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CHANGES IN BONE MINERAL PARAMETERS AFTER SLEEVE GASTRECTOMY: RELATIONSHIP WITH GHRELIN AND PLASMA ADIPOKINE LEVELS

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Abstract

Context. Metabolic surgery is currently the most efficient treatment for obesity, but concern is raised about the possible long-term nutritional side effects. Bone metabolism is often adversely affected after surgery, but literature data are contradictory.

Objective. The aim of this study was to evaluate the evolution of bone mass parameters in the first year after laparoscopic sleeve gastrectomy in relation to anthropometric and body composition parameters and specific hormones of obesity.

Design. We conducted a prospective study on 75 patients with obesity that underwent metabolic surgery over a course of 18 months at our center, with a follow-up period of 12 months.

Subjects and Methods. All patients underwent a complex preoperative assessment and were required to return for medical follow-up at 6 and 12 months after surgery. Each visit included anthropometric parameters, DEXA and determination of specific hormonal parameters.

Results. We noticed a significant improvement in anthropometric and body composition parameters after surgery. The value of adiponectin presented a significant increase after surgery and leptin showed a significant decrease at 6 and 12 months postoperative; ghrelin level decreased postoperative compared to preoperative, but without statistical significance. We observed no reduction in BMD after surgery, but a significant improvement in BMC at 12 months after surgery compared to preoperative. Ghrelin negatively correlated to BMD preoperative.

Conclusions. Despite the significant alterations in anthropometric, body composition and hormonal parameters, we found no negative effect on BMD and BMC in our study population.

Key words: laparoscopic sleeve gastrectomy, ghrelin, obesity, bone mineral density, bone mineral content.

INTRODUCTION

Obesity has become a global epidemic with over 650 million individuals affected by this disease worldwide (1). It leads to cardiometabolic complications and increased risk of cancer, that shortens life expectancy and decreases quality of life. The social and material impact is devastating. Unfortunately, most drug treatments are not effective. Aggressive diets, physical exercise, and cognitive behavioral approaches can result in about 5-15% loss of initial weight, rarely maintained over time and unaccompanied by the remission of complications (2, 3). Bariatric surgery has been officially approved for nearly three decades (4) and has strengthened its position over time through a variety of high performance, accessible (laparoscopic) and safe techniques. The number of surgeries has grown impressively (5), both the restrictive and malabsorptive (with or without restrictive component) ones, with comparable results. Studies have confirmed that bariatric surgery is superior to conventional medical treatments, both with regard to stable weight loss and especially to the regression of complications, reason why it gained the name of metabolic surgery (6). The multitude of evidence of dramatic weight reduction along with a reduction of obesity-related complications and comorbidities including type 2 diabetes mellitus, hypertension, dyslipidemia, nonalcoholic fatty liver disease and obstructive sleep apnea resulted in the recommendation of metabolic surgery in the guidelines for morbid or complicated obesity (7), sometimes as the only efficient treatment. However, there is evidence that bariatric procedures have nutritional and metabolic abnormalities still incompletely understood as adverse effects. Bone metabolism is often adversely affected, although the degree of this unwanted complication is not sufficiently estimated and the physiopathological mechanisms incompletely elucidated (8).

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Studies show that after bariatric surgery there is an increase in bone-turnover markers, and a continuous decrease in bone mass and its quality (9).

Sudden weight loss results in skeletal mechanical unloading with consequences on bone mass, strength and size. Mechanical loading results in bone formation by osteocytes (10), that act as a 'mechanostat'.

The easiest explanation would be the nutritional deficiency, the decrease in key osteogenic components such as calcium, phosphorus and vitamin D, but it turned out that these changes are just the "tip of an iceberg" of bone remodeling.

Ghrelin is a peptide that releases growth hormone, being synthesized primarily in the stomach. In humans, ghrelin is an appetite-stimulating hormone and at the same time has anabolic effect, decreasing energy expenditure (11). Thus, it stores energy, acting on the bone by increasing osteoblast proliferation and differentiation through its effects on growth hormone (12) and via mitogen-activated proteinkinase (MAPK) pathway (13). Ghrelin has an anabolic effect on bone tissue and positively influences trabecular bone density (14). However, literature data on the action of ghrelin on osteoclasts are contradictory, and even less data exist regarding the involvement of ghrelin in bone metabolism after bariatric surgery.

Leptin is an adipokine released from white adipose tissue in proportion to the size of fat depots, with the role of informing central nervous system about the body energy deposits. Leptin level decreases significantly after bariatric surgery in proportion to the loss of adipose tissue. This decrease affects bone metabolism by influencing both bone formation (upregulation of osteoblast function and bone formation) and bone resorption (increase in osteoclast production and activity by promoting bone resorption) (15, 16). Decreased leptin after bariatric surgery inversely correlates with increased bone formation and resorption markers, tipping the balance towards bone loss.

Adiponectin, expressed exclusively by adipocytes, has low circulating levels in obesity and increases after bariatric surgery. Adiponectin receptors have been identified on both osteoblasts and osteoclasts. At osteoblast level, these receptors will promote their differentiation, while suppressing the formation and activity of osteoclasts (15, 16). It is the most important adipokine that negatively correlates with BMD no matter of gender or post menopausal status (17).

Through multifactorial mechanisms, some incompletely known, obesity itself and its surgical treatment make the bone vulnerable and increase the risk of fracture. Although, especially after 2012, data from studies began to be systematically analyzed (18), no consensus has been reached regarding the risk groups, their preoperative assessment, and the type of intervention recommended.

The aim of this study was to evaluate the evolution of bone mass parameters in the first year after laparoscopic sleeve gastrectomy in relation to anthropometric and body composition parameters and specific hormones of obesity (ghrelin, adiponectin, leptin).

MATERIAL AND METHOD

Study population

We included in this study patients with obesity that were consecutively evaluated and underwent metabolic surgery over a course of 18 months at the Center for Obesity and Bariatric Surgery of "Sf. Spiridon" Emergency Hospital Iași.

In our center, patients are only considered for surgery if they meet the current criteria for metabolic surgery according to international guidelines and undergo the complex multidisciplinary assessment (19). After surgery, patients are required to return for nutritional and medical follow-up at 1, 3, 6, 12 months after surgery, then yearly, but, as in all bariatric centers, some patients are lost to follow-up (20). We included in this study all consecutive patients who gave informed consent, even if they did not present to the postoperative follow-ups; also, we included all patients who underwent bariatric surgery during the selected timeframe, which implied that not all patients had the 6 months and 12 months follow-up during the course of the study.

Studied variables

We measured all studied variables preoperative and at 6 and 12 months postoperative.

Anthropometric parameters (weight, height, and waist circumference - WC) were assessed according to the recommendations of the World Health Organization and allowed for the calculation of body mass index (BMI) (21). We also calculated excess weight (EW) using the difference between real weight of patients and ideal weight as defined by the Devine formula (22). The postoperative evolution of weight was described as percentage of excess weight loss

(%EWL), i.e. (weight (kg) lost over a period of time/ EW) x100.

Bone mineral density (BMD) and body composition (BC) were measured using dual-energy x-ray absorptiometry - DEXA (Hologic Delphi A; Hologic Inc., USA). Measurements were made according to the standard protocol and with daily calibration by two experienced technicians certified by the International Society for Clinical Densitometry (ISCD). BMD and whole body and regional body composition including fat mass and lean body mass were measured after an overnight fast, with the participant in the supine position and wearing a hospital gown. Body composition values were analyzed using software version 11.2 (Hologic) that allowed calculation of: bone mineral content (BMC-g), fat mass (g and %), lean mass (g), BMD (g/cm^2) - bone mineral content (g)/bone area (cm^2). Bone mineral density before and after bariatric surgery is usually assessed through imagistic methods and correlated with anthropometric parameters, metabolic, nutritional or hormonal parameters (25-hydroxyvitamin D, plasma PTH, ghrelin, leptin and adiponectin concentrations).

We determined the plasma values of specific hormonal parameters (acylated-ghrelin, adiponectin and leptin) by ELISA, using the equipment of the Genetics and Immunology Laboratory of "Sf. Spiridon" Hospital: ELISA reader, ELISA ASYS washer, SIGMA centrifuge and Zanussi fridge. Plasmatic acylated ghrelin was quantified using commercially available ELISA kits (BioVendor Laboratory, United States) based on a double-antibody sandwich technique.

Statistical analysis

Data was analyzed using Microsoft Office Excel and SPSS version 17.0. Numerical data were expressed as means and standard deviation (SD), minimum and maximum. Significant differences between numerical data were found using t student test and paired-samples t student (in this case descriptive statistics took into account only those pairs). We determined statistically significant correlations using Pearson's correlations, and we used a p value <0.05 to define statistical significance for all calculations.

Table 1. Clinical anthropometric and body composition parameters of study population prior to surgery

Parameter	Category	Mean±SD	Minimum	Maximum	p
Weight (kg) preop.	Men	147.577±20.56	110.0	180.0	**<0.001**
	Women	118.347±17.74	90.0	165.0	
	Total	123.413±21.26	90.0	180.0	
BMI (kg/m^2) preop.	Men	45.9007±4.65	38.06	54.34	0.667
	Women	45.0009±7.17	35.06	66.51	
	Total	45.1568±6.78	35.06	66.51	
WC (cm) preop.	Men	139.462±10.48	122.0	156.0	**<0.001**
	Women	123.550±14.4	101.0	170.0	
	Total	126.384±15.02	101.0	170.0	
EW (kg) preopr.	Men	67.389±16.89	37.8	97.2	**0.006**
	Women	52.355±17.34	26.0	91.0	
	Total	54.961±18.08	26.0	97.2	
BMD (g/cm^2) preop.	Men	1.23±0.23	0.858	1.6	0.108
	Women	1.16±0.08	0.994	1.36	
	Total	1.18±0.12	0.858	1.6	
BMC (kg) preop.	Men	3.22±0.4	2.34	3.64	**<0.001**
	Women	2.44±0.2	1.92	3.17	
	Total	2.57±0.4	1.92	3.64	
Fat (kg) preop.	Men	61.01±14.23	35.30	78.04	0.433
	Women	57.43±11.99	38.79	87.87	
	Total	58.04±12.33	35.30	87.87	
% fat preop.	Men	40.63±4.88	32.20	46.6	**<0.001**
	Women	46.96±3.83	40	56.9	
	Total	45.88±4.64	32.20	56.9	
Lean (kg) preop.	Men	84.14±7.8	71.9	97.63	**<0.001**
	Women	61.58±7.62	44.41	78.36	
	Total	65.41±11.4	44.41	97.63	

BMI=body mass index; WC=waist circumference; EW=excess weight; BMD=bone mineral density; BMC=bone mineral content.

Table 2. The evolution of clinical anthropometric and body composition parameters at 6 and 12 months after surgery

Parameter	Preoperative value	Value at 6 months postoperative	P*	Value at 12 months postoperative	P**	P***
Weight (kg)	124.135±20.85	91.48±16.84	**<0.001**	89.442±18.75	**<0.001**	**<0.001**
BMI (kg/m²)	45.43±7.14	33.06±5.69	**<0.001**	32.05±6.29	**<0.001**	**<0.001**
WC (cm)	127.36±15.26	101.69±14.38	**<0.001**	98.78±15.52	**<0.001**	**0.028**
%EWL		63.15±16.91		69.13±22.64		
% fat	45.72±5.4	36.79±6.34	**<0.001**	30.85±5.41	**<0.001**	**<0.001**
BMD (g/cm²)	1.169±.12	1.177±.07	0.781	1.21±.12	0.686	0.184
BMC (kg)	2.61±.425	2.55±.438	**0.035**	2.68±.541	**0.007**	0.075
Lean (kg)	65.58±12.42	55.91±11.22	**<0.001**	58.51±13.55	**<0.001**	0.259

BMI=body mass index; WC=waist circumference; EWL=excess weight loss; BMD=bone mineral density; BMC=bone mineral content.
*6 months postop. *vs*. preop.
**12 months postop. *vs*. preop.
***12 months postop *vs*. 6 months preop.

Table 3. The evolution of ghrelin, adiponectin and leptin after bariatric surgery

Parameter	Preoperative value	Value at 6 months postoperative	P*	Value at 12 months postoperative	P**	P***
Ghrelin (pg/mL)	54.31±23.5	52.21±22.8	0.417	48.30±18.2	0.275	0.244
Adiponectin (ug/mL)	11.41±3.44	15.05±4.69	**<0.001**	15.24±6.34	**0.014**	**0.021**
Leptin (ng/mL)	42.54±12.14	14.87±6.81	**<0.001**	15.7±8.29	**0.003**	0.552

*6 months postop. *vs*. preop.
**12 months postop. *vs*. preop.
***12 months postop *vs*. 6 months preop.

Ethical issues

The ethics committee of the "Grigore T. Popa" University of Medicine and Pharmacy approved the current study and all patients gave their informed consent prior to participation in the study.

RESULTS

We included 75 patients in the study (82.67% women), with and average age of 42.11±11.45 years (43.31±12.5 years in men, 41.85±11.3 years in women, p>0.05). Fifty-two patients were also evaluated at 6 months after surgery, and 26 patients were evaluated at 12 months after surgery as well. The average BMI before surgery was 45.15±6.78 kg/m², with patients presenting EW of 54.96±18.08 kg. There were no statistical differences between men and women in terms of BMI and BMD measured by DEXA, prior to surgery, but women presented a significantly higher percentage of body fat compared to men and also a significantly smaller WC. These data are presented in Table 1.

We noticed a significant improvement in anthropometric parameters after surgery (weight, BMI, WC), which leads to 63.15±16.91 %EWL at 6 months after surgery and 69.13±22.64 %EWL at 12 months after surgery. We also noticed a significant reduction in lean body mass in the first 6 months after surgery, but not afterwards (6 to 12 months after surgery). We observed no reduction in BMD after surgery and also a significant improvement in BMC at 12 months after surgery compared to preoperative (Table 2).

Our results showed that the value of adiponectin presented a significant increase after surgery (both at 6 and 12 months) and leptin showed a significant decrease at 6 months and 12 months postoperative compared to preoperative, but not at 12 months compared to 6 months postoperative. We observed a decrease in ghrelin level postoperative compared to preoperative, but this did not have a statistically significant value (Table 3). We found a significant inverse correlation between BMD and ghrelin preoperative (r=-0.448, p=0.009), but not with BMC, and the significance of this correlation did not persist postoperative; also, there were no significant correlations between adiponectin or leptin and BMC or BMD either preoperative or postoperative.

DISCUSSION

Human studies regarding the role of ghrelin on bone mineral density are limited and conducted on different study populations, leading to contradictory and inconsistent results. In one study on 137 older men, plasma ghrelin level correlated positively with BMD (23). Similar results were reported by Amini *et al.* which showed a significant positive correlation between plasma ghrelin level and BMD in women, suggesting that ghrelin would have a positive effect on BMD in women, independently of BMI, physical activity, age, smoking status or alcohol intake (24). Another study showed that both plasma ghrelin and BMD were significantly reduced at 11 months after sleeve gastrectomy (25). Different results were obtained in other studies on different populations (Korean middle-aged men, twins and older men and women), where there was no correlation between plasma ghrelin level and BMD (26-28). In contrast to these results, we found a negative correlation between plasma ghrelin and BMD preoperative, which was not maintained after surgery.

A meta-analysis published in 2011 showed that adiponectin correlated inversely, whereas leptin correlated positively with BMD especially in post-menopausal women. Increased levels of leptin are predictive of decreased risk of fracture, whereas an increased level of adiponectin is predictive for an increased risk of vertebrae fracture only in men. No significant association between ghrelin and BMD was demonstrated. Hence, adiponectin remains in this study the most relevant adipokine for BMD (17). Mpalaris *et al*. evaluated BMD together with assessing plasma concentration of leptin, adiponectin and ghrelin in 110 healthy post-menopausal women and showed an inverse correlation between adiponectin and BMD, independently of body weight; leptin was positively associated with BMD, but depending on weight, and ghrelin had no significant correlation with BMD (29). Other authors found a positive correlation between adiponectin and BMD in postmenopausal women (30). We found no significant correlations between adiponectin or leptin and BMD in our study population.

In bariatric surgery, laparoscopic sleeve gastrectomy (LSG) has gained much influence in recent years over Roux-en-Y gastric by-pass (RYGB): results on weight and metabolic parameters are encouraging, and numerous studies report significant and sustainable weight loss, with fewer adverse effects, among which we note reduced alteration in bone metabolism (31).

There is a relatively small number of studies reporting the possible relation between bone parameters (BMD, BMC) and the evolution of plasma ghrelin and adipokines after bariatric surgery, especially after LSG. Some studies demonstrated that the postoperative evolution of BMD was comparable between LSG and RYGB, the type of procedure having no influence on the bone mass (32, 33).

Some studies reported a reduction in BMD after surgery, but most studies referred in fact to the evolution after RYGB and other malabsorptive procedures, few authors studying particularly the effect of LSG on BMD and BMC (33). Carrasco *et al*. evaluated alterations in BMD after bariatric surgery (both procedures – LSG and RYGB) in relation to plasma ghrelin and adiponectin. Percentage of EWL was 79.1±3.8 at one year after RYGB and 74.9±4.1 at one year after LSG (no significant differences between these values). They showed a significant reduction in BMD only after RYGB and identified the significant reduction in ghrelin as the main factor which correlated with loss in BMD (34). Another study demonstrated that the effect of the two types of procedures on BMD was comparable at one year after the intervention, but menopausal women had a higher risk for low bone mass, even if osteoporosis was less frequent (31). However, Maghrabi *et al.* reported less reduction of BMD at 24 months after surgery for LSG compared to RYGB (35). Some studies reported an increase in BMC postoperative, a small insignificant reduction in BMD, simultaneously with the significant reduction in adipose tissue and lean body mass, suggesting that bone loss after bariatric surgery was related to the degree of weight loss and modification in body composition (36). Other authors reported a progressive increase in BMD in the first two years after LSG, but the modification in BMD was not associated with weight loss (37).

There were also some reports that the evolution of BMD after LSG depended on gender, with a significant reduction of BMD seen only in women (38). Our study brings valuable results, as it refers only to patients who underwent LSG and we showed that BMD and BMC were not decreased at 6 and 12 months after surgery, on the contrary, BMC presented a significant increase one year after surgery.

Numerous studies attempt to elucidate the causes of post-bariatric bone alteration, the relationship between them, and the possible protective factors. To date, comparing the results of these studies, due to the heterogeneity of the methods and study populations, brings a weak power of evidence.

Conflict of interest

The authors declare that they have no conflict of interest.

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