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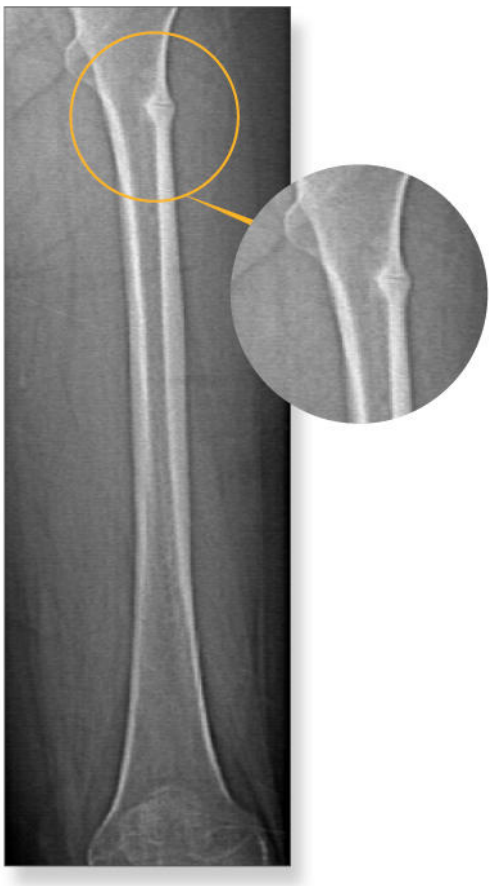

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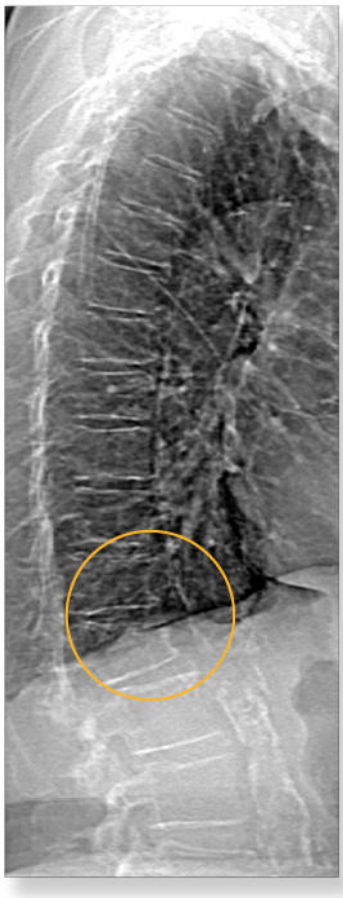

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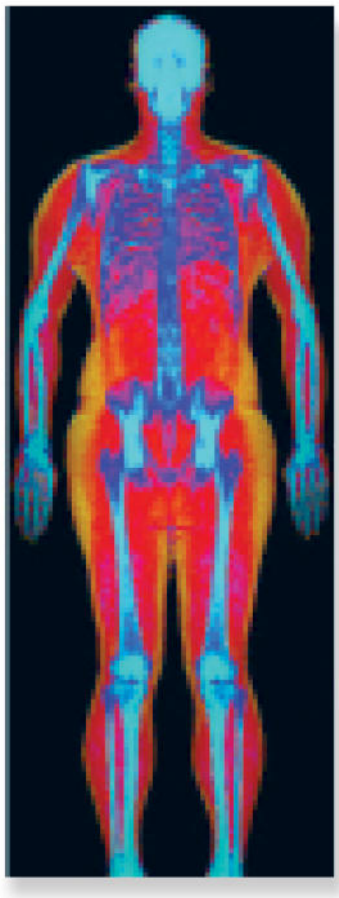

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ORIGINAL ARTICLE

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Changes in Bone Marrow Adipose Tissue One Year After Roux-en-Y Gastric Bypass: A Prospective Cohort Study

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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 1 year after RYGB and explore factors associated with BMAT change. Patients with morbid obesity scheduled for RYGB were examined preoperatively and 1 year after RYGB. Forty-four participants were included and preoperative examinations were possible in 35. Of these, 33 (94%) met for follow-up, 2 were excluded, and BMAT estimation was not possible in 1. 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 areal bone mineral density (aBMD). After RYGB, BMAT fraction decreased from $40.4 \pm 1.7\%$ to $35.6 \pm 12.8\%$, $p = 0.042$, or with mean percent change of 10.7% of preoperative BMAT fraction. Change in BMAT fraction was positively associated with change in body mass index (BMI) and total body fat. In females, we observed a mean percent reduction of $22.4 \pm 19.6\%$, whereas in males BMAT increased with a mean percent of $6.8 \pm 37.5\%$, $p = 0.009$. 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 $5.8 \pm 36.9\%$ and $13.5 \pm 28.0\%$, 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 sex specific. © 2019 The Authors. *Journal of Bone and Mineral Research* Published by Wiley Periodicals, Inc.

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 sex difference appears reversed.[2,3] 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 and 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 study 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 artifacts of obesity and weight loss are yet to be explored for noninvasive radiological methods of BMAT estimation.

Obesity and type 2 diabetes (T2D) are associated with increased fracture risk despite a normal areal bone mineral density

Received in original form March 1, 2019; revised form May 28, 2019; accepted June 4, 2019. Accepted manuscript online June 19, 2019.
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Journal of Bone and Mineral Research, Month 2019, pp 1–9
DOI: 10.1002/jbmr.3814

(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 and increase bone turnover and fracture rates.[21–24]

Studies indicate that a diet-induced weight loss is accompanied by a 1.1% to 3.5% reduction in MRI/MRS-estimated BMAT.[25,26] Unexpectedly, studies evaluating BMAT with MRS 6 to 12 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 1 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, sex, sex steroids, menopausal status, metabolic homeostasis (glycemic control, blood lipid levels, and T2D), body mass index (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 center for treatment of morbid obesity, were recruited. Eligibility criteria for RYGB were BMI $\geq$ 40 kg/m^2 or BMI $\geq$ 35 kg/m^2 with obesity-related comorbidity, aged 18 to 65 years, and failed attempts of sustained weight loss.[29] Patients with T2D were encouraged to participate. Participants were included from October 8, 2015 to January 27, 2017. Participants were excluded if they were unable to read Norwegian language or if they had severe psychiatric comorbidity, connective tissue disorders, or other hormonal diseases, kidney failure (glomerular filtration rate $<$ 30 mL/min/1.73 m^2), type 1 diabetes, BMI $>$ 47 kg/m^2, history of treatment with bone active substances (bisphosphonates, denosumab, hormone replacement, or parathyroid hormone), or if they were currently receiving anticoagulation or steroid treatment (estrogen, testosterone, or glucocorticoids). To avoid heterogeneity in our study population, non-whites were excluded.

Surgery, study visits, and follow-up

A laparoscopic RYGB with a gastric pouch of about 25 mL, a 150-cm antecolic alimentary, and a 50-cm biliopancreatic limb was performed in all participants.[30] Participants attended study visits preoperatively and 1 year after RYGB. Study visits included morning fasting blood samples, anthropometric measures, dual-energy absorptiometry (DXA) scan, impact microindentation, intravenous glucose tolerance test, euglycemic hyperinsulinemic clamp, indirect calorimetry, and bone marrow biopsy. In this article, the results of the blood samples, anthropometric measures, DXA, and bone marrow biopsy are presented. Baseline characteristics with comparison of participants with and without T2D and changes in impact microindentation, aBMD, and bone turnover markers will be published separately. The sex distribution and mean preoperative BMI was comparable among participants with and without T2D.

The participants also attended routine clinical follow-up with visits 6 weeks, 6 months, and 1 year after surgery. After surgery, all participants were advised and prescribed oral supplementation with 1000 mg calcium, 800 IU vitamin D, one multivitamin, 200 mg iron daily, and B12 injections 1 mg 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 anesthesia. 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 4 °C. 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 sizes 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, 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 ×230 magnification. The presented BMAT fraction is the mean of the estimated AdV/MarV from two levels of the biopsy. Biopsies obtained preoperatively and 1 year after RYGB were processed and analyzed 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 1 year after RYGB, respectively.

Bone mineral density

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 to L_4), hip, proximal femur, and total body were 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 August 26, 2016; from then on, GE Lunar iDXA was used. Body composition performed with GE Lunar Prodigy was reanalyzed with iDXA software to optimize comparability. The two DXA scanners were cross-calibrated by scanning 16 volunteers with both machines and revealed lumbar spine

(L_1 to L_4) intra-class correlation coefficient (ICC) (95% confidence interval [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 a.m. after an overnight fast. Serum for bone turnover markers (CTX-1 and PINP) was centrifuged and stored at −80 °C and analyzed after study follow-up was completed to avoid interassay 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 (Mannheim, Germany) electrochemiluminescence immunoassay (ECLIA). Serum 25(OH) vitamin D and testosterone levels were analyzed by liquid chromatography-mass spectrometry (LC-MS/MS) method; PTH by Immulite 2000 XPI (Siemens Healthineers, Erlangen, Germany), a noncompetitive chemiluminoimmunometric assay; FSH using Immulite 2000 XPI (Siemens Healthineers), a noncompetitive immunoluminometric assay; and estradiol with a competitive immunoluminometric assay Liaison XL kit from DiaSorin Inc. (Stillwater, MN, USA). The Central Laboratory of Oslo University Hospital analyzed HbA_{1c} using Tosoh G8 high-performance liquid chromatography, and 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 $\geq$ 40 kg/m^2 or BMI $\geq$ 35 kg/m^2 with obesity-related comorbidity.[29] T2D was defined as HbA_{1c} $\geq$ 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 participants with T2D preoperatively. Hypercholesterolemia was defined as low-density lipoprotein cholesterol $\geq$ 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) $\geq$ 25 IU/L.[32]

Study size

This was an explorative study and the first to evaluate BMAT fraction in bone marrow biopsy preoperatively and 1 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 after 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 loss to follow-up.

Statistical analysis

Normally distributed continuous variables are presented as mean $\pm$ SD; others are presented as median (range). Categorical data are presented as proportions (percentage). When comparing preoperative sex characteristics, independent sample *t* test or Mann–Whitney *U* test was used for continuous variables. Pearson chi-square or Fisher's exact test was used for categorical variables as appropriate. Intraclass 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, sex, 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% 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, USA).

Ethics

The study was conducted in accordance with the Declaration of Helsinki and was approved by the Northern Norway Regional Committee for Medical and Health Research Ethics 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 1-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 1-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 with 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 $\pm$ 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 $\pm$ 10.8% and 38.7 $\pm$ 8.1%, respectively, $p = 0.20$. Preoperative BMAT fraction was positively associated with HbA_{1c}; this association remained significant after adjustment for sex. 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 sex (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
Triglyceride (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

HbA_{1c} = glycosylated hemoglobin; LDL = low-density lipoprotein; CTX-1 = carboxyl terminal telopeptide of type 1 collagen; PINP = procollagen type 1 N-terminal propeptide.

Normally distributed continuous variables are presented as mean ± SD; others are presented as median (range). Categorical data are presented as proportions (percentage).

Any fracture except digit fractures are reported.

*Significant difference between sexes, $p < 0.05$.

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), whereas males lost 9.1 ± 3.0 units of BMI (kg/m^2), $p = 0.032$; however, this sex difference was no longer significant when adjusting for preopative BMI. Weight loss in kg and 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 1 were in diabetes remission 1 year after RYGB and the mean HbA1c decrease was 1.1 ± 0.76% 1 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 (Fig. 1). Example of a bone marrow biopsy taken preoperatively and 1 year after RYGB from the same participant is shown in Fig. 2.

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 nonsignificant, were 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 nonsignificant 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 43.7 ± 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 7 a decreased or unchanged BMAT fraction (Fig. 1). Males who increased in BMAT fraction had a

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* Value	β	95 % CI for β	*p* Value
Female sex	−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			

HbA_{1c} = glycosylated hemoglobin; aBMD = areal bone mineral density; CTX-1 = carboxyl terminal telopeptide of type 1 collagen; PINP = procollagen type 1 N-terminal propeptide.

Linear regression; HbA_{1c} was adjusted for sex. Lumbar spine and femoral neck aBMD were adjusted for age and sex. The results from the regression analysis are presented as regression coefficients (β) with 95 % confidence intervals (CI).

mean preoperative BMAT fraction of 36.4 ± 5.5%, compared with 45.8 ± 8.2% for the remaining males, $p = 0.051$.

The mean BMAT fraction decreased from 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 were 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 sex, preoperative BMI, and BMAT fraction. Lower preoperative BMAT fraction was associated with smaller changes in BMAT after RYGB, and this remained significant after adjusting for age, sex, and preoperative BMI (Table 3).

In males, we noted an association between changes in serum estradiol levels and change in BMAT fraction (Fig. 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

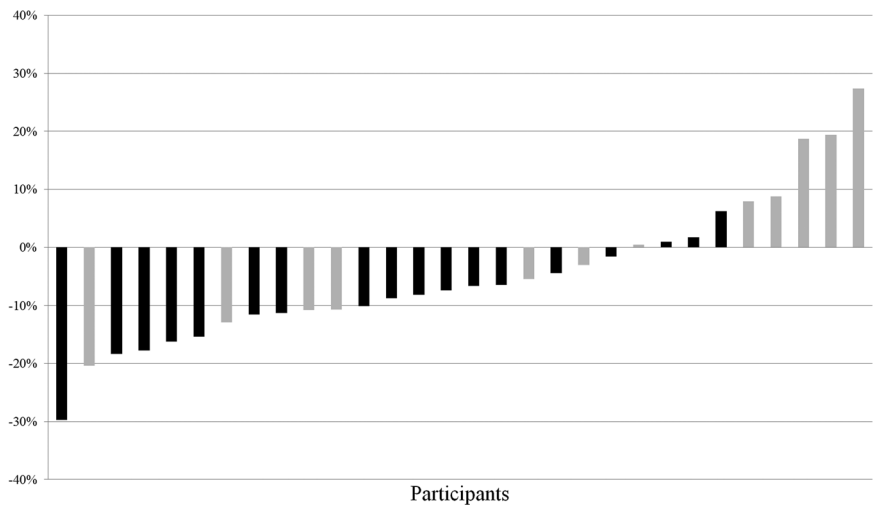

Fig. 1. Individual percent changes in bone marrow adipose tissue (BMAT) fraction in 30 participants after Roux-en-Y gastric bypass (RYGB). Female participants are marked with black bars and male participants are marked with gray bars.

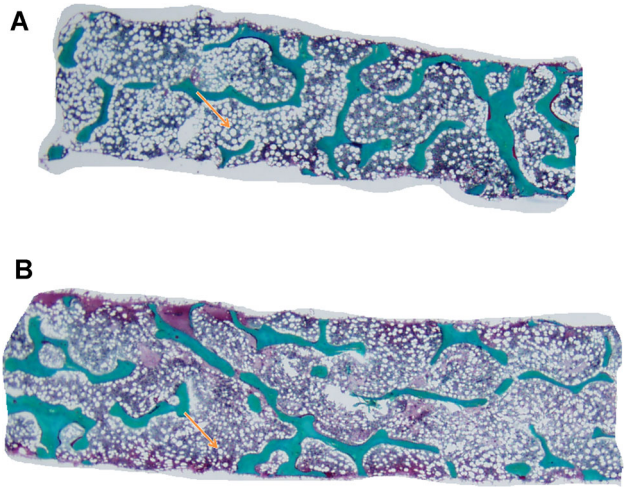

Fig. 2. Bone marrow biopsy from one participant preoperatively (*A*) and 1 year after Roux-en-Y gastric bypass (RYGB) (*B*). Body mass index preoperative and 1 year after RYGB was 39.8 kg/m^2 and 25.1 kg/m^2, respectively. Bone marrow adipose tissue fraction preoperatively was 41.1 % and 1 year after RYGB was 20.7 %. Arrows indicate bone marrow adipose tissue.

of premenopausal females, 18.8 ± 18.0% and 25.3 ± 21.3%, respectively, $p = 0.50$.

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% 1 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% to 26% higher BMAT fraction in osteoporotic subjects compared with controls.[6,7] Other studies indicate a 5.9% to 24% decrease in BMAT after osteoporosis treatment[33,35–37] and a 4% increase in BMAT after growth hormone treatment.[38] Although not directly comparable, our 10.7% decrease in BMAT fraction could be clinically relevant. Griffith and colleagues have 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 and colleagues, who observed a nonsignificant

Table 3. Factors Associated With Change in Bone Marrow Adipose Tissue (BMAT) Fraction 1 Year After Roux-en-Y Gastric Bypass

	Unadjusted			Adjusted		
	β	95 % CI for β	*p* Value	β	95 % CI for β	*p* Value
Female sex	**−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			

HbA_{1c} = glycosylated hemoglobin; aBMD = areal bone mineral density; CTX-1 = carboxyl terminal telopeptide of type 1 collagen; PINP = procollagen type 1 N-terminal propeptide.

Linear regression; female sex was adjusted for age and preoperative BMAT. BMAT (preoperative) was adjusted for age, sex, preoperative body mass index (BMI). **Δ** body mass index was adjusted for age, sex, preoperative BMI, and preoperative BMAT. **Δ** total body fat mass was adjusted for age, sex, and preoperative total body fat and BMAT. The results from the regression analysis are presented as regression coefficients (β) with 95 % confidence intervals (CI).

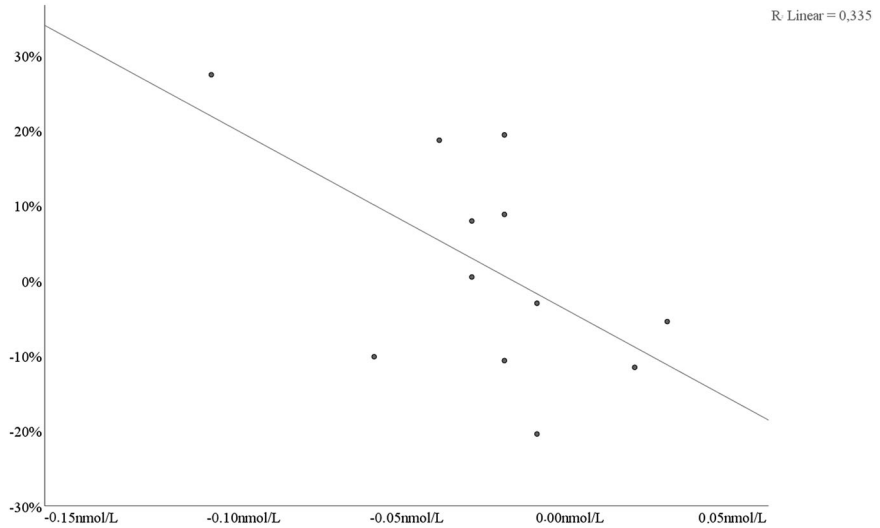

Fig. 3. Changes in serum estradiol in males was negatively associated with changes in bone marrow adipose tissue (BMAT) fraction after Roux-en-Y gastric bypass (RYGB). Linear regression analysis shows a regression coefficient (β) of −236.4 with 95 % confidence intervals for β −471.0 to −1.8, $p = 0.049$, 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.

decrease in L_1 to L_2 BMAT, assessed by MRS, 1 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 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 and colleagues, 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 and colleagues included only women and a mix of ethnicities, whereas we included both sexes and only whites.

BMAT and sex

Studies exploring groups with a large age span have not noted any sex differences in BMAT fraction.[7,40] However, a study limited to participants 30 to 60 years of age noted that males had 6% to 10% higher BMAT fraction compared with females.[2] Preoperatively, males and females in our study presented with comparable BMAT fractions. However, females lost more BMAT than males after RYGB. A sex-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 regulates BMAT in a sex-specific manner. However, pre- and postmenopausal females in our study had comparable serum estradiol values preoperatively and 1 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 with 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 was 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] After RYGB, reductions in aBMD have 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 and colleagues, who reported reductions of 6.5% in BMAT and 4.5% in volumetric BMD of the lumbar spine 6 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 sexes, and a low attrition rate. However, the study is limited by the restricted duration of follow-up. Because of 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, whereas 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% to 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 $\geq$ 50 kg/m^2 before 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 generalizability 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 sex specific.

Disclosures

IKBH, TM, JAK, EMH, CB, and HLG have nothing to declare. EFE consults for Shire, Amgen, Ascendis, Eli Lilly, Novartis, and Merck; has received grant support from Shire; and has received lecture fees from Eli Lilly, Novartis, Amgen, and Takeda.

Acknowledgments

We deeply appreciate the support from the staff at Department of Endocrinology, Morbid Obesity and Preventive Medicine, Oslo University Hospital. We are especially thankful for the support of Gøril Vinje, Åse Halsne, Inger Eribe, and Kristine Bech Holte. IKBH has received a PhD grant from South-Eastern Norway Regional Health Authority, project number 2014073.

Authors' roles: Study design: IKBH, TM, CB, HLG, and EFE. Study conduct: IKBH, TM, JAK, HLG, and EFE. Data collection: IKBH. Data analysis: IKBH, CB, and EFE. Data interpretation: IKBH, TM, JAK, CB, EMH, HLG, and EFE. Drafting manuscript: IKBH. Revising manuscript content: IKBH, MT, JAK, CB, EMH, HLG, and EFE. Approving final version of manuscript: IKBH, MT, JAK, CB, EMH, HLG, and EFE. IKBH and CB take responsibility for the integrity of the data analysis.

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