Bone Densitometry and Fat Index after Sleeve and Subsleeve Gastrectomy in Animal Model

Abstract

Background: Previous studies have reported that gastrectomy and fundectomy can induce osteopenia. Body fat index is a new index of obesity that shows central obesity and other risks of obesity. Sleeve gastrectomy (SG) is a bariatric surgery and a new technique introduced as subsleeve, which only resected fundus of the stomach. In this study, it has been shown the effect of subsleeve and SG on fat index and bone densitometry in an animal model. Materials and Methods: Rabbits were underlined SG, fundectomy (surgical removal of fundus), or sham-operated (controls without any resection), and after 12 weeks, fat index and bone densitometry were obtained. Results: Our study showed that there was no significant difference between SG and fundectomy groups in bone mass density and fat mass after surgery in comparison with presurgery condition. SG group were associated with lower fat index and bone density, and it showed significantly decrease in weight after 1.5 months. Conclusion: Sub-SG did not show any significant effect on fat index and bone densitometry in comparison with SG. However, we found lower fat index in sleeve group of rabbits, but it was not statistically significant.

Keywords: Body mass index, densitometry, gastrectomy, morbid obesities, rabbits

Introduction

However, sleeve gastrectomy (SG) may have different short-and long-term postoperative complications such as weight loss, disturbed nutrition intake, anemia, abnormal digestion, and absorption. Sub-SG is a new experimental technique that has been introduced recently. Its effectiveness is not clarified yet. Osteopenia is assessed after gastrectomy in experimental animals and humans. Previous reports have described the bone tissue in the men represented a significant decrease of volumetric bone mineral density (up to 16.8% within the trabecular and 10.0% in cortical bone compartments of lumbar spine) after total gastrectomy.

Overweight and obesity are the abnormal and high levels of fat accumulation in the body that endanger people’s health in many ways and are considered a public health difficult situation worldwide. Since 1975 thus far, obesity has nearly tripled.

Recent studies have shown that body mass index (BMI) itself could be a strong predictor of overall mortality. Attempts toward losing weight are unsuccessful in several patients. Bariatric surgery is a referral surgery for morbid obesity that is performed for at least 6–12 months after failing medical treatment and causes rapid weight loss. According to European guidelines on obesity management, bariatric surgery is recommended in patients with a BMI >50 kg/m² or those with a BMI ≥35 who have concomitant disease (such as psychological, dermatologic, orthopedic, cardiopulmonary, endocrine, and metabolic diseases). Surgical interventions to treat morbid obesity can be divided into two groups, including surgeries that limit gastric volume (such as adjustable gastric bandage and SG) and surgeries that reduce absorption (such as gastric bypass or biliopancreatic path deviation). Mid-term and long-term studies have shown that weight loss using bariatric surgery methods can cause nutrient and micronutrient deficiencies and decreased bone mineral density (BMD). Digestion of micronutrients such as calcium, Vitamin D, and essential proteins are also reduced. Rapid weight loss by reducing bone mechanical susceptibility can contribute to bone mass loss. Bone mass loss after

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bariatric surgeries, which has been mostly in the proximal part of the femur bone, has been reported in several studies.\textsuperscript{[11]}

Low BMI is additionally a crucial risk factor for osteoporosis, and moderate overweight and obesity is a protective issue for the development of osteoporosis. On the other hand, some studies have shown that morbid obesity (BMI > 35) has adverse effects on bone formation.\textsuperscript{[12]} Research has shown that patients undergoing bariatric surgery are at increased risk of secondary hyperparathyroidism and decreased BMD.\textsuperscript{[13]} There seems to be a direct relationship between the percentage of bone loss and the rate of weight loss.\textsuperscript{[14]}

It is troublesome to interpret bone condensation leads to the rapid weight loss phase due to technical errors related to reduce subcutaneous fat thickness. Since the second year after gastric bypass surgery, measuring of BMD has been the gold standard for assessing bone status.\textsuperscript{[15]}

In the SG procedure, 70%–75% of the gastric capacity decreased, but sub-SG might be more conservative\textsuperscript{[16]} because we hypothesized that only removing the gastric fundus did not have effect on bone loss. Therefore, we tested a new surgical procedure called sub-SG in obese rabbits. The new procedure was evaluated by comparing the body weights, fat index, and bone density among rabbits after 3 months. We decided to evaluate its effects on body fat index and bone densitometry.

**Materials and Methods**

Fifteen male New Zealand White rabbits were obtained from Animal Laboratory, Shiraz University of medical sciences, Shiraz, Iran. The rabbits were maintained in individual cages under standard conditions (temperature, humidity, and light) with free access to standard food and tap water.

Rabbits were divided into weight-matched groups (BMI approximately 10). Finally, 15 rabbits divided in three groups ($n = 5$): SG (2657.5 ± 208.0 g), fundus resection (2936.0 ± 142.5 g), and controls (sham-operated) (3133.3 ± 221.5 g) were included in this study. Control animals (sham-operated) were given the same amount of food eaten by the surgical groups, in order to measure the weight-loss effects of surgeries.

All procedures were approved by the Ethics Committee of Animal Research in Shiraz University of Medical Sciencs.

**Surgical procedures**

After 48 h fasting, rabbits were anesthetized with IM injection of ketamine (50 mg/kg) and xylazine (10 mg/kg). As prophylactic therapy, piperacillin (0.4 g/ml/kg) through IM injection was administered immediately after surgery and 3 following days.

In the SG, first, a 1.5-cm upper midline incision was made, then the stomach was externalized, and scissors were used to remove approximately 90% of the forestomach. The excised part was then sutured with 4-0 nonabsorbable polypropylene suture (Prolene, Ethicon). To perform the fundectomy, first, a 1.5-cm upper midline incision was made, then the stomach was externalized, and fundus of gastric curvature excised and sutured by 4-0 nonabsorbable polypropylene suture (Prolene, Ethicon). For the sham-operated groups, after making an incision, the stomach was externalized, manipulated, and then returned to the abdomen. Abdomen then closed by using 4-0 absorbable coated polyglactin (Vicryl, Ethicon) followed by 4-0 absorbable coated polyglactin (Vicryl Rapid, Ethicon) for the skin.

Rabbits were returned to their home cages, and they were restricted to only water–sugar diet on the first postoperative day and for 3 following days placed on milk and multi-vitamin (5 drops/500 cc milk), and afterward, all animals had access to standard rabbit food until the end of the trial.

Before and after surgery bodyweight of all rabbits was recorded. Whole-body composition reference values involving fat, lean, and bone from each rabbit were determined after 3 months and compared between three groups (HOLOGIC discovery Wi (S/N88108), version 13.4.2. Inc., Bedford, MA, USA). After measurements, the rabbits were sacrificed, and the different parts of their bodies were used for autopsy. Histopathology evaluation of the affected parts of their body was done.

**Statistical analysis**

Results are shown as means ± standard error mean. Kruskal–Wallis test was used for comparison of the means between the groups, and $P < 0.05$ was considered to be statistically significant.

**Results**

Rabbits in fundectomy and control groups displayed body weight gain after 3 months, but it was not statistically significant [$P > 0.05$; Figure 1]. There was no difference in relative body fat content, assessed by the percentage of body fat index among the three groups of rabbits [Table 1]. The weight of rabbits in subsleeve group became stable after 3 months. Figure 2 shows the sleeve and subsleeve surgery.

Body fat was not significantly different after 3 months among different groups ($P > 0.05$). Fat weight was also lower in rabbits submitted to SG compared with controls.

**Pathologic findings**

After measurements, the rabbits were sacrificed and the different parts of their bodies were used for histopathological study. Pathologic findings after 3 months showed that in fundectomised rabbits, gastric mucosal congestions were found in three of the rabbits. Histopathology sections of...
the rabbits’ liver show mild portal infiltration and kidneys of two of them show mild tubulo-interstitial nephritis. In the sleeve group, transmural necrosis in one of the rabbits’ stomach was seen, and it has not been shown any specific changes in others’ brain, spleen, kidney, liver, and stomach after 3 months.

There was no significant pathological difference in bony trabecular thickness and arrangement among the three groups.

Discussion

It is necessary to evaluate the body composition of patients in postoperative visits in order to diagnose the bone loss after bariatric surgeries. The dual-energy X-ray absorptiometry (DXA) is considered as the gold standard for the evaluation of body composition, but the use is limited due to its high cost.\[17\]

Comparing the mid-term and long-term effects of bariatric surgical procedures on laboratory animals can help prevent possible complications in patients with morbid obesity that is undergoing surgery. In this study, a comparison of weight loss among three groups of rabbits (fundectomy, sleeve, and sham) did not show a statistically significant difference [Figure 1].

The results of comparing body fat index showed that there was not a statistically significant difference in none of its factors, including bone mass content, fat mass, lean mass, total mass, fat percentage, and bone mass density, among the three groups of rabbits.

Studies have shown that the DXA in measuring bone density is more sensitive to added soft tissue and changes in body composition than computerized tomography, which can affect the ability to detect changes over time.\[18\] However, there was no distinction in bone mass density in our study. The study conducted by Adamczyk et al. on 36 patients who underwent SG surgery showed that after 12 months, and the total amount of BMD was decreased, but the amount of fat, lean body mass and total mass were decreased significantly.\[16\] There was an considerable decrease in bone density throughout the body and spine, which was not statistically significant. The overall rate of bone density in the hip bone and femoral head was significantly reduced in their study, which required a review of supplements used in patients and postoperative nutrition. Such as the Adamczyk study’s lack of precise control of dietary and physical activity has been mentioned as limitations of the study.\[6\]

Many studies have reported a reduction in bone density after bariatric surgeries.\[9,13,19\] In general, the baseline of BMD in obese patients is higher than in the general population, and a small decrease in BMD after weight loss can be justified in this way.\[11\] The Hsin et al. study focused on getting enough micronutrients to try to control bone density loss, but still found a decrease in BMD after weight loss.\[20\] Due to differences in diet and supplementation intake after surgery in patients, more comparative studies are needed in different groups of patients.

According to the reports on histological changes after bariatric operations in other studies, samples of brain, spleen, kidney, liver, stomach, and femoral bone were prepared and evaluated in this study. Apart from necrosis of gastric tissue, which was observed in one of the rabbits of the sleeve group, the histological changes in different organs such as stomach, kidney, and liver were more in the fundectomy group.

Table 1: Results of body fat index in rabbits 3 months after surgery (dual energy X-ray absorptiometry results summary)

| Groups    | Height (cm) | Bone mass content (g) | Fat mass (g) | Lean mass (g) | Total mass (g) | Percentage fat | Bone mass density (g/cm²) |
|-----------|-------------|-----------------------|-------------|---------------|---------------|----------------|--------------------------|
| Fundectomy| 51.25±2.4   | 89.96±4.34            | 818.37±99.68| 2221.6±52.99  | 3129.95±147.64| 25.87±2.0      | 0.37±0.02                |
| Sleeve    | 55.00±5.0   | 75.66±10.7            | 700.40±124.4| 2131.15±281.55| 2907.2±146.6  | 23.95±0.8      | 0.34±0.02                |
| Sham      | 45.0±5.0    | 79.25±13.5            | 713.35±175.3| 2339.3±0.1    | 3131.9±188.8  | 22.55±4.2      | 0.35±0.0                 |
| P         | 0.200       | 0.062                 | 0.081       | 0.069         | 0.108         | 0.165          | 0.081                    |

Figure 1: Total body weight (g ± standard error mean) of rabbits after surgeries (*P < 0.05)

Table 2: Comparison of body fat index in rabbits 3 months after surgery

| Groups    | Height (cm) | Bone mass content (g) | Fat mass (g) | Lean mass (g) | Total mass (g) | Percentage fat | Bone mass density (g/cm²) |
|-----------|-------------|-----------------------|-------------|---------------|---------------|----------------|--------------------------|
| Fundectomy| 51.25±2.4   | 89.96±4.34            | 818.37±99.68| 2221.6±52.99  | 3129.95±147.64| 25.87±2.0      | 0.37±0.02                |
| Sleeve    | 55.00±5.0   | 75.66±10.7            | 700.40±124.4| 2131.15±281.55| 2907.2±146.6  | 23.95±0.8      | 0.34±0.02                |
| Sham      | 45.0±5.0    | 79.25±13.5            | 713.35±175.3| 2339.3±0.1    | 3131.9±188.8  | 22.55±4.2      | 0.35±0.0                 |
| P         | 0.200       | 0.062                 | 0.081       | 0.069         | 0.108         | 0.165          | 0.081                    |

Figure 2: The sleeve gastrectomy (a) and subsleeve gastrectomy (b) surgery
Depending on the role of the stomach in maintaining calcium homeostasis in the body, different methods of bariatric surgeries can have different effects on bone density by creating changes in the structure of the stomach. In this study, femoral bone tissue was compared in three groups: sham, sleeve, and fundectomy groups; and there was no difference in their trabeculae and bone marrow space. In the study conducted by Lehto-Axtelius et al. on three rat groups, it was shown that osteopenic changes in calvaria in the fundectomy group is similar to the group in which gastrectomy was performed, but these changes are less in the group underwent antrectomy than in the other two groups, as well as enlarged bone marrow space in calvaria and loss of trabeculae in the tibia have also been reported after gastrectomy and fundectomy.[11] This difference in findings in the two studies could be due to differences in the time of investigation (Lehto et al. were examined at various times after the operations) and differences in animal model (Lehto et al. were used rats in their study).

Epidemiological studies have shown that obesity may be a risk issue for chronic nephropathy. Histological changes as secondary focal segmental sclerosis are more common in obese patients than in people who are in a normal range of weight.[21] In the present study, comparison of changes in renal histology was performed after bariatric surgeries, and no changes in renal tissue were observed in both sleeve and sham groups. In the group undergoing fundectomy, mild tubule interstitial nephritis was observed in 2 cases, and secondary focal segmental sclerosis was not reported in any groups.

Liver tissue pathological changes are present in a significant percentage of obese patients, and various studies have shown a comparative recovery after weight loss.[22,23] One of the shortcomings of this study was the lack of investigating pathological changes in the liver before and after surgery in each rabbit, and only a comparison of pathological changes among groups was performed, in which mild portal infiltration was shown in 3 rabbits of the fundectomy group. There was no difference among the three groups in terms of nonalcoholic fatty liver disease.

There are many differences in the results of various studies on bariatric surgical procedures regarding gastric histological changes after these surgeries.[24,25] In the present study, postoperative histological changes were observed only in the group of fundectomy as the mucosal congestion in the three rabbits. One of the rabbits in the sleeve group had some degrees of gastric necrosis. There were no significant histological changes in the sham group. Further studies should be performed to compare the results. The study conducted by Atef et al. on balloon placement in the stomach reported to reduce weight, local inflammation, and increased proliferative activity, which these were not observed in the present study.[24]

**Conclusion**

Sub-SG did not show any significant effect on fat index and bone densitometry in comparison with SG. However, we found lower fat index in the sleeve group of rabbits.

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**Conflicts of interest**

There are no conflicts of interest.

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