Type 2 diabetes ultimately results from pancreatic β-cell failure. Abnormally elevated intracellular regeneration of glucocorticoids by the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) in fat or liver may underlie pathophysiological aspects of the metabolic syndrome. Elevated 11β-HSD1 is also found in pancreatic islets of obese/diabetic rodents and is hypothesized to suppress insulin secretion and promote diabetes. To define the direct impact of elevated pancreatic β-cell 11β-HSD1 on insulin secretion, we generated β-cell-specific, 11β-HSD1-overexpressing (MIP-HSD1) mice on a strain background prone to β-cell failure. Unexpectedly, MIP-HSD11/1 mice exhibited a reversal of high-fat-induced β-cell failure through augmentation of the number and intrinsic function of small islets in association with induction of heat shock, protein kinase A, and extracellular signal–related kinase and p21 signaling pathways. 11β-HSD11/1 mice showed mild β-cell impairment that was offset by improved glucose tolerance. The benefit of higher β-cell 11β-HSD1 exhibited a threshold because homozygous MIP-HSD11/2 mice and diabetic Lepdb/db mice with markedly elevated β-cell 11β-HSD1 levels had impaired basal β-cell function. Optimal elevation of β-cell 11β-HSD1 represents a novel biological mechanism supporting compensatory insulin hypersecretion rather than exacerbating metabolic disease. These findings have immediate significance for current therapeutic strategies for type 2 diabetes. Diabetes 61:642–652, 2012

Type 2 diabetes prevalence has risen dramatically in parallel with the worldwide increase in obesity (1). Understanding and targeting the processes leading to β-cell exhaustion in the face of peripheral insulin resistance is therefore of major importance. Glucocorticoid hormones potently regulate metabolism and, in rare cases of excess (Cushing syndrome), cause metabolic syndrome (2). Elevated local tissue glucocorticoid excess, driven by increased levels of the intracellular glucocorticoid regenerating enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1), particularly in adipose tissue, is implicated in the development of idiopathic metabolic syndrome (3). This premise is strongly supported by the phenotype of transgenic mice overexpressing 11β-HSD1 in fat or liver, which recapitulates diabetes and insulin-resistant metabolic disease, and by the protection from metabolic disease exhibited by 11β-HSD1−/− mice (3).

11β-HSD1 is also found in pancreatic islets (4). 11β-HSD1 is elevated in islets of diabetic rodents (4–6), where it was hypothesized to promote β-cell failure by amplifying the suppressive effects of glucocorticoids on insulin secretion (7,8). Although this strengthens the growing contention that 11β-HSD1 inhibitors are an effective therapeutic treatment for metabolic syndrome through actions in multiple organ systems (9), any physiological role of 11β-HSD1, and indeed the potentially pathogenic role (5–8) of elevated 11β-HSD1 in islets in vivo, remains uncertain. To test the hypothesis that increased β-cell 11β-HSD1 is diabetogenic, we used the insulin-I promoter (10) to drive β-cell–specific 11β-HSD1 elevation in vivo in C57BL/KsJ mice, a strain prone to high-fat (HF) diet–induced β-cell failure (11).
stereomicroscope in Hanks’ balanced salt solution and 10% FBS (Lonza, Berkshire, U.K.). For 11β-HSD1 activity assay (13), batches of 100 islets/cell lines were incubated in RPMI 1640 medium, 10% FBS, and 2.8, 16.5, or 5.5 mM KCl for 24 h. For RNA extraction, islets were directly homogenized in TRIzol reagent and RNAs were isolated using RNeasy micro kit (Qiagen, Paisley, U.K.) or for insulin secretion analysis, islets were preincubated (2 h) with 0.5 μg/mL brefeldin A (BFA) and 15 μM IL-1 cytokine (CHX) (Applichem, Darmstadt, Germany) and then incubated for 30 min in basal Krebs. For potassium-stimulated insulin secretion (PSIS), islets were preincubated (2 h) in basal Krebs for 30 min and then for 30 min with 40 mM 11-dehydrocorticosterone (90:10 v/v) and immunostained using guinea pig anti-insulin, sheep anti-11β-HSD1 antibody (13), rabbit anti-glucagon, somatostatin, pancreatic polypeptide (Chemicon; Millipore, Billerica, MA), and Cy2-conjugated AffiniPure F(ab’2) fragment donkey anti-guinea pig IgG, rhodamine red X-conjugated AffiniPure F(ab’2) fragment donkey anti-sheep, fluorescein isothiocyanate-conjugated AffiniPure F(ab’2) fragment donkey anti-sheep, and rhodamine red X-conjugated AffiniPure F(ab’2) fragment donkey anti-rabbit (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) were used as secondary antibodies. Images were processed using KS3000 software (version 3.0; Carl Zeiss Vision, GmbH).

**Immunofluorescence staining.** Frozen sections (5 μm) of whole pancreata or single cells from trypsin-digested islets were fixed in acetone:methanol (90:10 v/v) and immunostained using guinea pig anti-insulin, sheep anti-11β-HSD1 antibody (13), rabbit anti-glucagon, somatostatin, pancreatic polypeptide (Chemicon; Millipore, Billerica, MA), and Cy2-conjugated AffiniPure F(ab’2) fragment donkey anti-guinea pig IgG, rhodamine red X-conjugated AffiniPure F(ab’2) fragment donkey anti-sheep, fluorescein isothiocyanate-conjugated AffiniPure F(ab’2) fragment donkey anti-sheep, and rhodamine red X-conjugated AffiniPure F(ab’2) fragment donkey anti-rabbit (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) were used as secondary antibodies. Images were processed using Leica confocal microscope linked to LSM Image Browser software.

**Insulin content.** Whole pancreata were homogenized in lysis buffer, and insulin was extracted with acid ethanol and measured by radioimmunounassay (Linco Research). Insulin was normalized to total protein content (Bio-Rad Laboratories, Hercules, CA).

**Statistics.** Data are expressed as mean ± SEM and were analyzed by unpaired Student’s t test, one-way ANOVA Newman-Keuls, or two-way ANOVA.

### RESULTS

**Generation of β-cell-specific 11β-HSD1 overexpression model.** The rat 11β-HSD1 cDNA was cloned downstream of the mouse insulin I promoter (Fig. 1A) that drives highly β-cell-specific expression (10,15). The construct was validated by demonstrating increased 11β-HSD1 activity after transfection into clonal INS1E and MIN6 cells (Supplementary Fig. 1A and B). Transgenic mice were generated on the β-cell, failure-susceptible C57BL/6J (Ksj) strain background (11). Southern blotting suggested integration of a low copy number (approximately two) of the transgene (Fig. 1B). In MIP-HSD1 transgenic (MIP-HSD1) and wild-type (MIP-HSD1+/−) islets, 11β-HSD1 mRNA was increased eightfold (Fig. 1C). 11β-HSD1 protein was elevated specifically in β-cells of MIP-HSD1 transgenics (Fig. 1D and Supplementary Fig. 2). Note that 11β-HSD1 is expressed in β-cells of normal islets, as shown by the colocalization with insulin-positive cells and in other islet cell subtypes (Supplementary Fig. 3), as found by others (16). 11β-HSD1 reductase (glucocorticoid reactivation) activity was approximated twofold higher in isolated primary MIP-HSD1 transgenic islets than littermate control islets and was glucose inducible (Fig. 1E). Hexose-6-phosphate dehydrogenase level, which drives 11β-reductase activity (17), was not limiting. 11β-HSD1 dehydrogenase (glucocorticoid inactivating) activity was 10-fold lower than reductase in islets and was unaffected by transgene in high glucose (Supplementary Fig. 1C and D). MIP-HSD1+− mice were viable and appeared grossly normal (Table 1). Fasting plasma insulin, glucose triglyceride, and NEFA levels were unaffected by genotype (Table 1), indicating similar peripheral insulin sensitivity and β-cell exposure to lipotoxicity with HF.

**MIP-HSD1+− mice are protected from HF diet–induced β-cell failure.** To determine transgene impact in vivo, mice were fed an HF diet for 12 weeks to induce β-cell failure (11). Plasma corticosterone levels were not significantly affected by diet or genotype (Table 1), indicating that transgene effects were β-cell specific and not due to enhanced chemiluminescence (Amersham, Little Chalfont, U.K.) and exposure to Amersham HyperfilmTm ECL autoradiographic film (Amersham). Blots were quantified using ImageJ software (www.nih.gov.nih.gov).

**Optical projection tomography.** Optical projection tomography (OPT) was performed on whole pancreata (14) using an OPT scanner (Biopticons 2001; Skyscan, Kontich, Belgium) and images were analyzed with Data Viewer version 1.3.2 (Biopticons).

**Immunostaining.** Whole pancreata were fixed (4% paraformaldehyde), wax embedded, and sectioned (5 μm) before being immunostained with sheep anti-11β-HSD1 antibody (13). For chromosome labeling with dianisominebenzidine (Dako/Cytomation, Inc., Carpinteria, CA), biotinylated anti-sheep (Abeam, plc, Cambridge, U.K.) was used as secondary antibodies. Images were processed using KS3000 software (version 3.0; Carl Zeiss Vision, GmbH).

**Microarray analysis.** Total islet RNA was processed through standard Affymetrix Mouse Genome 430 2.0 GeneChip protocols (ARK Genomics, Roslin Institute, Edinburgh, U.K.). Data were extracted through the GCOS software, and CEL files were imported into Bioconductor, normalized by RMA in the Affy module, and statistically analyzed using the Limma and Rank products (RankProd) packages. Gene ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was done with the DAVID tool. Data are available in the Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/gene/) under accession number GSE23161.

**Intravenous glucose tolerance test.** Mice were anesthetized by intraperitoneal injection of 1 mg/mL medetomidine (Domitor) and 100 mg/Kg ketamine (Vetalog) (0.1 mL/10 g body weight). The right jugular vein was cannulated with microaven thromb (0.25 inch outer diameter × 0.012 inch inner diameter; Sandown Scientific, Hampton, U.K.). The cannula was exteriorized on the back of the neck and mice were allowed to recover for 2 days. Mice were fasted for 4 h before saline (0.2 mL/min, 2 h) and then glucose (25% w/v) was infused (10 μL/min/30 g body weight) into the mice (CMA/102 microdialysis pump; CMA Microdialysis, Solna, Sweden). Glucose infusion was stopped at 60 min. At 0, 1, 3, 5, 10, 15, 30, 60, and 120 min, blood glucose was measured by using KS3000 software (version 3.0; Carl Zeiss Vision, GmbH).

**Intraperitoneal glucose tolerance test.** Mice were fasted for 6 h and then injected intraperitoneally with 2 mg/Kg body weight, n-glucose (25% w/v). Blood was sampled at 0, 2, 5, 10, 15, 30, 60, and 120-min intervals after glucose bolus. Blood glucose was determined using Infinity reagent (Thermotrace, Leeds, U.K.) and insulin by ELISA (CrystalChem, Chicago, IL).

**Plasma triglyceride and nonesterified fatty acid levels.** Triglyceride (Infinity; Thermo Fisher Scientific, Middletown, NY) and nonesterified fatty acid (NEFA) (Wako, Richmond, VA) were measured in plasma of fed or 6-h fasted mice after intraperitoneal injection with 2 mg/kg body weight of an overnight fast.

**Western blotting.** Batches of 60 islets were incubated in RPMI 1640 medium (11.1 mM/L glucose) and 10% FBS for 24 h with or without 1 μM/L U22316 11β-HSD1 inhibitor or 5 μM/L KT5720 cAMP-dependent protein kinase (PKA) phosphorylation inhibitor (Sigma-Aldrich). Islets were homogenized in lysis buffer (50 mM/L Tris, pH 7.4, 0.27 M/L sucrose, 1 mM/L sodium orthovanadate, pH 10, 1 mM/L EDTA, 1 mM/L EGTA, 10 mM/L sodium β-glycerophosphate, 10 nM/L NaF, 5 mM/L sodium pyrophosphate, 1% [w/v] Triton X-100, 0.1% [v/v] 2-mercaptoethanol, one tablet of complete TM protease inhibitor [Roche, Burgess Hill, U.K.]) and subjected to Western blotting. Primary antibodies were anti-PKAα (cat. (1:1000) (Santa Cruz Biotechnology, Santa Cruz, CA), anti-phospho-ERK thr202 tyr204 (1:1000), anti-ERK1/2 naïve (1:1000), and anti-β-actin (1:1000) (Cell Signaling Technologies, Beverly, U.K.). HRP–anti-rabbit IgG (1:1000) was used as a secondary antibody with enhanced chemiluminescence (Amersham, Little Chalfont, U.K.) and exposure to Amersham HyperfilmTm ECL autoradiographic film (Amersham). Blots were quantified using ImageJ software (www.nih.gov.nih.gov).
hypothalamic-pituitary-adrenal disturbance. To accurately assess insulin secretion, especially over the rapid response of the first phase (1–10 min), glucose was infused through a jugular cannula. CD-fed animals had comparable responses to glucose infusion (Fig. 2A and C). HF-fed KsJ mice, as expected (11), showed markedly attenuated GSIS indicative of β-cell failure and an associated prolonged elevation of plasma glucose levels in vivo (Fig. 2B and C). In contrast, HF-fed MIP-HSD1tg/+ mice exhibited profoundly elevated first- and second-phase GSIS that completely normalized glucose levels (Fig. 2B and C).

### MIP-HSD1tg/+ mice have increased adult islet number.

Protective MIP-HSD1tg/+ compensatory hyperinsulinemia can occur through two major pathways: increased β-cell number (neogenesis, β-cell replication, and reduced apoptosis) and increased β-cell function (insulin output per β-cell). To address β-cell/islet number, whole pancreas islet number was quantified by OPT (14) (Fig. 3A and B) and validated by conventional immunohistochemistry in pancreas sections (Fig. 3C). MIP-HSD1tg/+ had more islets (Fig. 3A–C) of smaller dimensions compared with littermates on control and HF diet (Fig. 3D). Average islet size increased

### TABLE 1

<table>
<thead>
<tr>
<th>Physiological parameters of MIP-HSD1 mice</th>
<th>KsJ CD</th>
<th>KsJ HF</th>
<th>MIP-HSD1tg/+ CD</th>
<th>MIP-HSD1tg/+ HF</th>
<th>MIP-HSD1tg/+ CD</th>
<th>MIP-HSD1tg/+ HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>29.1 ± 0.36</td>
<td>30.0 ± 0.38</td>
<td>30.0 ± 0.39</td>
<td>29.5 ± 0.42</td>
<td>30.2 ± 0.4</td>
<td>29.2 ± 0.3</td>
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<tr>
<td>Food intake (kcal/mice/day)</td>
<td>12.8 ± 3.1</td>
<td>11.1 ± 1.5</td>
<td>13.2 ± 2.0</td>
<td>11.9 ± 0.8</td>
<td>10.2 ± 1.3</td>
<td>11.8 ± 1.0</td>
</tr>
<tr>
<td>Epidelomal mass (mg/g body weight)</td>
<td>19.5 ± 1.9</td>
<td>28.3 ± 1.8*</td>
<td>17.1 ± 1.4</td>
<td>23.8 ± 1.7*</td>
<td>14.2 ± 1.4</td>
<td>22.6 ± 1.3*</td>
</tr>
<tr>
<td>Liver mass (mg/g body weight)</td>
<td>46.6 ± 1.4</td>
<td>44.2 ± 1.3</td>
<td>40.8 ± 2.1</td>
<td>45.1 ± 1.6</td>
<td>39.4 ± 2.16</td>
<td>41 ± 2.3</td>
</tr>
<tr>
<td>Fasting glucose (mM/L)</td>
<td>5.4 ± 0.32</td>
<td>5.6 ± 0.48</td>
<td>4.89 ± 0.6</td>
<td>5.2 ± 0.6</td>
<td>5.2 ± 0.24</td>
<td>5.3 ± 0.3</td>
</tr>
<tr>
<td>Fasting insulin (ng/mL)</td>
<td>0.85 ± 0.33</td>
<td>0.48 ± 0.27</td>
<td>0.56 ± 0.11</td>
<td>0.74 ± 0.33</td>
<td>0.56 ± 0.35</td>
<td>0.49 ± 0.17</td>
</tr>
<tr>
<td>Fasting NEFA (nmol/L)</td>
<td>0.94 ± 0.04</td>
<td>1.27 ± 0.18</td>
<td>1.10 ± 0.10</td>
<td>1.48 ± 0.20</td>
<td>1.08 ± 0.12</td>
<td>1.18 ± 0.05</td>
</tr>
<tr>
<td>Fed insulin (ng/mL)</td>
<td>0.33 ± 0.06</td>
<td>0.68 ± 0.06**</td>
<td>0.34 ± 0.06</td>
<td>0.58 ± 0.07*</td>
<td>0.27 ± 0.04</td>
<td>0.67 ± 0.14*</td>
</tr>
<tr>
<td>Fed glucose (mM/L)</td>
<td>0.97 ± 0.03</td>
<td>0.94 ± 0.03</td>
<td>0.88 ± 0.02</td>
<td>0.89 ± 0.02</td>
<td>0.96 ± 0.01</td>
<td>0.93 ± 0.01</td>
</tr>
<tr>
<td>Corticosterone level (ng/mL)</td>
<td>6.87 ± 1.5</td>
<td>9.46 ± 2.2</td>
<td>4.98 ± 0.9</td>
<td>9.60 ± 1.4</td>
<td>4.49 ± 0.6</td>
<td>5.11 ± 0.7</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. Body weight, food intake, tissue weights, fasting glucose, insulin, fasting and fed triglycerides, NEFA, and morning corticosterone levels in 3-month-old male KsJ, MIP-HSD1tg/+, and MIP-HSD1tg/+ mice after 12 weeks on CD or HF diet. *P < 0.05 vs. CD; **P < 0.01 vs. CD; n = 9–12.
in KsJ mice with chronic HF, indicating selective loss of small functional islets (Fig. 3A–D), a process driven at least in part by apoptosis (18), whereas MIP-HSD1tg/+ mice showed attenuation of β-cell loss. β-Cell replication (as indicated by Pdx1 and Ki67 costaining) was unaltered in MIP-HSD1tg/+ transgenics (Supplementary Fig. 4). The insulin promoter is expressed at an early stage of pancreatic islet development (19) when increased glucocorticoid exposure reduces β-cell mass (20). This would predict, if anything, lower islet mass in MIP-HSD1tg/+ mice. However, the MIP-HSD1 transgene had no discernible effect on the development of the endocrine pancreas (OPT in fetal day E18 islet bodies and newborn pancreas) (Supplementary Fig. 5), suggesting that a postnatal regulatory
FIG. 3. Augmented islet number and function in HF-fed MIP-HSD1<sup>Tg/+</sup> mice. A: Representative OPT images of whole pancreas from KsJ and MIP-HSD1<sup>Tg/+</sup> mice after 12 weeks on CD or HF diet. Insulin fluorescent immunostaining is represented by red coloring. B: Quantification by OPT of insulin-positive islets per whole pancreas in KsJ and MIP-HSD1<sup>Tg/+</sup> mice on CD or HF diet. *P < 0.05, KsJ CD vs. KsJ HF; ††P < 0.01, MIP-HSD1<sup>Tg/+</sup> CD vs. KsJ CD. C: The OPT method was validated by determining the ratio of islet number per pancreatic surface area in pancreatic sections immunostained for insulin. *P < 0.05, KsJ CD vs. KsJ HF; ††P < 0.05, MIP-HSD1<sup>Tg/+</sup> HF vs. KsJ HF; ‡†P < 0.01, MIP-HSD1<sup>Tg/+</sup> CD vs. KsJ CD. D: Islet size (ratio of islet area to total pancreatic surface area; mm<sup>2</sup>) in pancreatic sections immunostained for insulin. ***P < 0.001, KsJ CD vs. KsJ HF; †P < 0.05, MIP-HSD1<sup>Tg/+</sup> CD vs. KsJ CD. §§P < 0.01, MIP-HSD1<sup>Tg/+</sup> HF vs. MIP-HSD1<sup>Tg/+</sup> CD. E: GSIS from batches of 10 size-matched islets from CD-fed (left) or HF-fed (right) KsJ and MIP-HSD1<sup>Tg/+</sup> mice exposed to 2.8 mmol/L and then 16.8 mmol/L glucose. *P < 0.05, MIP-HSD1<sup>Tg/+</sup> HF vs. KsJ HF (n = 6). F: Basal insulin secretion by islets from KsJ and MIP-HSD1<sup>Tg/+</sup> mice on CD or HF diet were preincubated for 2 h in 2.8 mmol/L glucose with or without 0.5 μg/mL BFA and 15 μmol/L CHX and then incubated for 30 min with...
mechanism drives increased islet number in MIP-HSD1tg/+ mice.

MIP-HSD1tg/+ mice have augmented islet-autonomous secretory function. To test the additional possibility of enhanced islet insulin secretory function, we measured GSIS in carefully size-matched (average diameter 100 μm) isolated islets in vitro. In agreement with the findings in vivo, isolated islets from CD-fed KsJ and MIP-HSD1tg/+ mice showed similar GSIS (Fig. 3E, left), whereas islets from HF-fed MIP-HSD1tg/+ mice exhibited augmented GSIS (Fig. 3E, right).

MIP-HSD1tg/+ islets have augmented membrane-proximal secretory capacity. We next investigated whether the insulin secretory machinery was specifically enhanced in MIP-HSD1tg/+ islets. To assess this, insulin translation and Golgi trafficking was inhibited with CHX and brefeldin A (BFA), revealing relatively enhanced basal (unregulated) insulin secretion from islets of MIP-HSD1tg/+ mice on HF compared with control-fed mice (Fig. 3F). In addition, GSIS (glucose independent, membrane proximal) was significantly higher from islets of HF-fed MIP-HSD1tg/+ mice compared with HF-fed KsJ mice (Fig. 3G), consistent with a larger pool of secretory granules and/or a faster rate of exocytosis in islets of MIP-HSD1tg/+ mice on HF.

11β-HSD1/glucocorticoid-dependent effects on islet function are dose sensitive. To confirm glucocorticoid and 11β-HSD1 dependence of the transgene, islets were incubated acutely (2 h) in 20 nmol/L (—physiological) or 200 nmol/L (supraphysiological) 11-DHC. GSIS was significantly higher from islets from HF-MIP-HSD1tg/+ mice exposed to 20 nmol/L 11-DHC, and this was abolished by the 11β-HSD1 inhibitor U2316 (Supplementary Table 1) and the GR antagonist RU486. Supraphysiological 11-DHC completely blunted GSIS, as mentioned by others (4,16). Inhibition was also reversed in islets of HF-fed MIP-HSD1tg/+ mice by 11β-HSD1 and GR inhibitors.

Palmitate-stimulated GSIS is augmented in MIP-HSD1tg/+ islets in vitro. Fatty acids augment insulin secretion in the short term. To investigate the role of elevated β-cell 11β-HSD1 in this process, palmitate-stimulated GSIS was assessed in isolated islets from MIP-HSD1tg/+ after 24 h exposure to 20 nmol/L 11-DHC. Notably, long-term static exposure of islets to 20 nmol/L 11-DHC alone impaired insulin release in vitro (Supplementary Fig. 8). Palmitate-stimulated GSIS from MIP-HSD1tg/+ was reversed by 11β-HSD1 and GR inhibitors (Supplementary Fig. 8), suggesting a role for 11β-HSD1 in fatty acid-stimulated GSIS.

MIP-HSD1tg/+ islets show induction of genes of differentiation, secretory pathways, and cellular stress management. To better understand the mechanisms of improved islet function in HF-fed MIP-HSD1tg/+ mice, we performed a comparative transcriptomic analysis (Supplementary Table 2). MIP-HSD1tg/+ islets had higher mRNA levels for genes of the small GTPase pathway (Park2, Braf, Cdkn1a, and the GR antagonist RU486. Supraphysical 11-DHC completely blunted GSIS, as mentioned by others (4,16). Inhibition was also reversed in islets of HF-fed MIP-HSD1tg/+ mice by 11β-HSD1 and GR inhibitors (Supplementary Fig. 8), suggesting a role for 11β-HSD1 in fatty acid-stimulated GSIS.

BFA/CHX in 2.8 mmol/L glucose in order to stop both neosynthesis of insulin and neof ormation of newly formed insulin vesicles. *P < 0.05, MIP-HSD1tg/+ HF vs. MIP-HSD1tg/+ CD. †P < 0.01, treated MIP-HSD1tg/+ HF vs. treated MIP-HSD1tg/+ CD. n = 4. G: PSIS from islets of KsJ and MIP-HSD1tg/+ mice on CD or HF diet incubated for 30 min in 2.8 mmol/L glucose and then 30 min with 40 mmol/L KCl. *P < 0.05, MIP-HSD1tg/+ HF vs. KsJ HF. n = 6. H: GSIS from islets of KsJ and MIP-HSD1tg/+ mice on CD or HF diet preincubated for 2 h with 20 nmol/L 11-DHC (left) or 200 nmol/L 11-DHC (right) with or without 1 μmol/L U2316 or 1 μmol/L RU486 before incubation for 30 min with 2.8 mmol/L glucose followed by 30 min with 16.8 mmol/L glucose. *P < 0.05, MIP-HSD1tg/+ HF vs. KsJ HF. †P < 0.05, MIP-HSD1tg/+ HF vs. MIP-HSD1tg/+ CD. ‡P < 0.05, MIP-HSD1tg/+ HF treated vs. MIP-HSD1tg/+ HF. n = 5–6. (A high-quality digital representation of this figure is available in the online issue.)
FIG. 4. Validation of selected genes changes from microarray functional clusters at the mRNA, protein, and protein phosphorylation level associated with improved islet function in HF-fed MIP-HSD1\textsuperscript{tg/\textsuperscript{+}}. Prkacb (A), hspa1b (B), Braf (C), and Cdkn1a (D) mRNA expression was measured in isolated islets of KsJ and MIP-HSD1\textsuperscript{tg/\textsuperscript{+}} mice on CD or HF diet and normalized to gene expression of actin. *P < 0.05; **P < 0.01; MIP-HSD1\textsuperscript{tg/\textsuperscript{+}} HF vs. KsJ HF. #P < 0.05, KsJ HF vs. KsJ CD. †P < 0.05; ††P < 0.01, MIP-HSD1\textsuperscript{tg/\textsuperscript{+}} HF vs. MIP-HSD1\textsuperscript{tg/\textsuperscript{+}} CD. n = 6–8 (2–3 mice per islet preparation). AU, arbitrary units. Isolated islets from KsJ and MIP-HSD1\textsuperscript{tg/\textsuperscript{+}} mice on CD or HF diet were incubated in the absence (E and H) or presence of 1 \( \mu \)mol/L of the selective 11\( \beta \)-HSD1 inhibitor UE2316 (F and I) or 5 \( \mu \)mol/L PKA inhibitor KT5720 (G and J) for 24 h. Total extracts were subjected to immunoblotting analysis for PKA\( \beta \) catalytic subunit and \( \beta \)-actin (loading control) (E–G), th\( r \)202/tyr204ERK1/2 phosphorylation, and ERK1/2 native protein (H–J). Representative images of the blots are shown above the quantification. Quantitative analysis shows protein expression level normalized to the respective control proteins and then expressed relative to the values in the KsJ control mice. Note that proteins from CD mice were run on separate gels from HF mice together with the same internal control, which allowed signal comparison. *P < 0.05; **P < 0.01, MIP-HSD1\textsuperscript{tg/\textsuperscript{+}} HF vs. KsJ HF. †P < 0.05; ††P < 0.01, MIP-HSD1\textsuperscript{tg/\textsuperscript{+}} HF vs. MIP-HSD1\textsuperscript{tg/\textsuperscript{+}} CD. #P < 0.05; ###P < 0.001, KsJ HF vs. KsJ CD. §P < 0.05; §§§P < 0.001, MIP-HSD1\textsuperscript{tg/\textsuperscript{+}} CD vs. KsJ CD. n = 6.
maintained (Supplementary Fig. 7C, D, and F). However, MIP-HSD1<sup>tg/tg</sup> mouse GSIS was comparable to littermates on HF diet (Supplementary Fig. 7E, left, and F, left), reflecting a relative improvement in GSIS versus CD, and this partially normalized glucose tolerance (Supplementary Fig. 7E, right, and F, right). In agreement with this intermediate phenotype, GSIS from isolated islets of HF-fed MIP-HSD1<sup>tg/tg</sup> mice showed a higher basal and peak secretion, although this was not sustained over the whole second phase (Supplementary Fig. 7F).

**Islet 11β-HSD1 level is predictive of β-cell compensatory capacity and diabetes susceptibility in mice.** Considering the inverted U-shaped MIP-HSD1 gene dose-response effect on β-cell secretory function, we assessed islet 11β-HSD1 protein and activity levels in contrasting models of β-cell failure (HF-fed KsJ and Lep<sup>db/db</sup> mice) and β-cell compensation (C57BL/6J mice). HF induced a modest elevation of 11β-HSD1 in islets from robustly compensating C57BL/6J mice (Fig. 5A and B). In contrast, down-regulation of islet 11β-HSD1 was found in β-cell failure–susceptible, HF-fed KsJ mice (Fig. 5C and D). Markedly high 11β-HSD1 levels were found in diabetic Lep<sup>db/db</sup> islets (Fig. 5E and F).

**DISCUSSION**

Our data show that, for the first time, an unexpectedly beneficial metabolic outcome results from optimal elevation of β-cell 11β-HSD1, in contrast to the diabetogenic effects of elevated 11β-HSD1 in adipose tissue and liver (3). Moreover, this effect may be physiologically relevant, as normal mice that exhibit robust compensatory insulin secretion on HF exhibit a similar modest upregulation of β-cell 11β-HSD1. A higher or lower 11β-HSD1 level suppresses GSIS, consistent with an inverted U-shaped dose-response effect sometimes seen with glucocorticoids (31). This notion is supported by the stimulatory effect of physiological 11β-HSD1 substrate (11-DHC) but suppressive effects of high or indeed long-term exposure to 11-DHC in vitro. In agreement, diabetic Lep<sup>db/db</sup> mice (32) and MIP-HSD1<sup>tg/tg</sup> on CD have impaired GSIS in vivo associated with more marked elevation of β-cell 11β-HSD1. These data are also consistent with the association between increasing 11β-HSD1 levels and the severity of hyperglycemia in diabetic fa/fa rats (5). Conversely, 11β-HSD1<sup>−/−</sup> mice on CD and KsJ mice on HF diet have β-cell secretory defects associated with the absence or decrease of 11β-HSD1 activity, respectively. These findings may have implications
for the timing and dose of steroid or 11β-HSD1 inhibitor interventions in the therapeutic treatment of patients predisposed to β-cell failure. Defining the impact of altered islet 11β-HSD1 expression across the progression from glucose intolerance, prediabetes, and frank diabetes in both rodent models and humans, relative to peripheral tissue 11β-HSD1 levels, will be of future importance to assess any adverse risks of therapeutic inhibition. However, the dominant protection from glucose intolerance found in HF-fed 11β-HSD1−/− mice indicates that beneficial effects of 11β-HSD1 inhibition may outweigh any detrimental effect on β-cells.

The gene-dose, inverted-U effect of the MIP-HSD1 transgene may reconcile conflicting reports of suppressive (4–8) versus stimulatory effects (33–35) of glucocorticoids on insulin secretion. Thus, the prevailing effect is likely a function of dose and duration. Indeed, although our in vitro data are generally supportive of our in vivo findings, we note that long-term, but not short-term, static incubation with low 11-DHC is suppressive. We contend that static incubation with steroids and isolated islets in vitro does not reflect dynamic glucocorticoid exposure/metabolism patterns found in vivo. This merely serves to underline the importance of studying the impact of altered β-cell 11β-HSD1 in the whole animal chronic disease context. Of note, β-cell–specific GR overexpression (RIP-GR) causes direct suppression of insulin secretion (7). Beyond exposure and dose issues, this suggests that MIP-HSD1 effects could result from additional effects beyond GR activation. For example, physiological corticosterone binds with high affinity to mineralocorticoid receptors, which antagonizes GR-mediated suppression of insulin secretion and may thus contribute to beneficial (36) and dose-sensitive effects. Notably, the synthetic GR-selective glucocorticoid dexamethasone, commonly used in in vitro islet studies, is a poor 11β-HSD1 substrate, perhaps further explaining differences between solely GR- versus 11β-HSD1–mediated effects in islets. 11β-HSD1 reductase activity depletes endoplasmic reticulum (ER) NADPH, but this does not appear to affect the increased cytosolic NADPH levels required to activate GSIS (37). Elevated 11β-HSD1 may also preferentially amplify nongenomic effects of glucocorticoids (38) more than GR overexpression, or indeed non-GR effects due to alternative substrate metabolism (39) by 11β-HSD1. Indeed, alternate 11β-HSD1 metabolites (40) may affect GSIS through oxysterol activation of nuclear liver X receptor (41). Finally, RIP-GR mice may manifest impaired GSIS as a result of developmentally impaired β-cell mass (20), unlike the MIP-HSD1 model. In any case, there is more evidence to suggest that islet 11β-HSD1, rather than GR, levels are elevated in islets from diabetic mice (5,6, and the current study), suggesting the MIP-HSD1 mice are perhaps a more relevant in vivo model. Crucially, the unexpected augmentation of GSIS in the MIP-HSD1 mice due to chronic modest elevation of β-cell 11β-HSD1 in vivo has allowed us to challenge prevailing hypotheses on glucocorticoid-mediated diabetogenic effects in islets. Those hypotheses are derived from arguably artificial systems such as systemic administration of high-dose steroids in vivo, which induces confounding peripheral insulin resistance, as well as the effects of acute and often pharmacological steroid concentrations in islets in vitro.

Elevated expression of genes involved in differentiation, insulin secretion, and cellular stress protection and survival are associated with augmented MIP-HSD1186 islet function. Although similar effects of glucocorticoids have been noted in other cellular systems, our data show these effects for the first time, to our knowledge, in pancreatic islets, and highlight novel areas of cross-talk in this cell type. Thus, glucocorticoids curtail proliferation and induce differentiation, in part through the increased cyclin-dependent kinase inhibitor 1A (p21) (24,26,27,42) and enhanced differentiation through ERK1/2 (26). Increased v-raf murine sarcoma viral oncogene homolog B1 (B-Raf) signaling (43) and enhanced PKAβcat and small GTPase activation makes a clear mechanistic link with augmented insulin secretory capacity (21,25,43) as well as islet survival in the face of HP diet-induced ER stress (23); chronic exposure to elevated NEFA was comparable between genotypes, indicating a β-cell–specific protection from lipotoxicity in the MIP-HSD1186 mice. In contrast, we find a novel permissive role for 11β-HSD1 in short-term, fatty acid–induced GSIS in vitro, suggesting a physiological role for 11β-HSD1 in meal lipid–related GSIS processes. Further, a direct interaction between GR and PKA has been proposed in other cellular systems (40,44), suggesting a novel mechanism for functional augmentation of β-cells that can now be fully explored. Heat shock protein induction is consistent with increased GR activation (45,46) and, along with p21 in islets (29), is associated with cell survival and protection from apoptosis (28,47), echoing critical processes activated in β-cells that successfully adapt to ER stress (30,45). Note the impact of elevated 11β-HSD1 on β-cell apoptosis will need careful future exploration in model systems other than the HF-fed C57BL/KsJ mice where this process is more evident and quantifiable. Notably, we did not find suppression of inflammatory processes linked to islet damage in type 2 diabetes (48), as occurs when islets are exposed to high levels of corticosterone (45), perhaps reflecting the more physiological nature of β-cell protection in our model. The data therefore give insight into a novel mechanistic framework whereby optimally elevated local glucocorticoid action may facilitate safe execution of increased secretory demand, thus avoiding β-cell failure. Of particular note, given the topical nature of replication as a mechanism for compensation (49,50), the novel protective response of MIP-HSD1186 mice does not involve increased β-cell replication. Intriguingly, despite suppressed MIP-HSD1186 islet function under basal (CD) conditions, the markedly elevated β-cell 11β-HSD1 of MIP-HSD1186 elicits an HP diet–responsive β-cell rescue response that is adequate enough to normalize glucose homeostasis. This suggests the potential for mechanistic dissection of therapeutic glucocorticoid effects from those that are undesirable.

Transgenic elevation of β-cell 11β-HSD1 has revealed a novel physiological function for the enzyme in β-cell compensation that may open up new therapeutic opportunities. More broadly, coordinated regulation of local glucocorticoid regeneration in β-cells with dynamic changes in adipose tissue delineates a responsive physiological process that shapes metabolism to protect against metabolic disease. Of more immediate medical significance, our data critically inform imminent therapeutic strategies aimed at manipulation of tissue glucocorticoid action.

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