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MET-ENKEPHALIN-LIKE PEPTIDES AND GHRELIN MITIGATE NEGATIVE EFFECTS OF BARIATRIC SURGERY IN RATS

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Endogenous opioid peptides and ghrelin are synthesized in the gastro-intestinal tract and modulate its functions in human and animals in physiological and pathophysiological conditions. Opioids, mainly Met-enkephalin, and ghrelin are involved into regulation of appetite, gastric emptying, intestinal motility and intestinal juices secretion. Obesity is a metabolic disorder of energy homeostasis caused by the overdose of energy contained in food in relation to the needs of the organism. Bariatric surgery is often considered as a method of obesity treatment being more effective with a long-term. However, it seems probable that surgery markedly affects the interaction between these two endogenous peptides and their receptors. Thus the aim of the study was to compare the effect of two methods of bariatric surgery on the concentrations of Met-enkephalin and ghrelin in the stomach and other regions of the gastro-intestinal tract in rats. Experimentation was undertaken using 24 male Wistar rats divided into control and two experimental groups undergoing sleeve gastrectomy (GR) or gastric plication (GP). Three weeks after surgery blood and fragments of stomach, duodenum, jejunum, ileum and ascending colon were collected. Plasma glucose and triglycerides were significantly changed after the bariatric surgery. Both bariatric procedures significantly but differentially changed the Met-enkephalin and ghrelin concentrations in the most of tested tissues. The obtained results clearly showed involvement of Met-enkephalin and ghrelin in the regulation of post-bariatric metabolic processes.

Key words: Met-enkephalin, ghrelin, bariatric rats, gastro-intestinal

INTRODUCTION

Endogenous opioid peptides are widely distributed in the central nervous system and in peripheral organs such as adrenal, heart, pancreas, lungs, liver and all parts of gastrointestinal system (KOLTA et al., 1992; STERNINI et al., 2004). Physiologically active small peptides are released

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from their precursors which belong to three opioid families ß–endorphins, enkephalins and dynorphins. In the gastrointestinal tract, opioid peptides are present in neurons and endocrine cells of the mucosal layer. Opioids are synthesized in the enteric nervous system mainly in the dorsal root ganglion and transported to the nerve terminals. Released peptides act in sto-mach and intestine through G-protein coupled receptors – MOR, DOR and KOR for endorphin, enkephalin and dynorphin, respectively (Bagnol et al., 1997). The distribution of opioid receptors in the GI tract is uneven, MOR are present in the submucosal plexus, myenteric plexus and longitudinal muscle of small intestine; DORs were detected in stomach, intestines and are expressed in NPY submucosal neurons and in myenteric plexus together with SP expression. The presence of KORs in GI tract was found in myenteric and submucosal neurons, fibres in muscle layer and mucosa (Wood and Galligan, 2004; Sobczak et al., 2014).

Opioids, particularly ß-endorphin and Met-enkephalin modulate functions of gastro-intestinal (GI) tract in human and animals in physiological and pathophysiological conditions. Under physiological conditions, opioids inhibit gastric emptying and intestinal motility, decrease bile, pancreatic and intestinal juices secretions (Wood and Galligan, 2004)

Long term treatment with drugs based on opioid molecules slows motility of GI, mucosal transport of fluids and electrolytes and additionally have other side effects such as respiratory depression or even induction of opioid dependence. It was found that opioids act on the enteric neurons through the interaction with other neurotransmitters such as acetyl choline, substance P, ATP, vasoactive intestinal peptide, NPY or neurokinine A (Holzer, 2010; Brock et al., 2012; Holzer, 2014).

Additionally, opioids are involved into analgesic response and regulation of appetite (Parrish, 2008). The latter mechanism is directed by the interaction with ghrelin in stomach and intestines. Ghrelin is a 28 amino-acids peptide with an unique modification on the N-terminal third amino acid –serine. Ghrelin, identified by Kojima et al., (1999) as an endogenous ligand for growth hormone secretagogue receptors (GHSR-1a), is localized in the stomach and proximal GI tract and is involved into mediation of motility, gastric emptying, induction of migrating motor complexes (Date et al., 2000; Korbonits et al., 2004; Kojima and Kangawa, 2005; Tack, 2006; Sato et al., 2011).

Obesity is a metabolic disorder of energy homeostasis caused by the overdose of energy contained in food in relation to the needs of the organism. The results of energy imbalance are first stage of metabolic disorders manifested by an increase in the excess storage in adipose tissue. The primary method of treatment for obesity is a conservative regime (diet, physical activity, pharmacotherapy). The effectiveness is estimated at a low as 5% (long-term results of conservative treatment of obesity). Bariatric surgery is more effective with a long-term effectiveness of 80% (DeMaria, 2007). Its effectiveness can be decisive when selecting an effective method of treatment of obesity (Maggard et al., 2005; Abell and Minocha; 2006, Tucker et al., 2007).

However, the results of the clinical observations indicated that patients undergoing these treatments manifest metabolic disorders have not seen before surgery (Whitson et al., 2007). The studies have shown changes in the nervous Boeckxstaens and Jonge, 2009) hormonal and immune systems after bariatric surgery (Langer et al., 2005; Sundbom et al., 2007; Tymitz et al., 2011; Ivano et al., 2013).

Opioid agonists used for the treatment of postoperative pain may negatively contribute to postoperative ileus by stimulation of MOR in the GI tract and inhibit the intestinal motility. Bariatric surgery drastically decreases the gastric volume what can change concentrations of the endogenous opioid peptides and their receptors and also affects the ghrelin synthesis and secretion. It seems probable that surgery markedly affects the interaction between these two endogenous peptides and their receptors. Thus the aim of the study was to compare the effects of two methods of bariatric surgery on the concentrations of Met-enkephalin and ghrelin in the stomach and other regions of the gastro-intestinal tract in rats.

MATERIALS AND METHODS

Experimentation was undertaken using 24 male Wistar rats (Collegium Medicum UJ), weighing about 250 g. The rats were maintained

at a controlled temperature (20°C) and light regime (12L:12D). Rats had free access to water, but feed was carefully weighed before and after the experimental procedure. After 24 hours of feed deprivation animals were divided into three groups: control and one undergoing sleeve gastrectomy (GR) and the other undergoing gastric plication (GP). Protocol was approved by I Local Ethics Committee.

Sleeve gastrectomy

Surgery was performed with anesthesia (ketamine 10 mg/kg and droperidol 0.25 mg/kg b. wt administered intraperitoneally). The abdominal cavity was opened. Tube embolectomy 4 Ch was introduced into the stomach via the mouth. Then, the stomach was resected about three-quarters along the probe and stapled from the side of greater curvature.

Gastric plication

The procedure was similar to the sleeve gastrectomy but part of stomach was folded about three-quarters along the probe to inside instead being resected. Then it was stapled from the side of greater curvature.

The rats were kept in single cages for the next 21 days. Then, animals were sacrificed by decapitation after 24 hours of starving. The following were collected: blood and representative portions of the stomach, duodenum, jejunum, ileum and ascending colon.

Determination of Met enkephalin, ghrelin, glucose and triglycerides concentrations

Plasma concentrations of glucose and triglycerides were determined with commercial kits (Pointe Sci. USA). Tissue concentrations of both Met-enkephalin and ghrelin in the different regions of the intestine were determined by radioimmunoassay following the method described by Pierzchala and Van Loon, (1990). Briefly, this entailed the following. Met-enkephalin was isolated using Porapak Q: column chromatography and elution with ethyl alcohol (99.8%). After lyophilization, protein was dissolved with phosphate buffer pH=6.5, antibody to Met-enkephalin (1:8000) and iodinated with ^{125}I opioid (2500 cpm) were added and incubated at 4°C for 18-20 h. The next day, second antibody (0.1% anti-rabbit gamma-globulin) was added and mixture was incubated for 30 min. Separation of the antibody and peptide complex was achieved with polyethylene glycol 8000 (20%) and centrifugation.

Ghrelin was estimated in the fragments of gastrointestinal tract by RIA using commercial kits (Phoenix, USA).

STATISTICAL ANALYSIS

Data were analyzed by one way analysis of variance with means separated by *post hoc* Tukey's range text. Results are showed as $\bar{x}$±SEM (n=6).

RESULTS

Plasma concentrations of glucose and triglycerides (Table 1)

Both gastric procedures resulting in the drastic decrease of the stomach volume by almost 70% were accompanied by changes in the plasma concentrations of glucose and triglycerides. Plasma concentrations of glucose were depressed by 9.2% in rats following sleeve gastrectomy and decreased by 24.3% (P<0.01) after gastric plication compared to controls. In contrast, plasma concentrations of triglycerides were increased (P<0.01) after GR and GP by respectively 58.4% and 53.2% [from 3.01±0.07 mmol/l in control rats to 4.77±0.23 (GR) and to 4.61±0.15 mmol/l (GP)].

TABLE 1. The effect of sleeve gastrectomy and gastric plication on the rat plasma levels of glucose and triglycerides ($\bar{x}$ ± SEM, *P<0.01 compared with control)

	Control	Sleeve Gastrectomy	Gastric Plication
Glucose mmol/l	4.11 ± 0.11	3.73 ± 0.09	3.11 ± 0.08*
Triglycerides mmol/l	3.07 ± 0.07	4.77 ± 0.11*	4.66 ± 0.12*

Tissue concentrations of Met-enkephalin and ghrelin (Fig. 1-3)

There were marked changes in the tissue concentrations of both Met-enkephalin and ghrelin in all regions of the gastro-intestinal tract examined. The results for, respectively, the stomach, small intestine and colon are summarized in Figures 1, 2 and 3. As might be expected, tissue concentrations of ghrelin in control rats were greater (P<0.001) in the stomach than in the small intestine which in turn are higher than in the colon (P<0.001). In contrast, tissue concentrations of Met-enkephalin in control rats were highest in the duodenum, declining (P<0.05) along the small intestine into the colon. Moreover, tissue concentrations of Met-enkephalin in control rats were lower (P<0.01) in the stomach than in the duodenum.

Stomach (Fig. 1A,B)

Tissue concentrations of ghrelin were lower (P<0.01) in the stomach pouch from GR and GP compared to control rats. The tissue concentrations of Met-enkephalin were depressed by 20.0% in the GR treatment group [from 4.01±0.16 pmol/g in control rats to 3.21±0.11 pmol/g in GR rats] and by 25.4% in the GP rats [declining from 4.01±0.16 pmol/g in control rats to 2.99±0.10 pmol/g in GP rats].

Following gastric plication, stomach tissue concentrations of ghrelin were decreased by 83.3% (P<0.001) [from 278.5±11.9 pmol/g in control rats to 46.6±5.3 pmol/g in GP rats]. Unexpectedly in GR rats, tissue concentrations of ghrelin in the stomach were depressed by 18% (P<0.05).

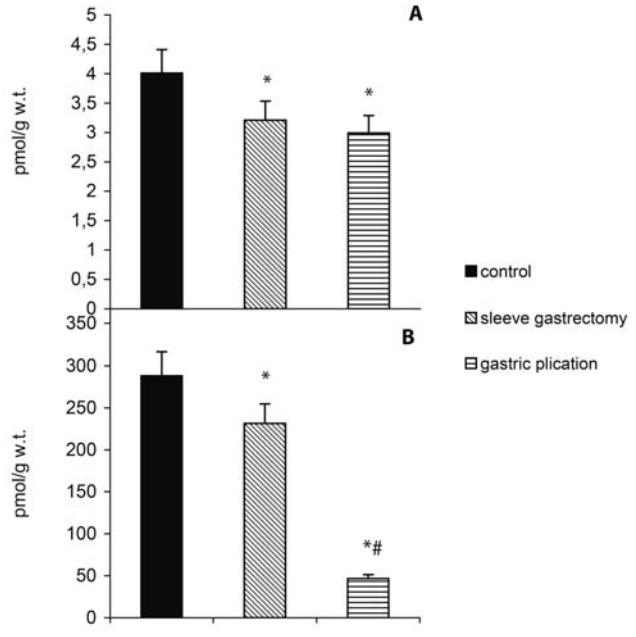

Fig.1. Met-enkephalin (A) and ghrelin (B) concentrations in rat stomach ($\bar{x}$±SEM, *P<0.05-0.001 compared with the control, #P<0.05 compared with sleeve gastrectomy).

Duodenum (Fig. 2A,B)

Tissue concentrations of Met-enkephalin in the duodenum of GP rats were somewhat elevated, albeit by only 6.9% [from 7.67±0.8 pmol/g in controls to 8.20±0.9 pmol/g in GP rats]. Similarly, tissue concentrations of ghrelin were increased by 57% (P<0.01) after gastric plication. There were no differences in duodenal concentrations of either ghrelin or Met-enkephalin after sleeve gastrectomy compared to control rats.

Jejunum (Fig. 2A,B)

Following sleeve gastrectomy, tissue concentrations of Met-enkephalin were slightly depressed (P<0.05) by 15.0% [declining from 6.01±0.3 pmol/g in control rats to 5.11±0.3 pmol/g in GR rats]. In contrast, after gastric plication, tissue concentrations of Met-enkephalin were increased (P<0.001) by 2.38 fold compared to those in the control rats [increasing from 6.01±0.3 pmol/g in control rats to 14.31±1.5 pmol/g in GP rats.

Jejunal concentrations of ghrelin were unaffected by sleeve gastrectomy. However, tissue concentrations of ghrelin were increased (P<0.001) by more than four-fold higher after plication [increasing from 21.14±1.23 pmol/g in control rats to 116.53±9.65 pmol/g in GP rats].

Ileum (Fig. 2A,B)

Ileal concentrations of Met-enkephalin were increased (P<0.001) in both GR and GP rats; the

increases being 202.13 % in GR [increasing from 3.75±0.29 pmol/g in control rats to 11.33±1.87 pmol/g in GR rats, P<0.001)] and 134.93 % in GP rats [increasing from 3.75±0.29 pmol/g in control rats to 8.81±0.91 pmol/g in GP rats (P<0.001)]. The difference between Met-enkephalin concentration in GR and GP rats was statistically significant (P<0.001). Tissue concentrations of ghrelin were greatly decreased in GR and GP rats; the decreases being 78.19 % in GR [decreasing from 66.11±5.93 pmol/g in control rats to 14.42±1.63 pmol/g in GR rats] and 84.98 % in GP rats [decreasing from 66.11±5.93 pmol/g in control rats to 9.93±1.15 pmol/g in GP rats].

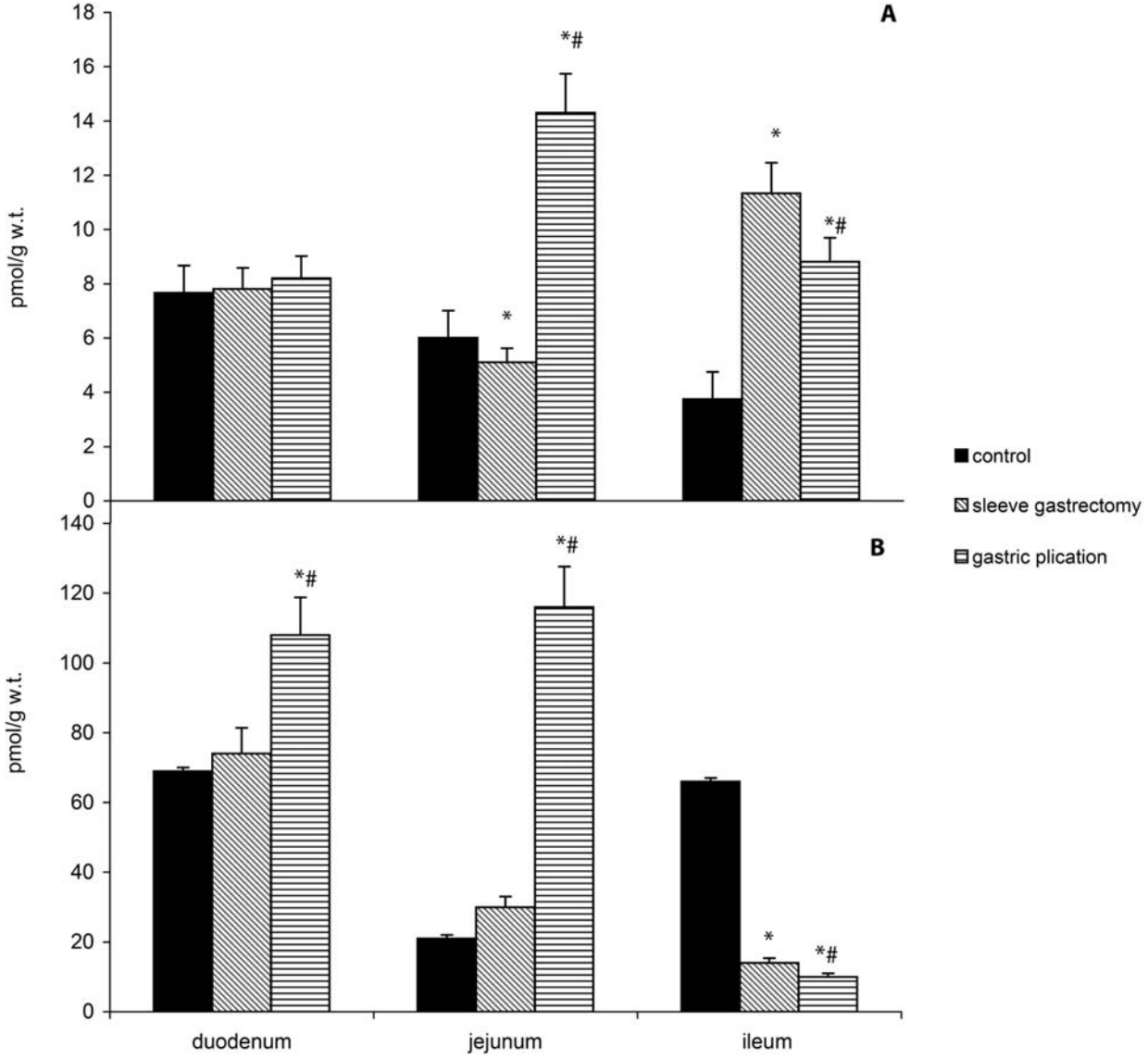

Fig. 2. Met-enkephalin (A) and ghrelin (B) concentrations in rat small intestine ($\bar{x}$±SEM, *P<0.05-0.001 compared with the control, #P<0.01 compared with sleeve gastrectomy).

Colon (Fig. 3A,B)

Tissue concentrations of Met-enkephalin in the colon were increased (P<0.001) by 2.18 fold following sleeve gastrectomy [increasing from 2.99±0.22 pmol/g in control rats to 6.53±0.60 pmol/g in GR rats]. There was no difference in the tissue concentration of Met-enkephalin in the GP and control rats. The tissue concentration of ghrelin was decreased by both surgical pro-

cedures by 58.93% in GR rats [decreasing from 7.11±0.59 pmol/g in control rats to 2.92±0.33 pmol/g in GR rats, (P<0.001)] and 30.37% in GP rats [decreasing from 7.11±0.59 pmol/g in control rats to 4.95±0.56 pmol/g in GP rats P<0.01)]. The difference between ghrelin concentration in GR and GP rats was statistically significant (P<0.05).

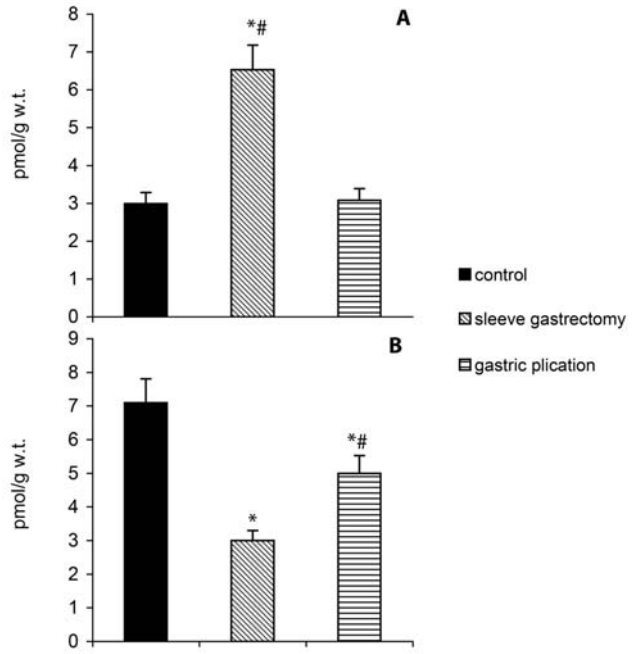

Fig.3. Met-enkephalin (A) and ghrelin (B) concentrations in rat ascending colon ($\bar{x}$±SEM, *P<0.05-0.001 compared with the control, #P<0.01 compared with sleeve gastrectomy).

DISCUSSION

Endogenous opioid peptides are synthesized and released from all parts of the gastrointestinal system and are involved into regulation of the intestinal motility, exogenous and endogenous gastrointestinal secretions and appetite. The highest concentration of Met-enkephalin has been found in the duodenum and was subsequently decreasing along the intestine by 22% in jejunum, by 21% in ileum and by 61% in colon of control rats. Concentration of opioid in the stomach was lower by 35% compare to the duodenum level. The uneven distribution of Met-enkephalin in tested fragments of intestines may suggest that these tissues process and/or metabolize enkephalin precursor to different degrees. Similar results were obtained by Kolta et al., (1992).

The ghrelin concentration was the highest in the stomach and similarly to the Met-enkephalin was decreasing along the intestine except the colon where the level was almost the same as in duodenum. It is probable that in addition to its roles in appetite regulation, immunomodulatory, anti-inflammatory and antioxidant actions ghrelin has protecive effects in the colon (Ceran et al.,2013).

Both bariatric surgeries significantly decreased the plasma level of glucose and unexpectedly increased plasma level of triglycerides. Daily feed intake was much higher in control animals than in rats after surgery [25.4 g/rat/day vs. 20.9 g/rat/day (unpublished data)]. Goldpaie et al., (2013) reported that bariatric surgery resulted in lowering plasma levels of fasting glucose and triglycerides in patients during six weeks period. Another study (Guimaraes et al., 2013) carried out on rats showed that 21 days after surgery, sleeve gastrectomy did not influenced the body composition and glucose, while gastric influence plication decreased body fat content.

Gastric sleeve gastrectomy lowered Met-enkephalin concentration in the remaining part of stomach and in jejunum, in contrast opioid level was increased in the ileum and colon what may suggest that in the distant parts of the intestine synthesis and processing of proenkephalin were increasing (Fig. 2A, 3A). Also, it seems probable that increased concentration of opioids may serve as: 1. analgesic; 2. antidiarrheal; 3. slowering motility and emptying stomach drug. All of these functions improve recovery of the gastro-intestinal tract after surgery and inhibit immune system preventing the inflammation (Holzer, 2010; Bochicchio et al., 2012; Holzer, 2014). Unexpectedly, gastric plication decreased Met-enkephalin concentration only in the stomach and increased in the duodenum, jejunum, and ileum what may suggest that such surgery is more severe than sleeve gastrectomy and stimulates synthesis and probably increases enzymatic hydrolysis of large enkephalin precursor. On the other hand, the activity of enkephalinases, which degrade endogenous opioids once they are secreted from neurons or other cells in the gastrointestinal tract might be decreased (Holzer, 2009).

There were large decrease in both stomach tissue concentrations of ghrelin (Figure 1B) and plasma concentrations of ghrelin following gastric plication. Similarly there was consistency between the small depression of stomach tissue concentrations of ghrelin and the absence of an effect on plasma concentrations of ghrelin following gastric sleeve gastrectomy in rats (Guimarães et al., 2013) and the small decrease in patients that had undergone laparoscopic Roux-en-Y gastric bypass surgeries (Leonetti et al., 2003).

There was some lack of consistency between the effects of sleeve gastrectomy and gastric plication on tissue concentrations of either ghrelin or Met-enkephalin. For instance, jejunal concentrations of Met-ekephalin were elevated by gastric plication but depressed by sleeve gastrectomy (Figure 2A). Similarly, colonic concentrations of Met-ekephalin were elevated by gastric plication but unaffected by sleeve gastrectomy (Figure 2A). The lack of an effect of sleeve gastrectomy on duodenal concentrations of ghrelin is similar to the recent report of a lack of a significant effect of sleeve gastrectomy on the number of ghrelin producing cells in the duodenum (Teive et al., 2012).

While gastric plication markedly decreased tissue concentrations of ghrelin in the stomach, there were reciprocal large increases in the tissue concentrations of ghrelin in the duodenum and jejunum. It is argued that the increase in duodenal and jejunal concentrations of ghrelin reflects a compensatory mechanism with potentially circulating and/or lumenal ghrelin of stomach origin suppressing ghrelin production in the upper small intestine. The physiological mechanisms underlying this remain to be elucidated. In line with the present data, plasma concentrations of acylated ghrelin are elevated in people with chronic atrophic gastritis (Campana et al., 2007).

It was interesting that gastric plication was associated frequently with changes in the tissue concentrations of both Met-enkephalin and ghrelin [e.g. increases in both in the jejunum and duodenum, large increases in tissue concentrations of Met-enkephalin and large decreases in tissue concentrations of ghrelin in the duodenum and decreases in both in the stomach (Figure 2)]. This is at least suggestive of some linkage between gastro-intestinal production of ghrelin and that of Met-enkephalin, perhaps involving the same control mechanism.

To conclude, our results indicate that Met-enkephalin and ghrelin synthesized at the gastro-intestinal tract might regulate the mechanism of recovery and mitigate the negative effects of gastric surgery.

To the best of our knowledge, this is the first report on changes in ghrelin and Met-enkephalin in different regions of the gastro-intestinal tract following sleeve gastrectomy and gastric plication in any species. However, the physiological mechanisms underlying their interaction remain to be elucidated.

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