Jejunal Pouch Reconstruction but not Preservation of Duodenal Passage after Total Gastrectomy Reduces Plasma Cholecystokinin and Pancreatic Polypeptide Long Term in Pigs

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Abstract. Aim: The long-term effects of reconstructions of the gastrointestinal tract after gastrectomy on plasma levels of gastrointestinal hormones that contribute to food intake controls were evaluated. Materials and Methods: Domestic pigs were randomly assigned to sham-surgery or total gastrectomy 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 jejunal pouch reservoir (J-pouch) on a Roux-en-Y loop. Plasma levels of peptides were analysed by radioimmunoassay (RIA). Results: Ten weeks after surgery, levels of cholecystokinin (CCK) and pancreatic polypeptide (PP) were significantly lowered (79.6% and 67.0%, respectively) in animals with a J-pouch, but not in sham-operated animals or animals with OJRY or OJD, as compared to preoperative levels. The levels of neuropeptide Y (NPY) and peptide YY (PYY) remained unchanged, irrespective of the mode of reconstruction. Conclusion: J-pouch, but not preservation of duodenal passage after total gastrectomy, lowers levels of CCK and PP, peptides that reduce food intake.

At present, the curative treatment for gastric cancer is surgery and the procedure will often be gastrectomy and an oesophageojunostomy Roux-en-Y. Some common and undesired long-term consequences of a total gastrectomy (TG) are weight loss and malnutrition. The reasons for the weight loss that frequently attend TG are not fully established. Some suggest that a reduced food intake results from the absence of the reservoir capacity of the stomach (1). Others have suggested other causes, such as pancreatic insufficiency, bacterial overgrowth, and/or shortened intestinal transit time (2-4), leading to inadequate digestion and/or absorption of nutrients. Postsurgical weight loss has also been attributed to a lack of hunger sensations resulting in changes of ingestive behaviour. This is supported by studies by Braga et al. (4), where body weight after TG was maintained as long as the caloric intake was sufficient.

After gastrectomy, there are several possible options for the reconstruction of the gastrointestinal (GI) tract, including an oesophagojejunostomy on a Roux-en-Y loop (OJRY), a jejunal small bowel pouch together with an oesophagojejunosnomastomy (J-pouch) (5), or interposition of a jejunal loop between the esophagus and duodenum such that duodenal passage is maintained (OJD) (Figure 1). It is possible that the different modes of reconstruction may affect plasma levels of the various GI hormones differently. Although an oesophagojejunosnomastomy on a Roux-en-Y loop is the most common reconstruction procedure, several studies indicate that patients with a pouch reservoir experience less weight loss during the first six months but not in the long term (6, 7). In addition, patients with a reservoir are suggested to experience fewer direct side-effects such as dumping syndrome-like symptoms and diarrhoea (8, 9), and report a better quality of life in general as compared to patients having a conventional oesophagojejunosnomastomy Roux-en-Y (6, 8-12). Others advocate a maintained duodenal passage as the method of choice and report evidence for better weight recovery and weight maintenance, lower reflux rate, slower oro-caecal transit time and less early retirement compared to patients having the conventional oesophagojejunosnomastomy Roux-en-Y procedure (13, 14).

It is widely acknowledged that several of the various GI hormones contribute to the complex controls of ingestion and body weight. As yet, little is known about how the plasma profiles of GI hormones are affected by various surgical reconstruction procedures of the upper alimentary tract after TG. Given that several of these peptides are located in and secreted from the stomach and/or intestine, it is plausible that...
surgical removal of the stomach and/or segments of the intestinal tract could change the GI endocrine plasma profile, which in turn may affect body weight. Several of the GI peptides that contribute to food intake controls, for example, cholecystokinin (CCK), pancreatic polypeptide (PP) and peptide YY (PYY), are considered to be released in response to nutrients present intraluminally in the GI tract. A bypassed or removed segment may subsequently result in less hormone being released. The present study was designed to evaluate the effects of restoration of the GI tract after total gastrectomy on plasma levels of CCK, PYY, neuropeptide Y (NPY) and PP in the pig. We hypothesized that basal plasma levels of gastrointestinal hormones known to contribute to controls of food intake and motility are changed after gastrectomy depending on the subsequent reconstruction procedure. Specifically, we compared the significance of a preserved duodenal passage, by interposing a jejunal segment between the oesophagus and duodenum, to a conventional oesophagojejunostomy bypassing the duodenum, and to an oesophagojejunostomy bypassing the duodenum with a jejunal pouch reservoir.

Materials and Methods

Animals. The experimental protocol was approved by the local ethical committee for experiments in animals at the University Hospital of Lund (BMC). We chose to use pigs because of the similarities in their GI physiology to humans. 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, then anaesthetized with Azaperon (R1929; intramuscularly, i.m.) and metomidate hydrochloride (by a 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 pre- and postoperative fluids. During surgery, which was carried out under sterile conditions, each pig received an infusion of 1,000 ml of Ringer-glucose once daily for 2 days and were allowed 7 days of recovery in the animal facility at the Lund University Hospital. 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. The animals were weighed once a week. 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, heparinised tubes from each anaesthetized pig just prior to the start of surgery. Ten weeks after surgery, the animals were again food deprived overnight, anaesthetized as before and jugular vein blood samples were drawn in a 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 RIA technique. Plasma levels of CCK were determined using an antibody that specifically detects the N-terminal portion of sulphated CCK-8 (courtesy of Dr. J.F. Rehfeld, Rigshospitalet, Copenhagen, Denmark) as described elsewhere (15). The limit of detection was <1.0 pmol/l and the intra-assay coefficient of variation was <15%. Plasma levels of NPY were determined as described by Edvinsson et al. (16). The antiserum used cross reacts with PYY (33%) but not with the C-terminal fragments of either NPY or PYY. The detection limit was 11 pmol/l and the intra-assay variation was <7%. Immunoassay of PYY was performed according to Ekman et al. (17). The limit of detection was 10 pmol/l and the intra-assay variation was <8.5%. For measuring levels of PP, a commercial kit was used (cat no. RB 316; Euro-Diagnostica, Malmö, Sweden), the detection limit of which was 8 pmol/l and the intra-assay variation was <4%.

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

**Results**

Cholecystokinin. A paired, two tailed *t*-test showed that there was a significant reduction of plasma levels of CCK three months after TG with a J-pouch (*p*<0.01) as compared to pre-surgical levels (Table I). However, CCK levels were unaffected by TG followed by sham surgery, OJRY or OJD, (*p*'s >0.05 (NS). One-way ANOVA of preoperative plasma levels showed no difference in levels of CCK (*p*=0.357).
A paired, two-tailed t-test showed that there was a significant reduction in levels of PP three months after TG in animals that had undergone J-Pouch reconstruction (p<0.05), whereas neither the sham-operated animals nor the animals with OJRY or OJD reconstruction exhibited any changes in PP levels as compared to pre-surgical levels (Table III). There was no effect of sham surgery or TG followed by any of the three reconstruction approaches on plasma levels of NPY (Table II) or PYY (Table IV) three months after surgery (p's>0.05, NS). None of the one-way ANOVA’s performed on the preoperative PP, PYY or NPY levels were significant, indicating that there were no pre-existing differences before surgery in basal NPY, PP or PYY levels. (p’s>0.05, NS).

Body weight maintenance. Body weight was recorded once weekly. In all the groups, the animals gained weight over time. There were no significant differences in body weights between the three TG groups before or after surgery. Although animals with TG, regardless of the mode of reconstruction, had gained weight slightly after 3 months as compared to prior to surgery, they did not gain as much as the sham-group.

Discussion

In the present study, we show that the mode of reconstruction after TG causes long-term changes in fasting levels of some GI hormones that are considered relevant for food intake control. Specifically, reconstruction with a pouch was shown to significantly reduce plasma levels of PP by 79.1% while not affecting the levels of the related peptides NPY and PYY. In addition, reconstruction with a J-pouch significantly reduced fasting plasma levels of CCK (67% reduction), but TG followed by either OJRY, or OJD caused no significant effects on CCK levels postsurgically.

Cholecystokinin is present in cells of the duodenum and released in response to food present intraluminally. Besides causing gallbladder contraction and stimulating exocrine secretion of the pancreas, it is known to inhibit food intake through an inhibition of gastric emptying (18) and by activating central nervous networks via a vagal afferent mechanism (19). Similar to CCK, PP is released for in response to food introduced intraluminally in the small intestine (20) and considered to cause suppression of food intake in mammals (21). In addition, PP has been shown to increase energy expenditure and to reduce expression or release of...
orexigenic factors such as ghrelin and leptin in the periphery, as well as to reduce expression of NPY, orexin and ghrelin in the hypothalamus (22).

The failure to maintain body weight and the loss of hunger sensations are common, serious and complex clinical problems that affect patients who undergo TG. Whether any reconstruction mode is more superior in terms of survival and/or weight maintenance is not fully established. Several authors report that patients with a jejunal pouch better maintain and even gain body weight. In addition, there appear to be fewer side-effects such as dumping syndrome and diarrhoea after jejunal pouch reconstruction vs. OIRY (8-11), possibly because a reservoir capacity of the upper GI tract is then recreated. From this perspective, our finding that levels of CCK and PP were changed in the group receiving a jejunal pouch, but not after OIRY or OJD, is highly interesting. Although physiological differences (humans with cancer vs. healthy pigs) must, of course, be taken into consideration, the possibility remains that changes in CCK and or PP levels postsurgically may contribute to different and perhaps improved long-term weight development after pouch reservoir reconstruction (6, 10, 11). CCK and PP each inhibit food intake and it is possible that a decrease in plasma levels of these peptides may contribute to sustaining a sufficient caloric intake by either decreasing satiety signals, modulating orexix factors, or altering energy expenditure (18, 19, 21, 22).

The gastrectomised animals increased slightly in body weights over the intervention period, but on average they did not follow the normal weight gain of the sham-operated animals. The finding that there were no major differences between body weights pre- or post surgically within each of the intervention groups may suggest that the decrease in CCK and PP in the J-pouch animals was most likely a direct effect of the changed anatomy of the gut and not indirectly due to effects caused by differences in body weights per se between the TG groups. From a strict weight development standpoint, however, none of the reconstruction methods improved weight gain after TG (23).

To fully understand the endocrine adaptation of the gut after significant surgery with quite profound anatomical modifications of the GI tract, it would be relevant to compare possible changes in levels of various satiety peptides in response to food ingested as well. This was not possible for two reasons, first, the ethical protocol did not allow for blood samples to be drawn in animals that were awake, or for us to house and feed the animals singly, which would then be necessary. Moreover, pigs are herd animals and they tend not eat if fed singly. Although there are reports on TG and effects on GI hormones in response to a meal in other species, it is difficult to directly compare results because of differences in the designs of the studies. For example, Bergh et al. compared levels of CCK in gastrectomised humans before and after a meal and found, in contrast to our results, that fasting CCK levels were elevated whereas there was no difference in postprandial levels (24). However, Bergh et al. did not describe which reconstruction procedures were used. Malesci et al. (25) examined release of PP after total gastrectomy with jejunal RY with or without duodenal bypass in humans and detected a reduction of the early biphasic postprandial PP secretion peak. The late, predominant PP peak was unchanged in response to food however (25), such that there was no change in the overall postprandial levels of the peptide. In contrast, Schwarz et al. (7) measured levels of PP and CCK after meal stimulation in patients that had undergone total gastrectomy with different modes of reconstructions: Roux-en Y oesophagojejunostomy vs. a jejunal pouch interposition between the oesophagus and duodenum or J pouch (5). After all types of reconstructions, peak levels of PP were lowered, and CCK levels increased, in response to food. We hesitate to directly compare our data, since Schwarz et al. (7) reported peak concentrations without specifying at which time point after food intake the peak value was detected, and reported no basal fasting levels. Our results do not conflict with these cited papers, but suggest that fasting levels of CCK and PP are suppressed after TG with jejunal pouch reconstruction in the long term.

In summary, the present study shows that total gastrectomy followed by an oesophagojejunostomy combined with a jejunal pouch Roux-en-Y, but not oesophagojejunostomy Roux-en-Y or an interposition of a jejunal loop between the oesophagus and duodenum, reduces basal plasma levels of CCK and PP, which are considered to reduce food intake through peripheral mechanisms. Whether such endocrine, adaptational changes account for postsurgical weight changes and/or help to attenuate satiety signalling remains to be established.

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