Deletion of Glutamate Dehydrogenase in β-Cells Abolishes Part of the Insulin Secretory Response Not Required for Glucose Homeostasis*§

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Insulin exocytosis is regulated in pancreatic β-cells by a cascade of intracellular signals translating glucose levels into corresponding secretory responses. The mitochondrial enzyme glutamate dehydrogenase (GDH) is regarded as a major player in this process, although its abrogation has not been tested yet in animal models. Here, we generated transgenic mice, named βGld1⁻/⁻, with β-cell-specific GDH deletion. Our results show that GDH plays an essential role in the full development of the insulin secretory response. In situ pancreatic perfusion revealed that glucose-stimulated insulin secretion was reduced by 37% in βGld1⁻/⁻ mice. Furthermore, isolated islets with either constitutive or acute adenovirus-mediated knock-out of GDH showed a 49 and 38% reduction in glucose-induced insulin release, respectively. Adenovirus-mediated re-expression of GDH in βGld1⁻/⁻ islets fully restored glucose-induced insulin release. Thus, GDH appears to account for about 40% of glucose-stimulated insulin secretion and to lack redundant mechanisms. In βGld1⁻/⁻ mice, the reduced secretory capacity resulted in lower plasma insulin levels in response to both feeding and glucose load, while body weight gain was preserved. The results demonstrate that GDH is essential for the full development of the secretory response in β-cells. However, maximal secretory capacity is not required for maintenance of glucose homeostasis in normo-caloric conditions.

Pancreatic β-cells produce the hormone insulin that is essential for glucose homeostasis. Upon nutrient stimulation, elevation of cytosolic calcium in the β-cell is the primary and necessary signal for insulin exocytosis (1). Then, increasing the calcium signal supported by metabolism-derived additive factors (2). The enzyme glutamate dehydrogenase (GDH, EC 1.4.1.3) has been proposed to participate to the development of the secretory response. GDH is a homohexamer located in the mitochondrial matrix that catalyzes the reversible reaction: α-ketoglutarate + NH₃ + NAD(P)H ⇄ glutamate + NAD(P)⁺; inhibited by GTP and activated by ADP (3, 4). Regarding β-cell, allosteric activation of GDH by ω-leucine or its non-metabolized analogue BCH has triggered most of the attention over the last three decades (5).

To date, the role of GDH in β-cell function remains unclear and debated. Specifically, GDH might play a role in glucose-induced amplifying pathway through generation of glutamate (6, 7). GDH is also an amino acid sensor triggering insulin release upon glutamine stimulation in conditions of GDH allosteric activation (8–10). Recently, the importance of GDH has been further highlighted by studies showing that SIRT4, a mitochondrial ADP-ribosyltransferase, down-regulates GDH activity and thereby modulates insulin secretion (11, 12).

GDH is encoded by a well-conserved 45-kb gene named GLUD1, which is organized into 13 exons (13). A decade ago, clinical data and associated genetic studies revealed GDH as a key enzyme for the control of insulin secretion. Indeed, mutations rendering GDH more active are responsible for a hyper-insulinism syndrome (14). Mutations producing a less active, or even nonactive, GDH enzyme have not been reported, leaving open the question if such mutations would be either lethal or asymptomatic. Despite these numerous reports on GDH and insulin secretion, abrogation of GDH in β-cells has not been tested in animal models until now. As a consequence, it is not yet established if GDH is in fact required for normal β-cell function and glucose homeostasis.

Here, we generated transgenic mice lacking GDH specifically in β-cells and questioned the putative requirement of GDH for β-cell function as well as the consequences of GDH abrogation on glucose homeostasis.

EXPERIMENTAL PROCEDURES

Transgene Cloning—By screening a RPCI-22 mouse 129 BAC library (MRC Genome Resource Facility, The Hospital of Sick
Deletion of GDH in β-Cells

Children, Toronto, Canada) with a cDNA probe corresponding to human GLUD1 exons 6–8, we obtained two fragments, respectively, of 6 and 10 kb comprising the sequence of Glud1 exon 7. Successively, three DNA fragments of the 129 SvJ Glud1 locus were inserted into the cloning sites of the targeting vector. They comprised 3 kb of the region upstream Glud1 exon 7 (short arm), 0.6 kb of the region containing Glud1 exon 7, and 5.6 kb of the region downstream Glud1 exon 7 (long arm). The three fragments were obtained by PCR using a RPCI-22 129 BAC clone as template and ligated into the KSloxPfrtNeoBS (3’-5’) vector (15). The resulting targeting construct contained two loxP sites flanking Glud1 exon 7, one frt-flanked neomycin resistance cassette, and two homology arms.

Generation of Glud1lox/lox Mouse—The targeting vector (15 μg) was linearized at the single SacII site and electroporated into three batches of 1.7 × 10^7 R1 ES cells, a cell line derived from (129/Sv × 129/Sv-Cp)F1 3.5-day blastocysts (16). Cells were then cultured in the presence of G418 (0.7 mg/ml) for 6 days. Homologous recombination events were identified by PCR and Southern blot analyses, see supporting information. Correctly targeted ES cell clones were injected into C57BL/6J blastocysts and implanted into recipient females (Karolinska Institutet, Stockholm, Sweden). Resulting chimeric mice (F0) were backcrossed to C57BL/6J mice and germline transmission was achieved by transducing islets isolated from control and transgenic Glud1lox/lox mice with the recombinant adeno virus Ad-GDH over a 90-min period as detailed previously (19) and islets were used the next day for secretion assays and control immunoblotting. For acute in vitro GDH knock-out, islets isolated from control and transgenic Glud1lox/lox mice were subjected to adeno viral treatment for 90 min with 0.4 μl/ml of the recombinant adenovirus rAdInsPNCre, thereby enabling expression of nuclear-localized Cre recombinase specifically in β-cells (20). Islets transduced with rAdInsPNCre were kept in culture for 3 days before insulin secretion assays.

Insulin Secretion and Measurements—Prior to the experiments, islets were maintained for 2 h in glucose-free culture medium. For static incubations, islets were then washed and preincubated further in glucose-free Krebs-Ringer bicarbonate HEPES buffer (KRHB, containing in mM: 135 NaCl, 3.6 KCl, 10 HEPES (pH 7.4), 5 NaHCO₃, 0.5 NaH₂PO₄, 0.5 MgCl₂, 1.5 CaCl₂, 0.1% bovine serum albumin). Then, batches of 10 islets were hand-picked and incubated as described (21) for 30 min at 37 °C at basal (2.8 mM) glucose and stimulated with 22.8 mM glucose or 5 mM glutamine plus 10 mM BCH (2-aminobicyclo[2.2.1]heptane-2-carboxylic acid). At the end of the assay period, islets were resuspended in acid-EtOH to determine their insulin contents. For islets perfusions (19), 10 hand-picked islets were put per chamber of 250 μl volume, thermostatted at 37 °C (Brandel, Gaithersburg, MD). The flux was set at 0.5 ml/min, and fractions were collected every minute after a 20-min washing period at basal 2.8 mM glucose. In situ pancreatic perfusion was performed in anesthetized mice as described (22) with a 1.5 ml/min perfusion rate. Pancreatic insulin content was determined in mouse pancreata that were first frozen in liquid nitrogen, then homogenized, resuspended in cold acid ethanol, and left at 4 °C for 48 h with sonication after 24 h. Insulin concentration in these different collected samples was measured by radioimmunoassay (Linco Research Inc., St. Charles, MO).

A bioluminescent assay kit (Roche Applied Science) was used to measure ATP levels in batches of 10 isolated islets following a 30-min incubation period at 37 °C in the presence of 2.8 and 22.8 mM glucose.

The enzymatic activity of GDH in isolated islets was measured as described (19) under the oxidative deamination direction as NADH fluorescence excited at 340 nm and measured at
migrating in SDS-PAGE shows a partial genomic map of wild-type Glud1 locus from exon 5 (E5) to 11 (E11). Individual letters represent restriction sites (B, BamHI; N, Nhel). The lower panel shows the targeted locus after excision of the neomycin-resistance selection cassette (Neo, dark green rectangle) that is flanked by two frt sites (light green rectangle); and replacement of the exon 7 (red rectangle) that is flanked by two loxP sites (orange arrowhead). B, transgenic Glud1lox/lox/lox mice were crossed with RIP-Cre mice resulting in constitutive ß-cell-specific Glud1 knock-out animals (βGlud1−/−). Tissues collected from these animals were analyzed by PCR to detect knock-out (278 bp) and wild-type (246 bp) alleles.

**FIGURE 1.** Conditional Glud1 targeting with ß-cell-specific constitutive Glud1 knock-out. A, upper panel shows a partial genomic map of wild-type Glud1 locus from exon 5 (E5) to 11 (E11). Individual letters represent restriction sites (B, BamHI; N, Nhel). The lower panel shows the targeted locus after excision of the neomycin-resistance selection cassette (Neo, dark green rectangle) that is flanked by two frt sites (light green rectangle); and replacement of the exon 7 (red rectangle) that is flanked by two loxP sites (orange arrowhead). B, transgenic Glud1lox/lox/lox mice were crossed with RIP-Cre mice resulting in constitutive ß-cell-specific Glud1 knock-out animals (βGlud1−/−). Tissues collected from these animals were analyzed by PCR to detect knock-out (278 bp) and wild-type (246 bp) alleles in different organs. Lanes show molecular weight markers (M); genomic DNA from the tail (Tt), brain (Br), liver (Li), pancreas (Pa), muscle (Mu), kidney (K), and negative control (C) without DNA. The 278-bp knock-out allele was detected in pancreas only (n = 3). C, immunoblot analysis was performed by migrating in SDS-PAGE 10 μg per lane of proteins extracted from isolated islets. GDH bands, contributed by both ß- and non-ß-cells, were much lower in βGlud1−/− (−/−) islets compared with control (+/+ and the lower nonspecific band exhibited similar intensity. As GDH standard (std), 50 ng of purified GDH were loaded. D, enzymatic activity of GDH in islets isolated from control and βGlud1−/− mice (n = 4, *, p < 0.05 versus Control).

**RESULTS**

**Generation of Transgenic Mice**—We generated transgenic mice with ß-cell-specific conditional abrogation of GDH using the loxP-flp-recombinase strategy. The targeting vector carried Glud1 (NCBI Access No. P26443) exon 7, comprising the NADH binding site essential for GDH activity (13), flanked by two loxP sites (Fig. 1A). We cloned 5’ and 3’ regions flanking exon 7 as 3-kb and 5.6-kb fragments, respectively. The targeting vector also contained a neo-cassette (positive marker) flanked by two Flp/frt sites. The resulting founder mice were crossed to obtain F1 mice carrying a mutant Glud1 genomic locus containing the exon 7 flanked by loxP sites and the neo-cassette (Glud1lox-neo-lox). Next, excision of the neo-cassette by crossing F1 with Flp deleter mice gave rise to mice carrying intact Glud1 exon 7 flanked by loxP sites only (Glud1lox/lox).

**Constitutive ß-Cell-specific GDH Knock-out**—Glud1lox/lox mice were crossed with mice expressing the Cre recombinase under the control of an insulin promoter (RIP), generating mice with ß-cell-specific GDH knock-out (βGlud1−/−). Allele analysis in different tissues revealed that the 278-bp knock-out
Deletion of GDH in β-Cells

Phenotype and Pancreatic Secretory Responses of β-Cell GDH Knock-out Mice—Surprisingly, β-cell-specific GDH knock-out mice were not diabetic, no glucosuria was detected, and the growth and organ weights were normal compared with control mice (Fig. 2, A and B).

Based on the unexpected normal development of the animals, despite deletion of GDH in β-cells, we measured insulin secretion in the in situ pancreatic perfusion preparation (Fig. 2C), a model that integrates paracrine and neuronal effectors of the β-cell. This physiological model demonstrated clear reduction of the secretory response evoked by 22.8 mM glucose in βGlud1−/− mice versus controls; both during the first (−48%, p < 0.01) and the second (−37%, p < 0.05) phases (Fig. 2D). The data show that abrogation of the mitochondrial enzyme GDH in β-cells limits both the phase of secretion commonly associated with the amplifying pathway (second phase) as well as the triggering pathway (first phase).

In good agreement with basal versus stimulated insulin secretion measured in the perfused pancreas (Fig. 2C), circulating insulin concentrations were not significantly different in the fasted state, but the increase associated with the fed state was reduced by 55% (p < 0.05) in βGlud1−/− mice (Table 1).

Islet Phenotype of Constitutive β-Cell-specific GDH Knock-out—Insulin secretory defects have been associated with modified islet organization in general and infiltration of α-cells within the core of the islet in particular (27). Accordingly, we analyzed islet cell distribution by immunostaining on pancreatic sections (Fig. 3, A and B). Most of the control islets (70%) exhibited the expected α- and β-cell distribution, i.e. insulin β-cells in the core of the islet and glucagon α-cells forming its periphery. In βGlud1−/− animals, the percentage of disorganized islets was as high as 67%, revealing α-cells mixed with β-cells in the core of the islet (Fig. 3C). It should be noted that RIP-Cre transgene does not by itself promote changes in islet morphology (18). Pancreatic insulin contents were not affected in βGlud1−/− animals compared with controls (124.1 ± 24.9 and 125.7 ± 9.1 ng/g pancreas of wet mass, respectively), and islets were similarly distributed in the whole pancreas. Functions of the α-cells were apparently not affected by GDH knock-out in β-cells, as revealed by similar plasma glucagon levels between the two groups under fasting (stimulatory) conditions (Table 1).

A primary function of mitochondrial metabolism is to produce ATP that is necessary in β-cells for triggering (first phase)
and maintaining (second phase) the secretory response. At this point, it could be hypothesized that β-cell GDH knock-out would impair the primary function of mitochondrial activation that is to generate ATP and thereby inhibit glucose-stimulated insulin secretion. ATP levels were slightly lower under basal glucose concentrations (2.8 mM) in islets of βGlud1<sup>−/−</sup> mice. However, upon glucose stimulation (22.8 mM), islet ATP concentrations increased similarly between the two groups (Fig. 3D). GDH knock-out did not affect glucose-induced ATP generation. Interestingly, data suggest that maintenance of basal housekeeping ATP levels under low glucose conditions might be normally contributed by GDH-dependent metabolism.

whether abrogation of GDH could induce compensatory pathways in the β-cells, we also performed acute in vitro knock-out in islets of transgenic floxed mice. Islets isolated from Glud1<sup>lox/lox</sup> mice were transduced with a recombinant adenovirus (rAdNspNCre) expressing the Cre recombinase specifically in β-cells (20). PCR analysis demonstrated in vitro homologous recombination resulting in the knock-out of GDH in β-cells of islets isolated from transgenic Glud1<sup>lox/lox</sup> mice but not in islets from wild-type animals (Fig. 5A).

In control islets, stimulatory glucose concentration (22.8 mM) induced a 5.7-fold (p < 0.01) increase in insulin secretion compared with basal release (Fig. 4A). However, constitutive abrogation of GDH in β-cells resulted in markedly reduced secretory responses, both during first phase (−68%, p < 0.01) and second sustained phase (−49%, p < 0.05). These data demonstrate that GDH is essential for the full development of the secretory response.

To test if the reduced glucose-evoked insulin release observed in βGlud1<sup>−/−</sup> islets was a direct consequence of the absence of GDH, we reintroduced GDH by means of adenoviral transduction (Fig. 4B). Insulin secretion stimulated with 22.8 mM glucose was increased 5.7-fold in control islets (p < 0.01) and only 2.0-fold in βGlud1<sup>−/−</sup> islets (p < 0.05). Following ectopic re-expression of GDH in βGlud1<sup>−/−</sup> islets, amplitude of the secretory response was rescued to similar levels compared with controls (4.9-fold, p < 0.05). As expected from a previous study (19), overexpression of GDH did not significantly modify glucose-induced insulin release compared with control islets (Fig. 4B).

**Deletion of GDH in β-Cells**

Secretonary Responses of Isolated Islets with Constitutive β-Cell GDH Knock-out—Kinetics of secretory responses were analyzed in perifusion experiments on islets isolated from βGlud1<sup>−/−</sup> compared with controls. Basal insulin release measured at 2.8 mM glucose was not affected by the absence of GDH. When stimulated with 22.8 mM glucose, control and βGlud1<sup>−/−</sup> islets exhibited glucose-stimulated insulin release (Fig. 4A). However, constitutive abrogation of GDH in β-cells resulted in markedly reduced secretory responses, both during first phase (−68%, p < 0.01) and second sustained phase (−49%, p < 0.05). These data demonstrate that GDH is essential for the full development of the secretory response.
Deletion of GDH in β-Cells

FIGURE 4. Constitutive β-cell-specific Glud1 knock-out impairs insulin secretion that is rescued by GDH ectopic re-expression. A, after an over-night culture, islets isolated from the two groups were hand-picked and distributed into perfusion chambers. Insulin release was measured in the effluent of chambers perfused first with basal 2.8 mM glucose before stimulation for 20 min with 22.8 mM glucose. Compared with controls, islets isolated from βGlud1<sup>−−/−</sup> mice exhibited marked reduction of the secretory responses during first phase (−68%, p < 0.01) and second sustained phase (−49%, p < 0.05). n = 7. B, islets isolated from βGlud1<sup>−−/−</sup> mice were transduced with adenovirus AdGDH carrying hGLUD1 to restore GDH expression. Insulin secretion was measured over a 15-min period at basal 2.8 mM (Glc 2.8) and stimulatory 22.8 mM glucose (Glc 22.8) on batches of 10 islets. *p < 0.05 versus control Glc 22.8, §p < 0.05 versus corresponding group at Glc 2.8 (n = 3).

FIGURE 5. Acute in vitro β-cell-specific Glud1 knock-out impairs insulin secretion. A, acute β-cell Glud1 knock-out assessed by PCR analysis of genomic DNA obtained from islets isolated from wild-type (Wt) and transgenic Glud<sup>lox/lox</sup> (Tg) mice 3 days after a 90-min transduction period with rAdInsPNCre adenovirus. The PCR 726-bp product corresponds to the wild-type allele in control islets. In Tg islets, the 828-bp fragment shows intactlox/lox allele contributed by non-β-cells as well as residual non-transduced β-cells. Successful homologous recombination is revealed by the lane showing the 278-bp band, indicating absence of Glud1 exon 7 in Tg islets. B, three days after acute in vitro β-cell-specific Glud1 knock-out, islets were washed, preincubated, and then incubated for 30 min at basal (2.8 mM) and stimulated with 22.8 mM glucose (Glc) or a mixture of 5 mM glutamine plus 10 mM BCH at basal glucose (Gln + BCH). n = 3 independent experiments; *, p < 0.04 versus basal Glc; §p < 0.03 versus stimulus-matched Control.

evoked by 22.8 mM glucose was reduced by 38% (p < 0.02) in islets with acute abrogation of GDH. These results are in accordance with knockdown of GDH by antisense approach in insulinoma INS-1E cells (2). Insulin release was also stimulated by a mixture commonly used to specifically induce GDH-dependent insulin secretion (5), i.e. 5 mM glutamine plus the l-leucine analogue BCH (10 mM) serving as an allosteric activator of GDH. The resulting amino acid dependent secretory response had a magnitude of 3.2-fold (p < 0.005) in control islets, that was inhibited by 47% (p < 0.03) in knock-out islets. As both acute (Fig. 5) and constitutive (Fig. 4) knock-outs exhibited similar secretion impairments, we can conclude that redundant mechanisms do not exist at the cellular level.

Glucose Homeostasis in β-Cell GDH Knock-out—Parameters related to the control of glucose homeostasis were measured at 2, 5, and 12 months of age. After a 15-h fasting period, glycemia were similar to controls in βGlud1<sup>−−/−</sup> mice of all tested ages. Intraperitoneal glucose tolerance tests (ipGTT) were performed on 2- and 5-month-old mice. Despite the severe reduction in glucose-stimulated insulin secretion in βGlud1<sup>−−/−</sup> animals (Fig. 2, C and D), blood glucose excursions were similar to controls (Fig. 6, A and E). At 2 months of age, plasma insulin levels measured 15 min following intraperitoneal glucose injection were reduced by 38% (p < 0.001) in βGlud1<sup>−−/−</sup> mice (Fig. 6C). When performing an insulin tolerance test (ITT) in 2-month-old mice, blood glucose lowering was faster in βGlud1<sup>−−/−</sup> mice compared with controls (Fig. 6B). Low circulating insulin concentrations associated with accelerated glucose clearance suggested higher sensitivity of peripheral tissues. However, ITT performed in 5-month-old mice did not show significant differences between knock-outs and controls (Fig. 6F). To further investigate insulin sensitivity in 2- and 5-month-old mice, we calculated the HOMA index as a surrogate of euglycemic clamp test (28). As expected, insulin resistance increased with age in both groups (Fig. 6D) and βGlud1<sup>−−/−</sup> mice exhibited similar HOMA-IR compared with controls.

It should be noticed that RIP-Cre transgenic mice used in the present study (18) exhibit normal glucose homeostasis (see Fig. 6, A and E and Refs. 29, 30), as opposed to one particular RIP-Cre line (31, 32) that has been associated with either glucose intolerance or diabetes (33).

In old animals of 12 months of age, both control and knock-out animals exhibited signs of glucose intolerance. Return to
Deletion of GDH in β-Cells

19.9 ± 4.0 mM in GDH-null mice (n = 5). Insulin tolerance test revealed similar insulin sensitivity between the groups in 12-month-old animals (Fig. 6F).

Taken together, data on glucose homeostasis in βGlud1−/− mice demonstrate that limited β-cell secretory response does not impair normal growth when animals are fed ad libitum a normo-calorie diet. Such physiological adaptation might be less efficient in old mice, along with development of age-dependent glucose intolerance (Fig. 6G).

DISCUSSION

Mitochondrial metabolism is crucial for the coupling of glucose recognition to insulin exocytosis in β-cells (2). In this context, importance of GDH has been well recognized in the past (5, 9, 34), although recent studies revisited its specific role and regulation in the β-cell (10, 11, 19, 35, 36). GDH might raise insulin release by participating to the amplifying pathway (6, 7) and/or by relaying signals of protein abundance to mitochondria (8–10). The present study was not designed to detail the amplifying pathway in particular, but rather investigated the consequences of the lack of GDH on β-cell insulin secretory response and on resulting glucose homeostasis.

The first conclusion of the present study is that GDH is necessary for the full development of the secretory response. The absence of this mitochondrial enzyme sets a limit (about 60%) to insulin release evoked by optimal glucose concentrations. Secondly, half of the amplitude obtained with optimal glucose-stimulated insulin secretion is not required to maintain glucose homeostasis. The βGlud1−/− mice demonstrate that partially reduced β-cell secretory response is asymptomatic under conditions of normocalorie feeding, pending metabolic adaptations. The fact that β-cell function was limited but not abrogated might explain the observed preservation of normal animal weight gain, not requiring maximal development of the secretory response when fed a normal diet.

normoglycemia was slower in βGlud1−/− mice (Fig. 6G). Indeed, 120 min following intraperitoneal glucose injection, blood glucose levels were 15.0 ± 2.0 mM in controls (n = 7) and
Deletion of GDH in β-Cells

The absence of GDH in β-cells inhibited glucose-stimulated insulin secretion. Results indicate that there are no alternative pathways or mechanisms compensating for the lack of GDH as both acute in vitro and constitutive GDH knock-outs resulted in similar reduction of the secretory response. Importantly, β-cells of βGlud1−/− mice were still able to partially achieve glucose-stimulated insulin secretion, although the amplitude was shifted down. Both first and second phases were similarly impaired, suggesting that GDH-dependent amplification of the secretory response would be involved in both phases. Therefore, our data reveal that GDH is not necessary for glucose recognition per se as secretion was indeed initiated up to a certain level, although the amplitude was strongly limited.

Pancreatic islet cells are organized with β-cells forming the core of the structure and most of glucagon secreting α-cells distributed at the surface of the islet (37). Insulin secretory defects have been associated with modified islet architecture in general and in particular with infiltration of α-cells within the core of the islet (27, 38). Such phenotype is reported here for βGlud1−/− mice exhibiting reduced secretory responses, although total pancreatic islet distribution and insulin content were not affected. This observation further suggests that β-cell function could participate to islet architecture.

The pancreatic β-cell has evolved over millions of years toward high performance to fully optimize energy storage during occasional short periods of food abundance to resist starvation periods. In the light of present and previous results, one can speculate that the amplifying pathway in β-cell appeared as a signal of exceptional abundance, induced in conditions of high nutrient supply to optimize storage of energy exceeding immediate requirements by the organism. Noteworthy, investigators typically study the amplifying pathway in experimental conditions where insulin release is evoked by highest physiological glucose concentrations. Such experimental paradigm might not reflect ordinary requirement of β-cell function in individuals with appropriate and regular nutrient intake. It is remarkable that such phenotype is reported here for βGlud1−/− mice, exhibiting half of the maximal insulin secretion amplitude, performed similar glucose homeostasis and growth as controls, pending access to balanced carbohydrate-rich diet.

Several studies provided indirect evidence pointing to GDH as a key enzyme in the control of insulin secretion in pancreatic β-cells. For instance, GDH is responsible for hyperinsulinemia in patients carrying activating mutations (14). More recently, a new regulatory role for GDH in β-cells has been revealed through its control by SIRT4, a mammalian sirtuin with ADP-ribosyltransferase activity (11, 12). Taking advantage of the first GDH knock-out model, we could directly challenge the role of GDH in β-cells in vivo. Our findings extend current knowledge by establishing the quantitative contribution of GDH in normal glucose-stimulated insulin secretion. Comparison of acute versus constitutive ablation of GDH suggests that no redundant mechanism exists in β-cells. However, the present data show that optimal β-cell function is not required for glucose homeostasis under standard conditions.

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