

## ORIGINAL ARTICLE

# Treatment of morbid obesity by intraparietogastric administration of botulinum toxin: a randomized, double-blind, controlled study

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**Objective:** The stomach is the main target organ for bariatric surgery, but no medical treatment has been developed to increase satiety and decrease food intake via gastric pathways. The aim of our study was to investigate whether or not the intraparietogastric administration of botulinum toxin A (BTX), able to modify the motility patterns of the stomach, could be useful for treatment of obesity.

**Design:** Double blind controlled study.

**Subjects:** Twenty-four morbidly obese patients (mean weight (s.e.m.)  $116.1 \pm 4.89$  kg, mean body mass index (BMI)  $43.6 \pm 1.09$  kg/m<sup>2</sup>) were blindly randomized to receive 200 IU BTX or placebo into the antrum and fundus of the stomach by intraparietal endoscopic administration.

**Measurements:** We evaluated weight loss, BMI changes, satiety score, the maximal gastric capacity for liquids and the gastric emptying time (octanoic acid breath test).

**Results:** The two groups were homogeneous for anthropometric characteristics. Eight weeks after treatment, BTX patients had significantly higher weight loss ( $11 \pm 1.09$  vs  $5.7 \pm 1.1$  kg,  $P < 0.001$ ) and BMI reduction ( $4 \pm 0.36$  vs  $2 \pm 0.58$  kg/m<sup>2</sup>,  $P < 0.001$ ) and a higher satiety score on a visual analogic scale ( $7.63 \pm 0.38$  vs  $4.72 \pm 0.44$ ,  $P < 0.001$ ) than controls. Furthermore, BTX patients showed a significantly greater reduction in maximal gastric capacity for liquids ( $266.6 \pm 48$  vs  $139 \pm 31$ ,  $P < 0.001$ ) and a greater prolongation in gastric emptying time ( $+18.93 \pm 8$  vs  $-2.2 \pm 6.9$  min,  $P < 0.05$ ). No significant side effects or neurophysiologic changes were found.

**Conclusions:** Topical intragastric BTX was effective in reducing food intake and body weight in morbidly obese patients. *International Journal of Obesity* (2007) 31, 707–712. doi:10.1038/sj.ijo.0803451; published online 26 September 2006

**Keywords:** botulinum toxin A; gastric emptying; weight loss; diet

## Introduction

Obesity is epidemic in the western countries. It affects more than 30% of the general population in USA and more than 10% in Italy.<sup>1,2</sup> Genetic, social, psychological and behavioural factors work in favour of obesity<sup>3</sup> and make it difficult both to prevent and to treat this condition. It is a paradox that,

despite the extensive research in the pharmacological treatment of obesity,<sup>4</sup> the number of drugs available for treatment in the past 10 years has decreased as the prevalence of obesity has increased.<sup>5</sup>

The target organ of pharmacological treatment is the central nervous system, and only recently research has focused on the gastrointestinal tract with the development of lipase inhibitors.<sup>6</sup> On the contrary, bariatric surgery is based on gut manipulation with restrictive or malabsorptive operations acting through reduction of the gastric reservoir or of nutrient transport across the intestinal barrier.<sup>7</sup> After bariatric surgery, morbidly obese patients experience reduced appetite and early satiety.<sup>7</sup> These effects are probably related to endocrine or paracrine effects of the operations. Vertical

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banded gastroplasty increases post-meal cholecystokinin plasma levels,<sup>8</sup> whereas Roux-en-Y gastric by-pass inhibits basal and post-prandial ghrelin plasma levels<sup>9</sup> and increases peptideYY (PYY) concentrations.<sup>10</sup> Jeuno-ileal by-pass increases cholecystokinin, motilin, glucagon-like peptide 1 and PYY,<sup>11</sup> delays gastric emptying and reduces hunger sensations. As cholecystokinin, ghrelin and PYY also influence gastrointestinal motility, it can be hypothesized that reduction of gastric emptying could contribute to the satiety effect of the operations. All these data suggest that reducing gastric emptying could be beneficial for body weight loss in patients who follow a strict hypocaloric diet.

Botulinum toxin A (BTX) has long been used to induce functional denervation of the voluntary muscles<sup>12</sup> and has recently been used to treat many gastrointestinal disorders, like achalasia and gastroparesis.<sup>13</sup> The effect of BTX is strictly related to the site of administration: when the oesophageal or the pyloric sphincter is injected, motility is increased. We postulated that administration of BTX in the antrum and fundus of the stomach of the obese patients could reduce gastric emptying and make it easier to adhere to dietary prescriptions. In this paper, we describe the results of a double-blind, placebo-controlled study describing the ability of BTX to increase satiety and induce weight loss in obese patients.

## Patients and methods

The study was performed at the L Sacco Hospital and the S Siro Clinical Institute between January and November 2004. All morbidly obese patients who required bariatric surgery to reduce body weight were examined according to the admission criteria. The protocol was approved by the Ethic Committee of the L Sacco Hospital and conducted according to the Declaration of Helsinki Principle. All patients gave their informed consent to the diagnostic and therapeutic procedures.

### Admission criteria

Thirty morbidly obese patients between 18 and 65 years, with body mass index (BMI)  $> 35 \text{ kg/m}^2$  and two complications of obesity or with BMI  $> 40 \text{ kg/m}^2$  without complications, were admitted to the study.

**Selection of patients.** A history of neoplasia, pregnancy (also potentially), surgery of the stomach or gastrointestinal diseases was considered an exclusion criterion. A recent ( $< 3$  months) antiobesity treatment was also considered a contraindication for participating to the study.

All patients had a preliminary interview by a dietitian to assess their eating behaviour and to exclude heavy binge eating. During the first week, they completed a diet diary to evaluate the amount of introduced calories and proportion of fat, protein and carbohydrate.

They had also a neurological evaluation to exclude patients with previous botulism, neurological or neuromuscular diseases.

All patients had a structured clinical interview conducted face to face by a psychiatrist, using the Italian version of the Mini International Neuropsychiatric Interview, to exclude severe psychiatric illnesses (including psychotic disorders, current mood and anxiety disorders, bulimia, alcohol or substance abuse or dependence) or heavy binge-eating disorder, as conditions reducing the ability to follow the prescribed diet. Finally, all patients had a clinical interview and specific blood tests to exclude any possibility of endocrine obesity. Two patients with minor hypothyroidism had replacement therapy before admission to the therapeutic procedure.

On the basis of the contraindications that emerged during the preliminary clinical examinations, five patients were excluded from the study. One more patient withdrew her consent. Twenty-four patients were admitted to the diagnostic and therapeutic procedures.

### Diagnostic procedures and evaluation criteria

**Clinical measurements.** Body weight and height were measured at the first visit with the patient wearing light clothing and no shoes. BMI was also calculated as body weight (kg)/height (m)<sup>2</sup>. Waist circumference was measured at the minimal circumference between iliac crest and last rib edge. Hip circumference was measured at the greatest circumference through the major trochanters. Blood pressure and heart rate were also assessed.

Routine blood tests were performed after a 12 h overnight fast, the day after. Serum levels of total cholesterol, high-density lipoprotein cholesterol, triglycerides, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transpeptidase, bilirubin, creatinine, alkaline phosphatase, total protein and red blood cells, white blood cells and platelet count were determined.

**Satiety evaluation:** The patients were asked to describe their satiety to the standard dinner prescribed for the day before the visit using a graphic rating scale that combined verbal descriptors on 0–100 mm scale: 0, hunger; 20, no satiety; 40, mild satiety; 60, moderate satiety; 80, high satiety; and 100, complete fullness. The dinner was equivalent in kilocalories to that prescribed during the period of diet restriction after treatment. The assessment was performed at home to avoid the influence of personal attitudes about time, flavouring and good cooking of foods, as these factors cannot be optimized for hospital meals.

On three separate days, the patients also performed the following tests as outpatients:

**Maximal liquid gastric capacity test:** As the patients had a liquid diet after the procedure, a maximal liquid gastric capacity test was performed. Patients were asked to drink a liquid solution (Osmolite HR, Abbott Park, IL, USA; (protein 30.3%, lipid 17.6%, carbohydrate 53%; osmolality

244 mosM/l), diluted 1:1 in water, 1 ml=0.5 kcal, at a constant rate (30 ml/min) until they felt a complete fullness sensation or abdominal discomfort. The results were expressed as kilocalories.

**Gastric emptying evaluation:** All tests were performed after an overnight fast. The test meal consisted in a standard bun (5.8% protein, 33.3% lipid and 57.5% carbohydrate, 378 kcal), with 100 mg of 2  $\mu$ Ci of octanoic acid ( $^{13}$ C) added. The test meal was followed immediately by 250 ml of water. Breath samples were taken immediately before the test meal and every 30 min thereafter for 3 h. Samples were analysed by a mass spectrometer (Breath mass plus, Finnigan, Germany) to measure  $^{13}$ CO<sub>2</sub>. The results were measured as half-emptying time (min) and lag-time (min).

**Neurophysiologic evaluation:** Distant effects of intragastric BTX administration were assessed by studying neuromuscular transmission. Abductor Digiti Minimi compound muscle action potential (CMAP) was recorded after stimulation of the right ulnar nerve according to Desmedt and Borenstein<sup>14</sup> Extensor digitorum (EDC) muscle fibre jitter was measured by single fibre electromyograph according to Ekstedt *et al.*<sup>15</sup> Jitter is the variability of the interpotential interval between two or more single muscle fibres belonging to the same motor unit. The jitter is expressed as the mean value of consecutive differences of successive interpotential intervals.

#### Study design

After completion of the diagnostic procedures, patients were randomized in a double-blind manner to receive intragastric administration of BTX (200 IU) or placebo (saline).

#### Therapeutic procedure

All therapeutic procedures were performed between 1400 and 1500 hours after a 6 h fasting. Under diazepam administration, an Olympus GIF IT 130 operative gastroscope was used for a preliminary evaluation of the oesophagus, stomach and duodenum. Seventeen patients had sliding hiatus hernia, in three cases associated with esophagitis, three patients had antral gastritis and one patient had pseudopolyps of the fundus. One patient had a gastric ulcer and another duodenitis suggesting Crohn's disease. These last two patients had negative biopsies and were treated with Proton Pump Inhibitors for 45 days before the procedure was successfully performed.

To inject BTX (Botox, Allergan, Irvine, CA, USA, 10IU in 0.5 saline) or saline (0.5 ml), we used an Endoflex needle, 2.3 mm in thickness and 230 cm in length. The microinjections were performed at the four cardinal points starting 3 cm from the pyloric ring, and repeated three times moving 2 cm towards the angulus each time. Four more microinjections were performed 2 cm below the cardias, and the last four doses were injected into the fundus of the stomach. The total dose was 200IU.

The time to accomplish the procedure was always less than 10 min. No significant acute side effects were recorded. All patients were observed for 1 h. Only one patient complaining of abdominal pain had to be observed for 18 h.

#### Post-endoscopic course

All patients had blood pressure and heart rate monitoring for 3 days. Fatigue, nausea, vomiting and diarrhoea were also investigated.

#### Diet

A 1200 kcal liquid diet (protein 15%, lipid 33%, carbohydrate 52%) was prescribed starting immediately after the endoscopic procedure. The diet was based on a personal choice between several market components for each, with a weekly balance between the daily meals. The patients were instructed to follow the diet for the 8 following weeks.

#### Follow-up

Every week, the patients attended our outpatient department to evaluate the satiety to the last standard dinner, the body weight, BMI, waist and hip circumference. The neurophysiologic test was performed 8 days after the injection of the tested substances. Final body weight measurement, anthropometric measures, maximal liquid gastric capacity test, octanoic acid breath test and blood tests were performed 8 weeks after treatment. All measurements were performed by an observer unaware of the treatment performed.

#### Statistical analysis

Results were expressed as mean  $\pm$  s.e.m. Differences between groups were compared using a two-tailed Student's *t*-test for unpaired data. Differences between times in the same group were compared using a two-tailed Student's *t*-test for paired data. Gastric emptying time differences between groups were evaluated using the rank test of Wilcoxon. Differences with  $P < 0.05$  were considered significant.

## Results

All the patients completed the diagnostic and therapeutic procedures. There were no withdrawals during the study.

The two treatment groups were homogeneous for age (BTX: 40.6  $\pm$  3.5 years; placebo: 45.2  $\pm$  3.7 years), sex ratio (BTX: M/F = 0.2; placebo: M/F = 0.3) and anthropometric characteristics (Table 1).

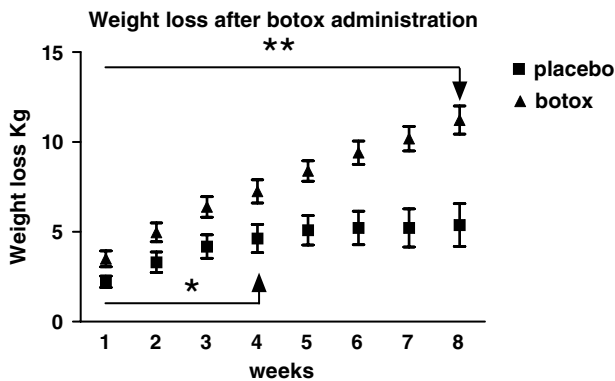
#### Acute side effects and neurophysiologic assessment

There were no acute side effects after BTX administration. Blood pressure was slightly decreased (-10 mm Hg) after treatment. Heart rate was unchanged. No patient experienced fatigue, nausea, vomiting or diarrhoea.

**Table 1** Anthropometric effects of BTX in obese patients

	BTX		Placebo	
	Pre	Post	Pre	Post
Weight (kg)	115.98±4.7	104.9±4.3*	116.25±5.2	110.77±4.88*
BMI (kg/m <sup>2</sup> )	42.5±1.4	38.5±1.4*	44.1±1.52	42.1±1.33*
Waist circumference (cm)	130.37±3.53	122.5±3.84*	129.75±4.17	125.2±3.38*
Hip circumference (cm)	125.67±2.65	121.92±2.57*	129.6±2.69	128.3±1.16 n.s.
Body weight reduction (kg)		11±1.09		5.7±1.1**
BMI reduction		4±0.36		2±0.58**
Waist circumference reduction (cm)		7.87±1.35		4.55±1.31
Hip circumference reduction (cm)		3.75±0.75		1.1±1.4

Abbreviations: BMI, body mass index; BTX, botulinum toxin A. \* $P < 0.001$  between times. \*\* $P < 0.001$  between groups.



**Figure 1** Reduction in body weight after treatment. Patients treated with BTX experienced weight loss throughout the study, with significant differences between each time of observation. Patients treated with placebo had a significant weight loss only in the first 4 weeks after treatment.

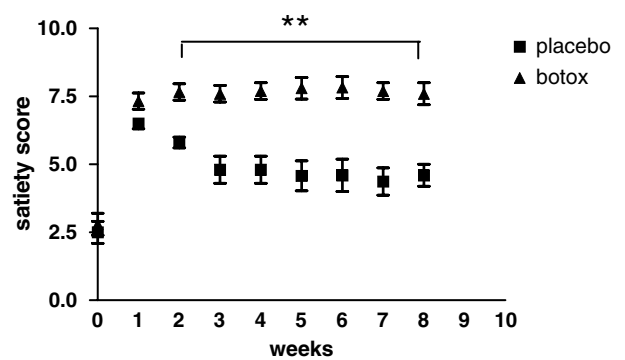
No subject in either BTX or placebo group presented abnormal values of CMAP decrement at any stimulation rate, CMAP incremental response during high-frequency stimulation or Jitter MCD before or after treatment.

#### Anthropometric changes

Significant reduction in body weight was evident in both groups from the first week after the procedure (Figure 1, Table 1). Patients treated with placebo had a significant weekly decrease of body weight for the first month, and patients treated with BTX for the whole study time. At the end of the study, patients treated with BTX had a greater reduction of body weight ( $11 \pm 1.09$  vs  $5.7 \pm 1.1$  kg,  $P < 0.0006$ ) and BMI ( $4 \pm 0.36$  vs  $2 \pm 0.58$  kg/m<sup>2</sup>,  $P < 0.001$ ) than the control group. There were no significant differences in waist and hip reduction between the two groups of treatment.

#### Satiety

Both treatments induces a higher satiety in comparison to the baseline values ( $P < 0.001$ ) (Figure 2). In BTX-treated patients, there were no differences between the scores



**Figure 2** Satiety, scored on a visual analogical scale, was significantly increased by the intraparietogastric injection of saline and BTX. Patients treated with BTX showed long-time significant effects, whereas patients treated with placebo experienced satiety only in the first 2 weeks after treatment. \*\* $P < 0.01$ .

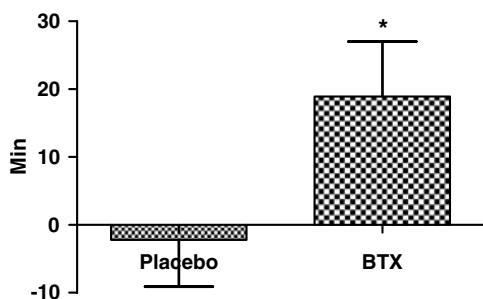
obtained at any time after treatment, whereas a significant reduction of effects was found in placebo-treated patients from the third week (3rd week vs 1st week,  $P < 0.01$ ). A significant difference between groups was evident after 2 weeks ( $P < 0.01$ ) and persisted throughout the study.

#### Gastric capacity test

The maximal gastric capacity for liquids was  $545 \pm 48$  kcal in BTX- and  $475.8 \pm 28$  kcal in placebo-treated patients. After 8 weeks, it was reduced to  $275.2 \pm 28.8$  and to  $332.64 \pm 34$  kcal, respectively, with a significant difference between times both in BTX- ( $P < 0.004$ ) and placebo- ( $P < 0.002$ ) treated patients. The reduction in the total liquid amount was in favour of BTX ( $266 \pm 48$  vs  $139 \pm 31$  kcal,  $P < 0.001$ ).

#### Gastric emptying test

The half-emptying time of the stomach 8 weeks after treatment was significantly delayed in patients treated with BTX ( $+18.93 \pm 8$  min) in comparison to patients treated with placebo ( $-2.2 \pm 6.9$  min,  $P < 0.05$ , rank test of Wilcoxon) (Figure 3).



**Figure 3** Gastric emptying, assessed by octanoic acid breath test, significantly increased in BTX patients ( $P < 0.05$ ) 8 weeks after treatment.

## Discussion

The gut plays a major role in the regulation of eating and energy balance,<sup>16</sup> mainly as a diffuse endocrine organ able to act on the central nervous system and the adipose tissue through a network of hormones, which are able to induce appetite or satiety while stimulating or inhibiting the motor activity of the gastrointestinal tract.

Cholecystokinin,<sup>17</sup> ghrelin<sup>18</sup> and PYY peptide<sup>19</sup> levels are related to the fasting–eating cycle and to the motor function of the gastrointestinal tract. Duodenal infusion of lipids, like triglycerides<sup>20</sup> or fatty acids,<sup>21</sup> induces early satiety and causes both gastric relaxation and reduction of gastric emptying at least by the release of cholecystokinin. These relationships suggest that satiety mediated by cholecystokinin<sup>22</sup> and PYY<sup>23</sup> administration is related to motility changes of the gastrointestinal tract. Inhibition of the motility of the stomach could be effective in reducing food intake. Gui *et al.*<sup>24</sup> demonstrated that BTX, a protein known to inhibit transport of the synaptic vesicles and to reduce striated muscle contraction, administered into the gastric wall of rats diminishes food intake and inhibits the increase of body weight with a statistically significant effect compared to the control (sham-operated) group. Coskun *et al.*<sup>25</sup> found that this effect on body weight was related to a significant reduction of gastric emptying.

In 2003, Rollnik *et al.*<sup>26</sup> reported a single case of a patient treated with intraparietogastric antral injection of BTXA (100 IU). After 4 months he had a 32.5% reduction in daily calorie intake and a 9% weight loss. Two further open studies<sup>27,28</sup> and a double-blind trial<sup>29</sup> extended this observation, but in the series of Garcia-Compean *et al.*<sup>27</sup> no significant effects on body weight and gastric emptying were observed. As the fundus of the stomach is the main source of ghrelin<sup>30</sup> and has a sensory activity<sup>31</sup> regulating the total gastric capacity, we modified the technique described by Rollnik and included the fundus as a target area for BTX injection. Our results confirm the observation of Rollnik *et al.*<sup>26</sup> and demonstrate, in a double-blind, placebo-controlled trial, that inhibition of the cholinomimetic

synapses of the stomach in the antrum and fundus cause a reduction of the threshold of satiety in obese patients. Motility of the stomach changed with emptying inhibition and early adaptation to reduced food intake. Satiety (measured in visual analogue scale) after a standard meal strongly increased after just a week and persisted during the follow-up. The strong difference in satiety between BTX- and placebo-treated patients caused a significant reduction of body weight, with a mean decrease  $> 1$  kg/week. Patients treated with placebo only also showed a significant weight reduction. The effect on BMI was also significant, as expected, and was higher for patients treated with BTX. On the contrary there were no differences between the two groups in waist and hip circumference.

The changes in the anthropometric measures of obesity of patients treated with BTX were related to a major change in their ability to feel satiety. We found that the maximal capacity of the stomach for liquid foods was reduced at a higher level when BTX was given. Although there is no agreement that the maximal gastric capacity of obese patients is increased,<sup>31–34</sup> it is likely that BTX acts also by reducing it. We think that BTX reduces the compliance of the gastric wall to the caloric load, but we did not study this aspect. Further investigations are needed to establish the effect of BTX on the tone of the gastric wall. Furthermore, the emptying test with monoctanoic acid demonstrated a significant increase of the emptying half-time in comparison to placebo. It is unlikely that prolongation of the half-emptying time as short as 18 min could be the cause of the increased post-prandial satiety and of the reduction in food intake. It is true that other topical therapies like gastric pacing<sup>32</sup> cause a significant delay in the gastric emptying while inducing a lower threshold for satiety. However, they probably act also on the capacity of the stomach. Finally, there is no correlation between a shorter gastric emptying time and body weight,<sup>34,35</sup> as many patients (also in our study) had a rather long emptying time. The mechanism of the reductive effect of BTX on food intake needs more investigations.

It is also important to stress that intraparietogastric administration of BTX to reduce body weight of obese patients is very safe. There were no significant side effects and, although distant effects have been reported for BTX administration into the striated muscle,<sup>36,37</sup> our neurophysiologic tests exclude that this is the case after intraparietogastric administration.

Finally, we would like to consider the main objection to the use of BTX to reduce the gastric capacity and to increase after-meal satiety: the short-lasting effect. In this study, we planned an observation time of 2 months, because in other therapeutic applications to gastrointestinal diseases, like achalasia and gastroparesis, BTX has a limited time of action.<sup>13</sup> The effect of BTX was fully evident for 8 weeks. Several patients agreed to extend the follow-up, but all except one lost no further weight after 3 months. Therefore, the effect of a one-time administration is limited. For this

reason, we are currently investigating whether or not repeated administrations could be effective in a long-term fight against obesity.

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## References

- Flegal KM, Carroll MD, Odgen CL, Johnson CL. Prevalence and trends in obesity among US adults 1999–2000. *JAMA* 2002; **288**: 1723–1772.
- Obesità. <http://www.obesita.org>.
- Friedman JH. A war on obesity, not the obese. *Science* 2003; **299**: 856–858.
- Bays HE. Current and investigational antiobesity agents and obesity therapeutic treatment targets. *Obes Res* 2004; **12**: 1197–1211.
- Mum EC, Blackburn GL, Matthews JB. Current status of medical and surgical therapy for obesity. *Gastroenterology* 2001; **120**: 669–681.
- Van Gaal L, Martens I, Bellaux D, Verdake HJ. Modern, new pharmacotherapy for obesity. A gastrointestinal approach. *Best Pract Res Clin Gastroenterol* 2004; **18**: 1049–1072.
- Cummings DE. Role for ghrelin in the regulation of appetite and body weight. *Arch Surg* 2003; **138**: 389–395.
- Foschi D, Corsi F, Pisoni L, Vago T, Bevilacqua M, Asti E *et al*. Plasma cholecystokinin levels after vertical banded gastroplasty. Effects of an acidified meal. *Obes Surg* 2004; **14**: 644–647.
- Cummings De, Weigle DS, Frayo S, Breen PA, Ma MK, Dellinger EP *et al*. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *New Engl J Med* 2002; **366**: 1623–1630.
- Korner J, Bessler M, Cirilo LJ, Cornwell IM, David A, Restuccia NL *et al*. Effects of Roux-en-y gastric by-pass surgery on fasting and postprandial concentrations of plasma ghrelin, peptide YY and insulin. *J Clin Endocrinol Metab* 2005; **90**: 359–365.
- Naslund E, Gryback P, Hellstrom PM, Jacobson H, Holst J, Theodorsson E *et al*. Gastrointestinal hormones and gastric emptying 20 years after jejunioileal by-pass for massive obesity. *Int J Obes* 1997; **21**: 387–392.
- Mahant N, Clouston PD, Lorentz IT. The current use of botulinum toxin. *J Clin Neurosci* 2000; **7**: 389–394.
- Zhao X, Pasricha PJ. Botulinum toxin for spastic GI disorders: a systematic review. *Gastrointest Endosc* 2003; **57**: 219–235.
- Desmedt JE, Borenstein S. Diagnosis of myasthenia gravis by nerve stimulation. *Ann NY Acad Sci* 1976; **274**: 174–188.
- Ekstedt J, Nillson G, Stalberg E. Calculation of the electromyographic jitter. *J Neurol Neurosurg Psychiatry* 1974; **37**: 526–539.
- Badman MK, Flier JS. The gut and energy balance: visceral allies in the obesity wars. *Science* 2005; **307**: 1909–1914.
- Debas HT, Farooq O, Grossman MI. Inhibition of gastric emptying is a physiological action of cholecystokinin. *Gastroenterology* 1975; **68**: 1221–1227.
- Asakawa A, Inni A, Kaga T, Yuzuriha H, Nagata T, Veno N *et al*. Ghrelin is an appetite-stimulatory signal from stomach with structural resemblance to motilin. *Gastroenterology* 2001; **120**: 337–345.
- Imamura M. Effects of surgical manipulation of the intestine on peptide YY and its physiology. *Peptides* 2002; **23**: 403–407.
- Barbera R, Peracchi M, Brighenti F, Cesana B, Bianchi PA, Basilisco G. Sensations induced by medium and long chain triglycerides: role of gastric tone and hormones. *Gut* 2000; **46**: 32–36.
- Lal S, Mc Laughlin J, Barlow J, D'Amato M, Giacobelli G, Varro A *et al*. Cholecystokinin pathways modulate sensations induced by gastric distension in humans. *Am J Physiol* 2004; **287**: G72–G79.
- Lieverse RJ, Jansen JB, Haslee AA, Lamers CB. Satiety effects of a physiological dose of cholecystokinin in humans. *Gut* 1995; **36**: 176–179.
- Batterham RL, Cohen MA, Ellis SA, Le Roux CV, Withers DJ, Frost GS *et al*. Inhibition of food intake in obese subjects by peptide YY (3–36). *New Engl J Med* 2003; **349**: 941–948.
- Gui D, De Gaetano A, Spada SL, Viggiano A, Cassetta E, Albanese A. Botulinum toxin injected in the gastric wall reduces body weight and food intake in rats. *Aliment Pharmacol Ther* 2000; **14**: 828–834.
- Coskun H, Duran Y, Dilege E, Mihmanli M, Seymen H, Demirkol MO. Effect on gastric emptying and weight reduction of botulinum toxin-A injection into the gastric antral layer: an experimental study in the obese rat model. *Obes Surg* 2005; **15**: 1137–1143.
- Rollnik J, Meier P, Manns M, Goke M. Antral injections of botulinum A toxin for the treatment of obesity. *Ann Intern Med* 2003; **138**: 359–360.
- Garcia-Compean D, Mendoza-Fuerte E, Martinez IA, Villareal I, Maldonado H. Endoscopic injection of botulinum toxin in the gastric antrum for the treatment of obesity. Results of a pilot study. *Gastroenterol Clin Biol* 2005; **29**: 789–791.
- Albani G, Petroni ML, Mauro A, Liuzzi A, Lezzi G, Verti B *et al*. Safety and efficacy of therapy with botulinum toxin in obesity: a pilot study. *J Gastroenterol* 2005; **40**: 833–835.
- Gui D, Mingrone G, Valenza V, Spada PL, Mutignani M, Runfola M *et al*. Effect of botulinum toxin antral injection on gastric emptying and weight reduction in obese patients: a pilot study. *Alim Pharmacol Ther* 2006; **23**: 675–680.
- Fruhbeck G, Diez-Caballero A, Gil MJ, Montero I, Gomez-Ambrosi J, Salvador J *et al*. The decrease in plasma ghrelin concentrations after bariatric surgery depends on the functional integrity of the fundus. *Obes Surg* 2004; **14**: 606–612.
- Kim DY, Camilleri M, Murray JA, Stephens DA, Levine JA, Burton DD. Is there a role for gastric accommodation and satiety in asymptomatic obese people? *Obes Res* 2001; **9**: 655–661.
- Xing J, Chen JDZ. Alterations of gastrointestinal motility in obesity. *Obes Res* 2004; **12**: 1723–1732.
- Geliebter A, Hassid G, Hashim SA. Test meal intake in obese binge eaters in relation to mood and gender. *Int J Eat Disord* 2001; **29**: 488–494.
- French JS, Murray B, Rumsy RDE, Sepple CP, Read NW. Preliminary studies on the gastrointestinal responses to fatty meals in obese people. *Intern J Obes* 1993; **17**: 295–300.
- Verdich C, Madsen JL, Toubro S, Buemann B, Holst JJ, Astrup A. Effect of obesity and major weight reduction on gastric emptying. *Int J Obes* 2000; **24**: 899–905.
- Backheit AMO, Ward CD, McLellan DL. Generalized botulism-like syndrome after intramuscular injections of botulinum type A: a report of two cases. *J Neurol Neurosurg Psychiatry* 1997; **62**: 198.
- Lange DJ, Brin MF, Warner CL, Fahan S, Lovelace RE. Distant effects of local injection of botulinum toxin. *Muscle Nerve* 1987; **10**: 552–555.