

Increase in Ghrelin Levels After Weight Loss in Obese Zucker Rats is Prevented by Gastric Banding

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Published online: 30 November 2007
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Abstract

Background Gastric banding is thought to decrease appetite in addition to the mechanical effects of food restriction, although this has been difficult to demonstrate in human studies. Our aim was to investigate the changes in orexigenic signals in the obese Zucker rat after gastric banding.

Methods Obese Zucker rats (*fa/fa*) were submitted to gastric banding (GBP), sham gastric banding fed ad libitum (sham), or sham operation with food restriction, pair-fed to the gastric banding group (sham-PF). Lean Zucker rats (*fa/+*) were used as additional controls. Body weight and food intake were daily recorded for 21 days after surgery when epididymal fat was weighed and fasting ghrelin and hypothalamic NPY mRNA expression were measured.

Results Gastric banding in obese Zucker rats resulted in a significant decrease of cumulative body weight gain and food intake. Furthermore, gastric banded rats were leaner than Sham-PF, as expressed by a significantly lower epididymal fat weight. Ghrelin levels of gastric banded rats were not increased when compared to sham-operated

animals fed ad libitum and were significantly lower than the levels of weight matched sham-PF rats (1116.9 ± 103.3 g GBP vs 963.2 ± 54.3 g sham, $3,079.5 \pm 221.6$ sham-PF and $2,969.9 \pm 150.9$ g lean rats, $p < 0.001$); hypothalamic NPY mRNA expression was not increased in GBP when compared to sham-operated rats.

Conclusion In obese Zucker rats, GBP prevents the increase in orexigenic signals that occur during caloric deprivation. Our data support the hypothesis that sustained weight loss observed after gastric banding does not depend solely on food restriction.

Keywords Obesity · Bariatric surgery · Gastroplasty · Food intake · Zucker rat

Introduction

Overweight and obesity have increased exponentially all over the world in the last few decades [1, 2]. As obesity is a chronic disease, often, there is weight regain after weight loss attained by most medical therapies [3, 4]. Bariatric surgery is the most effective treatment for severe obesity, as it allows sustained weight loss and improves most of the comorbidities associated with the disease [5].

Several studies have now shown that bariatric surgery, besides causing either food restriction and/or malabsorption, also induces a decrease in food intake [6–10] and changes metabolic pathways towards increased energy expenditure [11–13]. Moreover, there is growing evidence that bariatric surgery is able to interfere with the systems that regulate appetite and satiety [8–10, 14]. Gastrointestinal hormones are important signals that regulate food intake: changes in the levels of ghrelin, peptide YY (PYY), and glucagon-like peptide 1 (GLP-1) observed after

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obesity surgery have been hypothesized to mediate the anorexigenic effect of these surgical procedures [15]. However, results from several human studies have been contradictory [16–19]. There is, therefore, a need to test in animal models the hypothesis that decrease in orexigenic signals, coming from the gastrointestinal tract and hunger signals in the hypothalamus, mediate weight loss induced by gastric banding.

The obese Zucker rat (*fa/fa*) is the most widely used animal model of obesity. It is also better suited to study the effects of bariatric surgery than the normal weight Wistar rat, as it replicates most of the features observed in the common human obesity. The obese Zucker rat (*fa/fa*) is a spontaneous genetic model of obesity that exhibits hyperphagia, hyperinsulinemia, and hyperlipidemia without severe increase in plasma glucose levels. It carries a mutation of the gene encoding the leptin receptor that diminishes leptin signaling resulting in leptin resistance and consequently hyperphagia and positive energy balance [20–22]. Although mutations of the leptin receptor in the general human population are thought to be rare and unlikely to contribute to a significant proportion of cases of morbid obesity [23], for unknown reasons, leptin resistance is commonly found in most human obese individuals. The phenomenon is considered to be either polygenic, multifactorial, or acquired [24–26].

After having established an animal model of gastric banding in the normal weight Wistar rat [27, 28], our aim now was to apply the same surgical procedure to an obese animal and to investigate the metabolic and endocrine changes induced by gastric banding in the obese Zucker rat.

Materials and Methods

Animals

Forty male Zucker rats (8 weeks old), 28 obese (*fa/fa*), and 12 lean (*fa/+*) purchased from a commercial breeder (Charles River, Barcelona, Spain) were maintained in individual cages under controlled temperature (21–23°C), humidity, and light (12 h light, 12 h dark, lights on at 7:00 A.M.) with access to standard rat chow (A04, Panlab, s.l., Barcelona, Spain) and tap water. Animals were acclimatized to the local facilities for 7 days before surgery, and only healthy growing animals were used in the experiments. Obese Zucker rats were randomized into three weight-matched groups to be submitted either to gastric banding ($n=9$), sham gastric banding ($n=8$), and sham-operated pair-fed ($n=11$) to the gastric banding group, whereas all lean Zucker rats ($n=12$) were submitted to sham gastric banding to be used as lean controls. All procedures were approved by the local Ethics Board for

Animal Research and followed the European Union laws on animal protection (86/609/EC).

Gastric Banding

After an overnight 12-h fast, rats were anaesthetized by intraperitoneal injection of a mixture of ketamine 60 mg/kg (Imalgene 1000, Merial) and xylazine 12 mg/kg (Rompun, Bayer) according to body weight. Prophylactic antibiotherapy consisting of 1.25 mg ampicillin (Cilin, Quimedical) plus 1.25 mg flucloxacillin (Floxapen, GSK) diluted in 1 ml of sterile water was administered intraperitoneally and immediately before surgery. The surgical technique for gastric banding and sham surgery was performed as previously described for the Wistar rat [28]. Briefly, a midline abdominal incision was made and the stomach exposed to insert a 14-mm-long and 2-mm-wide silicon band, custom-made, at the glandular portion of the gastric fundus immediately below the rumen, partially restraining the stomach volume and creating upper and lower pouches in the stomach. To keep the band from sliding and from dislocation, two vertical stitches (Prolene 5/0, Ethicon, Edinburgh) were placed in the anterior view of the stomach wall near the lesser and the greater curvatures. The abdominal wall was closed with re-absorbable sutures (Vycril 3/0, Ethicon).

For the sham operation, the technique consisted of the same procedure described above except for the placing of a non-restraining band in the same location which was removed immediately before closure of the abdominal wall. Both groups of animals were given 5 ml sterile warmed saline subcutaneously to avoid dehydration and allowed to recover spontaneously from anesthesia and surgery. Rats were returned to their home cages which contained a pre-weighed amount of food.

Feeding Studies Protocols

Body weight was measured daily at 9:00 A.M. using a scale (Monobloc, Mettler, Toledo, USA) recording to the nearest 1 g, and the remaining food in the hopper was reweighed at the same time using a scale (Kern, KB 5000-1) recording to the nearest 0.1 g, which allowed daily food intake to be calculated. Feeding studies were started on the 14th day after surgery and were allowed an interval of three overnights in between studies for animal recovery from eventual study distress. All animals had ad libitum access to standard rat chow, except for the pair-fed group that was fed daily with the same amount eaten by animals submitted to gastric banding, except in the two feeding studies when they were fed ad libitum.

Nighttime feeding study: This animal study was carried in non-fasted rats starting out 1 h before the onset of dark phase

(6:00 P.M.); food intake was measured at 1 h before dark and 1, 2, 4, 8, 12 and 24 h after dark (7:00 P.M.). Fast and refeed study: Animals were fasted for 24 h before the study and refed in the early light phase with a pre-weighed amount of regular rat chow; food intake was measured 1, 2, 4, 8, and 24 h after refeeding by weighing the remaining food in the hopper.

Epididymal White Adipose Tissue Weight

At the end of the experiment, 21 days after surgery, 24 h fasted rats were killed by decapitation, and epididymal white adipose tissue pads were removed and weighed using a scale recording to nearest 0.001 g (Kern 440, Version 3.2).

Hormone Measurements

At time of killing, trunk blood was collected into chilled lithium heparin tubes containing a Kallikrein inhibitor, aprotinin (0.2 ml; Trasylol, Bayer, Portugal). Tubes were kept on melting ice until centrifuging at 3,000 rpm for 8 min at 4°C, and plasma was separated and kept at -20° until analysis. All samples were assayed in duplicate within one assay after a single freeze-thaw cycle. Hormone levels were analyzed by specific radioimmunoassays using commercially available kits for total ghrelin (GHRT-89HK, Linco Research, St. Charles, Mo., USA), PYY (RMPYY-68HK, Linco Research), GLP-1 (GLP1A-35HK, Linco Research), insulin (RI-13K, Linco Research), and leptin (RL-83K, Linco Research) according to the manufacturer's instructions. Glucose was analyzed in trunk blood before centrifuging by the glucose oxidase method using a glucometer (One Touch Ultra, Lifescan, Johnson and Johnson, Milipitas, CA). Triglycerides were analyzed by colorimetric test (VITROS Analyzer, Johnson & Johnson, UK).

Hypothalamic NPY Gene Expression

Brains were rapidly removed, after killing of the rats, and the basal hypothalamus was carefully dissected and frozen by immersion in liquid nitrogen. Samples were kept at -80°C until RNA extraction. Total RNA was extracted from frozen dissected hypothalami using Trizol Reagent (Invitrogen, Carlsbad, CA). Reverse transcription was carried out using optimized amounts (1 µg) of total RNA with a SuperScript first-strand synthesis system for reverse transcription-polymerase chain reaction (RT-PCR; Invitrogen) in a final volume of 20 µl. Semiquantitative RT-PCR was used to measure NPY mRNA in the basal hypothalamus. First-strand cDNA species were PCR amplified using the following primers, NPY, 5'-TGGACTGACCCTCGCTCTAT-3' and 5'-TGTCTCAGGGCTGGATCTCT-3'. Complementary DNA was also generated for the housekeeping gene β-actin using 5'-TGTCACCAACTGGGACGATA-3'

and 5'-TCTCAGCTGTGGTGGTGAAG-3'. For amplification of cDNA, PCR was performed with 1 µl template cDNA and 49 µl PCR mix. Optical density was calculated for the NPY gene product and the results expressed as the ratio of its density to that of the β-actin product.

Statistical Analysis

Analysis of variance was used for comparison of the means between the groups with post hoc least square difference test and Bonferroni correction to assess specific group comparisons where applicable. Calculations were made using the SPSS statistical package for Windows version 14.0. Results are shown as means±SEM, unless otherwise specified. A *p* value <0.05 was considered to be statistically significant.

Results

Body Weight and Food Intake After Gastric Banding

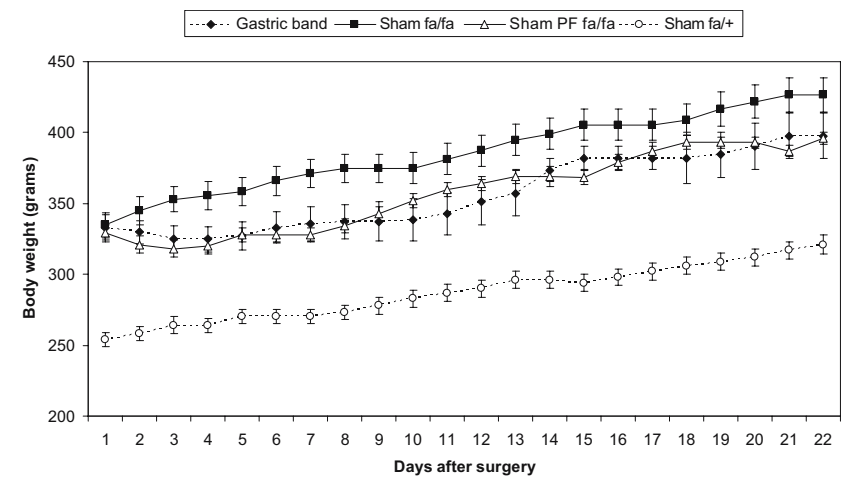
Gastric banding of obese Zucker rats resulted in a statistically significant decreases in body weight, in cumulative body weight gain (59.6±13.3 g vs 92.0±4.1 g for the control sham-operated rats; *p*=0.012; Fig. 1a and b) and in food intake (446.2±38.8 g vs 571.4±17.2 g for sham-operated rats; *p*=0.005; Fig. 2). Furthermore, the cumulative body weight gain of gastric banded obese Zucker rats was similar both to the one displayed by lean Zucker rats (*fa*/+) and by the food-restricted sham-operated obese rats pair-fed to the gastric banded animals (59.6±13.3 g vs 67.7±2.3 g for the sham-operated pair-fed rats and 66.4±2.0 g for lean controls).

Feeding Behavior: Nighttime and Fast and Refeed Feeding Studies

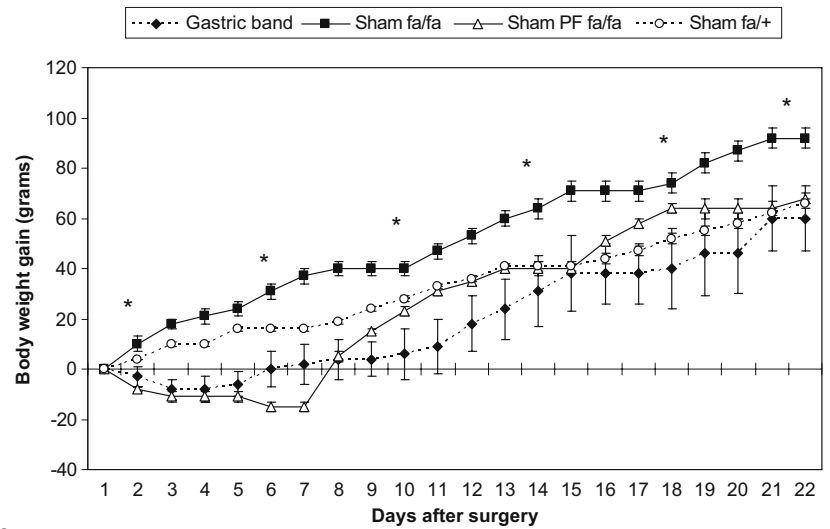
Under resting conditions, the rat feeding pattern of lean and obese controls was significantly different, as expected. The lean group had a lower food intake in comparison to that of obese animals of any other group (22.1±0.6 g vs 31.3±1.7 g for gastric banded rats, 31.4±0.8 g for sham-operated, 32.4±0.7 g for sham-operated pair-fed; *p*<0.05), except during peak feeding hours, i.e., in the first hour before and after dark. The food-restricted pair-fed obese rats, in the first peak feeding hour, showed higher food intake when compared to food intake of the other groups of animals, lean or obese (4.7±0.4 g vs 2.8±0.2 g for gastric banded rats, 3.0±0.4 g for sham-operated, 2.9±0.3 g for lean controls; *p*=0.032; Fig. 3a).

Obese rats submitted to bariatric surgery with gastric banding when compared to sham-operated animals had a different feeding behavior if refed after 24-h fasting. After

Fig. 1 a Absolute body weight of lean controls and obese Zucker rats after gastric banding, sham-operated fed ad libitum and sham-operated pair-fed to the gastric banded animals. **b** Cumulative body weight gain of lean controls and obese Zucker rats after gastric banding; sham operation fed ad libitum and sham operation pair-fed to the gastric banded animals. Body weight gain of obese Zucker rats was significantly higher than the other three groups of animals that displayed similar body weight gain (* $p < 0.05$)



a



b

refeeding, gastric banded obese rats showed statistically significant decrease in food intake during the first 2 h (5.3 ± 0.9 g vs 8.6 ± 0.6 g for sham-operated, 6.7 ± 0.5 g for sham-operated pair-fed; $p < 0.05$) and from 8 to 24 h after refeeding in comparison with sham-operated obese rats

(26.8 ± 3.6 g vs 33.9 ± 1.2 g for sham-operated, 32.4 ± 0.7 g for sham-operated pair-fed; $p < 0.05$). The feeding behavior displayed by the obese rats submitted to banded gastroplasty, after a 24-h fast, was similar to that of lean rats (Fig. 3b).

Fig. 2 Cumulative food intake of lean controls, obese Zucker rats gastric banded, and obese Zucker rats sham-operated fed ad libitum. Food intake of sham-operated rats fed ad libitum was significantly higher when compared to the other three groups of animals (** $p < 0.01$). Food intake of sham-operated pair-fed animals is not shown, as it was similar to the gastric banded rats

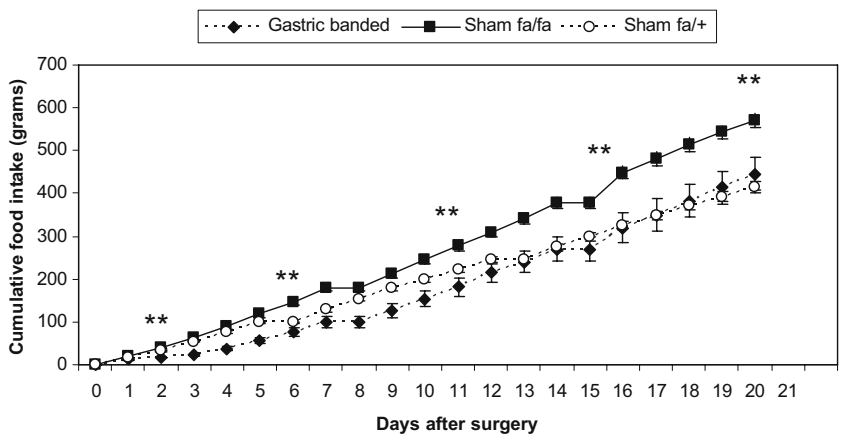
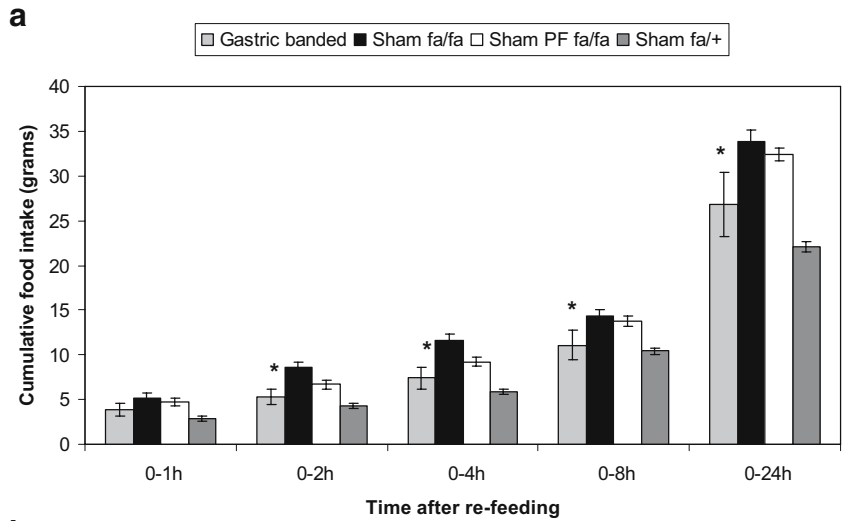
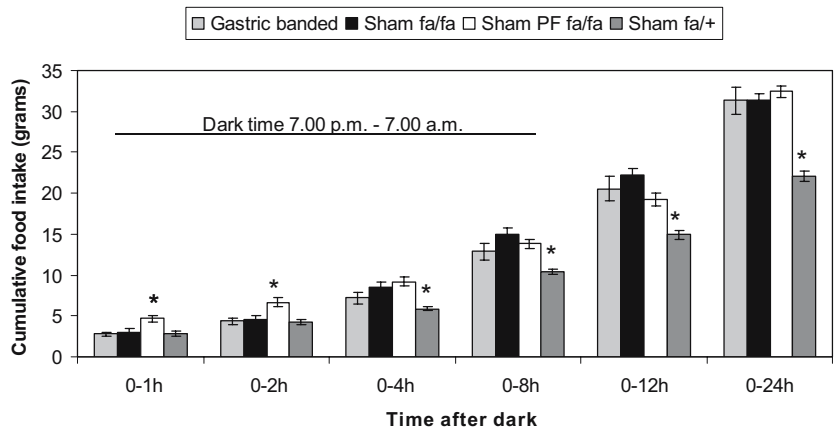


Fig. 3 a Twenty-four-hour cumulative food intake starting at peak feeding times, 1 h before dark phase, of lean controls and obese Zucker rats after gastric banding, sham-operation fed ad libitum and sham-operated pair-fed to the gastric banded animals ($*p < 0.05$). **b** Cumulative food intake after 24 h fast of lean controls and obese Zucker rats after gastric banding, sham operation fed ad libitum and sham-operated pair-fed to the gastric banded animals ($*p < 0.05$)



Body Composition

Fat mass, assessed by epididymal white adipose tissue (WAT) weight per 100 g of body weight, was lower in the lean rats (*fa/+*) than in obese Zucker rats (*fa/fa*; 0.67 ± 0.03 g vs 1.68 ± 0.1 g for gastric banded, 1.69 ± 0.1 g for sham-operated, and 1.92 ± 0.1 g for sham-operated pair-fed; $p < 0.001$). Epididymal WAT mass was higher in the sham-operated rats pair-fed than in gastric banded and sham-operated rats fed ad

libitum. There was a statistically significant difference in epididymal WAT between the gastric banded and pair-fed obese animals ($p = 0.036$; Fig. 4).

Hormone Measurements

Fasting ghrelin levels were significantly higher in lean Zucker rats (*fa/+*) than in the obese rats (*fa/fa*; $2,969.9 \pm 150.9$ g vs 963.2 ± 54.3 g for sham-operated rats and

Fig. 4 Epididymal WAT weight/100 g of body weight of lean controls and obese Zucker rats after gastric banding, sham operation fed ad libitum, and sham-operated pair-fed to the gastric banded animals 21 days after surgery

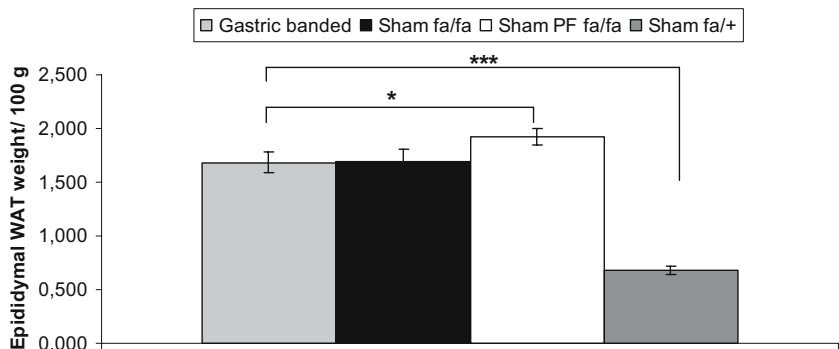
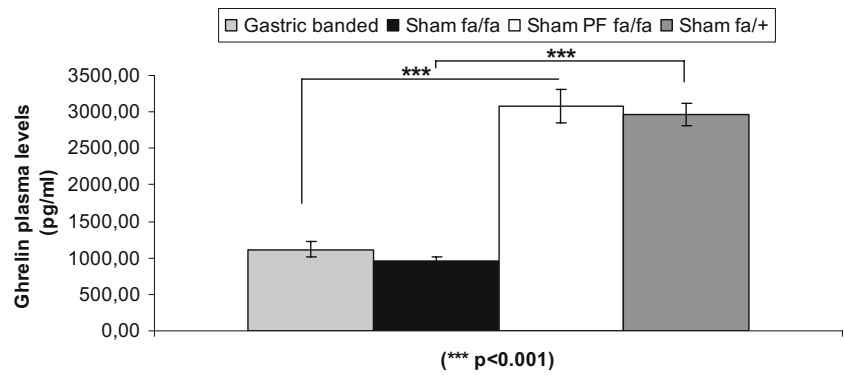


Fig. 5 Fasting plasma ghrelin levels of lean controls and obese Zucker rats after gastric banding, sham operation fed ad libitum, and sham-operated pair-fed to the gastric banded animals 21 days after the surgeries



1,116.9±103.3 g for gastric banded rats; $p<0.001$). Food-restricted obese rats pair-fed to the gastric banded presented a significant increase in fasting ghrelin levels towards similar values to those displayed by lean rats. This was not observed in obese animals after gastric banding in spite of similar decrease in food intake and body weight gain (3,079.5±221.6 g for pair-fed animals vs 1,116.9±103.3 g for gastric banded rats; $p<0.001$ and 2,969.9±150.9 g for lean rats; p value not significant; Fig. 5). Fasting levels of PYY and GLP-1 were similar in the four groups of animals (Table 1).

Lean Zucker rats (*fa/+*) had significantly lower fasting triglycerides, glucose, insulin, and leptin plasma levels when compared to all groups of obese Zucker rats (*fa/fa*), gastric banded, sham-operated fed ad libitum, and sham-operated pair-fed to the gastric banded ($p<0.001$). Furthermore, fasting triglycerides, insulin, and leptin plasma levels were higher in food-restricted sham-operated pair-fed animals than in gastric banded and sham-operated rats fed ad libitum, although it failed to reach statistical significance (Table 1).

NPY mRNA Expression in the Basal Hypothalamus

NPY/ β -actin mRNA expression in the basal hypothalamus of obese (*fa/fa*) Zucker rats submitted to gastric banding was lower than the one presented by sham-operated obese

animals (0.58±0.04 vs 0.61±0.02 for the sham-operated rats), but there was not a statistically significant difference (Fig. 6).

Discussion

We document here the metabolic and endocrine changes that occur in a rodent model of obesity after gastric banding. For that, we have used obese Zucker rats (*fa/fa*) submitted to bariatric surgery with gastric banding and compared them to two groups of sham-operated animals, one fed ad libitum and another food-restricted to the same amount eaten by the gastric banded animals (pair-fed). Rat gastric banding was herein performed according to a surgical procedure that we have recently introduced and applied to lean Wistar rats [27, 28].

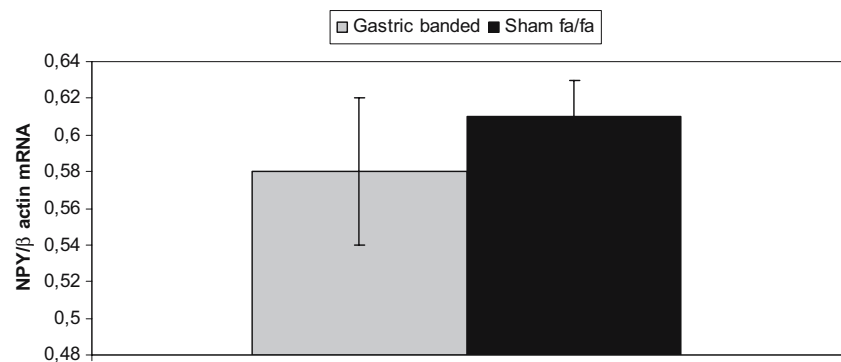
We have confirmed that obese animals had increased food intake, body weight gain, and fat mass as assessed by epididymal WAT weight when compared to lean rats (*fa/+*). Moreover, common features of the metabolic syndrome, such as fasting triglycerides, glucose, and insulin, were increased in the obese rats; as expected, leptin plasma levels, indicating increased adiposity and leptin resistance, were also elevated.

After bariatric surgery with gastric banding, the obese Zucker rat showed a decrease in food intake and body

Table 1 Gastrointestinal hormones and metabolic parameters in obese Zucker rats after gastric banding, sham-operated fed ad libitum, sham-operated pair-fed, and lean controls

	Gastric banded	Sham (<i>fa/fa</i>)	Sham PF (<i>fa/fa</i>)	Sham (<i>fa/+</i>)	Sig.
Glucose (mg/dl)	144.9±3.5	131.0±6.9	132.7±6.2	100.4±5.2	$p<0.001$
Triglycerides (mg/dl)	352.4±47.8	476.0±0.17	578.0±44.3	43.5±4.5	$p<0.001$
Insulin (ng/ml)	7.2±0.7	7.8±0.8	8.0±0.6	0.8±0.1	$p<0.001$
Leptin (ng/ml)	38.2±2.4	42.4±2.7	46.0±1.8	2.5±0.2	$p<0.001$
Ghrelin (pg/ml)	1,116.9±103.2	963.2±54.3	3,079.5±221.6	2,969.9±150.9	$p<0.001$
GLP-1 (pmol/l)	82.9±1.4	83.2±1.9	80.4±3.9	84.9±1.0	NS
PYY (pg/ml)	72.2±16.4	82.7±26.3	67.0±17.5	29.3±8.1	NS

Fig. 6 NPY/ β -actin mRNA expression in the basal hypothalamus of obese Zucker rats after gastric banding compared to sham-operated rats fed ad libitum 21 days after the surgeries



weight gain to levels that were similar to the ones observed in their lean counterparts, while also restoring a normal feeding behavior after fasting.

The obese Zucker rats (*fa/fa*) had significantly lower levels of circulating ghrelin than the lean controls (*fa/+*). Food-restricted rats pair-fed to the gastric banded ones had fasting ghrelin levels significantly higher than obese rats fed ad libitum, a feature that was not observed in gastric banded rats, in spite of similar decrease in food intake and weight loss. Ghrelin is a 28-amino-acid peptide hormone produced mainly in the stomach fundus and is the most potent appetite-stimulating hormone coming from the gastrointestinal tract [29]. Ghrelin is generally up-regulated under conditions of negative energy balance and down-regulated in the setting of positive energy balance; fasting serum ghrelin levels are usually low in obese subjects compared with lean individuals [30], and fasting plasma levels rise with diet induced weight loss [14, 31]. Ghrelin was shown to increase food intake dose-dependently in lean and obese Zucker rats [32]. Although there is an inverse correlation between ghrelin levels and body weight and ghrelin plasma levels are lower in the obese fatty Zucker rats when compared to the lean ones [33, 34], obese Zucker rats have an increased sensitivity to ghrelin feeding effects caused by enhanced expression of ghrelin receptor in the hypothalamus [32]. Hence, ghrelin is thought to have a role in the development of obesity of Zucker rats in the absence of leptin signaling, as leptin is able to inhibit the effects of ghrelin by diminishing the expression of its receptor [35–37].

Cummings et al. [14] were the first to report failure to increase ghrelin levels in weight loss attained after gastric bypass in humans. Since this first report, other investigators have found that the effect of bariatric surgery on ghrelin levels was highly variable. Fasting ghrelin levels have been found to increase 2 years after vertical banded gastroplasty [38] and in patients who attained 20% body mass index (BMI) loss [39]. Likewise, in obese patients 6 months [9, 16, 18, 19, 40, 41], 12 months [17, 19, 40], and 24 months [38, 40] after being submitted to adjustable gastric banding, fasting ghrelin levels have been shown to increase,

similarly to diet induced weight loss, although the increase in ghrelin levels was greater after surgery than after diet-induced weight loss, and this could be explained by the higher amount of weight loss achieved with surgery. Furthermore, postoperative ghrelin levels were also reported as not being different from those of non-operated BMI-matched patients [9]. However, some controversy remains, as other authors have found that ghrelin levels remain stable 6 months after surgery [17, 42] or return to near baseline levels from 24 to 36 months after surgery [19].

In our investigation, fasting levels of PYY and GLP-1 were not significantly different between the four groups of animals. PYY (3-36) and GLP-1 (7-36) are satiety signals released by the endocrine L cells of the gastrointestinal tract after meals that have been shown to reduce food intake and body weight gain in obese Zucker rats [43–45]. In humans, fasting and post-prandial plasma levels of PYY increase progressively 6 and 12 months after vertical banded gastroplasty surgery [46]. Some authors have found that the PYY area under the curve after a test meal was also superior in obese patients submitted to gastric banding when compared weight-matched obese controls [15], although in another similar study, this was not found to be the case [47]. Six months after vertical banded gastroplasty, fasting plasma GLP-1 concentrations did not change significantly, but the area under the curve during an oral glucose tolerance test strikingly increased [48]. However, in another study involving patients submitted to gastric banding, GLP-1 levels after a test meal were similar to body-weight-matched obese controls [15]. It is pertinent to add that fasting levels of PYY and GLP-1 are generally low, and changes in these anorexigenic signals occur in the post-prandial period.

We found that fasting NPY mRNA expression in the basal hypothalamus of gastric banded obese animals, in spite of decreased food intake and body weight, was lower than the observed in sham-operated rats, although this difference did not reach statistical significance. NPY is expressed in abundance in the arcuate nucleus (ARC) and paraventricular nucleus of the basal hypothalamus; it is the most potent stimulator of food intake and therefore plays a

major role in regulation of food intake [49]. NPY concentrations in the hypothalamus increase in food-deprived animals and are normalized by food ingestion [50, 51]. In the obese Zucker rat (*fa/fa*), NPY secretion is increased and does not decrease significantly with food ingestion, explaining the continuous stimulation of feeding behavior observed in these animals [52]. NPY mRNA expression in the ARC is generally in good agreement with peptide content [53], being increased in fed obese Zucker rats (*fa/fa*) when compared to lean Zucker rats and further increased after fasting [54].

Studies have shown that after gastric banding, patients experience increased fasting and post-prandial satiety levels [7] and decreased hunger scores [8–10]. Variations in gastrointestinal hormone levels have been proposed as a contributing mechanism for the weight reduction observed after bariatric surgery, and several clinical groups have investigated the effect of bariatric procedures on ghrelin, PYY, and GLP-1 levels. Overall, most of the available human data have so far provided conflicting results. This could be a consequence of underpowered studies, as most are cross-sectional, making it difficult to isolate the role of surgery from that of weight loss, and longitudinal studies include small numbers of patients with non-homogeneous populations, which are not readily comparable between each other because of different intervals after surgery, variable BMI or excess weight loss, and minor differences in surgical techniques. Thus, information regarding endocrine mechanism underlying obesity surgery remains to be substantiated. There are numerous methodological and ethical limitations inherent in performing detailed physiologic experiments in humans. Animal models provide an invaluable tool for the study of appetite regulation pathways and avoid the heterogeneity inherent to human studies.

In our rat model of bariatric surgery with gastric banding, we have documented that in the course of 21 days after operation, which is comparable to that observed over 2 years in human patients [55], animals reduced their food intake and body weight in comparison to obese controls while also restored normal feeding behavior after fasting. Furthermore, fasting ghrelin levels did not increase when compared to sham-operated animals fed ad libitum and were significantly lower than the levels presented by weight-matched food-restricted (pair-fed) rats. In addition, gastric banded rats were leaner than weight-matched pair-fed sham-operated animals as expressed by lower plasma leptin levels and by a significantly lower epididymal WAT weight, both indicators of adiposity. Moreover, there was also a non-significant decrease in fasting triglycerides and insulin levels of gastric banded rats when compared to sham-operated animals fed ad libitum and food-restricted sham-operated rats. Altogether, these results suggest that the metabolic and endocrine changes after gastric banding

do not depend solely on food restriction and weight loss and indicate that the mechanisms of weight loss in these two groups of rats may not be similar.

In conclusion, these data provide evidence that in spite of ongoing leptin resistance, gastric banding decreases food intake and body weight gain in obese Zucker rats to levels that are similar to their lean counterparts while also restoring normal feeding behavior after fasting. Furthermore, gastric banding leads to an improvement in metabolic features and prevents the increase in this counter regulatory orexigenic signals that occur during caloric deprivation, such as increase in fasting ghrelin plasma levels and NPY expression in the basal hypothalamus. Taken together, these changes suggest that gastric banding decreases the orexigenic signals to the brain centers, regulating food intake that normally occur after diet-induced weight loss.

Acknowledgments The authors are very grateful to Antonio Sergio, MD, a surgical expert in human gastric banding for the technical advice regarding how to perform bariatric surgery.

UMIB work is funded by grants from FCT (POCTI/FEDER), Portugal.

References

1. Silventoinen K, Sans S, Tolonen H, et al. Trends in obesity and energy supply in the WHO MONICA Project. *Int J Obes Relat Metab Disord* 2004;28:710–8.
2. do Carmo I, dos Santos O, Camolas J, et al. Prevalence of obesity in Portugal. *Obes Rev* 2006;7:233–7.
3. Schneider BE, Mun EC. Surgical management of morbid obesity. *Diabetes Care* 2005;28:475–80.
4. Kushner R. Diets, drugs, exercise, and behavioral modification: where these work and where they do not. *Surg Obes Relat Dis* 2005;1:120–2.
5. Martin LF, Tan TL, Horn JR, et al. Comparison of the costs associated with medical and surgical treatment of obesity. *Surgery* 1995;118:599–606.
6. Halmi KA, Mason E, Falk JR, et al. Appetitive behavior after gastric bypass for obesity. *Int J Obes* 1981;5:457–64.
7. Dixon AF, Dixon JB, O'Brien PE. Laparoscopic adjustable gastric banding induces prolonged satiety: a randomized blind crossover study. *J Clin Endocrinol Metab* 2005;90:813–9.
8. Lang T, Hauser R, Buddeberg C, et al. Impact of gastric banding on eating behavior and weight. *Obes Surg* 2002;12:100–7.
9. Schindler K, Prager G, Ballaban T, et al. Impact of laparoscopic adjustable gastric banding on plasma ghrelin, eating behaviour and body weight. *Eur J Clin Invest* 2004;34:549–54.
10. Komer J, Inabnet W, Conwell IM, et al. Differential effects of gastric bypass and banding on circulating gut hormone and leptin levels. *Obesity (Silver Spring)* 2006;14:1553–61.
11. Flancbaum L, Choban PS, Bradley LR, et al. Changes in measured resting energy expenditure after Roux-en-Y gastric bypass for clinically severe obesity. *Surgery* 1997;122:943–9.
12. Galtier F, Farret A, Verdier R, et al. Resting energy expenditure and fuel metabolism following laparoscopic adjustable gastric banding in severely obese women: relationships with excess weight lost. *Int J Obes (Lond)* 2006;30:1104–10.
13. Benedetti G, Mingrone G, Marcocchia S, et al. Body composition and energy expenditure after weight loss following bariatric surgery. *J Am Coll Nutr* 2000;19:270–4.

14. Cummings DE, Weigle DS, Frayo RS, et al. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *N Engl J Med* 2002;346(21):1623–30.
15. le Roux CW, Aylwin SJ, Batterham RL, et al. Gut hormone profiles following bariatric surgery favor an anorectic state, facilitate weight loss, and improve metabolic parameters. *Ann Surg* 2006;243:108–14.
16. Fruhbeck G, Rotellar F, Hernandez-Lizoain JL, et al. Fasting plasma ghrelin concentrations 6 months after gastric bypass are not determined by weight loss or changes in insulinemia. *Obes Surg* 2004;14:1208–15.
17. Hanusch-Enserer U, Cauza E, Brabant G, et al. Plasma ghrelin in obesity before and after weight loss after laparoscopic adjustable gastric banding. *J Clin Endocrinol Metab* 2004;89:3352–8.
18. Langer FB, Reza Hoda MA, Bohdjalian A, et al. Sleeve gastrectomy and gastric banding: effects on plasma ghrelin levels. *Obes Surg* 2005;15:1024–9.
19. Mariani LM, Fusco A, Turriziani M, et al. Transient increase of plasma ghrelin after laparoscopic adjustable gastric banding in morbid obesity. *Horm Metab Res* 2005;37:242–5.
20. da Silva BA, Bjorbaek C, Uotani S, et al. Functional properties of leptin receptor isoforms containing the gln→pro extracellular domain mutation of the fatty rat. *Endocrinology* 1998;139:3681–90.
21. Wang T, Hartzell DL, Flatt WP, et al. Responses of lean and obese Zucker rats to centrally administered leptin. *Physiol Behav* 1998;65:333–41.
22. Yamashita T, Murakami T, Iida M, et al. Leptin receptor of Zucker fatty rat performs reduced signal transduction. *Diabetes* 1997;46:1077–80.
23. Farooqi IS, Wangenstein T, Collins S, et al. Clinical and molecular genetic spectrum of congenital deficiency of the leptin receptor. *N Engl J Med* 2007;356:237–47.
24. Halaas JL, Gajiwala KS, Maffei M, et al. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 1995;269:543–6.
25. Oberkofler H, Beer A, Breban D, et al. Human obese gene expression: alternative splicing of mRNA and relation to adipose tissue localization. *Obes Surg* 1997;7:390–6.
26. Prolo P, Wong ML, Licinio J. Leptin. *Int J Biochem Cell Biol* 1998;30:1285–90.
27. Monteiro MP, Monteiro JD, Aguas AP, et al. Rats submitted to gastric banding are leaner and show distinctive feeding patterns. *Obes Surg* 2006;16:597–602.
28. Monteiro MP, Monteiro JD, Aguas AP, et al. A rat model of restrictive bariatric surgery with gastric banding. *Obes Surg* 2006;16:48–51.
29. Asakawa A, Inui A, Kaga T, et al. Antagonism of ghrelin receptor reduces food intake and body weight gain in mice. *Gut* 2003;52:947–52.
30. Small CJ, Liu YL, Stanley SA, et al. Chronic CNS administration of agouti-related protein (Agrp) reduces energy expenditure. *Int J Obes Relat Metab Disord* 2003;27:530–3.
31. Hansen TK, Dall R, Hosoda H, et al. Weight loss increases circulating levels of ghrelin in human obesity. *Clin Endocrinol (Oxf)* 2002;56:203–6.
32. Brown LM, Benoit SC, Woods SC, et al. Intraventricular (i3vt) ghrelin increases food intake in fatty Zucker rats. *Peptides* 2007;28:612–6.
33. Beck B, Max JP, Fernette B, et al. Adaptation of ghrelin levels to limit body weight gain in the obese Zucker rat. *Biochem Biophys Res Commun* 2004;318:846–51.
34. Ariyasu H, Takaya K, Hosoda H, et al. Delayed short-term secretory regulation of ghrelin in obese animals: evidenced by a specific RIA for the active form of ghrelin. *Endocrinology* 2002;143:3341–50.
35. Beck B, Richey S, Stricker-Krongrad A. Feeding response to ghrelin agonist and antagonist in lean and obese Zucker rats. *Life Sci* 2004;76:473–8.
36. Tovar SA, Seoane LM, Caminos JE, et al. Regulation of peptide YY levels by age, hormonal, and nutritional status. *Obes Res* 2004;12:1944–50.
37. Hewson AK, Tung LY, Connell DW, et al. The rat arcuate nucleus integrates peripheral signals provided by leptin, insulin, and a ghrelin mimetic. *Diabetes* 2002;51:3412–9.
38. Nijhuis J, van Dielen FM, Buurman WA, et al. Ghrelin, leptin and insulin levels after restrictive surgery: a 2-year follow-up study. *Obes Surg* 2004;14:783–7.
39. Foschi D, Corsi F, Rizzi A, et al. Vertical banded gastroplasty modifies plasma ghrelin secretion in obese patients. *Obes Surg* 2005;15:1129–32.
40. Stoeckli R, Chanda R, Langer I, et al. Changes of body weight and plasma ghrelin levels after gastric banding and gastric bypass. *Obes Res* 2004;12:346–50.
41. Ram E, Vishne T, Diker D, et al. Impact of gastric banding on plasma ghrelin, growth hormone, cortisol, DHEA and DHEA-S levels. *Obes Surg* 2005;15:1118–23.
42. Hanusch-Enserer U, Brabant G, Roden M. Ghrelin concentrations in morbidly obese patients after adjustable gastric banding. *N Engl J Med* 2003;348:2159–60.
43. Rodriguez de Fonseca F, Navarro M, Alvarez E, et al. Peripheral versus central effects of glucagon-like peptide-1 receptor agonists on satiety and body weight loss in Zucker obese rats. *Metabolism* 2000;49:709–17.
44. Stylopoulos N, Davis P, Pettit JD, et al. Changes in serum ghrelin predict weight loss after Roux-en-Y gastric bypass in rats. *Surg Endosc* 2005;19:942–6.
45. Pittner RA, Moore CX, Bhavsar SP, et al. Effects of PYY[3-36] in rodent models of diabetes and obesity. *Int J Obes Relat Metab Disord* 2004;28:963–71.
46. Alvarez Bartolome M, Borque M, Martinez-Sarmiento J, et al. Peptide YY secretion in morbidly obese patients before and after vertical banded gastroplasty. *Obes Surg* 2002;12:324–7.
47. Korner J, Bessler M, Cirilo LJ, et al. Effects of Roux-en-Y gastric bypass surgery on fasting and postprandial concentrations of plasma ghrelin, peptide YY, and insulin. *J Clin Endocrinol Metab* 2005;90:359–65.
48. Valverde I, Puente J, Martin-Duce A, et al. Changes in glucagon-like peptide-1 (GLP-1) secretion after biliopancreatic diversion or vertical banded gastroplasty in obese subjects. *Obes Surg* 2005;15:387–97.
49. Kalra SP, Dube MG, Sahu A, et al. Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased appetite for food. *PNAS* 1991;88:10931–5.
50. Lewis DE, Shellard L, Koeslag DG, et al. Intense exercise and food restriction cause similar hypothalamic neuropeptide Y increases in rats. *Am J Physiol* 1993;264:E279–84.
51. McKibbin PE, Cotton SJ, McMillan S, et al. Altered neuropeptide Y concentrations in specific hypothalamic regions of obese (fa/fa) Zucker rats. Possible relationship to obesity and neuroendocrine disturbances. *Diabetes* 1991;40:1423–9.
52. Beck B, Richey S, Stricker-Krongrad A. Ghrelin and body weight regulation in the obese Zucker rat in relation to feeding state and dark/light cycle. *Exp Biol Med (Maywood)* 2003;228:1124–31.
53. Sanacora G, Kershaw M, Finkelstein JA, et al. Increased hypothalamic content of preproneuropeptide Y messenger ribonucleic acid in genetically obese Zucker rats and its regulation by food deprivation. *Endocrinology* 1990;127:730–7.
54. Korner J, Savontaus E, Chua SC Jr, et al. Leptin regulation of Agrp and Npy mRNA in the rat hypothalamus. *J Neuroendocrinol* 2001;13:959–66.
55. Quinn R. Comparing rat's to human's age: how old is my rat in people years? *Nutrition* 2005;21:775–7.