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Version of Record: https://www.sciencedirect.com/science/article/pii/S1550728921005736
Manuscript_2f3a37c4c4c62202c0d725d27d30a017

A comparison of changes in bone turnover markers after Gastric Bypass and Sleeve Gastrectomy, and their association with markers of interest

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The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

Abbreviated title: bone turnover markers after bariatric surgery

Word count: 4015

Number of tables and figures: 6

Conflict of interest statement:

Julien Paccou, Dorothée Thuillier, Marion Courtalin, Pascal Pigny, Julien Labreuche, Bernard Cortet and François Pattou declare that they have no conflict of interest.

Funding source:

The authors are employed by their University and/or their hospital. These funding organizations did not suggest the subject of this study, and did not have access to the results before publication. This study received no external funding.

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ABSTRACT

Background: It is still debated whether differences in bone turnover markers (BTMs) exist between the two most popular bariatric surgery procedures (RYGB and SG).

Objectives: To compare changes in BTMs after RYGB and SG, and to investigate their association with pre-defined markers of interest.

Setting: University hospital, Lille, France.

Methods: An ancillary investigation of a prospective cohort was conducted. SG patients with severe obesity ≥ 40 years were matched one-to-one to RYGB patients for age, sex, body mass index (BMI) and menopausal status. BTMs, as well as pre-defined markers of interest, were measured at baseline, 12 and 24 months after bariatric surgery.

Results: Sixty-four patients (66% women) had a mean (SD) age of 49.6 years (5.1) and a mean (SD) BMI of 45.0 kg/m² (6.0). From baseline to 12 months, a significant increase in BTMs was observed in both groups (p-values<0.001). Moreover, RYGB was associated with a greater increase in C-terminal telopeptide (β-CTX) and procollagen type 1 N-terminal propeptide (PINP) compared to SG (p-values<0.0001). From 12 to 24 months, a significant decrease in BTMs was observed in both groups, but no significant differences were found between RYGB and SG. However, BTMs did not return to baseline levels. The changes in PINP and β-CTX at 12 months were independently associated with the type of surgical procedure, after adjusting for weight or each pre-defined marker of interest (all p<0.0001).

Conclusion: RYGB was associated with a greater increase in BTMs than SG at 12 and 24 months.

Keywords: Bone turnover markers; Bariatric surgery; Roux-en-Y gastric bypass; Sleeve gastrectomy; Sclerostin

INTRODUCTION

It is increasingly acknowledged that bariatric surgery adversely affects skeletal health **[1]**. Following bariatric surgery, the extent of high-turnover bone loss is much greater than would be expected in the absence of a severe skeletal insult **[1]**. Patients also exhibit a significant deterioration in bone microarchitecture and strength, as assessed by high resolution peripheral quantitative computed tomography (HR-pQCT) **[2, 3]**. A growing body of evidence suggests an association between bariatric surgery and higher fracture risk **[4–11]**. In a recent large-scale study of bariatric surgery patients in France – mainly women in their late 40s – we demonstrated that Roux-en-Y gastric bypass (RYGB), but not sleeve gastrectomy (SG) or other bariatric procedures, was clearly associated with a higher risk of major osteoporotic fracture **[12]**.

Although the mechanisms underlying the high-turnover bone loss and increase in fracture risk observed after bariatric surgery is not fully understood, many factors seem to be involved **[1]**. The usual suspects are nutritional factors (e.g. hyperparathyroidism secondary to vitamin D insufficiency and decreased calcium absorption) and mechanical unloading secondary to weight loss, and the roles of gut hormones, adipokines and bone marrow adiposity are still under investigation **[1, 13-15]**. However, most of the mechanisms that could explain the skeletal insult are purely hypothetical, and relatively little human data supporting the pathophysiological hypotheses are currently available **[1, 16-18]**.

It seems plausible that surgeries that include a malabsorptive component, such as RYGB, lead to greater high-turnover bone loss than SG, as they induce greater weight loss and are more likely to cause deficiencies in nutrients that are important for bone health, including calcium, vitamin D, and protein **[16–18]**. A few non-randomized clinical trials **[19, 20]**, but not all **[21]**, have reported a greater increase in bone turnover markers (BTMs) after RYGB than after SG. In an ancillary study to the Oseberg randomized controlled trial (RCT), Hofsø *et al.* reported in type 2 diabetic patients a greater increase in BTMs 12 months after RYGB than after SG **[22]**.

Muschitz *et al.* reported in premenopausal women similar increases in BTMs 24 months after both RYGB and SG **[21]**.

It is therefore still debated whether differences in BTMs exist between the two most popular bariatric surgery procedures (RYGB and SG), which account for 80% of all bariatric procedures performed **[19-24]**. Previous studies were limited by small study samples, report divergent results, and some studies have major flaws **[19-24]**.

Moreover, while the mechanisms underlying the increase in BTMs after bariatric surgery are not fully understood, elevated levels of BTMs might be associated with increased bone loss and increased fracture risk in obese patients after bariatric surgery.

We hypothesized that RYGB is associated with a greater increase in BTMs than SG. Our study objectives were **(1)** to prospectively evaluate the differential effects of RYGB and SG on BTMs over a period of 24 months in adult's ≥40 years old and **(2)** to explore potential mechanisms for changes in BTMs after bariatric surgery.

PATIENTS AND METHODS

Study design

The subjects enrolled in this study were participants in the ABOS cohort (Clinical Gov NCT01129297), an ongoing prospective cohort study for the longitudinal assessment of metabolic outcomes before and after weight-loss surgery. The study design has been previously described in detail elsewhere **[25]**. Participants in the ABOS cohort that were eligible for the ABOS-OS ancillary study were seen at Lille University Hospital from 2006 to 2017.

We proposed a prospective cohort with two matched groups of subjects: a group of severely obese men and women who had undergone RYGB, and another group who had undergone SG. In France, as is the case in other countries, current medical guidelines require that patients take

part in a medical management program before undergoing surgery. Eligibility was also based on body mass index (BMI) ≥ 40 or ≥ 35kg/m² with ≥ 1 comorbidity. Patients were matched (SG versus RYGB) (1:1) by age (± 5 years), sex, BMI (± 5 kg/m²) and menopausal status by using the global optimal matching algorithm without replacement. Women were considered postmenopausal if they have not had a menstrual period for >12 months. Moreover, no woman went through menopause during the study follow-up.

The study protocol was approved by the institutional review board and patients provided written informed consent prior to enrolment. All patients underwent an extensive preoperative multidisciplinary evaluation in line with French recommendations, which are similar to the National Institutes of Health's recommendations.

Study population

Inclusion criteria were men and women aged 40 years and older, with severe obesity (BMI ≥ 35kg/m²), no previous bariatric surgery, an indication for bariatric surgery using one of two surgical techniques (RYGB and SG), and quantity of frozen serum ≥ 600 µl at each visit (baseline, 12 and 24 months). Exclusion criteria were men and women who used medications known to impact bone metabolism – including bisphosphonates, teriparatide, denosumab, hormone replacement therapy (HRT) and oral glucocorticoids – in the last 3 months. Other exclusion criteria included prior bariatric surgery and glomerular filtration rate ≤ 30 ml/min/1.73m² (estimated using the modification of diet in renal disease (MDRD) formula).

Study assessments

The medical charts of all patients were reviewed at baseline and during follow-up by a physician (JP). Collected data included patients' demographic and clinical characteristics, and comorbidities. Comorbidities included cardiovascular disease (myocardial infarction, stroke, or

other cardiovascular disease), malignancy, hypertension, ulcer or other gastrointestinal disorders, diabetes mellitus, pulmonary problems, psychological disorders, and hepatic disorders.

Data on alcohol consumption, smoking status (current, past, and never) and concomitant medications, such as calcium and vitamin D supplementation, were also recorded.

Routine biochemical assays

Fasting serum high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), total cholesterol (TC), total calcium, glycated hemoglobin (HbA1C) and glycaemia were assessed by routine assays at baseline, 12 months and 24 months. Glomerular filtration rate (GFR) was estimated using the MDRD formula (ml/min/1.73m²). Intact parathormone (PTH) and insulin were measured by chemiluminescent immunoassay using an Abbott Architect analyzer (Abbott Laboratories, USA). 25-hydroxyvitamin D was measured by competitive chemiluminescent immunoassay using an IDS-*iSYS* analyzer (IDS, Pouilly en auxois, France).

Assays for BTMs, sclerostin and adipokines

Fasting serum samples were collected from all participant in the ABOS cohort and stored at −70°C. Fasting serum for BTMs was drawn prior to 10 am. Specific biochemical assays for the ABOS-OS ancillary study were performed in 2020 (e.g., at baseline, before surgery, specimens were collected from 2008 to 2017). BTMs are both Type 1 cross-linked C-terminal telopeptide (β-CTX) and serum procollagen type I N-terminal propeptide (PINP). Beta-CTX and PINP levels in serum samples were measured by chemiluminescent immunoassay using an IDS-*iSYS* analyzer (IDS, France). Leptin was measured by ELISA using the E07 kit provided by Mediagnost (Reutlingen, Germany). Sclerostin was measured by ELISA using the HS EIA

Sclerostin kit provided by TECO Medical (Sissach, Switzerland). Results were expressed in ng/ml. Total serum adiponectin was measured using the Human Adiponectin RIA kit (HADP-61HK) provided by Millipore (USA).

Statistical analysis

Categorical variables were expressed as frequencies and percentages, and quantitative variables as mean (standard deviation), or median (interquartile range) for variables with non-Gaussian distributions. The normality of distributions was assessed graphically and using the Shapiro-Wilk test. Baseline characteristics were described for the two matched intervention groups (RYGB vs. SG). Comparisons of the baseline characteristics between the two groups of patients were performed using a McNemar test for qualitative parameters, a paired Student test for Gaussian quantitative parameters (or a Wilcoxon sign rank test for non-Gaussian quantitative parameters).

BTMs (PINP and β-CTX), as well as other parameters of interests (total calcium, calciotropic hormones, sclerostin, body weight, adipokines and glucose homeostasis) were determined at baseline and at 12 and 24 months after surgery. Changes from baseline to 12-month follow-up and from 12- to 24-month follow-up were estimated and compared using a longitudinal analysis of covariance (ANCOVA) adjusted for baseline values. A linear mixed model, with 12- and 24-month changes from baseline as dependent variables, and surgical intervention, time (12 and 24 months), surgical intervention*time and baseline value of study parameter as fixed effects, was used. An unstructured covariance pattern was used to account for the correlation in repeated measures within the same patients, and a random effect was used to account for the matched design. The normality of model residuals was satisfied for all parameters, and linear contrasts were used to estimate and compare changes from 12 to 24 months.

The association between the 12-month change in each BTM (PINP and β-CTX) and the 12-month change in each of the other parameters of interests (total calcium, calciotropic hormones, sclerostin, body weight, adipokines and glucose homeostasis) was assessed using separate multivariable linear regression models, with change in BTM (PINP or β-CTX) as the dependent variable, and baseline value of BTMs, change and baseline value of each parameter of interest as independent variables. To assess the independent contribution of type of surgical procedure, further multivariate analyses were performed by including the surgical procedure groups as covariates in previous multivariable linear regression models; from these models, we reported the % of variance in 12-month change in BTMs explained by each individual variables by calculating the partial-R-square values (R^2).

Statistical testing was performed at the two-tailed α level of 0.05. Data were analyzed using the SAS software package, release 9.4 (SAS Institute, Cary, NC).

RESULTS

Patient flowchart

A total of 1,159 patients with severe obesity, no previous bariatric surgery and an indication for bariatric surgery using either of two surgical techniques (RYGB and SG) were seen at Lille University Hospital from 2006 to 2017. After applying the exclusion and inclusion criteria (**Supplemental Figure 1**), 201 patients (17%) were found to be eligible for the study. From the eligible patients (167 RYGB and 34 SG), 32 matched pairs were selected and included in the study.

Baseline characteristics

Table 1 shows the characteristics of the included patients. At baseline, the 64 patients (65.6% women (n=42), including 16 postmenopausal women) had a mean (SD) age of 49.6 years (5.1) and a mean (SD) BMI of 45.0 kg/m² (6.0). Values in both groups were comparable, except for fasting glucose who was higher in the RYGB group (7.4 versus 6.1 mmol/l, p=0.03). In parallel, the prevalence of diabetes mellitus was twofold higher in RYGB compared to SG but did not reach statistical significance (50% versus 25%, p=0.06). Bone turnover markers, adipokines and circulating sclerostin did not differ between the two groups.

Vitamin D and calcium supplementation

Prescription of vitamin D and calcium supplementation was low and remained below 50% over the entire duration of the study. At 24 months, 28 participants (44%) were receiving vitamin D and 27 (42%) calcium supplementation. Recommended vitamin D levels of 30 ng/mL were only obtained in 17% of the patients at baseline, 21% at 12 months, and 22% at 24 months.

Effects of bariatric surgery on BTMs

Changes in BTMs are shown in **Table 2** and **Figure 1**. From baseline to 12 months, a significant increase in BTMs was observed in both groups (p-values<0.001), with a greater overall increase being observed for β-CTX than for PINP. Moreover, RYGB was associated with a greater increase in β-CTX and PINP at 12 months than SG (p-values<0.0001). From 12 to 24 months, BTMs decreased significantly in both groups (p=0.041 and p=0.0001 respectively), with no between-group differences being found for changes in both β-CTX and PINP. However, BTMs did not return to baseline levels at 24 months. Thus, a high degree of bone turnover was maintained in both groups up to 24 months.

Effects of bariatric surgery on total calcium, calciotropic hormones and sclerostin

Changes in total calcium, calciotropic hormones and sclerostin are shown in **Table 3** and **Figure 1**. From baseline to 12 months, a significant increase in total calcium and 25-OH vitamin D was observed in both groups, whereas no significant changes in PTH and sclerostin were found. From 12 to 24 months, a significant decrease in calcium and sclerostin and a significant increase in PTH were observed in both groups, whereas 25-OH vitamin D remained unchanged. Furthermore, no between-group differences were observed for any of these parameters, either at 12 or 24 months.

Effects of bariatric surgery on body weight, adipokines and glucose homeostasis

Changes in body weight, adipokines and glucose homeostasis are shown in **Table 3** and **Figure 1**. Body weight was significantly lower at 12 months after RYGB than after SG (mean: -41.2 kg vs. -28.5 kg; $p<0.0001$). From 12 to 24 months, body weight did not change significantly after RYGB (–2.0 kg; $p=0.074$), whereas a slight increase was observed after SG (+2.3 kg; $p=0.04$), with between-group differences being found ($p=0.007$). From baseline to 12 months, leptin decreased whereas adiponectin increased (p-values<0.0001), and RYGB was associated with a greater change in leptin (-47.6 vs. -34.1 ng/ml; $p<0.0001$) and adiponectin (+10.3 vs. +6.7 mg/l; $p=0.001$) than SG. Adipokines changes were small between 12 and 24 months, with no between-group differences being observed. From baseline to 12 months, HbA1C and insulin decreased significantly (p-values<0.0001), with no between-group differences being found. From 12 to 24 months, changes were small, with a significant increase only being observed for HbA1C in the RYGB group (+0.2%; $p=0.0008$).

12-month changes in bone turnover markers and associations with 12-month changes in pre-specified markers of interest

Using separate multivariable linear regression models, we were able to show that changes in weight, sclerostin and adipokines were significantly associated with 12-month changes in both PINP and β-CTX (**Supplementary Tables 1 and 2**).

To assess the independent contribution of type of surgical procedure, further multivariate analyses were performed by including the surgical procedure groups as covariates in previous multivariable linear regression models. **Tables 4 and 5** show the effect of type of surgical procedure on 12-month changes in BTMs adjusted for changes in pre-specified markers of interest (in separate multivariate analysis). In all multivariate models, type of surgical procedure was found to be independently associated with higher 12-month changes in PINP and β-CTX (all $p<0.0001$). For the 12-month change in PINP, the % of variance explained by the surgical procedure varied from 19 to 32% after adjusting for pre-specified markers of interest **(Table 4)**, whereas for the 12-month change in β-CTX, the % of variance varied from 23 to 39% **(Table 5)**.

Among the pre-specified markers of interest, only the 12-month change in 25-OH vitamin D [β = -6.9 (-13.3 to -0.5), R^2=0.05, p=0.034] remained associated with the 12-month change in PINP after adjusting for type of surgical procedure **(Table 4)**. For the 12-month change in β-CTX, only the 12-month change in total calcium [β = 1233 (59 to 2407), R^2=0.04, p=0.040] and sclerostin [β = 587 (109 to 1065), R^2=0.05, p=0.017] remained associated after adjusting for type of surgical procedure **(Table 5)**. As illustrated, the % of variance explained by these pre-specified markers of interest (25-OH vitamin D, total calcium, and sclerostin), after adjusting for type of surgical procedure, was low (~5%).

Moreover, when 12-month changes in BTMs according to baseline serum 25(OH) vitamin D levels (< 20 vs. ≥ 20 ng/ml) were compared, no differences were found, after adjustment for baseline values and surgical intervention (RYGB vs. SG) **(Supplementary Figure 2)**.

DISCUSSION

In this prospective controlled two-arm study of bariatric surgery patients in France – mainly women in their late 40s – we showed that severely obese patients who had undergone RYGB exhibit a greater increase in BTMs, PINP and β-CTX at 12 and 24 months compared to patients who had undergone SG. Although BTMs decreased slightly – but significantly – from 12 to 24 months in both groups, they did not return to baseline levels, indicating that a high level of bone turnover was maintained in both groups through 24 months. Moreover, the 12-month change in PINP and β-CTX seemed to be mediated by the type of surgical procedure rather than by the 12-month change in the pre-specified markers of interest, i.e. body weight, total calcium, calciotropic hormones, sclerostin, and adipokine and glucose homeostasis.

Since RYGB was the most frequently performed bariatric procedure worldwide until very recently, most of the data on BTMs are from studies evaluating this surgical procedure. Bone turnover markers increase dramatically following RYGB, indicating a high level of bone turnover **[17, 18]**. Serum β-CTX elevation after RYGB has been documented as early as 10 days postoperatively **[26]**, with levels peaking by 6 to 12 months and remaining elevated for as long as 7 years **[17, 18, 26]**. During the first postoperative years, β-CTX typically increases by 50-300% **[27, 28]**. Conversely, markers of bone formation, such as PINP, increase, but generally to a lesser extent (20-150%) than β-CTX **[26-28]**. Sleeve gastrectomy is a relatively new bariatric procedure compared to RYGB and less is known of its effect on BTMs. When

SG is compared to RYGB, less pronounced increases in BTMs have been reported at 6 and 12 months in a few non-randomized non-controlled studies **[19, 20]** but not all **[21]**. Indeed, in the study conducted by Muschitz *et al.*, the authors reported similar increases in BTMs – both β-CTX and PINP – 24 months after RYGB and SG in 90 premenopausal women **[21]**. A sub-study of patients with uncontrolled diabetes mellitus included in the RCT STAMPEDE reported a high degree of bone turnover maintained at 5 years following RYGB, and to a lesser extent in SG patients **[29]**. In a recent RCT involving 92 patients (65 women and 27 men) with diabetes mellitus, Hofsø *et al.* reported a greater increase in BTMs after RYGB compared to SG, 12 months after bariatric surgery **[22]**. Our findings are somewhat consistent with those reported in this latter study, and we were able to confirm these results through 24 months in patients with and without diabetes mellitus. Thus, the finding that BTMs levels at 12 months are higher after RYGB than after SG is in accordance with most previous publications **[19, 20, 22]**. In accordance with the findings reported by Muschitz *et al.* **[21]**, we were able to demonstrate that BTMs increased and peaked at 12 months, and then decreased moderately at 24 months.

The mechanisms underlying the increase in BTMs after bariatric surgery are multifactorial, and while the usual suspects are probably involved, i.e. nutritional factors and mechanical unloading, a myriad of other factors – such as gut-derived hormones, adipokines and changes in body composition **[17, 18, 23]** – may also play a role. Vitamin D deficiency is common in the obese population **[30]**. If not addressed through adequate supplementation, vitamin D deficiency may be aggravated after bariatric surgery, and especially after procedures with a malabsorptive component. At baseline, subjects in our study were vitamin D deficient (mean levels ~18 ng/ml), and while serum levels of 25-OH vitamin D improved at 12 months (mean levels ~24 ng/ml), they failed to reach levels considered to be sufficient (at least 30 ng/ml **[31]**), highlighting the need for individualized repletion regimens in this population. In this study, the

12-month change in PINP seemed partly mediated by the 12-month change in vitamin D, after adjusting for type of surgical procedure. However, the % of variance explained by 25-OH vitamin D was low (5%). Therefore, this result should be interpreted with caution and confirmed by others. Secondary hyperparathyroidism is common in obese patients, and while we found high levels of PTH in both groups at baseline (mean levels ~59 pg/ml), we observed neither a change at 12 months nor an association with 12-month change in BTMs. Surprisingly, serum calcium levels (total), which were within the normal range, increased significantly at 12 months, and the 12-month change in serum calcium was positively associated with the 12-month change in β-CTX, after adjusting for type of surgical procedure. Again, the % of variance explained by total calcium was low (4%) and this result should be interpreted with caution. We hypothesize that accelerated bone resorption is the main factor leading to moderately elevated levels of serum calcium. Hofso *et al.* **[22]** also reported this finding, but they did not attempt to establish an association between serum calcium, 25-OH vitamin D and PTH levels, with BMD and BTMs. In the study conducted by Muschitz *et al.* **[21]**, serum calcium levels (total and adjusted for albumin) were within the lower normal range and declined significantly after 6 and 9 months, contrary to our results. Mechanical unloading secondary to weight loss is usually considered a factor in the high-turnover bone loss observed following bariatric surgery. However, in our study, the changes in BTMs were found to be associated with surgical procedure rather than weight loss. Interestingly, and in line with our findings, Hofsø *et al.* **[22]** and Ivaska *et al.* **[20]** found that surgical procedure predicted a greater increase in β-CTX and PINP, even after adjusting for weight loss. These findings suggest that bariatric surgery procedures induce a high level of bone turnover, and do not support the idea that the increase in BTMs after bariatric surgery is solely a physiological adaptation to lower body weight. Since levels of adipokines, such as leptin and adiponectin, are proportionally related to body fat mass, it is not surprising that decreases in leptin and increases in adiponectin levels have been reported

after bariatric surgery **[18, 26]**. We found similar changes in 12-month adipokine levels in both groups, in parallel with weight loss. This may explain why the 12-month changes in adipokines were not associated with 12-month changes in BTMs after adjusting for type of surgical procedure.

One would expect that the absence of mechanical loading due to rapid and excessive weight loss would be associated with increases in serum sclerostin levels. A few studies have reported a significant increase in post-operative levels of sclerostin **[21, 32]**, but not all **[33]**. In our study population, initial circulating sclerostin levels were within normal ranges and exhibited no significant changes at 12 months. Surprisingly though, significant decreases were observed from 12 to 24 months in both groups. Nevertheless, a weak association was found between the 12-month change in sclerostin and the 12-month change in β-CTX after adjusting for type of surgical procedure. Due to the low % of variance (5%), our results failed to illustrate the role of sclerostin as the main driver in the mechanisms underlying the high bone turnover levels observed after bariatric surgery.

Many additional factors could contribute to the observed changes in BTMs. These could be primary (i.e. as a direct result of changes in intestinal anatomy) or secondary (i.e. resulting from changes in systemic metabolism). Since insulin has an anabolic effect on bone **[34]**, we were interested in investigating the contribution of insulin to bone remodeling after bariatric surgery. However, insulin was not found to be associated with 12-month changes in BTMs. Gut-derived hormones, and changes in body composition and bone marrow adiposity were not assessed in this study, but further investigations are needed to gain a better understanding of the mechanisms underlying the high levels of bone turnover observed following bariatric surgery **[35-37]**.

Strengths and limitations

The major strengths of this study are: **(i)** the study population which comprised a/ mainly women in their late 40s, who are at risk of osteoporotic fractures and b/ patients with and without diabetes mellitus; **(ii)** the long-term follow-up (12 and 24 months for both group); **(iii)** the study design (all SG patients were matched one-to-one to RYGB for age, sex, BMI and menopausal status); **(iv)** use of recommended BTMs **[38]**; and **(v)** large number of measurements of pre-defined markers of interest in order to investigate their association with BTMs. We acknowledge several limitations. The primary limitation of our study is the relatively small sample size. However, even with a limited number of subjects in each group, we were able to detect differences in BTMs 12 and 24 months after bariatric surgery. Another limitation is the lack of data on bone mineral density (BMD) as a surrogate marker of bone metabolism. However, at least 8 studies (from 2013 to 2021) comparing RYGB and SG have reported changes in DXA-derived BMD at various sites following bariatric surgery **[19-21]**. We also acknowledge that the cutoff level of 20 ng/ml rather than 30 ng/ml or 40 ng/ml is a limitation in the 12-month changes in BTMs according to baseline serum 25(OH) vitamin D levels. Finally, the generalizability of our results is limited by its single-center design.

Conclusions

Despite these limitations, the findings of this study may have potential clinical implications. We found a greater increase in BTMs 12 and 24 months after RYGB than after SG. Moreover, the changes in BTMs were found to be associated with the type of surgical procedure rather than weight loss and other pre-specified markers of interest. These findings are insightful as they suggest that the mechanisms underlying the increase in BTMs may be mediated by the type of surgical procedure rather than by changes in body weight, calcium, PTH, 25-OH vitamine D, sclerostin, and adipokines and glucose homeostasis. Finally, the greater risk of

fracture, as well as the greater increase in BTMs observed with RYGB compared to SG, should be considered to determine the type of bariatric procedure. In patients at high risk of fracture, surgical intervention such as RYGB should be tailored to fracture risk, comorbidities, and desired weight loss. Whatever the chosen procedure, we stress out the importance of reaching adequate 25-OH vitamin D for values ≥ 30 ng/mL, ensuring adequate calcium supplementation (e.g. 1500 mg/day) and of having ideally a normal PTH.

Tables and figures

Table 1: Patients' general characteristics and biochemistry results at baseline

Table 2: Comparison of changes in PINP and β-CTX after RYGB and SG

Table 3: Comparison of changes in calciotropic hormones, bone turnover markers, sclerostin and adipokines after RYGB and SG

Table 4: Effect of bariatric surgical intervention on 12-month change in PINP adjusted for changes in pre-specified markers of interest

Table 5: Effect of bariatric surgical intervention on 12-month change in β-CTX adjusted for change in pre-specified markers of interest

Figure 1: Median and interquartile serum values of biochemistry parameters

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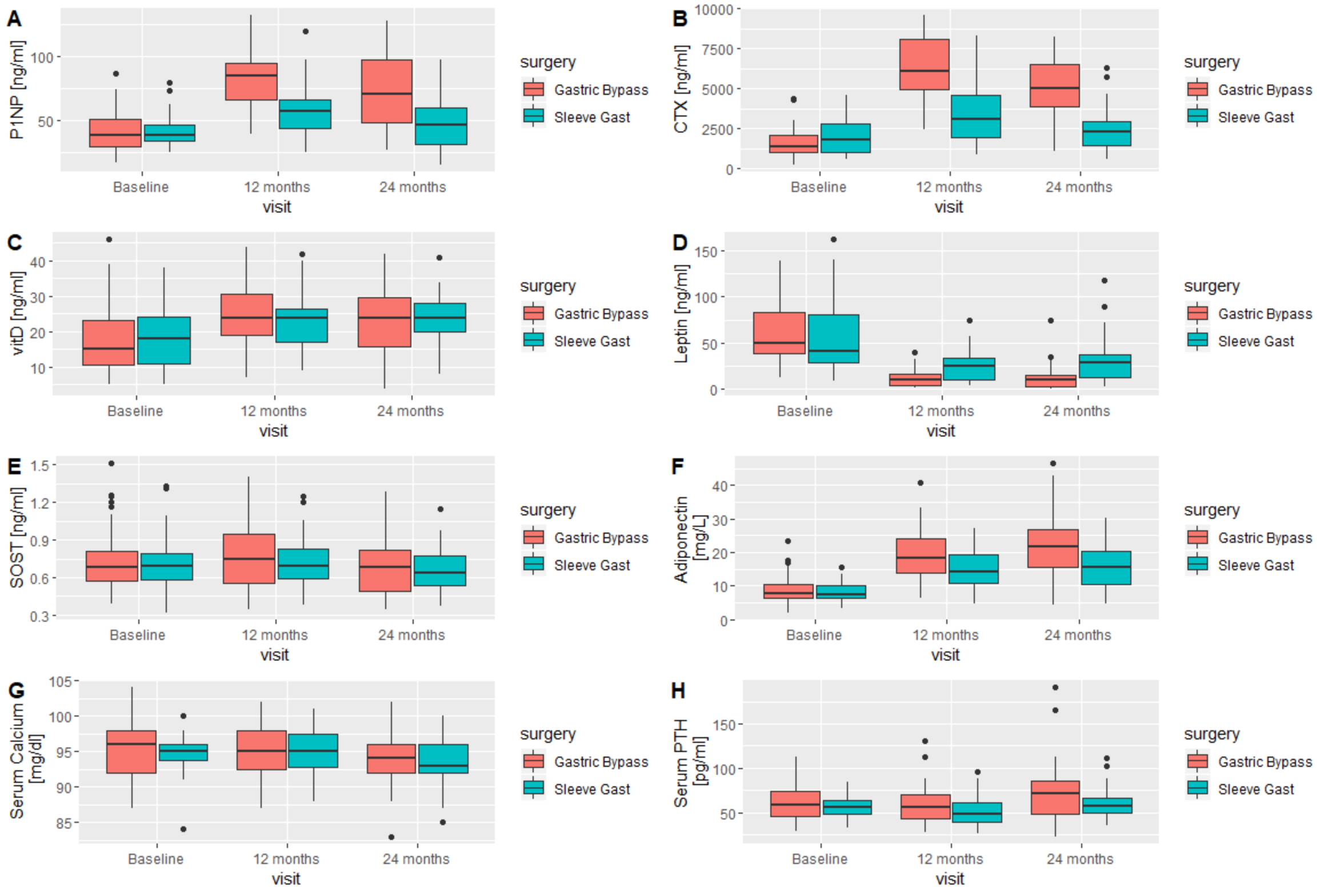
A
P1NP [ng/ml]
100
50
Baseline
12 months
24 months
visit
surgery
Gastric Bypass
Sleeve Gast
B
CTX [ng/ml]
10000
7500
5000
2500
0
C
vitD [ng/ml]
40
30
20
10
D
Leptin [ng/ml]
150
100
50
0
E
SOST [ng/ml]
1.5
1.2
0.9
0.6
0.3
F
Adiponectin [mg/L]
40
30
20
10
0
G
Serum Calcium [mg/dl]
105
100
95
90
85
H
Serum PTH [pg/ml]
150
100
50

Table 1: Patients' general characteristics and biochemistry results at baseline

	RYGB		SG		*p*-value
	N		N		
Age [years]	32	49.4 ± 4.6	32	49.8 ± 5.6	*
Women	32	21 (65.6)	32	21 (65.6)	*
Weight [kg]	32	125.9 ± 15.8	32	126.3 ± 17.4	0.83
BMI [kg/m²]	32	45.2 ± 5.9	32	44.8 ± 6.1	*
Waist circumference [cm]	31	131.3 ± 13.4	32	129.5 ± 12.3	0.37
Hip circumference [cm]	31	135.7 ± 14.7	32	137.3 ± 14.7	0.42
Type 2 diabetes	32	16 (50.0)	32	8 (25.0)	0.06
History of CVD	32	4 (12.5)	32	2 (6.3)	0.32
Hypertension	32	24 (75.0)	32	25 (78.1)	0.76
Hypercholesterolemia	32	26 (81.3)	32	23 (71.9)	0.37
Excessive alcohol consumption	29	18 (62.1)	31	21 (67.7)	1.00
Smoking status	32	20 (62.5)	32	19 (59.4)	0.95
HbA1c [%]	32	6.6 ± 1.3	32	6.0 ± 1.0	0.07
Fasting glucose [mmol/L]	32	7.4 ± 2.9	32	6.1 ± 1.8	**0.03**
Fasting insulin [mIU/ml]	32	28.0 ± 47.6	32	17.8 ± 12.5	0.23
Total chol. [mmol/L]	32	5.2 ± 1.1	32	5.2 ± 1.0	0.92
LDL chol. [mmol/L]	32	3.2 ± 0.9	32	3.2 ± 0.9	0.77
HDL chol. [mmol/L]	32	1.1 ± 0.2	32	1.2 ± 0.3	0.15
Triglycerides [mmol/L]	32	1.9 ± 1.3	32	1.7 ± 0.9	0.80
Hs-CRP [mg/l]	31	5.4 ± 3.5	32	6.2 ± 3.7	0.45
Serum calcium [mg/l]	32	93.5 ± 12.3	32	94.4 ± 2.9	0.71
Serum 25(OH) vitamin D [ng/ml]	32	18.0 ± 10.4	32	19.0 ± 9.1	0.62
Serum PTH [pg/ml]	32	61.0 ± 21.7	32	56.9 ± 14.6	0.42
Creatinine [mg/l]	32	8.1 ± 1.6	32	7.5 ± 1.5	0.14
Creatinine clearance (MDRD formula) [ml/mn]	32	92.1 ± 18.2	32	101.5 ± 23.6	0.08
P1NP [ng/ml]	32	40.2 ± 15.8	32	42.5 ± 13.3	0.55
CTX [pmol/l]	32	1609 ± 971.5	32	2646 ± 3706	0.11
SOST [ng/ml]	32	0.7 ± 0.2	32	0.7 ± 0.2	0.49
Leptin [ng/ml]	32	60.6 ± 33.3	32	58.8 ± 43.6	0.79
Adiponectin [mg/L]	32	9.0 ± 4.5	32	8.3 ± 3.0	0.49

Values expressed as numbers (%), mean ± SD

*Parameters used to matching

Abbreviations: SD = Standard Deviation; IQR = Interquartile Range; ASD = Absolute Standardized Difference; RYGB = Roux-en-Y gastric bypass; SG = sleeve gastrectomy; PTH = parathyroid

hormone; P1NP = procollagen type 1 N-terminal propeptide; CTX = collagen type 1 cross-linked C-telopeptide; HbA1c = glycated hemoglobin; hs-CRP = high-sensitivity C-reactive protein.

Table 2: Comparison of changes in PINP and CTX after RYGB and SG

	RYGB			SG			Difference in change between RYGB and SG	
	N	Mean (95%CI)	*p*-value	N	Mean (95%CI)	*p*-value	Mean (95%CI)	*p*-value
PINP [ng/ml]								
0	32	40.2 (34.4 to 46.0)		32	42.5 (37.6 to 47.4)			
12	32	83.6 (74.7 to 92.5)	**<0.0001***	32	57.6 (50.0 to 65.2)	**<0.0001***	27.8 (17.7 to 37.9)	**<0.0001***
24	32	72.1 (61.1 to 83.1)	**0.0004****	32	47.8 (40.8 to 54.8)	**0.002****	-1.6 (-10.4 to 7.2)	0.71**
CTX [pmol/l]								
0	32	1609 (1253 to 1965)		32	2646 (1288 to 4004)			
12	31	6236 (5479 to 6993)	**<0.0001***	32	4053 (2634 to 5472)	**0.0006***	3427 (2395 to 4459)	**<0.0001***
24	32	4911 (4222 to 5600)	**0.0001****	32	3390 (1522 to 5258)	**0.041****	-613 (-1517 to 291)	0.18**

Values are means (95%CI). Mean changes were estimated by using longitudinal ancova models ajusted for baseline values.

Abbreviations: RYGB = Roux-en-Y gastric bypass; SG = sleeve gastrectomy; P1NP = procollagen type 1 N-terminal propeptide; CTX = collagen type 1 cross-linked C-telopeptide

*Changes in PINP and CTX from baseline to 12 months; **Changes in PINP and CTX from 12 to 24 months

Table 3: Comparison of changes in calciotropic hormones, bone turnover markers, sclerostin and adipokines after RYGB and SG

	RYGB			SG			Difference in change between RYGB and SG	
	N	Mean (95%CI)	*p*-value	N	Mean (95%CI)	*p*-value	Mean (95%CI)	*p*-value
Weight [kg]								
0	32	125.9 (120.0 to 131.8)		32	126.3 (119.9 to 132.7)			
12	32	84.7 (79.5 to 89.9)	**<0.0001***	32	97.8 (90.6 to 105.0)	**<0.0001***	-12.75 (-18.23 to -7.27)	**<0.0001***
24	32	82.7 (76.0 to 89.4)	0.074**	32	100.1 (92.7 to 107.5)	**0.040****	-4.2 (-7.3 to -1.1)	**0.007****
HbA1c [%]								
0	32	6.6 (6.1 to 7.1)		32	6.0 (5.6 to 6.4)			
12	31	5.6 (5.3 to 5.9)	**<0.0001***	32	5.3 (5.1 to 5.5)	**<0.0001***	-0.1 (-0.4 to 0.2)	0.67*
24	29	5.8 (5.4 to 6.2)	**0.0008****	28	5.3 (5.1 to 5.5)	0.99**	0.2 (0.0 to 0.4)	**0.017****
Fasting insulin [mIU/ml]								
0	32	28.0 (10.5 to 45.5)		32	17.8 (13.2 to 22.4)			
12	31	7.5 (5.9 to 9.1)	**<0.0001***	30	8.7 (7.2 to 10.2)	**<0.0001***	-1.9 (-3.9 to 0.1)	0.062*
24	27	7.1 (5.6 to 8.6)	0.42**	31	9.7 (7.5 to 11.9)	0.27**	-1.4 (-3.4 to 0.6)	0.18**
Serum total calcium [mg/dl]								
0	32	93.5 (89.0 to 98.0)		32	94.4 (93.3 to 95.5)			
12	32	95.0 (93.7 to 96.3)	**0.061***	32	95.2 (93.9 to 96.5)	**0.038***	-0.1 (-1.8 to 1.6)	0.88*
24	32	93.9 (92.5 to 95.3)	**0.041****	32	93.3 (92.1 to 94.5)	**0.0006****	0.8 (-0.7 to 2.3)	0.31**
Serum 25(OH) vitamin D [ng/ml]								
0	32	18.0 (14.2 to 21.8)		32	19.0 (15.7 to 22.3)			
12	31	24.4	**<0.0001***	31	23.1	**0.002***	1.6	0.42*

		(20.9 to 27.9)			(20.1 to 26.1)		(-2.25 to 5.42)	
24	32	23.0 (19.4 to 26.6)	0.35**	32	24.0 (21.4 to 26.6)	0.46**	-2.1 (-5.6 to 1.4)	0.24**
Serum PTH [pg/ml]								
0	32	61.0 (53.1 to 68.9)		32	56.9 (51.5 to 62.3)			
12	31	59.9 (51.4 to 68.4)	0.96*	30	51.4 (44.45 to 58.41)	0.065*	6.1 (-2.9 to 15.1)	0.18*
24	31	72.6 (59.2 to 86.0)	**0.001****	32	60.1 (53.5 to 66.7)	**0.023****	3.8 (-6.4 to 14.0)	0.47**
Sclerostin [ng/ml]								
0	32	0.7 (0.6 to 0.8)		32	0.7 (0.6 to 0.8)			
12	32	0.8 (0.7 to 0.9)	0.097*	32	0.7 (0.6 to 0.8)	0.21*	0.1 (0.0 to 0.2)	0.49*
24	32	0.7 (0.6 to 0.8)	**0.0007****	32	0.6 (0.6 to 0.8)	**0.023****	-0.1 (-0.2 to 0.0)	0.40**
Leptin [ng/ml]								
0	32	60.6 (48.4 to 72.8)		32	58.8 (42.8 to 74.8)			
12	32	12.3 (8.6 to 16.0)	**<0.0001***	32	25.4 (19.0 to 31.8)	**<0.0001***	-13.5 (-18.7 to -8.3)	**<0.0001***
24	32	13.0 (7.7 to 18.3)	0.66**	32	30.4 (21.1 to 39.7)	**0.006****	-4.2 (-9.1 to 0.7)	0.094**
Adiponectin [mg/L]								
0	32	9.0 (7.3 to 10.7)		32	8.3 (7.2 to 9.4)			
12	32	19.4 (16.7 to 22.1)	**<0.0001***	32	14.9 (12.7 to 17.1)	**<0.0001***	3.6 (1.3 to 5.9)	**0.001***
24	32	22.2 (18.8 to 25.6)	**0.001****	32	15.8 (13.5 to 18.1)	0.31**	2.1 (-0.3 to 4.5)	0.10**

Values are means (95%CI). Mean changes were estimated by using longitudinal ancova models ajusted for baseline values.

Abbreviations: RYGB = Roux-en-Y gastric bypass; SG = sleeve gastrectomy; PTH = parathyroid hormone; HbA1c = glycated hemoglobin.

*Changes in parameters from baseline to 12 months; **Changes in parameters from 12 to 24 months

Table 4: Effect of bariatric surgical intervention on 12-month change in P1NP adjusted for changes in pre-specified markers of interest

12-month change in Covariates				Bariatric surgery intervention		
	β (95% CI)	R^2	*p*	β (95% CI)	R^2	*p*
Weight [kg]	-2.3 (-8.9 to 4.3)	0.005	0.48	25.4 (13.4 to 37.4)	0.19	<0.0001
HbA1c [%]	-0.5 (-10.7 to 9.7)	0.00	0.92	26.8 (15.9 to 37.7)	0.27	<0.0001
Fasting insulin [mIU/ml]	18.5 (-30.3 to 67.3)	0.007	0.45	28.2 (17.4 to 39.0)	0.32	<0.0001
Serum calcium [mg/dl]	3.6 (-10.0 to 17.2)	0.003	0.59	27.3 (17.1 to 37.5)	0.30	<0.0001
Serum 25(OH) vitamin D [ng/ml]	**-6.9 (-13.3 to -0.5)**	**0.05**	**0.034**	28.7 (18.5 to 38.9)	0.32	<0.0001
Serum PTH [pg/ml]	-0.4 (-6.2 to 5.4)	0.0002	0.89	26.8 (16.0 to 37.6)	0.27	<0.0001
Sclerostin [ng/ml]	4.2 (-1.3 to 9.7)	0.02	0.13	27.8 (17.7 to 37.9)	0.30	<0.0001
Leptin [ng/ml]	-2.3 (-18.5 to 13.9)	0.0009	0.78	26.5 (14.0 to 39.0)	0.19	<0.0001
Adiponectin [mg/L]	2.9 (-2.9 to 8.7)	0.01	0.32	25.2 (14.1 to 36.3)	0.22	<0.0001

β indicates the regression coefficient for the 12-month change in P1NP per one standard deviation in 12-month change in covariates or bariatric surgery intervention (RYGB vs. SG). β were estimated using separate multivariable linear regression models with change in P1NP as the dependent variable, and surgical intervention, changes in the studied covariates and baseline values of P1NP and the studied covariates as independent variables. R^2 indicated the partial R-squared value, which represents the % of variance explained by the corresponding variables.

Abbreviations: PTH = parathyroid hormone; P1NP = procollagen type 1 N-terminal propeptide; HbA1c = glycated hemoglobin.

Table 5: Effect of bariatric surgical intervention on 12-month change in CTX adjusted for change in pre-specified markers of interest

	12-month change in Covariates			Bariatric surgery intervention		
	β (95% CI)	R^2	*p*	β* (95% CI)	R^2	*p*
Weight [kg]	-202 (-752 to 348)	0.005	0.47	2931 (1863 to 3999)	0.26	<0.0001
HbA1c [%]	-498 (-1409 to 413)	0.01	0.28	3221 (2231 to 4211)	0.39	<0.0001
Fasting insulin [mIU/ml]	-644 (-5292 to 4004)	0.0007	0.78	3103 (2085 to 4121)	0.39	<0.0001
Serum calcium [mg/dl]	**1233 (59 to 2407)**	**0.04**	**0.040**	3101 (2176 to 4026)	0.39	<0.0001
Serum 25(OH) vitamin D [ng/ml]	16 (-604 to 636)	0.000	0.96	3108 (2106 to 4110)	0.39	<0.0001
Serum PTH [pg/ml]	-196 (-730 to 338)	0.005	0.46	3065 (2084 to 4046)	0.37	<0.0001
Sclerostin [ng/ml]	**587 (109 to 1065)**	**0.05**	**0.017**	3022 (2093 to 3951)	0.37	<0.0001
Leptin [ng/ml]	-433 (-1928 to 1062)	0.003	0.56	2928 (1740 to 4116)	0.23	<0.0001
Adiponectin [mg/L]	153 (-410 to 716)	0.003	0.59	3006 (1918 to 4094)	0.29	<0.0001

β indicates the regression coefficient for the 12-month change in CTX per one standard deviation in 12-month change in covariates or bariatric surgery intervention (RYGB vs. SG). β were estimated using separate multivariable linear regression models with change in CTX as the dependent variable, and surgical intervention, changes in the studied covariates and baseline values of CTX and the studied covariates as independent variables. R^2 indicates the partial R-squared value, which represents the % of variance explained by the corresponding variables.

Abbreviations: PTH = parathyroid hormone; CTX = collagen type 1 cross-linked C-telopeptide; HbA1c = glycated hemoglobin.