European Journal of Clinical Nutrition (2010) 64, 826–831

0954-3007/10
www.nature.com/ejcn

ORIGINAL ARTICLE

Long-term changes in gut hormones, appetite and food intake 1 year after subtotal gastrectomy with normal body weight

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Background/Objectives: No prospective study on the long-term effects of gastric resection on gastrointestinal hormonal changes in patients with normal body weight has been reported. The aim of this study was to evaluate the 1-year effect of subtotal gastrectomy on ghrelin and peptide YY $(PYY)_{3-36}$ levels.
Subjects/Methods: Eighteen patients with early gastric cancer underwent subtotal gastrectomy with Billroth I reconstruction. We assessed appetite, food intake, body composition, and ghrelin and PYY_{3-36} levels preoperatively and 1 year after surgery.
Results: There were no significant difference in the preoperative daily food intake and 1 year after subtotal gastrectomy. Weight loss occurred in all study subjects; 11.7% ($n=2$), 55.5% ($n=10$) and 33.3% ($n=6$) of the patients lost $<5\%$, 5–10% and $>10\%$ of their preoperative body weight, respectively. Body mass index, waist circumference and body fat significantly decreased 1 year after subtotal gastrectomy. There were no significant differences in the appetite visual analogue scale preoperatively and 1 year after subtotal gastrectomy. The plasma ghrelin concentration decreased significantly ($P=0.006$), whereas PYY_{3-36} did not show a significant change 1 year after subtotal gastrectomy.
Conclusions: Ghrelin levels and body fat decreased significantly, whereas PYY_{3-36} levels as well as appetite and food intake did not change significantly 1 year after subtotal gastrectomy with normal body weight. These findings suggest that decreased ghrelin might contribute directly to reduced body fat.
European Journal of Clinical Nutrition (2010) **64,** 826–831; doi:10.1038/ejcn.2010.83; published online 19 May 2010

Keywords: ghrelin; peptide YY; gastrectomy; appetite; body fat

Introduction

Ghrelin, a 28-amino-acid peptide with an n-octanoylation indispensable for its biological activity, is produced mainly by the stomach and contributes to 70% of the circulating concentration of ghrelin (Jeon *et al.*, 2004). Ghrelin has been shown to induce not only growth hormone release from the pituitary gland, but also to increase appetite and stimulate weight gain (Wren *et al.*, 2000) Peptide YY $(PYY)_{1-36}$ is released into the circulation from L-type endocrine cells of the distal intestine (Adrian *et al.*, 1985). After release, biologically active PYY_{3-36} is formed by cleavage of the N-terminal residues by dipeptidyl peptidase IV (le Roux and Bloom, 2005). In addition to various suppressive functions in the gastrointestinal tract, PYY_{3-36} induces physiologic post-prandial satiety by counterregulation of ghrelin release through the Y2-receptor in the hypothalamus (McGowan and Bloom, 2004).

Gastrointestinal hormones, such as ghrelin and PYY_{3-36}, have been reported to have important roles in determining food intake and body weight (Wren *et al.*, 2000; McGowan and Bloom, 2004). However, little is known about the long-term effects of gastric resection on these peptides. According to previous studies, removal of the entire stomach in

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Received 18 November 2009; revised 19 April 2010; accepted 19 April 2010; published online 19 May 2010

humans reduces the plasma ghrelin concentration by 65%, 1–8 years after surgery (Ariyasu, 2001); sleeve gastrectomy also reduces the plasma ghrelin level and increases $PYY_{3–36}$ in morbidly obese patients (Karamanakos *et al*., 2008). Of note, these findings reflect a retrospective cohort study in which patients who had undergone gastric resection and healthy controls or patients undergoing bariatric surgery for weight reduction in morbidly obese patients were compared. No prospective studies of the long-term effects of gastric resection on gastrointestinal hormonal changes in patients with normal body weight have been reported. Therefore, we prospectively evaluated the changes in ghrelin and $PYY_{3–36}$ concentrations preoperatively and 1 year after gastric resection in patients with early gastric cancer who underwent a subtotal gastrectomy.

Methods

Patients with early gastric cancer who underwent a subtotal gastrectomy at the Pusan National University Hospital between January 2004 and December 2005 comprised the study subjects. Early gastric cancer is defined by the Japanese Research Society for Gastric Cancer as cancer in which the tumor cells invade only the mucosal and submucosal layers (Japanese Gastric Cancer Association, 1998).

Subtotal gastrectomy was defined as the surgical removal of the distal two-thirds of the stomach; the restoration method of digestive continuity was a Billroth I gastroduodenostomy. Patients with endocrine diseases, such as diabetes mellitus, thyroid disease and pituitary disease, were excluded from this study, as were patients with tumors >2 cm in diameter. Written informed consent was obtained from each subject before enrollment in this study. The study was approved by the institutional review board of the Medical Research Institute of Pusan National University and was performed in accordance with the Declaration of Helsinki Principles.

All study subjects were evaluated for food intake, body composition, appetite and gastrointestinal hormones (ghrelin and PYY_{3-36}) preoperatively and 1 year after surgery. The average follow-up period was 12.1 months (s.d., 2.3 months). Of 21 patients, 3 (14.3%) were lost to follow-up during the study period. Eighteen patients with gastric cancer were studied (Table 1). However, no significant difference was found between inclusion and exclusion patients regarding their basal characteristics.

Daily energy and nutrient intake were assessed by a semiquantitative food frequency questionnaire. Subjects were asked to report the average frequency of consumption and portion size of each food on a weekly basis during the previous 3 months by an experienced dietitian (Lee *et al*., 2006). Subjects also were asked to maintain physical activity levels throughout the study period. The height, weight and waist circumference were measured with the subjects wearing a light gown, but no shoes. Body composition was measured using dual energy X-ray absorptiometry (Lunar Prodigy; GE Medical Systems, Waukesha, WI, USA), which is a sensitive test to quantify changes in lean and fat mass *in vivo* (Going, 1993) and assessing regional fat distribution (Taylor *et al*., 1998). All of the scans were analyzed with Lunar software, package l.35. The coefficients of variation were 2.6% for fat mass, 2.5% for total body fat percentage, <3% for regional fat measures and 0.88% for lean mass. Appetite was assessed using a horizontal visual analogue scale (VAS), 100 mm in length, with words anchored at each end, expressing the most positive (100 mm) and the most negative rating (0 mm) (Flint *et al*., 2000).

Table 1 Anthropometric characteristics and body composition data of patients ($n=18$)

Variable	*Value*
Age (years)	56.0 ± 11.1
Gender (male/female)	15/3
Height (cm)	163.8 ± 6.7
Weight (kg)	63.9 ± 9.0
Body mass index (kg/m^2)	23.8 ± 2.2
Waist circumference (cm)	83.4 ± 7.2
Total body fat (%)	16.4 ± 5.8
Fat-free mass (kg)	47.5 ± 8.0

Values are the mean ± s.d., except gender.

Blood samples were obtained at 0800 hours preprandially after an overnight fast for the determination of plasma ghrelin and PYY_{3-36}. Blood samples were drawn into chilled tubes containing Na_2EDTA (1 mg/ml) and aprotinin (500 U/ml). Plasma was immediately separated by centrifugation at 4°C and stored at −70°C until assayed. Plasma total ghrelin was measured with a commercially available radioimmunoassay kit (Phoenix Pharmaceuticals Inc., Belmont, CA, USA), using ^{125}I-labeled bioactive ghrelin as a tracer molecule and a polyclonal rabbit antibody against full-length octanoylated human ghrelin, which measures total circulating ghrelin levels. The lower and upper limits of detection were 10 and 1280 pg/ml, respectively. The mean intra- and inter-assay coefficients of variation were 4.8 and 3.5%, respectively. The radioimmunoassay did not show any cross-reaction with human leptin. Plasma PYY_{3-36} was measured with a commercially available kit (Linco Research Inc., St. Charles, MO, USA). The antibody, raised in guinea pigs, has 100% cross-reactivity with human PYY_{3-36}, but no cross-reactivity with human pancreatic polypeptide, NPY, and unrelated peptides, such as leptin and ghrelin. ^{125}I-PYY was used as a label; the labeled peptide was purified by HPLC (specific activity, 302 kCi/kg). The lowest level of PYY detected by this assay was 10 pg/ml using a 100 μl plasma sample. Intra- and inter-assay coefficients of variation were <9%. All procedures were performed at 4 °C.

The data are expressed as the mean ± s.d. A paired *t*-test was used to determine the statistical significance of the differences in calorie intake, appetite, body composition, ghrelin and PYY_{3-36} levels preoperatively and 1 year after

subtotal gastrectomy. We used SPSS 12.0 for Windows (SPSS Inc., Chicago, IL, USA) for all statistical analyses.

Results

Demographic data
The 18 study subjects had a mean age of 56 years, with a range of 28–74 years. The mean body mass index was 23.8 kg/m^2, with a range of 18.9–28.2 kg/m^2. The majority of the subjects (83.3%) were men (Table 1).

Evaluation of food intake
The average daily dietary intake is shown in Table 2. No significant differences existed in the daily total calorie, protein, fat and carbohydrate intake preoperatively and 1 year after subtotal gastrectomy (Table 2).

Evaluation of body composition
Weight loss occurred in all study subjects after subtotal gastrectomy. Of the study subjects, 11.7% ($n=2$), 55.5% ($n=10$) and 33.3% ($n=6$) lost <5, 5–10 and >10% of their preoperative body weight, respectively, 1 year after surgery. The body mass index, waist circumference and body fat percentage, as measured by dual energy X-ray absorptiometry, decreased to 90.4 ± 5.0, 89.7 ± 5.7 and 76.2 ± 9.7% after 1 year of subtotal gastrectomy, but fat-free mass did not result in a statistically significant change after surgery (Table 2).

Evaluation of appetite
There were no significant differences in the VAS appetite ratings preoperatively and 1 year after subtotal gastrectomy (Table 2 and Figure 1).

Evaluation of ghrelin and PYY_{3-36} levels
The total plasma level reached a nadir of 42.8% at 1 h after gastric resection and gradually increased to 85.8% by postoperative day 7. However, finally the total plasma ghrelin level decreased significantly (from 113.0 ± 46.1 to 65.2 ± 26.6 pg/ml; $P=0.006$) 1 year after subtotal gastrectomy (Figure 2). The PYY_{3-36} concentration decreased to 78.9% at postoperative day 7, but did not result in a statistically significant change within 1 year after subtotal gastrectomy (Figure 2).

Discussion

This is the first prospective study, to our knowledge, to evaluate long-term effects of gastric resection on gastrointestinal hormonal changes in patients with 'normal body weight'. Ghrelin is primarily produced in the stomach and transmits a peripheral appetite-stimulatory signal to the brain, thus inducing food intake (Wren *et al.*, 2000;

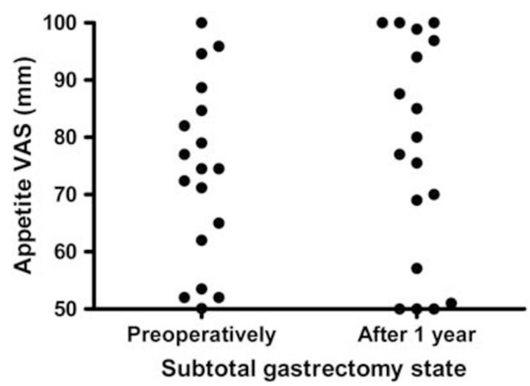

Figure 1 Comparison of appetite preoperatively and 1 year after subtotal gastrectomy.

Table 2 Comparison of appetite, food intake and body composition data preoperatively and 1 year after subtotal gastrectomy

	Subtotal gastrectomy state		P
	Preoperative (n = 18)	*Year 1* (n = 18)	
Appetite (VAS)	71.9 ± 18.7	77.2 ± 21.8	0.452
Food intake			
Total intake (kcal/day)	1751.2 ± 435.2	1734.2 ± 468.9	0.935
Protein intake (g/day)	68.9 ± 16.8	67.2 ± 28.7	0.865
Fat intake (g/day)	34.8 ± 12.1	33.0 ± 19.6	0.802
Carbohydrate intake (g/day)	290.3 ± 69.2	292.1 ± 71.1	0.960
Body composition			
Body weight (kg)	63.9 ± 9.0	57.1 ± 9.4	<0.001
Waist circumference (cm)	83.4 ± 7.2	77.0 ± 8.4	<0.001
Body mass index (kg/m^2)	23.8 ± 2.2	21.6 ± 2.4	<0.001
Body fat (%)	16.4 ± 5.8	13.2 ± 5.4	<0.001
Fat-free mass (kg)	47.5 ± 8.0	45.0 ± 7.7	0.006

Abbreviation: VAS, visual analogue scale.
Values are the mean ± s.d.; no significant differences (paired *t*-test) were detected before and 1 year after surgery.

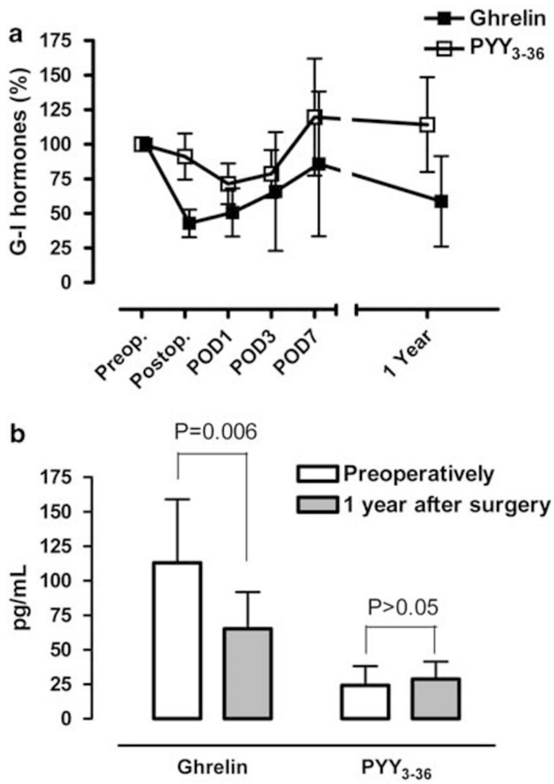

Figure 2 Changes in ghrelin and PYY_{3-36} concentrations (**a** and **b**; mean with s.d.) preoperatively and 1 year after subtotal gastrectomy (**b**; paired *t*-test). POD, postoperative day.

Jeon *et al*., 2004). The concentration of ghrelin increases in the fasting state or before meals and stimulates appetite by activation of neurons in the arcuate nucleus of the hypothalamus, whereas circulating concentrations decrease rapidly after the meal in response to food intake. The obese individuals have higher plasma ghrelin concentrations and lean individuals have lower plasma ghrelin concentrations than healthy controls. PYY_{3-36} is primarily secreted from the distal intestinal tract and induces postprandial satiety. The plasma PYY_{3-36} concentration remains low preprandially, but increases after food ingestion; PYY_{3-36} stimulates satiety and reduces hunger through hypothalamic Y2 receptors (Adrian *et al*., 1985; McGowan and Bloom, 2004). Plasma PYY_{3-36} concentrations are lower in obese people compared to normal weight controls and are inversely proportional to the body mass index. These findings suggest that ghrelin and PYY_{3-36} have important roles in appetite control, as well as energy homeostasis, in the regulation of body weight.

Gastric resection is the surgical removal of part or all of the stomach, and is usually performed in the treatment of gastric cancer or intractable peptic ulcers. Patients who undergo gastric resection commonly lose body weight. A reduced caloric intake and malabsorption are considered the most important factors contributing to weight loss after gastric resection (Braga *et al*., 1988). Because ghrelin is produced mainly by the stomach, weight loss after gastric resection may be accompanied by impaired ghrelin production. Thus, gastrointestinal peptide changes may also be involved in weight loss after gastric resection. However, no prospective studies of the long-term effects of gastric resection on gastrointestinal hormonal changes in patients with normal body weight have been reported.

In this study, there were no significant differences in the daily intake of total calorie, protein, fat and carbohydrate preoperatively and 1 year after subtotal gastrectomy (Table 2). One could expect that after total gastrectomy, loss of the gastric reservoir reduces calorie intake, which is a major cause of weight loss (Liedman *et al*., 1996). However, after subtotal gastrectomy, no significant difference in food intake was found preoperatively and 1 year after surgery. This might be due to partial restoration of the reservoir function of the remnant stomach and the increased frequency of food intake.

It has been reported that weight loss usually occurs early, especially within the first 6 months after surgery, although data on changes in body composition after subtotal gastrectomy are limited (Liedman *et al*., 1997). Our study showed that body weight, body mass index, waist circumference and body fat percentage were decreased 1 year after subtotal gastrectomy, whereas fat-free mass did not change (Table 2). These findings suggest that reduced body fat is the most important factor contributing to weight loss after gastrectomy. The reduction of body fat results in weight loss and reduced body mass index after subtotal gastrectomy. In a study about changes in body composition after gastrectomy, it has been reported that approximately 90% of weight loss is caused by reduced body fat (Liedman *et al*., 1997). However, the reason why the decrease in body fat is most sensitive among compartments of body composition after gastrectomy is unknown.

The stomach is the principal site of ghrelin production, which contributes 70% of the ghrelin levels in humans (Jeon *et al*., 2004). Therefore, we hypothesized that ghrelin levels would decrease after gastrectomy, which results in loss of appetite and body weight. However, contrary to our expectations, no significant differences in VAS appetite ratings occurred before and 1 year after subtotal gastrectomy (Figure 1). The underlying mechanism is unknown, but appetite and satiety controls are mediated by a complicated interplay of neuroendocrine signaling pathways involving a great variety of peptide hormones and neuropeptides, including ghrelin and PYY_{3-36} (Romijn *et al*., 2008) Although appetite-stimulating plasma ghrelin concentrations decrease after gastrectomy, appetite would not have been changed due to compensation of other hormones involved in the control of appetite and food intake. Positive emotions to overcome the cancer in patients with gastric cancer who underwent gastrectomy may positively influence the subjective VAS appetite ratings 1 year after surgery (Boström *et al*., 2003).

We reported that compensatory ghrelin production occurred in the remnant stomach after the surgical removal of part of the stomach as well as other tissues or organs such as the bowel, pancreas, kidney and placenta in postoperative day 7 (Jeon *et al*., 2004). In this study, however, finally plasma ghrelin concentrations were significantly decreased 1 year after subtotal gastrectomy. Plasma PYY_{3-36} concentrations also decreased gradually according to the changes in the plasma concentration of ghrelin, but did not change 1 year after subtotal gastrectomy. Therefore, it seems unlikely that compensatory ghrelin production remains so in the long-term, although it can occur transiently. Ghrelin is known to enhance appetite and increase food intake in the short term and maintain body fat in the long term (Dornonville de la Cour *et al*., 2005). In this study, appetite did not change 1 year after subtotal gastrectomy, but body fat decreased significantly compared to before surgery, which is considered to be closely related to reduced ghrelin concentrations. These observations also indicate that ghrelin may act directly on fat metabolism by a peripheral mechanism rather than through a central mechanism. Ghrelin is known to maintain a positive energy balance by suppression of fat utilization without a change in energy expenditure (Tschöp *et al*., 2000). Thus, low levels of circulating ghrelin in subtotal gastrectomized patients appear to contribute to a loss of body fat and body weight. The mechanism underlying fat loss by reduced ghrelin levels is unknown. Recently, one human study showed a significant positive correlation between the total plasma ghrelin level and the whole-body respiratory quotient (Doucet *et al*., 2004). The respiratory quotient has been used as an indicator of the glucose-to-fat oxidation rate, reflecting macronutrient oxidation. Ghrelin increases the respiratory quotient in mice, suggesting an increased use of carbohydrates and decreased use of fat as an energy source (Frayn, 1983). These results suggest that the effect of ghrelin on body composition may be mediated by changes in fuel preference rather than food intake. Although the reduced circulating ghrelin concentration may contribute to body fat loss, future studies should further clarify the mechanisms of action of ghrelin in fat metabolism.

Our study has some limitations, including the relatively small size of the study sample and the fact that no attempt was made to measure acylated and de-acylated ghrelin. The other limitations are the lack of data about physical activity levels, which may also affect changes in body weight and ghrelin, although we asked the subjects to maintain physical activity levels throughout the study period. Considering these limitations, further research is needed to reconfirm these findings.

In summary, we have shown that the plasma concentration of ghrelin is decreased 1 year after subtotal gastrectomy in early gastric cancer patients with normal body weight. However, the plasma concentration of PYY_{3-36}, as well as appetite and food intake, did not show a significant change 1 year after subtotal gastrectomy. These findings suggest that decreased ghrelin might contribute directly to reduced body fat.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by a Medical Research Institute Grant (2005-01) from Pusan National University, Busan, Korea.

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