SUMOylation reduces oxidative stress and preserves islet mass at the expense of robust insulin secretion. To investigate a role for the deSUMOylating enzyme sentrin-specific protease 1 (SENP1) following metabolic stress, we put pancreas/gut-specific SENP1 knockout (pSENP1-KO) mice on a high-fat diet (HFD). Male pSENP1-KO mice were more glucose intolerant following HFD than littermate controls but only in response to oral glucose. A similar phenotype was observed in females. Plasma glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) responses were identical in pSENP1-KO and wild-type littersmates, including the HFD-induced upregulation of GIP responses. Islet mass was not different, but insulin secretion and \(\beta\)-cell exocytotic responses to the GLP-1 receptor agonist exendin-4 (Ex4) and GIP were impaired in islets lacking SENP1. Glucagon secretion from pSENP1-KO islets was also reduced, so we generated \(\beta\)-cell–specific SENP1 KO mice. These phenocopied the pSENP1-KO mice with selective impairment in oral glucose tolerance following HFD, preserved islet mass expansion, and impaired \(\beta\)-cell exocytosis and insulin secretion to Ex4 and GIP without changes in cAMP or Ca\(^{2+}\) levels. Thus, \(\beta\)-cell SENP1 limits oral glucose intolerance following HFD by ensuring robust insulin secretion at a point downstream of incretin signaling.

Glucose metabolism is the primary driver for insulin secretion, stimulating electrical activity and Ca\(^{2+}\) entry to trigger the exocytosis of insulin granules. Multiple additional factors serve to either maintain or augment the pool of secretory granules available to respond to the Ca\(^{2+}\) increase (1). These pathways could be required for robust insulin secretory responses to receptor-mediated secretagogues, such as the incretin hormones glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) (2). The action of the incretins to facilitate insulin secretion is impaired in type 2 diabetes (T2D), although the underlying mechanism appears complex (3) and could involve altered receptor signaling (4). Incretin-induced insulin secretion from human islets correlates with the ability of glucose to augment depolarization-induced insulin exocytosis in single \(\beta\)-cells (5). In mice with impaired metabolism–insulin granule coupling, achieved by \(\beta\)-cell–specific deletion of the sentrin-specific protease 1 (SENP1), we showed that the \(\beta\)-cell response to the GLP-1 receptor agonist exendin-4 (Ex4) was impaired and that the ability of dipeptidyl peptidase 4 inhibition to improve oral glucose tolerance was greatly reduced (5).

Glucose metabolism drives the export mitochondrial reducing equivalents, and the resulting redox signal relay increases the small ubiquitin-like modifier (SUMO) protease activity of SENP1 (6). A resulting deSUMOylation of exocytotic proteins, such as synaptotagmin VII, facilitates Ca\(^{2+}\)–dependent insulin granule exocytosis (7–11). This redox-dependent pathway appears impaired in T2D, likely as a result of upstream mitochondrial dysfunction, and loss of islet SENP1 results in moderate oral glucose
intolerance with impaired insulin secretion (7). Somewhat contradicting this, overexpression of SENP1 induces apoptosis in β-cells (12), and mice lacking the SUMO-conjugating enzyme Ubc9 develop diabetes as a result of β-cell death (13). Effectively, SUMOylation appears required for β-cell viability at the cost of β-cell function (14). It remains unknown whether the deSUMOylating enzyme SENP1 is required for the facilitation of insulin secretory responses and glucose tolerance under metabolic stress, such as high-fat diet (HFD), or conversely, whether loss of SENP1 would protect against glucose intolerance by preserving β-cell mass and insulin secretion.

Here, we investigated two interrelated questions in both pancreas/gut-specific SENP1 knockout (pSENP1-KO) and β-cell–specific SENP1 KO (βSENP1-KO) mice. We asked whether the loss of β-cell SENP1 sensitizes mice to HFD-induced glucose intolerance and whether altered incretin responsiveness contributes to this. We find that following HFD, both pSENP1-KO mice and βSENP1-KO mice show a worsening of oral glucose intolerance. This is accompanied by a decreased insulin secretory response to glucose and incretin receptor activation without any difference in β-cell mass and little or no effect on cAMP or Ca2+ responses to incretin receptor activation. Our findings support a model whereby SENP1 is required to ensure the availability of releasable insulin granules. This is important for limiting oral glucose intolerance following HFD where incretins, notably plasma GIP, are increased and require a robust pool of release-competent insulin granules on which to act.

RESEARCH DESIGN AND METHODS

Animals, Diets, and In Vivo Studies

Pdx1-Cre mice [B6.FVB-Tg (Pdx1-cre)6 Tuv/J, 014647; The Jackson Laboratories] on a C57BL/6 background and Ins1-Cre mice on a mixed C57BL/6 and SV129 background (15) were crossed with Senp1-floxed mice on a C57BL/6 background (7) to generate Pdx1-Cre+/Senp1fl/fl KO (pSENP1-KO) and Ins1-Cre+/Senp1fl/fl KO (βSENP1-KO) mice (5,7). Pdx1-Cre−;Senp1−/− and Ins1-Cre−;Senp1−/− mice were used as heterozygotes (pSENP1-HET and βSENP1-HET, respectively). Pdx1-Cre−;Senp1+/− and Ins1-Cre−;Senp1+/− mice were used as wild-type (WT) littermate controls (pSENP1-WT and βSENP1-WT, respectively). Genotypes were confirmed from ear notches (7). Loss of expression was confirmed by nested PCR and Western blot. At 12 weeks of age, mice were fed an HFD (60% fat, CA89067-471; Bio-Serv) for 8–10 weeks.

Mice were fasted 4–5 h prior to oral glucose tolerance test (OGTT) (16), intraperitoneal (IP) glucose tolerance test (IPGTT) (17), or insulin tolerance test (ITT) (18). OGTT and IPGTT were with dextrose at doses indicated in the figures, and ITT was with IP injection of 1 units/kg Humulin R (Eli Lilly). The timelines of OGTT, IPGTT, and ITT are shown in the figures. Tail blood was collected for glucose and insulin measurement (7). To measure total plasma GLP-1, GIP, and glucagon, before or after oral glucose gavage (2 g/kg dextrose), tail blood was collected at the indicated times, and plasma was frozen until assay (Multi Species GLP-1 Total ELISA Kit [Millipore], Mouse GIP ELISA Kit [Crystal Chem], U-Plex Mouse Glucagon ELISA Kit [Mesoscale Discovery]).

Western Blotting

SENP1 antibody-C12 (1:500, sc-271360; Santa Cruz Biotechnology), SUMO1 antibody (1:1,000, ab133352; Abcam), and β-actin antibody (1:2,000, sc-47778; Santa Cruz Biotechnology) were used as primary antibodies. Anti-mouse (1:5,000, NA934V; GE Healthcare) or anti-rabbit (1:5,000, NA931V; GE Healthcare) were used as secondary antibodies. Mouse islets, gut, and brain were homogenized with 7 mol/L guanidine HCl. The protein was precipitated by addition of methanol, chloroform, and water in a 4:1:3 (v/v) ratio, and the pellet was recovered (10,000 rpm for 10 min) and dissolved in 1% SDS, 0.2 mol/L Tris, 10 mmol/L dithiothreitol, pH 6.5. Protein concentration was estimated by absorbance at 280 nm. Fifty, 10, and 10 μg protein from islets, intestine, and brain, respectively, were separated by SDS-PAGE (7.5% gel), transferred to polyvinylidene fluoride membrane, and probed with primary antibody in the presence of 5% skin milk. For SUMOylation (19), islets were incubated in Krebs-Ringer bicarbonate HEPES buffer with 2.8 mmol/L glucose for 2 h followed by 16.7 mmol/L glucose for 15 min. Islets were washed with cold PBS and put in lysis buffer with 10 mmol/L N-ethylmaleimide (128-53-0; MilliporeSigma), 100 μL PhosStop (1 tablet in 1 mL lysis buffer; MilliporeSigma), and 10 μL protease inhibitor (P8340; MilliporeSigma). Ten micrograms of protein were loaded for SDS-PAGE (10% gel).

Nested Quantitative PCR of SENP1

To evaluate KO efficiency, the cDNA encoding exons 14 and 15 was quantified by nested quantitative PCR (qPCR). Total RNA was extracted from kidney, brain, stomach, intestine, and islets using TRIzol reagent (15596018; Thermo Fisher Scientific). cDNA was prepared from total RNA (50–100 ng) with All-In-One 5X RT Master Mix (G486; Applied Biological Materials Inc.). The cDNAs of Senp1 and Ppia were amplified using preamplification primers and Platinum Taq DNA polymerase (10966-018; Thermo Fisher Scientific) with the following cycling parameters: 15 cycles of 94°C for 30 s, 60°C for 10 s, 55°C for 10 s, and 72°C for 25 s. To remove Taq DNA polymerase and the primers, the PCR fragment was incubated with 4 mol/L guanidine HCl for 10 min at room temperature and precipitated with 80% ethanol in the presence of glycogen (50 mg/mL). The nested qPCR was carried out with the qPCR primers Fast SYBR Green Master Mix (438512; Applied Biosystems), 7900HT Fast Real-Time PCR System (Applied Biosystems), and the preamplified cDNAs as the templates with the following cycling parameters: 40 cycles of 95°C for 5 s and 60°C for 20 s. All primers are listed in Supplementary Table 1.
Pancreatic Islet Isolation, Insulin, and Glucagon Secretion

Islets were isolated by collagenase digestion of the pancreas (20) and cultured overnight. Insulin secretion was measured by perfusion (7). Briefly, 25 (insulin) or 75–85 (glucagon) islets were preperifused for 30 min at 2.8 mmol/L glucose before sample collection and treated with 16.7 mmol/L glucose, Ex4 (10 mmol/L; MilliporeSigma), GIP (100 nmol/L; AnaSpec), alanine (10 mmol/L; MilliporeSigma), oleate (0.5 mmol/L, MilliporeSigma) (21,22), or KCl (30 mmol/L). Samples were collected every 1–5 min at a flow rate of 100 μL/min, and then islets were lysed in acid/ethanol. All samples were stored at −20°C until assayed for insulin (STELLUX Chemi Rodent Insulin ELISA; ALPCO) and glucagon (Rodent Glucagon Assay or U-PLEX Mouse Glucagon Assay; Mesoscale Discovery). For the glutamine- and leucine-stimulated insulin secretion (10 mmol/L each; MilliporeSigma), static insulin secretion was performed (23).

Patch Clamp Analysis

Islets were handpicked and incubated with Ca2+-free buffer at 37°C for 10 min before being shaken and dispersed into single cells (9). Cells were incubated in 35-mm dishes (430165; Thermo Fisher Scientific) in RPMI medium (11875; Thermo Fisher Scientific) with 11.1 mmol/L glucose, 10% FBS, and 100 units/mL penicillin/streptomycin, and 2.8 mmol/L glucose for 1 h prior to switching to bath solution. For the remaining experiments (5 mmol/L glucose), cells were switched to the bath without preincubation. Bath solution contained 118 mmol/L NaCl, 5.6 mmol/L KCl, 20 mmol/L tetraethylammonium, 1.2 mmol/L MgCl2·6H2O, 2.6 mmol/L CaCl2, and 5 mmol/L HEPES with glucose, Ex4 (10 mmol/L; MilliporeSigma), and/or GIP (100 mmol/L; AnaSpec) as indicated (pH 7.4 adjusted with NaOH) at 32−35°C. The pipette solution contained 125 mmol/L Cs-glutamate, 10 mmol/L NaCl, 10 mmol/L CsCl, 1 mmol/L MgCl2·6H2O, 0.05 mmol/L EGTA, 5 mmol/L HEPES, and 3 mmol/L MgATP (solution pH 7.15 adjusted with CsOH) with or without 0.1 mmol/L cAMP as indicated in the figure legends. Patch clamp was performed in the standard whole-cell configuration with sine+DC (direct current) Lockin function of an EPC 10 amplifier (HEKA Electronik) 5−30 min after switching cells to the bath solution. As such, cells were preexposed to bath glucose, Ex4, or GIP prior to establishment of the whole-cell configuration. Exocytotic responses and inward Ca2+ currents were measured 1−2 min after obtaining the whole-cell configuration in response to 10 500-ms depolarizations to 0 mV from a holding potential of −70 mV. Changes in capacitance and integrated Ca2+ charge entry were normalized to cell size (fF/pF and pC/pF, respectively). Mouse β-cells were identified by cell size (>4 pF) and half-maximal inactivation of Na+ currents near −90 mV (24).

β-Cell cAMP and Intracellular Ca2+ Imaging

The cAMP biosensor (Epac-εH187, Kd = 4 μmol/L) (25) under control of the rat insulin promoter was expressed by adenoviral expression (26). Islets were infected immediately postisolation with 1.5 μL of high-titer adenovirus for 2 h at 37°C and then moved to fresh media overnight. Islets from BSEN1-WT and KO mice were imaged simultaneously; one group was prelabeled with 1 μg/mL DiR (1,1-dioctadecyl-3,3,3,3-tetramethylindocarbocyanine iodide) (Molecular Probes, Eugene, OR) for 10 min. DiR labeling had no effect on islet metabolic or Ca2+ oscillations (data not shown). For measurements of cytosolic Ca2+, islets were preincubated in 2.5 μmol/L FuraRed (F3020; Molecular Probes) at 37°C for 45 min before they were placed in a glass-bottomed imaging chamber (Warner Instruments) and mounted on an ECLIPSE Ti inverted microscope with a 10×/0.50 numerical aperture SuperFluor objective (Nikon Instruments). The chamber was perfused with external solution containing 135 mmol/L NaCl, 4.8 mmol/L KCl, 2.5 mmol/L CaCl2, 1.2 mmol/L MgCl2, and 20 mmol/L HEPES (pH 7.35). The flow rate and temperature were maintained at 0.25 mL/min and 33°C using feedback control (MFCS-EZ; Fluigent). Excitation was by a SOLA SEII 365 (Lumencor) at 10% output. Single DiR images used a Chroma Cy7 cube (710/75×, T760lpxr, 810/90 m). For FuraRed, excitation (430/24× and 500/20×, ET type; Chroma Technology Corporation) and emission (650/60 m) filters (BrightLine; Semrock) were used with an FF444/521/608-Di01 dichroic mirror (Semrock) and reported as an excitation ratio (R430/500). The same dichroic mirror was used for cAMP biosensor fluorescence resonance energy transfer imaging, with cyan fluorescent protein excitation provided by an ET430/24× filter and emission filters for cyan fluorescent protein and Venus emission (ET470/ 24m and ET535/30m; Chroma Technology) reported as an emission ratio (R470/535). Fluorescence emission was collected with an ORCA-Flash4.0 V2 Digital CMOS camera (Hamamatsu) every 6 s. A single region of interest was used to quantify the average response of each islet using Nikon Elements and custom MATLAB software (MathWorks).

Histological Analysis

Pancreata were weighed prior to being fixed in Z-fix (VWR International) and embedded in paraffin. Blocks were sectioned at 5-μm thickness with a total of three to five slides (each separated by 500 μm) for immunostaining and imaging (7). Paraffin sections were rehydrated, permeabilized, blocked, and incubated with guinea pig polyclonal insulin antibody (1:60, A0564; Dako) and mouse polyclonal glucagon antibody (1:1,000; MilliporeSigma) overnight. Sections were washed and incubated
Figure 1—Normal glucose tolerance, but impaired insulin responses, of pSENP1-KO mice on CD. A: i) Western blot of SENP1 expression in tissues from pSENP1-WT and -KO mice. ii) SUMOylation profiles of islet lysates from pSENP1-WT and -KO mice showing numerous SUMOylated proteins. B: Representative traces (left) and average total responses of β-cell exocytosis elicited by a series of 500-ms membrane depolarizations from −70 to 0 mV at 2.8 and 10 mmol/L glucose (n = 28, 22, 22, and 20 cells; n values correspond to graph bars from left to right, respectively). The pipette solution included 0.1 mmol/L cAMP. C: Representative traces, and average β-cell voltage-dependent Ca^{2+} currents elicited by a single 500-ms membrane depolarization from −70 to 0 mV at 2.8 and 10 mmol/L glucose (n = 26, 18, 25, and 16 cells). The pipette solution included 0.1 mmol/L cAMP. D: Schematic diagram of experiments on CD-fed mice. E: OGTT in male pSENP1-WT, -HET, and -KO mice (n = 6, 6, and 6 mice). F and G: IPGTT in male pSENP1-WT, -HET, and -KO mice (n = 8, 13, and 10 mice) (F) and associated plasma insulin responses (n = 8, 9, and 8 mice) (G). H: OGTT in female pSENP1-WT, -HET, and -KO mice (n = 8, 9, and 9 mice). I and J: IPGTT in female pSENP1-WT, -HET, and -KO mice (n = 9, 7, and 7 mice) (I) and associated plasma insulin
with Alexa Fluor 488 goat anti-guinea pig IgG (1:500, A11073; Thermo Fisher Scientific) and Alexa Fluor 594 goat anti-mouse IgG (1:500 A11037; Thermo Fisher Scientific) for 1 h, washed, and mounted in ProLong Gold Antifade Mountant with DAPI (Life Technologies). To determine islet area, insulin-positive cells were identified with software tools from ImageJ (National Institutes of Health) and normalized to total pancreas area. The islet mass was calculated as pancreas weight \times \text{relative islet area} (as a proportion of pancreas section area). Islet size was determined by manual outlining in a blinded fashion using ZEN Pro (Zeiss) and ImageJ software.

Statistical Analysis

GraphPad Prism 8 for Mac OS X software was used for one-way or two-way ANOVA followed by Bonferroni post-test to compare means between groups. Unbiased robust regression followed by outlier identification analysis was used for outlier identification and removal.

Data and Resource Availability

All data generated or analyzed during this study are included in the published article and its online supplementary files. Resources generated in the current study are available from the corresponding author upon reasonable request.

RESULTS

Mice Lacking Islet SENP1 Develop Worsened Oral Glucose Intolerance After HFD

We previously showed that SENP1 is required for insulin exocytosis and that male pSENP1-KO mice are mildly intolerant of oral glucose (7). Because of loss of Cre expression in the previous colony, we made the pSENP1-KO mice by crossing Pdx1-Cre<sup>i</sup> mice from The Jackson Laboratories [B6.FVB-Tg (Pdx1-cre)6 Tuv/J] and Pdx1-Cre<sup>−/−</sup> Senp1<sup>i</sup>/<sup>r</sup> and confirmed loss of SENP1 (Fig. 1A) and increased SUMOylation (Fig. 1Aii, arrows) in islets. β-Cells from pSENP1-KO mice had impaired glucose-dependent facilitation of exocytosis (Fig. 1B) and unaffected voltage-dependent Ca<sup>2+</sup> currents (Fig. 1C). The pSENP1-KO mice had modest fasting hyperinsulinemia compared with littermates (Supplementary Fig. 1). We performed OGTT, IPGTT, and ITT on male and female mice at 10–12 weeks of age (Fig. 1D). Male pSENP1-KO mice were not obviously intolerant to oral or IP glucose (Fig. 1E and F and Supplementary Fig. 1A–D), despite reduced glucose-stimulated plasma insulin (Fig. 1G). Female mice exhibited a similar phenotype, with some indication of IP glucose intolerance (Fig. 1H–J and Supplementary Fig. 1E–H).

After HFD (Fig. 2A), there was no difference in insulin tolerance or fasting insulin (Fig. 2B and C), but male pSENP1-KO mice exhibited elevated fasting glucose and body weight (Fig. 2D and E). IP glucose intolerance was only modestly worsened in the pSENP1-KO mice (Fig. 2F), but these mice were clearly more intolerant of an oral glucose challenge with a decreased plasma insulin response compared with littermate controls (Fig. 2G and H). With a higher dose of glucose, there was still no worsened IP glucose intolerance in the pSENP1-KO mice (Fig. 2I), but a larger oral glucose challenge resulted in severely impaired glucose tolerance and plasma insulin (Fig. 2J and K). Similar to other reports (27), female mice were relatively resistant to HFD, and we did not observe any worsening of oral glucose intolerance in the female pSENP1-KO mice (Supplementary Fig. 2A–F).

A previous study showed that increasing SUMOylation protects from oxidative stress and preserves islet mass (13). Following HFD, we found no differences in β-cell mass, islet number, and islet size in either male (Fig. 3A) or female (Supplementary Fig. 3) pSENP1-KO mice compared with controls. Since OGTT, but not IPGTT, showed impairment in pSENP1-KO mice after HFD, this prompted us to examine the response of islets from these mice to incretin signaling. Single β-cells from pSENP1-KO mice fed HFD showed lower exocytotic responses, and this was more obvious in the presence of the GLP-1 receptor agonist Ex4 (10 nmol/L) or GIP (100 nmol/L) (Fig. 3B). Furthermore, voltage-dependent Ca<sup>2+</sup> currents were similar between pSENP1-WT and -KO β-cells (Fig. 3C). Although incretin receptor activation still increased insulin secretion from pSENP1-KO islets, the secretory response to glucose, Ex4, and GIP remained much lower in islets from HFD-fed pSENP1-KO mice compared with islets from pSENP1-WT littermates (Fig. 3D–F).

Loss of Islet SENP1 Impairs Glucagon Secretion

With the Pdx1 promoter as the driver of Cre expression, loss of SENP1 will not be restricted to β-cells (28) (Fig. 4A). Indeed, Senp1 was ∼80% lost in the proximal intestine and absent from islets of pSENP1-KO mice (Fig. 4B), suggesting that oral glucose tolerance might be impacted by deletion of SENP1 from incretin-producing intestinal cells or glucagon-producing α-cells. However, total plasma GLP-1 and GIP levels during an oral glucose challenge were similar between pSENP1-WT and -KO mice on either chow diet (CD) or HFD (Fig. 4C and D). Fasting plasma glucagon appears decreased in the pSENP1-KO, and this is significant after HFD (Fig. 4E). In vitro glucagon secretion from pSENP1-KO islets is reduced compared with control littermates (Fig. 4F and G), including at
low glucose and in response to GIP alone (29) or with alanine, which we used together to potently activate α-cells (30). Similar results were observed from islets of pSENP1-KO mice following HFD (data not shown). Although GIP-dependent α-to-β-cell communication primarily impacts the incretin response to a mixed meal, rather than to oral glucose (31), another reduction in intra-islet glucagon could reduce insulin secretion by lowering β-cell cAMP tone (26).

We wanted to focus on the role for β-cell SENP1 and so generated βSENP1-KO mice (Fig. 4A). Approximately 80% of Senp1 was lost in islets from βSENP1-KO but was unaffected in other tissues (Fig. 4H), fasting plasma glucagon is not decreased in βSENP-KO mice on CD or HFD (Fig. 4I), and in vitro glucagon secretion from βSENP-KO islets was not different from littermate controls (Fig. 4J and K).

**β-Cell–Specific Deletion of SENP1 Leads to Worsened Oral Glucose Tolerance After HFD**

We observed no differences in insulin tolerance, fasting insulin, fasting glucose, and body weight in these mice on CD or HFD (Fig. 5A and B and Supplementary Figs. 4 and 5). Both oral and IP glucose tolerance were similar in βSENP1-KO and WT littermates on CD in both males (Fig. 5C and D) and females (Supplementary Fig. 5E and F). After HFD, male βSENP1-KO mice were more glucose intolerant than βSENP1-WT littermates to oral (Fig. 5E) but not IP (Fig. 5G) glucose. Plasma insulin responses to oral glucose were impaired (Fig. 5F) but not in response to IP glucose (Fig. 5H). Similar, but less striking, differences were observed in females (Supplementary Fig. 5G–K). We found no difference in β-cell mass, islet number, or islet size in βSENP1-KO mice compared with littermate controls (Fig. 6A–D) and confirmed impaired single-β-cell exocytosis (Fig. 6A) with no significant difference in Ca2+ currents (Fig. 6B). Glucose-stimulated insulin secretion from islets of chow-fed βSENP1-KO mice was reduced (Fig. 6C). After HFD, insulin secretion from islets of βSENP1-KO mice and littermate controls was impaired to the same degree (Fig. 6D). However, insulin secretion from HFD βSENP1-WT islets was potentiated.
to a greater degree by Ex4 and GIP than from βSENP1-KO islets (Fig. 6G). Consistently, Ex4 and GIP were unable to increase exocytosis to the same extent from β-cells of HFD βSENP1-KO mice compared with littermate controls (Fig. 6F), independent of any changes in Ca\(^{2+}\) currents (Supplementary Fig. 7D and E).

Although SUMOylation may inhibit GLP-1 receptor activity (32,33), we found no difference in the cAMP response to Ex4 or GIP between βSENP1-WT and -KO islets expressing the β-cell–specific cAMP sensor (Fig. 6H and I and Supplementary Fig. 8). Even though our experiments include cAMP in the patch pipette, exocytosis from β-cells of βSENP1-KO mice following HFD was still much lower than in littermate controls (Fig. 6J and K). While the cycling period of Ca\(^{2+}\) oscillations was higher in βSENP1-KO β-cells, the time in the active phase remained the same (Fig. 6L and M), suggesting that SENP1 acts downstream of the Ca\(^{2+}\) response and that the increased cycling period may be secondary to the reduced workload (ATP hydrolysis) in the βSENP1-KO β-cells because of loss of exocytosis (34). Ex4 and GIP maintained their effect on Ca\(^{2+}\) responses (Fig. 6L and M and Supplementary Fig. 7D and E), and βSENP1-KO islets exhibited impaired oleate-stimulated and glutamine/leucine-stimulated insulin secretion (Supplementary Fig. 7B and C). This all points to a mechanism downstream of cAMP and Ca\(^{2+}\) responses (9,10,35).

**DISCUSSION**

We aimed to investigate the role of the deSUMOylating enzyme SENP1 within the β-cell following HFD-induced metabolic stress. In two models, we confirmed that loss of β-cell SENP1 results in impaired exocytosis and reduced insulin secretion, consistent with the SUMOylation-dependent inhibition of insulin secretion in insulinoma cells and human and mouse β-cells (7,9–11,13). DeSUMOylation, likely of multiple targets (6), is an important mechanism that facilitates insulin secretion. In vivo, overexpression of the SUMO-conjugating enzyme Ubc9 within β-cells leads to an obvious IP glucose intolerance (13). While we see impaired insulin secretion, we do not find robust glucose intolerance in young chow-fed mice following islet or β-cell SENP1 KO. Thus, insulin secretion may not be sufficiently limited in vivo to...
consistently impact glucose homeostasis in the absence of a stressor. At present, we also cannot rule out potential compensatory mechanisms in vivo, such as increased insulin sensitivity, that might be difficult to detect in our ITTs. Nonetheless, high-fat feeding revealed the importance SENP1-dependent insulin secretion in the maintenance of glucose homeostasis.

A worsening of HFD-induced glucose intolerance in both the pSENP1-KO and βSENP1-KO mice was observed selectively in response to oral, but not IP, glucose. This suggests an important interaction with the incretin response, which is upregulated in HFD (36–38) consistent with the enhanced GIP response we observed. Recent work suggested an important interaction among incretin responses, glucagon secretion, and insulin release (39). GIP-induced glucagon secretion supports insulin secretion following a mixed meal (26,40–42) but does not likely contribute in the response to oral glucose alone (31). Thus, the reduced glucagon response to GIP + alanine in the pSENP1-KO islets used here as a strong stimulus of α-cell function likely does not contribute to worsened oral glucose intolerance. It is possible, however, that reduced glucagon "tone" in the pSENP1-KO islets could impact insulin secretion by lowering baseline β-cell cAMP (26) and contribute to the lower insulin response in the pSENP1-KO compared with the βSENP1-KO model. Regardless, the observation that HFD-induced oral glucose intolerance persists in the βSENP-KO model suggests that loss of SENP1 in the β-cell, rather than in α-cells or in the intestine, is primarily responsible for the impaired oral glucose tolerance following HFD.

But why is IP glucose tolerance after HFD not worsened in these models, particularly given the role for SENP1 in glucose-stimulated insulin secretion? In βSENP1-KO, this
is easier to explain since glucose-stimulated insulin secretion is similarly impaired after HFD in both KO and WT islets. In pSENP1-KO, a lower glucose-stimulated insulin secretion in vitro after HFD has only a small impact, if any, to worsen IP glucose intolerance. The reasons for this are likely twofold. First, after the 8-week HFD, insulin secretion from the pSENP1-WT islets is already impaired (a peak of ~0.4% of content as in Fig. 3D and E) compared with chow-fed WT mice in our hands (peak of ~1.5% as in Fig. 6C or elsewhere [7]). The additional reduction of
Figure 6—Impaired insulin secretion and exocytosis to glucose and incretins from βSENP1-KO islets following HFD. A: Representative traces (left) and averaged exocytosis elicited by a series of 500-ms membrane depolarizations from −70 to 0 mV in β-cells from male βSENP1-WT and -KO mice on CD at 10 mmol/L glucose (n = 55 and 61 cells from 5 and 7 mice). The pipette solution included 0.1 mmol/L cAMP. B: Representative Ca²⁺ current traces and average integrated Ca²⁺ entry of β-cell elicited by a single 500-ms membrane depolarization from −70 to 0 mV at 10 mmol/L glucose (n = 51 and 57 cells). The pipette solution included 0.1 mmol/L cAMP. C: Insulin secretion from male βSENP1-WT and -KO islets from mice on CD in response to glucose (n = 8 and 7). D and F: Side-by-side insulin secretion from βSENP1-WT and -KO islets from male mice following HFD in response to glucose alone (n = 9 and 12) (D) or together with Ex4 (10 nmol/L) (n = 8 and 9) (D) or GIP (100 nmol/L) (n = 7 and 7) (F) and respective area under the curve (AUC) during the glucose stimulation (F). E: Exocytosis in β-cells of male βSENP1-WT and -KO mice, following HFD, elicited by a series of 500-ms membrane depolarization from −70 to 0 mV at 5 mmol/L glucose alone or with Ex4 (10 nmol/L) or GIP (100 nmol/L) (n = 44, 46, 47, 38, 40, and 41 cells from 5 mice per group). CAMP 0.1 mmol/L was omitted from pipette solution. H and I: The cAMP response at 10 mmol/L glucose to Ex4 (10 nmol/L) (n = 4 pairs of mice, 68 and 74 islets) (H) or GIP (100 nmol/L) (n = 4 pairs of mice, 73 and 88 islets) (I). J: Exocytosis in β-cells of male βSENP1-WT and -KO mice following HFD at 2.8 and 10 mmol/L glucose, with 0.1 mmol/L cAMP included in the pipette solution (n = 26, 35, 29, and 38 cells from 3, 4, 3, and 4 mice). K: Average integrated Ca²⁺ charge entry during voltage-dependent Ca²⁺ currents elicited from β-cells by a single 500-ms membrane depolarization from −70 to 0 mV at 2.8 and 10 mmol/L glucose (n = 26, 30, 30, and 38 cells from 3, 4, 3, and 4 mice). The pipette solution included 0.1 mmol/L cAMP. L and M: Representative Ca²⁺ responses (left), oscillation period, and Ca²⁺ plateau fraction at 10 mmol/L glucose to Ex4 (n = 4 pairs of mice, 73 and 88 cells) (L) and to GIP (n = 4 pairs of mice, 68 and 74 cells) (M). Data are mean ± SEM and were compared using Student t test or one-way or two-way ANOVA followed by Bonferroni post-test. *P < 0.05, **P < 0.01, ***P < 0.001 between βSENP1-WT and -KO under the same condition.
glucose-stimulated secretion may be insufficient to worsen
an already-impaired IP glucose tolerance. Second, plasma
glucose clearance following IP administration is more
dependent on insulin-independent mechanisms than glu-
cose through the oral route (43). Glucose effectiveness, the
ability of glucose to promote its own insulin-independent
clearance, contributes two-thirds of clearance following IP
glucose in mice (44), and this proportion increases sub-
stantially following high-fat feeding (45,46). This, coupled
with upregulation of incretin responses upon high-fat feed-
ing, may explain why we see a stronger effect of islet
SENP1 KO on oral glucose tolerance in our HFD mice.

Although SUMOylation is suggested to reduce GLP-1
receptor activity (32,33), cAMP and Ca2+ responses to
Ex4 and GIP were similar between βSENP1-WT and -KO
islets. Therefore, SENP1 does not appear to control incre-	in receptor activity directly, although it is possible that
other SUMO proteases (47) may be key determinants of
upstream incretin signaling. Also, β-cells lacking SENP1
show decreased exocytosis, even though most patch clamp
experiments included high cAMP in the pipette solution,
which should bypass the need for incretin receptor activa-
tion. This suggests that the impaired response upon loss
of SENP1 resides downstream of receptor signaling and
cAMP, although it could be possible that effectors such as
cAMP-dependent protein kinase or exchange protein
directly activated by cAMP are directly impacted by loss
of SENP1. The observation that insulin secretion from
βSENP1-KO islets was also impaired upon fatty acid or
amino acid stimulation suggests that SENP1 acts far
downstream in the regulation of insulin granule fusion.
Although we see reduced Ca2+ oscillation frequency in
the βSENP1-KO, this is likely secondary to the reduced
workload resulting from a loss of exocytosis (34). It
seems, therefore, that SENP1 is required to ensure the
availability of insulin granules on which cAMP-dependent
signals act or that these converge on common exocytotic
protein targets, such as synaptotagmin VII, shared by
other metabolic pathways (9,35).

As such, we do not believe that SENP1 activity
"mediates" incretin signaling but rather serves an impor-
tant role in maintaining, or augmenting, the pool of
release-ready insulin granules on which incretins ulti-
mately act. Interestingly, the activity of SENP1 is linked
to metabolism and the mitochondrial export of reducing
equivalents (6,7) to mediate an amplification of insulin
secretion. SUMOylation blocks granule fusion at a very
distal step in the secretory pathway, producing a "traffic
jam" of insulin granules at the plasma membrane (9).
Thus, SENP1 acts to maintain, or perhaps amplify, the
availability of secretory granules for subsequent release
whether in response to glucose, incretins, or other stim-
uli. This suggests that a glucose-dependent effect to
enhance the secretory granule pool will augment the
secretory response to incretins. Indeed, the ability of

glucose to facilitate β-cell exocytosis is correlated with
Ex4-dependent insulin secretion from human islets (5).

The SENP1 pathway itself may be impaired by HFD
possibly because of inactivation of SENP1 by oxidative
stress (48) and similar to what occurs in islets from
human donors with T2D (7). The export of mitochondrial
reducing equivalents activates SENP1 by reducing a thiol
group through a redox relay involving NADPH and reduc-
tion of glutathione (7,48). On the other hand, H2O2,
which can oxidize and inactivate SENP1 (8), is also pro-
duced from NADPH through NOX4, and this is also
required for glucose-stimulated insulin secretion (49,50).
It remains unclear how SENP1 may evade H2O2-induced
inactivation and compete with NOX4 for NADPH. One
possibility may involve either spatial or temporal com-
partmentalization. SENP1 potentiates insulin secretion by
modulating exocytotic proteins (6,9–11), while NOX4-
induced H2O2 primarily acts through KATP channel
inhibition (49). Here, excessive oxidative stress induced
by HFD could lead to basal hyperinsulinemia and defec-
tive glucose-stimulated insulin secretion (51), as occurs
in islets from donors with impaired glucose tolerance or
T2D (52). Indeed, NOX4-induced islet H2O2 may drive
β-cell dysfunction and glucose intolerance after HFD (53),
and it is interesting to speculate that overproduction of
H2O2 could spill over and limit SENP1 activity and
increase SUMOylation. While inhibition or loss of SENP1
may protect against β-cell apoptosis (13), it comes at the
cost of robust incretin-induced insulin secretion in the
face of metabolic stressors.

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Y.J., A.B., and Y.W.W. researched and analyzed data. H.L., J.E.M.F., M.J.M.,
P.E.M. and, as such, had full access to all the data in the study and
and, as such, had full access to all the data in the study and
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