ORIGINAL CONTRIBUTIONS





Short-Term Changes on Body Composition and Bone Mass After One-Anastomosis Gastric Bypass: a Prospective Observational Study

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Published online: 21 April 2020 © Springer Science+Business Media, LLC, part of Springer Nature 2020

Abstract

Purpose Bariatric surgery generates a large weight loss. It is considered a successful surgery when 50% of the excess weight loss is reached. However, this measure does not include some variables that may have a direct impact on a patient's health, such as fat-free mass (FFM) or bone mass. Therefore, the aim of this study is to evaluate body composition and bone mass in patients undergoing one-anastomosis gastric bypass (OAGB).

Methods A prospective observational study was performed in patients undergoing OAGB. Body composition and bone mass were evaluated by bioelectrical impedance analysis at baseline (1 day prior to surgery), at 6 and 12 months after surgery.

Results A total of 94 patients (67% females and 33% males) were included in the study. The excess BMI loss at 6 and 12 months after surgery was $97.9 \pm 20.1\%$ and $110.2 \pm 30.5\%$ respectively. The FFM showed a reduction of $6.6 \pm 4.8 \text{ kg} (p < 0.01)$ 6 months after surgery and of $7.9 \pm 4.9 \text{ kg} (p < 0.01)$ at 12 months, meaning a decrease of $10.5 \pm 7.3\%$ and a $12.9 \pm 6.6\%$ respectively. The bone mass decrease was $10.1 \pm 6.9\%$ (p < 0.01) and $12.9 \pm 6.5\%$ (p < 0.01) at 12 months after OAGB.

Conclusions OAGB obtains a relevant weight loss in patients with morbid obesity, mainly, due to fat mass reductions. However, this procedure also provokes FFM and bone mass decreases, especially in females, but not significantly greater than other restrictive or mixed procedures.

Keywords One-anastomosis gastric bypass · Body composition · Bioelectrical impedance · Fat-free mass · Fat mass · Bone mass

Introduction

Bariatric surgery (BS) is an effective method to generate significant weight loss and remission of comorbidities in morbidly obese patients [1, 2], expecting increases in life expectancy of approximately 7 years [3]. Usually, the results obtained after BS are expressed by body mass index (BMI) and percentage of excess weight loss, considering that surgery has

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been successful when the patients lose 50% or more of their excess weight loss (EWL) and achieve a BMI below 35 kg/m^2 [4]. However, in this criterion, only the total weight of the patient is analyzed, obviating other variables that may have a direct impact on the patients' health, such as body composition.

After surgery, most of the weight loss corresponds to fat mass, but several studies have also shown significant reductions in fat-free mass (FFM) [5], leading to sarcopenic status [6], which has certain drawbacks. The FFM is composed of approximately 40% of skeletal muscle, which is a metabolically active tissue [7]. Thus, FFM loss contributes to the decrease of basal metabolic rate, predisposing to weight regain, which is a relevant drawback in bariatric patients. Furthermore, skeletal muscle is the main responsible for the homeostasis of glucose and peripheral insulin sensitivity. In addition, between 80 and 90% of glucose elimination stimulated by insulin is produced by this tissue [8]. As a result, the FFM loss after BS might predispose the patient to weight regain and relapse in certain comorbidities. After Roux-en-Y gastric bypass (RYGB), calcium and vitamin D deficiencies have been reported in over 10% and 50% of the patients, respectively [9]. These deficits, together with a lower mechanical load after BS (due to weight loss) and different neurohormonal mechanisms, are factors that contribute to a reduction in bone mineral density [10, 11]. Some studies have obtained decreases in bone mineral density both at 6 months and at 12 months after surgery, showing reductions of 10.5% and 7.4% of the bone mineral density of the pelvis and the spine [12].

One-anastomosis gastric bypass (OAGB) is a malabsorptive bariatric procedure and consequently at an eventual higher risk for nutritional deficiencies than restrictive or mixed procedures. However, a recently published paper of our group has demonstrated that with correct vitamin and mineral supplementation, the incidence of nutritional deficiencies is similar to that observed after RYGB [13].

The aim of this study was to assess the variations in fat-free mass and bone mass at 6 months and 1 year after (OAGB), as measured by bioelectrical impedance analysis.

Materials and Methods

Participants and Study Design

A prospective observational study of patients undergoing OAGB at a single institution, between September 2017 and June 2018, was performed. Inclusion criteria were BMI \geq 40 kg/m² or BMI \geq 35 kg/m² associated with obesity-related comorbidities. Exclusion criteria were pregnancy, age less than 18 years, patients undergoing revisional surgery, patients undergoing any other surgical procedure added to the bariatric approach, patients with a previous history of cancer disease, and patients with the inability to understand the nature and purpose of the study and/or to accept written participation in the study or with the impossibility to comply with pre-established clinical follow-up, including the attendance to the outpatient clinic 6 and 12 months after surgery and the performance of the bioelectrical impedance analysis.

All participants signed informed consent before starting the study. The study was approved by the local ethics committee and conformed to the Declaration of Helsinki.

Preoperative Evaluation

A multidisciplinary team performed a combined medical and nutritional workup to evaluate potential surgical candidates. A weight loss of at least 10% of the patient's weight was considered an indispensable condition to undergo the selected bariatric technique, as a previous study of our group has demonstrated that this weight loss reduces the operative risk before an OAGB [14]. Patients received information about possible perioperative complications and necessary postoperative nutritional supplementation.

Surgical Procedure

As previously described [15, 16], 6 ports were placed: right and left flank (12 mm), supraumbilical (11 mm), right and left hypochondrium, and right iliac fossa (5 mm). A 20-cm-long gastric pouch, calibrated with a 36 Fr bougie, was constructed. Termino-lateral gastrojejunal anastomosis with a linear stapler (I-Drive with Tri-staple cartridges, Medtronic, USA) was performed. The enterotomies and gastrotomies were sutured with continuous barbed suture V-Loc 2/0 (Medtronic, USA). The total bowel length was determined: the biliopancreatic limb length ranged between 200 and 350 cm long and the common limb between 180 and 250 cm. After the assessment of the total bowel length, the appropriate length of the limbs was determined following the ratio biliopancreatic limb 60%/common limb 40%; this ratio was established as the most accurate parameter to predict a 5-year postoperative BMI \leq 25 kg/m², in a recently published paper of our group [17].

Before hospital discharge, all the patients received identical postoperative counseling, support, diet, and exercise instructions. Multivitamin and mineral supplements (WLS Maximum®, Fitforme®, Portugal) were uniformly prescribed (1 tablet/day). Additional calcium supplements were not prescribed.

Follow-up

All the patients were followed up by the surgeon and endocrinologist at 3, 6, and 12 months after surgery. The follow-up rate at 6 and 12 months was 100%; this means that all the patients attended to their appointments in the outpatient clinic. During the follow-up, anthropometric parameters and comorbidities resolution were evaluated.

Medical treatment, such as antidiabetic, antihypertensive, and hypolipemiant drugs, was adjusted according to the current needs of the patient. The nutritional status of the patients was evaluated by the endocrinologist with analytical blood tests. Deficiencies were supplemented, according to the results obtained.

Anthropometry and Body Composition

Body composition and anthropometry measures were evaluated between 8:00 and 10:00 a.m., after at least 8 h of fasting, with an empty bladder. Alcohol consumption and exercise were forbidden 8 h before the test [18]. Bioelectrical impedance analysis (Tanita BC-420MA, Tanita, Tokyo, Japan) was used to assess body weight, body composition, and bone mass. The accuracy of bioelectrical impedance analysis has been validated in bariatric patients by several studies [19, 20]. The guidelines of the International Society for the Advancement of Kinanthropometry was used to measure height, waist, and hip circumferences [21].

Variables

Anthropometric parameters (BMI), excess BMI loss (EBMIL), total weight loss (TWL), and body composition analysis, including fat mass, fat-free mass, and bone mass, were obtained at baseline (1 day prior to surgery), at 6 and 12 months after OAGB.

Nutritional deficiencies were determined by blood analysis obtained at 3, 6, and 12 months after surgery. Total proteins; hemoglobin; iron; ferritin; calcium; and vitamins A, D, and B12; and folic acid were determined. Calcium serum values were adjusted depending on total protein levels before establishing a diagnosis of hypocalcemia. Similarly, iron deficiencies were determined based on iron and ferritin levels and the presence of microcytic anemia.

Statistical Analysis

All data were analyzed using the statistical package SPSS 22.0 (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to verify the normality of the distributions. A paired *t* test was used to compare baseline data with results at 6 and 12 months after surgery. The associations between body composition variables were performed using Pearson's bivariate correlation analysis. Delta values and percent of change were for each variable between baseline data and results at 6 and 12 months after surgery. Data are expressed as mean \pm standard derivation. Significant differences were considered when p < 0.05. Cohen's *d* was used to calculate effect size (ES) and was interpreted as follows: 0.20–0.50 (small), 0.50–0.80 (medium), > 0.80 (large) [19].

Results

In this study, 94 patients were included (67% females and 33% males) with a mean age of 43.7 ± 10.6 years. The patients' baseline values showed a height of 166.7 ± 8.2 cm, and a preoperative weight and BMI of 114.9 ± 19.5 kg and 41.6 ± 6.3 kg/m², respectively.

Anthropometric Measurements and Body Composition

Baseline anthropometric measurements, body composition values, and the effects of OAGB on them can be observed in Table 1. At 6 months of surgery, patients presented a mean BMI of $26.4 \pm 3.4 \text{ kg/m}^2$ with a mean EBMIL of $97.9 \pm 20.1\%$ and a total weight loss (TWL) of $36.2 \pm 5.2\%$. Twelve months

Table 1 Body composition and bone mass

	Preoperative	6 months	12 months
Weight (kg)	114.9 ± 19.5	$72.8 \pm 10.3^{\rm a}$	67.4 ± 14.9^{a}
BMI (kg m ⁻²)	41.6 ± 6.3	26.4 ± 3.4^{a}	23.8 ± 3.9
EWL (%)		97.9 ± 20.1^{a}	110.2 ± 30.5^a
FM (kg)	54.1 ± 12.0	18.6 ± 6.7^{a}	14.0 ± 6.3^a
FM (%)	46.9 ± 5.3	25.5 ± 8.1^{a}	20.5 ± 7.8^a
FFM (kg)	60.8 ± 10.9	54.2 ± 9.6^{a}	$53.4\pm12.3^{\rm a}$
FFM (%)	53.1 ± 5.3	74.5 ± 8.1^{a}	79.4 ± 7.8^a
TBW (kg)	45.0 ± 9.3	38.2 ± 6.9^{a}	36.3 ± 10.0^a
TBW (%)	39.3 ± 4.1	52.5 ± 5.8^{a}	55.9 ± 6.7^a
Bone mass (kg)	3.05 ± 0.5	2.73 ± 0.4^{a}	2.67 ± 0.6^a

Data are mean \pm SD; *BMI*, body mass index; *EWL*, excess weight loss; *FM*, fat mass; *FMM*, fat-free mass; *TBW*, total body water

^a Significant difference from preoperative values, p < 0.01

after surgery, mean BMI was 23.8 ± 3.9 kg/m² with a mean EWL of $110.2 \pm 30.5\%$ and a TWL of $38.8 \pm 7.7\%$.

Six months after surgery, patients showed a fat mass reduction of $65.2 \pm 11.1\%$, and 12 months postoperatively, mean fat mass reduction was $70.7 \pm 11.6\%$.

OAGB also provoked reductions on FFM, and significant decreases were observed at 6 and 12 months after surgery, with a reduction of 6.6 ± 4.8 kg (p < 0.01) and 7.9 ± 4.9 kg (p < 0.01). The FFM reduction was $10.5 \pm 7.3\%$ and $12.9 \pm 6.6\%$ at 6 and 12 months after surgery, respectively. This reduction represented $15.7 \pm 10.6\%$ and $19.2 \pm 12.2\%$ of the total weight loss at 6 and 12 months after OAGB. Equally, bone mass decreased by $10.1 \pm 6.9\%$ 6 months after surgery, and $12.9 \pm 6.5\%$ at 12 months after the surgical procedure. Changes in total weight were associated with changes in bone mass 6 months after surgery (r = 0.326, p = 0.009), while changes in FFM reported positive and significant correlations with changes in bone mass in all measures (6 months r = 0.985, p = 0.000; 12 months r = 0.980, p = 0.000).

Body composition changes were greater during the first 6 months after surgery, as 88.6% of the total weight lost during the study occurred 6 months after surgery. Similarly, 89.2% and 84.2% of the total reductions of FFM and bone mass during the follow-up study occurred 6 months after surgery (Table 1).

Vitamin and mineral deficiencies at 12 months postoperatively are summarized in Table 2. The most remarkable deficiencies include vitamin D (deficiency in 34% of the patients), iron (15% of the patients), and folic acid (8.5% of the patients). Once the specific deficiency has been documented in the laboratory data, a proactive specific supplementation is prescribed additionally to the routine multivitamin and mineral complex. The specific supplementation is maintained until analytical values are within the normal range. There were no cases of hypoproteinemia.

Table 2 Nutritional deficiencies, requiring specific supplementation

12 months postoperatively	N (%)
Calcium	5 (5.3)
Vitamin D	32 (34)
Iron	14 (15)
Vitamin B12	6 (6.4)
Folic acid	8 (8.5)
Vitamin A	1 (1.1)

Calcium deficiencies associated with vitamin D ones were supplemented with calcium citrate 250 mg and cholecalciferol 1.5 µg (Calcium Plus, Fitforme, Portugal), 3 tabs/day. Isolated vitamin D deficiencies were supplemented with calcifediol 266 µg (Hidroferol, Faes Farma, Spain), 1 blister/week. All the patients reported complete compliance with the prescribed supplementation.

Sex Differences

Sex differences can be observed in Fig. 1. At 6 months, the relative change in total weight, FFM, and bone mass did not show significant changes between sexes. However, males had greater relative fat mass losses (male $-72.6 \pm 7.7\%$, female - $62.2 \pm 10.9\%$; p = 0.000). At 12 months, females showed a tendency to a greater relative decrease of FFM (male -10.8 \pm 9.4%, female – 14.4 \pm 3.1%; d = 0.55) and bone mass (male $-10.5 \pm 10.1\%$, female $-14.5 \pm 3.0\%$; d = 0.64) (Fig. 1).

Discussion

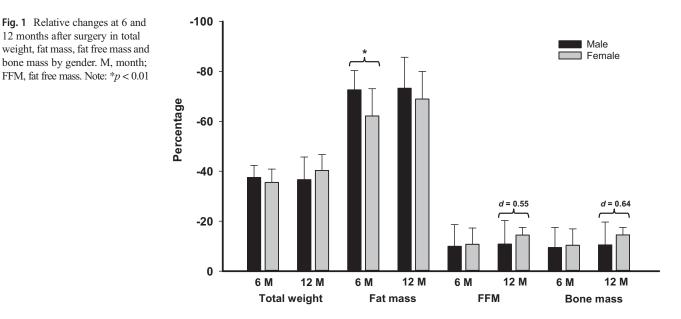
12 months after surgery in total

Bariatric surgery causes large weight reductions, mainly the first year after surgery, with different EBMIL depending on

the bariatric technique used; malabsorptive approaches obtain greater EBMIL than restrictive or mixed ones [22-25]. As a malabsorptive technique, in the present study, the EBMIL reaches up to 119.1%. Other studies on OAGB have reported reductions ranging from 75-89% of EWL 12 months after OAGB [24-26].

In 2018, the International Federation for Surgery of Obesity (IFSO) published a position statement, referring to OAGB. In this statement, they decided to include all the bariatric procedures with a single gastroenteric anastomosis under the acronyms OAGB, abolishing the terms "mini-gastric bypass (MGB)," "single-anastomosis gastric bypass," or "omega-loop gastric bypass" [27]. The OAGB we describe in this paper has important differences with other techniques actually also denominated "OAGB," such as a longer pouch of 20 cm (the stomach is sectioned in the middle of the distance between the pylorus and the incisura angularis), a calibrated 2.5-cm-long gastroenterotomy, and the measurement of the total bowel length, in contrast to a shorter gastric pouch, a 5-cm-wide anastomosis, and the measurement of only the biliopancreatic limb in the other techniques. The first modifications are focused on reducing the biliary reflux and the latter on decreasing the risk of malnutrition [15, 16]. Therefore, other studies have reported completely different results than the present one, because the technique they define as OAGB is different than the one we describe here. In our study, 12 months after surgery, EBMIL was greater than those reported in previous papers. These greater weight loss may be due to a longer biliopancreatic limb length used in our patients, which is only possible to perform in a safe manner, after the measurement of the total bowel length [28].

In bariatric patients, dual-energy X-ray absorptiometry (DXA) is considered the gold standard to evaluate body composition. Studies that have used this method to assess body



composition after sleeve gastrectomy and RYGB show reductions in FFM between 5 and 8.6 kg (between 9.4 and 14%) 6 months after surgery [29-32], and decreases between 4.4 and 10.7 kg (8.6–18.6%) at 12 months [12, 29, 32–35]. However, due to the high cost of the material used, DXA is an expensive technique and requires trained personnel for its use. Bioelectrical impedance is presented as a suitable alternative to evaluate body composition, since it is a reliable method, has a low cost, and is easy to apply. The bioelectrical impedance has demonstrated significant correlations with the DXA method in bariatric patients before and after surgery, both to determine the fat mass $(r^2 = 0.94)$ and fat-free mass $(r^2 = 0.82)$, demonstrating the reliability of this method [18]. High accuracy has been reported even in phases of weight loss [7]. Several studies have used this method to assess body composition after sleeve gastrectomy, reporting decreases between 6.1 and 9.5 kg (decrease of 10.2 and 11.4%, respectively) of FFM 6 months after surgery [5, 36, 37]. Likewise, after RYGB, reductions of 5.9-8.9 kg (8.9-12.5%) have been obtained at 6 months [5, 34, 35], while 12 months after surgery these reductions were between 7.8 and 10 kg (12.4-14.9%) [5, 35, 38, 39].

Our results show that the OAGB produces a large reduction of adipose tissue. This tissue is associated with high risks for health and the development of a large number of comorbidities [40]. Therefore, the reduction of fat mass provides great benefits, since it decreases the incidence of cardiovascular diseases, such as dyslipidemia, type 2 diabetes mellitus, or hypertension. However, in the same way that studies previously showed, our patients also report significant reductions in FFM, both at 6 and 12 months after surgery. Although, to our knowledge, the effects of the OAGB on body composition has scarcely been studied, these reductions are similar to the results obtained in other studies on RYGB. These studies have reported decreases in FFM of, approximately, 6 kg 6 months after surgery [37] and reductions of 13% of FFM at 12 months postoperatively [41].

The reduction of the FFM can be mainly due to the high caloric deficit to which the patients are submitted after BS, together with low protein intake. After surgery, an intake of 60 g/day and up to 1.5 g/kg ideal body weight per day is recommended [42]. However, some studies conclude that 45% of patients do not follow these recommendations 4 months after surgery, while only 32% of patients exceed the intake of 1.2 g/kg of ideal body weight per day 12 months after BS [38]. Losses of FFM have different disadvantages. On the one hand, after bariatric surgery, large decreases in basal metabolic rate are produced [43], which is associated with reductions in FFM [44] since it is composed of tissues that consume a large amount of energy [7]. A low basal metabolic rate will predispose to weight regain, which is a common problem in bariatric patients [36, 37], while negative associations have been found between FFM and weight regain after surgery [45].

On the other hand, FFM is largely composed of skeletal muscle [46], which in recent decades has been recognized as an endocrine organ [47, 48]. Among other functions, skeletal muscle is responsible for metabolizing approximately 75% of glucose after a meal [49] and has an important storage function, since 80% of the body's glycogen is stored in the skeletal muscle [50]. Therefore, preserving fat-free mass can be important to maintain weight loss in the mid and long term after surgery and can prevent the relapse in comorbidities associated with obesity. In the present study, we observed FFM loss similar to the results reported by other groups on RYGB [36, 41], suggesting that FFM loss is not increased after malabsorptive procedures.

Regarding the bone mass, our results show a decrease of 10.1% and 12.9% at 6 and 12 months after surgery. The reduction of bone mineral density is common after BS. Diverse studies showed decreases of between 8 and 11% and 3–8% of the bone mineral density of the hip and waist, respectively, at 12 months of Roux-en-Y gastric bypass [51, 52]. Unfortunately, in the scientific literature, there are few studies that evaluate bone mass by bioelectrical impedance.

The pathways suggested in bone loss after surgery are, mainly, malabsorption of calcium and vitamin D, changes in adipose and gut hormones, and a lower mechanical load [10]. However, the mechanical load seems to be especially important in the variation of bone mass. In our study, significant correlations are reported between changes in total weight, changes in FFM, and bone mass changes. This coincides with the results reported by other authors, who found that reductions in bone mineral density were associated with weight loss and not with variations in vitamin D [31]. These correlations suggest that the large reductions in weight caused by BS imply a lower mechanical load, which may be a relevant factor for bone mineral content reduction.

Although little information is currently available about this topic, some studies show that fracture probability within 10 years increases from 1.5 to 2.1% at 12 months after surgery [30], while other studies concluded that the relative risk of suffering a fracture was 2.3 times greater after surgery [53]. Previously, Peppa et al. showed that bioelectrical impedance is a reliable tool to detect osteopenia and osteoporosis and concluded that subjects with a normal bone density showed 3.2 kg of bone mass (measured by bioelectrical impedance), while subjects with 2.7 kg and 2.6 kg suffered osteopenia and osteoporosis, respectively [54]. In our study, bariatric patients decreased bone mass up to 2.73 kg and 2.67 kg at 6 and 12 months of BS, reporting that patients are likely to suffer from osteoporosis. In fact, at 6 months of surgery, 60.3% of the patients showed values ≤ 2.7 kg bone mass, increasing to 64.5% 12 months after surgery. These data do not significantly differ from the reported results after RYGB or SG.

Most changes in total weight and body composition occur in the short term after BS. Our results report that an 89.2% reduction of the FFM and 84.2% of the decrease in bone mass occurs during the first 6 months after surgery. According to the literature, most reductions in bone mineral density occur in the short term [55]. In turn, Ciangura et al. showed how, during the first 3 months of surgery, 2.3 kg per month of lean body mass was lost, while this trend was reduced to 0.5 kg per month between 3 and 6 months after surgery and 0.2 kg per month between 6 and 12 months after BS [32].

Health professionals should use strategies to prevent these disadvantages, and these should be applied mainly during the first 6 months of the surgery since it is at this point when major reductions in FFM and bone mass occur. Although there are currently several treatments to prevent these reductions, such as calcium, vitamin D, and protein supplementation, one that seems to be especially effective is to perform exercise after surgery. Performing exercise reduces the loss of bone mineral density and FFM [56, 57]. In addition, the type of exercise that is performed is determinant, since it seems that resistance training takes on great importance for preventing losses of FFM and bone mass [58].

Limitations

One of the main strengths of the present study is the high follow-up rate, similar to that reported in previous studies of our group. In our opinion, a close follow-up is mandatory after all bariatric procedures, and even more after malabsorptive ones, as an important amount of patients will present nutritional deficiencies, which should be early diagnosticated and treated in order to prevent further metabolic sequelae [13, 15].

This study has several limitations. First, the gold standard for measuring bone mass is by using bone densitometry scans; however, it has been measured by means of bioelectrical impedance, which is a validated method for this aim in the bariatric population. However, some authors have identified some variability in individual repeat measures, when using foot-only electrode devices. In addition, Savastano et al. used bielectrical impedance devices with both hand and foot electrodes and acknowledged a likelihood of overestimating FFM. Additionally, the DXA bone density function of this scale has still low evidence of validity [18, 19].

Second, physical activity levels performed after surgery and protein intake, factors that could have influenced changes in FFM, were not collected. Third, baseline measurements were obtained after the mandatory weight loss of 10% of total body weight.

We have to assume that during the follow-up, we did not routinely supplement with calcium, as the deficiency rate without this supplementation was relatively low among our patients (5.3%). However, calcium deficiency was diagnosed using corrected calcium, which would only be seen in advanced calcium deficiencies. The assessment of parathyroid hormone levels would have been a more sensitive indicator of dietary calcium intake. Thus, our hypocalcemia rates were probably underestimated. In addition, in cases with calcium deficiencies, repletion doses were also below recommendations (750 mg/day). Given the results obtained in the present study referring to bone mass reduction, we would probably recommend a routine calcium supplementation after all OAGB, and in cases of deficiencies to prescribe higher doses for repletion (1200–2000 mg/day). Moreover, the routine evaluation of calcium metabolism should also include parathormone levels [42].

Further limitations include that preoperative sedentarism, sun exposure, or postmenopausal status in females were not assessed. Finally, preoperative serum vitamin D levels were not routinely measured and consequently preoperative vitamin D deficiencies could not be determined. Postoperative values were measured and considered for the evaluation of calcium and vitamin D deficiencies. However, these laboratory values were not collected in the database for the present study.

Conclusion

OAGB causes large reductions in fat mass, but it also generates mild reductions in the FFM and bone mass, especially in females. Major reductions in FFM and bone mass occur during the first 6 postoperative months. FFM and bone mass decreases do not significantly differ from data about Rouxen-Y gastric bypass reported on literature. Further longitudinal observations and comparative studies with other techniques are still needed to verify the effects of OAGB on body composition and bone mineral content and to find strategies that can prevent these disadvantages generated by surgery.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Statement of Informed Consent Informed consent was obtained from all individual participants included in the study.

Statement of Human Rights All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments.

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