Changes in bone marrow adipose tissue one year after Roux-en-Y gastric bypass - a prospective cohort study.

Abstract

Bone marrow adipose tissue (BMAT) has been postulated to mediate skeletal fragility in type 2 diabetes (T2D) and obesity. Roux-en-Y gastric bypass (RYGB) induces a substantial weight loss, and resolution of comorbidities. However, the procedure induces increased bone turnover and fracture rates. No previous study has evaluated biopsy measured BMAT fraction preoperatively and after RYGB. In this study we aimed to investigate BMAT fraction of the hip in participants with and without T2D preoperatively and one year after RYGB, and explore factors associated with BMAT change. Patients with morbid obesity scheduled for RYGB where examined preoperatively and one year after RYGB. 44 participants were included and preoperative examinations were possible in 35. Of these 33 (94%) met for follow-up, two were excluded and BMAT estimation was not possible in one. Eighteen (60%) of the participants were females and 11 (37%) had T2D. Preoperative BMAT fraction was positively associated with glycosylated hemoglobin and negatively associated with bone mineral density (aBMD). After RYGB BMAT fraction decreased from 40.4 ±1.7% to 35.6 ±12.8%, p=0.042, or with mean percent change of 10.7% of preoperative BMAT fraction. Change in BMAT fraction was positively associated with change in BMI and total body fat. In females we observed a mean percent reduction of 22.4 ±19.6%, while in males BMAT increased with a mean percent of 6.8 ±37.5%, p=0.009. For males changes in estradiol were associated with BMAT change, this was not observed for females. In participants with and without T2D, the mean percent BMAT reduction was 5.8±36.9% and 13.5±28.0%, respectively, p=0.52. We conclude that a high BMAT seems to be associated with lower

aBMD and poorer glycemic control in obese subjects. After RYGB we observed a significant decrease in BMAT. The reduction in BMAT did not differ between participants with and without T2D, but appeared gender specific.

Key words: Morbid obesity, Roux-en-Y gastric bypass, Weight loss, Bone marrow adipose tissue, Bone marrow fat, Bone mineral density.

Introduction

The gradual replacement of the hematopoetic bone marrow with bone marrow adipose tissue (BMAT) starts in the appendicular skeleton during childhood and continues to increase with age [1]. In early adulthood males seem to have a higher BMAT fraction, however after menopause this gender difference appears reversed [2,3]. Bone marrow adipose tissue (BMAT) has been considered an inactive fat depot, but was recently recognized as an endocrine organ with local and systemic effects [4]. Increased BMAT fraction has been associated with increased fracture rates in conditions like anorexia nervosa, postmenopausal and idiopathic osteoporosis [5-7]. Although the function of BMAT remains to be fully understood it has been postulated that BMAT plays a role in lipid storage, metabolic homeostasis, hematopoietic regulation, mechanical function, thermogenesis, skeletal remodeling and fragility [8]. BMAT may be quantified histologically based on examinations of bone marrow biopsies or by imaging modalities like magnetic resonance spectroscopy (MRS). One paper has published acceptable correlations between lumbar spine BMAT, evaluated by MRS, and posterior superior iliac spine BMAT, estimated from bone marrow biopsy. However, this study was performed in lean premenopausal women and MRS was noted to report approximately 10% higher BMAT fraction [9]. Thus potential artefacts of obesity and weight loss are yet to be explored for non-invasive radiological methods of BMAT estimation.

Obesity and type 2 diabetes (T2D) are associated with increased fracture risk despite a normal areal bone mineral density (aBMD) [10-12]. Increased BMAT may be a contributing mediator of this skeletal fragility. Positive associations have been described between glycosylated hemoglobin (HbA_{1c}) and BMAT [13,14] and visceral/subcutaneous/total fat and BMAT [14,15]. However, studies diverge with regard to whether subjects with T2D [13,14,16] or obesity [17] have relatively more BMAT than controls.

Roux-en-Y gastric bypass (RYGB) is offered to patients with morbid obesity [18]. RYGB induces a large and persistent weight loss and remission of obesity related comorbidities, most notably T2D [19,20]. On the other hand RYGB appears to induce bone loss, increase bone turnover and fracture rates [21-24].

Studies indicate that a diet-induced weight loss is accompanied by a 1.1-3.5% reduction in MRI/MRS estimated BMAT [25,26]. Unexpectedly, studies evaluating BMAT with MRS six to twelve months after RYGB did not note any change [27,28]. However, one of the studies reported that the subpopulation of subjects with preoperative T2D experienced a 6.5% decline in BMAT [27]. To our knowledge no previous study has evaluated bone marrow biopsy measured BMAT preoperatively and one year after RYGB.

We aimed to explore potential changes in BMAT after RYGB and search for possible associated factors. Specifically, we wanted to investigate if such changes in BMAT fraction differed in participants with and without T2D. Secondly, we wanted to explore factors associated with BMAT including age, gender, sex steroids, menopausal status, metabolic homeostasis (glycemic control, blood lipid levels and T2D), BMI and body composition, bone mineral density and bone turnover, in a morbidly obese population. We hypothesized that the BMAT fraction would decrease after RYGB and that participants with T2D would have a larger decrease in BMAT fraction than participants without T2D.

Materials and Methods

Study population

Patients scheduled for RYGB at the Department of Morbid Obesity and Bariatric Surgery, Oslo University Hospital, a tertiary referral centre for treatment of morbid obesity, were

92 recruited. Eligibility criteria for RYGB were body mass index (BMI) ≥ 40 kg/m^2 or BMI ≥ 35
93 kg/m^2 with obesity-related co-morbidity, age between 18 to 65 years, and failed attempts of
94 sustained weight loss [29]. Patients with T2D were encouraged to participate. Participants were
95 included from 08.10.15 to 27.01.17. Participants were excluded if they were unable to read
96 Norwegian language or if they had severe psychiatric comorbidity, connective tissue disorders
97 or other hormonal diseases, kidney failure (glomerular filtration rate < 30 mL/min/1,73m^2),
98 type 1 diabetes, BMI > 47 kg/m^2, history of treatment with bone active substances
99 (bisphosphonates, denosumab, hormone replacement or parathyroid hormone), or if they were
100 currently receiving anticoagulation or steroid treatment (estrogen, testosterone or
101 glucocorticoids). To avoid heterogeneity in our study population non-Caucasians were
102 excluded.

103 *Surgery, study visits and follow-up*

104 A laparoscopic RYGB with a gastric pouch of about 25 ml, a 150 cm antecolic alimentary and
105 a 50 cm biliopancreatic limb was performed in all participants [30]. Participants attended study
106 visits preoperatively and one year after RYGB. Study visits included morning fasting blood
107 samples, anthropometric measures, dual-energy absorptiometry scan, impact
108 microindentation, intra-venous glucose tolerance test, euglycemic hyperinsulinemic clamp,
109 indirect calorimetry and bone marrow biopsy. In this paper the results of the blood samples,
110 anthropometric measures, DXA and bone marrow biopsy are presented. Baseline
111 characteristics with comparison of participants with and without T2D and changes in impact
112 microindentation, aBMD, and bone turnover markers has been previously published. The
113 gender distribution and mean preoperative BMI was comparable between participants with
114 and without T2D .

The participants also attended routine clinical follow-up with visits six weeks, six months and one year after surgery. After surgery all participants were advised and prescribed oral supplementation with 1000 mg of calcium, 800 IE vitamin D, one multivitamin, 200 mg of iron daily and B12 injections 1mg every third month. At routine clinical visits vitamin levels were monitored and additional supplements were advised on demand.

Bone marrow biopsies

Preoperative and follow-up bone marrow biopsies were taken from the right posterior superior iliac spine (except for in one participant where both biopsies were taken from the left) after injection of local anaesthesia. The posterior superior iliac spine was identified by palpation. Bone marrow biopsies were obtained using an 8G T-LokTM Jamshidi crista biopsy needle from Argon Medical Devices (Stenløse, Denmark), fixed in 70% ethanol directly, and stored at 4ºC. For histological analysis the biopsies were embedded undecalcified in methylmetacrylate [(31)]. After embedding 7 µm sections were cut using a Jung microtome model K (R. Jung GmbH, Heidelberg, Germany) equipped with a tungsten knife. To achieve a largest possible area the biopsies were cut through the middle. Then two levels were cut with a distance of 100 µm. These sections were stained with Masson Goldner Trichrome. BMAT fraction was quantified as adipocyte volume (AdV) relative to marrow volume (MarV) using grid based point-counting. Grid size were 0,03 mm^2 and 0,06 mm^2, where the smaller grid was used for lower BMAT fractions and the larger for higher BMAT fractions. We used a light microscope (Nikon Eclipse 80I, Tokyo, Japan) equipped with a motorized specimen stage (Prior Proscan 11 TM, Rockland, MA, USA), and a digital video camera (Olympus DP72, Tokyo, Japan) connected to a PC running the NewCast interactive stereology software (Visiopharm, Hørsholm, Denmark). The estimates were performed at x 230 magnification. The presented BMAT fraction is the mean of the estimated AdV/MarV from two levels of the biopsy. Biopsies obtained preoperatively and one year after RYGB were processed and

analysed in batches by one lab technician blinded for all clinical data. Coefficient of variation was calculated by recounting 5 randomly selected biopsies and the mean value was 2.8% and 3.3% preoperatively and one year after RYGB, respectively.

Bone mineral density

Dual energy x-ray absorptiometry (DXA) scans including whole body scans were performed for assessment of body composition, including whole body fat and lean mass. aBMD, g/cm^2, of the lumbar spine (L_1-L_4), hip, proximal femur and total body was assessed. The lumbar vertebra with the lowest aBMD was used in the analysis. All scans were performed by the same nurse. GE Lunar Prodigy was used until 26.8.2016 from then on GE Lunar iDXA's was used. Body composition performed with GE Lunar Proliogy was reanalysed with iDXA software to optimise comparability. The two DXA scanners were cross-calibrated by scanning 16 volunteers with both machines and revealed lumbar spine (L1-L4) intra-class correlation coefficient (ICC) (95% CI) of 0.989 (0.968 to 0.996), and for femoral neck and total hip, ICC (95% CI), was 0.994 (0.982 to 0.998) and 0.996 (0.988 to 0.999), respectively. The DXA machine was calibrated daily against the standard calibration phantom, supplied by the manufacturer, and the estimated short-term precision errors for aBMD at the lumbar spine and at the femoral neck was < 1.0%.

Blood samples

All blood samples were taken before 10 am after an overnight fast. Serum for bone turnover markers (CTX-1 and PINP) was centrifuged and stored at -80ºC and analyzed after study follow-up was completed to avoid inter-assay variation. All other study blood sample analyses were made shortly after retrieval.

The Hormone Laboratory, Oslo University Hospital analyzed carboxyl terminal telopeptide of type 1 collagen (CTX-1) and procollagen type 1 N-terminal propeptide (PINP)

using Roche electrochemiluminescence immunoassay (ECLIA), serum 25(OH) vitamin D and testosterone levels was analyzed by liquid chromatography-mass spectrometry (LC-MS/MS) method, PTH by Immulite 2000 XPI, Siemens Healthineers a non-competitive chemiluminoimmunometric assay, FSH using Immulite 2000 XPI, Siemens Healthineers, a non-competitive immunluminometric assay and estradiol with a competitive immunluminometric assay Liaison XL kit from Diasorin Inc. The Central Laboratory of Oslo University Hospital analyzed HbA_{1c} using Tosoh G8 high-performance liquid chromatography, total cholesterol, low density lipoprotein cholesterol and triglycerides were analyzed with a Cobas 6000 from Roche using an enzymatic colorimetric method.

Clinical parameters

Morbid obesity was defined as BMI ≥ 40 kg/m^2 or BMI ≥ 35 kg/m^2 with obesity-related co-morbidity [29]. T2D was defined as HbA_{1c} > 6.5% or use of one or more oral glucose lowering drug with or without insulin treatment. Diabetes remission was defined as HbA_{1c} < 6.5% without the use of glucose lowering drugs in participant with T2D preoperatively. Hypercholesterolemia was defined as low density lipoprotein cholesterol ≥ 3 mmol/L or use of statins. All fractures except digit fractures are reported. Hormonal intrauterine devices made clinical evaluation of menstrual cycle difficult. For this reason a postmenopausal status was defined as a serum follicle stimulating hormone (FSH) > 25 IU/l [32].

Study size

This was an explorative study and the first to evaluate BMAT fraction in bone marrow biopsy preoperatively and one year after RYGB. However, sample size estimation was performed using BMAT as the primary endpoint based on data from a previous study evaluating change in BMAT following teraparatide treatment [33]. Given a mean change in BMAT of 5.5% between baseline and follow-up with an estimated standard deviation of 9.0%, type I error of

5% and power of 90%, a total of 31 participants should be included. Additional 10% was added to account for possible technical difficulties with bone marrow biopsies and lost to follow-up.

Statistical analysis

Normally distributed continuous variables are presented as mean and standard deviation ±SD others are presented as median (range). Categorical data are presented as proportions (percentage). When comparing preoperative gender characteristics, independent sample t-test or Mann-Whitney U test were used for continuous variables. Pearson Chi-square or Fisher's exact test were used for categorical variables as appropriate. Intra-class correlation coefficient (ICC) with 95% CI was used to assess concordance between the two DXA scanners (GE Lunar Prodigy and GE Lunar iDXA). ICC values of 0.75 or higher were considered excellent [34]. For evaluation of changes from pre- to post RYGB, paired-sample t–tests or Wilcoxon signed-rank tests were used. To explore differences in changes from pre- to post RYGB between subgroups delta values were compared with independent sample t-test or Mann-Whitney U test. Adjustments for confounding factors were performed using multiple linear regression analyses. Only variables with significant relationships with both the exposure and the outcome variables were considered as possible confounders in addition to variables of known clinical importance. Possible confounding variables were age, gender, BMI and preoperative BMAT fraction. Confounders that correlated, $r > 0.7$, were not adjusted for in order to avoid multicollinearity. The results from the regression analyses are presented as regression coefficients (β) with 95% confidence intervals (CI). Two tailed p-values < 0.05 were considered statistically significant. All statistical analyses were made using the IBM SPSS statistics version 25.0 (IBM SPSS Inc., Armonk, NY: IBM Corp).

Ethics

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Regional Committee for Medical and Health Research Ethics North Norway; 2015/604. Written informed consent was obtained from all participants.

Results

A total of 44 participants were included. Preoperative bone marrow biopsies were possible in 35, and 33 of these (94%) met for the one year follow-up visit. Two participants were excluded at follow-up due to sex reassignment and glucocorticoid treatment, respectively. BMAT fraction was not possible to estimate in one biopsy at one year follow-up. Thus the study population ultimately consisted of 30 participants.

Preoperative participant characteristics

Preoperative characteristics are presented in Table 1. Males had a higher body weight, more lean mass and a higher fraction reported previous fractures compared to females. Eleven participants had T2D, median duration since diagnosis of T2D was 5 years (range 1 to 18 years) and a mean preoperative HbA_{1c} of 6.9 ± 0.70%. Eight (73%) were treated with oral glucose lowering drugs and one with oral glucose lowering drugs and insulin. BMAT fractions in participants with and without T2D were 43.3 ± 10.8% and 38.7 ± 8.1%, respectively, p=0.20. Preoperative BMAT fraction was positively associated with $HbA_{1c,}$ this association remained significant after adjustment for gender. Both lumbar spine and femoral neck aBMD were negatively associated with BMAT fraction, but only the association between lumbar spine aBMD and BMAT fraction remained significant after adjustment for age and gender (Table 2).

Table 1: Preoperative characteristics in 30 participants with morbid obesity scheduled for Roux-en-Y gastric bypass

	All subjects n=30	Females n=18	Males n=12	
Age, years	46.3 ± 9.6	44.8 ±8.5	48.5 ± 11.1	
Postmenopausal		8 (44%)		
Smoking, current or previous	18 (60%)	11 (61%)	7 (58%)	
Any previous fracture	17 (57%)	7 (39%)	10 (83%)	*
Type 2 diabetes	11 (37%)	7 (39%)	4 (33%)	
Hypercholesterolemia	10 (33%)	5 (28%)	5 (42%)	
Weight, kg	120.1 ± 15.3	113.2 ± 11.7	130.4 ± 14.5	*
Body mass index, kg/m^2	40.7 ± 3.6	41.6 ± 3.3	39.4 ± 3.6	
Total body fat, kg	54.5 ± 8.5	56.2 ± 8.0	54.4 ± 9.4	
Total body lean mass, kg	61.8 ± 11.5	54.3 ± 4.4	71.8 ± 9.0	*
Systolic blood pressure, mmHg	126.0 ±11.8	125.4 ±10.0	126.9 ± 13.5	
Diastolic blood pressure, mmHg	82.0 ± 7.8	82.3 ± 6.4	81.4 ± 9.8	
HbA_{1c}, %	6.0 ± 0.83	5.9 ± 0.66	6.1 ± 1.1	
Total cholesterol, mmol/L	4.3 ± 0.73	4.5 ± 0.63	4.0 ± 0.79	
LDL cholesterol, mmol/L	2.8 ± 0.69	2.9 ± 0.64	2.6 ± 0.78	
Triglyseride, mmol/L	1.5 ± 0.64	1.4 ± 0.63	1.6 ± 0.64	
25(OH) vitamin D, nmol/L	56.1 ± 20.2	55.4 ± 19.8	57.2 ± 21.7	
Parathyroid hormone, pmol/L	4.7 ± 2.0	4.6 ± 1.8	4.8 ± 2.3	
Areal bone mineral density, g/cm^2				
Lumbar spine	1.11 ± 0.13	1.13 ± 0.12	1.09 ± 0.15	
Femoral neck	1.08 ± 0.12	1.09 ± 0.12	1.07 ± 0.12	
Total hip	1.16 ± 0.12	1.18 ± 0.13	1.13 ± 0.11	
Total body	1.33 ± 0.087	1.31 ± 0.090	1.35 ± 0.081	
Bone marrow adipose tissue, %	40.4 ± 9.3	39.4 ± 9.9	41.9 ± 8.4	
CTX-1, µg/L	0.34 ± 0.14	0.31 ± 0.13	0.39 ± 0.15	
P1NP, µg/L	47.3 ± 20.7	44.0 ± 18.4	52.2± 23.7	
Testosterone, nmol/L		0.59 ± 0.35	13.8 ± 4.1	*
Estradiol, nmol/L		0.11 ± 0.14	0.078 ± 0.030	

*Significant difference between genders, $p < 0.05$. Normally distributed continuous variables are presented as mean and standard deviation ±SD others are presented as median (range). Categorical data are presented as proportions (percentage). Abbreviations: Glycosylated hemoglobin (HbA_{1c}), low density lipoprotein (LDL) carboxyl terminal telopeptide of type 1 collagen (CTX-1) and procollagen type 1 N-terminal propeptide (PINP). Any fracture except digit fractures are reported.

Table 2: Factors associated with bone marrow adipose tissue fraction in 30 subjects with morbid obesity scheduled for Roux-en-Y gastric bypass.

	Unadjusted			Adjusted		
	β	95% CI for β	p	β	95% CI for β	p
Female gender	-2.5	-9.6 to 4.7	0.48			
Postmenopausal	6.0	0.21 to 15.8	0.21			
Age	0.286	-0.072 to 0.64	0.11			
Type 2 diabetes	4.6	-2.5 to 11.7	0.20			
Hypercholesterolemia	4.7	-2.6 to 12.0	0.20			
HbA_{1c}	**4.4**	**0.41 to 8.3**	**0.032**	**4.3**	**0.22 to 8.3**	**0.039**
Body mass index	-0.93	-1.87 to 0.013	0.053			
Total body fat mass	-0.44	-0.83 to -0.055	0.078			
Total body lean mass	0.025	-0.29 to 0.34	0.83			
Lumbar spine aBMD	**-31.1**	**-55.1 to -7.1**	**0.013**	**-27.5**	**-52.8 to -2.3**	**0.034**
Femoral neck aBMD	**-30.8**	**-58.4 to -3.2**	**0.030**	-25.7	-58.7 to 7.2	0.12
Total hip aBMD	-23.2	-51.3 to 4.9	0.10			
Total body aBMD	-18.3	-58.9 to 22.3	0.36			
aBMD t-score < -1.0	8.0	-0.23 to 16.3	0.056			
CTX-1	18.0	-6.4 to 42.5	0.14			
PINP	0.025	-0.15 to 0.20	0.77			

Linear regression; HbA_{1c} was adjusted for gender. Lumbar spine and femoral neck aBMD was adjusted for age and gender.
The results from the regression analysis are presented as regression coefficients (β) with 95% confidence intervals (CI).
Abbreviations: Glycosylated hemoglobin (HbA_{1c}) areal bone mineral density (aBMD), carboxyl terminal telopeptide of type 1 collagen (CTX-1) and procollagen type 1 N-terminal propeptide (PINP).

Participant characteristics after RYGB

All participants lost weight after RYGB. Mean weight loss was 32.6 ± 10.8 kg or 27.2 ± 8.7% of total preoperative weight. Fat mass decreased with 26.2 ± 9.4 kg and lean mass decreased with 6.2 ± 2.6 kg, all $p < 0.001$. Females lost 12.4 ± 4.4 units of BMI (kg/m^2), while males lost 9.1 ± 3.0 units of BMI (kg/m^2), p=0.032, however this gender difference was no longer significant when adjusting for preopative BMI. Weight loss in kg, decrease in lean mass and fat mass were not different between males and females. One year after RYGB aBMD decreased with 4.3 ± 5.9% in the lumbar spine, 8.2 ± 4.8% in the femoral neck, 11.8 ±4.9% in total hip and 9.4 ± 3.9% in total body. Of the 11 participants with T2D preoperatively, all except one were in diabetes remission one year after RYGB and the mean HbA1c decrease was 1.1 ± 0.76% one year after RYGB. BMAT fraction decreased from 40.4 ± 1.7% preoperatively to 35.6 ± 12.8 % at follow-up, p=0.042, or with mean percent change of 10.7%

of preoperative BMAT fraction (Figure 1). Example of a bone marrow biopsy taken preoperatively and one year after RYGB from the same participant is shown in Figure 2.

Figure 1: Individual percent changes in bone marrow adipose tissue (BMAT) fraction in 30 participants after Roux-en-Y gastric bypass (RYGB). Female participants are marked with black bars and male participants are marked with grey bars.

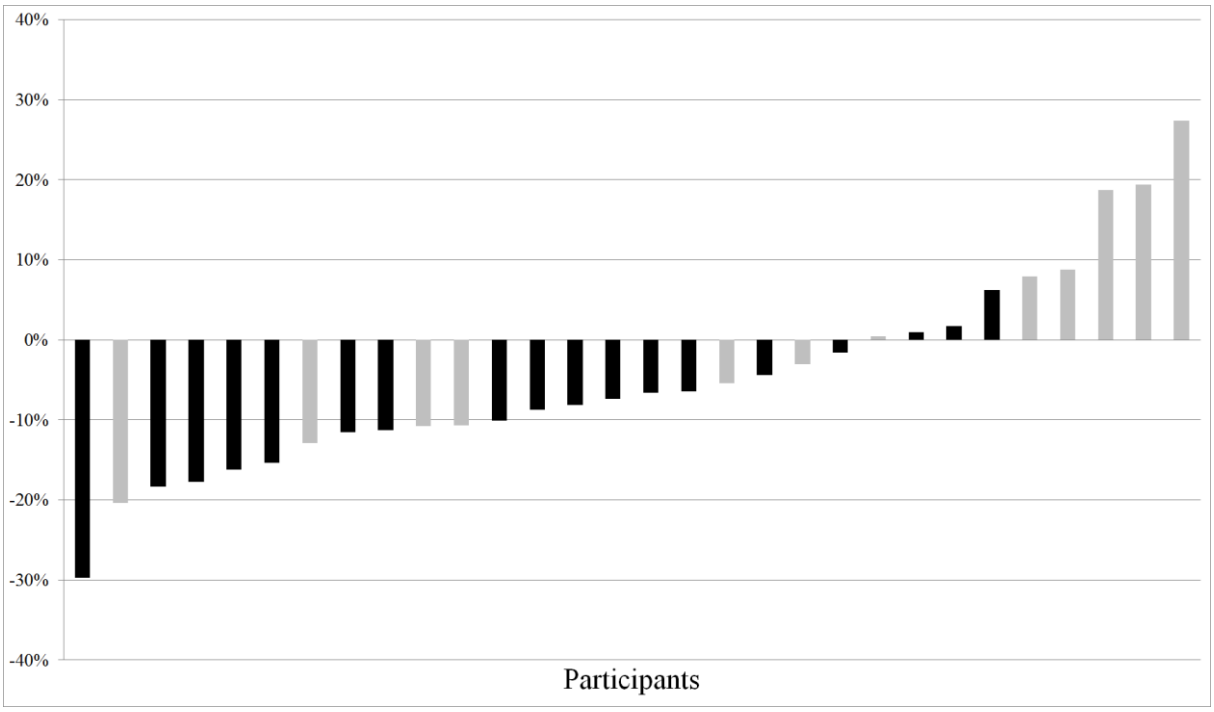
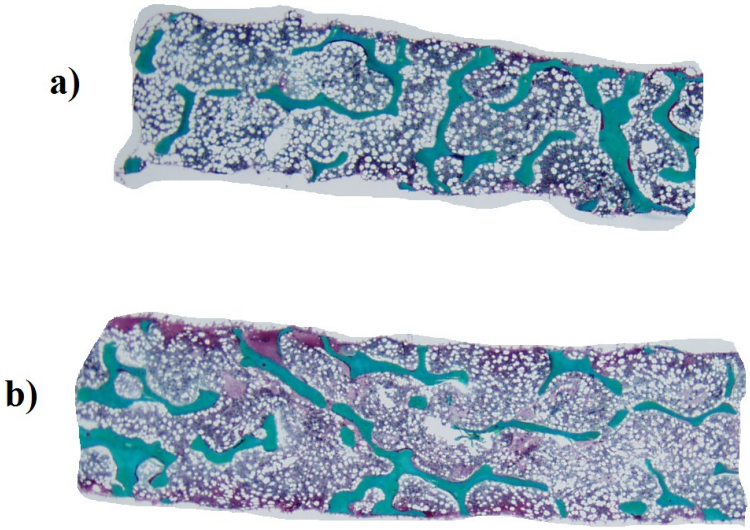


Figure 2: Bone marrow biopsy from one participant preoperatively (a) and one year after Roux-en-Y gastric bypass (RYGB) (b). Body mass index preoperative and one year after RYGB was 39.8 kg/m^2 and 25.1 kg/m^2, respectively. Bone marrow adipose tissue fraction preoperatively was 41.1% and one year after RYGB was 20.7%. Arrows indicate bone marrow adipose tissue.



Serum testosterone levels increased in males with a mean 4.7 ± 3.4 nmol/L, p <0.001, and serum estradiol levels decreased by 0.025 ± 0.036 nmol/L, p=0.035. In postmenopausal females similar trends, although non-significant, was observed; serum testosterone increased 0.14 ± 0.20 nmol/L, p=n.s and serum estradiol decreased 0.070 ± 0.097 nmol/L, p=n.s. In premenopausal females we observed non-significant decreases in both serum testosterone and serum estradiol; 0.078 ± 0.19 nmol/L and 0.024 ± 0.12 nmol/L, p=n.s for both.

Factors associated with changes in BMAT fraction after RYGB

In females BMAT fraction changed from 39.4 ± 9.9% preoperatively to 30.1 ± 9.0 % at follow-up, p <0.001, or with mean percent change of 22.4 ± 19.6% of the preoperative BMAT

fraction. In males BMAT fraction changed from 41.9 ± 8.4% preoperatively to 33.7 ± 13.8 % at follow-up corresponding to a mean percent change of 6.8 ± 37.5% of the preoperative BMAT, p=n.s. The mean between group difference (95% CI) was -11.1 (-19.8 to -2.4), p= 0.014. This difference remained significant after adjusting for age and preoperative BMAT fraction and BMI. Five of the 12 males demonstrated an increase in BMAT fraction after RYGB and seven a decreased or unchanged BMAT fraction (Figure 1). Males who increased in BMAT fraction had a mean preoperative BMAT fraction of 36.4 ± 5.5%, compared to 45.8 ± 8.2% for the remaining males, p=0.051.

The mean BMAT fraction decreased form 43.3 ± 10.9% to 40.3 ± 15.3% in participants with preoperative T2D and from 38.7 ± 8.1% to 32.8 ± 10.7% in participants without T2D, the changes in BMAT fraction was comparable between the two groups. No associations were observed between changes in BMAT and changes in HbA_{1c}, aBMD (all measured regions), or bone turnover markers (Table 3).

Participants who lost more BMI units or decreased more in total body fat mass decreased more in BMAT fraction, and this remained significant after adjusting for gender, preoperative BMI and BMAT fraction. Lower preoperative BMAT fraction were associated with smaller changes in BMAT after RYGB, and this remained significant after adjusting for age, gender, and preoperative BMI (Table 3).

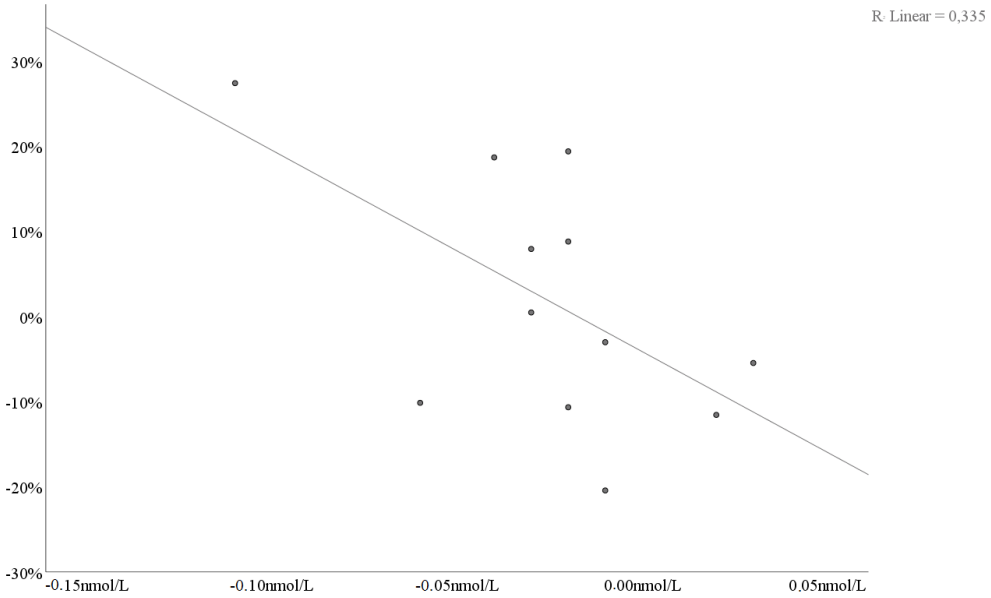
Table 3: Factors associated with changes in bone marrow adipose tissue (BMAT) fraction one year after Roux-en-Y gastric bypass.

	Unadjusted			Adjusted		
	β	95% CI for β	p	β	95% CI for β	p
Female gender	**-11.1**	**-19.8 to -2.4**	**0.014**	**- 8.4**	**-16.1 to -0.65**	**0.035**
Postmenopausal	2.2	-6.6 to 11.0	0.60			
Age	0.20	-0.30 to 0.70	0.42			
Type 2 diabetes (preoperative)	2.9	-6.8 to 12.7	0.54			
Hypercholesterolemia (preoperative)	0.078	-10.0 to 10.1	0.99			
BMAT (preoperative)	**-0.45**	**-0.94 to 0.045**	**0.074**	**-0.60**	**-1.1 to -0.12**	**0.017**
Δ HbA_{1c}	-0.78	-8.5 to 6.9	0.84			
Δ Body mass index	**1.3**	**0.31 to 2.4**	**0.013**	**1.7**	**0.72 to 2.7**	**0.002**
Δ Total body fat mass	**0.50**	**0.027 to 0.98**	**0.039**	**0.59**	**0.14 to 1.0**	**0.013**
Δ Total body lean mass	0.77	-1.1 to 2.6	0.40			
Δ Lumbar spine aBMD	-3.9	-82.9 to 75.0	0.92			
Δ Femoral neck aBMD	-7.6	-103.6 to 88.4	0.87			
Δ Total hip aBMD	41.5	-58.7 to 141.6	0.40			
Δ Total body aBMD	-18.0	-113.7 to 77.8	0.70			
Δ CTX-1	3.4	-22.4 to 29.3	0.79			
Δ P1NP	-0.16	-0.41 to 0.098	0.22			

Linear regression; Female gender was adjusted for age and preoperative BMAT. BMAT (preoperative) was adjusted for age, gender, preoperative BMI. **Δ** body mass index was adjusted for age, gender, preoperative BMI and preoperative BMAT. **Δ** total body fat mass was adjusted for age, gender and preoperative total body fat and BMAT. The results from the regression analysis are presented as regression coefficients (β) with 95% confidence intervals (CI). Abbreviations: Glycosylated hemoglobin (HbA_{1c}) areal bone mineral density (aBMD), carboxyl terminal telopeptide of type 1 collagen (CTX-1) and procollagen type 1 N-terminal propeptide (PINP).

In males we noted an association between changes in serum estradiol levels and change in BMAT fraction (Figure 3). This association remained significant after adjustment for age. In females no association between change in serum estradiol levels and BMAT fraction was noted. Postmenopausal females revealed a mean percent decrease in BMAT comparable to that of premenopausal females, 18.8 ± 18.0% and 25.3 ± 21.3%, respectively, p=0.50.

Figure 3: Changes in serum estradiol in males was negatively associated with changes in bone marrow adipose tissue (BMAT) fraction after Roux-en-Y gastric bypass (RYGB). Linear regression analysis show a regression coefficient (β) of -236.4 with 95% confidence intervals for β -471.0 to -1.8, p=0.049. Indicating that for every 1 unit change in delta estradiol the delta BMAT fraction decreased with 236.4. This finding remained significant after adjustment for age.



Discussion

This is the first study to describe BMAT fraction preoperatively and after RYGB assessed by bone marrow biopsies. We observed that BMAT decreased with a mean percent of 10.7% one year after RYGB, but there was no statistical difference in BMAT reduction in participants with and without preoperative T2D.

Reductions in BMAT fraction

Studies have shown 15-26% higher BMAT fraction in osteoporotic subjects compared to controls [6,7]. Other studies indicate 5.9-24% decrease in BMAT following osteoporosis treatment [33,35-37] and a 4% increase in BMAT following growth hormone treatment [38]. Although not directly comparable, our 10.7% decrease in BMAT fraction could be clinically relevant. Griffith et al. has previously reported that BMAT fraction may predict future aBMD loss [39] giving the results possible clinical importance. Our results differ from the study from Bredella et al who observed a non-significant decrease in L1-L2 BMAT, assessed by MRS, one year after RYGB in 11 patients [28].

BMAT and T2D

Participants with preoperative T2D had comparable BMAT fraction to participants without diabetes; however, we observed a significant an association between preoperative BMAT and HbA_{1c}. This supports a potential association between glycemic control and BMAT in subjects with morbid obesity. One year after RYGB all but one of the participants with preoperative T2D were in diabetes remission. Despite a high diabetes remission rate we did not observe additional reductions in BMAT fraction in participants with preoperative T2D, as we had hypothesized. This observation is in contrast to the results described by Kim et al, who report a significant difference in BMAT change after RYGB between participants with and without T2D and only observed a reduction in BMAT in participants with preoperative T2D [27].

Notably, Kim et al included only women and a mix of ethnicities while we included both genders and only Caucasians.

BMAT and gender

Studies exploring groups with a large age span have not noted any gender differences in BMAT fraction [7,40]. However, a study limited to participants 30 to 60 years of age noted that males had 6-10% higher BMAT fraction when compared to females [2]. Preoperatively males and females in our study presented with comparable BMAT fractions. However, females lost more BMAT than males after RYGB. A gender specific BMAT response to intervention has to our knowledge not been shown before. Notably, the majority of human studies evaluating BMAT response to interventions have been performed in females.

Our study is the first to show an association between change in endogenous serum estradiol and change in BMAT. Estrogen deficiency has been linked to higher BMAT fraction and in postmenopausal females exogenous estradiol supplementation has been reported to reduce in BMAT [36]. Endogenous estradiol levels, however, have only been shown to be associated with BMAT in older males, where an inverse association was noted [41]. In line with this study we observed an inverse association between change in estradiol and BMAT fraction in males only. In light of these findings one might suspect that endogenous estradiol regulate BMAT in a gender-specific manner. However, pre- and postmenopausal females in our study had comparable serum estradiol values preoperatively and one year after RYGB. Study blood samples were drawn at random times during the menstrual cycle, confounding sex hormone interpretation in premenopausal subjects. Regardless, the lack of significant changes in serum estradiol levels in females is viewed a more likely explanation of why we did not observe any association between change in estradiol and change in BMAT fraction in these participants.

BMAT fraction change, total body fat change and preoperative BMAT fraction

An association between larger degree of caloric restriction in patients with anorexia nervosa and higher BMAT fraction has been reported [42]. This appears to contradict our findings of reduction in BMAT fraction after loss of 30% of total weight and 45% of total fat mass. This could support a hypothesis of a U-shaped association between total body fat and BMAT, where BMAT is elevated in circumstances of high or low total body fat and normalizes with normalization of total body fat. Participants with lower preoperative BMAT fraction experienced smaller decrease or increase in BMAT fraction after RYGB. Furthermore, male participants who increased in BMAT fraction after RYGB had a tendency of lower preoperative BMAT fraction when compared to those who decreased or experienced minimal changes. Individual differences in BMAT response to intervention should be the focus of future studies.

BMAT and BMD

We observed that preoperative BMAT fraction were inversely associated with aBMD, in line with previous studies of subjects with morbid obesity [13] and subjects with increased fracture rates [5-7]. Interventional studies in osteoporotic pre- and postmenopausal women have shown an inverse association between change in aBMD and changes in BMAT fraction [33,36,37]. Following RYGB reductions in aBMD has been consistently observed [21,22], in line with our findings. However, aBMD decreased in parallel to a decrease in BMAT fraction. This finding is in line with the subpopulation with T2D in the study by Kim et al. who reported reductions of 6.5% in BMAT and 4.5% in volumetric BMD of the lumbar spine six months after RYGB [27], but opposing the trends reported in studies evaluating treatment of osteoporosis.

Strength and limitations

Strengths of our study include the use of bone marrow biopsies to evaluate BMAT fraction, inclusion of both genders and a low attrition rate. However the study is limited by the restricted duration of follow-up. Due to limited sample size the negative finding of no differences in BMAT fraction between participants with and without T2D should be interpreted with care. The area of bone marrow investigated histologically is smaller than the area sampled using MRS. Our intra-observer variation was lower than the detected difference between preoperative and postoperative BMAT fraction and the bone marrow biopsy technique standardized. However we did not have data on BMAT fraction variation between two repeated bone marrow biopsies taken from the same participant at same point in time. In the setting of morbid obesity and large weight loss DXA assessment of aBMD might be affected by imaging artifacts [21]. The introduction of a new DXA machine during the study could affect the aBMD results, albeit probably insignificantly, as proper cross calibration was performed. For 23 participants the preoperative DXA examination was performed using the GE Lunar Prodigy, while the follow up examination was performed using the GE Lunar iDXA. In 11 participants GE Lunar iDXA was used for both pre and postoperative analyses. Patients with T2D were encouraged to participate in the study, thus the fraction of participants with T2D (38%) exceeds the fraction in patients seeking RYGB (25-30%) at our institution in general [43]. For technical reasons, concerning the bone marrow biopsy subjects with a preoperative BMI > 47 kg/m^2 were excluded. The prevalence of subjects with ≥ 50 kg/m^2 prior to RYGB surgery has been reported between 21 and 32% [43,44]. Thus possibly one third of the bariatric population was potentially excluded. Perhaps affecting the generability of the study results.

Conclusion

Our findings indicate that a high BMAT fraction seems to be associated with lower aBMD and poorer glycemic control in subjects with morbid obesity. One year after RYGB we observed a 10.7% decrease in BMAT fraction. This reduction was comparable in participants with and without T2D, but appeared to be gender specific.

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References

1. Veldhuis-Vlug AG, Rosen CJ. Clinical implications of bone marrow adiposity. Journal of internal medicine. Feb 2018;283(2):121-39. Epub 2017/12/07.
2. Kugel H, Jung C, Schulte O, Heindel W. Age- and sex-specific differences in the 1H-spectrum of vertebral bone marrow. Journal of magnetic resonance imaging : JMRI. Feb 2001;13(2):263-8. Epub 2001/02/13.
3. Griffith JF, Yeung DK, Ma HT, Leung JC, Kwok TC, Leung PC. Bone marrow fat content in the elderly: a reversal of sex difference seen in younger subjects. Journal of magnetic resonance imaging : JMRI. Jul 2012;36(1):225-30. Epub 2012/02/18.
4. Cawthorn WP, Scheller EL, Learman BS, Parlee SD, Simon BR, Mori H, et al. Bone marrow adipose tissue is an endocrine organ that contributes to increased circulating adiponectin during caloric restriction. Cell metabolism. Aug 5 2014;20(2):368-75. Epub 2014/07/08.
5. Ecklund K, Vajapeyam S, Mulkern RV, Feldman HA, O'Donnell JM, DiVasta AD, et al. Bone marrow fat content in 70 adolescent girls with anorexia nervosa: Magnetic resonance imaging and magnetic resonance spectroscopy assessment. Pediatric radiology. Jul 2017;47(8):952-62. Epub 2017/04/23.
6. Cohen A, Dempster DW, Stein EM, Nickolas TL, Zhou H, McMahon DJ, et al. Increased marrow adiposity in premenopausal women with idiopathic osteoporosis. The Journal of clinical endocrinology and metabolism. Aug 2012;97(8):2782-91. Epub 2012/06/16.
7. Justesen J, Stenderup K, Ebbesen EN, Mosekilde L, Steiniche T, Kassem M. Adipocyte tissue volume in bone marrow is increased with aging and in patients with osteoporosis. Biogerontology. 2001;2(3):165-71. Epub 2001/11/16.
8. Scheller EL, Cawthorn WP, Burr AA, Horowitz MC, MacDougald OA. Marrow Adipose Tissue: Trimming the Fat. Trends in endocrinology and metabolism: TEM. Jun 2016;27(6):392-403. Epub 2016/04/21.
9. Cohen A, Shen W, Dempster DW, Zhou H, Recker RR, Lappe JM, et al. Marrow adiposity assessed on transiliac crest biopsy samples correlates with noninvasive measurement of marrow adiposity by proton magnetic resonance spectroscopy ((1)H-MRS) at the spine but not the femur. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. Oct 2015;26(10):2471-8. Epub 2015/05/20.
10. Bergkvist D, Hekmat K, Svensson T, Dahlberg L. Obesity in orthopedic patients. Surgery for obesity and related diseases : official journal of the American Society for Bariatric Surgery. Nov-Dec 2009;5(6):670-2. Epub 2009/08/07.
11. Johansson H, Kanis JA, Oden A, McCloskey E, Chapurlat RD, Christiansen C, et al. A meta-analysis of the association of fracture risk and body mass index in women. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. Jan 2014;29(1):223-33. Epub 2013/06/19.
12. Vestergaard P, Rejnmark L, Mosekilde L. Relative fracture risk in patients with diabetes mellitus, and the impact of insulin and oral antidiabetic medication on relative fracture risk. Diabetologia. Jul 2005;48(7):1292-9. Epub 2005/05/24.
13. Yu EW, Greenblatt L, Eajazi A, Torriani M, Bredella MA. Marrow adipose tissue composition in adults with morbid obesity. Bone. Apr 2017;97:38-42. Epub 2017/01/04.
14. Baum T, Yap SP, Karampinos DC, Nardo L, Kuo D, Burghardt AJ, et al. Does vertebral bone marrow fat content correlate with abdominal adipose tissue, lumbar spine bone mineral density, and blood biomarkers in women with type 2 diabetes mellitus? Journal of magnetic resonance imaging : JMRI. Jan 2012;35(1):117-24. Epub 2011/12/23.
15. Bredella MA, Torriani M, Ghomi RH, Thomas BJ, Brick DJ, Gerweck AV, et al. Vertebral bone marrow fat is positively associated with visceral fat and inversely associated with IGF-1 in obese women. Obesity (Silver Spring, Md). Jan 2011;19(1):49-53. Epub 2010/05/15.

16. Sheu Y, Amati F, Schwartz AV, Danielson ME, Li X, Boudreau R, et al. Vertebral bone marrow fat, bone mineral density and diabetes: The Osteoporotic Fractures in Men (MrOS) study. Bone. Apr 2017;97:299-305. Epub 2017/02/10.
17. Ermetici F, Briganti S, Delnevo A, Cannao P, Leo GD, Benedini S, et al. Bone marrow fat contributes to insulin sensitivity and adiponectin secretion in premenopausal women. Endocrine. Feb 2018;59(2):410-8. Epub 2017/06/19.
18. Angrisani L, Santonicola A, Iovino P, Vitiello A, Zundel N, Buchwald H, et al. Bariatric Surgery and Endoluminal Procedures: IFSO Worldwide Survey 2014. Obesity surgery. Sep 2017;27(9):2279-89. Epub 2017/04/14.
19. Courcoulas AP, King WC, Belle SH, Berk P, Flum DR, Garcia L, et al. Seven-Year Weight Trajectories and Health Outcomes in the Longitudinal Assessment of Bariatric Surgery (LABS) Study. JAMA surgery. May 1 2018;153(5):427-34. Epub 2017/12/08.
20. Adams TD, Davidson LE, Litwin SE, Kim J, Kolotkin RL, Nanjee MN, et al. Weight and Metabolic Outcomes 12 Years after Gastric Bypass. The New England journal of medicine. Sep 21 2017;377(12):1143-55. Epub 2017/09/21.
21. Yu EW, Bouxsein ML, Roy AE, Baldwin C, Cange A, Neer RM, et al. Bone loss after bariatric surgery: discordant results between DXA and QCT bone density. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. Mar 2014;29(3):542-50. Epub 2013/08/10.
22. Lindeman KG, Greenblatt LB, Rourke C, Bouxsein ML, Finkelstein JS, Yu EW. Longitudinal 5-Year Evaluation of Bone Density and Microarchitecture After Roux-en-Y Gastric Bypass Surgery. The Journal of clinical endocrinology and metabolism. Nov 1 2018;103(11):4104-12. Epub 2018/09/17.
23. Axelsson KF, Werling M, Eliasson B, Szabo E, Naslund I, Wedel H, et al. Fracture Risk After Gastric Bypass Surgery: A Retrospective Cohort Study. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. Dec 2018;33(12):2122-31. Epub 2018/07/17.
24. Yu EW, Lee MP, Landon JE, Lindeman KG, Kim SC. Fracture Risk After Bariatric Surgery: Roux-en-Y Gastric Bypass Versus Adjustable Gastric Banding. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. Jun 2017;32(6):1229-36. Epub 2017/03/03.
25. Cordes C, Dieckmeyer M, Ott B, Shen J, Ruschke S, Settles M, et al. MR-detected changes in liver fat, abdominal fat, and vertebral bone marrow fat after a four-week calorie restriction in obese women. Journal of magnetic resonance imaging : JMRI. Nov 2015;42(5):1272-80. Epub 2015/04/14.
26. Bosy-Westphal A, Later W, Schautz B, Lagerpusch M, Goele K, Heller M, et al. Impact of intra- and extra-osseous soft tissue composition on changes in bone mineral density with weight loss and regain. Obesity (Silver Spring, Md). Jul 2011;19(7):1503-10. Epub 2011/03/05.
27. Kim TY, Schwartz AV, Li X, Xu K, Black DM, Petrenko DM, et al. Bone Marrow Fat Changes After Gastric Bypass Surgery Are Associated With Loss of Bone Mass. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. Nov 2017;32(11):2239-47. Epub 2017/08/10.
28. Bredella MA, Greenblatt LB, Eajazi A, Torriani M, Yu EW. Effects of Roux-en-Y gastric bypass and sleeve gastrectomy on bone mineral density and marrow adipose tissue. Bone. Feb 2017;95:85-90. Epub 2016/11/23.
29. NIH conference. Gastrointestinal surgery for severe obesity. Consensus Development Conference Panel. Annals of internal medicine. Dec 15 1991;115(12):956-61. Epub 1991/12/15.
30. Schauer PR, Ikramuddin S, Hamad G, Eid GM, Mattar S, Cottam D, et al. Laparoscopic gastric bypass surgery: current technique. Journal of laparoendoscopic & advanced surgical techniques Part A. Aug 2003;13(4):229-39. Epub 2003/10/17.

31. Erben RG. Embedding of bone samples in methylmethacrylate: an improved method suitable for bone histomorphometry, histochemistry, and immunohistochemistry. The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society. Feb 1997;45(2):307-13. Epub 1997/02/01.
32. Harlow SD, Gass M, Hall JE, Lobo R, Maki P, Rebar RW, et al. Executive summary of the Stages of Reproductive Aging Workshop + 10: addressing the unfinished agenda of staging reproductive aging. The Journal of clinical endocrinology and metabolism. Apr 2012;97(4):1159-68. Epub 2012/02/22.
33. Cohen A, Stein EM, Recker RR, Lappe JM, Dempster DW, Zhou H, et al. Teriparatide for idiopathic osteoporosis in premenopausal women: a pilot study. The Journal of clinical endocrinology and metabolism. May 2013;98(5):1971-81. Epub 2013/04/02.
34. Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. Psychological bulletin. Mar 1979;86(2):420-8. Epub 1979/03/01.
35. Limonard EJ, Veldhuis-Vlug AG, van Dussen L, Runge JH, Tanck MW, Endert E, et al. Short-Term Effect of Estrogen on Human Bone Marrow Fat. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. Nov 2015;30(11):2058-66. Epub 2015/05/20.
36. Syed FA, Oursler MJ, Hefferanm TE, Peterson JM, Riggs BL, Khosla S. Effects of estrogen therapy on bone marrow adipocytes in postmenopausal osteoporotic women. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. Sep 2008;19(9):1323-30. Epub 2008/02/16.
37. Yang Y, Luo X, Xie X, Yan F, Chen G, Zhao W, et al. Influences of teriparatide administration on marrow fat content in postmenopausal osteopenic women using MR spectroscopy. Climacteric : the journal of the International Menopause Society. Jun 2016;19(3):285-91. Epub 2016/01/09.
38. Bredella MA, Gerweck AV, Barber LA, Breggia A, Rosen CJ, Torriani M, et al. Effects of growth hormone administration for 6 months on bone turnover and bone marrow fat in obese premenopausal women. Bone. May 2014;62:29-35. Epub 2014/02/11.
39. Griffith JF, Yeung DK, Leung JC, Kwok TC, Leung PC. Prediction of bone loss in elderly female subjects by MR perfusion imaging and spectroscopy. European radiology. Jun 2011;21(6):1160-9. Epub 2011/01/13.
40. Verma S, Rajaratnam JH, Denton J, Hoyland JA, Byers RJ. Adipocytic proportion of bone marrow is inversely related to bone formation in osteoporosis. Journal of clinical pathology. Sep 2002;55(9):693-8. Epub 2002/08/27.
41. Mistry SD, Woods GN, Sigurdsson S, Ewing SK, Hue TF, Eiriksdottir G, et al. Sex hormones are negatively associated with vertebral bone marrow fat. Bone. Mar 2018;108:20-4. Epub 2017/12/16.
42. Abella E, Feliu E, Granada I, Milla F, Oriol A, Ribera JM, et al. Bone marrow changes in anorexia nervosa are correlated with the amount of weight loss and not with other clinical findings. American journal of clinical pathology. Oct 2002;118(4):582-8. Epub 2002/10/12.
43. Aftab H, Risstad H, Sovik TT, Bernklev T, Hewitt S, Kristinsson JA, et al. Five-year outcome after gastric bypass for morbid obesity in a Norwegian cohort. Surgery for obesity and related diseases : official journal of the American Society for Bariatric Surgery. Jan-Feb 2014;10(1):71-8. Epub 2013/11/05.
44. Suter M, Calmes JM, Paroz A, Romy S, Giusti V. Results of Roux-en-Y gastric bypass in morbidly obese vs superobese patients: similar body weight loss, correction of comorbidities, and improvement of quality of life. Archives of surgery (Chicago, Ill : 1960). Apr 2009;144(4):312-8; discussion 8. Epub 2009/04/22.