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Publication Date

2022-12-01

DOI

10.1016/j.bonr.2022.101596

Bone Reports 17 (2022) 101596

Contents lists available at ScienceDirect

Bone Reports

journal homepage: www.elsevier.com/locate/bonr

Bone marrow adipose tissue composition and glycemic improvements after gastric bypass surgery

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ARTICLE INFO

Keywords:
Marrow adipose tissue
Unsaturated lipids
Diabetes
Gastric bypass surgery

ABSTRACT

Fracture risk is increased in type 2 diabetes, which may in part be due to altered bone marrow adiposity. Cross sectional studies have reported that people with type 2 diabetes have lower unsaturated BMAT lipid levels than people without diabetes, although there are limited data on longitudinal changes. We hypothesized that Roux-en-Y gastric bypass (RYGB), which dramatically improves glycemic status, would have differential effects on BMAT composition, with increases in the unsaturated lipid index in people with diabetes. Given reports that axial BMAT is responsive to metabolic stimuli while appendicular BMAT is stable, we hypothesized that BMAT changes would occur at the spine but not the tibia. We enrolled 30 obese women, stratified by diabetes status, and used magnetic resonance spectroscopy to measure BMAT at the spine in all participants, and the tibia in a subset ($n = 19$). At baseline, BMAT parameters were similar between those with and without diabetes, except tibial marrow fat content was lower in women with diabetes (97.4 % ± 1.0 % versus 98.2 % ± 0.4 %, $p = 0.04$). Six months after surgery, both groups experienced similar weight loss of 27 kg ± 7 kg. At the spine, there was a significant interaction between diabetes status and changes in both marrow fat content and the unsaturated lipid index ($p = 0.02$, $p < 0.01$ for differences, respectively). Women with diabetes had a trend towards a decline in marrow fat content (−4.3 % ± 8.2 %, $p = 0.09$) and increase in the unsaturated lipid index (+1.1 % ± 1.5 %, $p = 0.02$). In contrast, BMAT parameters did not significantly change in women without diabetes. In all women, changes in the unsaturated lipid index inversely correlated with hemoglobin A1c changes ($r = -0.47$, $p = 0.02$). At the tibia, there was little BMAT change by diabetes status. Our results suggest that vertebral BMAT composition is responsive to changes in glycemic control after RYGB.

1. Introduction

Adipose tissue within the bone marrow cavity is a dynamic fat depot that may reflect or influence both bone and systemic metabolism. Greater amounts of bone marrow adipose tissue (BMAT) are reported in skeletal disorders such as osteoporosis (Griffith et al., 2005; Griffith et al., 2006), and also in metabolic disorders, including type 2 diabetes (Sheu et al., 2017; Yu et al., 2017) and anorexia nervosa (Bredella et al., 2009); all of these are associated with increased fracture risk (Fan et al., 2016; Vestergaard et al., 2002). The negative relationship between BMAT and bone may be mediated through the common mesenchymal stem cell precursor in the bone marrow, which could differentiate into an adipocyte or osteoblast. Diseases such as diabetes mellitus with abnormal fat metabolism may promote adipogenesis over osteoblastogenesis, and marrow adipocytes may secrete adipokines, cytokines, or other factors that negatively affect bone. While initial work in this area has provided critical insights into underlying BMAT physiology, a deeper understanding of its role in metabolic bone disease may provide clarity to mechanisms underlying increased fracture risk in diabetes.

There is growing evidence that the composition of BMAT, in addition to BMAT content or overall amount, is an important factor for metabolic health. In cross-sectional studies, lower levels of BMAT unsaturated

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https://doi.org/10.1016/j.bonr.2022.101596
Received 26 May 2022; Accepted 3 June 2022
Available online 6 June 2022

lipids are associated with osteoporosis, fracture, and type 2 diabetes (Yeung et al., 2005; Patsch et al., 2013; Baum et al., 2012; Woods et al., 2022). The marrow adipocyte population is heterogenous and lower levels of unsaturated lipids may reflect differences in the marrow microenvironment. Preclinical animal studies have reported two different populations of marrow adipocytes, regulated and constitutively active, which have differences in development, size, location, gene expression, and lipid composition (Scheller et al., 2015). While regulated marrow adipocytes are generally located in the axial skeleton and respond to changes in aging, caloric restriction, estrogen deficiency, and other metabolic changes, constitutively active marrow adipocytes are located in the appendicular skeleton and are stable (Scheller et al., 2016). There are few data on longitudinal changes in BMAT composition, which have been published in people who have had sleeve gastrectomy (Bredella et al., 2020) or with acute overfeeding and fasting (Bredella et al., 2021).

Roux-en Y gastric bypass (RYBG) surgery has profound metabolic effects and is also an opportunity to observe dramatic changes in skeletal metabolism (Schafer et al., 2018; Gagnon and Schafer, 2018). We previously reported 6-month changes in BMAT content in a prospective cohort of 30 obese women with and without diabetes who had RYGB surgery. We found that changes in vertebral marrow fat content differed by diabetes status and that improved glycemic control – rather than weight loss – was associated with BMAT reductions (Kim et al., 2017). While our previous work provided critical insights regarding dynamic changes in vertebral marrow fat content with RYGB, the measurement of total marrow fat content was limited to saturated lipids. We sought to investigate whether the lipid composition, particularly the unsaturated lipid level, is altered as well. In a subset ($n = 19$), we obtained tibial marrow adiposity measurements to further determine whether marrow adiposity content and composition change based on anatomic location.

We conducted a study in women undergoing RYGB surgery, with the objectives to examine (1) whether changes in BMAT composition differ by diabetes status, and (2) whether changes at the vertebrae and distal tibia are distinct. We hypothesized that BMAT composition would differ by diabetes status, with increases in the unsaturated lipid index in women with diabetes, and that these changes would occur at the spine but not the tibia.

2. Methods

2.1. Study population

Participants were recruited from a larger study of the calcium metabolism and skeletal outcomes after RYGB (Schafer et al., 2015a). Previous findings on changes in BMAT content after RYGB have been published (Kim et al., 2017; Schafer et al., 2015b), and we now report the data for BMAT composition changes.

Adult women over the age of 25 years were recruited from the University of California, San Francisco, and the San Francisco Veterans Affairs Health Care System. The participant had to be scheduled for an upcoming gastric bypass procedure to be eligible for the study. Enrollment was stratified by a history of diabetes, with an HbA1c $\geq$6.5 % or a documented history of diabetes with current use of an antidiabetic medication. Perimenopausal women, whose last menstrual cycle was between 3 months and 5 years, were not eligible, given declines in sex hormones have known independent effects on skeletal metabolism. Stable hormone use was allowed, including premenopausal women on hormone contraception and postmenopausal women on hormone therapy. Other exclusion criteria included estimated glomerular filtration rate $<$ 30 mL/min, prior weight loss procedures, and use of medications that influence skeletal or BMAT metabolism (ex: osteoporosis pharmacotherapy, chronic $>$5 mg prednisone daily or equivalent, pioglitazone).

Throughout the study, we assessed dietary calcium intake and provided chewable calcium citrate supplements to attain a total daily calcium intake of 1200 mg. Similarly, we monitored 25-hydroxyvitamin D levels and supplemented to a target of $>$30 ng/mL, both preoperatively and postoperatively.

The study protocol was approved by the University of California, San Francisco institutional review board, and all participants gave written informed consent. The study was enrolled at www.clinicaltrials.gov (NCT01330914).

Proton magnetic resonance spectroscopy (^{1}H-MRS) acquisition and quantification.

Participants had magnetic resonance imaging performed prior to surgery and 6 months after surgery. All scans were obtained with a GE MR750 3 Tesla scanner (GE Healthcare, Milwaukee, WI, USA) with embedded posterior phased array coils (GEM suite, GE Healthcare). In order to view the lumbar vertebrae for positioning of the spectral acquisition box, we used the following standard clinical sagittal T2-weighted Fast Spin Echo (FSE) sequence: repetition (TR)/echo time (TE) = 5000/87 ms, echo train length = 32, field of view 22 mm, slice thickness = 6 mm. We performed single-voxel MRS using the Stimulated Echo Acquisition Mode (STEAM) sequence with the parameters: TR/TE = 3000/20 ms (L3, L4) or 3000/30 ms (tibia), data points = 4096, without water suppression. For vertebral scans, the volume of interest (15 × 15 × 20 mm) was located in the center of the L3 and L4 vertebral bodies. For distal tibial scans, the 15 × 10 × 10 mm single voxel was located with the center 27 mm proximal to the joint line. Outer volume saturation bands were utilized to exclude contamination of outside signals.

In our previously published cohort study describing changes in marrow fat content after RYGB, we used jMRUI and time domain fitting with Lorentzian models to fit a lipid peak at 1.3 ppm and a water peak at 4.7 ppm (Kim et al., 2017). For this analysis, we used in-house developed software and time domain fitting with Voigt models (Fig. 1). Compared to our previous use of the Lorentzian model that fits one lipid peak, the Voigt model fits multiple lipid peaks, including the unsaturated lipid peak, which may have biological significance. MRS quantification methods using the Voigt line shape model and time-domain analyses have been described previously (Xu et al., 2018). As expected, results for total marrow fat content were higher with the new method due to the inclusion of additional small lipid peaks. Spectral peak assignment was based on published data: (Noula et al., 2000; Chi and Gupta, 1998; Karampinos et al., 2014) water peak at 4.7 ppm and six lipid peaks at 0.9, 1.3, 2.1, 2.8, 4.2, 5.3 ppm, respectively. Specifically, the unsaturated lipid peak, or olefinic lipid peak, was assigned to the resonance of lipids at 5.3 ppm (–CH=CH–). The amplitude of each peak

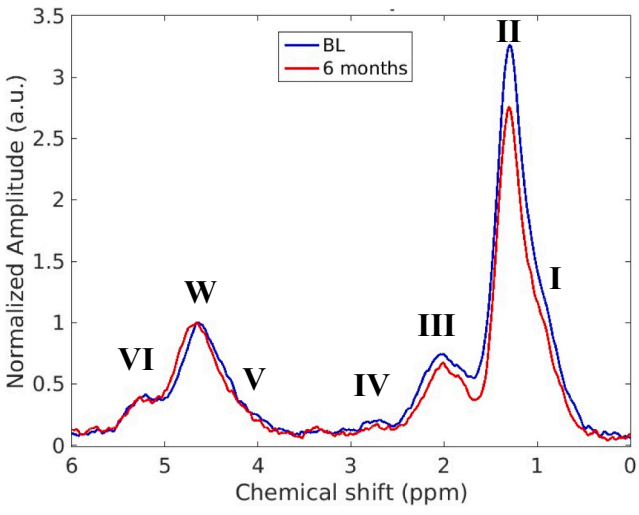

Fig. 1. Representative pre-operative (blue) and 6-month postoperative (red) L4 spectra, normalized to the water peak (W). Lipid peaks are labeled I-VI at 0.9, 1.3, 2.1, 2.8, 4.2, and 5.3 ppm (ppm), respectively. Peak VI (olefinic proton) was used to calculate the unsaturated lipid index. BL, baseline.

was calculated, and marrow fat content was calculated as (total lipids / (water + total lipids)) × 100 %. The unsaturated lipid index was calculated as (olefinic lipids / total lipids) × 100 %. The mean L3 and L4 values were used in this analysis. The unsaturated lipid resolution was scored based on the quality of resolution of the peaks. Excellent scan/rescan reproducibility was reported previously for this algorithm, with coefficient of variation (CV) 1.5 % for total BMAT content and 5.1 % for the BMAT unsaturated lipid index (Xu et al., 2018). Study personnel who performed these analyses were blinded to diabetes status and other participant attributes.

2.2. Areal and volumetric bone mineral density

We measured BMD at baseline and 6 months after gastric bypass surgery. Areal BMD (g/cm^2) was assessed by dual-energy x-ray absorptiometry (DXA; Hologic Discovery W and Horizon A densitometers; Bedford, MA, USA). Paired baseline and postoperative scans were performed on the same machine. We assessed volumetric BMD (g/cm^3) at the L3 and L4 vertebrae by quantitative computed tomography (QCT; GE VCT64 scanner; General Electric, Milwaukee, WI, USA), which was then analyzed using previously published methods (Mindways Software; Austin, TX, USA) (Lang et al., 1999; Khoo et al., 2009). Volumetric BMD at the tibia was measured by high-resolution peripheral quantitative computed tomography (HR-pQCT; XtremeCT, Scanco Medical, Brüttisellen, Switzerland), using the manufacturer's standard in vivo protocol. The ankle on the side of the nondominant forearm was scanned, with fixed scanned regions starting at 22.5 mm proximal to the mid joint line and extending proximally for 9.02 mm (110 slices). HR-pQCT images were analyzed using the manufacturer's standard clinical evaluation protocol in Imaging Processing Language (IPL v5.08b, Scanco Medical) (Boutroy et al., 2008; Khosla et al., 2006; Laib et al., 1998).

2.3. Other measures

We collected body composition measures at baseline and 6 months after gastric bypass surgery, including body mass index (BMI; weight/height^2, kg/m^2). Whole body fat (kg) and lean mass (kg) were assessed by DXA. If the width of the participant was greater than the scanning area, modified half body scans were applied (Tataranni and Ravussin, 1995). Visceral adipose tissue area (cm^2) was assessed by CT with a single axial slice at the mid-L4 vertebra. We manually traced fascial borders of the internal abdominal wall using previously published methods (Harris et al., 2007). Visceral adipose tissue area was calculated by multiplying the number of pixels within the adipose attenuation threshold by the pixel area.

Fasting serum specimens were obtained at baseline and 6 months after gastric bypass surgery. We measured serum chemistries, hemoglobin A1c (HbA1c), 25 hydroxyvitamin D (25OHD, liquid chromatography tandem mass spectrometry), and sclerostin (ELISA, R&D). Sera was also banked at −70 °C.

2.4. Statistical analysis

Baseline characteristics were assessed for normality and means ± standard deviations were calculated. We analyzed for differences between women with and without diabetes by applying chi-square and independent samples *t*-tests. For assessing individual-level longitudinal change, we used paired *t*-tests for all participants and for two groups divided by diabetes status. We then utilized independent samples *t*-tests to analyze differences in longitudinal outcomes between the groups. We assessed the association between longitudinal changes in BMAT and changes in metabolic and skeletal parameters by performing Pearson's correlation tests. Although multiple comparisons were examined, no formal adjustments were made. Instead, we interpreted the magnitude and direction of estimated effects considering relevant biology to avoid over-interpretation of isolated findings of statistical significance. Finally, we estimated adjusted associations between changes in BMAT and changes in metabolic and skeletal parameters using linear models. We adjusted for age and menopause status, given their associations with BMAT in other studies (Griffith et al., 2012; Kugel et al., 2001). Data were analyzed using Stata 14 software (StataCorp, College Station, TX, USA).

3. Results

3.1. Baseline characteristics

We enrolled 30 participants, who were aged 48 years ±12 (mean ± SD) years (Table 1). There were 14 women with type 2 diabetes; 11 of these women were prescribed antidiabetic medication including metformin, sulfonylureas, and insulin, and one woman was prescribed canagliflozin. Mean HbA1c was 7.6 % in participants with diabetes. Participants with diabetes had lower BMI and total body fat than participants without diabetes, which likely reflects lower BMI thresholds for surgery in people with metabolic disease. Baseline BMD at all sites did not differ by diabetes status.

At baseline, mean marrow fat content was 66.3 % at the lumbar spine for all participants and 97.8 % at the distal tibia in 19 participants with tibial data. Participants without diabetes had slightly higher levels of marrow fat content at the distal tibia compared to those with diabetes (98.2 % versus 97.4 %, $p = 0.04$). The overall mean BMAT unsaturated

Table 1
Baseline characteristics before surgery.

	All subjects ($n = 30$)	Diabetic subjects ($n = 14$)	Nondiabetic subjects ($n = 16$)	p value
Age, year	48.2 ± 11.7	48.2 ± 12.0	48.1 ± 11.9	0.98
Postmenopausal, n	11 (37 %)	4 (29 %)	7 (44 %)	0.39
Race, n				
White	13 (43 %)	6 (43 %)	7 (44 %)	0.57
Black	10 (33 %)	4 (29 %)	6 (38 %)	
Hispanic	6 (20 %)	4 (29 %)	2 (13 %)	
Asian	1 (3 %)	0 (0 %)	1 (6 %)	
Weight, kg	117.5 ± 17.1	109.3 ± 16.3	124.7 ± 14.9	0.01
BMI, kg/m^2	43.7 ± 5.7	41.6 ± 5.3	45.5 ± 5.4	0.05
Total body fat, kg	56.9 ± 10.6	51.3 ± 10.1	61.8 ± 8.6	<0.01
Total body lean mass, kg	56.6 ± 8.0	54.3 ± 8.1	58.5 ± 7.6	0.15
Visceral fat, cm^2	177.9 ± 79.3	197.3 ± 96.9	161.0 ± 58.0	0.22
HbA1c, %	6.6 ± 1.3	7.6 ± 1.2	5.7 ± 0.5	<0.01
eGFR, mL/min/1.73 m^2	60.2 ± 3.1	59.9 ± 4.3	60.6 ± 1.5	0.55
25OHD, ng/mL	41.3 ± 12.2	44.9 ± 13.3	38.1 ± 10.7	0.13
Sclerostin, pg/mL	162.6 ± 67.2	182.0 ± 82.6	145.6 ± 46.4	0.16
Areal BMD, g/cm^2				
Femoral neck	0.908 ± 0.144	0.917 ± 0.169	0.900 ± 0.124	0.75
Total hip	1.073 ± 0.151	1.115 ± 0.192	1.035 ± 0.095	0.18
Lumbar spine	1.134 ± 0.137	1.177 ± 0.151	1.096 ± 0.114	0.11
Volumetric BMD, g/cm^3				
Lumbar spine	0.161 ± 0.033	0.167 ± 0.036	0.156 ± 0.031	0.37
Marrow fat content, %				
Lumbar spine	66.3 ± 13.7	65.7 ± 13.0	66.8 ± 14.7	0.82
Distal tibia	97.8 ± 0.9 (n = 19)	97.4 ± 1.0 ($n = 10$)	98.2 ± 0.4 ($n = 9$)	0.04
Marrow fat unsaturated index, %				
Lumbar spine	4.9 ± 1.6	4.6 ± 0.8	5.3 ± 2.0	0.24
Distal tibia	4.3 ± 1.4 (n = 19)	4.0 ± 1.0 (n = 10)	4.7 ± 1.7 (n = 9)	0.31

Values are means ± SD or counts (percentages).
P-values are for between group differences.

index was 4.9 % at the spine and 4.3 % at the distal tibia; differences between women with and without diabetes were not statistically significant.

3.2. Marrow fat content and composition over 6 months

At the 6-month follow up visit, 25 of the initial 30 participants returned for repeat measures. Of the 5 participants who did not follow up: 2 women had sleeve gastrectomy instead of RYGB, 2 women moved away, and 1 woman was unable to attend the follow up study visit. All participants lost a significant amount of weight: average weight loss was 27.3 kg, with similar degrees of weight loss between women with and without diabetes (Table 2). All participants with diabetes had improvements in glycemic control, and 10 of the 13 women achieved remission of diabetes, as defined by A1c < 6.5 % without the use of antidiabetic medication.

At the lumbar spine, changes in marrow fat content and lipid composition differed by diabetes status (Fig. 2, Table 3). There were trends for decreases in marrow fat content in those with diabetes (absolute change of −4.3 % ± 8.2 %, $p = 0.09$), in contrast to stable levels in women without diabetes (+2.6 % ± 5.5 %, $p = 0.13$; $p = 0.02$ for differences between the groups). Conversely, the BMAT unsaturated index increased in those with diabetes (absolute change of +1.1 % ± 1.5 %, p = 0.02), with a trend towards a decrease in those without diabetes (−1.0 % ± 1.7 %, $p = 0.06$; $p < 0.01$ for differences between the groups). In other words, women with diabetes tended to have decreases in marrow fat content at the spine with increases in the unsaturated lipid index after RYGB. These changes were distinct from women without diabetes, who generally had stable levels after gastric bypass surgery. Hormone use was stable over 6 months, except one premenopausal woman without diabetes who stopped hormonal contraception by the follow up timepoint. A sensitivity analysis excluding this participant yielded similar results. We also performed a sensitivity analysis excluding 6 women with the poorest unsaturated lipid peak resolution, and our findings persisted. There was no relationship between changes in marrow fat content at the spine and changes in the unsaturated lipid index (Table 4).

Table 2
Six-month changes in metabolic and skeletal parameters after Roux-en-Y gastric bypass surgery.

	All subjects ($n = 25$)	Diabetic subjects ($n = 13$)	Nondiabetic subjects (n = 12)	Between-group p-value
Weight, kg	−27.3 ± 6.8*	−27.3 ± 7.2*	−27.4 ± 6.6*	0.96
Total body fat, kg	−19.3 ± 4.8*	−19.3 ± 4.9*	−19.3 ± 4.9*	1.00
Total body fat, %	−35.2 ± 9.7*	−38.4 ± 10.5*	−31.7 ± 7.7*	0.08
Total body lean mass, %	−12.8 ± 4.9*	−13.6 ± 5.1*	−11.8 ± 4.8*	0.37
Visceral fat, %	−43.9 ± 17.5*	−48.7 ± 18.0*	−38.6 ± 15.9*	0.15
HbA1c, absolute %	−1.2 ± 1.1*	−1.9 ± 1.1*	−0.4 ± 0.4*	<0.01
25OHD, ng/mL	−9.7 ± 15.6*	−8.7 ± 16.9	−10.9 ± 14.8*	0.73
Sclerostin, pg/mL	59.0 ± 70.4*	68.4 ± 85.6*	48.7 ± 50.8*	0.49
Areal BMD, %				
Femoral neck	−4.3 ± 4.1*	−2.5 ± 4.3	−6.2 ± 2.9*	0.02
Total hip	−4.1 ± 2.8*	−4.0 ± 3.0*	−4.2 ± 2.7*	0.82
Lumbar spine	−0.7 ± 3.5	+0.4 ± 2.8	−2.0 ± 4.0	0.09
Volumetric BMD, %				
Lumbar spine	−6.4 ± 5.9*	−4.2 ± 6.5*	−8.7 ± 4.2*	0.05
Distal tibia	−0.6 ± 1.6 ($n = 12$)	−1.0 ± 2.0 ($n = 7$)	−0.2 ± 0.6 ($n = 5$)	0.38

Values are means ± SD.
* $p < 0.05$.

At the distal tibia, we did not detect significant changes in BMAT content after RYGB, with similar findings between women with and without diabetes. For all women, there were declines in the unsaturated lipid index (−0.4 % ± 0.7 %, $p = 0.04$), and the small differences between women with and without diabetes were not significant. Similar to the spine, there was no relationship between changes in marrow fat content at the tibia and changes in the unsaturated lipid index (Table 4).

3.3. Body composition and metabolic parameters over 6 months

All participants lost a substantial amount of weight by 6 months after RYGB. Changes in total body fat among women with and without diabetes were − 38.4 % ± 10.5 % versus −31.7 % ± 7.7 %, respectively ($p < 0.05$ for both, $p = 0.08$ for difference). Women with diabetes had 48.7 % ± 18.0 % loss of visceral fat, and women without diabetes had a 38.6 % ± 15.9 % loss (p < 0.05 for both, $p = 0.15$ for difference). Women with diabetes had greater declines in HbA1c compared to women without diabetes (−1.9 % ± 1.1 % versus −0.4 % ± 0.4 %, respectively, $p < 0.01$ for difference). There was an increase in serum sclerostin that did not differ in women with and without diabetes.

At the lumbar vertebrae, 6-month changes in the unsaturated lipid index correlated with changes in HbA1c ($r = −0.47$, $p = 0.02$; Fig. 3). After adjusting for age and menopausal status, we found for every 1 % absolute decrease in HbA1c, vertebral unsaturated lipid index increased by 1.0 % (p < 0.01). We performed a sensitivity analysis removing an individual who had an A1c decrease of 4.4 % and increase in the unsaturated index by 3.9 %, and found similar results: in the adjusted model, for every 1 % absolute decrease in HbA1c, vertebral unsaturated lipid index increased by 0.8 %, $p = 0.045$. There was no relationship between changes in the vertebral unsaturated lipid index and changes in weight, total body fat, or visceral fat (Table 4).

At the distal tibia, changes in the unsaturated lipid index correlated with weight loss ($r = −0.59$, $p = 0.02$) and total body fat loss ($r = −0.56$, $p = 0.03$). These BMAT composition correlations with weight and total body fat loss were not seen at the vertebrae. Changes in the tibial unsaturated lipid index did not correlate with changes in HbA1c or visceral fat (Table 4).

3.4. Skeletal parameters over 6 months

After RYGB, there was a decline in hip BMD by DXA in all subjects: −4.3 % ± 4.1 % ($p < 0.01$) at the femoral neck and − 4.1 % ± 2.8 % (p < 0.01) at the total hip. There was no significant change in spine areal BMD by DXA, but spine volumetric BMD declined by −6.4 % ± 5.9 % (p < 0.01). There was no significant change in tibial volumetric BMD. Women with diabetes had less BMD decline at the femoral neck than women without diabetes (−2.5 % ± 4.3 % versus −6.2 ± 2.9 % respectively, $p = 0.02$ for difference) with similar trends with spine volumetric BMD (−4.2 % ± 6.5 % versus −8.7 ± 4.2 %, $p = 0.05$ for difference).

Changes in spine marrow fat content correlated with changes in spine volumetric BMD ($r = −0.44$, $p = 0.03$), similar to our previously published findings (Kim et al., 2017). There was no relationship with changes in the unsaturated lipid index at the spine with any BMD outcomes. In the subset with tibial data, neither changes in marrow fat content nor the unsaturated lipid index correlated with BMD outcomes.

4. Discussion

In a prospective cohort study of obese women undergoing RYGB,

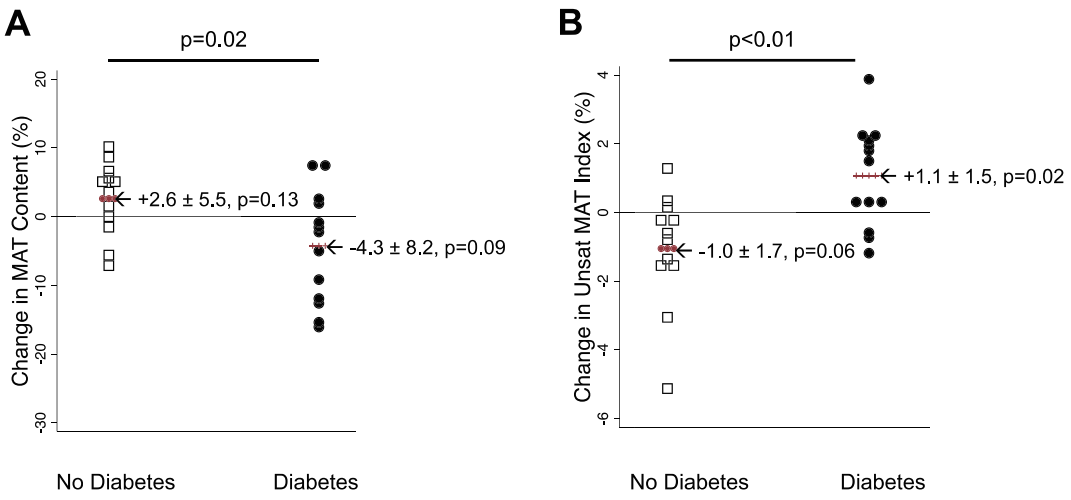

Fig. 2. Six-month changes in spine marrow adipose tissue (MAT) (A) and the spine MAT unsaturated index (B) after Roux-En-Y gastric bypass surgery. White squares represent participants without preoperative diabetes, black circles represent participants with preoperative diabetes.

Table 3
Six-month changes in marrow adiposity after Roux-en-Y gastric bypass surgery.

	Baseline (%)	6-Months (%)	Absolute Change (%)	Between-group *p* value
Spine: Fat content				
Combined (*n* = 25)	65.7 ± 13.9	64.7 ± 13.7	−1.0 ± 7.7, *p* = 0.54	
Diabetes (*n* = 13)	66.4 ± 13.2	62.1 ± 13.9	−4.3 ± 8.2, *p* = 0.09	p = 0.02
No diabetes (n = 12)	64.9 ± 15.2	67.5 ± 13.5	+2.6 ± 5.5, *p* = 0.13	
Spine: Unsaturated index				
Combined (n = 25)	5.1 ± 1.7	5.1 ± 1.6	+0.1 ± 1.9, *p* = 0.90	
Diabetes (n = 13)	4.5 ± 0.8	5.6 ± 1.5	+1.1 ± 1.5, p = 0.02	p < 0.01
No diabetes (n = 12)	5.7 ± 2.2	4.6 ± 1.5	−1.0 ± 1.7, p = 0.06	
Tibia: Fat content				
Combined (n = 15)	97.8 ± 1.0	97.5 ± 1.0	−0.2 ± 0.9, *p* = 0.32	
Diabetes (n = 9)	97.4 ± 1.1	97.0 ± 1.0	−0.4 ± 1.1, *p* = 0.34	*p* = 0.39
No diabetes (*n* = 6)	98.3 ± 0.5	98.2 ± 0.6	0 ± 0.3, *p* = 0.86	
Tibia: Unsaturated index				
Combined (n = 15)	4.2 ± 1.2	3.9 ± 1.2	−0.4 ± 0.7, p = 0.04	
Diabetes (n = 9)	4.1 ± 1.0	3.8 ± 1.2	−0.2 ± 0.6, p = 0.24	p = 0.34
No diabetes (n = 6)	4.5 ± 1.5	3.9 ± 1.3	−0.6 ± 0.8, *p* = 0.11	

Values are means ± SD.

Table 4
Correlations between changes in BMAT composition and changes in metabolic and skeletal outcomes after Roux-en-Y gastric bypass surgery.

6-month change in metabolic and skeletal outcomes	6-month change spine marrow fat unsaturated index, % (n = 25)	6-month change tibia marrow fat unsaturated index, % (*n* = 15)
Weight, kg	*r* = −0.32, *p* = 0.12	**r = −0.59, p = 0.02**
Total body fat, %	*r* = −0.24, *p* = 0.24	**r = −0.56, p = 0.03**
Visceral fat, %	r = −0.24, p = 0.24	*r* = −0.35, *p* = 0.20
HbA1c, absolute %	**r = −0.47, p = 0.02**	*r* = −0.18, *p* = 0.51
Femoral neck areal BMD, %	*r* = 0.27, *p* = 0.18	*r* = 0.28, *p* = 0.31
Total hip areal BMD, %	*r* = −0.13, p = 0.54	*r* = −0.42, p = 0.12
Spine areal BMD, %	*r* = 0.11, *p* = 0.60	*r* = 0.03, p = 0.90
Spine volumetric BMD, %	*r* = 0.10, *p* = 0.65	*r* = 0.18, *p* = 0.53
Tibia volumetric BMD, %	*r* = −0.33, *p* = 0.28	*r* = −0.05, *p* = 0.88
Spine marrow fat content, %	r = −0.18, *p* = 0.38	r = −0.18, *p* = 0.52
Tibia marrow fat content, %	*r* = −0.15, *p* = 0.57	*r* = −0.14, p = 0.60

Correlations with p<0.05 have been highlighted.

stratified by preoperative diabetes status, novel changes in BMAT composition were observed in relation to changes in metabolic outcomes. We found that 6-month changes in the BMAT unsaturated index were distinct from changes in marrow fat content and also distinct by anatomic site. At the spine, BMAT changes differed by diabetes status: women with preoperative diabetes tended to have declines in marrow fat content but an increase in the unsaturated index, while women without diabetes had more stable levels. At the distal tibia, marrow fat content was stable but there was a decrease in the unsaturated index, with no difference by diabetes status.

Our results suggest BMAT composition can reflect dynamic metabolic changes. After RYGB, the diabetes group on average experienced improvements in glycemic control and an increase in the unsaturated index at the spine. Indeed, this was supported by the correlation with A1c, meaning greater declines in A1c were associated with greater increases in the unsaturated index. Women with diabetes had a mean 1.1 % increase in the unsaturated lipid index, which may be biologically significant as cross-sectional studies have reported a 1.2–1.3 % difference in the unsaturated lipid index in postmenopausal women with and without type 2 diabetes (Patsch et al., 2013; Baum et al., 2012). At baseline in our cohort, there was no statistically significant difference between the vertebral unsaturated lipid index of women with versus without diabetes (4.6 % versus 5.3 %), but the between-group difference in means was comparable (0.7 %, 95 % confidence interval -0.5 % to 1.8 %). Although the physiologic relevance of BMAT composition is unknown, it may be a marker for BMAT quality or adipocyte function. There is recent evidence that bone marrow adipocytes have distinct

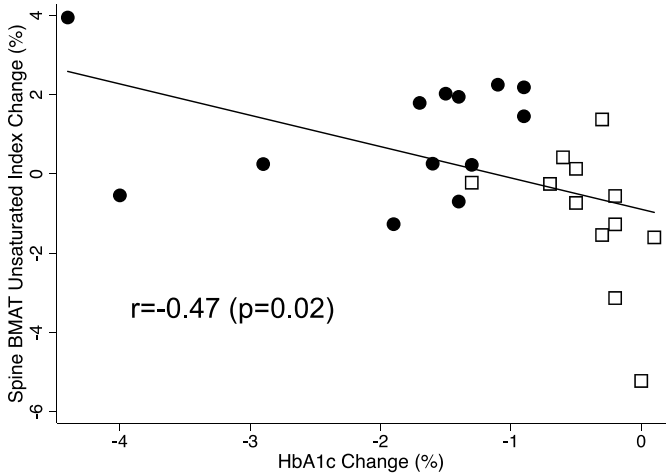

Fig. 3. 6-month changes in the spine marrow fat unsaturated index correlated with changes in HbA1c after Roux-En-Y gastric bypass surgery. White squares represent participants without preoperative diabetes, black circles represent participants with preoperative diabetes.

glucose metabolic profiles compared to white adipocytes and that BMAT is a major site of basal glucose uptake in humans (Suchacki et al., 2020). Our results support the concept that marrow adipocytes can respond to improved glycemic control, and BMAT composition may be a non-invasive biomarker.

In addition to BMAT parameters at the spine, we also measured BMAT parameters at the tibia. We found that changes in marrow fat content differed by site, with significant differences between women with and without diabetes at the spine but comparable levels at the tibia. This is consistent with the concept of regulated BMAT in the axial skeleton, responding to different metabolic stimuli, in contrast to constitutive BMAT in the appendicular skeleton, which is more stable (Scheller et al., 2015). Suchaki et al. recently demonstrated that the axial skeleton, including the spine, has higher basal glucose uptake compared with the appendicular skeleton (Suchacki et al., 2020). At the tibia, we found no differences between women with and without diabetes in changes to either marrow fat content or composition. For the combined group, although tibial marrow fat content was stable after RYGB, unsaturated levels declined, which correlated with weight loss. Different sites may respond differently to stimuli; trabecular bone at the spine may be more responsive to changes in glycemic control, and the appendicular skeleton may be more responsive to changes in weight bearing.

The majority of the literature on BMAT composition is cross-sectional, therefore it is valuable to understand longitudinal changes, especially in the setting of metabolic alterations. One other study of predominantly non-diabetic adolescents who had sleeve gastrectomy surgery has also reported changes in BMAT composition (Bredella et al., 2020). In that study, Bredella et al. also reported distinct vertebral and tibial changes, but while they found increases in the unsaturated lipids at the spine, we found a trend towards declines among the older women without diabetes we enrolled. These differences in findings may be due to the different study population in terms of age, sex, and important comorbidities. Also, the type of bariatric surgery likely plays a role, as Bredella and colleagues have demonstrated that BMAT changes differ between RYGB and sleeve gastrectomy surgery (Bredella et al., 2017). Nevertheless, results from both studies support the concept that BMAT composition changes in response to metabolic alterations.

Changes in BMAT content and composition were not correlated in our study and likely reflect distinct properties of marrow adipocytes. There was a negative correlation between vertebral BMAT content and volumetric BMD. Given the finding that women with diabetes had less femoral neck areal BMD loss and similar trends with vertebral volumetric BMD compared to women without diabetes, this suggests that BMAT content decreases may play a potential role in attenuation of bone loss. However, a large study reported no difference in fracture risk by diabetes status after gastric bypass surgery (Axelsson et al., 2018), therefore diabetes improvement and BMAT content may play a small role in the multifactorial changes that occur in post bariatric skeletal metabolism. In contrast, a novel finding is that we did not detect a relationship between changes in BMAT composition and BMD. Other studies have reported a relationship between BMAT composition and skeletal outcomes such as BMD, and prevalent and incident vertebral fracture (Yeung et al., 2005; Patsch et al., 2013; Woods et al., 2022). Our study was likely underpowered to detect a relationship between BMAT composition and BMD. While changes in BMAT composition do not directly correlate with bone loss after RYGB, the relationship with changes in metabolic parameters provides important insights into diabetic skeletal health. As a metabolic marker, BMAT composition may be more sensitive for diabetic bone disease than BMAT content. While BMAT content is significantly elevated in animal models of diabetes (Devlin et al., 2014), findings are not as consistent in humans. In studies reporting similar BMAT content levels between people with and without diabetes, the BMAT composition provided distinct information (Patsch et al., 2013; Baum et al., 2012), and has been proposed to be a more sensitive marker. Indeed in our study, changes in BMAT composition were more consistently observed than changes in marrow fat content. A strength of our study is the use of the Voight line shape model to quantify multiple lipid peaks—thereby providing a more comprehensive quantification of total BMAT—including the unsaturated lipid peak.

Our study has several limitations. While gastric bypass surgery is a useful model for studying dramatic metabolic change including improvements in glycemic control, there are numerous other metabolic changes occurring that may contribute to changes in BMAT and skeletal outcomes. In addition there could be an independent effect of antidiabetic medication, although no participants were prescribed thiazolidinediones and there is not a clear relationship between other antidiabetic medication and marrow adiposity. Our follow-up was limited to six months, although a recent study has demonstrated that BMAT can dynamically respond after 10 days of a high calorie diet and 10 days of fasting (Fazeli et al., 2021). Our modest sample size limits generalizability but provides hypothesis-generating data on the role of BMAT composition in diabetes. Our findings are unlikely to be due to age-related changes, as marrow fat content increases with age and the unsaturated levels decline; women with diabetes had changes in the opposite direction, which strengthens our findings.

In conclusion, we found that longitudinal changes in spinal BMAT content and composition after gastric bypass-induced weight loss were different for women with versus without diabetes, and that change in the unsaturated lipid index at the spine correlated with glycemic improvements. The observed site-specific changes at the spine and tibia are supportive of the concept of regulated versus constitutive BMAT. The unsaturated lipid index may be a biomarker for different adipocyte profiles, and its elucidation may be necessary to understand the biological significance of BMAT, including its role in diabetic bone disease.

Declaration of competing interest

Anne Schafer receives grant support from Amgen. All other authors have no conflicts of interest.

Acknowledgements

The authors thank Barbara Arnold for her work in study coordination and data collection; Viva Tai, RD, MPH, for DXA scan acquisition; Lisa Palermo, MA, and Sheena Patel, MPH for their roles in data management; Thomas Lang, PhD, and Aldric Chau for CT image analysis; and Melissa Lu, Lucy Wu, Mariko Kamiya, and Nicole King for their roles in study support and data coordination.

This study was supported by Department of Veterans Affairs, Veterans Health Administration, Clinical Science Research and Development Service, Career Development Award-2 (5 IK2 CX001984 and 5 IK2 CX000549); the American Society for Bone and Mineral Research (Junior Faculty Osteoporosis Clinical Research Award); and the UCSF Diabetes Center. Additional support was provided by the National Center for Advancing Translational Science of the National Institutes of Health (NIH) through UCSF-CTSI grant UL1 TR000004; the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK), NIH (5T32 DK007418-35 and R01 DK107629); the National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH (R01 AR055588, R01AR069670, and P30AR075055); and the Wilsey Family Foundation. Manuscript content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. Calcium citrate supplements were supplied by Bariatric Advantage.

CRediT authorship contribution statement

Study design: ALS, AVS, and DMS. Study conduct and data collection: TYK and ALS. Data analysis: TYK, XL, KX, and ALS. Data interpretation: all authors. Drafting manuscript: TYK. Revising manuscript content: all authors. Approving final version of manuscript: all authors. TYK takes responsibility for the integrity of the data analysis.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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