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Full Length Article

Bone metabolism, bone mineral density and low-energy fractures 10 years after Roux-en-Y gastric bypass

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ABSTRACT

Background: Roux-en-Y gastric bypass (RYGB) is a common surgical procedure for treatment of morbid obesity. RYGB induces considerable and sustained weight loss, and remission of obesity related-comorbidities. While studies have suggested negative effects of RYGB on bone health, long-term data are lacking. We aimed to evaluate the prevalence of aBMD below the expected range for age, osteopenia, osteoporosis and low-energy fractures in a defined patient cohort 10 years after RYGB. Secondly, we wanted to identify factors associated with increased risk of aBMD z-score or t-score of −1.1 or lower 10 years after RYGB.

Methods: Patients undergoing RYGB surgery from June 2004 to December 2006 at the Department of Morbid Obesity and Bariatric Surgery, Oslo University Hospital, a tertiary referral centre for treatment of morbid obesity, were invited to a 10 year follow-up. Follow-up visits included morning fasting blood samples, clinical examination, anthropometric measures and dual energy X-ray absorptiometry (DXA).

Results: Out of 194 patients eligible for the study, 124 attended the 10 year follow-up and 122 (63%) were examined with DXA. Mean (SD) age was 50.3 (9.0) years, 118 (97%) were of Caucasian ethnicity, 94 were females (77%), of whom 41 (44%) were postmenopausal.

Secondary hyperparathyroidism (SHPT) was noted in 37 participants (31%) and vitamin D deficiency (value below 50 nmol/L) and insufficiency (value below 75 nmol/L) in 40 (33%) and 91 (75%), respectively. Among the 63 participants who were premenopausal females or males 49 years or younger the prevalence of areal bone mineral density (aBMD) in the lower range of normal (z-score −1.1- to −1.9) was 30% (n = 19) and aBMD below the expected range for age (z-score ≤ −2.0) was noted in 8% (n = 5). Among the 59 participants who were postmenopausal females or males 50 years or older, the prevalence of osteopenia (t-score −1.1 to −2.4) was 51% (n = 30) and osteoporosis (t-score ≤ −2.5) was 27% (n = 16). The bone resorption markers CTX-1 and PINP were higher in participants with aBMD z-score or t-score of −1.1 or lower compared to participants with aBMD z-score or t-score of −1.0 or higher. Preoperative hypothyroidism, or higher age, postmenopausal status, BMI < 35 kg/m^2, SHPT or higher PINP levels at 10 year follow-up were independently associated with aBMD z-score or t-score of −1.1 or lower 10 years after RYGB. Eighteen participants (15%) reported a clinical low-energy fracture after RYGB. In addition, vertebral fracture assessment by DXA revealed that 10 participants (8%) had experienced at least one moderate to severe morphometric vertebral fracture.

Conclusion: Ten years after RYGB 27% of postmenopausal females and males 50 years or older were osteoporotic, and 8% of premenopausal females and males 49 years or younger exhibited aBMD below the expected range for age. The prevalence of fragility fractures was high. SHPT, higher age, postmenopausal status or higher

Abbreviations: aBMD, Areal bone mineral density; BALP, Bone specific alkaline phosphatase; CKD, Chronic kidney disease; CTX-1, Carboxyl terminal telopeptide of type 1 collagen; eGFR, estimated glomerular filtration rate; FSH, Follicular stimulating hormone; DXA, Dual energy X-ray absorptiometry; PINP, Procollagen type 1 N-terminal propeptide; RYGB, Roux-en-Y gastric bypass; SHPT, Secondary hyperparathyroidism; T2D, Type 2 diabetes; TSH, Thyroid stimulating hormone; 25-hydroxyvitamin D, 25(OH) vitamin D

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PINP levels at 10 years and preoperative hypothyroidism were all independent risk factors for aBMD z-score or t-score of −1.1 or lower 10 years after RYGB.

1. Introduction

Laparoscopic Roux-en-Y gastric bypass (RYGB) surgery is a widely applied surgical technique for treatment of morbid obesity following failed attempts of sustained weight loss [1]. RYGB induces considerable and sustained weight loss, remission of obesity related comorbidities and reduces mortality [2–4]. However, changes to gastrointestinal anatomy and physiology may pose negative effects on bone health.

Shortly after RYGB, increased levels of bone turnover markers and reduced bone mineral density, assessed by dual energy X-ray absorptiometry (DXA) or quantitative computed tomography, have been noted [5–8]. Persistent elevation of serum bone turnover markers and continuous reductions in areal bone mineral density (aBMD) following weight stabilization indicate effects of RYGB on bone metabolism beyond that of adaptation to a reduced weight [9]. In line with the high turnover state increased fracture rates have been reported in several studies [10,11]. The high turnover state after RYGB is probably multifactorial. The high prevalence of secondary hyperparathyroidism (SHPT) [7,12], likely contributes to this state of high bone turnover.

These findings emphasize the need for long-term evaluations of bone health after RYGB. Unfortunately studies describing bone health five to 10 years after RYGB are few, characterised by small samples of females only, and with low or undefined rates of follow-up [13,14].

We aimed to evaluate the prevalence of aBMD below the expected range for age, osteopenia, osteoporosis and low-energy fractures in a defined patient cohort 10 years after RYGB. Secondly, we wanted to identify factors associated with an increased risk of aBMD z-score or t-score of −1.1 or lower 10 years after RYGB. We hypothesized that SHPT would be associated with an increased risk of aBMD z-score or t-score of −1.1 or lower 10 years after RYGB.

2. Methods

2.1. Study population

Patients undergoing RYGB surgery between June 2004 and December 2006 at the Department of Morbid Obesity and Bariatric Surgery, Oslo University Hospital, a tertiary referral centre for treatment of morbid obesity, were invited to a 10 year follow-up. Eligibility criteria for RYGB were body mass index (BMI) $\geq 40\ kg/m^2$ or BMI $\geq 35\ kg/m^2$ with obesity-related comorbidity, age between 20 and 60 years, and failed attempts of sustained weight loss. Results from the five year follow-up have been published previously [15]. Ten year follow-up visits were conducted from June 2016 to March 2018.

2.2. Surgery, follow- up and study visits

Prior to surgery all patients had one or more individual consultations with a nurse, a dietician and a surgeon. If indicated they also had consultations with an internist or a psychologist. Preoperative data were registered on predefined forms. Until December 2005 preoperative data were registered retrospectively, with subsequent prospective registration. Patients underwent a laparoscopic RYGB with a gastric pouch of about 25 mL, a 150 cm antecolic alimentary limb and a 50 cm biliopancreatic limb [16]. Postoperative routine clinical follow-up visits were conducted six-eight weeks, six months, one, two and five years after surgery. After surgery all patients were prescribed oral supplementation therapy consisting of daily doses of calcium carbonate (500 mg × 2 daily), vitamin D3 (400IU × 2 daily), Nycoplus multivitamin – Nycomed® (one tablet), iron (100–200 mg) as well as vitamin B12 injections (1 mg) every three months. At routine clinical visits, vitamin levels were monitored and additional supplements were recommended if appropriate.

Ten year follow-up visit included morning fasting blood samples, clinical examination, anthropometric measures and DXA. All, but four, 10 year follow-up visits were performed by one clinician (SH). Data concerning comorbidity, medications, nutritional supplements, menstrual status and fracture history were recorded in a predefined case report form.

DXA scans were performed for assessment of lumbar spine (L_1-L_4), hip, proximal femur and total body aBMD and body composition (including percent body fat). Vertebral fracture assessment by DXA was evaluated for the presence of vertebral fractures. All DXA scans were performed by the same nurse. GE Lunar Prodigy was used until August 26, 2016 when it was replaced by GE Lunar iDXA. The two DXA scanners were cross-calibrated by scanning 16 volunteers with both machines and revealed lumbar spine (L1-L4) intra-class correlation coefficient (ICC) (95% CI) of 0.989 (0.968 to 0.996), and for femoral neck and total hip, ICC (95% CI), was 0.994 (0.982 to 0.998) and 0.996 (0.988 to 0.999), respectively. Furthermore, studies have shown excellent *in vivo* precision of the GE Lunar iDXA for the measurement of lumbar spine, hip and total body aBMD in adults with normal and obese BMI [17,18]. Our DXA machine is calibrated daily against the standard calibration phantom, supplied by the manufacturer, with estimated short-term precision errors of < 1.0% for aBMD at the lumbar spine and at the femoral neck.

Blood samples were drawn before 10 am after an overnight fast, centrifuged and analyzed shortly after retrieval. The Hormone Laboratory, Oslo University Hospital analyzed: thyroid stimulating hormone (TSH) using a non-competitive immunofluorometic method from DELFIA (reference range *TSH mLU/L*: 0.5–3.6). Carboxyl terminal telopeptide of type 1 collagen (CTX-1) and procollagen type 1 N-terminal propeptide (PINP) using Roche® electrochemiluminescence immunoassay (ECLIA) (reference ranges based on information from the manufacturer; *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 and *PINP µg/L*: females > 25 years: 11–94, males > 25 years: 20–91). Bone specific alkaline phosphatase (BALP) and osteocalcin using LIAISON® chemiluminescence immunoassay (CLIA) (reference range based on information from the manufacturer; *BALP* µg*/L:* 5.5–24.6, *osteocalcin nmol/L*: females ≥21 years: 1.5–5.4, males ≥21 years 1.6–4.3 [19]). Serum 25-hydroxyvitamin D (25(OH) vitamin D) levels were analyzed by liquid chromatography-mass spectrometry (LC-MS/MS) method, Calcitriol ($1.25(OH)_2D3$) vitamin D with enzymatic immunoassay from IDS Nordic (reference range $1.25(OH)_2D3$ *pmol/L*: 39–193). Serum parathyroid hormone (PTH) by Immulite 2000 XPI, Siemens Healthineers a chemiluminoimmunometric assay (reference range *PTH pmol/L*: 1.5–7.0). Serum ionized calcium using Roche® Cobas b221 (reference range *ionized calcium mmol/L*: 1.15–1.33). FSH was analyzed using Immulite 2000 XPI, Siemens Healthineers, a non-competitive immunluminometric assay. The coefficient of variance for CTX-1, PINP, osteocalcin, 25(OH) vitamin D and PTH is 5%, 5%, 6%, 11% and 7%, respectively.

The Central Laboratory of Oslo University Hospital analyzed HbA_{1c} using Tosoh G8 high-performance liquid chromatography and calculated estimated glomerular filtration rate (eGFR) using CKD-EPI formula (*mL/*min*/1.73m²*); were categorized into the following stages of chronic kidney disease (CKD): CKD stage I: > 90, CKD stage II: 60–90, CKD stage III: 30–59, stage IV: 5–29, stage V: < 15.

2.3. Outcomes

2.3.1. Osteopenia, osteoporosis and aBMD below expected range for age

Areal BMD t-scores represent the number of standard deviations an actual aBMD deviates from the peak bone mass of young women. The aBMD z-score represents the number of standard deviations an actual BMD deviates from the expected aBMD of age, gender and ethnicity. Both scores are based on the reference population from the NHANES and Lunar studies given by the manufacturer. T-scores and *Z*-scores of the lumbar spine L1-L4 were calculated after exclusion of vertebrae with osteoarthritic changes (spondylosis) or compression fractures. The percent estimates of aBMD z-score were referred to as percent of expected aBMD.

In order to explore aBMD outcomes the population was split according to gender, age and menopausal status:

i. Premenopausal females and males 49 years or younger

aBMD in the higher range of normal: 1.0 standard deviations lower than the expected aBMD of age, gender and ethnicity or higher (z-score > -1.0).

aBMD in the lower range of normal: 1.1 to 1.9 standard deviations lower than the expected aBMD of age, gender and ethnicity (z-score < -1.0 to > -2.0).

aBMD below expected range for age: 2.0 standard deviations lower than the expected aBMD of age, gender and ethnicity (z-score ≤ -2.0) [20].

ii. Postmenopausal females and males 50 years or older [21]:

Normal aBMD: 1.0 standard deviations lower than the peak bone mass of young women or higher (t-score > -1.0).

Osteopenia: 1.1 to 2.4 standard deviations lower than the peak bone mass of young women (t-score < -1.0 to > -2.5).

Osteoporosis: aBMD 2.5 standard deviations or more lower than the peak bone mass of young women (t-score ≤ -2.5).

2.3.2. Fractures

Vertebral fractures were assessed on lateral X-rays recorded on the DXA scanner and by visual semiquantitative technique (VFA). Moderate (reduction of vertebral height of > 25–40%) and severe (reduction of vertebral height of > 40%) vertebral fractures are reported [22]. Vertebral fractures revealed by DXA are referred to as morphometric vertebral fractures.

Table 1
Participant characteristics 10 years after Roux-en-Y gastric bypass (RYGB).

	All n = 122	Premenopausal females and males younger than 50 years of age n = 63	Postmenopausal females and males 50 years or older n = 59	p-Value
Caucasian, n	118 (97%)	62 (98%)	56 (95%)	0.47
Age, years	50.3 ± 9.0	44.2 ± 6.7	56.8 ± 6.1	< 0.001
Body mass index, kg/m^2	35.6 ± 7.2	37.4 ± 7.0	33.6 ± 6.6	0.003
% fat mass	45.4 ± 7.8	46.4 ± 7.2	44.2 ± 7.1	0.043
% total weight loss after RYGB	24.6 ± 13.6	22.6 ± 11.5	26.8 ± 15.4	0.093
Loss of height, cm	1.5 ± 1.9	0.91 ± 1.6	2.1 ± 2.0	0.001
First degree relative with osteoporosis	22 (18%)	8 (13%)	14 (24%)	0.11
First degree relative with hip fracture	17 (14%)	6 (10%)	11 (19%)	0.15
TSH, mlU/L	1.7 (< 0.001–7.4)	1.5 (< 0.001–7.4)	1.9 (0.2–4.8)	0.026
eGFR, mL/min/1,73m^2	98.4 ± 16.0	104.8 ± 12.8	91.5 ± 16.4	< 0.001
Ionized calcium, mmol/L	1.2 ± 0.039	1.2 ± 0.032	1.2 ± 0.045	0.70
25(OH) vitamin D, nmol/L	60.0 ± 22.9	56.1 ± 21.8	64.2 ± 23.5	0.052
1.25(OH)$_2$D3, pmol/L	135.1 ± 41.9	139.0 ± 38.9	133.2 ± 41.6	0.44
PTH, pmol/L	5.9 ± 2.8	5.9 ± 2.4	5.9 ± 3.2	0.96
CTX-1, µg/L	0.48 ± 0.24	0.41 ± 0.18	0.54 ± 0.27	0.002
PINP, µg/L	55.4 ± 26.3	58.6 ± 28.5	52.1 ± 23.5	0.19
BALP, µg/L	14.3 ± 5.4	14.0 ± 5.5	14.6 ± 5.3	0.51
Osteocalcin, nmol/L	4.1 ± 1.2	3.9 ± 1.1	4.2 ± 1.4	0.22
Areal bone mineral density				
Lumbar spine (L1-L4), g/cm^2	1.17 ± 0.16	1.21 ± 0.14	1.12 ± 0.17	0.001
Femoral neck (left), g/cm^2	0.92 ± 0.13	0.97 ± 0.11	0.86 ± 0.13	< 0.001
Total hip, g/cm^2	0.97 ± 0.16	1.02 ± 0.13	0.93 ± 0.18	0.002
Total body, g/cm^2	1.17 ± 0.15	1.22 ± 0.12	1.11 ± 0.16	< 0.001
T-score				
Lumbar (L1-L4)[a]	−0.9 ± 1.3	−0.55 ± 1.17	−1.3 ± 1.34	0.001
Femoral neck (left)	−0.8 ± 0.98	−0.36 ± 0.85	−1.19 ± 0.96	< 0.001
Total hip	−0.4 ± 1.1	0.07 ± 1.01	−0.79 ± 1.09	< 0.001
Total body	0.64 ± 1.4	1.19 ± 1.2	0.016 ± 1.3	< 0.001

The results are given as number (proportion in per cent) for categorical variables, mean (SD) for continuous variables with normal distribution and median (range) for other variables ≥ .

Loss of height is the difference between the measured preoperative height and the measured height at 10 year follow-up.

Abbreviations; Thyroid stimulation hormone (TSH), estimated glomerular filtration rate (eGFR), 25-hydroxyvitamin D (25(OH) vitamin D), Calcitriol (1.25(OH)$_2$D3), parathyroid hormone (PTH), carboxyl terminal telopeptide of type 1 collagen (CTX-1), procollagen type 1 N-terminal propeptide (PINP), bone specific alkaline phosphatase (BALP).

Missing (n): FSH (4), % total weight loss (3), TSH (2), eGFR (1), ionized calcium (2), 1.25(OH)$_2$ vitamin D (5), PTH (1), CTX-1 (4), PINP (5), BALP (7), Osteocalcin (10), aBMD lumbar spine (1), aBMD left femoral neck (6), aBMD total hip (5), aBMD total body (3).

Reference ranges: *TSH mlU/L*: 0.5–3.6; *eGFR (mL/min/1,73 m2)*: Stages of chronic kidney disease (CKD): CKD stage I: > 90, CKD stage II: 60–90, CKD stage III: 30–59, stage IV: 5–29, stage V: < 15; *ionized calcium mmol/L*: 1.15–1.33; *25(OH) vitamin D nmol/L*: 37–132; *1.25(OH)$_2$D3 pmol/L*: 39–193; *PTH pmol/L*: 1.5–7.0; *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; *BALP µg/L:* 5.5–24.6; *osteocalcin nmol/L*: Females ≥21 years: 1.5–5.4, males ≥21 years 1.6–4.3.

[a] Vertebrae with osteoarthritic changes (spondylosis) or compression fractures were excluded from calculation.

Clinical fractures were self-reported and registered in predefined case report forms. A low energy fracture was defined as a fracture resulting from minimal trauma quantified as forces equivalent to a fall from standing height or less [21]. All low energy fractures, except digital fractures, acquired during the 10 years after RYGB are reported.

2.3.3. Calciotropic hormones and supplements

Vitamin D deficiency was defined as serum 25(OH) vitamin D levels below 50 nmol/L and insufficiency as levels below 75 nmol/L [23,24]. Seasons were defined as; Summer: June to August, fall: September to November, winter: December to February and spring: March to May. 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. Intake of vitamin supplements was self-reported. Patients were classified as taking calcium and vitamin D supplements if they reported intake of 1000 mg and 800 IU, respectively, or more, at least five days a week.

2.3.4. Menopausal status

Hormonal intrauterine devices made clinical evaluation of menstrual cycle challenging. For this reason a postmenopausal status was defined as a serum follicle stimulating hormone (FSH)of > 25 IU/L or higher [25].

2.3.5. Comorbidities

Preoperative type 2 diabetes (T2D) was defined as $HbA_{1c} \geq 6.5\%$ and/or the use of one or more oral glucose lowering drugs with or without the use of insulin. Preoperative hypothyroidism was based on information given by the participant preoperatively, stated in referrals or based on use of levothyroxine substitution treatment.

2.4. Statistical analysis

Normally distributed continuous variables are presented as mean ± SD, others are presented as median (range). Categorical data are reported as proportions (percentage). Subgroups were compared with an independent sample *t*-test, the Chi Square test or Fisher exact test as appropriate. Intra-class correlation coefficient (ICC) with 95% CI was used to assess concordance between the two DXA scanners (GE Lunar Prodigy and GE Lunar iDXA). ICC values of 0.75 or higher were considered excellent [26]. One way-ANOVA was used to explore possible seasonal differences in 25(OH) vitamin D values. To explore the association between PTH and aBMD z-scores, linear regression analyses were applied. The results from the linear regression analyses are presented as regression coefficients (β) with 95% CI.

Logistic regression analyses were performed to identify factors associated with aBMD z-score or t-score of −1.1 or lower 10 years after RYGB. All variables with a $p < 0.25$ in the univariable analysis were entered into the multivariable logistic regression model using a manual backward stepwise elimination procedure. Multivariable analyses were preceded by estimation of correlation between risk factors. Predictors that correlated > 0.7, were not included in the model in order to avoid multicollinearity. The associations between factors and aBMD z-score or t-score of −1.1 or lower 10 years after RYGB at 10 year follow-up visit were quantified by calculating odds ratios (OR) with 95% CI. In linear regression analysis all continuous variables were checked for deviation from normality, non-linear effects, multicollinearity, and homoscedasticity. For logistic regression analysis they were checked for deviation of linearity of the logit and multicollinearity. Evaluation of the models predictive accuracy was assessed by calibration (Hosmer and Lemeshow goodness-of-fit test) and by the discriminatory capability (area under the ROC curve). Two tailed *p*-values < 0.05 were considered statistically significant. All statistical analyses were performed using the IBM SPSS statistics version 25.0 (IBM SPSS Inc., Armonk, NY: IBM Corp).

2.5. Ethics

The study was conducted in accordance with the Declaration of Helsinki and approved by the Regional Committee for Medical and Health Research Ethics South-East Norway; 2015/142. Written informed consent was obtained from all participants. Preoperative data were registered in a database licensed by the Norwegian Data Inspectorate.

3. Results

Nine out of 203 patients died prior to the 10 year follow-up; thus 194 were eligible for the study. Of these, 124 attended the 10 year follow-up, and 122 (63%) were examined with DXA scans and were included in this study.

3.1. Participant characteristics

Participant characteristics are presented in Table 1. Mean age was 50.3 (SD 9.0) years, 118 (97%) were of Caucasian ethnicity. There were 94 females (77%), of whom 41 (44%) were postmenopausal. Of the 122 participants 63 (52%) were premenopausal females or males 49 years or younger, and 59 (48%) were postmenopausal females or males 50 years or older.

At 10 year follow-up, five participants received bone specific treatment for osteoporosis (oral bisphosphonates (2), intravenous bisphosphonates (2) or denosumab (1): one male older than 50 years of age and four postmenopausal females, they were all classified as osteoporotic). These participants had lower CTX-1 levels, 0.23 ± 0.11 μg/L, compared to 0.49 ± 0.23 μg/L for the remaining cohort, p = 0.034, but their PINP levels of 51.0 ± 14.7 μg/L were comparable to 55.6 ± 26.6 μg/L for the remaining cohort, p = n.s. Three participants reported treatment for rheumatoid arthritis, one had been treated for prostate cancer and one had undergone surgery for primary hyperparathyroidism. No participants had been diagnosed with breast cancer or Cushing's disease. Twenty-five participants (21%) had CKD stage II and three had CKD stage III (3%), no participant had CKD stage IV or higher. At the 10 year follow-up seven participants presented with low TSH levels (< 0.5 mLU/L), five had known preoperative hypothyroidism, while two had no known thyroid condition.

3.2. Calcium and calciotropic hormones

A total of 29 participants (24%) reported regular intake of calcium supplements and 38 (31%) reported regular intake of vitamin D supplements. Individual measurements of serum ionized calcium, PTH and 25(OH) vitamin D are shown in Fig. 1. Forty participants (33%) were vitamin D deficient and 91 (75%) insufficient, respectively. Among the participants reporting regular intake of vitamin D supplements, five (12.5%) had vitamin D deficiency, a significantly lower fraction when compared to participants not taking supplements where 33 participants (41%) were deficient, p = 0.002. 25(OH) vitamin D levels drawn during summer, fall, winter and spring season were 61.9 ± 20.5, 65.4 ± 22.4, 52.8 ± 24.4 and 56.8 ± 22.9, respectively. No statistical difference was noted between the 25(OH) vitamin D levels of the different seasons.

No participant had serum ionized calcium or 25(OH) vitamin D levels above the reference range. SHPT was noted in 37 participants (31%). Participants with SHPT exhibited numerically lower levels of 25(OH) vitamin D compared to those without SHPT, 54.0 ± 3.9 and 62.7 ± 2.4, respectively, p = 0.055.

3.3. Markers of bone turnover

Mean values for the bone turnover markers; CTX-1, PINP, BALP and osteocalcin, are given in Table 1 and individual measurements are

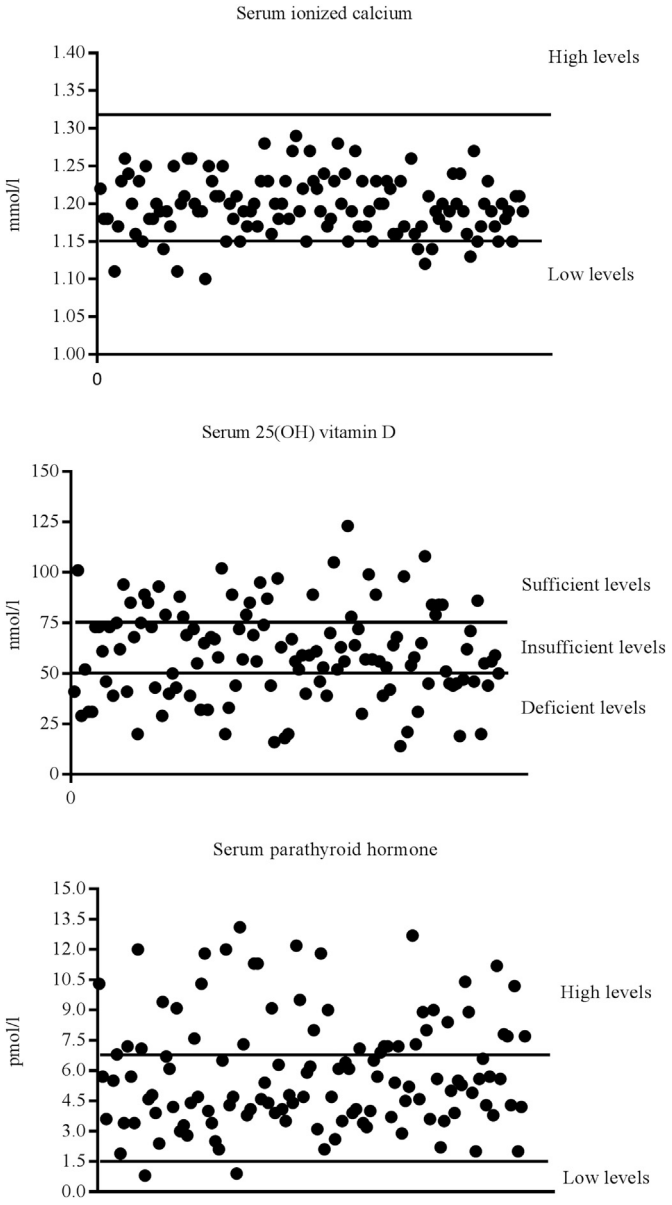

Fig. 1. Individual measurements of serum ionized calcium, parathyroid hormone and 25(OH) vitamin D in 122 participants 10 years after Roux-en-Y gastric bypass. Reference range is marked with lines.
Reference ranges: Serum ionized calcium mmol/L: 1.15–1.33; 25(OH) vitamin D nmol/L: 37–132; PTH pmol/L: 1.5–7.0.

presented in Fig. 2. Participants with an aBMD z-score or t-score of −1.1 or lower 10 years after RYGB revealed higher levels of CTX-1 and PINP compared to participants with an aBMD z-score or t-score of −1.1 or lower 10 years after RYGB. However, only the association with PINP remained significant in the multivariable analysis (Table 2).

3.4. Bone mineral density

The percent estimates of aBMD z-scores, relative to expected aBMD of age, gender, and ethnicity, were below 90% in 35%, 43% and 27% of participants, in the total hip, femoral neck and lumbar spine, respectively (Fig. 3). An inverse association was noted between PTH and aBMD z-score of femoral neck; β −0.051 95% CI (−0.099 to −0.002), p = 0.040, while the association with lumbar spine and total hip aBMD z-score were non-significant (Fig. 4).

3.4.1. Osteopenia, osteoporosis and aBMD below expected range for age

3.4.1.1. Premenopausal females and males 49 years or younger. Of the 63 participants who were premenopausal females or males 49 years or younger, 5 (8%) revealed aBMD below the expected range for age (z-score ≤ −2.0) and 19 (30%) had osteopenia (z-score −1.1- to −1.9) of the spine, femoral neck or total hip 10 years after RYGB.

3.4.1.2. Postmenopausal females and males 50 years or older. Of the 59 participants who were postmenopausal females or males 50 years or older 16 (27%) had osteoporosis (t-score ≤ −2.5) and 30 (51%) had aBMD in the osteopenic range (t-score −1.1 to −2.4) of the spine, femoral neck or total hip 10 years after RYGB.

The prevalence of aBMD below the expected range for age and/or osteoporosis in the different skeletal locations (spine, femoral neck and total hip) are presented in Table 3. Of the total cohort 70 (57%) participants exhibited an aBMD z-score or t-score of −1.1 or lower. Preoperative hypothyroidism, or higher age, postmenopausal status, BMI < 35 kg/m^2, higher PINP value or SHPT at 10 year follow-up were all independent risk factors for aBMD z-score or t-score of −1.1 or lower 10 years after RYGB 10 years after RYGB (Table 2). The Hosmer-Lemeshows goodness of fit test for the multivariable model indicated a satisfactory fit of the model (p = 0.35). The discriminatory capability (area under the ROC) between participants with and without a z-score or t-score of −1.1 10 years after RYGB was 0.81 (95% CI:0.73–0.89). When exploring risk factors for aBMD below the expected range for age, osteoporosis and/or fragility fractures (low energy fracture and morphometric vertebral fractures) only age was detected as a risk factor (Supplementary Table 1).

3.5. Fractures

Clinical low-energy fractures after RYGB were reported by 18 participants (15%) during the 10 years of follow-up, seven (11%) premenopausal females or males 49 years or younger and 11 (19%) postmenopausal females or males 50 years or older. Lower limb fractures were the most prevalent, followed by rib fractures (Table 4). The mean duration from RYGB to first low-energy fracture was 8.4 years ± 1.8. Participants with osteoporotic aBMD had a significantly higher prevalence of low energy fractures when compared to participants with normal or osteopenic aBMD (p = 0.023). Vertebral fracture assessments of DXA revealed that three male (11%) and seven female (7%) participants had experienced one or more moderate to severe morphometric vertebral fractures. Of these 10 participants, one had presented with symptoms of a vertebral fracture.

4. Discussion

4.1. Areal bone mineral density below the expected range for age and osteoporosis

Among premenopausal females and males 49 years or younger the prevalence of aBMD below expected range for age was 8% and among the postmenopausal females and males 50 years or older the prevalence of osteoporosis was 27%. Duran et al. observed osteoporosis (t-score ≤ −2.5) in 13% in a study of 30 females, with a median age of 46 years, eight years after RYGB [13]. However, without knowing the prevalence of postmenopausal status it is difficult to directly compare this study to ours. The Tromsø study, a Norwegian population based study, reported a prevalence of osteoporosis of the femoral neck of 4.8% in males and 6.1% in females for subjects aged 50–69 years in contrast to our findings of 13% and 33%, respectively [27]. Furthermore, we noted that more than one in three had aBMD measures below 90% of expected for age, gender and ethnicity. Collectively, our findings suggest that RYGB may be a risk factor for aBMD below expected range for age and osteoporosis.

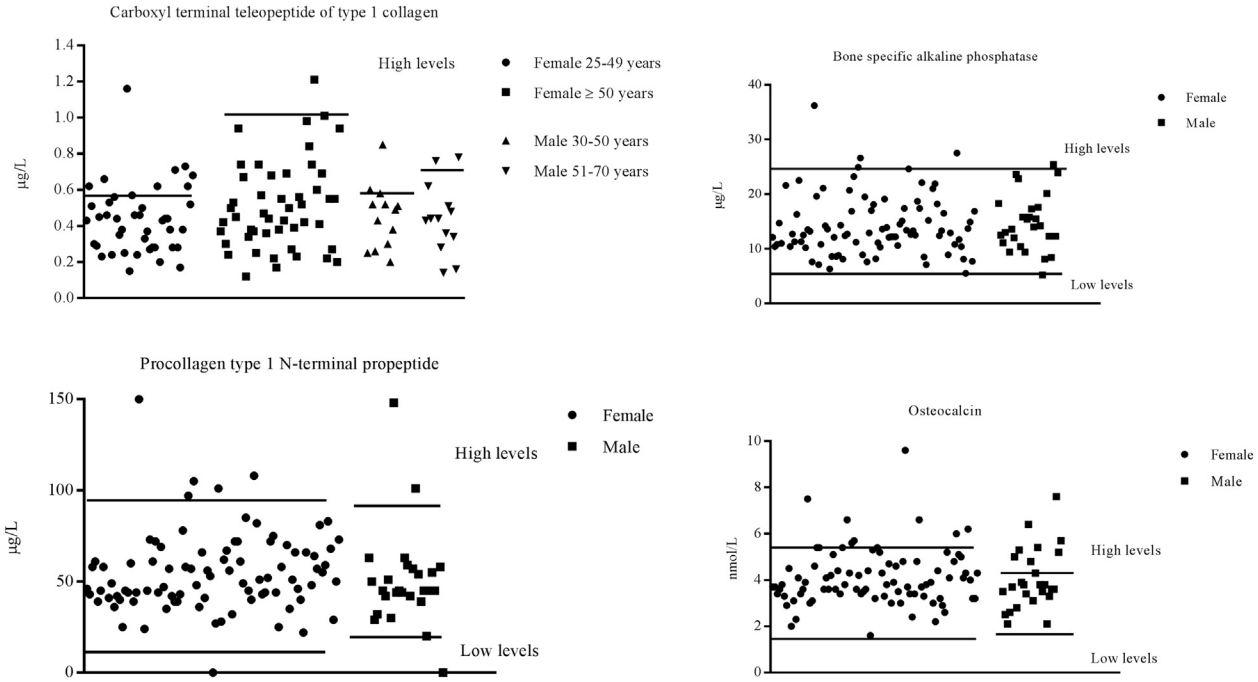

Fig. 2. Individual measurements of bone turnover markers in 122 participants 10 years after Roux-en-Y gastric bypass. Reference range is marked with lines.
Reference ranges: Carboxyl terminal telopeptide of type 1 collagen (CTX-1), µg/L: females 25–49 years: ≤ 0.57, ≥50 years: ≤1.01, males 30–50 years: ≤ 0.58, 51–70 years: ≤ 0.7; procollagen type 1 N-terminal propeptide (PINP), µg/L: females > 25 years: 11–94, males > 25 years: 20–91; bone specific alkaline phosphatase (BALP), µg/L: 5.5–24.6; osteocalcin, nmol/L: females ≥21 years: 1.5–5.4, males ≥21 years 1.6–4.3.
Missing (n): CTX-1 (4), PINP (5), BALP (7), Osteocalcin (10).

4.2. Bone turnover

We observed higher levels of bone turnover markers (CTX-1 and PINP) than those reported in preoperative cohorts of similar age [6,28,29] indicating elevated bone turnover 10 years after RYGB. Participants with an aBMD z-score or t-score of −1.1 or lower 10 years after RYGB 10 years after RYGB had higher levels of both CTX-1 and PINP, suggesting that longstanding accelerated bone turnover is a central mechanism underlying the development of decreasing aBMD after RYGB. Several studies comparing changes in aBMD and bone turnover markers, have shown that high turnover states increase bone loss and fracture rates [30].

4.3. Mechanisms

The pathophysiology underlying the high rates of an aBMD z-score or t-score of −1.1 or lower 10 years after RYGB is likely multifactorial. We noted that participants with SHPT had approximately three times higher odds for having an aBMD z-score or t-score of −1.1 or lower 10 years after RYGB 10 years after RYGB, and we also observed an inverse association between PTH and aBMD z-scores of the femoral neck. Together these findings support our hypothesis that SHPT cause negative effects on skeletal health after RYGB. Studies have revealed significantly reduced intestinal calcium absorption after RYGB [7,31]. To what extent regular intake of calcium and vitamin D supplements could have prevented SHPT in our population is beyond the scope of the study. Notably, the standard recommendation of calcium supplementation was in the form of calcium carbonate, in contradiction to recent European and American recommendations of calcium citrate. Furthermore, the standard doses of 800 IU vitamin D3 daily is in line with Norwegian and European guidelines for post-bariatric management [32,33], but are considerably lower than the 3000 IU daily recommended by the American guidelines [24]. Additionally, we found that only one of three participants reported regular intake of the recommended dose of calcium and vitamin D supplements, that vitamin D non-compliance was associated with vitamin D deficiency, and that participants with SHPT had numerically lower 25(OH) vitamin D levels compared to the remaining cohort. Other cohorts have also reported limited compliance in regard to use of recommended supplements after bariatric surgery [34,35].

Participants with preoperative hypothyroidism revealed four times higher odds for having an aBMD z-score or t-score of −1.1 or lower 10 years after RYGB. In adulthood, the thyroid hormone is important for bone mass maintenance and strength [36]. Untreated hypothyroidism is associated with reduced bone turnover and a positive bone balance [37]; which generally protects against bone loss and fracture. Due to simple, reliable biochemical diagnostic tools, patients are quickly diagnosed and put on thyroid substitution therapy. Studies of subjects receiving substitution treatment for hypothyroidism have revealed discordant results regarding whether or not it is associated with a lower aBMD [38,39], but supraphysiological doses of substitution therapy have been shown to exert negative effects on bone in hypothyroid patients [40]. In addition a hyperthyroid state preceding hypothyroidism could have negatively affected bone health in this group. We therefore favor the notion that excessive doses of thyroid hormone or possible hyperthyroidism preceding hypothyroidism, after thyroiditis or surgical/radioiodine treatment of hyperthyroidism, constitute plausible reasons for our findings.

Mechanical loading of bone plays a key role in determining bone mass, strength and size [41,42]. In line with this we noted that participants with BMI < 35 kg/m^2 10 years after RYGB had a doubled risk of having an aBMD z-score or t-score of −1.1 or lower 10 years after RYGB, however this association did not remain significant in the multivariable analysis, indicative of limited importance in the long-term after RYGB. As for the general population, postmenopausal status and older age were associated with an increased risk of having an aBMD z-score or t-score of −1.1 or lower 10 years after RYGB [21]. Our findings are supported by Schafer et al. who described that postmenopausal females had a larger decrease in bone mineral density after RYGB when compared to premenopausal females and males [29]. Older age was the

Table 2
Factors associated with aBMD z-score or t-score −1.1 or lower 10 years after Roux-en-Y gastric bypass (RYGB).

	Univariable				Multivariable	
	aBMD z-score or t-score of −1.0 or higher10 years after RYGB n = 52	aBMD z-score or t-score of −1.1 or lower 10 years after RYGB n = 70	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-Value
Gender, female	39 (75%)	55 (79%)	1.2 (0.52–2.9)	0.64		
Heredity osteoporosis	5 (10%)	17 (24%)	3.0 (1.0–8.8)	**0.044**		
Preoperative						
Hypothyroidism	**4 (8%)**	**18 (27%)**	**4.4 (1.4–14.0)**	**0.012**	**4.1 (1.1–16.3)**	**0.041**
Type 2 diabetes	13 (26%)	21 (34%)	1.5 (0.66–3.4)	0.34		
BMI > 50	19 (37%)	21 (31%)	0.79 (0.37–1.7)	0.55		
10 years after RYGB						
Age, years	**47.4 ± 8.0**	**52.5 ± 9.1**	**1.1 (1.0–1.1)**	**0.002**	**1.1 (1.0–1.1)**	**0.016**
Postmenopausal	**7 (14%)**	**35 (52%)**	**6.7 (2.6–16.9)**	**< 0.001**	**4.7 (1.6–14.1)**	**0.005**
Δ BMI	−10.0 ± 6.6	−11.6 ± 6.7	0.96 (0.91–1.0)	0.18*		
BMI < 35 kg/m^2	20 (39%)	40 (57%)	2.1 (1.0–4.4)	**0.043**		
Loss of height, cm	−1.4 ± 1.9	−1.6 ± 1.9	0.95 (0.78–1.1)	0.57		
Smoking	15 (29%)	26 (37%)	1.5 (0.67–3.2)	0.34		
Muscular or skeletal pain	32 (62%)	47 (67%)	1.3 (0.63–2.8)	0.52		
Secondary hyperparathyroidism	**13 (25%)**	**24 (35%)**	**1.6 (0.72–3.6)**	**0.25**	**2.8 (1.0–7.9)**	**0.045**
25(OH) vitamin D insufficiency	41 (79%)	50 (71%)	0.67 (0.29–1.6)	0.35		
Low-energy fracture after RYGB	7 (13%)	11 (16%)	1.2 (0.43–3.3)	0.73		
CTX-1, μg/L	**0.39 ± 0.17**	**0.54 ± 0.26**	**34.1 (3.9–295)**	**< 0.001**		
PINP, μg/L	**48.1 ± 17.2**	**60.7 ± 30.3**	**1.0 (1.0–1.0)**	**0.016**	**1.0 (1.0–1.1)**	**0.006**

aBMD z-score or t-score of −1.1 or lower 10 years after includes:
i. Premenopausal females and males 49 years or younger
aBMD in the lower range of normal: 1.1 to 1.9 standard deviations lower than the expected aBMD of age, gender and ethnicity (z-score < −1.0 to > −2.0).
aBMD below expected range for age: 2.0 standard deviations lower than the expected aBMD of age, gender and ethnicity (z-score ≤ −2.0) [20].
ii. Postmenopausal females and males 50 years or older [21]
Osteopenia: 1.1 to 2.4 standard deviations lower than the peak bone mass of young women (t-score < −1.0 to > −2.5).
Osteoporosis: aBMD 2.5 standard deviations or more lower than the peak bone mass of young women (t-score ≤ −2.5).
Heredity of osteoporosis was defined as having a first degree relative with osteoporosis diagnosis.
The results are given as number (proportion in per cent) for categorical variables, mean (SD) for continuous variables with normal distribution. Patient characteristics associated with aBMD < −1.0 10 years after RYGB were studied with univariate regression. Any variable associated with $p \leq 0.25$ from the univariable analysis were entered into a multivariable logistic regression model using a manual backward stepwise elimination procedure. In total, 8 potential predictors were examined (*p*-values written in bold). * Δ BMI not included in multivariable analysis due to high degree of association with BMI < 35 kg/m^2 at 10 year.
Abbreviations; Body mass index (BMI), carboxyl terminal telopeptide of type 1 collagen (CTX-1), procollagen type 1 N-terminal propeptide (PINP), bone specific alkaline phosphatase (BALP).
Missing: CTX-1 (4), PINP (5), BALP (7), Osteocalcin (10).
Definitions: Postmenopausal; follicular stimulating hormone > 25 IU/L, 25(OH) vitamin D insufficiency; serum 25(OH) vitamin D < 75.0 nmol/L.
Postmenopausal females are compared to premenopausal females and men.

only independent risk factor for having aBMD below the expected range for age, osteoporosis or a fragility fracture, stressing the importance of screening older subjects in a bariatric population.

4.4. Fractures

Emerging data indicate that obesity is associated with a site specific increased fracture rate [43,44] despite a normal aBMD [45], implicating that obesity is associated with reduced bone quality. A study comparing subjects pursuing bariatric surgery to obese controls revealed an increased fracture rate in the bariatric population, prior to the surgical intervention [46]. To what extent the RYGB surgery may have contributed to the high fracture rate noted cannot be fully answered by our study. However, two years or more after RYGB patients have a 43%

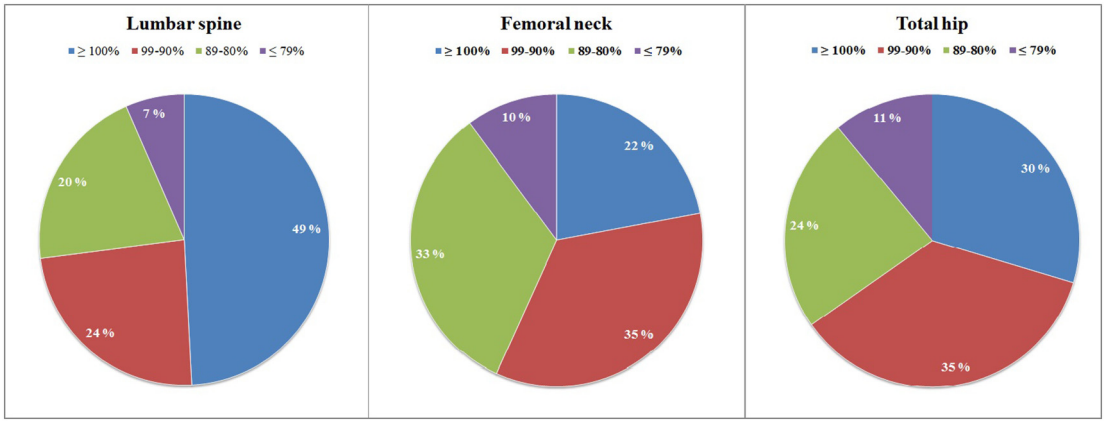

Fig. 3. Percent of expected areal bone mineral density in 122 patients 10 years after Roux-en-Y gastric bypass when matched with a reference population (NHANES and Lunar given by manufacturer GE Lunar) in regard to age, gender and ethnicity.

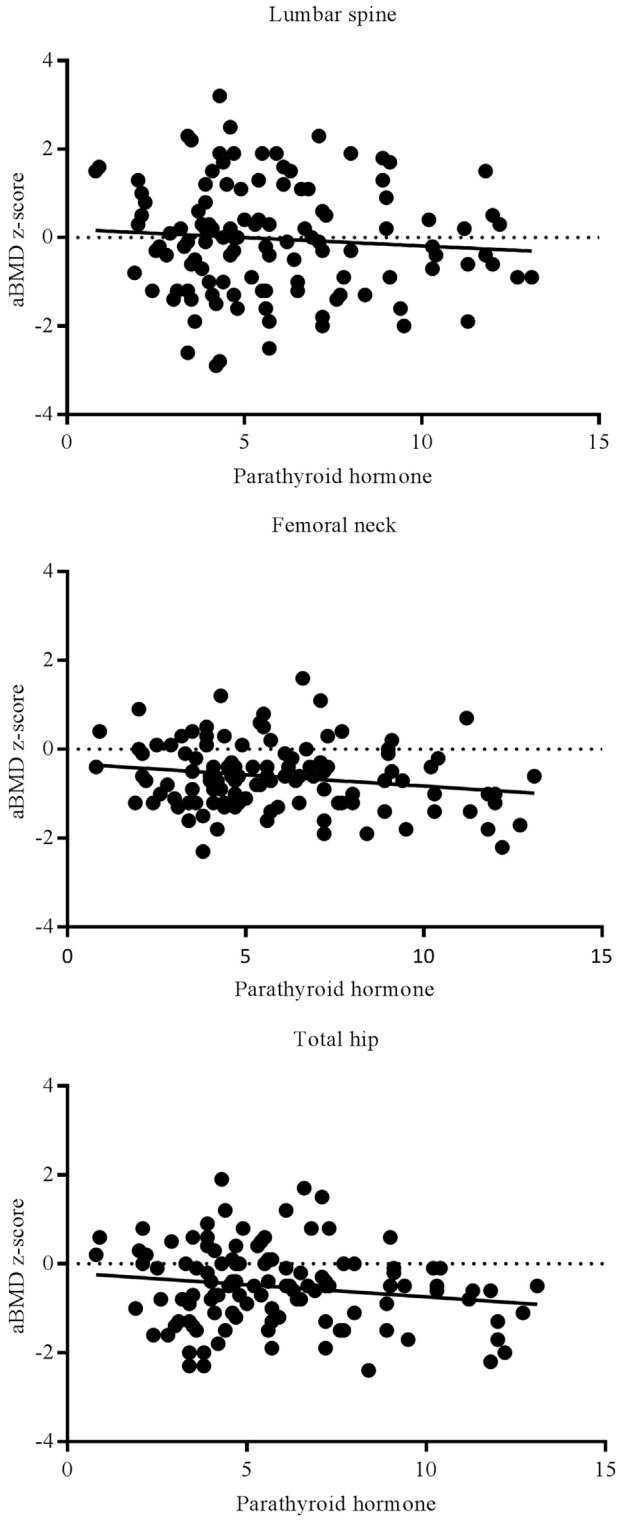

Fig. 4. Inverse association between parathyroid hormone and areal bone mineral density (aBMD) z-scores of lumbar spine (β −0.38 95% CI (−0.12 to 0.044), p = 0.36), femoral neck (β −0.051 95% CI (−0.099 to −0.002), p = 0.040) and total hip (β −0.054 95%CI (−0.11 to 0.005), p = 0.072) in 122 participants 10 years after Roux-en-Y gastric bypass.

higher risk of skeletal fractures compared to subjects after adjustable gastric banding surgery, supporting the notion that RYGB itself may pose a negative effect on bone health [10].

During follow-up 15% of our participants experienced clinical low-energy fractures, similar rates have been reported for subjects exposed

Table 3
Prevalence of areal bone mineral density below the expected range for age or osteoporosis assessed by dual energy X-ray absorptiometry in 122 patients 10 years after Roux-en-Y gastric bypass for subgroups based on menopausal status or age, subdivided for gender.

	Areal bone mineral density below the expected range for age in premenopausal females and males younger than 50 years of age n = 63						Osteoporosis in postmenopausal females and males 50 years or older n = 59					
	Total		Male		Female		Total		Male		Female	
	n	%	n	%	n	%	n	%	n	%	n	%
Any skeletal location	5/63	8%	1/12	8%	4/51	8%	16/59	27%	2/16	13%	14/43	33%
Lumbar spine[a]	3/63	5%	1/12	8%	2/51	4%	11/59	19%	1/16	6%	10/43	23%
Femoral neck	0/61		0/11		0/50		6/57	9%	1/15	7%	5/42	12%
Total hip	2/61	3%	0/11		2/50	4%	5/57	9%	0/15		5/42	12%

Premenopausal females and males younger than 50 years of age: Areal bone mineral density below expected range for age; z-score −2.0 or lower.
Postmenopausal females and males 50 years or older: Osteoporosis; t-score −2.5 or lower.
Missing: Femoral neck and total hip; total n = 4, male younger than 50 years of age (1), male 50 years or older (1), premenopausal female (1), postmenopausal female (1).
[a] Vertebrae with osteoarthritic changes (spondylosis) or compression fractures were excluded from calculation.

to long-term glucocorticoid treatment [47]. In addition 11% of male participants and 7.4% of females had a moderate or severe morphometric vertebral fracture detected by vertebral fracture assessments of DXA. These rates are higher compared to prevalence of 7.5% in males and 3% in females reported in the general Norwegian population (38–59 years) [48]. The presence of morphometric vertebral fractures is a known risk factor for future fracture [49], yielding clinical relevance to our finding. Median duration to low energy fracture post RYGB was more than eight years. Together, our findings indicate an increased risk of fracture 10 years after RYGB and support the need for long term follow-up focusing on bone health.

4.5. Strengths and limitations

Strengths of our study include the large and well defined cohort, the comprehensive clinical examination during individual consultations, clinical interviews according to predefined case report forms, DXA scans and retrieval of bone turnover markers of all participants. The single center design may be a strength of the study as all patients received the same type of surgery, supplementation, counseling, and follow up, but this design may also limit the external validity of our findings.

The study is limited by the lack of preoperative bone specific medical history, bone turnover markers and DXA measurements. Possible effects of changes in neuro-hormonal axis on bone health were not evaluated. A total of 37% of potential participants were lost to follow-up. In the letter inviting patients to participate in the study we informed that the 10 year follow-up would include evaluation of bone health. This might have influenced subjects suspecting bone disease to participate in the study. The introduction of a new DXA machine during the study could have affected the aBMD results. Of the 122 participants, 51 had their examination with the GE Lunar Prodigy and 71 with the GE Lunar iDXA, but proper cross calibration was performed during transition from one machine to another. Additionally, the definition of aBMD z-score of −1.1 to −1.9 as aBMD in the lower range of normal

Table 4
Prevalence of low-energy fractures acquired during 10 years of follow up after Roux-en-Y gastric bypass (RYGB) in 122 participants.

Fracture site	Premenopausal females and males younger than 50 years of age n = 63			Postmenopausal females and males 50 years or older n = 59		
	aBMD higher range of normal n = 39	aBMD in the lower range of normal n = 20	aBMD below expected range for age n = 4	Normal aBMD n = 13	Osteopenia n = 30	Osteoporosis n = 16
Spine	1	1	0	–0	–0	2
Femoral neck/hip	–0	–0	–0	–0	1	1
Rib	–0	–0	–0	–0	1	1
Upper limb	1	–0	–0	–0	–0	2
Lower limb	2	–0	1	1	1	–0
More than one location	1	–0	–0	1	–0	–0
Proportion of participants with clinical low energy fracture after RYGB	*5 (13%)*	*1 (5%)*	*1 (25%)*	*2 (15%)*	*3 (10%)*	*6 (38%)*
Morphometric fractures of spine	*2 (%)*	*3 (15%)*			*3 (10%)*	*2 (13%)*

Definitions: aBMD: areal bone mineral density.
Premenopausal females and males younger than 50 years of age.
aBMD in the higher range of normal: 1.0 standard deviations lower than the expected aBMD of age, gender and ethnicity or higher (z-score > -1.0).
aBMD in the lower range of normal: 1.1 to 1.9 standard deviations lower than the expected aBMD of age, gender and ethnicity (z-score < -1.0 to > -2.0).
aBMD below expected range for age: 2.0 standard deviations lower than the expected aBMD of age, gender and ethnicity (z-score ≤ -2.0).
Postmenopausal females and males 50 years or older:
Normal aBMD: 1.0 standard deviations lower than the peak bone mass of young women or higher (t-score > -1.0).
Osteopenia: 1.1 to 2.4 standard deviations lower than the peak bone mass of young women (t-score < -1.0 to > -2.5).
Osteoporosis: aBMD 2.5 standard deviations or more lower than the peak bone mass of young women (t-score ≤ -2.5).
Upper limb fractures: Distal radius (n = 1), humerus (n = 2).
Lower limb fractures: Proximal tibia (n = 1), ankle (n = 1), tarsal (n = 1), metatarsal (n = 2).
More than one location: Ankle and distal radius (n = 1), carpal bone and costa (n = 1).
Morphometric fractures of spine revealed by vertebral fracture assessments by DXA.

was defined by the authors and is not a part of ICDS guidelines. This new definition may have inflated the number of patients considered to have abnormal aBMD. Lastly, information concerning etiology of hypothyroidism and repeated TSH measurements could have further elaborated the association between an aBMD z-score or t-score of -1.1 or lower 10 years after RYGB and preoperative hypothyroidism.

5. Conclusion

Ten years after RYGB 27% of postmenopausal females and males 50 years or older were osteoporotic, and 8% of premenopausal females and males 49 years or younger exhibited aBMD below the expected range for age. The prevalence of low-energy fractures was high. Hypothyroidism prior to, or SHPT, higher PINP levels, older age or postmenopausal status at 10 year follow-up were all risk factors for aBMD z-score or t-score of -1.1 or lower 10 years after RYGB.

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Authors' roles

Conceptualization; IKBH, TM, SH, MCK, and JAK. Data curation; SH, IKBH and MCK. Formal analysis; IKBH and CB. Funding acquisition; EFE. Investigation; IKBH. Methodology; IKBH. Project administration; MCK, TM, SH, JAK, IKBH and HLG. Supervision; TM, JAK, EFE, HLG, Visualization; IKBH, Writing - original draft; Writing; IKBH, review & editing; IKBH, SH, MCK, HLG, EFE, TM, JAK, CB.

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