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Changes in bone quality after Roux-en-Y gastric bypass: A prospective cohort study in subjects with and without type 2 diabetes

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ABSTRACT

Background: Obesity and type 2 diabetes (T2D) are associated with an increased risk of skeletal fractures despite a normal areal bone mineral density (aBMD) and low bone turnover, possibly due to reduced bone material strength. Roux-en-Y gastric bypass (RYGB) enables a substantial and persistent weight loss and resolution of obesity related comorbidities such as T2D. However, the procedure induces a decrease in aBMD and increased bone turnover and fracture rate. To our knowledge, changes in bone material strength after RYGB have not been explored. This study aimed to evaluate changes in factors influencing bone quality; bone material strength, aBMD and bone turnover markers, in a population with morbid obesity undergoing RYGB and whether these changes differed in participants with and without T2D. We also sought to assess factors associated with bone material strength and bone mineral density in obese subjects before and after RYGB.

Methods: We examined 34 participants before and one year after RYGB, of whom 13 had T2D. Bone material strength index (BMSi) was evaluated by impact microindentation, aBMD and body composition by Dual energy X-ray absorptiometry, levels of bone turnover markers and calciotropic hormones were estimated from fasting serum samples. Participants with and without T2D were comparable before surgery, with the exception of glycosylated hemoglobin (HbA_{1c}).

Results: Preoperatively, BMSi was inversely associated with BMI, $\beta_{unadjusted}$ -1.1 (-1.9 to -0.28), $R^2 = 0.19$, $p = 0.010$, and this association remained significant after adjusting for age and gender. After RYGB the participants had lost a mean ± SD of 33.9 ± 10.9 kg, 48.7 ± 14.2 % of total body fat, increased physical activity, unchanged vitamin D levels, and all but one of the 13 participants with T2D were in diabetes remission. BMSi increased from 78.1 ± 8.5 preoperatively to 82.0 ± 6.4 one year after RYGB, corresponding to an increase of 4.0 ± 9.8 in absolute units or 6.3 ± 14.0 %, $p = 0.037$. The increase was comparable in participants with and without T2D. In subjects with T2D, a larger decrease in HbA_{1c} was associated with a larger increase in BMSi $\beta_{unadjusted}$ -9.2 (-16.5 to -1.9), $R^2 = 0.47$, $p = 0.019$. Bone turnover markers (CTX-1 and PINP) increased by 195.1 ± 133.5 % and 109.5 ± 70.6 %, respectively. aBMD decreased by 3.9 ± 5.5 % in the lumbar spine, 8.2 ± 4.6 % in the femoral neck, 11.6 ± 4.9 % in total hip and 9.4 ± 3.8 % in total body.

Conclusion: Our findings indicate that bone material strength improves despite an increase in bone turnover and a decrease in aBMD one year after RYGB. Trends were statistically comparable in participants with and without T2D. However, improved glucose control was associated with improved bone material strength in participants with T2D.

Abbreviations: aBMD, Areal bone mineral density; BMI, Body mass index; BMSi, Bone material strength index; CTX-1, Carboxyl terminal telopeptide of type 1 collagen; FSH, Follicular stimulating hormone; DXA, Dual energy X-ray absorptiometry; HRpQCT, High-resolution peripheral quantitative computed tomography; PINP, Procollagen type 1 N-terminal propeptide; RYGB, Roux-en-Y gastric bypass; SHPT, Secondary hyperparathyroidism; T2D, Type 2 diabetes; 25-hydroxyvitamin D, 25(OH) vitamin D

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1. Introduction

Fracture risk is most commonly evaluated based on areal bone mineral density (aBMD), despite aBMD being only a modest risk factor for fracture [1]. Epidemiological studies demonstrate a considerable overlap in aBMD values between fracture and fracture-free populations [2]. Bisphosphonate treatment induces a reduced fracture risk out of proportion to the observed increase in aBMD [3]. This discrepancy may be due to a difference or change in bone quality. Bone quality is described as the totality of features and characteristics that influence a bone's ability to resist fracture. Collectively, aBMD, bone architecture, and bone material properties interact to define bone quality; and all three are affected by bone turnover [4]. Obesity and type 2 diabetes (T2D) are associated with site specific increased incidence of fracture [5–7], despite a normal aBMD [8] and low bone turnover [9,10]. An important factor proposed to explain this discrepancy is reduced bone material strength and thus compromised bone quality [11–13].

Roux-en-Y gastric bypass (RYGB) is among the most commonly performed surgical procedures for weight loss offered to patients with morbid obesity. RYGB enables a substantial and persistent weight loss and resolution of obesity related comorbidities such as T2D [14–16]. The procedure is incorporated in the American Diabetes Associations treatment guidelines for selected patients with T2D and Class I-III obesity [17].

After RYGB, a reduction in aBMD and an increase in bone turnover and fracture rate are observed [18–27]. Possible mechanisms include weight loss mediated reduced stimulation of osteocyte mechanoreceptors, disturbances of the calcium and vitamin D, balance and changes in gastrointestinal- and sex hormone levels. Following weight stabilization a continued aBMD decrease and persistent elevated serum bone turnover markers has also been observed, indicating possible effects of RYGB on bone beyond adaptation to a reduced weight [28]. However, studies utilizing high-resolution peripheral quantitative computed tomography (HRpQCT) to estimate failure load have revealed diverging results one year after RYGB [22,24] and, to our knowledge, changes in tissue level bone material strength have not been previously explored.

This study aimed to evaluate changes in factors influencing bone quality; bone material strength, aBMD and bone turnover markers, in a population with morbid obesity undergoing RYGB, and whether these changes differed in participants with and without T2D. We also sought to assess factors associated with bone material strength and aBMD in subjects with obesity and after RYGB. Our hypothesis was that bone material strength would deteriorate, aBMD would decrease and bone turnover increase after RYGB and collectively pose a negative effect on bone quality.

2. Methods

2.1. Study population

We recruited patients referred for RYGB at the Department of Morbid Obesity and Bariatric Surgery, Oslo University Hospital, a tertiary referral center for treatment of morbid obesity. Eligibility criteria for RYGB were body mass index (BMI) $\geq$ 40 kg/m^2 or BMI $\geq$ 35 kg/m^2 with obesity-related co-morbidity, age between 18 to 65 years, and previously failed attempts of sustained weight loss. Patients with T2D were encouraged to participate in the study. The inclusion period was from October 8th, 2015, to January 27th, 2017. Participants were excluded if they were unable to read Norwegian language, 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-Caucasians were excluded.

2.2. Surgery and study visits

All participants had a laparoscopic RYGB with a gastric pouch of about 25 ml, a 150 cm antecolic alimentary and 50 cm biliopancreatic limb [29]. Participants attended study visits preoperatively and one year after RYGB. Study visits included blood samples, anthropometric measures, dual-energy absorptiometry scan (DXA), impact microindentation, intravenous glucose tolerance test, euglycemic hyperinsulinemic clamp, indirect calorimetry, and bone marrow biopsy. In this paper results of the blood samples, anthropometric measures, DXA measurements and impact microindentation are presented. Results of bone marrow biopsy measurements of bone marrow adipose tissue in this population has been previously published [30].

In addition to study visits, the participants also attended regular clinical follow-up visits six to eight weeks, six months, and one year after surgery. After surgery, all participants were advised oral supplementation with 1000 mg of calcium, 800 IU vitamin D, one multivitamin, 200 mg of iron daily and B12 injections 1 mg every three months. At clinical visits, vitamin levels were monitored and additional supplements were advised as appropriate.

2.3. Clinical parameters

T2D was defined as glycosylated hemoglobin (HbA_{1c}) $\geq$ 6.5 %, or use of one or more oral glucose lowering drug (GLD). Diabetes remission was defined as HbA_{1c} < 6.5 % without GLD in participant with T2D preoperatively. Vitamin D deficiency was defined as serum 25(OH) vitamin D levels below 50 nmol/L. Secondary hyperparathyroidism (SHPT) was defined as a serum concentration of PTH above 7.0 pmol/L in the absence of serum ionized calcium above 1.33 mmol/L. All previous fractures are reported, except digit fractures. 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 [31]. Physical activity was reported by the participants on a predefined non-validated form and categorized as (i) 0–1 hour; (ii) 1–2 hours or (iii) $\geq$ 3 h per week.

2.4. Blood samples

Blood samples were taken before 10 a.m. after an overnight fast, centrifuged, and stored in refrigerator or freezer. Serum samples for evaluation of bone turnover markers were stored at -80 C and analyzed at the end of the study 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), procollagen type 1 N-terminal propeptide (PINP) using Roche® electrochemiluminescence immunoassay (ECLIA), and osteocalcin using LIAISON® chemiluminescence immunoassay (CLIA). Serum 25(OH) vitamin D levels were analyzed by liquid chromatography-mass spectrometry (LC–MS/MS) method, serum parathyroid hormone (PTH) by Immulite 2000 XPI, Siemens Healthineers a chemiluminoimmunometric assay, serum ionized calcium using Roche® Cobas b221, and C-peptide using Modular E170 Roche® ECLIA. FSH was analyzed using Immulite 2000 XPI, Siemens Healthineers, a non-competitive immunluminometric assay.

The Central Laboratory of Oslo University Hospital analyzed HbA_{1c} using Tosoh G8 high-performance liquid chromatography. Phosphate and magnesium were analyzed using Cobas 6000 Roche® photometry.

The reference range for the ten variables reported were: CTX-1 μg/L: females 25–49 years: ≤ 0.57, ≥ 50 years: ≤ 1.01, males 30–50 years: ≤ 0.58, 51–70 years: ≤ 0.7; PINP μg/L: females > 25 years: 11–94, males > 25 years: 20–91; osteocalcin nmol/L: females ≥ 21

years: 1.5–5.4, males ≥ 21 years 1.6–4.3; 25(OH) vitamin D nmol/L: 37–132; PTH pmol/L: 1.5–7.0; ionized calcium mmol/L: 1.15–1.33; C-peptide pmol/L: 300–1480; HbA_{1c} %: < 6; phosphate mmol/L: females ≥ 16 years: 0.9–1.7, males 16–49 years: 0.8–1.7; magnesium mmol/L: 0.71-0.94. The coefficient of variance was 5 % for CTX-1, 5 % for PINP and 6 % for osteocalcin.

2.5. Impact microindentation

Tissue level bone material strength of cortical bone was assessed by impact microindentation using a commercially handheld device (OsteoProbe®, Active Life Scientific, Santa Barbara, California). The impact microindentation was performed on the anterior surface of the mid-shaft of the right tibia (with the exception of one participant where the examinations were performed on the left) 10 cm below the inferior margin of the patella after injection of local anesthetics. To avoid overlying skin and subcutaneous tissue opposing the measurement, an insertion channel was made with a sharp needle (BD Microlance™ 3, 21 G) prior to indenting the cortical bone surface. To ensure sufficient width of the insertion channel the needle was moved in circular movements expanding the insertion channel. The first eight indentations were made in vivo, with 2 mm separating two measurements. Subsequently, five indentations against a phantom of poly-methyl methacrylate were performed for calibration of participant measurements. Indentations with obvious operator errors were removed. The output for OsteoProbe® is the Bone Material Strength index (BMSi), which is a normalized measure of indentation depth [32]. To minimize inter-observer variations, all measurements were made by the same investigator (I.K.B.H).

2.6. Areal bone mineral density

DXA scan including whole body scans for assessment of body composition, including whole body fat and lean mass, was performed. aBMD, g/cm^2 of the lumbar spine (L_1-L_4), total hip, proximal femur, and total body was assessed. T-scores and Z-scores of the lumbar spine L1-L4 were calculated after exclusion of vertebrae with osteoarthritic changes (spondylosis) or compression fractures. All scans were performed by the same nurse. GE Lunar Prodigy was used until August 26th 2016, when it was replaced by GE Lunar iDXA (Lunar Corporation, Madison, WI, USA). Body composition performed with GE Lunar Prodigy was reanalyzed with iDXA software, to optimize comparability. Cross calibration between the measurements of two DXA machines has been published previously [30]. 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 is < 1.0 %.

2.7. Statistical analysis

Participant characteristics are presented as mean values ± standard deviation (SD), median (range), or as proportions (percentage). When comparing preoperative characteristics of participants with and without T2D, independent sample *t*-test or Mann-Whitney U test were used for continuous variables, and Pearson Chi-square or Fisher's exact test for categorical variables. Correlations were assessed with Pearson (r) or Spearman (rsp) correlation coefficients, as appropriate. When exploring changes between preoperative and one year after RYGB, paired-sample t–tests or Wilcoxon signed-rank test were used for continuous variables and McNemar's test for paired proportions was used for evaluation of changes in categorical variables. To explore difference in changes from preoperative to one year after RYGB between participants with and without T2D, 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 change, and preoperative value. In order to avoid multicollinearity, confounders that correlated, r > 0.7, were not adjusted for. The results from the regression analyses are presented as regression coefficients (β) with 95 % confidence intervals (CI) and R square (R^2). 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).

2.8. Ethics

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

3. Results

A total of 44 participants were included. Preoperative study examinations were possible in 38, and 36 (92%) of these met for follow-up one year after RYGB. Two of the 36 participants were excluded from the study at follow-up due to sex reassignment and glucocorticoid treatment, respectively. Thus the study population ultimately consisted of 34 participants. Of these BMSi estimation was not possible in one participant preoperatively; due to edema of the lower extremity, and in three participants postoperatively; as the OsteoProbe apparatus was not available.

3.1. Preoperative characteristics

Preoperative characteristics are presented in Table 1. Nineteen participants had undergone one or more skeletal fracture, fracture site, time, and energy is presented in supplementary Table 1. Thirteen participants (38 %) had T2D at study inclusion, eight (62 %) of these were treated with one or more oral GLD, and one with both insulin and oral GLD. Median (range) duration of T2D was 4 years (5 months to 18 years). Except for HbA_{1c}, preoperative characteristics including body composition, aBMD, BMSi values, calciotropic hormones, and bone turnover markers did not differ statistically between participants with and without T2D (Table 1).

3.2. Changes in body composition and physical activity

All participants had lost weight one year after RYGB. Mean ± SD weight loss was 33.9 ± 10.9 kg or 28.3 ± 8.9 % of total weight. BMI decreased with 11.6 ± 4.3 points, fat mass decreased with 48.8 ± 14.2 % or 27.4 ± 9.5 kg, and lean mass decreased with 10.5 ± 4.1 % or 6.3 ± 2.5 kg, all p < 0.001. The mean tissue fat of the lower limb decreased from 17.0 ± 5.1 kg preoperatively to 8.7 ± 2.9 kg postoperatively, p < 0.001. Participants with and without T2D had comparable changes in body composition. The proportion of participants reporting less than one hour of hard physical activity a week decreased from 55.8% preoperatively to 23.5% one year after RYGB. Participants reporting 1–2 hours or more than 3 h a week increased from 17.6% to 32.4% and 26.5 % to 38.2 %, respectively.

3.3. Changes in T2D status and related parameters

All but one of the thirteen participants with T2D was in diabetes remission at study follow-up. One year after RYGB, we observed a decrease in C-peptide levels for all participants combined, and an HbA_{1c} reduction for all but two participants. For participants with T2D, HbA_{1c} decreased from 6.7 % to 5.6 % one year after RYGB. For participants without T2D, HbA_{1c} decreased from 5.5 % to 5.2 %. C-peptide levels

Table 1
Baseline characteristics of 34 participants with morbid obesity awaiting Roux-en-Y gastric bypass surgery.

Characteristics	All participants	With type 2 diabetes (n = 13)	Without type 2 diabetes (n = 21)	p-value T2D vs. non T2D
Age, years	45.2 ± 9.7	48.3 ± 7.9	43.2 ± 10.4	0.14
Female gender	21 (63%)	8 (62%)	13 (62%)	0.98
Postmenopausal*	8 (36%)	5 (62%)	3 (23%)	0.12
Smoking, current or previous	21 (60%)	6 (43%)	15 (71%)	0.20
Previous fracture	19 (56%)	9 (64%)	10 (48%)	0.33
Weight, kg	120.0 ± 14.9	117.5 ± 13.3	121.5 ± 15.9	0.47
BMI, kg/m^2	40.9 ± 3.5	40.3 ± 4.0	41.2 ± 3.1	0.47
Fat mass, kg	55.2 ± 8.6	52.6 ± 9.1	56.8 ± 8.0	0.17
Lean mass, kg	61.1 ± 11.2	61.0 ± 11.4	60.6 ± 10.2	0.90
Impact microindentation, BMSi	78.1 ± 8.4	78.6 ± 7.4	77.7 ± 9.1	0.78
Lumbar spine aBMD, g/cm^2	1.18 ± 0.16	1.15 ± 0.14	1.20 ± 0.17	0.41
Lumbar spine t-score	0.1 ± 1.3	−0.1 ± 1.1	0.2 ± 1.4	0.49
Femoral neck aBMD, g/cm^2	1.09 (0.78-1.3)	1.04 (0.78-1.3)	1.11 (0.88-1.3)	0.12
Femoral neck t-score	0.6 ± 1.1	0.2 ± 1.1	0.8 ± 1.0	0.09
Total hip aBMD, g/cm^2	1.17 ± 0.1	1.14 ± 0.13	1.19 ± 0.18	0.22
Total hip t-score	1.2 ± 1.1	0.9 ± 1.1	1.3 ± 1.1	0.33
Whole body BMD, g/cm^2	1.35 ± 0.10	1.31 ± 0.10	1.36 ± 0.10	0.16
Whole body t-score	2.2 ± 1.1	1.8 ± 0.70	2.4 ± 1.2	0.16
HbA_{1c}, %	5.8 (5.0-8.0)	6.7 (5.9-8.0)	5.5 (5.0-6.2)	< 0.001
C-peptide, pmol/L	1394 (680-2436)	1379 (837-2436)	1402.0 (680-1994)	0.70
Serum ionized calcium, mmol/L	1.2 ± 0.13	1.2 ± 0.04	1.2 ± 0.16	0.47
Parathyroid hormone, pmol/L	4.2 (1.7-9.8)	4.1 (1.7-8.9)	4.3 (1.9-9.8)	0.53
25(OH)Vitamin D, nmol/L	61.0 ± 22.0	65.2 ± 16.5	55.6 ± 25.0	0.23
CTX-1, µg/L	0.33 ± 0.14	0.37 ± 0.13	0.30 ± 0.14	0.19
PINP, µg/L	47.1 ± 20.1	53.8 ± 23.7	43.0 ± 16.7	0.13
Osteocalcin, nmol/L	3.1 ± 0.66	3.4 ± 0.71	3.0 ± 0.60	0.11

Abbreviations: body mass index (BMI), bone mineral density (BMD), bone material strength index (BMSi), carboxyl terminal telopeptide of type 1 collagen (CTX-1), procollagen type 1 N-terminal propeptide (PINP), type 2 diabetes (T2D).
Continuous variables with normal distribution are presented as mean ± SD and analyzed with independent tvariables with normal distribution are presented as mean ± SD and analyzed with independent *t*-test. Other continuous variables are presented as median (range) and analyzed with Mann-Whitney U test.
Categorical variables are presented as n (%) and analyzed with Person Chivariables are presented as n (%) and analyzed with Person Chi-square or Fisher exact test if less than 5 expected frequencies.
Missing values: BMSi n = 1, Serum ionized calcium: n = 2.
* Proportion of postmenopausal females is given as proportion of total number of females.

decreased from median (range) 1394 (680–2436) pmol/L to 747 (426–1115) pmol/L after RYGB. The decrease was comparable for participants with and without T2D.

3.4. Changes in calciotropic hormones and bone turnover markers

Mean 25(OH) vitamin D levels remained unchanged for both participants with and without T2D (Table 2). However, the number of participants with vitamin D deficiency decreased from 11 preoperatively to seven one year after RYGB, p = 0.34. PTH levels increased from a median (range) 4.2 pmol/L (1.7–9.8) preoperatively to 4.7 pmol/L (2.1–15.2), p = 0.024, and the number of participants with SHPT increased from four to six, p = 0.69. The increase in PTH levels for the total study population seemed to be driven by a significant increase in PTH for participants with T2D, whereas the increase was non-significant for participants without T2D. A similar pattern was noted for ionized calcium levels (Table 2). CTX-1 increased by 195.1 ± 133.6 %, PINP by 109.5 ± 70.6 % and Osteocalcin by 52.2 ± 28.0 %. The observed increase in bone turnover markers was comparable between participants with and without T2D (Table 2).

3.5. Changes in bone material strength of cortical bone

BMSi (preoperative and one year after RYGB) did not correlate with aBMD (all measured locations), or tissue fat of the lower limb, r < 0.30, p > 0.05 for all. Preoperatively, BMSi was inversely associated with BMI ($\beta_{unadjusted}$ -1.1 (-1.9 to -0.28), R^2 = 0.19, p = 0.010), and this association remained significant after adjusting for age and gender ($\beta_{adjusted}$ -1.5 (-2.3 to -0.65), R^2 =0.35, p = 0.001). One year after RYGB, BMI did not correlate with BMSi (r = 0.12, p = 0.51), also changes in BMSi did not correlate with weight loss after RYGB (r = 0.15, p = 0.44). BMSi increased from 78.1 ± 8.4 preoperatively to 82.0 ± 6.4 one year after RYGB, corresponding to a mean difference of 4.0 ± 9.8 in absolute units, or 6.3 ± 14.0% increase, p = 0.037. The change was not statistically different in participants with and without T2D (Fig. 1).

For all participants combined, the change in HbA_{1c} was not associated with change in BMSi, $\beta_{unadjusted}$ -2.1 (-8.4–4.2), R^2 = 0.017, p = 0.50. When the cohort was divided based on presence or absence of preoperative T2D we observed that for participants without T2D, a larger decrease in HbA1c was associated with a smaller improvement or deterioration of BMSi levels ($\beta_{unadjusted}$ 16.7 (0.26–33.2), R^2 = 0.23, p = 0.047). This association remained significant after adjustment for BMI change and age ($\beta_{adjusted}$ 18.4 (1.7–35.0), R^2 = 0.33, p = 0.033). In participants with T2D, however, a larger decrease in HbA_{1c} was associated with a larger increase in BMSi $\beta_{unadjusted}$ -9.2 (-16.5 to -1.9), R^2 = 0.47, p = 0.019 (Fig. 2), this association remained significant after adjusting for change in BMI and age ($\beta_{adjusted}$ -7.8 (-15.2 to -0.38), R^2 = 0.68, p = 0.042).

Preoperative BMSi values were not associated with postoperative BMSi values (r = 0.15, p = 0.43). However, preoperative BMSi values were negatively associated with delta BMSi $\beta_{unadjusted}$ -0.89 (-1.2 to -0.59), R^2 = 0.60, p < 0.001. This association remained significant after adjustment for age, gender and BMI change ($\beta_{adjusted}$ -0.90 (-1.2 to -0.60), R^2 = 0.63, p < 0.001). We observed no association between age, gender, and menopausal status and change in BMSi.

3.6. Changes in areal bone mineral density

One year after RYGB, aBMD decreased with 3.9 ± 5.5% in the lumbar spine, 8.2 ± 4.6 % in the femoral neck, 11.6 ± 4.9 % in total hip, and 9.4 ± 3.8 % in total body. This corresponded to a decrease in

Table 2
Changes in bone mineral density, markers of bone turnover and calcium metabolism one year after Roux-en-Y gastric bypass surgery, in participants with and without type 2 diabetes.

	Participants with type 2 diabetes (n = 13)				Participants without type 2 diabetes (n = 21)				
	Preoperatively	One year after RYGB	Difference	p-value within group	Preoperatively	One year after RYGB	Difference	p-value within group	p-value between groups
Impact microindentation, BMSi	78.6 ± 7.4	81.5 ± 5.6	2.8 ± 10.0	0.38	77.7 ± 9.1	82.2 ± 7.0	4.7 ± 9.8	0.059	0.63
Lumbar spine, aBMD	1.15 ± 0.14	1.09 ± 0.16	−0.063 ± 0.052	0.001	1.20 ± 0.17	1.16 ± 0.17	−0.033 ± 0.060	0.021	0.15
Lumbar spine, t-score	−0.1 ± 1.1	−0.5 ± 1.3	−0.42 ± 0.50	0.010	0.2 ± 1.4	0.02 ± 1.3	−0.21 ± 0.60	0.12	0.30
Femoral neck, aBMD	1.0 (0.78-1.3)	0.93 (0.71-1.3)	−0.11 (-0.16 to -0.02)	0.001	1.1 (0.88-1.3)	1.0 (0.84-1.2)	−0.073 (-0.18 to 0.01)	< 0.001	0.16
Femoral neck, -score	0.23 ± 1.1	−0.65 ± 1.1	−0.88 ± 0.43	< 0.001	0.81 ± 1.0	−0.048 ± 0.76	−0.86 ± 0.42	< 0.001	0.92
Total hip, aBMD	1.1 ± 0.13	1.0 ± 0.13	−0.12 ± 0.047	< 0.001	1.2 ± 0.12	1.1 ± 0.11	−0.12 ± 0.047	< 0.001	0.77
Total hip, t-score	0.93 ± 1.0	0.062 ± 1.0	−0.87 ± 0.49	< 0.001	1.3 ± 1.1	0.51 ± 0 .89	−0.82 ± 0.40	< 0.001	0.74
Total body, aBMD	1.3 ± 0.10	1.2 ± 0.10	−0.12 ± 0.036	< 0.001	1.4 ± 0.10	1.3 ± 0.090	−0.11 ± 0.049	< 0.001	0.67
Total body, t-score	1.8 ± 0.70	0.66 ± 0.64	−1.2 ± 0.35	< 0.001	2.4 ± 1.2	1.2 ± 1.2	−1.1 ± 0.48	< 0.001	0.77
CTX-1	0.37 ± 0.13	0.91 ± 0.26	0.54 ± 0.19	< 0.001	0.30 ± 0.14	0.82 ± 0.31	0.52 ± 0.29	< 0.001	0.81
PINP	54.8 ± 23.7	93.5 ± 19.6	39.7 ± 24.9	< 0.001	43.0 ± 16.7	86.4 ± 26.3	43.4 ± 20.1	< 0.001	0.64
Osteocalcin	3.4 ± 0.71	4.9 ± 0.99	1.6 ± 0.79	< 0.001	3.0 ± 0.60	4.5 ± 0.68	1.49 ± 0.60	< 0.001	0.80
Parathyroid hormone	4.1 (1.7-8.9)	5.1 (2.1-13.8)	1.3 (-0.3-5.5)	0.011	4.3 (1.9-9.8)	4.4 (2.1-15.2)	0.50 (-4.4-9.3)	0.34	0.58
25(OH) vitamin D	65.2 ± 16.5	66.3 ± 16.4	1.2 ± 24.6	0.87	55.6 ± 25.0	66.7 ± 23.4	11.0 ± 27.7	0.083	0.32
Ionized calcium	1.2 ± 0.041	1.2 ± 0.032	0.022 ± 0.020	0.002	1.2 ± 0.16	1.2 ± 0.032	−0.031 ± 0.15	0.39	0.22
Phosphate	0.99 ± 0.14	1.2 ± 0.16	0.17 ± 0.17	0.004	0.95 ± 0.14	1.2 ± 0.14	0.23 ± 0.17	< 0.001	0.30
Magnesium	0.83 ± 0.057	0.86 ± 0.050	0.032 ± 0.085	0.20	0.84 ± 0.044	0.84 ± 0.045	0.0019 ± 0.044	0.84	0.18

Abbreviations; areal bone mineral density (aBMD), carboxyl terminal telopeptide of type 1 collagen (CTX-1), procollagen type 1 N-terminal propeptide (PINP).
Units of measurement: Impact microindentation; bone material strength index (BMSi), aBMD; g/cm^3, CTX-1; μg/L, P1NP; μg/L, osteocalcin; nmol/L, 25(OH) vitamin D; nmol/L, ionized calcium; mmol/L, phosphate; mmol/L, magnesium; mmol/L.
All measures are normally distributed parameters are presented as mean ± SD and analyzed with pared *t*-test. Parameters with non-linear distribution are presents as median (range), and analyzed with Wilcoxon Signed Rank test. Analyses between groups were made with independent sample *t*-test (normal distribution) or Mann Whitney U test (not normally distributed). Missing values preoperatively: BMSi; n = 1, Serum ionized calcium; n = 2. One year after RYGB: BMSi; n = 4.

t-score at all evaluated skeletal locations (Table 2). Higher age was associated with a greater decrease of the lumbar spine aBMD $\beta_{unadjusted}$ -0.004 (-0.005 to -0.002), $R^2 = 0.37$, $p < 0.001$, and this remained significant when adjusted for gender and preoperative aBMD ($\beta_{adjusted}$ -0.004 (-0.006 to -0.003), $R^2 = 0.48$, $p < 0.001$). Postmenopausal women exhibited a higher lumbar spine aBMD loss compared to

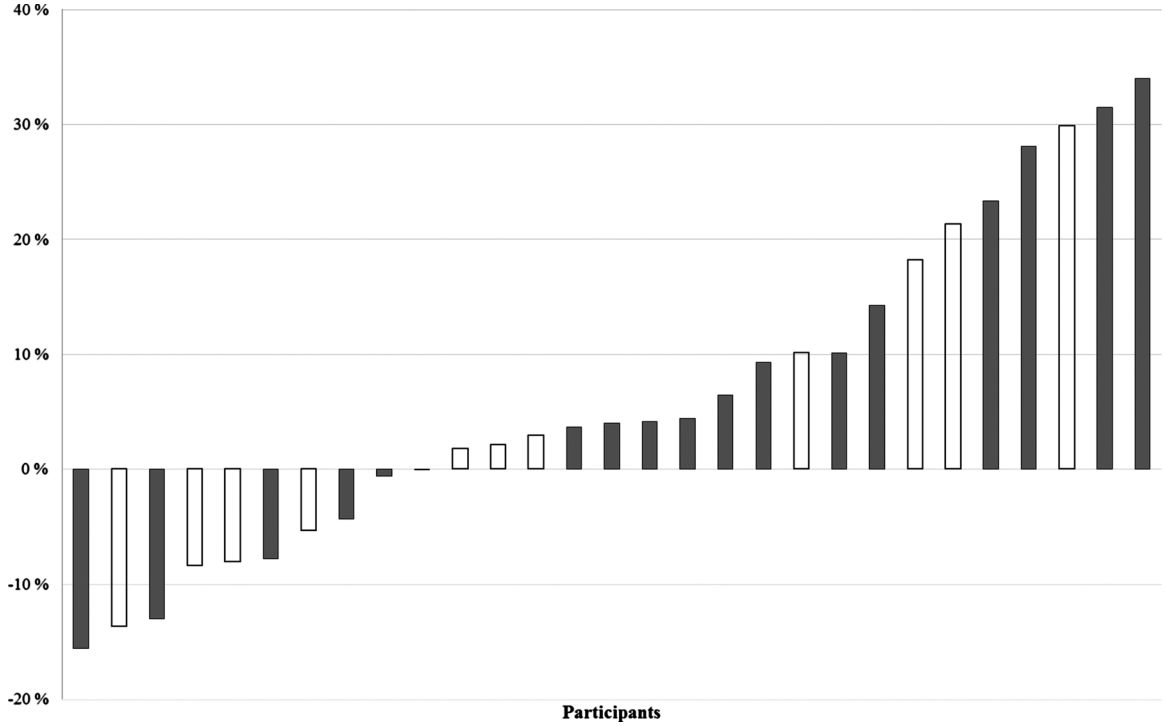

Fig. 1. Percent change in bone material strength index (BMSi) one year after RYGB in 30 participants with and without type 2 diabetes (T2D). Participants with T2D are marked with white columns. Overall mean (SD) BMSi increased from 78.0 (8.4) at baseline to 82.2 (6.3) after RYGB, corresponding to a mean difference of 4.23 (9.7) or 6.6% increase, p = 0.024. Mean changes in BMSi were comparable in participants with and without T2D. BMSi was measured with impact microindentation on the anterior surface of the mid-shaft of the tibia using an OsteoProbe®.

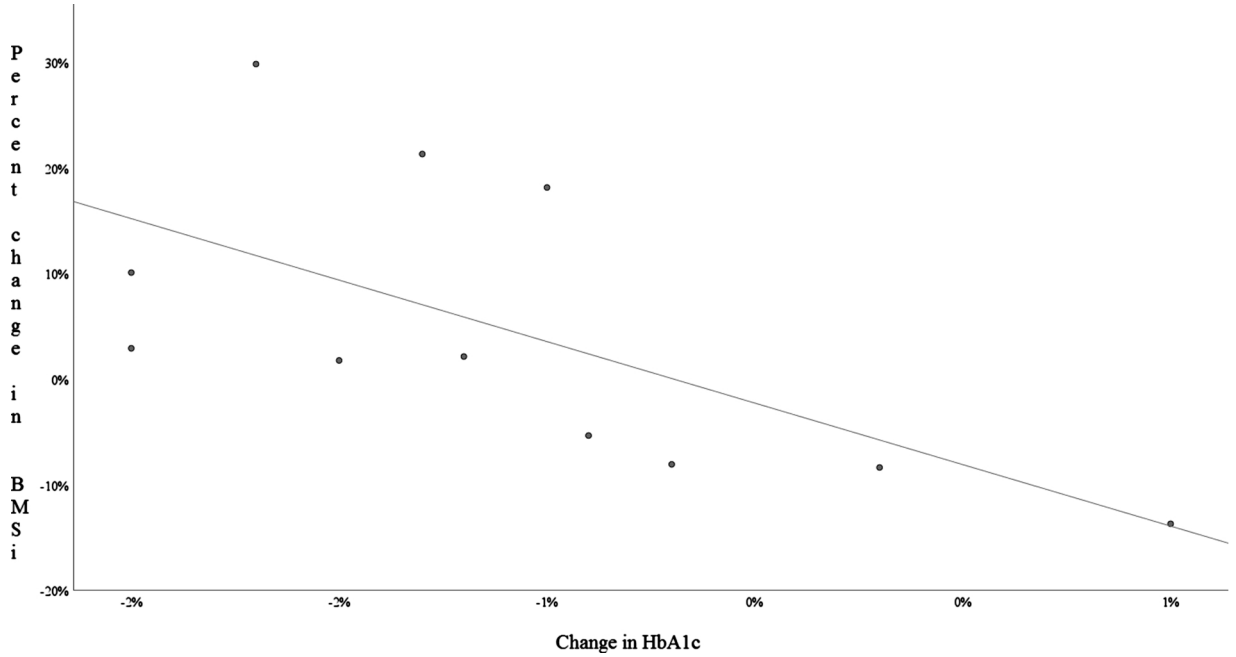

Fig. 2. Association between change in HbA_{1c} and percent change in bone material strength index (BMSi) one year after Roux-en-Y gastric bypass (RYGB) in 11 participants with type 2 diabetes (T2D). A larger decrease in HbA_{1c} was associated with a higher increase in bone material strength measured by impact microindentation (OsteoProbe®); $\beta_{unadjusted}$ -9.2 (-16.5 to -1.9), $R^2 = 0.47$, $p = 0.019$.

premenopausal women $\beta_{unadjusted}$ -0.073 (-0.11 to -0.032), $R^2 = 0.42$, $p = 0.001$. However, this was no longer significant when adjusted for age and preoperative aBMD ($\beta_{adjusted}$ -0.031 (-0.78 to 0.017), $R^2 = 0.66$, $p = 0.19$). The aBMD loss in the lumbar spine, femoral neck, total hip, and total body did not differ statistically between participants with and without T2D (Table 2).

4. Discussion

4.1. Bone material strength after RYGB

We conducted a prospective cohort study evaluating skeletal health after RYGB. This is the first study to describe in vivo measurements of cortical bone material strength in a bariatric surgery population, and changes induced after RYGB. We observed that for participants with morbid obesity, BMSi was inversely associated to BMI, and the mean BMSi increased one year after RYGB. Our findings support the hypothesis that higher BMI is associated with decreased bone material strength, and implicate that surgically induced weight loss has a positive effect on bone quality. Studies comparing patients with and without fragility fractures have described 4–4.5% lower bone material strength in the fracture population [33,34]. Our observed increase of 6.3% therefore seems clinically important.

The relative preservation of lean mass, maintenance of calcium, vitamin D homeostasis, and the increased physical activity noted in our population might contribute to the observed improvement in bone material strength. Our findings are in line with a study reporting that high-intensity loading leads to increased bone material strength [35]. The presented finding of improved bone material strength after RYGB challenges preceding studies describing failure load to decrease or remain unchanged one year after RYGB [20,22,24]. Studies reporting failure load, however, are based on microfinite element analysis of microstructure images (HRpQCT) and not in vivo bone material strength measurements, and are thus not directly comparable.

4.2. Bone material strength measurements in a bariatric population

The use of the impact mircroindentation to evaluate bone material strength in humans is a relatively new method, and our study the first to use this technique in a bariatric population. Potential biases of obesity and weight loss on BMSi measurements remains to be explored. In a study of more than 200 elderly women, Sundh et al observed a negative correlation between tibial subcutaneous fat and BMSi [11]. Whether this finding represents a bias due to larger amounts of pretibial subcutaneous fat on the BMSi measurements, or a direct negative effect of local adipose tissue on bone material strength, remains unknown. In this context, we analyzed the relation between the lower extremity fat assessed by DXA and BMSi prior to and one year after RYGB. We found no correlation, be it at baseline ($r^2 = 0.006$) or one year postoperatively ($r^2 = 0.020$). Notably, these results should be interpreted with caution as they are based on measurements of entire lower extremity fat and not solely pretibial fat.

Although preoperative BMSi values were not correlated with postoperative BMSi values, preoperative BMSi were negatively associated with delta BMSi values, implying that participants with higher preoperative BMSi values experienced smaller increase or decrease in BMSi values after RYGB. Individual difference in BMSi response to intervention should be emphasized in future studies.

4.3. Bone material strength and type 2 diabetes

Studies have shown that subjects with T2D have lower bone material strength compared to controls [12,13]. We did not observe any difference between preoperative BMSi values, or changes of bone material strength after RYGB in participants with and without T2D. Nevertheless, the association between the decrease in HbA_{1c} and the improvement in bone material strength, observed in participants with T2D supports the notion that glucose control influences bone health. A larger sample size might have identified relevant differences not identified in our series.

4.4. Bone mineral density changes after RYGB

RYGB induces a large and rapid weight loss that is accompanied by an increased bone turnover and reduction in bone mineral density. Our findings are in line with other evaluations of bone turnover and aBMD

after RYGB [18–24]. Notably, studies comparing changes in volumetric BMD (QCT) and aBMD (DXA) after bariatric surgery may be affected be technical reproducibility issues that in part may be related to imaging artifacts in the setting of morbid obesity and large weight loss [18,21,22]. Earlier studies comparing aBMD changes after RYGB in participants with and without T2D have been conflicting. In their pilot study, Schafer et al showed a non-significantly larger aBMD decrease in total hip in participants with T2D than controls six months after RYGB [19]. However, in the final study, with a 2 fold higher number of participants, this finding was not reproduced. Actually, they noted that T2D participants had a smaller femoral neck aBMD loss than participants without T2D, with similar trends in the lumbar spine and total hip [23]. Our study is the first to compare aBMD changes in participants with and without T2D one year after RYGB, and we observed comparable changes in aBMD. Our study diverges from previous studies as both men and women are included.

4.5. Long-term bone quality changes after RYGB

The presented improvements in bone material strength are based on early findings post RYGB. Studies evaluating participants in the years following weight stabilization have noted persistent elevated bone turnover markers, continued bone loss, and estimated failure load decrease [20,24], corresponding to an increased fracture rate observed by recent studies [25–27]. In light of this knowledge, it is unlikely that bone material strength continues to increase in the years following weight stabilization. However, this is beyond the scope of our study and remains to be further explored.

4.6. Strengths and limitations

Strengths of our study include the use of in vivo measurement of tissue level bone material strength of cortical bone, and a low rate of participants lost to follow-up. However, the study is limited by the restricted duration of follow-up, limited number of participants, and lack of a control group. The evaluation of unopposed penetration of tissue overlying the bone surface was based on tactile sensations of the investigator. The introduction of a new DXA machine during the study could affect the aBMD results, albeit probably insignificantly, as proper cross calibration was performed. Twenty-three of the participants had their preoperative examination on the GE Lunar Prodigy and the follow-up with the GE Lunar iDXA, and eleven had both with the iDXA. Patients with diabetes were encouraged to participate in the study, thus the fraction of participants with diabetes in this study (38%) exceeds the fraction in patients seeking RYGB (25–30 %) at our institution [36].

5. Conclusion

In conclusion, our study shows that a higher BMI is associated with a lower bone material strength in a morbid obese population before RYGB. One year after RYGB, we observed improved bone material strength despite, an increase in bone turnover and decrease in aBMD. Bone changes were comparable in participants with and without T2D, however, improved glucose control was associated with improved bone material strength in participants with T2D.

Declaration of Competing Interest

Blom-Høgestøl IK: Nothing to declare
Mala T: Nothing to declare
Kristinsson JA: Nothing to declare
Brunborg C: Nothing to declare
Gulseth HL: Nothing to declare
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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.bone.2019.115069.

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