*Original Article*

# STAT5 Activity in Pancreatic β-Cells Influences the Severity of Diabetes in Animal Models of Type 1 and 2 Diabetes

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**Pancreatic β-cell growth and survival and insulin production are stimulated by growth hormone and prolactin through activation of the transcription factor signal transducer and activator of transcription (STAT)5. To assess the role of STAT5 activity in β-cells in vivo, we generated transgenic mice that expressed a dominant-negative mutant of STAT5a (DNSTAT5) or constitutive active mutant of STAT5b (CASTAT5) under control of the rat insulin 1 promoter (RIP). When subjected to a high-fat diet, RIP-DNSTAT5 mice showed higher body weight, increased plasma glucose levels, and impairment of glucose tolerance, whereas RIP-CASTAT5 mice were more glucose tolerant and less hyperleptinemic than wild-type mice. Although the pancreatic insulin content and relative β-cell area were increased in high-fat diet–fed RIP-DNSTAT5 mice compared with wild-type or RIP-CASTAT5 mice, RIP-DNSTAT5 mice showed reduced β-cell proliferation at 6 months of age. The inhibitory effect of high-fat diet or leptin on insulin secretion was diminished in isolated islets from RIP-DNSTAT5 mice compared with wild-type islets. Upon multiple low-dose streptozotocin treatment, RIP-DNSTAT5 mice exhibited higher plasma glucose levels, lower plasma insulin levels, and lower pancreatic insulin content than wild-type mice, whereas RIP-CASTAT5 mice maintained higher levels of plasma insulin. In conclusion, our results indicate that STAT5 activity in β-cells influences the susceptibility to experimentally induced type 1 and type 2 diabetes. *Diabetes* 55:2705–2712, 2006**

Decrease in functional β-cell mass appears to play a role in the development of both type 1 and type 2 diabetes. While the β-cells are almost completely eliminated through autoimmune destruction in type 1 diabetes, type 2 diabetes arises from a combination of insulin resistance and inadequate functional β-cell mass. Obesity is often associated with an increased β-cell mass that initially compensates for the insulin resistance; however, β-cell compensation may eventually fail, resulting in type 2 diabetes (1).

In addition to nutrients such as glucose and amino acids, several factors stimulate replication, neogenesis, and survival of β-cells (1,2). Among the most potent β-cell growth factors are growth hormone, prolactin, and placental lactogen, which stimulate proliferation, insulin release, and insulin gene expression in cultured β-cells (2,3). Placental lactogen and prolactin both activate the prolactin receptor (3), which is expressed together with the growth hormone receptor in pancreatic β-cells (4). Transgenic mice in which expression of placental lactogen was targeted to the β-cells exhibited increased β-cell proliferation and islet mass together with resistance to β-cell apoptosis induced by streptozotocin (STZ) (5,6). Targeted deletion of prolactin receptor in mice resulted in reduced β-cell mass and impaired glucose tolerance (7), while mice lacking growth hormone receptor showed decreased β-cell mass and proliferation (8).

Growth hormone receptor and prolactin receptor belong to the cytokine receptor superfamily, which signals through activation of the Janus kinase/signal transducer and activator of transcription (STAT) pathway (9,10). In rat insulinoma cells, stimulation by growth hormone or prolactin leads to activation of STAT5a and -5b (11,12), which are encoded by two different genes that show 96% sequence similarity at the protein level (13). Induction of insulin gene transcription and β-cell proliferation by growth hormone or prolactin is mediated through STAT5 activation in rat insulinoma cells (11,14). Moreover, we have recently shown that growth hormone protects against apoptosis induced by proinflammatory cytokines or fatty acids through activation of STAT5 in insulin-producing INS-1E cells (15 and L.T. Dalgaard, P.T., L.W. Gaarn, Y.C.L., K. Capito, J.H.N., unpublished results).

Because growth hormone levels are reduced during obesity (16), other growth factors are responsible for the compensatory β-cell hyperplasia associated with insulin resistance (1). Various cytokines, such as leptin, interleukin-6, and tumor necrosis factor α, are elevated in obesity-associated diabetes and may play a role in the pathogenesis of type 2 diabetes (17). Leptin has been shown to activate STAT3 and -5b in rat insulinoma cells (18,19). In rodent β-cells, leptin stimulates proliferation (20,21), protects against fatty acid–induced apoptosis (22,23), and inhibits insulin gene expression and secretion (24–27).

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AUC, area under the curve; CASTAT5, constitutive active mutant of signal transducer and activator of transcription 5b; DNSTAT5, dominant-negative mutant of signal transducer and activator of transcription 5a; GLP, glucagon-like peptide; RIP, rat insulin 1 promoter; STAT, signal transducer and activator of transcription; STZ, streptozotocin.

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To investigate the functional role of STAT5 in islets in vivo, we generated transgenic mice in which expression of either the dominant-negative mutant of STAT5a (DNSTAT5) or the constitutive active mutant of STAT5b (CASTAT5) was directed to the pancreatic β-cells using the rat insulin 1 promoter (RIP). By subjecting the mice to a high-fat diet or multiple low doses of STZ, we evaluated the importance of β-cell STAT5 activity for the susceptibility of the mice to experimentally induced diabetes.

## RESEARCH DESIGN AND METHODS

**Generation of transgenic mice.** For creation of the transgenic constructs, mouse cDNA encoding CASTAT5 or DNSTAT5 was inserted directly after a 410-bp fragment of RIP that was constructed in the mammalian expression vector pCI, as previously described (28). CASTAT5 (originally denoted STAT5b1*6) was kindly provided by Dr. T. Kitamura (Minato-ku, Tokyo, Japan). This mutant contains two amino acid substitutions (H299R and S711F) that render the mutant constitutively phosphorylated (29). The COOH-terminal deletion mutant of STAT5a, DNSTAT5 (also known as STAT5aΔ749), which lacks the functional transactivating domain and inhibits the activity of both STAT5a and -5b, was kindly provided by Dr. B. Groner (Freiburg, Germany) (30). The DNA fragments containing the RIP-CASTAT5-polyA or RIP-DNSTAT5-polyA transgene were excised, purified, and microinjected into fertilized eggs of C57Bl/6JxDBA2/J mice using standard procedures performed by Dr. J.P. Hjorth (University of Aarhus, Aarhus, Denmark). Founder mice were backcrossed with C57Bl/6J mice at Taconic Europe (Ry, Denmark) to established the RIP-CASTAT5 and RIP-DNSTAT5 transgenic mouse lines. Genotyping was performed by standard PCR on tail genomic DNA using STAT5a/b-specific primers (5′-CATGGAGCTGGTTCGCTGTAT-3′ and 5′-GTACTCTTGGGTCTGCTGCAG-3′) for the RIP-CASTAT5 line and STAT5a-specific primers (5′-ATCGAGGTCCGGCACTACCTG-3′ and 5′-CTGGAGCTGT GTGGCATAGTG-3′) for the RIP-DNSTAT5 line. Both primer sets are placed in two different exons flanking a short intron and give rise to short PCR fragments of ~200 bp derived from the CASTAT5 or DNSTAT5 transgene and longer PCR fragments (1,065 or 458 bp) derived from the endogenous STAT5a/b genes. All experiments were performed with male single transgenic mice and wild-type littermates that were obtained from the F2-F5 generation. The mice were housed in a normal cycle of 12 h light/12 h dark and had free access to food and water. The animal studies were conducted in accordance with institutional guidelines and approved by the Animal Experiments Inspectorate in Denmark.

**RT-PCR.** Islets were isolated by collagenase digestion of pancreatic tissue. Total RNA was extracted from the islets using RNAzol (Campro Scientific, Veenendaal, the Netherlands), and cDNA synthesis and PCR were performed as previously described (14). For amplification of prolactin receptor, the following primers were used: 5′-GCCACTGTCTGCTCTTTTCAG-3′ and 5′-GGATTTCAGGTTTTCCAGGTG-3′. The primers for STAT5a/b and STAT5a were similar to those used for genotyping of RIP-CASTAT5 and RIP-DNSTAT5 mice, respectively. The primer sequences for cyclin D2 and TATA-box–binding protein were as described previously (14).

**High-fat diet treatment.** From the age of 5–6 weeks, mice were fed either a high-fat diet containing 35% fat (no. D12492; Research Diets, New Brunswick, NJ) or standard diet containing 5% fat (Altromin, Lage, Germany) for a period of 20 weeks. Body weights were recorded and plasma samples were obtained from the orbital sinus between 12:00 and 2:00 P.M. every 4th week.

**Multiple low-dose STZ treatment.** At the age of 11–15 weeks, mice received daily intraperitoneal injections of 50 mg/kg STZ freshly dissolved in 10 mmol/l citrate buffer, pH 4.5, for 5 consecutive days. Before the first STZ injection, and weekly thereafter, plasma samples were obtained from the orbital sinus of nonfasted mice between 12:00 and 2:00 P.M.

**Plasma glucose, insulin, and leptin assays and glucose tolerance tests.** Plasma glucose concentrations were measured enzymatically using a colorimetric kit (Chema Diagnostica, Jasi, Italy). Plasma insulin and leptin concentrations were determined using radioimmunoassay kits (Linco Research, St. Charles, MO). For glucose tolerance tests, mice were fasted overnight and received an oral glucose load (2 g/kg body wt). Before and 15, 30, 60, and 120 min after glucose administration, glucose levels were measured in blood samples obtained from the orbital sinus using a Glucometer Elite (Bayer, Lyngby, Denmark).

**Pancreatic insulin content.** Pancreatic tissue was weighted, sonicated, and extracted in 3 mol/l acetic acid. Insulin content was determined by radioimmunoassay using guinea pig anti-insulin serum, monoiodinated human insulin as tracer, rat insulin as standard, and ethanol to separate antibody-bound insulin from free insulin (31).

**Immunohistochemistry and morphometry.** Pancreatic tissue was fixed overnight in 4% paraformaldehyde and cut into 20 parts that were divided randomly into two paraffin blocks. Two sections of 5 μm separated by at least 100 μm were deparaffinized and incubated in methanol containing 0.3% $H_2O_2$. Following boiling for 10 min in a modified citrate buffer (Dako, Glostrup, Denmark) in a microwave oven, the sections were blocked with 10% normal horse serum and exposed overnight at 4°C to guinea pig anti-insulin diluted 1:4,000 (Dako) and rabbit anti-Ki67 diluted 1:1,000 (Novocastra, Newcastle, U.K.). The primary antibodies were detected by alkaline phosphatase–labeled donkey anti–guinea pig Ig and biotin-labeled donkey anti-rabbit Ig followed by peroxidase-conjugated streptavidin and successive developments in Vector Red alkaline phosphatase substrate (Vector Laboratories, Burlingame, CA) and diaminobenzidine-$H_2O_2$ mixture. All secondary antibodies were from Jackson ImmunoResearch (West Grove, PA). Specimens were examined under a microscope, photographed by digital camera, and quantified using the Image-Pro Plus software (Media Cybernetics, Silver Spring, MD). The relative β-cell area was determined by measurement of the area occupied by all insulin cells by analysis of 44–117 microscopic images (10× objective) from each pancreas, while the tissue section area of the same microscopic slides was measured from 5 to 20 microscopic images (1.25× objective) covering all tissue. The β-cell proliferation was measured by quantifying the number of Ki67-positive insulin cells in relation to the area of all insulin cells.

**Insulin secretion.** Islets were prepared by collagenase digestion of pancreatic tissue from mice that were fed a high-fat or standard diet for 9–11 weeks from the age of 5–7 weeks. Islets were kept in tissue culture for 24 h in RPMI-1640 medium containing 10% newborn calf serum, as previously described (32). Insulin release from islets was determined by perifusion in a noncirculating system with beads of 0.25 ml Bio-Gel P2 (Bio-Rad Laboratories, Rockville Center, NY) as a supporting medium. Twenty-five islets per chamber were perifused at 37°C at a flow rate of 0.26 ml/min in Krebs-Ringer medium supplemented with 20 mmol/l HEPES, 5 mmol/l $NaHCO_3$, 2 mg/ml BSA, and 3.3 mmol/l glucose. Islets were initially perifused to obtain a basal release rate and then challenged with 16.7 mmol/l glucose and 0.1 μmol/l glucagon-like peptide (GLP)-1 in the absence or presence of 10 nmol/l leptin. The effluent medium was collected for periods of 5 or 10 min. Insulin was determined by radioimmunoassay.

**Statistics.** Data are expressed as means ± SE. For comparison of the mean between two groups, Student's unpaired two-tailed $t$ test was used. To compare the mean of more than two samples, one-way ANOVA with Neuman-Keuls posttest was applied. Two-way ANOVA with Bonferroni posttest was used to measure differences between glucose tolerance tests and plasma glucose levels following STZ injections. To quantify differences in glucose tolerance tests and insulin secretion, the area under the curve (AUC) was measured using Prism (version 4; GraphPad, San Diego, CA).

## RESULTS

**Generation of transgenic mice that express CASTAT5 or DNSTAT5 in β-cells.** To determine the role of STAT5 activity in β-cells, we generated transgenic mice, in which expression of CASTAT5 or DNSTAT5 was targeted to β-cells, using RIP. In one of the RIP-CASTAT5 lines, twofold-higher expression of total STAT5a/b mRNA was detected in transgenic islets compared with wild-type islets, together with increased expression of two STAT5 target genes, prolactin receptor and cyclin D2 (Fig. 1). Islets from transgenic mice from one of the RIP-DNSTAT5 lines showed almost fourfold-higher STAT5a mRNA expression than islets from wild-type littermates, while gene expression of prolactin receptor and cyclin D2 was reduced in RIP-DNSTAT5 islets. RIP-CASTAT5 islets exhibited normal expression levels of STAT5a, which is expressed at a lower level than STAT5b in wild-type islets. However, total STAT5a/b expression was only slightly increased in RIP-DNSTAT5 islets, indicating that a compensatory downregulation in endogenous STAT5a/b expression occurred in these islets (data not shown). Using transgene-specific primers, expression of the transgene was not detected in hypothalamus, muscle, or liver in transgenic mice from the RIP-CASTAT5 and RIP-DNSTAT5 lines (data not shown). These two transgenic lines were used in the subsequent studies.

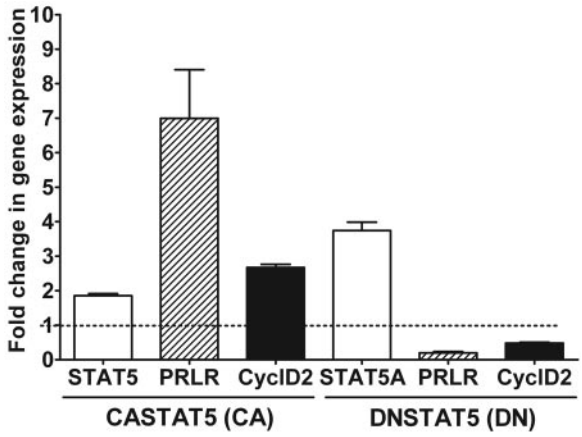


**FIG. 1. Gene expression in islets from RIP-CASTAT5 and RIP-DNSTAT5 mice. mRNA levels of STAT5a/b, STAT5a, and two STAT5 target genes, prolactin receptor (PRLR) and cyclin D2 (CyclD2), were analyzed by RT-PCR and expressed in relation to TATA-box–binding protein. Fold change in relative gene expression is shown for transgenic islets normalized against wild-type islets.**

**Effect of β-cell STAT5 activity on high-fat diet–induced body weight gain.** When RIP-CASTAT5, RIP-DNSTAT5, and wild-type mice were fed a standard diet, there was no difference in body weight between the transgenic and wild-type mice at ≤6 months of age. High-fat diet resulted in increased body weight in both transgenic and wild-type mice. However, RIP-DNSTAT5 mice were more susceptible to the effect of high-fat diet, as the mean body weight was 40% higher in RIP-DNSTAT5 mice maintained on high-fat diet for 16 weeks compared with similar transgenic mice on standard diet, while high-fat diet resulted in 18 and 20% higher body weight in RIP-CASTAT5 and wild-type mice, respectively (Fig. 2*A*). After 16 weeks on high-fat diet, the body weight in RIP-DNSTAT5 mice was significantly higher than in RIP-CASTAT5 and wild-type mice (Fig. 2*A*).

**Effect of β-cell STAT5 activity on plasma glucose, insulin, and leptin.** When fed standard diet, plasma glucose, insulin, and leptin levels were similar in transgenic and wild-type mice. After 16 weeks on high-fat diet, plasma glucose concentrations in RIP-DNSTAT5 mice were significantly increased compared with mice on standard diet, while high-fat diet only slightly affected blood glucose levels in wild-type and RIP-CASTAT5 mice (Fig. 2*B*). In RIP-DNSTAT5 mice, plasma glucose levels were significantly higher than in wild-type or RIP-CASTAT5 mice on high-fat diet (Fig. 2*B*). Plasma insulin concentrations and plasma insulin-to-glucose ratio were also significantly elevated in RIP-DNSTAT5 mice on high-fat diet compared with similar transgenic mice on standard diet, while high-fat diet resulted in a small but nonsignificant increase in plasma insulin concentrations in RIP-CASTAT5 and wild-type mice (Fig. 2*C* and *D*). Plasma insulin levels were significantly higher in RIP-DNSTAT5 than in RIP-CASTAT5 transgenic mice on high-fat diet. Moreover, high-fat diet affected plasma leptin levels, as elevated plasma concentrations of leptin were observed in both RIP-DNSTAT5 and wild-type mice compared with mice of similar genotype fed a standard diet (Fig. 2*E*). RIP-CASTAT5 mice displayed significantly lower plasma leptin levels than RIP-DNSTAT5 and wild-type mice on high-fat diet.

**Effect of β-cell STAT5 activity on glucose tolerance.** Transgenic and wild-type mice were subjected to oral glucose tolerance tests after 20 weeks on high-fat or standard diet. In mice maintained on standard diet, blood glucose levels were significantly lower in RIP-CASTAT5 mice compared with RIP-DNSTAT5 ($P < 0.01$) and wild-



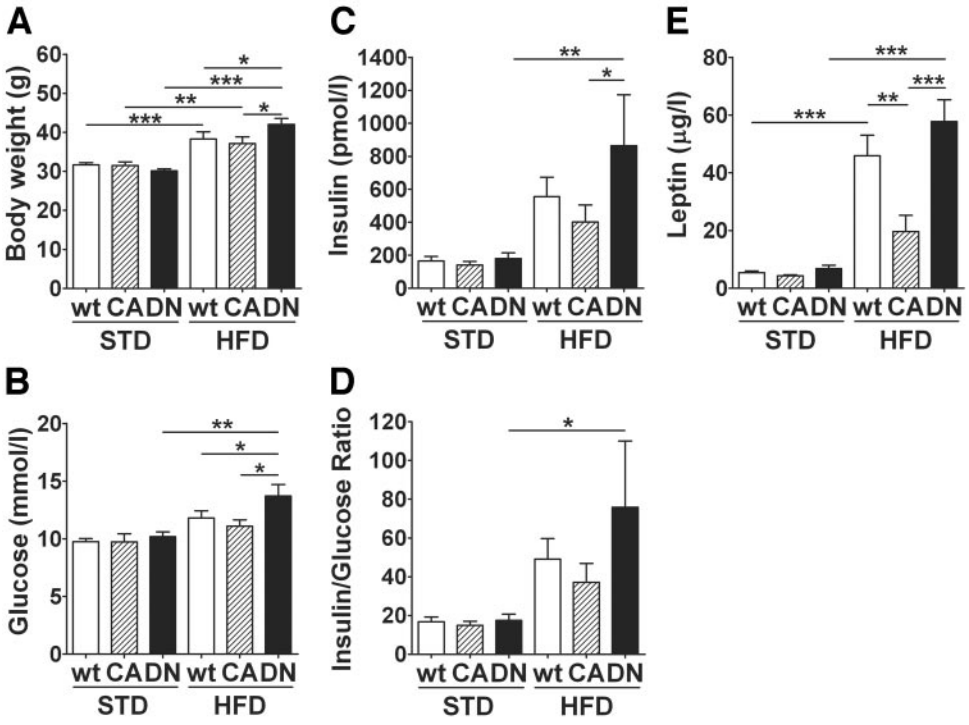


**FIG. 2. Body weight (*A*) and plasma levels of glucose (*B*), insulin (*C*), and leptin (*E*) of wild-type (wt), RIP-CASTAT5 (CA), and RIP-DNSTAT5 (DN) mice fed standard diet (STD) or high-fat diet (HFD) for 16 weeks. The ratio of picomoles insulin to millimoles glucose in plasma is shown in *D*. Data are shown as means ± SE of 8–18 mice/group. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.**

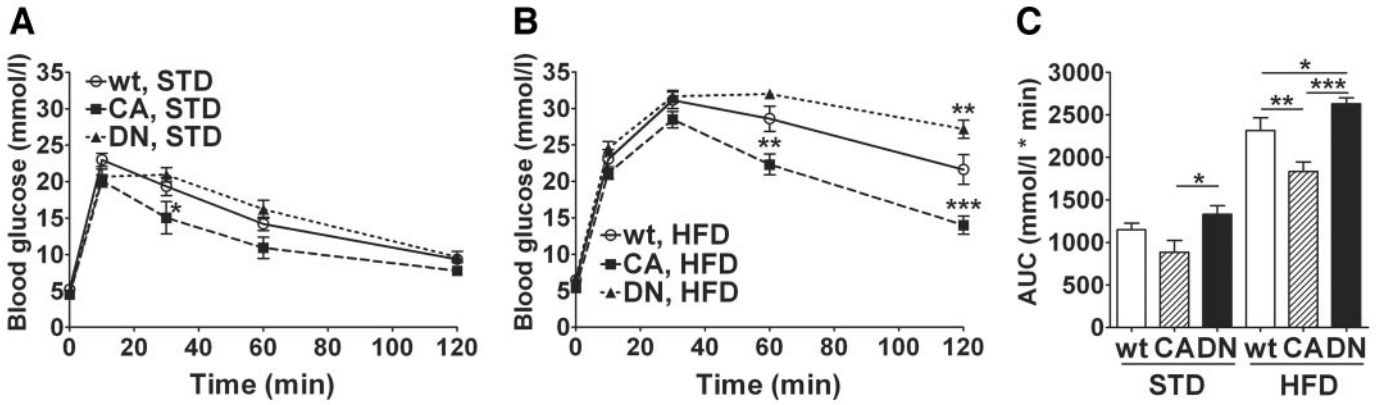


**FIG. 3. Oral glucose tolerance in wild-type (wt), RIP-CASTAT5 (CA), and RIP-DNSTAT5 (DN) mice fed standard diet (STD) (*A*) or high-fat diet (HFD) (*B*) for 20 weeks. *A* and *B*: Blood glucose concentrations were measured before and after oral glucose load at the indicated time points. For mice on both diets, glucose clearance was significantly improved in RIP-CASTAT5 mice compared with wild-type or RIP-DNSTAT5 mice (two-way ANOVA). Significant differences between transgenic and wild-type mice are marked at the time points noted by asterisks. *C*: AUC during the 120-min period of the glucose disposal curves above glucose levels at $t = 0$. Data are presented as means ± SE of 9–16 mice/group. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.**

type ($P < 0.05$) mice at 30 min after glucose administration (Fig. 3*A*). The AUC for the blood glucose response was significantly reduced in standard diet–fed RIP-CASTAT5 mice compared with RIP-DNSTAT5 mice (Fig. 3*C*). In both transgenic mice and wild-type mice, high-fat diet induced glucose intolerance ($P < 0.001$). When comparing glucose disposal in transgenic and wild-type mice fed high-fat diet, lower blood glucose levels were found in RIP-CASTAT5 mice than in RIP-DNSTAT5 and wild-type mice both at 60 and 120 min after glucose load (Fig. 3*B*). RIP-DNSTAT5 mice on high-fat diet showed reduced glucose clearance compared with wild-type mice, as blood glucose levels were significantly higher at 120 min after glucose administration (Fig. 3*B*). When comparing the AUC for the blood glucose responses of mice on a high-fat diet, glucose tolerance was significantly more impaired in RIP-DNSTAT5 mice compared with wild-type and RIP-CASTAT5 mice, whereas RIP-CASTAT5 mice showed less impaired glucose clearance compared with wild-type mice (Fig. 3*C*).

**Effect of β-cell STAT5 activity on pancreatic insulin, β-cell area, and proliferation.** Following 20 weeks on high-fat or standard diet, the pancreas was analyzed for insulin content, relative β-cell area, and proliferating β-cells. In both transgenic and wild-type mice, high-fat diet feeding significantly increased pancreatic insulin content compared with similar mice on standard diet (Fig. 4*A*). In RIP-DNSTAT5 mice, insulin content of the pancreas was significantly higher compared with both wild-type and RIP-CASTAT5 mice fed a high-fat diet (Fig. 4*A*). High-fat diet feeding also caused an increase in the percentage of the pancreatic area occupied by β-cells in RIP-DNSTAT5 transgenic mice (Fig. 4*B*). A small nonsignificant increase in relative β-cell area was found in high-fat diet–fed wild-type and RIP-CASTAT5 mice compared with mice of similar genotype on standard diet. In addition, the relative β-cell area was significantly higher in RIP-DNSTAT5 mice compared with RIP-CASTAT5 mice on high-fat diet. β-Cell proliferation was quantified by measuring the percentage of Ki67-positive β-cells (Fig. 4*C* and *D*). In both transgenic and wild-type mice, β-cell proliferation was similar in mice fed high-fat and standard diet (Fig. 4*D*). When comparing β-cell proliferation in transgenic and wild-type mice, irrespective of type of diet, RIP-DNSTAT5 mice displayed significantly lower β-cell proliferation (8.8 ± 1.6, $n = 10$) than both wild-type (20.1 ± 4.9, $n = 10$; $P < 0.05$) and RIP-CASTAT5 (23.7 ± 4.7, $n = 10$; $P < 0.01$) mice.

**Effect of β-cell STAT5 activity on insulin secretion in isolated islets.** To further examine the role of STAT5 in islet function, we investigated glucose- and GLP-1–stimulated insulin secretion from perifused islets isolated from mice fed high-fat or standard diet for 9–11 weeks. High-fat diet feeding resulted in reduced first-phase insulin secretion in response to glucose in wild-type islets and a tendency toward decreased first-phase insulin secretion in

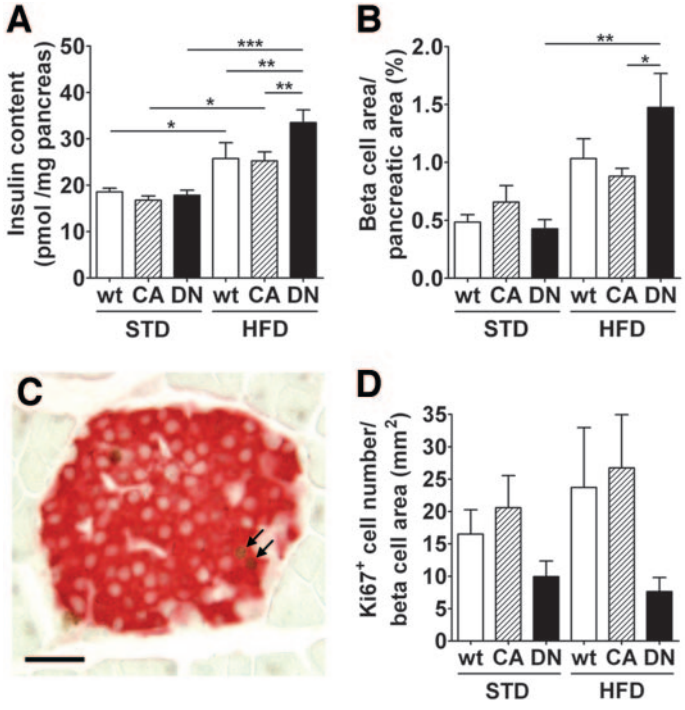


**FIG. 4. Analysis of pancreata of wild-type (wt), RIP-CASTAT5 (CA), and RIP-DNSTAT5 (DN) mice fed standard diet (STD) or high-fat diet (HFD) for 20 weeks. *A*: Insulin content in pancreatic extracts. *B*: Percentage of the area of pancreatic sections occupied by β-cells. *C*: Pancreatic islet immunostained for insulin (red) and Ki67 (brown). Arrows indicate two insulin cells with Ki67-positive nuclei. *D*: Number of Ki67-positive β-cells expressed in relation to total β-cell area. Data are presented as means ± SE of 5–9 mice/group. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.**

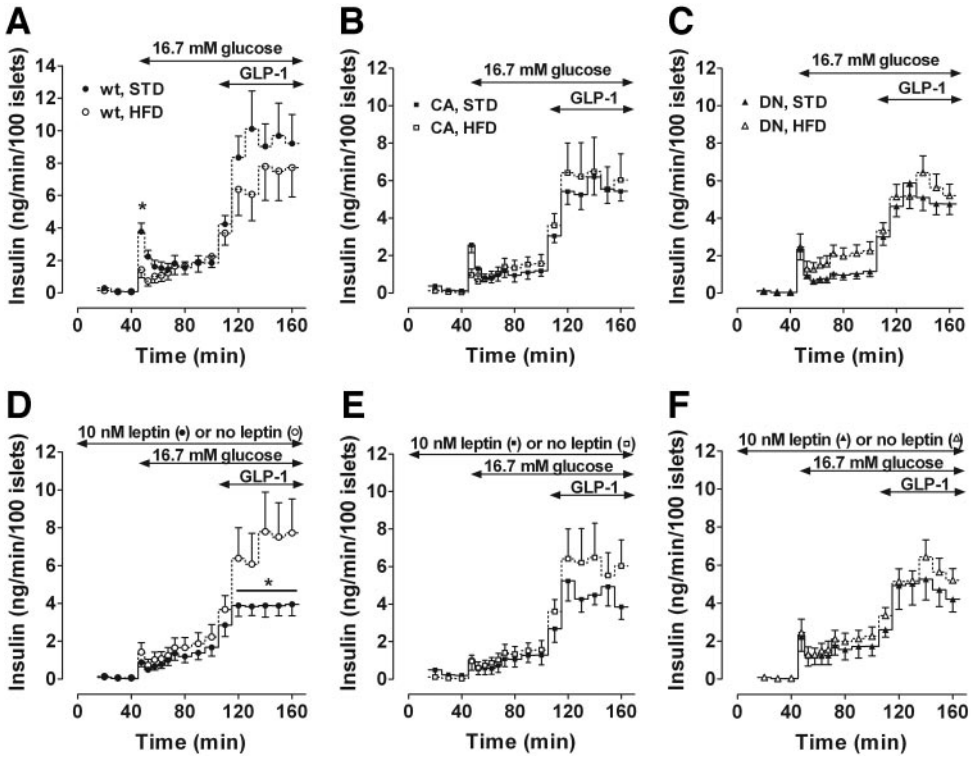


**FIG. 5. Insulin secretory response in isolated islets from wild-type (wt) (*A* and *D*), RIP-CASTAT5 (*B* and *E*), and RIP-DNSTAT5 (*C* and *F*) mice. *A–C*: Islets from mice fed a standard diet (STD) or high-fat diet (HFD) for 9–11 weeks. *D–F*: Islets from high-fat diet–fed mice, stimulated or not stimulated by leptin. Islets were perifused with 16.7 mmol/l glucose and 0.1 μmol/l GLP-1 in the absence or presence of 10 nmol/l leptin as indicated. Results are means ± SE ($n$ = 4–7). *$P$ < 0.05.**

RIP-CASTAT5 islets (Fig. 5*A* and *B*). In contrast, islets from high-fat diet–fed RIP-DNSTAT5 mice showed normal first-phase insulin response and a tendency to increased second-phase glucose-induced insulin release compared with islets from standard diet–fed mice (Fig. 5*C*). There was no difference in insulin secretory response to glucose and GLP-1 in islets from high-fat diet–fed RIP-DNSTAT5, RIP-CASTAT5, and wild-type mice. Addition of leptin significantly reduced GLP-1–induced insulin secretion from wild-type islets, while a small, but nonsignificant, inhibitory effect of leptin was observed in RIP-CASTAT5 islets (Fig. 5*D* and *E*). In contrast, insulin secretion from RIP-DNSTAT5 islets was unaffected by leptin (Fig. 5*F*).

**Role of β-cell STAT5 activity in diabetes induced by multiple low-dose STZ.** To assess the effect of β-cell STAT5 on diabetes induced by multiple low-dose STZ, RIP-DNSTAT5 and RIP-CASTAT5 mice were subjected to injections of low-dose STZ for 5 consecutive days in parallel with wild-type mice in two separate series of experiments. The difference in the severity of STZ-induced diabetes in the wild-type mice in the two series of experiments is in accordance with the known sensitivity of this model to subtle experimental conditions (K. Buschard, personal communication). When comparing fed plasma glucose weekly after the first STZ injection, plasma glucose concentrations were significantly increased in RIP-DNSTAT5 mice compared with wild-type mice (Fig. 6*A*). RIP-DNSTAT5 mice further displayed reduced plasma insulin levels at day 14 and decreased pancreatic insulin content at day 35 after STZ (Fig. 6*B* and *C*). In RIP-CASTAT5 mice, the STZ-induced increase in plasma glucose levels was slightly, but nonsignificantly, lower than in wild-type mice (Fig. 6*D*). Four weeks after STZ injection, RIP-CASTAT5 mice displayed significantly higher plasma insulin concentrations (Fig. 6*E*) and a tendency toward increased pancreatic insulin content was observed (Fig. 6*F*).

## DISCUSSION

To study the role of STAT5 activity in pancreatic β-cells, we have generated two new transgenic mouse lines that specifically expressed either CASTAT5 or DNSTAT5 in β-cells. Because DNSTAT5 inhibits the activity of STAT5a and -5b (30), the individual functions of the STAT5 isoforms cannot be studied in RIP-DNSTAT5 mice. When subjecting the mice to high-fat diet, the RIP-DNSTAT5 mice showed increased body weight, higher insulin and leptin levels, and more severely impaired glucose tolerance compared with wild-type mice. In agreement with previous studies (27,33), high-fat diet resulted in reduced first-phase insulin secretion in response to glucose in isolated wild-type islets, while leptin suppressed GLP-1–induced insulin secretion. In contrast, insulin secretion from RIP-DNSTAT5 islets was unaffected by leptin and slightly stimulated by high-fat diet feeding. As leptin activates STAT3 and -5b in β-cells (18,19), expression of DNSTAT5 in β-cells may abolish the inhibitory effect of leptin on insulin secretion and thereby promote the hyperinsulinemia in high-fat diet–fed mice. The elevated plasma insulin levels may further increase adiposity and plasma leptin in RIP-DNSTAT5 mice. In contrast, the RIP-CASTAT5 mice displayed less impaired glucose clearance and lower plasma leptin levels than wild-type mice fed a high-fat diet. The lower plasma leptin levels in RIP-CASTAT5 mice may reflect the lower body weight and plasma insulin levels than those seen in RIP-DNSTAT5 mice. The inhibitory effect of leptin on insulin release in

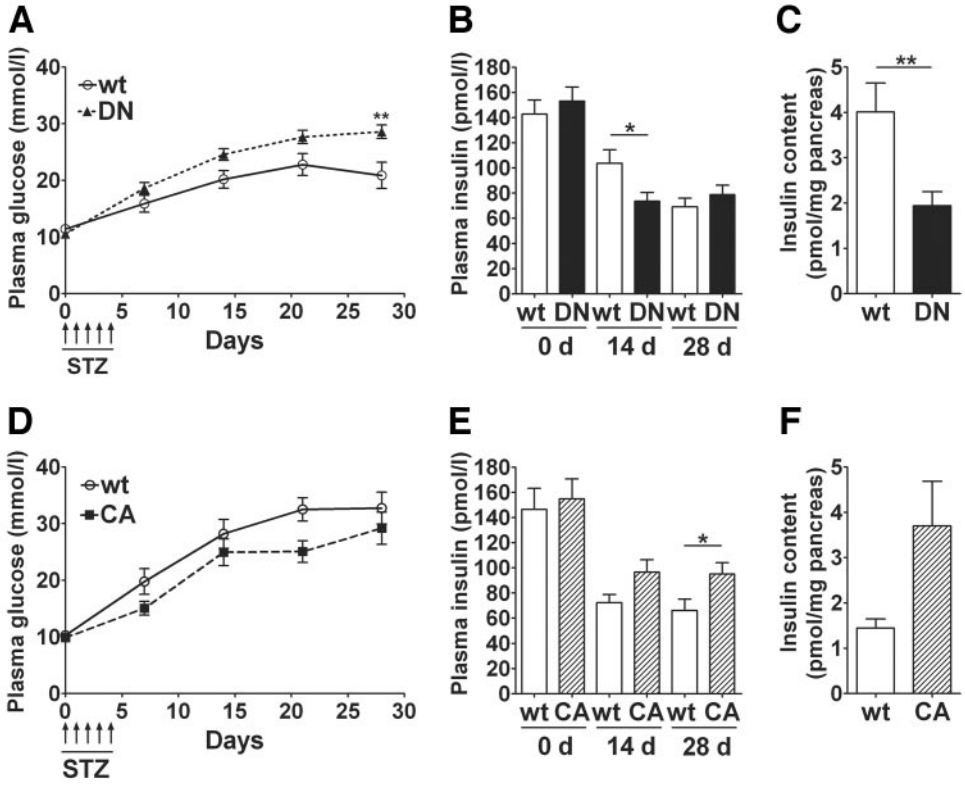


**FIG. 6. Effect of STZ injection in RIP-DNSTAT5 (DN; *A–C*) and RIP-CASTAT5 (CA; *D–F*) mice compared with wild-type (wt) mice analyzed in the same experiment. *A* and *D*: Plasma glucose concentrations before and after multiple low-dose STZ injections ($n$ = 9–16). Glucose levels were significantly increased in RIP-DNSTAT5 mice compared with wild-type mice when analyzed by two-way ANOVA ($P < 0.01$). *B* and *E*: Plasma insulin concentrations before and 14 or 28 days after STZ injection ($n$ = 8–17). *C* and *F*: Insulin content in pancreatic extracts on day 35 following STZ injection ($n$ = 4–8). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.**

RIP-CASTAT5 islets was also slightly reduced, which may be due to induction of SOCS3, a negative feedback regulator of cytokine signaling (19,28). Alternatively, STAT5 activation in islets may prevent islet lipid accumulation and thereby protect the β-cells against the adverse effects of lipids (L.T. Dalgaard, P.T., L.W. Gaarn, Y.C.L., K. Capito, J.H.N., unpublished results). As insulin is known to mediate an adipogenic effect, hypersecretion of insulin is not only a compensatory response to decreased insulin sensitivity but may also exaggerate the progression of obesity, insulin resistance, and type 2 diabetes (34).

The hyperinsulinemia observed in high-fat diet–fed mice correlated with increased pancreatic insulin content and relative β-cell area. The pancreatic insulin content and the relative β-cell area were significantly higher in RIP-DNSTAT5 mice than in RIP-CASTAT5 mice on high-fat diet. This unexpected finding contrasts our previous in vitro data showing increased β-cell proliferation by CASTAT5 in isolated islets (35). The compensatory expansion of the β-cell mass associated with obesity is believed to result from a combination of increased β-cell proliferation, neogenesis, and hypertrophy. In our study, β-cell replication was lower in RIP-DNSTAT5 mice, which is associated with decreased islet expression of cyclin D2, which has been shown to be important for postnatal β-cell replication (36,37). Increased β-cell replication may have occurred in high-fat diet–fed RIP-DNSTAT5 mice at an earlier stage, as high-fat diet was reported to lead to increased β-cell proliferation in mice after short duration of feeding, whereas long-term high-fat feeding resulted in reduced proliferation and increased apoptosis of β-cells (38). However, obesity-induced β-cell hyperplasia may also occur through β-cell neogenesis, as increased percentage of insulin-positive duct cells was observed in both high-fat diet–fed mice and obese humans (38,39). In several rodent models of obesity, compensatory increase in β-cell mass occurred at the initial phase, followed by decompensation and decline in β-cell function (1). The lower β-cell proliferation in RIP-DNSTAT5 mice may indicate that decompensation is more advanced in these mice than in wild-type and RIP-CASTAT5 mice after 20 weeks on high-fat diet. The occurrence of β-cell hyperplasia in RIP-DNSTAT5 mice on high-fat diet suggests that obesity-induced islet growth is induced by nutrients, such as glucose, or β-cell growth factors, such as GLP-1 and glucose-dependent insulinotropic peptide (40), acting through other signaling molecules than STAT5 and cyclin D2.

Two recent studies have reported that mice devoid of STAT3 in β-cells became hyperphagic, obese, and glucose intolerant due to loss of STAT3 in the hypothalamus (41,42). These mice were created using RIP2-directed expression of cre. As expression of the transgenes was not detected in the hypothalamus of the present STAT5 transgenic mice generated using RIP1, including the promoter elements required for pancreas specificity (43), we do not suspect that transgenic expression affected leptin sensitivity at the level of the hypothalamus in our mice.

To investigate the importance of β-cell STAT5 activity for the susceptibility to type 1 diabetes, transgenic mice were exposed to multiple injections of low-dose STZ. This model for pathogenesis of type 1 diabetes is characterized by slow progression of hyperglycemia accompanied with insulitis and β-cell loss mediated by apoptotic processes

(44,45). Our data demonstrate that transgenic expression of CASTAT5 in β-cells partly protected against multiple low-dose STZ injections, while RIP-DNSTAT5 mice were more susceptible to STZ-induced diabetes. This is in agreement with in vitro studies showing that growth hormone abrogates cytokine-induced apoptosis in INS-1 cells through activation of STAT5 (15). Moreover, protection against STZ-induced diabetes was also observed in mice with transgenic expression of placental lactogen in β-cells (5,6) or by injection of prolactin (46). The mechanism for the protective effect of STAT5 against β-cell destruction may involve members of the Bcl-2 family, as growth hormone stimulates the β-cell expression of Bcl-$X_L$ (15), which is a target gene of STAT5 involved in antiapoptosis (47). Alternatively, induction of SOCS3 expression by CASTAT5 in β-cells (Y.C.L., personal communication) may protect against cytokine-induced apoptosis by inhibition of interleukin-1β signaling, as reported by Karlsen et al. (48). Thus, although CASTAT5 expression was low in transgenic islets, the expression was sufficient enough to exert a protective effect against STZ-induced β-cell destruction.

In summary, we have generated and characterized two new transgenic lines of mice that express either CASTAT5 or DNSTAT5 in pancreatic β-cells. Expression of CASTAT5 in β-cells partly counteracted the diabetogenic effect of both high-fat diet and of multiple low-dose STZ treatment, whereas reduction of β-cell STAT5 activity increased the susceptibility to both obesity-induced diabetes and β-cell loss induced by low-dose STZ. Therefore, pharmacological or genetic interventions that lead to increased STAT5 activity in β-cells may protect against both type 1 and type 2 diabetes.

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