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Gastric Bypass Surgery Induces Changes in Gut Hormone-Producing Cell Populations in a Porcine Model

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Abstract

Background: In most patients gastric bypass (GBP) causes remission of type 2 diabetes. It is established that plasma levels of gut hormones are affected by GBP, but it is not well understood how the enteroendocrine cells producing these gut hormones are affected by GBP.

Objectives: We set out to investigate the effect of GBP on enteroendocrine cells in the stomach and intestine of pigs.

Methods: Lean non-diabetic pigs were subjected to either GBP or sham-surgery and immunocytochemistry and morphometry for all major gut hormones in all parts of the GI-tract was performed. Sham-operated, pair-fed pigs were used as controls.

Results: Postoperatively in the antrum, the density of gastrin-cells was lower (GBP 12.8±2.1 cells/μm^2 versus sham 21.3±2.6 cells/μm^2) while density of serotonin-cells was higher in GBP-pigs (GBP 21.6±2.3 cells/μm^2 versus sham 10.6±0.7 cells/μm^2). In the fundus, no effect of GBP was observed on any cell population. In the duodenum, densities of CCK- (GBP 5.1±1.0 cells/μm^2 versus sham 2.6±0.4 cells/μm^2), ghrelin- (GBP 3.4±0.5 cells/μm^2 versus sham 1.4±0.2 cells/μm^2), GIP- (GBP 5.5±0.3 cells/μm^2 versus sham 2.3±0.3 cells/μm^2) and neurotensin-cells (GBP 3.5±0.7 cells/μm^2 versus sham 0.5±0.2 cells/μm^2) were higher in the GBP-pigs. In the distal jejunum, density of ghrelin-cells was lower (GBP 0.7±0.2 cells/μm^2 versus sham 2.3±0.4 cells/μm^2) while densities of GIP- (GBP 3.5±0.3 cells/μm^2 versus sham 2.4±0.2 cells/μm^2) and secretin-cells (GBP 3.4±0.7 cells/μm^2 versus sham 1.6±0.3 cells/μm^2) were higher in GBP-pigs compared to sham-pigs. In the ileum, densities of GIP-cells (GBP 5.4±0.4 cells/μm^2 versus sham 3.9±0.4 cells/μm^2) and somatostatin-cells (GBP 3.3±0.4 cells/μm^2 versus sham 2.1±0.3 cells/μm^2) were higher, while densities of GLP-1-cells (GBP 5.0±0.5 cells/μm^2 versus sham 8.8±1.4 cells/μm^2) and PYY-immunoreactive cells (GBP 3.8±0.1 cells/μm^2 versus sham 5.9±0.8 cells/μm^2) were lower in the GBP-pigs. In the colon, densities of GIP- (GBP 2.8±0.3 cells/μm^2 versus sham 1.4±0.2 cells/μm^2), serotonin- (GBP 6.7±0.3 cells/μm^2 versus sham 4.8±0.5 cells/μm^2) and somatostatin-cells (GBP 1.9±0.2 cells/μm^2 versus sham 1.3±0.1 cells/μm^2) were higher in the GBP-pigs. GBP had no effect on villi length or total mucosa height in any of the intestinal segments analyzed, whereas duodenum (GBP 37.6±3.4 cells/μm^2 versus sham 26.5±2.8 cells/μm^2) and ileum (GBP 127±1.2 cells/μm^2 versus sham 84.7±9.9 cells/μm^2) of the GBP-pigs displayed higher proliferation, as assessed by Ki67 immunoreactivity.

Conclusions: We conclude that GBP induces rapid and profound changes in the densities of gut hormone-producing cells throughout the GI-tract in pigs. These changes seem to be the result of GBP *per se* and not a result of body weight or food intake. Also, GIP was increased in the GBP-pigs in all the intestinal segments analyzed.

Keywords: Gastric bypass surgery; Pig; Porcine; Enteroendocrine cells; Gut hormones; Body weight; Glucose homeostasis

Introduction

Currently, gastric bypass surgery (GBP) is the most commonly used weight-loss treatment for the morbidly obese. In most cases, GBP leads to rapid remission of type 2 diabetes (T2D) [1-3]. The degree of remission is dependent on disease duration [4], and although some patients relapse, 60% of the patients remain in T2D remission after 5 years [5]. Furthermore, factors such as family history of T2D and prediabetes [6,7] may also affect the remission of T2D. The effect of surgery is also very strong in reducing the incidence of new T2D cases in humans [2]. The underlying mechanisms for the remission of T2D remain to be elucidated. If these were to be resolved new treatment regimens for T2D could be developed.

A number of factors have been proposed to contribute to the T2D remission. These include improved insulin sensitivity, presumably in the liver [8], altered bile acid composition [9,10], altered gut nutrient sensing [11], altered gut microbiota [12-14], altered secretory pattern of gut hormones in response to a meal [15,16] and the possible removal of anti-incretins in the upper intestine [17]. Another possible contributing factor to remission of T2D is increased β-cell mass and improved β-cell function, as we recently reported in a porcine model of GBP [18]. Speck

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et al. reported similar findings in a rat model of duodeno-jejunal bypass (DJB) [19]. Furthermore, our data show that the insulinotropic and glucose-lowering effect of GBP cannot entirely be explained by weight loss or reduced food intake, since GBP-pigs had similar body weight as the control pigs and since both groups consumed an equal amount of food [18]. Also, subjecting GBP-patients to a mixed-meal challenge orally yields a more robust hormone response than does a mixed-meal administered via a gastrostomy catheter [20-22].

The gastrointestinal (GI)-tract harbors eleven distinct endocrine cell types [23-30], many of which produce and secrete hormones involved in the regulation of appetite, body weight, glucose homeostasis, and insulin secretion [31]. Thus in the stomach gastrin-producing G-cells are present in the antrum and ghrelin-producing P/D1-cells and histamine-producing ECL-cells are present in the fundus. CCK-producing I-cells, secretin-producing S-cells, ghrelin-producing P/D1-cells, neurotensin-producing NT-cells, GIP-producing K-cells mainly reside in the proximal part of the intestine (duodenum and jejunum) whereas GLP-1-producing L-cells, which also contain PYY, are enriched in distal parts of the intestine. Serotonin-producing EC-cells and somatostatin-producing D-cells are present in all segments of the GI-tract. The secretion and expression of enteroendocrine hormones is largely influenced by luminal stimuli, e.g. food/macronutrients entering the intestine and eliciting a hormonal response. The influence of luminal stimuli is evident e.g. in patients and animals receiving total parenteral nutrition, which causes gut atrophy and lower gut hormone secretory response [24,32,33].

Several reports have shown effects of various bariatric surgery techniques in rodents on glucose homeostasis and gut-hormone secretion (e.g. [11,34-36]). Much attention has been given to how GBP affects plasma levels of gut hormones [15]. However, little attention has been given to the effects of GBP on the distribution of gut hormone-producing cells in the GI-tract. Speck *et al.* reported trends towards increased K- and L-cell densities and increased number of GIP and GLP-1 co-expressing cells after DJB in rats, and Mumphrey et al. reported increased number of L-cells in a rat model of GBP. However, it is hard to extrapolate these data to humans due to considerable differences in GI-tract anatomy between humans and rodents. The pig, however, serves as an appropriate animal model for studying GBP. The pig shares a number of similarities with humans; both species are omnivorous and have similar GI-tract and pancreas physiology and anatomy.

Here, we studied the effects of GBP on all enteroendocrine cell populations in segments representing the entire GI-tract in a porcine model.

Materials and Methods

Animals and surgery

Castrated male pigs (Swedish Landrace X Yorkshire X Hampshire) weighing approximately 25 kg at the start of the study, were randomly selected from the University herd at Odarslöv research farm (Swedish Agricultural University) for the study and transported to the animal facilities at the Department of Cell and Organism Biology (Lund University, Lund, Sweden) where they were kept in individual pen with wood chips as bedding material. All pens were equipped with a dry feeding trough, a drinking nipple and a constant heating lamp (150 W). All surgery was performed under aseptic conditions. For the gastric bypass, seven pigs were operated through an upper midline incision under sterile conditions and general halothane anesthesia. Standard instruments for porcine surgery were used. All anastomoses were performed using commercially available devices intended for human use. The gastric pouch (12-15 ml volume) was constructed using linear staplers (Covidien, Mansfield, MA) using 3-4 cm of the upper stomach [18]. The pouch was carefully and completely separated from the remaining main stomach, with great care taken not to interfere with the vagus trunks. The jejunum was divided 60 cm from the duodeno-jejunal transition and a jejuno-jejunostomy was created 150 cm more distally. The jejunal end of the Roux limb (alimentary limb, 150 cm) was brought up and anastomosed to the lowest part of the gastric pouch. For the sham-operation, eight pigs were operated through an upper midline incision under sterile conditions and general halothane anesthesia. The bowel was gently manipulated but not transected. The pigs were kept under anesthesia for the same length of time as the average GBP-operation lasted (approximately 70 minutes). The pigs were closely monitored and treated with ampicillin (Doktacillin, 15 $mg*kg^{-1}$) and Temgesic (Buprenorphine) for three days after surgery. After surgery, all pigs were given three meals per day (at 0800, 1300, and 1800) of low-calorie diet (250 ml, Modifast, Stocksund, Sweden; 220 kcal, 25E% protein, 52E% carbohydrates, and 21E% fat [18].

Tissue collection

Overnight-fasted pigs were euthanized three weeks after surgery using an overdose of halothane. Tissues were collected and kept in 4% paraformaldehyde until analysis. It should be noted that distal jejunum biopsies were obtained 130 cm distal to the ligament of Treitz in the sham-operated pigs and in the corresponding area in the GBP-pigs segment, i.e. 10 cm distal to the jejunojejunostomy.

Measurements of villi length and total mucosa height

Villi length was measured at 10X magnification using the NIS-Elements AR-software (Nikon, Tokyo, Japan). Approximately 50 villi were measured from each animal. Villi were measured in duodenum, distal jejunum and ileum. Total mucosa height was defined as the height from the tip of approximately 50 villi to the *muscularis mucosae* in the duodenum, jejunum and ileum, and at approximately 50 sites in the stomach and colon, and measured using NIS-Elements AR-software.

Immunohistochemistry

Antibodies were diluted in PBS (pH 7.2) containing 0.25% BSA and 0.25% Triton X-100. Sections (6 µm) were incubated with primary antibodies (Table 1) overnight at 4°C, followed by rinsing in PBS with Triton X-100 for 2 × 10 min. Thereafter, secondary antibodies with specificity for rabbit coupled to Cy2 (Jackson, West Grove, PA) were applied on the sections. Incubation period was for 1 h at room temperature. Sections were again rinsed and then mounted in PBS-glycerol (1:1).

Cell density quantification

The density of immunoreactive (IR) cells was quantified in an epifluorescense microscope (Olympus BX60) with a filter for Cy2 (492 nm) (visual field=0.63 mm^2) as described previously [37]. Briefly, the number of IR cells was counted in transversely sectioned mucosa, with the entire depth of the mucosa visible in three separate sections from each tissue specimen. Images were taken with a digital camera (Nikon DS-2Mv).

Statistics

All data are presented as mean±SEM. Statistical significance was assessed by the unpaired Student's t-test. A $p<0.05$ was considered statistically significant.

Antigen	Code	Dilution	Source
CCK	1564/12	1: 1280	Gift from Prof J. Rehfeld (Copenhagen, Denmark)
Gastrin	4562	1: 1200	Gift from Prof J. Rehfeld (Copenhagen, Denmark)
Ghrelin	R726-2	1: 2560	Phoenix (Belmont, CA)
GIP	RD11/19/77	1: 640	Gift from Prof. TO'dorisio (Columbus, OH)
GLP-1	7811	1: 10000	Euro Diagnostica, Malmo, Sweden
Ki67	MAB1445	1: 300	CloneTec, Shiga, Japan
PYY	8415/2	1: 1: 640	Euro Diagnistica
Secretin	7875	1: 640	Euro Diagnistica
Serotonin	N-Ser	1: 3200	Immunonuclear, Stillwater, MN, USA

Table 1: Details of the different antisera used.

Results

Body weight, glucose and insulin

Body weight development and glucose and insulin response to an intravenous glucose tolerance test in the two groups of pigs have been reported elsewhere [18]. In brief, both GBP-pigs and sham-pigs had similar body weight development throughout the study period, but GBP-pigs displayed lower plasma glucose, improved β-cell function and β-cell mass.

Villi height and total mucosa height

First we analyzed whether GBP affects gross morphology of the GI-tract. The effect of GBP on villi height in duodenum (Figure 1A), distal jejunum (Figure 1B) and ileum (Figure 1C) was analyzed in GBP-pigs and sham-pigs. GBP did not affect villi length in any of the intestinal segments. Furthermore, total mucosa height was unaffected by GBP in antrum (Figure 1D), fundus (Figure 1E), duodenum (Figure 1F), distal jejunum (Figure 1G), distal jejunum (Figure 1H) and colon (Figure 1I).

The primary goal of the study was to analyze all major enteroendocrine cell populations known to reside in the stomach (antrum and fundus), small intestine (bypassed duodenum, non-bypassed jejunum and ileum) and large intestine (non-bypassed colon).

Stomach

In the antrum, the density of gastrin-cells was 40% lower (Figure 2A), but the density of serotonin-cells was 2-fold higher in the GBP-pigs (Figure 2B). No difference was observed for antral somatostatin-cells (Figure 2C). In the fundus, the density of ghrelin-cells trended towards an increase in the GBP-pigs (Figure 2D; p=0.061), whereas leptin-IR cells and somatostatin-cells were unaffected by the GBP-procedure (Figure 2E and F, respectively).

Duodenum

Higher densities of CCK-, ghrelin-, GIP- and neurotensin-cells (Figure 3A, B, C and E, respectively) were observed in the GBP-pigs. Densities of GLP-1-, secretin-, serotonin- and somatostatin-cells were unaffected by GBP (Figure 3D, F, G and H).

Distal jejunum

Densities of GIP- and secretin-cells were higher in the GBP-pigs, (Figure 4C and F, respectively). On the other hand, GBP-pigs displayed lower density of ghrelin cells (Figure 4B). Densities of CCK-, GLP-1-, neurotensin-, serotonin- and somatostatin-cells were unaffected by GBP (Figure 4A, D, E, G and H, respectively)

Ileum

Higher densities of GIP-cells (Figure 5A) and somatostatin-cells (Figure 5E), but lower densities of GLP-1-cells and PYY-IR cells was evident in the GBP-pigs (Figure 5B and C, respectively). Cells producing serotonin were unaffected by GBP (Figure 5D).

Colon

Densities of GIP-, serotonin- and somatostastin-cells were higher in GBP-pigs compared to sham-pigs (Figure 6A, D and E, respectively), whereas densities of GLP-1-cells and PYY-IR cells were unaffected by GBP (Figure 6B and C, respectively).

Proliferation

Since we found GBP to impact on cell density we next studied whether this could be explained by changed proliferative activity. To this end we assessed densities of cells with Ki67-positive nuclei as a measure of general tissue proliferation. This revealed that distal jejunum (Figure 7B) and colon (Figure 7D) had similar densities of Ki67-postive cells in both groups, but GBP-pigs displayed higher densities of Ki67-postive cells in the duodenum (Figure 7A) and ileum (Figure 7C) compared to sham-pigs.

Discussion

It is well established that GBP results in improved glycaemia and remission of T2D in most cases. To provide mechanistic explanation for this effect, a major focus has been on how GBP affects plasma levels of gut hormones [15]. However, the impact of GBP on the enteroendocrine cell populations producing these hormones has so far only been studied in rodents [38, 39]. Rodents and humans differ considerably in GI-tract physiology and anatomy, as well as in dietary composition. The human and porcine GI-tract is similar and both species are omnivorous. Thus, pigs are suitable model animals for studies of mechanistic events, e.g. possible alterations in enteroendocrine cell populations, potentially underlying effects of GBP on glucose homeostasis.

Here we studied the effect of GBP on all major enteroendocrine cell populations in segments representing the entire GI-tract. Our data show that GBP has select effects on subpopulations of enteroendocrine cells along the length of the GI-tract.

A key finding was that GBP-pigs had higher density of GIP-cells in all intestinal segments studied. Our data are in line with Speck *et al.*, who reported a trend towards an increase in K-cells per villus [19] in DJB-rats. On the other hand, the effect of GBP on circulating GIP levels is not so clear-cut [40]. This may be due to differences in study design and study subjects, e.g. prandial state, obese or lean and T2D or not. Although Rubino and coworkers have shown reduced fasting levels of GIP after GBP in T2D patients [41], our data (submitted manuscript) and those of others [42] indicate that postprandial GIP levels are increased after GBP.

Despite a body of evidence showing increased plasma levels of GLP-1 in response to GBP (e.g. [15,16,43,44]), our present data show GLP-1-cell density in the ileum of the GBP-pigs to be slightly lower than in sham-pigs. Our data are in contrast with Hansen et al. [45] who were unable to find any effect of GBP on L-cell density in a rat model of

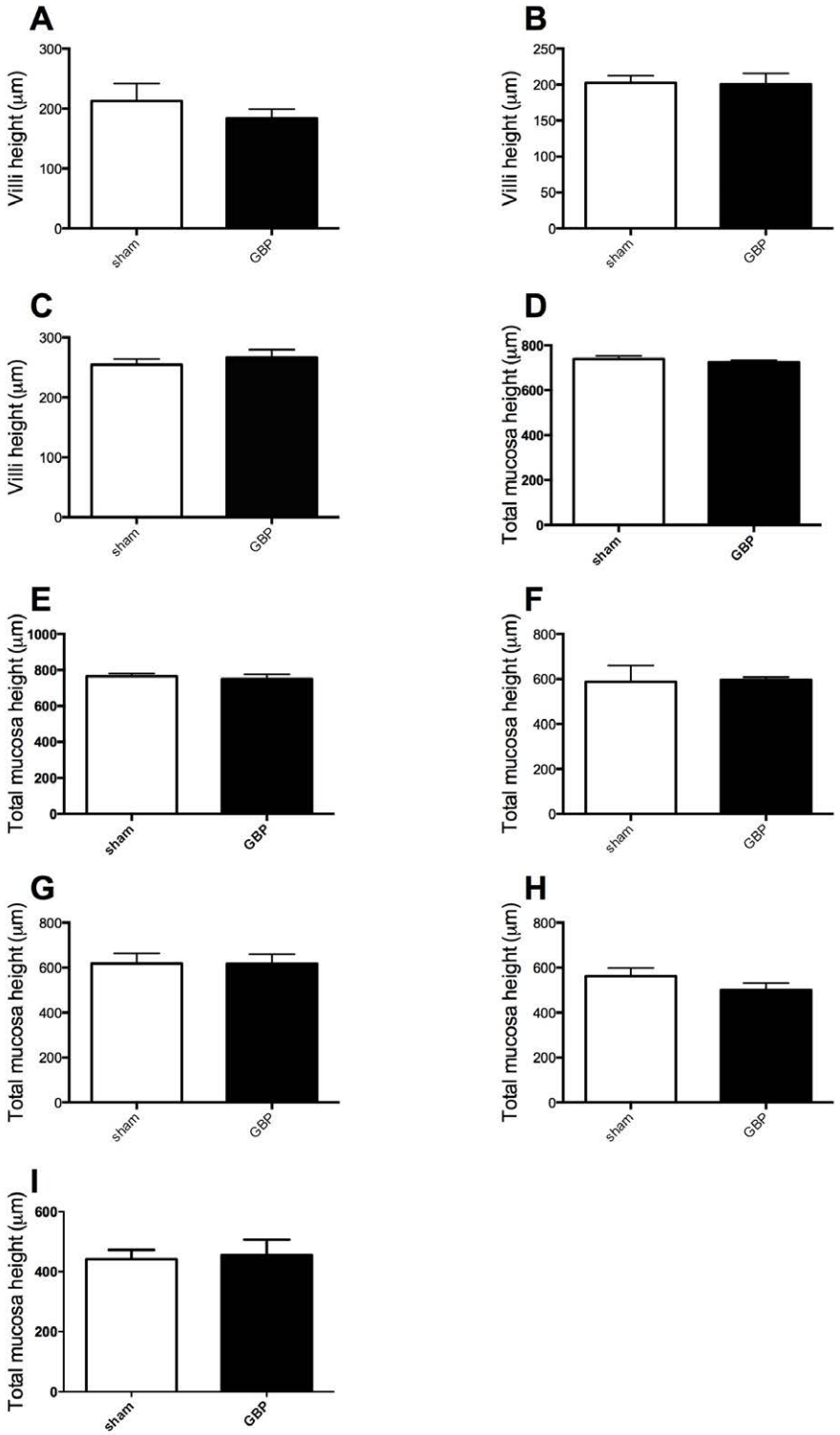

Figure 1: Villi length was analyzed in duodenum (A), distal jejunum (B) and ileum (C) of the pigs. GBP did not affect villi height compared to sham-operation. GBP-pigs and sham-pigs had similar mucosa height in antrum (D), fundus (E), duodenum (F), distal jejunum (G), ileum (H) and colon (I). Data is presented for n=7 for GBP-pigs and n=8 for sham-pigs.

GBP. The present finding of reduced GLP-1-cell density may provide explanation for our recent data showing that GBP in pigs does not provoke elevated plasma levels of active GLP-1 (submitted manuscript) as is evident in humans [15].

PYY is known to be produced by both L-cells and K-cells [46]. This could be a possible explanation as to why our data show lower density of PYY-IR cells in the ileum (in which L-cells were fewer in the GBP pigs), but not in the colon (where L-cells were unchanged in the

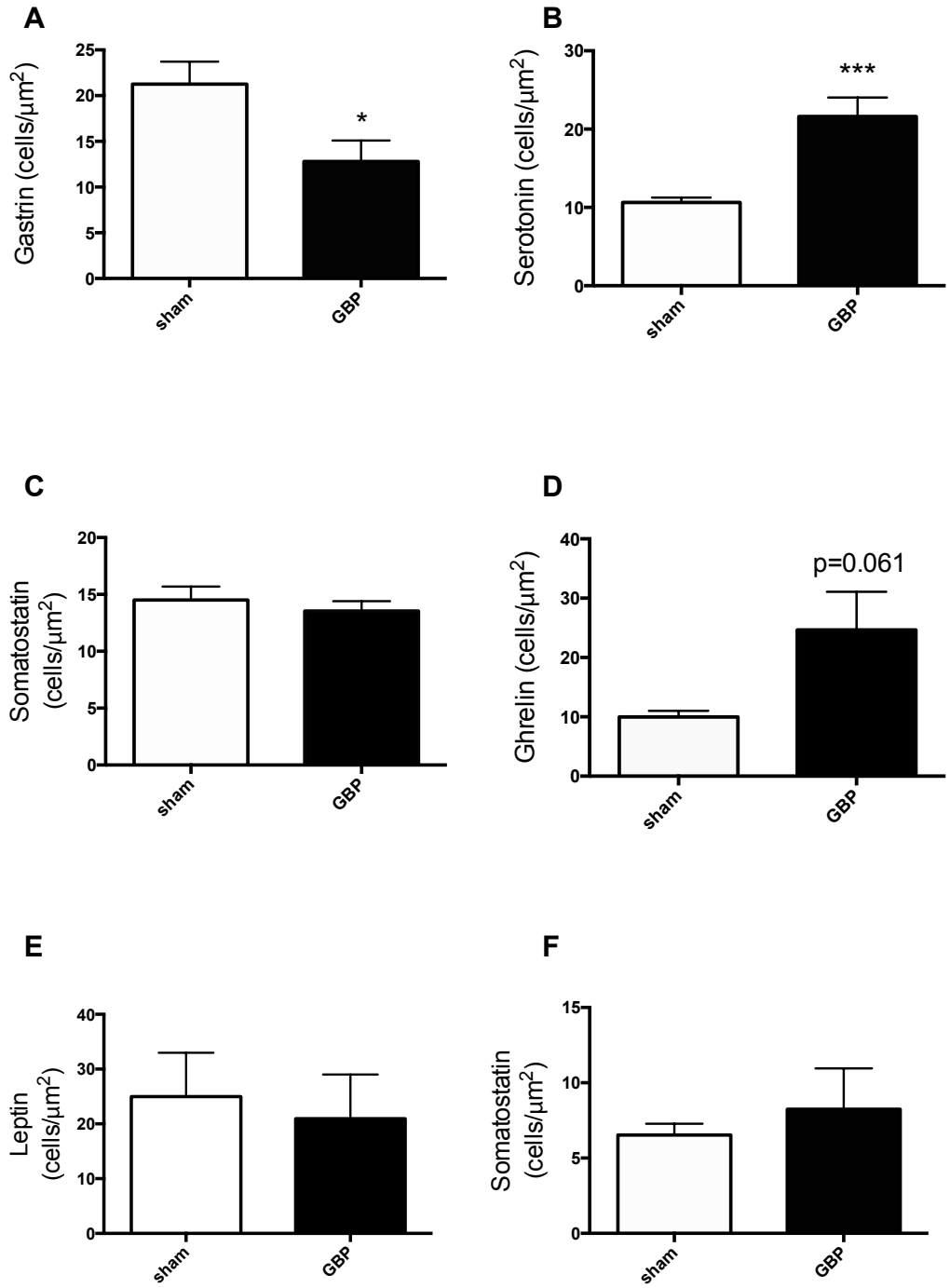

Figure 2: In the antrum density of gastrin-cells was decreased after GBP (A), while density of serotonin-cells was increased (B) and density of somatostatin-cells was unaffected (C). In the fundus, density of ghrelin- cells trended towards an increase after GBP (D) whereas densities of leptin-cells and somatostatin-cells were unchanged (E and F, respectively). Data is presented for n=7 for GBP-pigs and n=8 for sham-pigs. *, p<0.05; ***, p<0.001.

GBP-pigs compared to sham-pigs). In agreement with most studies in humans [47] elevated plasma levels of PYY have been reported after GBP in pigs [48] and in rodents [49].

Our data showing lower density of gastrin-cells in the antrum contrasts a previous study reporting unchanged levels of gastrin cell density six months after GBP in pigs [50]. Nevertheless, our observation fits well with the fact that the antrum is bypassed after surgery and the stimuli for gastrin release may be lowered, as the ingested food is not introduced into this part of the stomach. A positive correlation between gastrin serum levels and gastrin cell density has been shown in short-term in the rat [51]. Whether changes in cell density are translated into differences in circulating levels in other species and over long-term remains to be investigated. However, Jacobsen et al. report that within two weeks after GBP gastrin levels are markedly reduced and less responsive to a mixed-meal test in non-diabetic subjects [52].

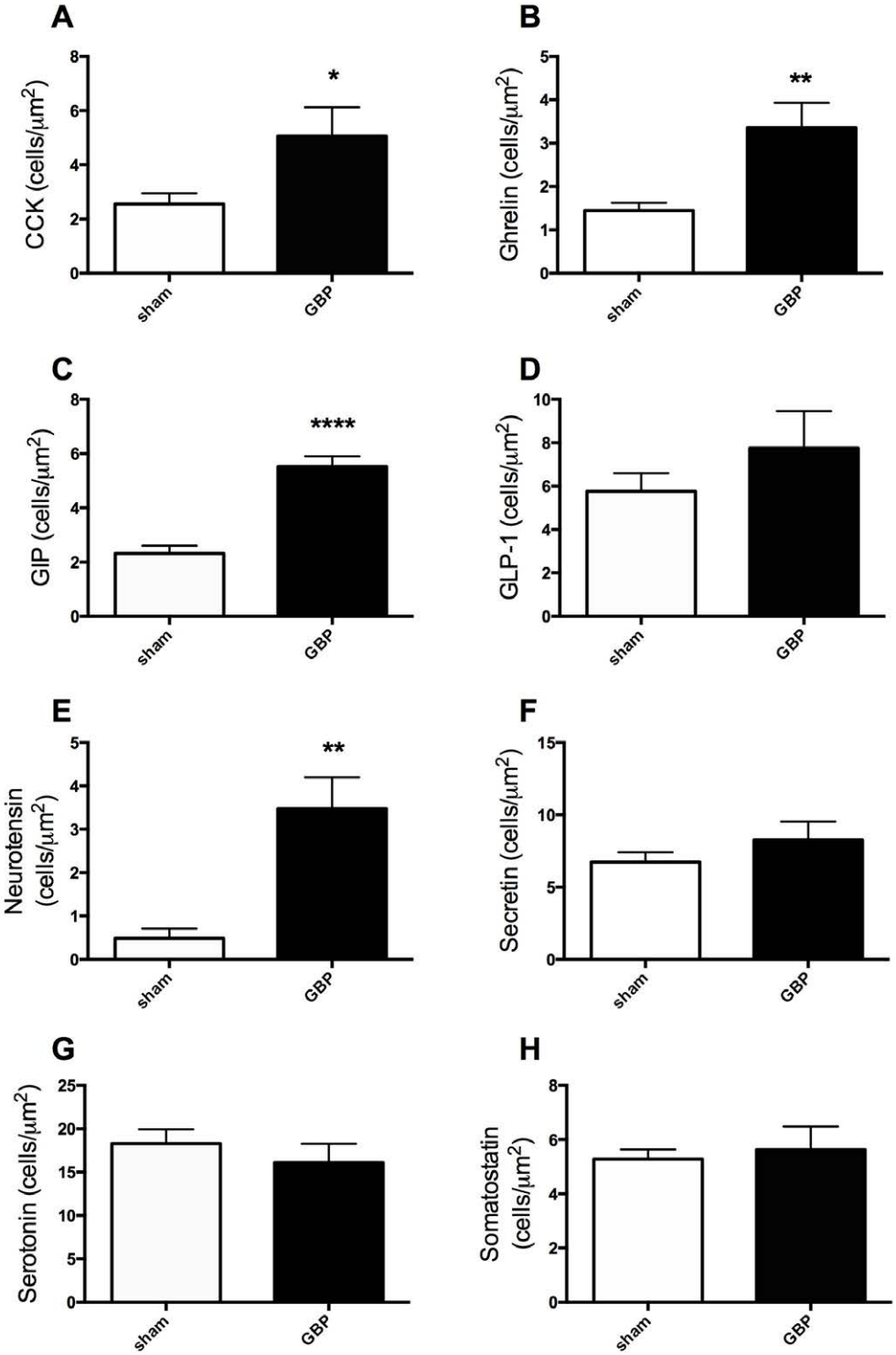

Figure 3: In the duodenum, the densities of CCK- (A) ghrelin- (B), GIP- (C) and neurotensin-cells (E) were increased in GBP-pigs compared to sham pigs. Densities of GLP-1-, secretin-, serotonin- and somatostatin-cells were unaffected by GBP (D, F, G and H, respectively). Data is presented for n=7 for GBP-pigs and n=8 for sham-pigs. *, p<0.05; **, p<0.01; ****, p<0.0001.

In the duodenum ghrelin-cells were observed more frequently in the GBP-pigs whereas ghrelin cell density was lower in distal jejunum. Whether this difference in ghrelin cell density between bypassed (duodenum) and non-bypassed segments (distal jejunum) is a consequence of compensatory mechanisms or lack of luminal stimuli in the bypassed segments remains to be investigated. Cummings et al. showed that the 24-h ghrelin profile for GBP-patients was markedly lower compared to normal-weight and matched obese

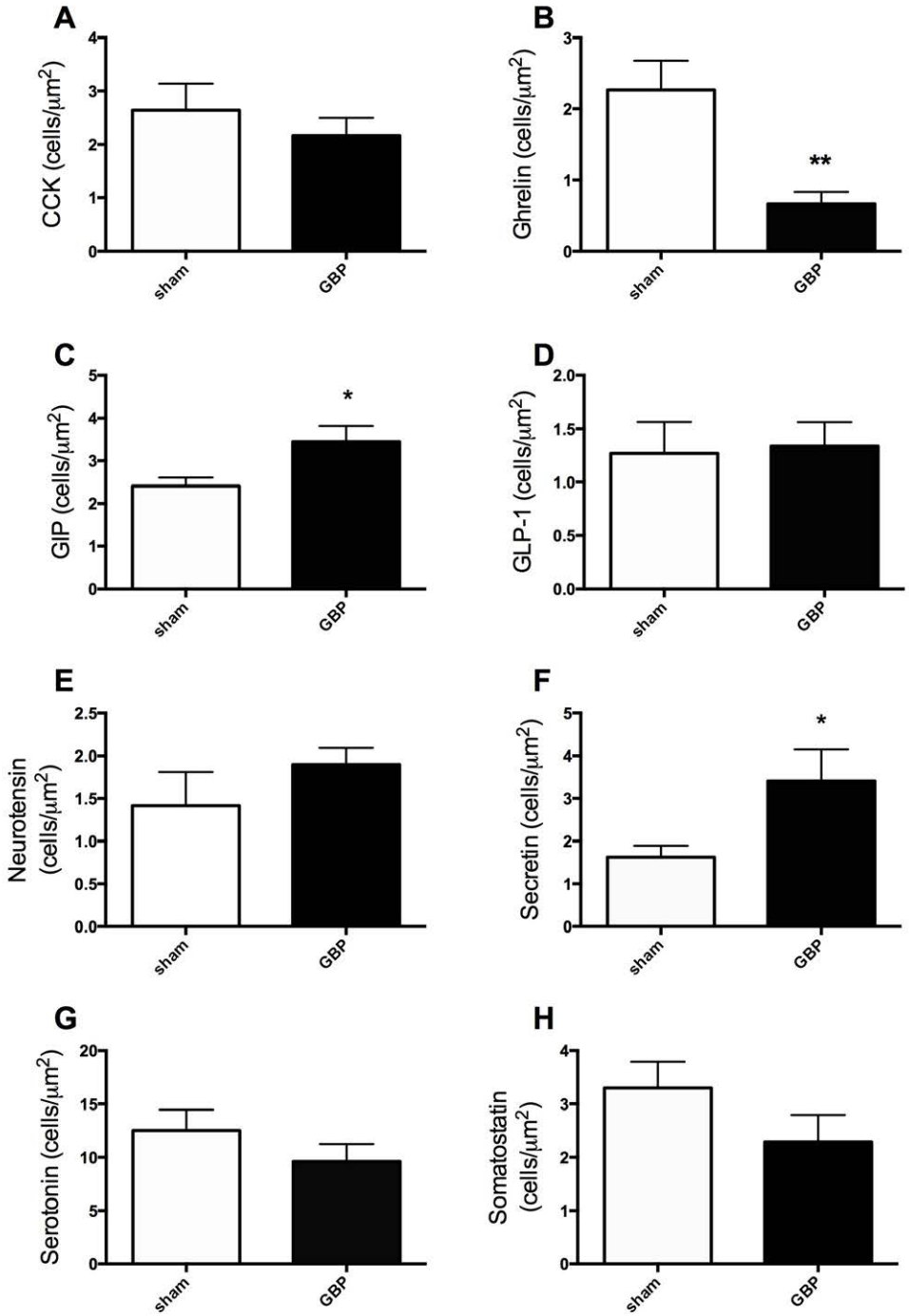

Figure 4: In the distal jejunum, density of ghrelin-cells (B) was decreased while densities of GIP- (C) and secretin-cells (F) were increased. Densities of CCK- (A), GLP-1- (D), neurotensin- (E), serotonin- (G) and somatostatin-cells (H) did not differ between the two surgical groups. Data is presented for n=7 for GBP-pigs and n=8 for sham-pigs. *, p<0.05; **, p<0.01.

controls, suggesting absence of meal-related fluctuations and diurnal rhythm of ghrelin after GBP [53]. However, other studies show that the effect of GBP on ghrelin plasma levels is not so clear-cut. Thus, early after GBP, ghrelin levels seem to be unaltered [16]; one year after GBP, normoglycemic superobese patients have increased levels [54], while T2D-patients have decreased levels [16]. Whether the reported differences in ghrelin plasma levels after GBP are related to ghrelin cells reacting differently in different parts of the GI-tract or other circulating factors or other cell populations throughout the GI-tract contribute remains to be elucidated.

Our data on markedly higher density of duodenal neurotensin-cells in GBP-pigs is in line with histological observations made in rats [39]. Recently, elevated fasting levels of proneurotensin were associated with the development of diabetes in humans [55] and neurotensin was shown to stimulate insulin secretion from isolated rat islets [56]. It

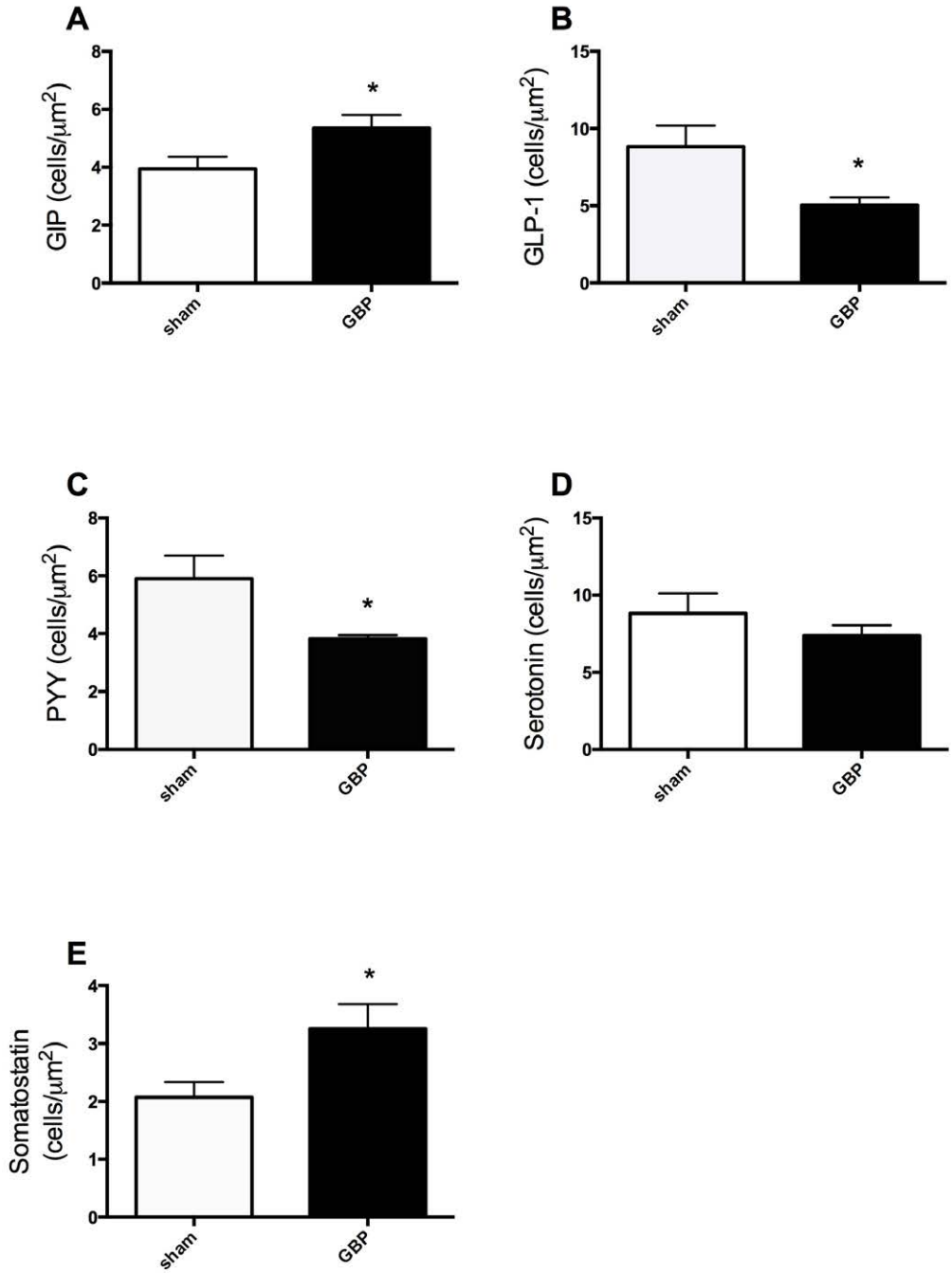

Figure 5: In the ileum, densities of GIP-cells (A) and somatostatin-cells (E) were increased while densities of GLP-1- (B) and PYY-immunoreactive cells (C) were decreased in the GBP-pigs. Density of serotonin- (D) was unaffected by GBP. Data is presented for n=7 for GBP-pigs and n=8 for sham-pigs. *, p<0.05.

remains to be established whether our present morphological findings translate into circulating neurotensin levels. Nevertheless, increased insulinotropic action of neurotensin may be a potential mechanism contributing to the remission of T2D induced by GBP.

CCK plasma levels have been shown to increase in response to a mixed-meal test within two weeks after GBP-surgery in non-diabetic subjects [52]. This fits with our observation made in the present study that duodenal CCK-cell density was higher in GBP-pigs.

Mumphrey *et al.* reported increased amount of serotonin-cells in the Roux- and the common limb in GBP-rats compared to obese sham-operated rats [39]. This is in contrast to our present data on increased density of antral and colonic serotonin-cells as a response to GBP. Whether adiposity or species differences are responsible for the discrepancy in intestinal segments exhibiting GBP-induced increase in serotonin-cells remains to be elucidated.

Somatostatin-cell densities were higher in the ileum and the colon of GBP-pigs. In GBP patients circulating levels of somatostatin were unaffected by a mixed-meal two weeks after surgery [52]. Furthermore, jejuno-ileal bypass in rats has been shown to have little effect on the

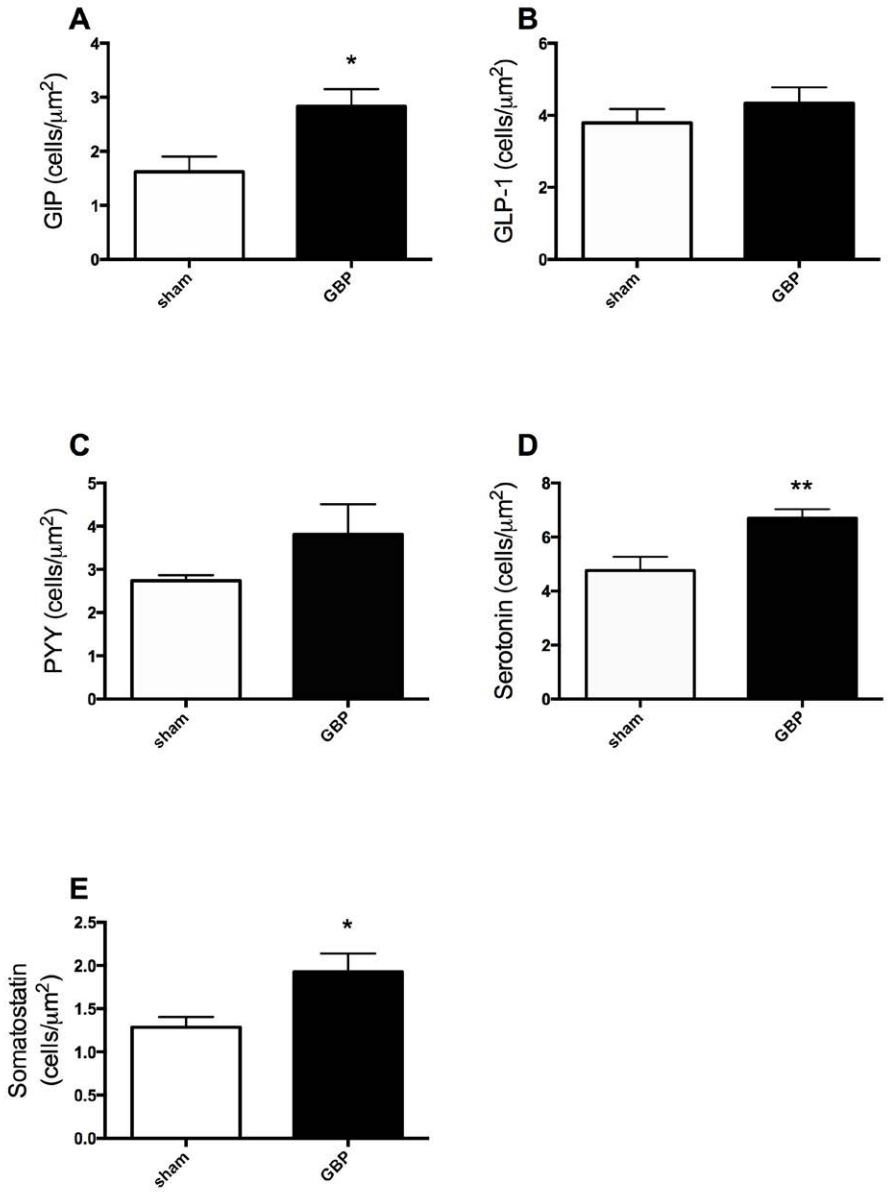

Figure 6: In the colon, densities of GIP- (A), serotonin- (D) and somatostatin- cells (E) were increased in the GBP-pigs compared to the sham-pigs. Densities of GLP-1- (B) and PYY-immunoreactive cells (C) were unaffected by GBP. Data is presented for n=7 for GBP-pigs and n=8 for sham-pigs. *, p<0.05; **, p<0.01.

density of somatostatin-cells in the functional and bypassed segments of the bowel [57]. However, circulating somatostatin levels may not reflect GI-tract expression since somatostatin may emanate from other sites as well. Of note is that densities of somatostatin-cells were unaffected in the bypassed parts of the GI-tract (fundus and duodenum) in the GBP-pigs.

No effect of GBP was observed on villi length or total mucosa height in the present study. This is consistent with previous findings by Speck *et al.* [19]. Thus it is unlikely that the observed differences in cell densities are explained by gross morphological changes. Instead specific changes in each cell population seem to occur. Nevertheless, we found increased density of Ki67-postive cells (in the duodenum and the ileum) suggesting increased proliferation. The fact that this did not translate into differences in villi or total mucosa lengths may be related to the relatively short study period (3 weeks) compared to previous studies [39, 45, 50, 58]. In another study we have evidence for increased cell density of jejunal L- and K-cells in humans 12 months after GBP [59]. Similar observations were recently reported also by Rhee *et al.* [60]. Together with the present data this suggest that GBP provokes both rapid and sustained changes in enteroendocrine cell populations. Importantly, GBP-pigs were compared with pair-fed, sham-operated pigs. Furthermore pigs had similar body weight development in both groups. Hence, differences in food intake and weight loss could be ruled out as confounding factors for the observed changes in enteroendocrine cell populations.

In the present study we have used the classic one cell-one hormone nomenclature, however recent evidence [61-63] suggest that this may be a simplified view. Rather a large degree of coexpression of several

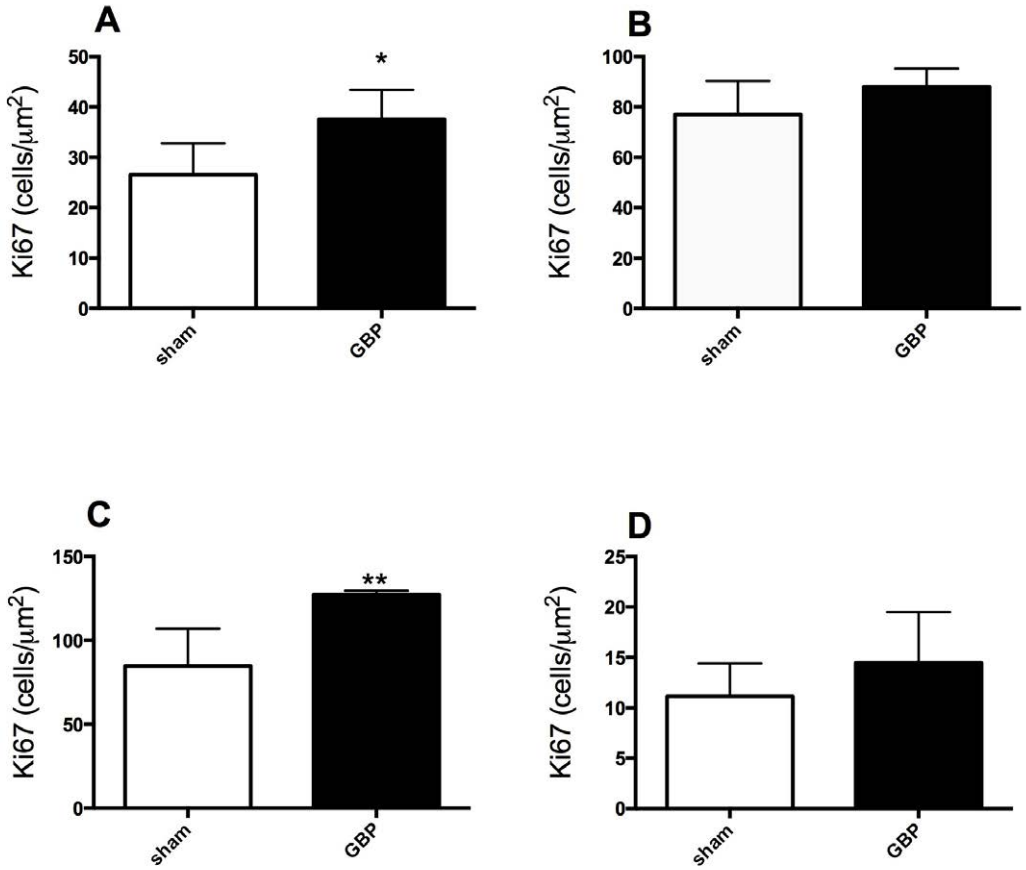

Figure 7: GBP-pigs had increased density of Ki67-immunoreactive cells in the duodenum (A) and ileum (C) whereas the density of Ki67-immunoreactive cells in the distal jejunum (B) and the colon (D) did not differ between the two groups of pigs. Data is presented for n=7 for GBP-pigs and n=8 for sham-pigs. *, p<0.05; **, p<0.01.

hormones is evident at least in humans and mice. Although we showed that ghrelin and motilin are highly coexpressed and co-secreted [37], hormone coexpression patterns have not yet been described in detail in the porcine GI-tract. Nevertheless it is to be noted that in the present study, cells expressing CCK, GIP, and neurotensin showed similar density increases in duodenum. These hormones are together with secretin, GLP-1 and PYY believed to be coexpressed in a distinct lineage of enteroendocrine cells [61]. In the other GI-segments studied this pattern could not be traced to the same extent.

Our data show that GBP has profound effects on endocrine cells in all GI segments, both bypassed segments (stomach and duodenum) and non-bypassed segments. It should be mentioned that our observations may, to some extent be affected by cellular protein levels and therefore cells with low expression may have been underestimated. A consistent finding was increased density of GIP-cells in all intestinal segments included in the study. Furthermore, no clear pattern with respect to whether the GI-segment was bypassed or not was obvious for any enteroendocrine cell population.

Conclusion

We conclude that the rearrangement of the GI-tract after GBP provokes a complex pattern of changes in several gut hormone-producing cell populations. Whether this relates to circulating hormone levels remains to be established. But our data suggest that many players in addition to the incretin hormones may contribute to the GBP-induced remission of T2D.

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