The Amplifying Pathway of the β-Cell Contributes to Diet-induced Obesity*

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Efficient energy storage in adipose tissues requires optimal function of the insulin-producing β-cell, whereas its dysfunction promotes diabetes. The associated paradox related to β-cell efficiency is that excessive accumulation of fat in adipose tissue predisposes for type 2 diabetes. Insulin exocytosis is regulated by intracellular metabolic signal transduction, with glutamate dehydrogenase playing a key role in the amplification of the secretory response. Here, we used mice with β-cell-selective glutamate dehydrogenase deletion (βGlud1−/−), lacking an amplifying pathway of insulin secretion. As opposed to control mice, βGlud1−/− animals fed a high calorie diet maintained glucose tolerance and did not develop diet-induced obesity. Islets of βGlud1−/− mice did not increase their secretory response upon high calorie feeding, as did islets of control mice. Inhibited adipose tissue expansion observed in knock-out mice correlated with lower expression of genes responsible for adipogenesis. Rather than being efficiently stored, lipids were consumed at a higher rate in βGlud1−/− mice compared with controls, in particular during food intake periods. These results show that reduced β-cell function prior to high calorie feeding prevented diet-induced obesity.

Pancreatic β-cells produce the hormone insulin that is essential for glucose homeostasis. Upon nutrient stimulation, elevation of cytosolic calcium in the β-cell results from the closure of potassium channels and is the primary and necessary signal for insulin exocytosis (1). Then increasing the magnitude of insulin secretion in response to glucose stimulation requires amplification of the calcium signal supported by metabolism-derived additive factors (2). The mitochondrial enzyme glutamate dehydrogenase (GDH), encoded by the Glud1 gene (3), has been shown to participate to the development of the secretory response (4). Specifically, GDH is required for the amplifying pathway of glucose-stimulated insulin secretion in the β-cell (5), providing permissive levels of glutamate to the cytosolic compartment and the exocytotic machinery (6–11). The amplifying pathway, formerly referred to as the K-ATP-independent pathway, was uncovered more than two decades ago (12). However, its physiological function remains unclear, in particular because it is activated by rather high glucose concentrations. Moreover, mice lacking β-cell GDH and maintained on normal chow diet are asymptomatic, although the secretory response of their β-cells is limited (4).

A related open question is how efficient a β-cell should be for maintenance of energy homeostasis. Obesity is associated with hyperinsulinemia typically because of insulin resistance (13), which may lead to diabetes in case of subsequent β-cell failure (14). However, mice can be genetically protected against obesity either by the reduction of insulin gene dosage (15, 16) or by the ablation of insulin signaling in adipose tissue (17). One can postulate that the maximal potential of insulin production by the β-cell might not be necessary under the normal energy homeostatic state, as shown in mice having genetically reduced insulin production (15). Such assumption is also supported by clinical data showing maintenance of glucose tolerance in donors of islets who underwent partial pancreatectomy (18). However, preservation of normoglycemia, while having half of the full potential for insulin release, might be compromised in conditions requiring increased insulin production, such as obesity-associated insulin resistance or pregnancy (19). Pharmacologically, inhibition of insulin secretion can be achieved by use of the somatostatin analogue octreotide (20) or the non-selective potassium channel opener diazoxide (21). Obese patients who received octreotide for 6 months lost weight compared with placebo (22). In a pilot study conducted on obese subjects, high dose diazoxide treatment over a 6-month period reduced their fasting insulin and fat mass (23). Thus, on a background of obesity and hyperinsulinemia, reducing the β-cell efficiency could favorably impact on body weight. Whether in the case of impaired β-cell amplifying pathway a high fat diet prevents obesity or promotes diabetes is unknown.

Here, we used mice lacking GDH in β-cells and investigated the consequences of genetically limited insulin secretion on energy homeostasis in mice fed a high calorie diet. The results show that inhibition of an amplifying pathway of the secretory response in the β-cells completely protected against diet-induced obesity.

Experimental Procedures

Generation and Treatment of Mouse Models—Glud1floxed animals (Glud1flo2/+, MGI:3835667) were crossed with Rip-Cre mice expressing the Cre recombinase under the rat insulin promoter. As Rip-Cre mice, we used the TgIns2creHerv line (MGI: 2387567), avoiding undesired brain (4) or hypothalamic recom-b
bination of the floxed gene (24–26). Heterozygous βGlud1fl/fl were then bred with homozygous Glud1lox/lox to obtain homozygous βGlud1−/− mice (4). For each experiment, we used control and knock-out male mice from the same litter to optimize standardization of the genetic background between the two groups of animals maintained on C57BL/6 × 129/Sv background to avoid inbred strain-specific phenotypes (27). For time-controlled β-cell-selective GDH deletion, Glud1lox/lox floxed animals were crossed with Rip-CreER mice (Tg(Ins2-cre/ERT)Oswm, MGI:3720973), allowing tamoxifen-induced Cre recombinase expression and floxed gene knock-out in β-cells (28). Tamoxifen was injected at the indicated time for 5 consecutive days as previously described (28). Mouse breeding and handling was carried out in our local certified animal facility according to procedures that were approved by the animal care and experimentation authorities of the Canton of Geneva. Where indicated, mice were fed a high fat diet (HFD) in which 58, 26, and 16% of the calories came from fat, carbohydrate, and protein, respectively (D12331; Research Diets, New Brunswick, NJ).

**Genotyping and Immunoblotting**—Transgenic animals were identified by PCR on genomic DNA extracted from tail biopsies (Genelute Mammalian Genomic DNA kit; Sigma). The primers (purchased from Microsynth GmbH, Baglach, Switzerland) used to genotype floxed mice were 5′-TGTAATGTGTCTGTGTGCAC and 5′-CTAAGGACCCAGACGTGG (86-bp fragment). The primers to genotype Rip-Cre mice were 5′-TAAGGCTAAGTAGGTGT and 5′-TCCATGGTGATACAAGGGGC (350-bp fragment). The primers to genotype Rip-CreER mice were 5′-TGCCACGACCAATGACGC and 5′-CCAGGTTACGGATATTGTCATG (750-bp fragment). Deletion of the floxed Glud1 exon 7-mediated tamoxifen-induced Rip-CreER recombination was verified using primers: 5′-TGTAATGTGTCTGTGTGCAC and 5′-CTAAGGTTTCTGCCACAAAG (278-bp fragment). A fragment of 828-bp is produced in the absence of deletion, corresponding to the intact floxed Glud1 allele.

**Metabolic Analyses**—Calorimetric parameters including heat production, locomotor activity, O2 consumption (VO2), CO2 production (VCO2), and respiratory exchange ratio (RER, defined as VCO2/VO2) were monitored over 48 h after 8 days adaptation of the mice to individual metabolic cages (LabMaster; TSE Systems, Bad-Homburg, Germany). Triglyceride levels in plasma or Folch extracts (29) from liver and muscles were determined using a commercial kit (Roche Diagnostics). Ketone bodies in the form of 3-hydroxybutyrate and non-esterified fatty acids were measured in plasma using kits from Wako Diagnostics (Neuss, Germany). Plasma leptin, glucagon, and insulin concentrations were determined on multiplex suspension array system (Bio-Plex; Bio-Rad) using LincoPlex kits for mouse hormones (Linco Research Inc., St. Charles, MO).

Epididymal fat sections were stained with hematoxylin and eosin as described (30). Sections were analyzed on a Zeiss Axioshot microscope equipped with an Axioscan color CCD camera (Carl Zeiss, Feldbach, Switzerland). Body composition was assessed by EchoMRI quantitative magnetic resonance body composition analyzer (Echo Medical Systems, Huston, TX) with direct measurements of total body fat, lean mass, free water, and total body water.

**Glucose and Insulin Tolerance Tests**—Mice fasted overnight were injected intraperitoneally with glucose (3 g/kg body weight). Whole blood was collected from tail vein at times 0, 15, 30, 60, 120, and 180 min for glucose level measurements using a glucometer (Glucotrend; Roche). Additionally, plasma insulin levels were determined at times 0 and 15 min using an ultrasensitive mouse insulin ELISA (Merckodia AB, Uppsala, Sweden). For insulin tolerance test, 4-h fasted mice were injected intraperitoneally with human recombinant insulin (1:400) and mouse anti-glucagon (1:500) primary antibodies as detailed previously (31). Fluorochrome-linked secondary antibodies were diluted 1:1000 and incubated for 1 h at room temperature. Sections were analyzed on a Zeiss Axioshot microscope equipped with an Axioscan color CCD camera.

**Insulin Secretion Assay**—For insulin secretion assay, mouse pancreatic islets were isolated by collagenase digestion as described (32) and cultured free-floating in RPMI 1640 medium before use. Prior to the experiments, islets were preincubated for 1 h at 2.8 mM glucose in Krebs-Ringer bicarbonate HEPES buffer (KRBH, containing 135 mM NaCl, 3.6 mM KCl, 10 mM HEPES (pH 7.4), 5 mM NaHCO3, 0.5 mM NaH2PO4, 0.5 mM MgCl2, 1.5 mM CaCl2, 0.1% bovine serum albumin) for 30 min. For perfusion experiments, 10 hand-picked islets were put on chamber kept at 37 °C (Brandel, Gaithersburg, MD) as detailed previously (32). The flux was set at 0.5 ml/min, and fractions were collected every min after a 30-min washing period at basal 2.8 mM glucose. Fractions were collected when glucose was sequentially set at 2.8, 11.8, and 22.8 mM and then back at 2.8 mM. For static incubations, batches of 10 islets were handpicked after the preincubation period and incubated in KRBH for 1 h at 37 °C (2.8 mM glucose). Fractions were collected every min for 15 min. The values were normalized to values of the reference cyclophilin mRNA.

**Gene Expression Analysis**—Total RNA was isolated using either TRIzol reagent (Invitrogen Life Technologies) or, in the case of islets, RNeasy MicroKit (Qiagen, Venlo, Netherlands) and next purified with Nucleospin RNA II kit (Macherey-Nagel, Düren, Germany). The primers were designed using the Primer Express Software (Applera Europe) (see supplemental Table S1). Real time PCR was performed using an ABI 7000 sequence detection system (Applied Europe), and PCR products were quantified fluorometrically using the SYBR Green core reagent kit. The values obtained were normalized to values of the reference cyclophilin mRNA.

**Statistical Analysis**—Statistics were done using the SPSS 15.0 statistical package (SPSS, Chicago, IL). Unless indicated, the data are represented as the means ± S.E. for at least three independent experiments, each performed in triplicate. Differences between
groups were assessed by the two-tailed unpaired t test for single comparison or by one-way ANOVA, using post hoc multiple comparison procedure (Fischer’s least significant difference method). The results were considered statistically significant at \( p < 0.05 \).

Results

\( \beta \text{Glud}^{1/-} \) Mice Are Resistant to Diet-induced Obesity—Taking advantage of the \( \beta \text{Glud}^{1/-} \) mice lacking an amplifying pathway of glucose-stimulated insulin secretion (5), we questioned whether early limitation of \( \beta \)-cells performance, prior to high calorie intake and insulin resistance, would either prevent or favor obesity-associated glucose intolerance. To this end, 3-month-old control and \( \beta \text{Glud}^{1/-} \) mice were fed \textit{ad libitum} a HFD for 20 weeks. Control mice developed obesity reaching \( \approx 45 \) g and were \( \approx 10 \) g heavier than lean controls maintained on standard chow diet (45.2 g versus 35.3 g, \( p < 0.01 \); Fig. 1A). On a regular chow diet, we previously reported that \( \beta \text{Glud}^{1/-} \) mice gain similar weight compared with control

\[ \text{FIGURE 1.} \ \beta \text{Glud}^{1/-} \text{ mice are resistant to diet-induced obesity.} \ \text{A, body weight of control (} n = 11 \text{) and } \beta \text{Glud}^{1/-} \text{ (} n = 12 \text{) mice recorded over the 20-week period of HFD (started at 12 weeks of age). Control lean mice were kept on a regular chow diet (} n = 11 \text{). B, body weight gain of control (} n = 11 \text{) and } \beta \text{Glud}^{1/-} \text{ mice (} n = 12 \text{) over the 20-week period on HFD. C, daily food intake of control (} n = 11 \text{) and } \beta \text{Glud}^{1/-} \text{ mice (} n = 12 \text{) during the first 11 weeks on HFD. D, overall food intake over the 20-week period on HFD. E, metabolic efficiency of control (} n = 9 \text{) and } \beta \text{Glud}^{1/-} \text{ mice (} n = 8 \text{) expressed as the gain in body weight per energy food intake (MJ) over the 20-week period on HFD. F, plasma levels of triglycerides (TG), non-esterified fatty acids (NEFA), and 3-hydroxybutyrate (ketone bodies, KB) of control and } \beta \text{Glud}^{1/-} \text{ mice after 20 weeks on HFD (} n = 4-7 \text{/group). The values are means } \pm \text{ S.E.}, \ * p < 0.05; **, p < 0.01 \text{ (Student’s t test testing control versus } \beta \text{Glud}^{1/-} \text{ mice).} \]
animals (4). On a HFD, βGlud1−/− mice were totally resistant to diet-induced obesity, because their body weights overlapped lean controls (Fig. 1A). Body weight differences first emerged at week 4 on HFD (p < 0.01; Fig. 1A). At the end of the 20-week period on a HFD, the total body weight gain of control mice was 16.4 g versus 7.1 g for βGlud1−/− mice (p < 0.01; Fig. 1B). This 57% lower weight gain observed in βGlud1−/− mice was not explained by changes in calorie intake because daily food consumption was similar between the two groups fed the HFD (Fig. 1, C and D). This suggested alternative mechanisms for lower weight gain, such as reduced storage efficiency.

Metabolic efficiency represents the fraction of ingested calories that is stored as extra energy substrates, in contrast to the major part that is directly used for cellular function and heat production (33). Metabolic efficiency over the 20 weeks on HFD was reduced by 57% in βGlud1−/− mice compared with control mice (0.69g/MJ versus 1.6g/MJ, respectively, p < 0.01; Fig. 1E). As shown later on, this lower metabolic efficiency of βGlud1−/− mice suggested that calories contributed by lipids contained in the HFD were preferentially consumed as direct energy substrates rather than efficiently stored. This is also supported by the fact that the concentrations of circulating lipids were not different between control and knock-out animals (Fig. 1F).

High fat feeding over 20 weeks resulted in higher fat depots in control mice compared with mice kept on standard chow diet, whereas βGlud1−/− mice on HFD maintained a lean phenotype (Fig. 2, A–C). In particular, epididymal fat weights were markedly lower in βGlud1−/− mice compared with corresponding controls on HFD (Fig. 2C). At the histological level, this observation correlated with preservation of small size adipocytes in βGlud1−/− compared with hyperplastic adipocytes observed in obese controls (Fig. 2A). More specifically, HFD increased the size of adipocytes by 37% in control mice compared with those fed a normal chow diet, whereas HFD βGlud1−/− mice maintained their adipocytes at a normal lean size (Fig. 2B). The increased fat mass in HFD control animals correlated with marked elevation of circulating leptin levels (34). Metabolic efficiency over the 20-week period on a HFD, the total body weight gain of HFD was reduced by 57% in βGlud1−/− compared with hyperplastic adipocytes (33). Metabolic efficiency over the 20 weeks on HFD was reduced by 57% in βGlud1−/− mice compared with control mice versus normal chow diet, whereas HFD βGlud1−/− mice remained below this glycemia threshold (Fig. 3A). Fasting (6 h) plasma insulin levels were maintained lower in HFD βGlud1−/− mice compared with HFD controls (Fig. 3B). However, 2 h after refeeding, circulating insulin concentrations reached similar levels in both groups. Insulin-induced lowering of glycemia in HFD control mice could not go below the 7 mM range, whereas HFD βGlud1−/− mice efficiently reduced blood glucose levels down to 4 mM in response to insulin injection (Fig. 3C). Higher insulin sensitivity of βGlud1−/− mice is also observed when fed a chow diet (4). Fasting plasma glucagon levels were not different between control and knock-out animals (Fig. 3D).

Glucose tolerance test performed on control HFD obese mice uncovered their glucose intolerance. These mice exhibited hyperglycemia maintained over 2 h following glucose injection (Fig. 3E), confirming insulin resistance. HFD βGlud1−/− mice had preserved glucose excursion, showing insulin sensitivity similar to lean control mice fed normal chow diet (Fig. 3E). At 15 min following the glucose load, plasma insulin levels were 32% lower in HFD βGlud1−/− mice compared with HFD controls (Fig. 3F). These data indicate that a primary limitation in β-cell function (4), preceding the start of HFD feeding, prevents obesity and insulin resistance without favoring development of diabetes.

The performance of pancreatic β-cells following HFD feeding was then tested by measuring glucose-stimulated insulin secretion on perfused islets. Obesity in general and insulin resistance in particular is known to enhance the secretory response of the β-cell (36). Accordingly, we observed that, compared with islets isolated from lean control mice fed standard chow diet, islets from obese HFD controls had much more robust responses to both 11.8 and 22.8 mM glucose stimulations (Fig. 4A). Islets isolated from βGlud1−/− mice fed the HFD retained a non-exacerbated secretory profile, exhibiting half of the secretory response observed in HFD controls (Fig. 4B) with similar insulin content per islet. This shows that the limited β-cell function already documented in βGlud1−/− mice fed normal chow diet (4) is preserved upon HFD exposure. Pancreatic sections revealed similar organization (Fig. 4C) and size distribution (Fig. 4D) of islets in control and βGlud1−/− mice fed the HFD.

**Glucose Tolerance Is Preserved in βGlud1−/− Mice Fed a HFD—Because fasting blood glucose >7.0 mM qualifies as diabetes in human subjects (35), we arbitrarily established this cutoff for mice. After 20 weeks on HFD, control mice became diabetic, whereas HFD βGlud1−/− mice remained below this glycemia threshold (Fig. 3A). Fasting (6 h) plasma insulin levels were maintained lower in HFD βGlud1−/− mice compared with HFD controls (Fig. 3B). However, 2 h after refeeding, circulating insulin concentrations reached similar levels in both groups. Insulin-induced lowering of glycemia in HFD control mice could not go below the 7 mM range, whereas HFD βGlud1−/− mice efficiently reduced blood glucose levels down to 4 mM in response to insulin injection (Fig. 3C). Higher insulin sensitivity of βGlud1−/− mice is also observed when fed a chow diet (4). Fasting plasma glucagon levels were not different between control and knock-out animals (Fig. 3D).**

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FIGURE 2. βGlu1<l>−<l>−</l> mice on a HFD have reduced capacity of fat storage. A, picture of representative control and βGlu1<l>−<l>−</l> mice after 20 weeks on HFD and their corresponding epididymal fat sections stained by hematoxylin-eosin. Scale bar, 100 μm. B, sizes of adipocytes measured on sections of fat tissues collected from control and βGlu1<l>−<l>−</l> mice after 20 weeks on HFD and control CD mice (n = 10/group). C, epididymal fat weights of control (n = 5) and βGlu1<l>−<l>−</l> (n = 6) mice after 20 weeks under HFD and control CD mice (n = 5). D, plasma leptin levels of control (n = 6) and βGlu1<l>−<l>−</l> (n = 6) mice after 20 weeks on HFD and control CD (n = 5), normalized to cyclophilin and expressed as relative values versus control CD mice. The values are means ± S.E. *, p < 0.05; **, p < 0.01 versus control CD.
derived energy mostly from lipids, as demonstrated by the shift of the RER toward lower values compared with mice on normal high carbohydrate chow diet (Fig. 5, C and F). Of note, mice on chow diet exhibited marked day versus night fluctuations, with high RER during eating period at night and lower RER during fasting day time, reflecting, respectively, alimentary carbohydrate consumption and gradual mobilization of stored fat as a fuel source (Fig. 5C). Such fluctuation was attenuated in mice on HFD, consuming more lipids whatever the time period (Fig. 5F). Interestingly, βGlud1−/− mice fed the HFD exhibited even lower RER values during eating period compared with HFD controls (Fig. 5F). This shows that whole body fatty acid oxidation was increased during this period in βGlud1−/− mice, indicating direct usage of ingested fat for energy production rather than efficient lipid storage. However, the overall energy expenditure was similar between the two groups (data not shown). Because a significant fraction of whole body O2 consumption and CO2 production in animals results from physical movements, we measured locomotor activity showing that HFD βGlud1−/− mice had slightly higher activity over the 24-h
2.1-fold in control animals and 1.6-fold in βGld1<sup>−/−</sup> mice compared with animals on chow diet (Fig. 6B). No significant changes were observed in the muscles (Fig. 6B). Thus, in HFD βGld1<sup>−/−</sup> mice, there was reduced fat storage specifically in adipose tissue (Fig. 2C) despite unchanged circulating triglycerides levels (Fig. 1F) and marginal differences in the liver (Fig. 6B). Overall, the resistance in βGld1<sup>−/−</sup> mice to diet-induced obesity is consistent with lower chronic exposure to insulin signaling that normally promotes fat storage mainly in adipocytes (37).

Next, gene expression profile was investigated in skeletal muscles and liver of control and βGld1<sup>−/−</sup> mice fed HFD for 20 weeks, as well as control mice kept on regular chow diet. In the muscles of control mice, HFD increased expression of catabolic genes, such as CPT-1 (1.3-fold) controlling β-oxidation and UCP3 (2.8-fold), possibly inducing mitochondrial uncoupling and energy waste (Fig. 6C). Conversely, HFD induced down-regulation of Glut-4 (−25%), SREBP (−28%), PPARδ (−79%), and PGC-1α (−94%) in the muscles of control animals (Fig. 6C), indicating reduced mitochondria biogenesis. In the liver of the same HFD control animals, we observed induction of CPT-1 (2.9-fold) and glucokinase GK (2.2-fold) and down-regulation of glucose-6-phosphatase G6Pase (−46%) and SREBP (−63%) compared with chow diet controls (Fig. 6D). HFD βGld1<sup>−/−</sup> mice preserved part of the expression profile of lean controls, in particular PPARδ in skeletal muscles (3.7-fold versus HFD controls; Fig. 6C) and CPT-1 and GK in the liver (−48% and −62% versus HFD controls, respectively; Fig. 6D).

Deletion of GDH in β-Cells during Obesity Development Prevents Further Fat Storage and Glucose Intolerance—Because βGld1<sup>−/−</sup> mice lacking GDH-dependent β-cell amplifying pathway were resistant to diet-induced obesity (Fig. 1A), we next tested the consequences of a limited β-cell function induced once mice were overweighted secondary to HFD feeding. To do so, we generated tamoxifen-inducible in vivo knock-out of Glud1 in β-cells (Rip-CreER;Glud1<sup><sub>fl/fl</sub></sup>) and induced Glud1 recombination at week 14 of HFD feeding by tamoxifen injections. The mice were then kept for another 10-week period on the same HFD. At the end of the whole period, islets isolated from Rip-CreER;Glud1<sup><sub>fl/fl</sub></sup> mice treated with tamoxifen 10 weeks earlier exhibited a 76% reduction in Glud1 expression compared with tamoxifen-treated Glud1<sup>−/−</sup> floxed controls (Fig. 7A). Insulin secretion tested in these control islets showed a 4.4-fold response to glucose stimulation (Fig. 7B). In tamoxifen-induced βGld1<sup>−/−</sup>-islets, the secretory response was reduced by 52% (Fig. 7A), consistent with constitutive deletion of GDH in β-cells of lean βGld1<sup>−/−</sup> mice (4).

After 14 weeks on HFD, before tamoxifen treatment, non-recombined Rip-CreER;Glud1<sup><sub>fl/fl</sub></sup> mice and Glud1<sup><sub>fl/fl</sub></sup> floxed controls had gained similar extra weight compared with lean controls, i.e. 7.8 ± 2.5 g (<i>p</i> < 0.05) and 7.4 ± 1.6 g (<i>p</i> < 0.05), respectively (Fig. 7C). As lean controls on normal chow diet, we used Rip-CreER;Glud1<sup><sub>fl/fl</sub></sup> mice also treated with tamoxifen at week 14, which therefore became βGld1<sup>−/−</sup> for the remaining 10-week period. Of note, βGld1<sup>−/−</sup> mice on normal chow diet develop weight as wild type mice (4).
FIGURE 5. Metabolic rate and energy partitioning in response to HFD. A–F, calorimetric parameters monitored in control and βGlud1−/− mice fed either standard chow diet (A–C) or a HFD (D and E) for 20 weeks (n = 6/group): O₂ consumption (A and D), CO₂ production (B and E), and RER (C and F). The values are means ± S.E., *p < 0.05 versus control HFD (ANOVA).
Quantitative magnetic resonance revealed that HFD controls kept on enlarging their fat mass after week 14 (Fig. 7D). This resulted in continuous body weight gain in HFD controls, not in chow diet controls, whereas knock-out of GDH in HFD βGlud1−/− at week 14 partially reduced further weight gain (Fig. 7E). At week 24 on a HFD, the fat mass of HFD controls was doubled compared with chow diet controls along with 18% lower lean mass (Fig. 7F). Deletion of β-cell GDH for the last 10 weeks on HFD resulted in lower fat mass (−36%, p < 0.05) in HFD βGlud1−/− week 14 mice compared with HFD controls (Fig. 7F).

Intraperitoneal glucose tolerance tests showed that, as opposed to lean controls, HFD controls developed glucose intolerance over the 24 weeks on HFD (Fig. 8). Remarkably, knock-out of β-cell GDH at week 14 preserved glucose tolerance in HFD βGlud1−/− week 14 (Fig. 8C). These data show that limiting the β-cell function during development of obesity protected against further fat storage and accompanying glucose intolerance.
Deletion of GDH in β-cells during obesity development prevents further fat storage. Mice with tamoxifen-inducible in vivo knock-out of Glud1 in β-cells (Rip-CreER;Glud1<sup>fl/fl</sup>) were generated to assess the role of GDH-dependent insulin secretion during obesity progression. A, 10 weeks after in vivo tamoxifen treatment, Glud1 mRNA levels were measured by quantitative RT-PCR in islets isolated from control and Rip-CreER;Glud1<sup>fl/fl</sup> (β-Glut1<sup>−/−</sup>-Tam) mice (n = 3 in each group). *, p < 0.01 versus control. B, glucose-stimulated insulin secretion measured in islets isolated from control (n = 4) and β-Glut1<sup>−/−</sup>-Tam (n = 5) mice 10 weeks after in vivo tamoxifen treatment. Islets were exposed to basal 2.8 mM or stimulatory 22.8 mM glucose (Glc) for 1 h. §, p < 0.05 versus 2.8 mM Glc of the same genotype; *, p < 0.05 versus control at 22.8 mM Glc. C, body weight of control (HFD control, cyan diamonds, n = 5) and inducible β-Glut1<sup>−/−</sup> (HFD β-Glut1<sup>−/−</sup>-Tam) mice recorded over the 24-week period of HFD (started at 12 weeks of age). Control lean inducible β-Glut1<sup>−/−</sup> mice were kept on regular chow diet (CD β-Glut1<sup>−/−</sup>-Tam, gray triangles, n = 4). Knock-out of the Glud1 gene was induced by a 5-day tamoxifen treatment at week 14 after the initiation of the HFD. Animals from all groups were injected with tamoxifen at the indicated week 14. D, fat mass progression measured by quantitative magnetic resonance in live HFD control mice following tamoxifen treatment at week 14. §, p < 0.05 versus week 13 (n = 5). E, body weight gain of lean knock-out control mice CD β-Glut1<sup>−/−</sup>-Tam (n = 4), HFD control (n = 5), and HFD β-Glut1<sup>−/−</sup>-Tam (n = 5) mice recorded over the 10-week period following tamoxifen treatment at week 14. §, p < 0.05 for HFD control versus HFD β-Glut1<sup>−/−</sup>-Tam at week 14. F, fat and lean masses measured by quantitative magnetic resonance in live mice at week 24 of HFD. §, p < 0.05 versus lean control CD β-Glut1<sup>−/−</sup>-Tam (n = 4); *, p < 0.05 comparing HFD β-Glut1<sup>−/−</sup>-Tam at week 14 (n = 5) versus HFD control (n = 5), ANOVA.
Full β-Cell Response Favors Obesity

FIGURE 8. Deletion of GDH in β-cells during obesity development prevents glucose intolerance. Mice with tamoxifen-inducible knock-out of Glud1 in β-cells (Rip-CreER;Glud1fl/fl) were deprived of GDH in β-cells after 14 weeks on HFD and then kept for another 10-week period. A, glucose tolerance test (3g/kg) was performed at week 13 before tamoxifen (Tam) treatment on fasted control (CD, gray triangles) and non-recombined β-Glud1fl/fl (orange squares) mice on HFD, as well as on control lean β-Glud1fl/fl mice on chow diet (CD, gray triangles). B, glucose tolerance test at week 24 on HFD, 10 weeks after tamoxifen treatment, performed fasted on control (cyan diamonds) and β-Glud1−/− (knock-out induced at week 14, orange squares) mice on HFD, as well as on control lean β-Glud1−/− mice (knock-out induced at week 14) on CD (gray triangles). C, area under the curve analysis of the glucose tolerance test shown in (B) and performed at week 24 on the HFD. The values are means ± S.E. *p < 0.05 versus lean control CD β-Glud1−/− @wk14; **p < 0.05 versus HFD control, ANOVA (n = 4–5).

Discussion

Previous studies have shown that GDH is necessary for the full development of the secretory response, and the absence of this mitochondrial enzyme sets a limit (~50%) to maximal insulin release (4). In particular, lack of GDH in the β-cell disrupts an amplifying pathway normally induced by robust stimulation of the secretory response (5). Noteworthy, reduced β-cell secretory response is asymptomatic, pending specific metabolic adaptations and under conditions of normo-calorie feeding (4). The fact that β-cell function was limited but not abrogated might explain the observed preservation of normal animal growth. This is the case when mice are fed ad libitum a normal diet not requiring extraordinary signals of abundance in the form of high insulin. However, limiting β-cell secretory response protected against diet-induced obesity. In particular, induction of fat storage in adipocytes of βGlud1−/− mice under HFD diet was prevented, pointing to limited insulin signaling (37). This effect does not rule out other putative contributions, such as central effects or subtle changes in thermogenesis potentially masked by the inherent multifactorial balance of energy expenditure (33), although βGlud1−/− mice on the chow diet did not exhibit changes in weight gain.

Protection against obesity has been reported previously in knock-out mouse models with either lower insulin supply (15, 16) or impaired insulin action on peripheral tissues (38, 39). Mice with adipose tissue-selective insulin receptor knock-out (FIRKO mice) are protected against obesity (17). The associated absence of insulin signaling in adipose tissue represses Glut-1 and enhances adiponectin expression (17). In the present study, β-cell-selective GDH knock-out mice maintained lower expression of Glut-1 and ERK1 in adipose tissue compared with control obese animals, thereby preventing adipogenesis. Expression of the adipocyte-derived anti-diabetic hormone adiponectin (40) was also preserved in our βGlud1−/− mice, in good agreement with higher insulin sensitivity compared with controls. Our data demonstrate that the primary limitation of the β-cell response recapitulates effects observed downstream in insulin signaling and points to insulin as a key signal governing peripheral tissue adaptation.

In a previous study (41), the authors investigated glucose homeostasis in a prediabetes state of Goto-Kakizaki rats, a model of spontaneous type 2 diabetes developing glucose intolerance (42, 43). In 3-week-old prediabetic Goto-Kakizaki rats, the authors observed glucose tolerance despite markedly reduced β-cell secretory response upon glucose stimulation (41). The βGlud1−/− mice exhibited similar adaptation to low β-cell function with preserved glucose homeostasis. Our data are also in agreement with those obtained from mice lacking the G protein-coupled receptor GPR40 activated by free fatty acids and expressed in β-cells. Such GPR40-deficient mice secrete less insulin in response fatty acids and partially maintain glucose homeostasis and insulin sensitivity upon high fat diet (44). Altogether, these data indicate unexpected efficient adaptation of peripheral tissues to β-cell performance.

According to the World Health Organization (fact sheet no. 311), at the present time more people worldwide suffer from excessive rather than limited energy supply. Under this new paradigm, it is an intriguing concept whether high β-cell efficiency, acquired through evolution, could participate to obesity predisposition. The pancreatic β-cell has evolved over millions of years toward high performance to fully optimize energy stor-
age 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. It is noteworthy that investigators typically study the amplifying pathway in experimental conditions where insulin release is evoked by the highest physiological glucose concentrations. Such an experimental paradigm might not reflect ordinary requirement of β-cell function in individuals with appropriate and regular nutrient intake.

We previously reported that βGlut1−/− mice, exhibiting half of the maximal insulin secretion amplitude, perform similar energy homeostasis and nutrient storage as controls, pending access to balanced carbohydrate rich diet (4). On a high calorie diet, limitation of excessive fat deposit in adipose tissues protected knock-out mice from high fat diet-induced obesity and the accompanying insulin resistance. At the clinical level, a few studies have been conducted in obese subjects using diazoxide to inhibit insulin secretion from the β-cell. Short term treatments showed contradictory results with either no change in body weight (45) or anti-obesity effect in hyperinsulinemic obese adults (46). A 6-month diazoxide treatment of change in body weight (45) or anti-obesity effect in hyperinsulinemic obese adults (46). A 6-month diazoxide treatment of type 2 diabetes. Diazoxide induces hyperglycemia, which is not observed in βGlut1−/− mice possibly because, to some extent, their β-cells remained sensitive to glucose. In our study, limitation of the β-cell secretory response prior to obesity induction completely prevented overweight gain, whereas limiting insulin release in obese mice had marginal effects on body weight while preserving glucose homeostasis. Taken together, pilot clinical studies, as well as present mechanistic data, call for better understanding of the contribution of insulin release by the β-cell toward development of obesity, opening new avenues for the prevention of type 2 diabetes.

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