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Rats Submitted to Gastric Banding are Leaner and Show Distinctive Feeding Patterns

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Background: Bariatric surgery is expanding to meet the global epidemic of morbid obesity, because this surgery is successful in achieving sustained weight loss. After having recently established a rat model of gastric banding, our aim now was to investigate the relative fat mass content and the feeding patterns of gastric banded rats.

Methods: Two groups of Wistar rats, submitted either to gastric banding or to sham surgery, were followed-up for 26 days regarding weight, daily food intake and feeding patterns both under resting conditions and when refed after fasting. Weight of the epididymal fat pad was used as a measure to evaluate changes in white adipose tissue in the rats.

Results: 10 days after surgery and thereafter, rats submitted to gastric banding showed the same daily food intake that was observed in sham-operated rats. Nevertheless, gastric banded rats kept lower body weights and were leaner than controls. These differences were associated with distinctive feeding patterns, both under resting conditions and when refed after fasting, suggesting that gastric banded rats present a significant increase in feeding frequency when compared with controls.

Conclusion: This data is the first experimental evidence that an increase in feeding frequency is associated with weight loss after gastric banding, even if there is no decrease in total energy intake. Thus, medical advice on the advantages of fractionating daily caloric intake into multiple meals is further supported by the herein new information obtained in an animal model of gastric banding.

Key words: Obesity, bariatric surgery, gastric banding, food intake, adipose tissue, rat

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Introduction

Currently, there is a growing world epidemic of overweight, obesity, and morbid obesity.[1] Surgical treatments are more effective than nonsurgical treatments for weight loss and control of co-morbid conditions frequently associated with obesity.[2] For morbid obesity, bariatric surgical procedures are the only effective therapy, and provide greater and more durable weight reduction than behavioral and pharmacological interventions.[3,4] Understandably, the use of bariatric surgery is expanding exponentially to meet the global epidemic of morbid obesity.[1,2]

Bariatric surgery with gastric banding induces a substantial weight loss due to a decrease in fat mass with relatively less reduction in the components of fat-free mass, and without significant deleterious effects on body composition.[5,6] In the phase of rapid weight loss that follows gastric banding, there is a preferential mobilization of visceral fat, compared with total and subcutaneous adipose tissue.[7] Restrictive obesity surgery has been shown to result not only in sustained weight loss, but also in improvement in eating behavior.[8-11] After being submitted to restrictive procedures, patients report a new experience of fullness and a reduction in their hunger sensation.[8,11]

After having recently established a rat model of bariatric surgery with gastric banding,[12] we have now investigated the eating patterns displayed by Wistar rats submitted to gastric banding in comparison with those of sham-operated rats, and we have also monitored changes in relative fat mass content of the rats.

Material and Methods

Animals

Eighteen male Wistar rats (125-150 g) purchased from a local 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 07:00 h), with *ad libitum* 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. Rats were randomized into two weight-matched groups to be submitted either to gastric banding (n=4) or sham gastric banding (n=14). 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 anesthetized by intra-peritoneal injection of a mixture of ketamine 60 mg/kg (Imalgene 1000, Merial) and xilazine 12 mg/kg (Rompun, Bayer) according to body weight. Prophylactic antibiotherapy consisting of 1.25 mg ampicilin (Cilin, Quimedical) plus 1.25 mg flucloxacilin (Floxapen, GSK) diluted in 1 ml of sterile water was administered intraperitoneally immediately before surgery. The surgical technique for gastric banding and sham surgery was performed as previously described.[12] Both groups of animals were given 5 ml of 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 09:00 h using a scale (Monobloc, Metterr, 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 20th day after surgery, and were allowed an interval of three overnights in-between studies, for animal recovery from eventual study distress.

Night-time Feeding Study

These animal studies were carried out in non-fasted rats, starting out 1 hour before the onset of the dark phase (18:00 h); food intake was measured at 1 hour before dark and 1, 2, 4, 8, 12 and 24 hours after dark (19:00 h).

Fast and Re-feed Study

Animals were fasted for 24 hours before the study and re-fed 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 re-feeding by weighing the remaining food in the hopper.

Epididymal White Adipose Tissue Weight

At 26 days after surgery, all rats were sacrificed. Epididymal white adipose tissue pads were removed and weighed using a scale recording to the nearest 0.001 g (Kern 440, Version 3.2).

Statistical Analysis

Results are shown as means ± SEM, unless otherwise specified. Unpaired *t*-test was used for comparison of the means between the two unpaired treatment groups. $P<0.05$ was considered to be statistically significant.

Results

Male Wistar rats were submitted either to gastric banding or to sham surgery and were followed-up for 26 days after the surgical procedure. Only rats submitted to surgery that recovered successfully and survived until the end of the experiment were used in the statistical analysis presented in this report.

Mean body weight was comparable in the two groups of rats before they were submitted to surgery: gastric banded (175.5 ± 6.6 g) and sham-operated (171.4 ± 3.1 g) rats, respectively ($P=0.54$). After surgery, gastric banded rats showed a decrease in weight gain when compared to sham-operated rats. This difference was statistically significant throughout the time-span of this investigation. The cumulative weight gain 26 days after the procedure was 126.6 ± 12.7 g for the gastric banded rats and 169.6 ± 4.8 g for the control rats (Figure 1), a difference that was statistically significant (*P*=0.001).

Gastric banded rats showed a statistically significant lower absolute daily food intake during the first 10 days after surgery, in comparison to sham-operated rats. Thereafter, both groups of rats displayed similar daily food intakes (Figure 2A). This was reflected in the finding that the cumulative food intake for the 26 days was 441.0 ± 53.0 g for the gastric banded rats and 580 ± 12.6 g for the sham-operated rats, a difference that was also statistically significant (*P*=0.001) (Figure 2B).

Night-time Feeding Study

In rats, peak feeding hours occur during the first hour before and after dark. A feeding study was therefore performed with frequent night-time food reweighs in the two groups of rats, 20 days after surgery. This study also involved the evaluation of the 24-h food intake pattern of the rats. Gastric banded animals showed a decrease in food intake in the first hour before dark compared to sham-operated animals (i.e. 1.3 ± 0.35 g for gastric banded rats and 2.3 ± 0.20 g for controls), a difference that was statistically significant (*P*= 0.03). In the first hour after dark, there was a similar difference regarding food intake (i.e. 0.7 ± 0.46 g for gastric banded compared to 2.3 ± 0.24 g for sham-operated rats) that was also

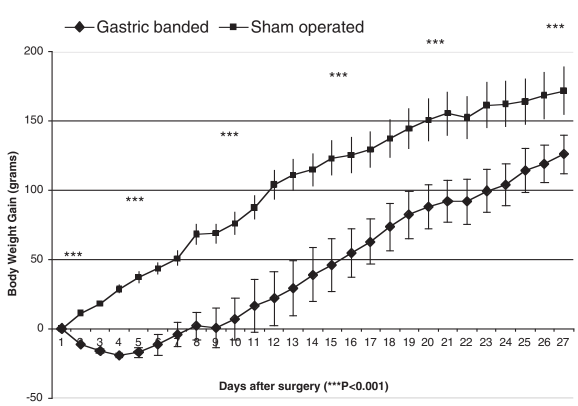

Figure 1. Graph displaying cumulative body weight gain in gastric banded and sham-operated rats. Before surgery, mean body weight was comparable between the two groups of rats. After surgery, gastric banded rats showed a lower cumulative weight gain compared to sham-operated rats, a difference that was statistically significant throughout the 26 days.

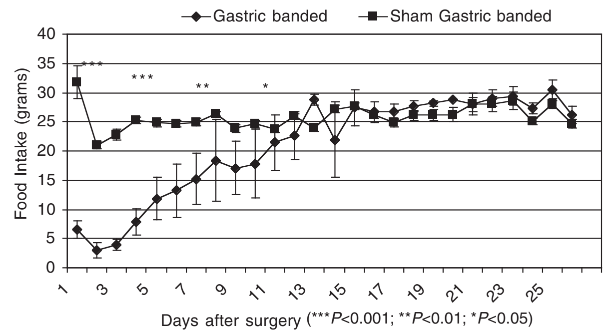

Figure 2A. Absolute daily food intake progression chart. During the first 10 days after surgery, gastric banded rats showed a decrease in absolute daily food intake, in comparison to sham-operated rats, although afterwards both groups displayed similar daily food intake.

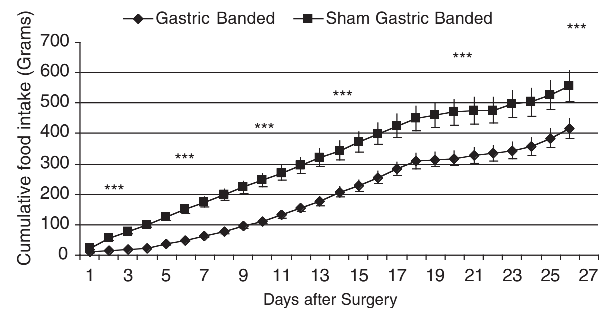

Figure 2B. Cumulative food intake during 26 days after surgery in gastric banded and sham-operated rats.

statistically significant (*P*= 0.016). For the following 3 hours after dark, there was a trend for an increase in food intake in the gastric banded rats, although without reaching statistical significance. During the day light phase, gastric banded animals showed higher food intake (5.8 ± 0.06 g) than controls (3.6 ± 0.38 g), a finding that was statistically significant (*P*=0.018) (Figure 3A). Concerning the cumulative food intake, the values were lower for gastric banded rats in comparison to sham-operated ones during the first 2 hours after dark, but the 24-h food intake was similar for both groups (i.e. 24.2 ± 1.15 g for gastric banded and 24.6 ± 0.77 g for sham-operated rats) (Figure 3B).

Fast and Re-feeding Study

In order to evaluate the food intake pattern of rats after fasting, a feeding study was carried out, starting in early day light phase and after 24 hours of fasting. Re-fed gastric banded rats showed a trend

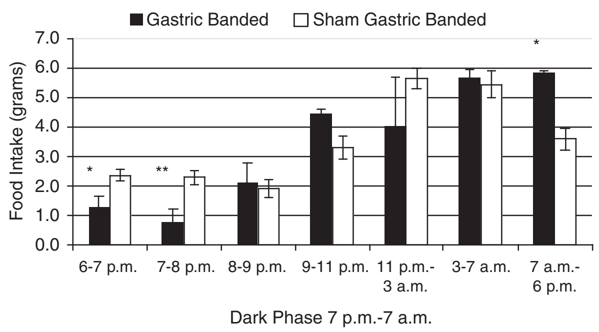

Figure 3A. Dark-phase/light-phase feeding study of rats. Gastric banded rats showed statistically significant lower values in food intake during the first hours before and after dark. During the 3 hours that followed darkness, there was a trend for a higher food intake in the gastric banded rats, although without reaching statistical significance. During the light-phase, gastric banded rats showed significantly higher food intake when compared to controls.

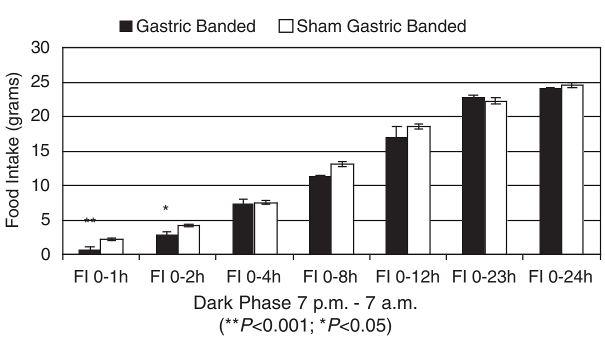

Figure 3B. The cumulative food intake was lower in the gastric banded rats compared to sham-operated ones during the first 2 hours after dark, but the 24-h food intake was similar in both groups.

for a lower food intake during the first 2 hours after fasting in comparison with sham-operated rats, without reaching statistical significance. In the following 6 hours, gastric banded rats showed higher food intake than sham-operated rats: food intake values for the period of time between the fourth and eighth hour after re-feeding were 4.4 ± 1.3 g for gastric banded rats compared to 1.75 ± 0.33 g for sham-operated rats (a statistically significant difference, P=0.009) (Figure 4). The cumulative 24-h food intake was again similar in both groups of rats, being 34.9 ± 2.87 g for gastric banded animals and 36.2 ± 0.85 g for sham-operated ones.

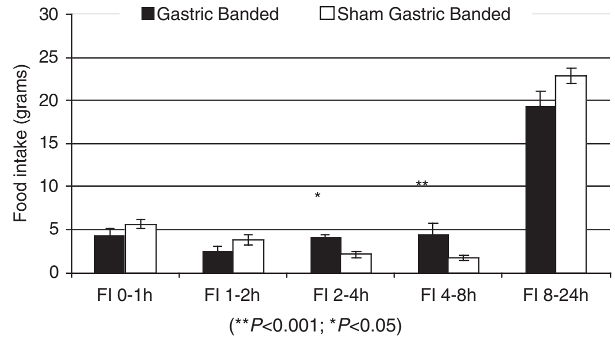

Figure 4. Fast and re-feeding study – Re-fed gastric banded rats showed a trend for lower food intake in the first 2 hours after fasting compared to sham-operated rats. In the following 6 hours, there was higher food intake in gastric banded rats than in sham-operated rats, a difference that was statistically significant between the fourth and eighth hour after re-feeding.

White Adipose Tissue Weight

Gastric banded rats had a mean epididymal fat-pad weight of 3.2 ± 0.5 g, while the same fat-pad weighed 4.6 ± 0.1 g in sham-operated rats, a difference that reached statistical significance (P=0.03). This difference was still present when the white adipose tissue weight was adjusted for body weight (Figure 5).

Discussion

A rat model of gastric banding was recently introduced by us.[12] We have shown that, similar to what is observed in humans submitted to bariatric surgery

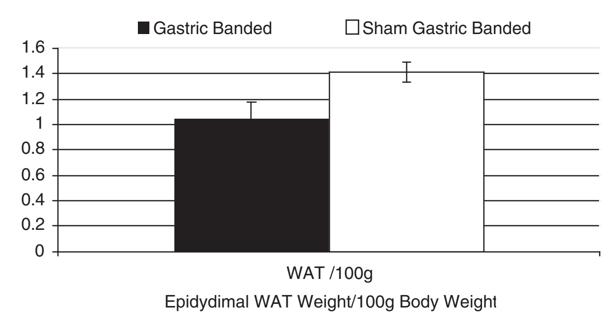

Figure 5. Comparison of white adipose tissue (WAT) epididymal fat-pad weight /100 grams of body weight between gastric banded and sham-operated rats. The amount of WAT was significantly lower in gastric banded rats than in controls.

with gastric banding, rat gastric banding leads to a decrease both in food intake and in body weight.[12] This accounts for the decreased weight gain observed in this rat model of gastric banding. We document now that this restrictive effect of the gastric banding wanes progressively with time and that the gastric banded rats present a progressive increase in food intake until reaching, 10 days after the surgery, a similar amount as that observed in sham-operated rats. Nevertheless, rats submitted to gastric banding keep weighing less than controls and they are also leaner, as shown by the lower weight of the epididymal fat-pad, a parameter that is commonly used in the rat as a surrogate marker of white adipose tissue mass.[13-15]

Although eating the same daily amount as sham-operated rats, gastric banded rats displayed distinct feeding patterns, both under resting conditions and when re-fed after being fasted. In fact, when compared to control animals, there was a decrease in food intake in the late day light phase and early dark-phase, corresponding to peak feeding hours in the rat and an increase in day light-phase food intake. After fasting, the feeding pattern displayed by the gastric banded rats also differed from that of sham-operated ones, since the banded rats showed a trend for decrease in food intake during the first 2 hours and this was followed by an increase in food intake during the next 6 hours. Both of these studies on feeding patterns (night time and fast/re-feed) suggest an increase in feeding frequency in gastric banded rats, although with the same total daily energy intake in both groups of rats.

In humans, there is evidence that increasing feeding frequency, i.e. keeping the same total daily energy intake but dividing it into more frequent meals, results in nutritional benefits, namely on metabolism and body weight management.[16] In fact, increased feeding frequency leads to a reduction in the total secretion of insulin, an improvement in insulin resistance and a better blood glucose control, as well as an improvement in the blood lipid profile.[16] Also, there is a significant negative correlation between eating frequency and body weight, and an inverse relationship with body mass index.[17] An irregular meal frequency causes a lower postprandial energy expenditure compared with a regular meal frequency, for the same mean caloric intake. The reduced thermogenic postprandial effect with the irregular meal frequency is associated, in the long-term, with weight gain.[1] Frequent eating helps appetite control, thus preventing overeating at meals. It has been also suggested that eating 'little and often' may be a more compatible pattern of eating for a physically-active lifestyle than eating large meals.[19] Increased feeding frequency leads to decreased food intake at a subsequent *ad libitum* meal because of a prolonged but attenuated increase in serum insulin concentration that may facilitate this acute reduction in appetite.[20] The greater control of satiety derived from consuming multiple smaller meals may be linked to attenuation in insulin response, although other physical (gastric stretch) and physiological (release of gastric hormones) factors may also be affected by the periodicity of eating.[21]

In conclusion, this investigation offers the first experimental evidence that demonstrates that gastric banding changes feeding frequency, and that these changes are a major factor in the weight loss observed after this type of surgery, even in the absence of a decrease in energy intake.

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