Intestinal adaptation and Reg gene expression induced by antidiabetic duodenal-jejunal bypass surgery in Zucker fatty rats

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The antidiabetic mechanism of bariatric surgery includes specific changes in the secretion of incretins. To identify additional players originating from the gut, we evaluated the effects of duodenal-jejunal bypass (DJB) in morbidly obese Zucker fatty rats. A fast relief of hyperglycemia and hyperinsulinemia was achieved even before a significant weight loss occurred. Fourteen days after DJB, we characterized the changes in intestinal histochemistry in the bypassed duodenum and shortcut jejunum that was reanastomosed directly to the starting point of the duodenum and compared with the corresponding regions of sham-operated rats. The bypassed duodenum exhibited mucosal atrophy and apoptosis and decreased proliferative renewal. In shortcut jejunum, DJB resulted in 40% significantly enlarged intestinal circumference and increased epithelial proliferation, especially in putative transit-amplifying (TA) cells and the crypt. Because Reg family proteins promote cell growth and survival, we explored their expression in the intestine. With the use of immunohistochemistry, Reg1, -3, and -3β were normally expressed in intestinal mucosa. After DJB, the level of Reg1 protein was reduced, whereas Reg3α and -3β were not changed in bypassed duodenum. Downstream in shortcut jejunum, the levels of Reg1 and -3β were greatly induced and especially concentrated in the putative TA cells. Our results revealed significant changes in the integrity and proliferation of the intestinal mucosa as a consequence of DJB, and in cell- and isoform-specific expression of Reg proteins within the replicating mucosal epithelium, and provide evidence indicating that the activation of Reg proteins may contribute to intestinal compensation against increased load and/or to improving insulin sensitivity.

Reg family proteins; immunohistochemistry; type 2 diabetes; obesity; cell proliferation; cell death; apoptosis

HUMAN AND ROGENT STUDIES HAVE demonstrated that bariatric (metabolic) surgery is a very effective treatment for morbid obesity that causes steady weight loss and ameliorates type 2 diabetes (T2D) by improving insulin secretion and sensitivity (3, 28, 30). The antidiabetic mechanism has been shown to be independent of weight loss at least in part and may include specific changes in the intestines such as increased secretion of gut hormones glucagon-like peptide-1 (GLP-1), gastric inhibitory peptide (GIP), and peptide YY (YY) that boost the β-cell function and/or improve insulin actions (32, 33, 37, 45). More recently, jejunal glucose sensing has been shown to be essential for controlling glucose production via a gut-brain-liver neurocircuit (2). While factors such as incretins and PYY have been implicated in the glycemic control, little is known about other molecular players involved in the entero-islet responses to bariatric operations. Reg family proteins are normally expressed in the pancreas and gastrointestinal tract and have been proposed to promote cell proliferation and/or inhibit apoptosis. Reg1 promoted cell replication in the pancreatic islets (13, 41, 43) and gastric and intestinal mucosa; Reg1 deletion resulted in reduced epithelial repair and regeneration upon gastric and intestinal injuries (6, 9, 31, 34). We reported that forced expression of Reg3α accelerates the proliferation of insulin-producing cells (7). Reg3β played an important role in the development and regeneration of motor neurons (14) and stimulated liver regeneration after partial hepatectomy (1, 10, 35). In the pancreas and our recent report, β-cell-specific overexpression of Reg3β induced proislet gene expression and protected the islet cells (46). Both Reg3α and -3β were also expressed in the gastrointestinal tract, including in humans (5, 22, 42); however, their functions in the regeneration of intestinal epithelium have not been explored.

To investigate intestinal adaptation to bariatric surgery and the involvement of Reg proteins in the antidiabetic mechanism, we have studied Zucker fatty rats, which carry mutant leptin receptors and display hyperphagia, obesity, diabetes, and/or hypertension (19, 24, 39). In previous reports, these rats responded well to various forms of bariatric surgery, e.g., gastric banding decreased food intake and body weight (11); duodenal-jejunal bypass (DJB) improved glucose tolerance (32); and Roux-en-Y gastric bypass decreased food intake and body weight and improved insulin sensitivity and insulin production (24). We have surgically bypassed a segment of the small intestine, 15 cm, including the entire duodenum and proximal jejunum from food contact (but maintained normal flow of bibiopancreatic juice) in the DJB group. As a consequence, the bypassed intestinal segment displayed autolysis and atrophy, whereas the shortcut jejunum exhibited increased proliferation and enlargement in response to increased food flux. In the meantime, Reg protein expression was significantly upregulated in the shortcut segment and was particularly high in the putative transit-amplifying (TA) cells, indicating that these proteins may function as growth factors to mediate intestinal mucosal proliferation after DJB.

MATERIALS AND METHODS

**DJB surgery on Zucker fatty rats.** Male Zucker fatty rats, purchased from Charles River, were individually housed and had free access to food and water unless specified otherwise. All animal-handling procedures were approved by the Research Institute Animal Care Committee of McGill University Health Centre. Male rats of 3.5 mo old

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were randomized into sham or DJB groups by body weight, blood glucose, and serum insulin levels. As shown in Fig. 1, top, for DJB, rats were anesthetized with isoflurane. The abdominal cavity was exposed through a ventral midline incision at 0.75 cm below the starting point of the duodenum (pylori), a suture was tightly tied the duodenum, and then an incision was made immediately upstream. Bowel continuity was interrupted at the level of the distal jejunum (15 or 10 cm from the pylori or ligament of Treitz, respectively). The distal limb was directly connected to the duodenal incision (duodenal-jejunal anastomosis), and the proximal limb carrying the biliopancreatic juices was reconnected downward to the alimentary limb at a distance of the same length of bypassed duodenum and proximal jejunum from the duodenal-jejunal anastomosis. For sham operation, rats underwent a similar surgical procedure; however, all resections were reanastomosed to maintain the physiological flow of food through the bowel as shown in Fig. 1, bottom. Rats were returned to normal feeding 5 days after surgery.

Postoperative observation of blood glucose, insulin, and glucose tolerance. Rats were kept for 2 mo, and body weight, blood glucose, and serum insulin levels were monitored continuously. Eleven days after DJB surgery, an intraperitoneal glucose tolerance test was performed with d-glucose at 1 g/kg body wt after 16 h fasting, and blood glucose level was measured from the tail vein at 0, 15, 30, 60, and 120 min. The difference between glucose tolerance was calculated by area under the curve. To explore the changes in Reg gene expression and islet function, rats were killed 14 and 60 days after the surgery, respectively, and segments of intestine and pancreas were removed for immunohistochemistry. The experiment was repeated three times: n = 3 for 60 days; n = 3 for 14 days; and n = 5 for 14 days (3) or 60 days (2).

**Histology and immunohistochemistry.** Intestinal segments and pancreas were fixed in 4% paraformaldehyde and embedded in paraffin. Pancreatic and intestinal cross sections were cut at 5 μm thickness. The intestinal circumference and wall thickness were measured based on hematoxylin- and eosin-stained histology. To detect apoptotic cells, the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL; Millipore, Billerica, MA) assay was performed. For immunohistochemistry, the primary antibodies against Ki67 and insulin (Millipore), Reg1 (Dr. H. Okamoto) (21), Reg3α and Reg3β (R&D Systems, Minneapolis, MN), or Reg3α (INGAP; Dr. M. Petropavlovskaiia of McGill) (42) were incubated overnight at 4°C, followed by horse peroxidase-conjugated secondary antibodies. The signals were visualized by diaminobenzidine substrate (Vector Labs, Burlingame, CA). To quantify cell proliferation in Fig. 4C, 15 crypts from intestinal sections were randomly selected, and the number of Ki67-positive vs. total cells was counted; the percentage of Ki67-positive cells was calculated and averaged in each rat. For dual-labeled immunofluorescence, pancreatic sections were incubated with anti-glucagon (Santa Cruz Biotechnology, Santa Cruz, CA) and Alexa594-conjugated secondary antibody (Invitrogen). To evaluate the extent of islet fibrosis that occurred in obesity and diabetes, pancreatic sections were incubated with 0.1% Direct Red 80 dissolved in aqueous saturated picric acid (Sigma-Aldrich, St. Louis, MO) for 75 min followed by two washes with acidified water. Collagen was visualized as red filaments under a light microscope (47). To quantify the difference, 10–15 imaging fields (x400) containing islet clusters were randomly selected from each pancreas of three sham- and three DJB-operated rats, and the thickness of collagen fibers and the extent of collagen deposition were scored from 1 to 4 with 1 as the least and 4 the worst fibrosis. The average score from each pancreas/rat was considered and tested by Student’s t-test. The percent area of insulin, glucagon, and fibrosis was quantified using Northern Eclipse software as reported (20, 37).

**RESULTS**

**DJB caused early relief of hyperglycemia and hyperinsulinemia before weight loss.** We studied bariatric intervention against obesity and diabetes in Zucker fatty rats. Starting from the same body weight before surgery, DJB rats show a tendency of less weight gain 7 days after surgery compared with sham-operated rats; the weight difference became significant from 21 days onward and reached 80 g after 40 days (P < 0.05 by t-test; Fig. 2A). The blood glucose level of DJB rats was decreased from 7 days and maintained at 20–40 mg/dl lower than sham control at each time point (P < 0.05 by ANOVA; Fig. 2B). Almost immediately after the surgery, from 3 days onward, serum insulin level at the random fed state was dramatically decreased by 30–50% in DJB rats compared with sham-operated animals (P < 0.05 when the two curves were
proliferation was assessed by immunostaining of nuclear anti-
resulting in a loss of TUNEL-positive cells. The rate of cell
attached from the villi as a result of atrophy or autolysis,
right), approximately one-third of the mucosal layer was de-
epithelial cells in sham-operated rats. In DJB rats (Fig. 4
mucosal villi, representing active and normal turnover of
TUNEL-positive cells were concentrated at the tips of the
proliferation, we performed immunohistochemistry against
a tendency of decreased intestinal circumference (Table 1).

DJB caused mucosal atrophy and autolysis in the bypassed
duodenum. To evaluate morphological change in the small
intestine affected by DJB, we performed histology 14 days
after the surgery. As shown in the cross sections of sham-
operated rats in Fig. 3, top left, the dense and tall mucosal villi
were mainly composed of enterocytes that were evenly stained
by hematoxylin-eosin and include scattered mucus-secreting
goblet cells (better viewed in Fig. 4A, left, scattered dots in
higher magnification). After DJB (Fig. 3, top right), the du-
odenal villi were mostly broken and/or shortened, as measured
by a 31% significant decrease in intestinal wall thickness and a
tendency of decreased intestinal circumference (Table 1).

To further evaluate changes in the rates of cell death and
proliferation, we performed immunohistochemistry against
TUNEL and Ki67, respectively. As shown in Fig. 4A, left,
TUNEL-positive cells were concentrated at the tips of the
mucosal villi, representing active and normal turnover of
epithelial cells in sham-operated rats. In DJB rats (Fig. 4A,
right), approximately one-third of the mucosal layer was de-
tached from the villi as a result of atrophy or autolysis,
resulting in a loss of TUNEL-positive cells. The rate of cell
proliferation was assessed by immunostaining of nuclear anti-
gen Ki67, which is present during all active phases of the cell
cycle and absent in the quiescent state (17). As shown in Fig.
4B, left, continuous columns of enterocytes were labeled by
Ki67, together with the TUNEL staining at the tip of mucosal
villi (Fig. 4A, left), representing normal and active cellular
renewal in sham-operated rats. In bypassed duodenum of DJB
rats (Fig. 4B, right), the columns of Ki67-positive cells
marked by red lines) were shortened significantly, with the
mucosal villi disorganized. The bypassed duodenum exhibited
more quiescent cells (arrow) and interrupted epithelial replica-
tion as a consequence of diminished requirement for digestion
and absorption.

DJB induced adaptive mucosal proliferation and enlarge-
ment of the shortcut jejunum. The general morphological
feature of the distal jejunum was rather similar to that of the
duodenum in sham-operated rats (Fig. 3, left), in the tall and
dense mucosal villi representing healthy enterocytes. In con-
trast to bypassed duodenum, however, there were two obvious
changes in the shortcut jejunum after DJB, i.e., the intestinal
circumference was increased 40%, and the villous folding
became more complex; there were horizontal layers of entero-
cytes (Table 1 and Fig. 3, bottom right). Together they would
significantly increase the absorptive surface to accommodate
the increased digestive load caused by DJB. TUNEL staining
revealed scattered epithelial apoptosis in the mucosa to a
similar extent in both DJB and sham-operated rats (data not
shown). Virtually all mucosal enterocytes were stained posi-
tive for Ki67 in sham-operated rats, again indicating constant
and normal cellular renewal, but the putative stem cell popu-
lation in the crypt was mostly quiescent (arrow in Fig. 4C, left).
After DJB, the Ki67-positive cells were expanded to include
most of the crypt, including the putative TA and stem cells

Fig. 2. DJB caused early relief of hyperglycemia and hyperinsulinemia, followed by weight loss and improved glucose tolerance. A: change in body weight. *P < 0.05 from 21 days vs. sham-operated rats using t-test. B: change in blood glucose level measured in fed status. *P < 0.05 vs. sham-operated rats tested by one-way ANOVA. C: change in serum insulin level measured in fed status. *P < 0.05 vs. sham-operated rats by ANOVA. D: results of ip glucose tolerance test (GTT) 11 days after DJB surgery. **P < 0.01 vs. sham-operated rats by t-test and area under the curve. Data in A-C were based on two experiments using a total of 5 sham-operated and 5 DJB rats; data in D were based on two experiments using a total of 6 sham-operated and 6 DJB rats.
Fig. 3. Histological changes of the small intestines after DJB, 14 days after surgery. Hematoxylin-eosin staining showed autolysis and atrophy of bypassed duodenum (segment A, top right) and intestinal distension of shortcut jejunum (segment B, bottom right) in DJB rats. Each panel was representative of 10 images taken from 3 rats. The experiments were done two times. The result of dimensional quantification was summarized in Table 1. The scale bar was 1 mm.

(Fig. 4C, right), indicating an increased rate of cell replication compared with sham-operated rats. The double arrows point to expanded layers of mucosal (including putative stem) cells that were replicating, averaged at 88 ± 9% of total cells for DJB vs. 43 ± 4% for sham operated (n = 3, P < 0.01, shown in Fig. 4C, bottom). As a result, DJB rats exhibited higher density of the mucosal villi and enlargement of this portion of the intestine (Fig. 4C, right, and Fig. 3, bottom right). The opposite characteristics of bypassed and shortcut intestinal segments in DJB rats, consistent with an early report of human jejunooileal bypass for obesity (8), clearly demonstrated an adaption and compensation to altered food flux caused by the surgery.

Decreased level of Reg1 immunostaining in the bypassed duodenum after DJB. Reg family proteins promote cell proliferation and regeneration in a number of tissues and could be involved in DJB-induced intestinal adaptation (7, 38). We next examined possible changes in Reg1, -3α, -3β, and -3γ expression by immunohistochemistry (Fig. 5). The staining of Reg1, -3α, and -3β was detected as brown pigmentation at various levels in the intestinal mucosa. In the duodenum of sham-operated rats, Reg1 was normally expressed at a high level across the mucosal villi, Reg3α protein was expressed at a low level, and Reg3β expression was very rarely detected in patches of enterocytes (better viewed in higher magnification in Fig. 5D). However, in DJB rats, the level of Reg1 expression was reduced, especially from the layer of putative TA cells (arrow in Fig. 5A). In an attempt to quantify the decrease, Reg1 protein was barely detectable using Western blot and protein isolated from the paraffin blocks using the Qproteome FFPE tissue kit (Qiagen; data not shown) (44). No obvious change in the patterns of either Reg3α or Reg3β staining was observed (Fig. 5, B-D). The reduced Reg1 level correlated with decreased proliferation and increased cell death in the bypassed duodenum (Fig. 4), supporting a role of Reg1 as a growth factor for intestinal epithelial cells. However, Reg3α immunoreactivity was not detected in either the duodenum or jejunum compared with its positive staining in the pancreatic islet α-cells and ductal cells (data not shown) (42). The only other two Reg protein isoforms in rats, Reg3γ and Reg4, were not studied here.

Increased Reg1, Reg3α, and Reg3β levels in the shortcut jejunum after DJB. In contrast to mucosal atrophy of the bypassed duodenum, the shortcut jejunum displayed adaptive proliferation; opposite changes in the levels of Reg1, -3α, and -3β proteins were expected in DJB vs. sham-operated rats. In sham-operated animals, Reg1 was widely expressed across the mucosal layers with a relatively high level in the putative TA cells but only sporadically in the crypt (Fig. 6A, left). In DJB rats, Reg1 staining was greatly enhanced with predominant induction in the putative TA cells (arrow, Fig. 6A, right). Reg3α was expressed in all mucosal cells, and the level appeared much higher than in the proximal duodenum in sham-operated rats (Fig. 6B vs. Fig. 5B, left). In DJB rats, although the overall level of Reg3α expression in the shortcut segment did not seem to elevate from sham-operated rats, it was more concentrated in the putative TA cells (arrow in Fig. 6B). Reg3β was normally dispersed in a few individual cells in the jejenum of sham-operated rats. In DJB rats, it was visibly induced in the putative TA cells (arrow, Fig. 6C, right). All three Reg proteins studied exhibited specific induction in the putative TA cells in the shortcut jejunum after DJB. To quantify the overall increase and/or cell-specific induction, Reg1 and Reg3β proteins were barely detectable using Western blot, whereas the sample variability in total tissue Reg3α level did not support a meaningful conclusion either (data not shown).
Fig. 4. DJB-induced changes in cellular apoptosis and proliferation in the intestinal mucosa. 

A: in bypassed duodenum (segment 1 or A), terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining using diaminobenzidine substrate (brown) was localized at the tip of villi in sham-operated rats but more widely distributed in the broken villi in DJB rats. 

B: changes in the pattern of enterocyte proliferation marked by nuclear staining of Ki67. Decreased mucosal proliferation in bypassed duodenum (segment 1 or A) in DJB rats vs. sham operated are marked by red lines representing the average heights of Ki67-positive columns and especially in putative TA cells marked by arrows.

C: increased mucosal proliferation marked by Ki67 staining especially in the putative transit-amplifying (TA) cells in shortcut jejunum (segment 3 or B), illustrated in ×100 (top) and ×400 (bottom) magnifications. Double arrows highlight expanded layers of Ki67-positive putative TA and stem cells. Based on two experiments using 3 sham- and 3 DJB-operated rats each time; representative images of more than five in each group were illustrated. The scale bar was 250 or 60 µm as marked.
Intestinal stem cells are proposed to reside at the base of the crypts of Lieberkühn; from the bottom to the top, they give rise to the TA cells, which further divide and differentiate into absorptive enterocytes and mucus-secreting goblet and enteroendocrine cells in sham-operated rats (23, 40). Normally, the putative stem cells were not very active in cell cycle progression (largely negative for Ki67 labeling; Fig. 4, C, left, marked by arrow) and did not exhibit Reg protein expression (Figs. 5 and 6, below the layers marked by arrows). In the shortcut jejunum after DJB, these cells exhibited clearly increased proliferation by Ki67 staining and signs of expansion (Fig. 4C, right, marked by arrows). Above the stem cell layer, the putative TA cells exhibited increased expression of Reg1, -3α, and -β genes, shown by the arrows in Fig. 6, right, indicating possible involvement of these proteins in mucosal proliferation.

**Decreased intraislet fibrosis in the pancreas after DJB.** As part of the diabetic mechanism, significant islet hyperplasia and fibrosis have been reported in Zucker fatty rats and other T2D models (4, 27, 47). The antidiabetic gastric bypass and DJB have been proposed to improve pancreatic islet function directly, or as a result of food restriction, weight loss, and/or improvement of insulin sensitivity (26, 29, 36). We thus evaluated pancreatic islet morphology 2 mo after DJB or sham operation (Fig. 7, top). Dual-labeled immunofluorescence of insulin and glucagon confirmed islet hyperplasia; however, there was no significant difference in the islet density and the relative ratio of insulin and glucagon staining in DJB vs. sham-operated animals. Islet fibrosis was evident in both sham-operated and DJB rats (arrows in Fig. 7, middle). Normally collagen was mainly present in the islet capsule as visualized by Sirius Red staining (47). In sham-operated Zucker fatty rats, thick collagen fibers in red were dispersed within the hyperplastic islet clusters, demonstrating intraislet fibrosis (Fig. 7, bottom left, marked by arrows). In DJB rats, the extent and thickness of the intraislet collagen filaments were significantly diminished. In Direct Red 80-stained sections, the area of fibrosis was decreased from 15.3 ± 0.5 to 7.1 ± 0.3% (P < 0.02) and average collagen deposition score from 3.0 ± 0.4 to 2.1 ± 0.2 (P = 0.047; n = 3), confirming improved islet fibrosis (Fig. 7, bottom right), which was consistent with the reported finding in the nonobese diabetic Goto-Kakizaki (GK) rats 1 yr after the surgery (37). Thus, although there was no obvious change in islet cell mass caused by DJB, diminished fibrosis was evident after 2 mo.

**DISCUSSION**

In this study we demonstrated a significant antidiabetic effect of DJB in correcting hyperglycemia and hyperinsulinemia in Zucker fatty rats, which is in agreement with previously reported findings (11, 24, 32). In response to the surgical rerouting, bypassed duodenum exhibited clear mucosal atrophy associated with decreased proliferation and increased cell death. On the contrary, the shortcut jejunum displayed the opposite changes, e.g., enlarged intestinal circumference, increased mucosal proliferation, expanded proliferating zone, and more folded mucosal villi in compensation to expedited food load. To explore putative proliferative roles of the Reg proteins to either intestinal mucosa or pancreatic islets, we detected decreased Reg1 expression in the bypassed duodenal mucosa but significantly increased Reg1, -3α, and -β expression in the shortcut jejunal mucosa, especially in the putative layer of TA cells. The close association of isof orm- and segment-specific changes of Reg protein expression with the intestinal adaptation suggests a role of Reg proteins in this process and/or indirectly in the antidiabetic effects. Our data further indicate that not only Reg1 but also Reg3α and -β may function as growth factors to promote the proliferation of intestinal mucosa.

DJB diverted food flux from the stomach directly to the distal jejunum and caused adaptive changes in the small intestines. The abolished demand in the segment of duodenum and proximal jejunum for digestion and absorption led to increased apoptosis, decreased proliferation, and mucosal atrophy. Morphologically, this segment displayed a thinner intestinal wall and a diminished circumference. In contrast with increased load of less digested food flux, the reanastomosed distal jejunal mucosa increased the ability for digestion and absorption through compensatory hyperplasia in the mucosa. Morphologically, the shortcut jejunum displayed increased villus density, a thicker wall, and circumference enlargement. To our knowledge, these morphological characterizations after DJB, supported by specific markers of cellular proliferation and apoptosis, have not been reported.

Bariatric surgery is a very effective treatment for morbid obesity that causes steady weight loss and ameliorates T2D by improving insulin secretion and sensitivity (3, 28, 30). At least part of the antidiabetic effect has been shown to be independent of weight loss and may have to do with increased secretion of GLP-1 and GIP (32, 33, 37, 45). According to the hindgut hypothesis, the expedited delivery of nutrient chyme to the distal intestines and the earlier presentation of undigested food trigger greater production of GLP-1 and PYY, enhancing physiological signals that improve glucose metabolism (12, 15). Gastric bypass was also found to activate gluconeogenesis in distal small intestine and release increased glucose into the portal vein. A proposed detection system in the portal vein transmits the glucose signal to the brain via the afferent nervous system (40), which boosts satiety and an eff'erent signal to cause decreased glucose production in the liver and improved insulin sensitivity (25, 41). A recent report further established an essential role of jejunal glucose sensing in the control of hepatic glucose production (2). Additional considerations were given to impaired ghrelin secretion, increased serum bile acids level, and alternations in additional gut fac-

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Values are means ± SE; n = 3 rats in each group. DJB, duodenal-jejunal bypass; NS, not significant. P values were derived from unpaired t-tests.
tors, such as the numerous bioactive peptides normally produced in the intestines (16, 18).

The DJB procedure has been indicated to improve glucose homeostasis independent of weight loss and/or food restriction in various animal models. In diabetic Zucker fatty rats, DJB greatly normalized the blood glucose level to the level of the nondiabetic controls (33). Even in nonobese diabetic GK rats, DJB ameliorated hyperglycemia, increased postprandial GLP-1 secretion, and increased the enteroendocrine cells coexpressing GLP-1 and GIP in the distal jejunum shortcut to the stomach.

Fig. 5. Immunohistochemical changes of Reg1, -3α, and -3β in bypassed duodenum 14 days after DJB. A: the level of Reg1 staining was decreased in the bypassed segment, especially in the putative TA cells (arrow) of DJB rats. B: Reg3α staining was weak and not altered in DJB rats. C: Reg3β staining was barely detectable in both DJB and sham-operated rats, magnification ×100. D: Reg3β staining at ×400 magnification showing brown pigmentation in the mucosal cells of both DJB and sham-operated rats. Based on two experiments using 3 sham- and 3 DJB-operated rats each time; representative images of more than five in each group were illustrated. The scale bar was 250 or 60 μm as marked.
In the long term, DJB increased β-cell mass and decreased islet fibrosis in GK rats and contributed to a persistent improvement of glucose homeostasis (32, 37). In our study, DJB-caused improvement in glycemic parameters was consistent with a previous report using a similar model (33). At 60 days after DJB, there was no change in the relative ratio of β- and α-cells in the islets of our rats, similar to the findings of a mouse study (45). Nevertheless, there seemed to be decreased collagen deposition in between the hyperplasic islet clusters that thus relieved intraslet fibrosis in the pancreas of DJB rats (Fig. 7, bottom). It is established that the β-cell area is inversely correlated with the extent of islet fibrosis, as reported in GK rats (37). Similarly in Zucker fatty rats, DJB exerted its beneficial effects on the pancreas by attenuating islet fibrosis, which may aid the preservation of islet mass in the long run beyond 2 mo.

Reg proteins have been proposed as growth factors to promote cell replication and regeneration in various tissues, including pancreas, liver, motor neurons, and gastrointestinal tracts. This study explored whether their expression is affected by the adaptive changes of the small intestines, and the induction of Reg proteins in the shortcut intestines may promote mucosal replication and adaptation. With the use of immunohistochemistry, Reg1, -3α, and -3β were localized to mucosal...
epithelium at different levels, whereas Reg3β was not detected. Reg1 acted as a growth factor to stimulate islet β-cell regeneration through the phosphatidylinositol 3-kinase/ATF-2/cyclin D1 signaling pathway and prevented the development of diabetes after 90% pancreatectomy in rats (13, 38). In the gastrointestinal tract, Reg1 promoted gastric mucosal proliferation and regeneration after injury (9, 31). Furthermore, data from knockout mice suggested that Reg1 is required for normal cell proliferation and intestinal turnover, and the treatment of recombinant Reg1 inhibited indomethacin-induced injury in the small intestine (6, 34). In nongastrointestinal systems, Reg3β was proposed as a growth factor mediating ciliary neurotrophic factor-mediated survival signaling in motor neurons (14); transgenic overexpression in the liver accelerated hepatocyte regeneration after partial hepatectomy and protected drug-induced hepatitis; and Reg3β gene deletion delayed liver regeneration and enhanced liver sensitivity to oxidative stress (1, 10, 35). For another member of the family, Reg3α was reported by us to increase insulinoma cell growth and cellular CDK4/cyclin D1 level (7). In the small intestine, Reg3α and Reg3β were expressed in an age-dependent fashion (5, 22, 42); however, their role in normal intestinal development and the adaptive response to DJB was elusive. Here we showed increased Reg1 and Reg3β expression in the distal jejunum that was positively correlated with increased mucosal proliferation. Although the overall level of Reg3α was not increased, its expression seemed to be more inducible in the replicating and putative TA cells after DJB, supporting a functional involvement. The mucosal stem cells are normally located at the crypt base and actively divide to maintain rapid turnover of the epithelial enterocytes. In the shortcut intestine, we provided evidence consistent with increased stem cell replication and differentiation into the epithelium in adaption to increased food load after DJB. The parallel upregulation of Reg1, -3β, and -3α genes in the putative TA cells suggests that these proteins may play a common role of promoting intestinal mucosal replication and adaptation to DJB-induced changes.

In summary, we demonstrate that antidiabetic DJB caused distinct changes in the bypassed and reanastomosed intestinal segments of Zucker fatty rats. The duodenum and proximal jejunum being bypassed underwent autolysis and atrophy, whereas the shortcut distal jejunum exhibited greatly increased...
mucosal cell proliferation. Several isoforms of Reg proteins were normally expressed by the mucosal cells in both segments but were differentially affected by the operation. In the bypassed segment, Reg1 expression was decreased with reduced mucosal replication. In the reanastomosed intestinal segment, Reg gene expression was markedly enhanced, especially in the putative TA cells with concurrent increase in the rate of epithelial proliferation. Reg1 has been proposed to be a growth factor for intestinal cells; based on our observation, Reg3a and -3b showed similar changes consistent for them to be involved in the generation and/or maintenance of intestinal mucosa, too. However, whether the induced Reg proteins in the intestinal cells affected islet function as endocrine factors, or intestinal adaptation only, remained to be determined.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

REFERENCES


