional difference between TSC1 and TSC2, although it is generally believed that TSC1 and TSC2 function together as a complex. Alternatively, the Rip/Cre expression, especially in the hypothalamus, may be influenced by genetic background. Also, if the Tsc2cKO animals displayed some mild, undetected increase in adiposity in the older animals studied, it is possible that the stimulus of mild insulin resistance may have promoted some increase in β-cell proliferation.
Shegeyama et al. (35) reported that Tsc2 knockout by Rip/Cre resulted in mice that had epileptic seizures and died around 3 wk of age. It is not clear why the Tsc2 Rip/Cre knockout by two groups produced vastly different phenotypes. Our Tsc1 knockout mice by Rip/Cre have normal viability up to 6 mo of age. Consistent with our observations, Tsc2 knockout increased cell size but not cell numbers at young ages (31, 35). Furthermore, transgenic mice that express Rheb under the control of the rat insulin promoter were reported to have a similar phenotype with increased β-cell mass but without increased β-cell proliferation (8). Therefore, our data with Tsc1 and the majority of other observations with Tsc2 suggest that mTOR plays a major role in β-cell size but not proliferation.

In the prediabetic stage, before β-cell failure and progression to type 2 diabetes, islet β-cells normally produce more insulin to compensate for the insulin resistance in the peripheral tissues and maintain euglycemia. Indeed, mTOR plays an essential role in promoting pancreatic β-cell function in response to growth factors and nutrients in vitro (44), and the process of prediabetic compensation is similar to that observed here in Rip-Tsc1 cKO mice with constitutive activation of Tsc1.
mTOR. Together, these observations indicate that cellular mTOR signaling promotes β-cell growth and function and likely plays an important role in the compensatory response to insulin resistance.

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REFERENCES