Total Gastrectomy Causes a Sustained, Long-term Elevation of Somatostatin in Plasma, Independent of the Mode of Reconstruction in Pigs

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Abstract. *Aim: The long-term effects of gastrectomy and various reconstructions of the gastrointestinal tract on fasting plasma levels of gastrointestinal hormones known to contribute to the control of gastrointestinal motor function were evaluated in pigs. Materials and Methods: Domestic pigs were randomly selected to sham surgery or total gastrectomy (TG) followed by reconstruction with oesophago-jejunostomy on a Roux-en-Y loop (OJRY), jejunal interposition between the oesophagus and the duodenum (OJD), or an oesophagojejunostomy with a proximal jejunal pouch reservoir (J-pouch) on a Roux-en-Y loop. Blood was collected just before surgery and ten weeks later and peptide levels were analysed by radioimmunoassay. Results: Somatostatin levels were sustained at a high level after TG, regardless of the mode of reconstruction, but were significantly lower in sham-operated animals. Levels of vasoactive intestinal peptide (VIP), neurotensin and motilin were unchanged. Conclusion: TG by itself leads to high levels of somatostatin long term, however, somatostatin, motilin, neurotensin and VIP are unaffected by the mode of reconstruction.*

The life-saving intervention for cancer of the stomach is a total gastrectomy (TG) followed by reconstruction of the gastrointestinal (GI) tract, typically by an oesophago-jejunostomy Roux-en-Y (OJRY). This type of major surgery has been associated with a high degree of mortality and morbidity. Continuing efforts to improve surgical techniques, for example through stapling of anastomoses, extensive lymph node dissection, improved peri- and postoperative

care along with the addition of adjuvant chemotherapy has helped to reduce morbidity and to increase survival in gastric cancer. Since an increasing number of patients survive the cancer disease, the need for reducing the common and often serious side-effects caused by the surgery and subsequently changed physiology of the GI tract has increased.

The removal of the stomach as such and bypassing the duodenum results in the loss of a reservoir function of the GI tract, as well as the loss of a segment of the GI tract that plays a crucial role in the control of food ingestion, digestion, motility and absorption. The radical surgery carries a range of unwanted side-effects, among which are changes in GI motility, weight loss and loss of hunger sensations. A serious and common side-effect of TG and OJRY that involves severe changes GI motility and transit is the so-called dumping syndrome, first described by Mix in 1922 (1). This entails a variety of symptoms, including vasomotor symptoms and abdominal bloating, cramping and profound diarrhoea following a meal. The exact mechanism underlying dumping syndrome is unknown, but some GI hormones secreted from the upper GI tract that are altered after TG have been suggested to contribute to it (2-4).

In the present study, we examine plasma levels of some relevant GI hormones, namely somatostatin, neurotensin, motilin and vasoactive intestinal peptide (VIP), in a pig model (5, 6) before and after TG. These peptides are released from the upper GI tract and contribute to the controls of gastrointestinal motor function (7-11). We compared reconstruction with a standard OJRY to two other reconstruction procedures that have previously been suggested as more physiological: jejunal interposition between the oesophagus and duodenum such that duodenal passage is preserved (OJD); and an oesophagojejunostomy with a proximal jejunal pouch (12), where the pouch is anastomosed to the oesophagus such that a reservoir substitute is created (J-pouch). There is accumulating support for these two procedures having fewer unwanted direct side-effects, leading to improved quality of life, less weight loss

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and less dumping syndrome-like symptoms after TG, and as such, these procedures are to be favored over OJRY (13-19). Whether such a reduction in unwanted effects after TG may correlate to differences in the plasma profiles of relevant GI hormones related to a specific reconstruction is not known. The aim of the present study was to examine whether any of these reconstruction methods specifically alter plasma levels of GI motor-related peptides over the long term.

Material and Methods

Animals. The experimental protocol was approved by the Ethical Committee for experiments in animals at the University Hospital of Lund (BMC). Swedish domestic pigs of random sex were used. The mean body weight was 21.9 (±3.5) kg at the time of surgery.

Surgery. Prior to surgery, the pigs were food deprived overnight, and anaesthesized with Azaperon (R1929; intramuscularly, *i.m.*) and metomidate hydrochloride (*via* continuous intravenous infusion, *i.v.*) (Janssen Pharmaceutical, Beerse, Belgium). After this, an endotracheal intubation was performed and the animals were artificially ventilated with a 70/30% mixture of nitrous oxide and oxygen. The left jugular vein was exposed and a catheter was introduced into the lumen to allow for collection of blood samples as well as for administration of peri- and postoperative fluids. During surgery, which was carried out under sterile conditions, each pig received an infusion of 1,000 ml of Ringer-glucose *i.v.* (Kabi Baxter Infusion AB, Stockholm, Sweden). A laparotomy was performed through a midline incision. The pigs were randomly assigned to undergo either sham surgery, or a TG with reconstruction of the alimentary tract using one of the following three methods:

a) Oesophago-jejunostomy on a Roux-en-Y loop (Figure 1 a): The jejunum was divided with a TLC55 stapler (Ethicon Ltd. Somerville, New Jersey, USA) approximately 20 cm below the ligament of Treitz and a Roux-en-Y loop was prepared. An oesophagojejunostomy was created end to side with an ILS stapler (Ethicon Ltd). After this, an enteroanastomosis was created side to side, 40 cm distal to the oesophagojejunostomy with a TLC stapler and the opening was closed with an RL 60 stapler.

b) Jejunal interposition between the oesophagus and the duodenum (Figure 1 b): The jejunum was divided 20 cm distal to the ligament of Treitz. Forty cm distal of the division site, the intestine was divided again and an oesophagojejunostomy was created with an ILS 21 stapler. An anastomosis between the distal part of the interposed intestine and the duodenum was performed in a similar way. Later in the study, both anastomoses were sutured with a running 4-0 PDS suture. This was done to avoid causing a possible obstruction of the ductus choledochus, which emanates very closely to the pylorus in the pig. No such bile duct obstruction was detected in any of the tested animals of the present study upon post-mortem exam, however.

c) Jejunal pouch on a Roux-en-Y loop (Figure 1 c): Twenty cm below the ligament of Treitz, the jejunum was divided and a Roux loop prepared. From the proximal end of the Roux loop, a pouch was created with two TLC 55 staplers. An oesophagojejunostomy was created with an ILS 21 through the open end, which was closed with an RL 60 stapler. After this, an entero-anastomosis was created side to side to the distal end of the Roux-loop with a TLC 55 stapler

40 cm distal to the oesophagojejunostomy. Finally, the opening was closed with an RL 60 stapler.

After surgery, each animal received an additional *i.v.* infusion of 1,000 ml Ringer-glucose once daily for 2 days and were allowed 7 days of recovery in the animal facility at the University Hospital of Lund. After this, they were moved to their original farm and housed under their normal living conditions, with free access to food and drinking water. The animals were assessed daily during the recovery period and throughout the experiment. After 10 weeks of intervention, the pigs were moved back into the animal facility at the Lund University hospital and blood samples were again collected after an overnight fast.

Collection of blood samples. Venous blood samples were collected from the jugular vein into chilled, heparinized tubes from each anaesthetized pig just prior to the start of TG surgery. Ten weeks later, the animals were again food deprived over night, anaesthetized in the similar manner and jugular vein blood samples were drawn in the similar way. Immediately after blood collection, the samples were centrifuged (4,000 rpm; 10 min, +4°C) and plasma was removed. The collected plasma samples were stored (–80°C) until analysis of the respective hormone.

Analysis of gastrointestinal hormones. Analyses of each peptide were performed using a radioimmunoassay (RIA) technique. Somatostatin was determined in plasma according to Wallengren *et al*. (20). The detection limit was 5 pmol/l and the intra-assay variation was <12%. Immunoassay of motilin was performed according to Sjölund *et al*. (9); the limit of detection was 7 pmol/l and the intra-assay variation was <11%. Neurotensin was immunoassayed according to Leander *et al*. (8); the detection limit of the assay was 10 pmol/l and the intra-assay variation was <10%. Finally, plasma levels of VIP were measured as described by Wallengren *et al*. (21); the limit of detection for the assay was 6 pmol/l and the intra-assay variation was <8.5%.

Data evaluation. For data evaluation, paired comparisons of plasma levels of each hormone before *vs*. after surgery was performed using two-tailed paired *t*-test. In addition, the pre-surgical plasma levels of each hormone across intervention groups were analyzed separately with a one-way ANOVA so that any possibility of pre-existing confounding group differences in basal plasma levels for each hormone could be detected.

Results

Weight development. As described elsewhere (5), body weights increased over time in all the groups, but after TG, regardless of the type of reconstruction, weight gain was significantly lower than in the sham-operated group.

GI hormones. A paired two-tailed *t*-test showed that basal levels of somatostatin were lowered 10 weeks after sham surgery ($p<0.05$). In contrast, in animals that had undergone TG, somatostatin levels remained elevated, such that there was no decrease from basal preoperative levels after 10 weeks, regardless of reconstruction procedure (Table I). Neurotensin (Table II), motilin (Table III) and VIP (Table
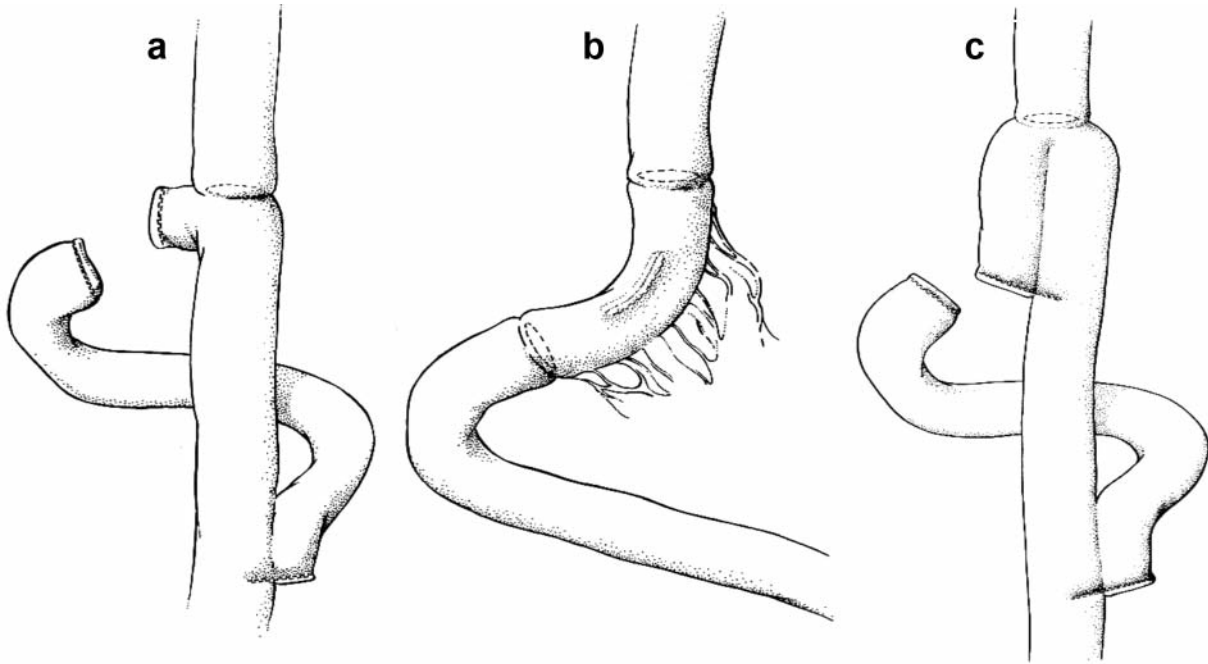
Figure 1. *The different reconstruction procedures after total gastrectomy. a, Oesophago-jejunostomy Roux-en-Y; b, interposition of a jejunal loop between the oesophagus and the duodenum; c, oesophago-jejunostomy Roux-en-Y with a proximal jejunal pouch.*

IV), remained unaffected by TG with subsequent surgical reconstructions ($p>0.05$; NS). There was no significant variation in basal fasting levels between any of the 4 groups before surgery as reflected by one-way ANOVA $p>0.05$ for each of the individual groups.

Discussion

In the present paper we found that TG as such causes a sustained, long-term elevation of plasma levels of somatostatin, whereas there appeared to be no change in somatostatin levels in response to any specific reconstruction method. The plasma levels of motilin, neurotensin and VIP remained unchanged after TG, regardless of the reconstruction method.

Somatostatin is a peptide that displays a variety of mainly inhibitory effects in the GI tract, for example inhibition of motility (7) and exocrine and endocrine secretion, including release of growth hormone (GH) and of orexigenic agents such as insulin (22, 23). Functional studies have indicated that the main pool of circulating somatostatin is likely to arise from the stomach (24), whereas pancreatic somatostatin is released locally within the pancreas. Cellular studies have shown that the distribution of somatostatin-containing cells are similar in pigs and in man: the somatostatin-containing cells are dense in the stomach, as well as in the duodenum and pancreas, but present to a minor degree in the remaining GI tract (25). We found, interestingly, that somatostatin

levels remained at a high level after the stomach was removed (Table I). This suggests that in the pig, a significant pool of the somatostatin released into the circulation not only arises from the stomach but from other sites as well, for example the duodenum (25). There are of course, regulatory factors other than an increased release that may explain the sustained, high steady-state plasma levels of somatostatin after TG observed here, for example, loss of vagal nerve activity or a reduced hepatic clearance. Somatostatin release into the circulation is stimulated by sympathetic nerve activity and beta-receptor activation, and a loss of vagal tone should therefore lead to increased basal plasma levels (26). Other explanations could be that the plasma levels of somatostatin were affected by increasing age (27) and reduced food intake (28). In the pig, somatostatin plasma levels were lowered in the sham-operated animals after 10 weeks, when body weight had greatly increased (5). It remains to be established whether "normalization" of weight gain after TG, by for example, additional nutritional support or pair-feeding, would reduce the plasma levels of somatostatin similarly to sham operated animals, over the long term.

Several reports in man support the notion that the type of reconstruction may reduce morbidity and unwanted side-effects related to changes in intestinal motility after TG (13, 19, 29). In the pig model, we were unable to detect any differences in body weight development with regard to any particular reconstruction method (5), but did show that reconstruction

Table I. *Effects of different reconstruction procedures after total gastrectomy on fasting plasma levels of somatostatin. Medians and range are shown. Paired t-test was used for comparisons of plasma levels before and 10 weeks after surgery.*

Somatostatin (pmol/l)	Before surgery	10 weeks post surgery
Oesophagojejunostomy (n=5)	89 (40-191)	111 (41-200) *NS*
Oesophagoduodenostomy (n=5)	63 (34-110)	86 (25-144) *NS*
J-Pouch (n=5)	103 (30-166)	98 (36-130) *NS*
Sham surgery (n=6)	98 (48-148)	68 (45-80)*

*p<0.05; NS, not significant.

Table II. *Effects of total gastrectomy and reconstruction procedures on fasting plasma levels of neurotensin. Medians and range are shown. Two-tailed, paired t-test was used for comparisons of pre-surgical vs. 10 week post-surgical levels.*

Neurotensin (pmol/l)	Before surgery	10 weeks post surgery
Oesophagojejunostomy (n=5)	5 (3-6)	7 (4-13) *NS*
Oesophagoduodenostomy (n=5)	9 (6-11)	18 (9-59) *NS*
J-Pouch (n=5)	9 (3-14)	11 (7-16) *NS*
Sham surgery (n=6)	7 (5-9)	9.7 (5-12) *NS*

NS, not significant.

Table III. *Effects of total gastrectomy and reconstruction procedures on fasting plasma levels of motilin. Medians and range are shown. Two-tailed, paired t-test was used for comparisons of plasma levels before and 10 weeks after surgery.*

Motilin (pmol/l)	Before surgery	10 weeks post surgery
Oesophagojejunostomy (n=5)	41 (26-52)	32 (18-65) *NS*
Oesophagoduodenostomy (n=5)	38 (11-51)	31 (5-51) *NS*
J-Pouch (n=5)	52 (36-69)	34 (11-61) *NS*
Sham surgery (n=6)	51 (22-79)	28 (11-61) *NS*

NS, not significant.

Table IV. *Effects of total gastrectomy and reconstruction procedures on fasting plasma levels of VIP. Medians and range are shown. Two-tailed Mann-Whitney U-test was used for comparisons of pre-surgical vs. 10 week post-surgical levels.*

Vasoactive intestinal peptide (pmol/l)	Before surgery	10 weeks post surgery
Oesophagojejunostomy (n=5)	8 (7-9)	8 (5-12) *NS*
Oesophagoduodenostomy (n=5)	6 (5-7)	7 (6-10) *NS*
J-Pouch (n=5)	7 (6-10)	8 (6-9) *NS*
Sham surgery (n=6)	6 (5-8)	8 (5-14) *NS*

NS, not significant.

with a J-pouch significantly reduces plasma levels of cholecystokinin and pancreatic polypeptide, which may reflect a compensatory attenuation of an anorexigenic response (5). The finding that somatostatin levels remain elevated in the long-term after TG could perhaps carry a similar compensatory effect on postoperative GI motility. Somatostatin has powerful inhibitory effects on GI motility and transit time, and somatostatin agonists can be used clinically to treat motility disturbances in the dumping syndrome (2).

Although we were unable to detect changes in circulating plasma levels of some of the GI hormones measured in the present study, this does not conclusively show that these peptides are unaffected by the respective reconstruction modes. Many GI hormones, including somatostatin, act locally *via* paracrine, as well as *via* endocrine, mechanisms. For example, circulating somatostatin is rapidly degraded from the main circulation through a high hepatic clearance rate. Peptide levels may be significantly increased locally and exert powerful paracrine effects on motility and secretion, without this being reflected by increases in plasma levels. Although we did not detect changes in plasma levels of VIP, motilin or neurotensin after surgery (Tables II-IV), there may still be differences in peptide levels at the site of action in response to a specific reconstruction method. In conclusion, plasma levels of somatostatin are increased after total gastrectomy regardless of the reconstruction method in

the pig, whereas plasma levels of VIP, motilin and neurotensin remain unchanged. Whether similar changes occur in man remains to be established.

Acknowledgements

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