High beta-cell mass prevents streptozotocin-induced diabetes in thioredoxin-interacting protein-deficient mice


To cite this version:

HAL Id: hal-01187491
https://hal.science/hal-01187491v2
Submitted on 28 Aug 2015

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
High β-cell mass prevents streptozotocin-induced diabetes in thioredoxin-interacting protein-deficient mice


Department of Medicine, Mount Sinai Hospital, Toronto General Research Institute, Banting and Best Diabetes Centre, University Health Network and Department of Physiology, University of Toronto, Toronto, Ontario, Canada

Submitted 9 July 2008; accepted in final form 10 February 2009

Masson E, Koren S, Razik F, Goldberg H, Kwan EP, Sheu L, Gaisano HY, Fantus IG. High β-cell mass prevents streptozotocin-induced diabetes in thioredoxin-interacting protein-deficient mice. Am J Physiol Endocrinol Metab 296: E1251–E1261, 2009. — Thioredoxin-interacting protein (TxNIP) is an endogenous inhibitor of thioredoxin, a ubiquitous thiol oxidoreductase, that regulates cellular redox status. Diabetic mice exhibited increased expression of TxNIP in pancreatic islets, and recent studies suggest that TxNIP is a proapoptotic factor in β-cells that may contribute to the development of diabetes. Here, we examined the role of TxNIP deficiency in vivo in the development of insulin-deficient diabetes and whether it impacted on pancreatic β-cell mass and/or insulin secretion. TxNIP-deficient (Hcb-19/TxnIP-/-) mice had lower baseline glycemia, higher circulating insulin concentrations, and higher total pancreatic insulin content and β-cell mass than control mice (C3H). Hcb-19/TxnIP-/- did not develop hyperglycemia when injected with standard multiple low doses of streptozotocin (STZ), in contrast to C3H controls. Surprisingly, although β-cell mass remained higher in Hcb-19/TxnIP-/- mice compared with C3H after STZ exposure, the relative decrease induced by STZ was as great or even greater in the TxNIP-deficient animals. Consistently, cultured pancreatic INS-1 cells transfected with small-interfering RNA against TxNIP were more sensitive to cell death induced by direct exposure to STZ or to the combination of inflammatory cytokines interleukin-1β, interferon-γ, and tumor necrosis factor-α. Furthermore, when corrected for insulin content, isolated pancreatic islets from TxNIP-/- mice exhibited reduced glucose-induced insulin secretion. These data indicate that TxNIP functions as a regulator of β-cell mass and influences insulin secretion. In conclusion, the relative resistance of TxNIP-deficient mice to STZ-induced diabetes appears to be because of an increase in β-cell mass. However, TxNIP deficiency is associated with sensitization to STZ- and cytokine-induced β-cell death, indicating complex regulatory roles of TxNIP under different physiological and pathological conditions.

Thioredoxin interacting protein; pancreatic β-cell; apoptosis; insulin secretion

Address for reprint requests and other correspondence: I. G. Fantus, Dept. of Medicine, Mount Sinai Hospital, 60 Murray St., Lebovic Bldg, Rm. 5028, Toronto, ON, Canada MST 3L9 (e-mail: gfantus@mtsinai.on.ca).

Plasma was collected and frozen at the STZ 2/H11003 "STZ 50" groups and 2 wk after the second set of STZ injections for endpoint was reached 6 wk after STZ injections for the control and mice in EDTA tubes and centrifuged at 5,000 g. Later (6 h after death of the study), blood samples were collected on 6-h-fasted mice administered 50 mg/kg body wt BrdU by intraperitoneal injection. Analysis of serial consecutive sections stained with either one of the four markers cited above or insulin enabled identification of apoptotic or proliferative cells as insulin-immunopositive -cells. The number of TUNEL, cleaved caspase-3 staining of two pancreas sections per mouse were performed. To detect proliferation, two sections per mouse were stained for Ki-67. Bromodeoxyuridine (BrdU) staining was performed on pancreas sections from mice administered 50 mg/kg body wt BrdU by intraperitoneal injection, 6 h before death. Analysis of serial consecutive sections stained with either one of the four markers cited above or insulin enabled identification of apoptotic or proliferative cells as insulin-immunopositive -cells. The number of TUNEL, cleaved caspase-3, Ki-67, or BrdU-positive -cells was determined per 100 islets.

**Materials and Methods**

**Animal Experiments**

Hcb-19 and control C3H/DiSnA mice (generous gift of Dr. R. A. Davis, San Diego State University) were maintained under standardized conditions at the University Health Network animal facility (Toronto, Canada) and fed and provided water ad libitum. All procedures and experiments were approved by the University Health Network Animal Care Committee and carried out in accordance with the guidelines of the Canadian Council on Animal Care. The multiple low-dose STZ protocol recommended by the Animal Models of Diabetes Complications Consortium (http://www.amdcc.org/) was used to induce diabetes in 8-wk-old C3H and Hcb-19 male mice. STZ (0.1 M sodium citrate buffer, pH 4.5) (Sigma-Aldrich, St. Louis, MO) was injected intraperitoneally after a 6-h fast, at a dose of 50 mg/kg of body wt daily for five consecutive days. Control animals received the same volume per body weight of citrate buffer. Later (6 wk), a group of STZ-treated C3H and Hcb-19 mice received a second set of five STZ injections ("STZ 2×50" group). The experimental endpoint was reached 6 wk after STZ injections for the control and "STZ 50" groups and 2 wk after the second set of STZ injections for the STZ 2×50 group. Six hour fasting glycemia was monitored weekly throughout the experimental period via the tail vein using a glucometer (One Touch Ultra; Life Scan, Milpitas, CA). At the endpoint of the study, blood samples were collected on 6-h-fasted mice in EDTA tubes and centrifuged at 5,000 g for 5 min at 4°C. Plasma was collected and frozen at −80°C. Mice were subsequently euthanized with CO2, and pancreases were removed and weighed. A small piece from the pancreatic tail was collected, weighed, and snap-frozen for measurement of total insulin content. The rest of the pancreas was cut into multiple pieces, fixed in 10% formalin for 24 h, and embedded in paraffin for histological analyses.

**Measurement of Plasma Insulin Levels and Total Pancreatic Insulin Content**

The piece of pancreas tail, or alternatively the entire pancreas, was homogenized in acid-ethanol (0.2 mmol/L HCl in 75% ethanol) to extract insulin. Insulin concentration of the pancreatic extract as well as plasma insulin levels were measured by enzyme-linked immnosorbent assay (ELISA), using a rat insulin kit (Linco Research, St. Charles, MO). Total pancreatic insulin content was calculated from the insulin concentration in the extracts corrected for total pancreas weights.

**Immunohistochemistry**

To estimate -cell mass, two pancreatic sections from each mouse (200 µm apart) were stained for insulin using a 1:200 dilution of rabbit antibody (Biomedica, Plovdiv, Bulgaria) for 1 h after pepsin antigen retrieval and counterstained with hematoxylin. Slides were then digitized using a brightfield scanner (ScanScope XT; Aperio Technologies, Vista, CA). The proportion of the pancreas occupied by the -cells (relative -cell area) was determined by distinguishing stained tissue (-cells) from unstained (non -cell area) using image analysis software (Aperio Image Scope; Aperio Technologies). -Cell mass was calculated by multiplying the relative -cell area by the wet weight of the pancreas as described (3). To detect apoptosis, TdT-dUTP nick end-labeling (TUNEL) and cleaved caspase-3 staining of two pancreas sections per mouse were performed. To detect proliferation, two sections per mouse were stained for Ki-67. Bromodeoxyuridine (BrdU) staining was performed on pancreas sections from mice administered 50 mg/kg body wt BrdU by intraperitoneal injection, 6 h before death. Analysis of serial consecutive sections stained with either one of the four markers cited above or insulin enabled identification of apoptotic or proliferative cells as insulin-immunopositive -cells. The number of TUNEL, cleaved caspase-3, Ki-67, or BrdU-positive -cells was determined per 100 islets.

**Inlet Isolation, Perfusion Secretory Assays, and Measurement of Apoptosis**

Pancreatic islets were isolated as previously described (18). Briefly, a collagenase solution was injected in the common bile duct, and the pancreas was collected and digested by incubation at 37°C. Islets were handpicked in the pancreatic lysate and maintained overnight in 11
mM glucose RPMI 1640, supplemented with 10% FBS and penicillin/streptomycin. Batches of 50–60 islets were placed in a perfusion chamber (capacity 1.3 ml) at 37°C and perfused at a flow rate of ~1 ml/min with a Krebs-Ringer bicarbonate buffer (KRB). Islets were first equilibrated for 30 min in KRB HEPES buffer supplemented with 2.8 mmol/l glucose. They were then stimulated with 16.7 mmol/l glucose for a 30-min period. Fractions were collected at different time points for insulin determination using a radioimmunoassay (Linco Research). At the end of the perfusion, islets were collected and lysed in acidic ethanol for measurement of insulin content. Results are presented as insulin secreted normalized per islet and per total insulin content. Alternatively, after the overnight recovery period, batches of 50 islets were placed in 5 mM glucose RPMI medium and treated with a combination of the proinflammatory cytokines IL-1β (2 ng/ml), IFN-γ (100 ng/ml), and TNF-α (10 ng/ml) (R & D Systems, Minneapolis, MN) for 48 h, 3 days, or 5 days or with 1 or 2 mM STZ for 24 h. At the end of the treatment periods, islets were lysed, and apoptosis was assessed by determination of mono- and oligonucleosomes using an enzyme immunoassay (Cell Death Detection ELISA-PLUS; Roche Diagnostics, Mannheim, Germany) according to the manufacturer’s instructions.

**Cell Culture**

INS-1 832/13 cells (rat pancreatic β-cell line originally described in Ref. 1) were maintained in RPMI 1640 medium (11.1 mM glucose, 1 mM sodium pyruvate, and 10 mM HEPES) (GIBCO-Invitrogen, Carlsbad, CA) supplemented with 10% FBS, 2 mM l-glutamine, 55 μM l-mercaptoethanol, and penicillin/streptomycin at 37°C and 5% CO₂. For the experiments, INS-1 cells cultured in 5 mM glucose medium were exposed to 25 mM glucose for 24 h, or 0.1, 0.5, or 1 mM STZ for 1 h, followed by a 6-h recovery period. Alternatively, cells were treated with a combination of 1 ng/ml IL-1β, 50 ng/ml IFN-γ, and 5 ng/ml TNF-α for 24 h to 3 days. At the end of the

![Fig. 2. Hcb-19/TxNIP−/− mice have higher insulin levels, total pancreatic insulin content, and β-cell mass than C3H mice. C3H and Hcb-19/TxNIP−/− mice were injected ip with either citrate buffer or 50 mg/kg of body wt STZ daily for 5 consecutive days. The 2 × 50 groups received a second set of 5 STZ injections 6 wk later. A: blood samples were collected on 6-h-fasted mice at 6 wk after STZ injections for the control and 50 groups and at 2 wk after the second set of injections for the 2 × 50 groups. Plasma was used to measure insulin levels by enzyme-linked immunosorbent assay (ELISA; n = 5–8). B: a small piece of the pancreas tail was used to determine the total insulin content by ELISA after acidic ethanol extraction as described in MATERIALS AND METHODS (n = 7–13). Total insulin content was also measured on extracts of the entire pancreas of mice under control conditions (inset; n = 7). C: pancreas was fixed, and 2 sections from each mouse were stained for insulin to assess β-cell mass, using automated image analysis software (n = 5–9). Bars represent means ± SD. *P < 0.05 vs. C3H group. D: representative photomicrographs.](E1253RESISTANCE TO DIABETES IN Hcb-19/TxNIP−/− MICE AJP-Endocrinol Metab • VOL 296 • JUNE 2009 • www.ajpendo.org)
treatment times indicated, the cell culture supernatant was collected, and floating and attached cells were washed with PBS and lysed in RIPA buffer containing protease inhibitors for 30 min on ice. Cell lysates were centrifuged at 16,000 g for 10 min at 4°C. The supernatant was used to determine protein concentration using the Bradford assay (Bio-Rad Laboratories, Hercules, CA) and analyzed by Western blotting. For RNA interference experiments, INS-1 cells were forward transfected with small-interfering RNA (siRNA) control or directed against TxNIP (Stealth RNAi; Invitrogen) using Lipofectamine RNAiMax, according to the manufacturer’s instructions (Invitrogen). As a preliminary experiment, three different sequences at increasing concentrations (1, 5, and 10 nM) were tested for their efficiency to decrease TxNIP expression by Western Blotting in INS-1 cells 3 days after transfection. One of them was selected and used at the lowest efficient dose of 1 nM. Transfected cells were then exposed to cytokines or STZ as described above. Attached cells were collected using Trypsin and pooled with the floating cells contained in the culture medium, washed, and stained with annexin V and propidium iodide using a cell death kit according to the manufacturer’s instructions (BioLegend, San Diego, CA). Apoptosis and necrosis were studied by flow cytometry using a BD FACS Aria cell sorter (BD Biosciences, San Jose, CA). Results were analyzed using FlowJo software (Tree Star, Ashland, OR).

**Western Blotting**

Protein lysates were separated by SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked using PBS-0.05% Tween 20 with 5% nonfat milk for 1 h, then incubated overnight at 4°C with the indicated primary antibody. Protein bands were detected with secondary antibodies conjugated to horseradish peroxidase (Santa Cruz Biotechnology, Santa Cruz, CA) and an enhanced chemiluminescence system (LumiGlo; Mandel, Guelph, ON, Canada). The following primary antibodies were used: mouse -tubulin (1:1,500 dilution; Santa Cruz Biotechnology). kim without insulin (Hcb-19) and wild-type control (C3H) mice received five consecutive daily intraperitoneal injections of STZ at a dose of 50 mg/kg body wt. Controls for both groups were injected with citrate, the STZ vehicle. As shown in Fig. 1, and in agreement with previously published data (7, 12, 16, 30), Hcb-19/TxNIP mice are “resistant” to STZ-induced diabetes. A second set of five STZ injections was required in the mutant mice to induce high blood glucose levels similar to those measured in the C3H control strain (STZ 2×50 groups, Fig. 1). These observations indicate that Hcb-19/TxNIP mice are “resistant” to STZ-induced diabetes.

**Statistical Analyses**

Results are given as means ± SD. Statistical significance was assessed by two-tailed unpaired t-test. P < 0.05 was considered to be significant.

**RESULTS**

**Hcb-19/TxNIP−/− Mice do not Develop Diabetes in Response to Low-Dose STZ Administration**

The multiple low-dose STZ administration protocol has been widely used in rodents to induce insulin-deficient diabetes similar to type 1 diabetes while minimizing generalized nonspecific toxic effects of this chemical agent (19, 23, 35). TxNIP−/− (Hcb-19) and wild-type control (C3H) mice received five consecutive daily intraperitoneal injections of STZ at a dose of 50 mg/kg body wt. Controls for both groups were injected with citrate, the STZ vehicle. As shown in Fig. 1, and in agreement with previously published data (7, 12, 16, 30), Hcb-19/TxNIP−/− mice had lower glucose levels than C3H control mice under basal conditions, i.e., in the absence of STZ exposure (5.6 ± 1.0 vs. 10 ± 1.1 mmol/l for Hcb-19 and C3H, respectively, P < 0.05). Interestingly, Hcb-19/TxNIP−/− mice did not develop hyperglycemia while the C3H control mice were overtly diabetic 6 wk after STZ injections (STZ 50 groups: 6.8 mmol/l for Hcb-19 and C3H, respectively, P < 0.05). All C3H mice developed diabetes to varying degrees in response to one set of five STZ injections. Only 4 out of 26 Hcb-19 mice exhibited hyperglycemia under these conditions. Fig. 3. Hcb-19/TxNIP−/− mice have more and larger pancreatic islets than C3H mice. Pancreas of C3H and Hcb-19/TxNIP−/− mice were fixed, sectioned, and stained for insulin. Islets (1,101) from 10 C3H mice and islets (1,245) from 9 Hcb-19 mice were counted and sized. The no. of islets/pancreas (A) and the average size of individual islets (B) are shown, as well as the quartile distribution of islet size (C). Quartile 1 = 15–31; quartile 2 = 31–66; quartile 3 = 66–154; and quartile 4 = 154–1,601 μm². Results are expressed as means ± SD. *P < 0.05 vs. C3H group.
In Vivo and Ex Vivo Assessment of Apoptosis in Hcb-19/TxNIP<sup>−/−</sup> β-Cells

Because changes in β-cell mass are dependent on the balance between the rate of β-cell death and proliferation (or neogenesis), we investigated whether the STZ effect on β-cell apoptosis and proliferation was different between the two strains.

Hcb-19/TxNIP<sup>−/−</sup> Mice Have a Greater Insulin Reserve Than C3H Controls

We next assessed some of the potential causes of the relative resistance to diabetes exhibited by the Hcb-19/TxNIP<sup>−/−</sup> mice. At the experimental endpoint, plasma was collected after a 6-h fast to measure insulin levels. In addition, pancreatic tissue was harvested to determine total insulin content and β-cell mass by immunohistochemistry. Under basal conditions, in the absence of STZ administration, Hcb-19/TxNIP<sup>−/−</sup> mice received 5 consecutive daily injections of 50 mg/kg body wt STZ or citrate buffer and were euthanized 1 day later. Pancreas was collected and fixed, and serial sections were stained for TdT-dUTP nick end-labeling (TUNEL) or cleaved caspase-3 to detect apoptosis and for insulin to localize the positive cells to insulin-immunopositive β-cells. The number of β-cells positive for TUNEL (A) and cleaved caspase-3 (B) staining/100 islets is shown. Results are expressed as means ± SD (n = 5–8). *P < 0.05 vs. C3H group.

**Assessment of Apoptosis in Hcb-19/TxNIP<sup>−/−</sup> β-Cells In Vivo and Ex Vivo**

---

![Graph A](image1.png)

**Fig. 4.** Apoptosis of β-cells 1 day after the last of 5 STZ injections. C3H and Hcb-19/TxNIP<sup>−/−</sup> mice received 5 consecutive daily injections of 50 mg/kg body wt STZ or citrate buffer and were euthanized 1 day later. Pancreas was collected and fixed, and serial sections were stained for TdT-dUTP nick end-labeling (TUNEL) or cleaved caspase-3 to detect apoptosis and for insulin to localize the positive cells to insulin-immunopositive β-cells. The number of β-cells positive for TUNEL (A) and cleaved caspase-3 (B) staining/100 islets is shown. Results are expressed as means ± SD (n = 5–8). *P < 0.05 vs. C3H group.

---

![Graph B](image2.png)

---

**Fig. 5.** STZ and inflammatory cytokines induce similar degrees of apoptosis in pancreatic islets isolated from Hcb-19/TxNIP<sup>−/−</sup> and C3H mice. Pancreatic islets were isolated from C3H and Hcb-19/TxNIP<sup>−/−</sup> mice, and batches of 50 islets were treated with a combination of interleukin (IL)-1β, interferon (IFN)-γ, and tumor necrosis factor (TNF)-α for increasing periods of time (0–5 days), or with 1 or 2 mM STZ for 24 h, as described in MATERIALS AND METHODS. At the end of the treatment periods, apoptosis was assessed by determination of mono- and oligonucleosomes using an enzyme immunoassay. Results are expressed as means ± SD (n = 3–6).
In vivo studies. Pancreatic tissue was collected 1 day after the last STZ injection, and serial sections were stained for insulin, TUNEL assay, or cleaved caspase-3. Only rare apoptotic β-cells were detected in histological sections from control pancreas tissue (mice injected with vehicle alone). The number of both TUNEL and cleaved caspase-3 positive β-cells was markedly increased in sections from STZ-treated mice (Fig. 4). However, the overall percentage of apoptotic cells detected in pancreatic sections of STZ-treated mice appeared to be relatively few considering the marked decrease in β-cell mass observed. This may have been a consequence of the fact that this measure of apoptosis provides only a single snapshot of a dynamic process and that dead cells are quickly cleared in vivo by macrophages. The number of apoptotic β-cells detected in Hcb-19/TxNIP−/− pancreatic sections was significantly lower, 33 and 50% of that observed in C3H mice as assessed by TUNEL (Fig. 4A) and cleaved caspase-3 staining (Fig. 4B), respectively. In addition, pancreatic tissue collected 1 day and 2 wk after the last STZ injection was stained for insulin and Ki-67 or BrdU to evaluate proliferation. Although an increase in the proportion of proliferative β-cells in response to STZ was observed 2 wk after the last STZ injection, in contrast to apoptosis, there were no differences between the two strains in these markers of proliferation (data not shown). Although these data suggest that Hcb-19/TxNIP−/− mouse β-cells may be less sensitive to STZ-induced apoptosis than those in the C3H wild type, this conclusion would be inconsistent with the fact that the relative reduction in β-cell mass in response to STZ exposure was greater in Hcb-19/TxNIP−/− mice (Fig. 2C).

Ex vivo studies. To investigate this phenomenon further, we performed ex vivo measurements of apoptosis in pancreatic islets isolated from C3H control and Hcb-19/TxNIP−/− mice. In STZ-injected mice, β-cell death results from both an initial, direct cytotoxic effect of STZ and a subsequent immune reaction mediated by inflammatory cytokines IL-1β, IFNγ, and TNFα (23). Therefore, isolated islets were treated directly with STZ or with a combination of these three cytokines for different time periods. Apoptosis was then assessed by determination of mono- and oligonucleosome levels. Results presented in Fig. 5 show that every treatment tested induced a significant degree of apoptosis in pancreatic islets. The induction ranged from ~2.5 (48 h cytokine treatment)- to 5 (2 mM STZ)-fold. However, we observed no statistically significant differences in apoptosis levels between the two strains of mice under any of these conditions. Of note, basal levels of apoptosis (without any treatment) were slightly higher in Hcb-19/TxNIP−/− islets compared with C3H controls [arbitrary units: 0.220 ± 0.097 vs. 0.191 ± 0.069, P = not significant (NS)]. These ex vivo data do not support the notion that TxNIP deficiency provides resistance against β-cell apoptosis induced by STZ or inflammatory cytokines.

Effects of STZ and Inflammatory Cytokines in Cultured INS-1 cells: Role of TxNIP

To determine whether TxNIP expression was affected by STZ and/or proinflammatory cytokines, cultured INS-1 β-cells were treated with increasing concentrations of STZ for 1 h followed by 6 h recovery or with a combination of IL-1β, IFNγ, and TNFα for 24 h to 3 days. Cells were also exposed to high glucose, STZ, or the combination of IL-1β, IFNγ, and TNFα induces TxNIP protein expression in INS-1 cells. INS-1 cells were cultured in 5 mM glucose medium (control) or exposed to various treatments (25 mM glucose for 24 h and 0.1, 0.5, or 1 mM STZ for 1 h), followed by a 6-h recovery period or a combination of 1 ng/ml IL-1β, 50 ng/ml IFNγ, and 5 ng/ml TNFα for 24 h, 48 h, or 3 days. Protein lysates were analyzed by immunoblotting for TxNIP and β-tubulin, to verify equal loading, as described in MATERIALS and METHODS. A: representative immunoblots of 3 independent experiments. In immunoblot A2, the bands at the 24- and 48-h time points were separated from the original image to be displayed in chronological sequence. B: results are expressed as means ± SD of fold changes vs. control (no treatment). *P < 0.05 vs. control group.
to high glucose concentrations (25 mM), as a positive control (8, 22). Western blot analyses showed that both STZ and cytokine exposure significantly induced TxNIP protein expression, although to a lesser extent than high glucose, with a maximum induction of 23- and 11-fold for STZ and cytokine treatment, respectively, vs. 142-fold for high glucose (Fig. 6).

We next aimed to investigate the significance of the STZ- and cytokine-induced increase in TxNIP expression in cultured β-cells and determine the role that TxNIP deficiency might play in pancreatic β-cell death. To this end, TxNIP expression was downregulated in INS-1 cells using RNA interference. As shown in Fig. 7A, TxNIP siRNA was able to efficiently decrease TxNIP protein expression and inhibit its induction by STZ and cytokine treatment. INS-1 cells transfected with control (scrambled) or TxNIP siRNA were then exposed, as described above, to various concentrations of STZ or a combination of IL-1β, IFNγ, and TNF-α for the times indicated, and cell death was investigated by flow cytometry using annexin V and propidium iodide as markers of apoptosis and necrosis, respectively. As expected, the different treatments caused a significant amount of cell death, increasing with duration and concentration (Fig. 7B). Although cytokine exposure led mainly to a marked augmentation of the annexin V-positive, propidium iodide-positive cell population (late apoptotic cells), STZ treatment resulted in an additional significant increase of highly propidium iodide-positive (necrotic) cells. Of particular interest, the results indicated that the proportion of dying/dead cells was greater in TxNIP-deficient cells. Of particular interest, the results indicated that the proportion of dying/dead cells was greater in TxNIP-deficient compared with control cells under every treatment condition (Fig. 7C). It should be noted that, in the case of STZ, this was not accounted for by the increased necrosis, since the apoptotic cell populations were also elevated (Fig. 7B). These data are consistent with the in vivo observation of STZ-induced loss of β-cell mass and indicate that TxNIP-deficient cells are more sensitive to STZ- and cytokine-induced cell death. We noted that the basal level of cell death in TxNIP siRNA transfected cells, in the absence of any treatment, appeared to be slightly higher (P = NS) than that seen in control siRNA transfected cells. While we cannot exclude nonspecific effects of RNA interference, this observation suggests that abnormally low levels of TxNIP may impair INS-1 pancreatic β-cell viability.

Hcb-19/TxNIP−/− Pancreatic Islets Manifest Decreased Glucose-Induced Insulin Secretion

The hyperinsulinemia combined with lower glycemia observed in the present study, as well as in previous reports (7, 16, 30), may be interpreted as indicative of a greater insulin secretory capacity of Hcb-19/TxNIP−/− islets. This question has not been specifically investigated in previous studies. To examine this directly, pancreatic islets were isolated from Hcb-19/TxNIP−/− and C3H control mice and perfused. Batches of 50 to 60 islets were stimulated with glucose to determine the insulin secretory capacity of individual islets ex vivo. First, the results showed that Hcb-19/TxNIP−/− islets had a higher insulin content than C3H controls (48 ± 15.6 vs. 30 ± 8.6 ng/islet for Hcb-19 and C3H, respectively, P < 0.05) (Fig. 8A). Second, mutant Hcb-19 islets exhibited a small decrease in glucose-induced insulin secretion when corrected for islet number (P = NS; Fig. 8, B and C). However, when the data were normalized for total insulin content, glucose-induced secretion was markedly reduced in Hcb-19/TxNIP−/− islets (area under the curve = 4.2 ± 3.4 vs. 10.9 ± 5.5 pg·min·ml−1·ng−1 for Hcb-19 and C3H, respectively, P < 0.05; Fig. 8, D and E). Of note, both the initial peak (first phase) and the subsequent sustained insulin secretion (second phase) were affected. It should also be noted that basal insulin secretion was similar when corrected for islet insulin content. These data strongly suggest that the higher circulating insulin levels exhibited by Hcb-19/TxNIP−/− mice are due to the greater islet cell mass, rather than increased β-cell secretory capacity. Combined with increased peripheral glucose uptake, and/or impaired hepatic glucose production, this would contribute to the lower glycemia observed in this strain.

DISCUSSION

The biological functions of TxNIP are not completely defined. Based on its binding and inhibitory action on thioredoxin, it has been suggested to regulate cellular redox state and promote oxidative stress (28, 31). In various cell types, TxNIP has been found to inhibit proliferation and/or promote apoptosis (6, 14, 22, 27, 33, 34). The observation that TxNIP gene expression is markedly upregulated by glucose has led to investigation of its role in glucose metabolism and the pathophysiological effects of hyperglycemia. The availability of TxNIP-deficient mouse models has proven valuable to obtain further insight into the biological roles of TxNIP in vivo. In the present study, we used the Hcb-19/TxNIP−/− mouse model, which harbors a TxNIP gene nonsense mutation, and showed that these mice exhibit a higher insulin reserve (circulating insulin levels, total pancreatic insulin content, and β-cell mass) in the basal state compared with strain-matched C3H controls. TxNIP deficiency was associated with a relative resistance to STZ-induced diabetes mellitus. Thus, whereas C3H controls developed marked hyperglycemia after receiving the standard five injections of the low-dose STZ protocol, the TxNIP-deficient mice remained normoglycemic. A second set of STZ injections was required to induce significant hyperglycemia. After this second set and concomitant with the rise in circulating glucose concentrations, circulating insulin levels, pancreatic insulin content, and β-cell mass was increased to similar levels in the Hcb-19/TxNIP−/− animals as in the C3H wild type. These observations are consistent with the recent study of Chen et al. (7), reported while this work was under- way. Indeed, the authors observed similar findings of higher β-cell mass and resistance against STZ-induced diabetes in the Hcb-19/TxNIP−/− mouse model, as well as in a β-cell specific TxNIP knockout, confirming that the observed resistance is a pancreatic effect and not the consequence of the unique metabolic phenotype of the Hcb-19 model (16). In addition, they showed that TxNIP deficiency protects the leptin-deficient ob/ob mouse from the development of type 2 diabetes.

Unexpectedly, we observed that the β-cell mass was more dramatically affected in Hcb-19/TxNIP−/− mice compared with C3H after STZ exposure. This strongly suggests that the normoglycemia and higher β-cell mass exhibited by TxNIP-deficient mice after the standard low-dose STZ protocol is the result of the higher initial β-cell mass and that TxNIP-deficient β-cells may be paradoxically more sensitive to STZ-induced cell death. However, this appeared to be in contradiction with the fact that fewer apoptotic β-cells were detected in pancreatic
sections of Hcb-19/TxNIP−/− mice compared with C3H after STZ injection. Furthermore, a reduction in β-cell apoptosis was reported to be associated with TxNIP deficiency in the β-cell-specific knockout model exposed to STZ, as well as in the ob/ob model (7). It is important to note that staining of pancreatic sections in these studies gave only a single time point or snapshot of the apoptotic process and that dead cells are completely and rapidly cleared in vivo. Thus the lower
number of detected apoptotic cells in the Hcb-19/TxNIP−/− mice observed in the present study 1 day after the last STZ injection does not prove that fewer cells actually died. The timing and/or rate of β-cell death and clearance may also be different between the two strains. For example, TxNIP-deficient β-cells could have undergone apoptosis before control cells and have already been cleared at the time of measurement. Of note, Chen et al. (7) did not quantify β-cell mass before and after STZ exposure. Therefore, although these investigators proposed that TxNIP deficiency enhanced β-cell mass and protected against β-cell apoptosis, whether overall fewer or more β-cells were destroyed by STZ exposure in their models was not documented.

A potentially greater sensitivity of TxNIP-deficient β-cells to STZ-induced cell death in vivo was consistent with our in vitro data obtained in INS-1 cells. In the context of STZ, it should be noted that there are similarities to the clinical and immunohistological features seen in human type 1 diabetes. Thus β-cell death results from both an initial, direct, cytotoxic effect (generation of free radicals and DNA strand breaks) and a subsequent immune reaction directed against the β-cell in response to tissue damage and protein modification (23, 35). Cytokine and chemokine expression by inflammatory cells, T cells, and macrophages is proposed to be the major pathogenetic mechanism leading to insulitis and β-cell apoptosis (15, 17, 19–21, 23, 35, 36). To link our in vivo observations to this proposed STZ-induced pathophysiology, we downregulated TxNIP expression in cultured INS-1 β-cells using SiRNA and exposed them to STZ or the cytokines IL-1β, IFNγ, and TNF-α. The results indicated that TxNIP−/− cells are more sensitive to STZ- and cytokine-induced death. Although TxNIP overexpression has been associated with cell death/apoptosis in various cell types (8, 14, 27, 33, 34), it appears that abnormally low levels of TxNIP expression might also be deleterious for cell survival. In addition, we observed that, although STZ and inflammatory cytokines induced a large amount of cell death in INS-1 cells, these agents increased TxNIP expression to a much lower extent than high glucose.

Moreover, despite the blockade of increased TxNIP expression to a much lower extent than high glucose.

Furthermore, the effects of TxNIP on insulin secretion have not been extensively investigated. In the current study, we show that glucose-stimulated insulin secretion corrected for total insulin content of individual islets from Hcb-19/TxNIP−/− mice is reduced when compared with C3H wild-type mouse islets. The perfusion experiments suggest that TxNIP signaling might affect both the initial primed pool of insulin granules accounting for first phase release as well as the subsequent recruitment of granules that accounts for the second phase. In contrast, Chen et al. (8) reported that insulin secretion was unchanged in Hcb-19/TxNIP−/− islets. This discrepancy is likely due to the fact that, in the latter study, insulin secretion was expressed per islet and not corrected for islet insulin content, which was higher in the Hcb-19/TxNIP−/− mice. In fact, the higher circulating insulin levels we, and others (16, 30), observe in Hcb-19/TxNIP−/− mice is likely the result of the higher β-cell mass and total islet insulin content, which more than compensate for the impaired insulin secretory capacity of the β-cells.

Recently, TxNIP deficiency was found to be associated with increased protein kinase B (Akt) signaling. Thus Akt mRNA and protein levels, as well as phosphorylation, were increased in isolated islets of both Hcb-19/TxNIP−/− and β-cell-specific TxNIP knockout mice (7). Relevant to insulin secretion, the phenotype of transgenic mice overexpressing active Akt1 in β-cells has been shown to be similar to TxNIP-deficient mice; namely, they exhibit an increased islet mass, hyperinsulinemia, and a resistance to STZ-induced diabetes. However, they also demonstrate an impaired insulin secretion in response to glucose when corrected for β-cell mass (32). Together, these observations raise the possibility that TxNIP deficiency may impair insulin secretion of pancreatic islets by inducing Akt signaling.

In summary, the present data indicate that TxNIP is involved in the constitution of β-cell mass and that the greater β-cell mass observed in TxNIP−/− mice accounts for their resistance to STZ-induced diabetes. The development of islets and islet cell mass are determined, in part, during a neonatal remodeling period, when there is a high degree of β-cell replication and apoptosis (3). This may influence individual susceptibility to diabetes by limiting β-cell mass early in life (11). Considering the complex roles TxNIP appears to play in β-cell survival and function, further investigation is warranted to determine the involvement of TxNIP in islet cell development.

ACKNOWLEDGMENTS

We thank Dr. R. A. Davis, San Diego State University, for providing us with the C3H/Hcb-19 mouse model.

GRANTS

This work was supported by Grant Nos. MOP 38009 and MOP 49409 from the Canadian Institutes of Health Research to L. G. Fantus. E. Masson was supported by a Canadian Diabetes Association postdoctoral fellowship, an award from the Faculty of Medicine, and a Banting and Best Diabetes Centre postdoctoral fellowship, University of Toronto. F. Razik was supported by a Summer Studentship from the Samuel Lunenfeld Research Institute, Mount Sinai Hospital.

REFERENCES

7. Chen J, Hui ST, Couto FM, Mungrue IN, Davis DB, Attie AD, Luisis AJ, Davis RA, Shalev A. Thiooxidin-interacting protein deficiency
RESISTANCE TO DIABETES IN Hcb-19/TxNIP<sup>−/−</sup> MICE

E1261


