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**Research Article**

# Alteration in the gut microbiome is associated with changes in bone metabolism after laparoscopic sleeve gastrectomy

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## Abstract

Laparoscopic sleeve gastrectomy (LSG), the most common bariatric surgical procedure, leads to durable weight loss and improves obesity-related comorbidities. However, it induces abnormalities in bone metabolism. One unexplored potential contributor is the gut microbiome, which influences bone metabolism and is altered after surgery. We characterized the relationship between the gut microbiome and skeletal health in severe obesity and after LSG. In a prospective cohort study, 23 adults with severe obesity underwent skeletal health assessment and stool collection preoperatively and 6 mo after LSG. Gut microbial diversity and composition were characterized using 16S rRNA gene sequencing, and fecal concentrations of short-chain fatty acids (SCFA) were measured with LC-MS/MS. Spearman's correlations and PERMANOVA analyses were applied to assess relationships between the gut microbiome and bone health measures including serum bone turnover markers (C-terminal telopeptide of type 1 collagen [CTx] and procollagen type 1 N-terminal propeptide [P1NP]), areal BMD, intestinal calcium absorption, and calciotropic hormones.

Six months after LSG, CTx and P1NP increased (by median 188% and 61%, $P < .01$) and femoral neck BMD decreased (mean −3.3%, $P < .01$). Concurrently, there was a decrease in relative abundance of the phylum *Firmicutes*. Although there were no change in overall microbial diversity or fecal SCFA concentrations after LSG, those with greater within-subject change in gut community microbial composition ($\beta$-diversity) postoperatively had greater increases in P1NP level ($\rho = 0.48$, $P = .02$) and greater bone loss at the femoral neck ($\rho = -0.43$, $P = .04$). In addition, within-participant shifts in microbial richness/evenness ($\alpha$-diversity) were associated with changes in IGF-1 levels ($\rho = 0.56$, $P < .01$). The lower the postoperative fecal butyrate concentration, the lower the IGF-1 level ($\rho = 0.43$, $P = .04$). Meanwhile, the larger the decrease in butyrate concentration, the higher the postoperative CTx ($\rho = -0.43$, $P = .04$). These findings suggest that LSG-induced gut microbiome alteration may influence skeletal outcomes postoperatively, and microbial influences on butyrate formation and IGF-1 are possible mechanisms.

**Keywords:** gut microbiome, obesity, bariatric surgery, bone turnover markers, bone mineral density, DXA

## Lay Summary

Laparoscopic sleeve gastrectomy (LSG), the most common bariatric surgical procedure, is a highly effective treatment for obesity because it produces dramatic weight loss and improves obesity-related medical conditions. However, it also results in abnormalities in bone metabolism. It is important to understand how LSG affects the skeleton, so that bone loss after surgery might be prevented. We studied adult men and women before and 6 mo after LSG, and we explored the relationship between the altered gut bacteria and bone metabolism changes. We found that:

- Those with greater shifts in their gut bacterial composition had more bone loss.
- Butyrate, a metabolite produced by gut bacteria from fermentation of dietary fiber, was associated with less bone breakdown and higher IGF-1 level (a bone-building hormone).

We conclude that changes in the gut bacteria may contribute to the negative skeletal impact of LSG and reduced butyrate production by the gut bacteria leading to lower IGF-1 levels is a possible mechanism.

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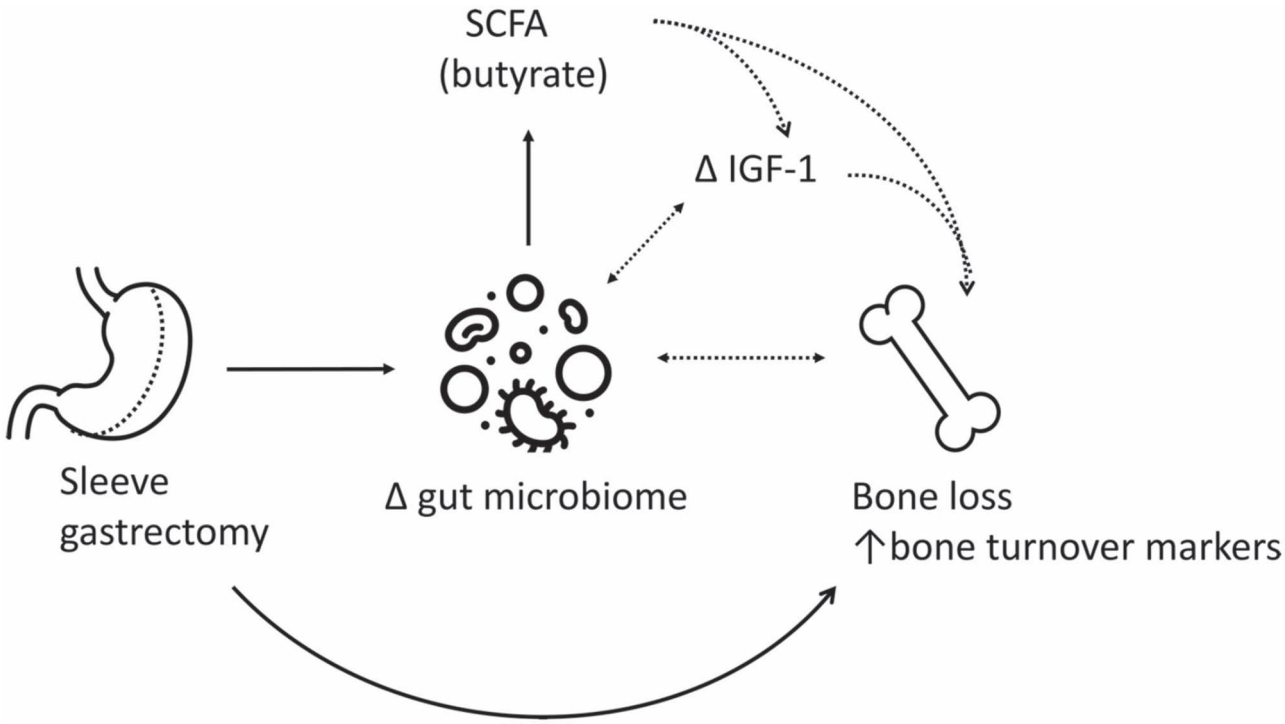
## Graphical Abstract




Sleeve gastrectomy leads to abnormal bone metabolism and induces alteration in the gut microbiome. This altered gut microbiome is associated with changes in bone turnover marker levels and bone loss at the femoral neck 6 mo postoperatively and thus may be a contributor to negative skeletal consequences of sleeve gastrectomy. Microbial influences on butyrate and IGF-1 production are possible mechanisms.

## Introduction

Bariatric surgery has proven to be a highly effective intervention for severe obesity (BMI $\geq$ 40 kg $m^{-2}$), resulting in body weight loss of >20%-30% and improvement in obesity-related conditions.[1,2] With the rise of the obesity epidemic, there is a growing demand for bariatric surgery. Laparoscopic sleeve gastrectomy (LSG) has emerged as the most performed procedure due to its perceived safety and nearly comparable weight loss and metabolic benefits compared to the previous "gold-standard" Roux-en-Y gastric bypass (RYGB).[3,4] However, recent studies suggest that like RYGB, LSG has the unintended consequence of inducing abnormalities in bone metabolism, leading to marked increases in bone turnover marker levels and rapid decreases in BMD.[5–9] The skeletal effects are likely multifactorial, with contributing factors including changes in calcium homeostasis and calciotropic hormones, nutritional factors including calcium malabsorption,[9] mechanical unloading, and hormonal changes.[10] Alteration in the gut microbiome is an unexplored potential contributor.

There is emerging evidence that the gut microbiome regulates bone metabolism. The human gut microbiome represents a dynamic ecosystem of microbes that are involved in several essential host functions and can regulate bone homeostasis via its effects on nutrient absorption, vitamin synthesis, immunity, and hormone modulation.[11–14] Animal experiments with both germ-free animals and perturbation of microbiota by antibiotics have demonstrated bone mass alteration that is reversed with colonization.[15–18] In both animal and human studies, supplementation with prebiotics or probiotics to alter the gut microbiome has been shown to improve calcium absorption and protect against bone loss.[19–25]

LSG leads to a rapid and sustained effect on the gut microbiome.[26–28] Although inconsistently described between studies, LSG is generally associated with an increase in microbial $\alpha$-diversity (richness and evenness) and a decrease in the *Firmicutes:Bacteroidetes* ratio.[26,27,29] Many members of the phylum *Firmicutes* ferment dietary undigested carbohydrates and fibers to produce the end-product short-chain fatty acids (SCFA). Indeed, recent studies have also shown a reduction in fecal SCFA concentration after LSG.[30,31] SCFA are known to improve intestinal mucosal integrity and increase calcium absorption, reduce inflammation, influence bone cell differentiation (osteoblasts and osteoclasts), and modulate hormones including IGF-1,[32–34] which has a role in maintenance of bone health in adulthood.[35] Members of the phylum *Bacteroidetes* are the main contributors to proinflammatory LPS biosynthesis in the human gut microbiome.[36] Prior studies have found an enrichment of *Bacteroides* in postmenopausal women and older men with osteoporosis.[37–39] Therefore, the postoperative changes in the gut microbiome composition and function may have a negative impact on skeletal health, though no study has yet investigated the role of the gut microbiome in bone metabolism after LSG.

The aim of this study was to determine the relationship between the gut microbiome and skeletal health in a cohort of pre- and postmenopausal women and men with severe obesity undergoing LSG. We hypothesized that gut microbiome alterations contribute to LSG's skeletal effects.

## Materials and methods

### Study design and population

This was an ancillary investigation to a larger prospective pre-post cohort study that examined calcium metabolism and skeletal health after LSG.[9] Women and men aged 24 to 70 yr with severe obesity undergoing LSG were recruited from an academic bariatric surgery center (the University of California, San Francisco) between 2016-2021. Participants were eligible if they were scheduled for an upcoming LSG procedure and were excluded if they were perimenopausal (defined as last menses >3 mo but <4 yr ago), had prior bariatric surgery or intestinal malabsorption, had conditions or were taking medications known to impact bone and mineral metabolism (eg, primary hyperparathyroidism, Paget's disease, hyperthyroidism; on osteoporosis pharmacotherapy, glucocorticoids, thiazolidinediones, aromatase inhibitors, or androgen deprivation therapy), had significant comorbid conditions such as liver and kidney failure, used illicit drugs or alcohol >3 drinks/d, or weighed >200 kg (the weight limit for our DXA scanner). To minimize selection bias upon enrollment, extensive chart review was performed to identify all preoperative patients meeting inclusion criteria to assess eligibility.

### Study protocol

Participants in the parent cohort study underwent study measurements within 3 mo before LSG and again 6 and 12 mo postoperatively. LSG was performed using minimally invasive surgical techniques to remove 60% to 80% of the greater curvature of the stomach by staples from approximately 6 cm proximal to the pylorus to about 1 cm below the gastroesophageal junction. Calcium intake and vitamin D status were standardized throughout study participation. Individualized calcium citrate and vitamin D supplements were supplied at least 2 wk prior to the preoperative study measurements to achieve a daily calcium intake (diet + supplement) of approximately 1200 mg $d^{-1}$ and to target a 25OHD level $\geq$ 30 ng $mL^{-1}$. Calcium intake and 25OHD levels were monitored and supplement doses adjusted throughout the study period. Scheduled check-ins with participants were done at 3, 6, 9, and 12 mo postoperatively to minimize loss to follow-up. Real-time data entry with regular audits was performed to ensure full and accurate data capture.

Of the 55 participants who contributed pre- and postoperative data to the larger parent cohort study, this ancillary study included the 23 participants who provided stool samples at both preoperative and 6-mo postoperative time points. For those who agreed to participate in the ancillary study, additional exclusion criteria included antibiotic therapy or regular pre- or probiotic use $\leq$3 mo before each specimen collection. A separate consent was obtained for the ancillary study. Participants were instructed to collect the first bowel movement of the day each time. Participants stored their samples in their home freezers (−20 °C) until delivering them on ice to the study team. Samples were then stored at −80 °C until processing.

The protocol was approved by the Institutional Review Board at the University of California, San Francisco, and all participants provided written informed consent. The study was registered at the US National Institutes of Health (ClinicalTrials.gov, NCT02778490).

### Study measures

#### Biochemical assays

Serum samples were collected after an overnight fast and were immediately analyzed for calcium, creatinine, albumin, phosphate, 25OHD, and PTH at a commercial laboratory (Quest Diagnostics). Remaining sera were stored at −80 °C until batch analysis in a central laboratory (Maine Medical Center Research Institute) for measurement of bone turnover marker levels (serum C-terminal cross-linked telopeptide (CTx) and procollagen type 1 N-terminal propeptide (P1NP)), $1,25[OH]_2D$, and IGF-1 with chemiluminescence on an autoanalyzer (iSYS, Immunodiagnostic Systems). The inter- and intra-assay coefficients of variation were 6.0% and 3.2% for CTx, 5.0% and 2.9% for P1NP, 11.1% and 6.4% for $1,25(OH)_2D$, and 5.0% and 2.16% for IGF-1.

#### Body composition and skeletal imaging

Weight and height were measured. Waist circumference was measured at the level directly below the lowest rib, and hip circumference at the maximum extension of the buttocks, viewed from the side. BMI (kg $m^{-2}$) was calculated. Areal BMD at the lumbar spine and proximal femur and estimated total and regional body composition were assessed with whole-body DXA (Horizon A, Hologic).[40] If a participant's body dimensions exceeded the scanning field, modified half-body scans were used.[41]

#### Gut microbial profiling

DNA was extracted from all stool samples using a modified cetyltrimethylammonium bromide buffer extraction protocol as previously described.[42] Every extraction batch had a minimum of one negative control (PBS) per plate, placed in the middle of the plate. These control extractions were taken through the full sequencing pipeline. PCR amplification of the V4 region of the 16S rRNA gene was amplified using 515F and 806R primer pairs on the Illumina NextSeq 500 Platform on a 153 bp × 153 bp sequencing run (Illumina, San Diego).[43] Bacterial reads were demultiplexed by barcode using QIIME scripts (v1.9.1).[44] Sequence data underwent Divisive Amplicon Denoising Algorithm 2 (DADA2; v1.20) processing within each run.[45] Briefly, sequences were filtered if they had any N's, had a maximum expected error greater than 2, a quality score <2, or matched to the PhiX genome, filtering approximately 20% of sequences per sample. The software then learned errors, dereplicated sequences, performed denoising, merged reads with a minimum overlap of 25 bp, and checked for chimeras. Taxonomic classifications were assigned using the SILVA database v138. Phylogenetic trees were constructed using the phangorn (v2.8.1), msa (v1.24), and ape (v5.5) packages in R (v4.0.3). Sequence variants (SV) associated with negative controls were removed outright if they were present in $\geq$15% of negative controls and < 15% of samples; the mean of remaining variants was subtracted from samples. The resulting SV tables were combined and representatively rarefied to 18 000 reads per sample, resulting in all samples retained for analysis (Supplementary Figure S1).

$\alpha$-Diversity, a within-sample diversity measure, was determined by Shannon diversity index, Faith's phylogenetic diversity, Chao1 index (richness), and Pielou's evenness index using phyloseq package (version 1.34) in R. $\beta$-Diversity, a between-samples community compositional dissimilarity measure, was calculated using Bray-Curtis (highlighting dissimilarities in

higher abundance taxa), Canberra distance, and weighted (accounting for abundance of taxa) and unweighted UniFrac distances—both measures of phylogenetic relatedness—with phyloseq (version 1.34) and vegan package (version 2.5-7) in R.

### Fecal short-chain fatty acids profiling

Dry fecal samples were analyzed for eight SCFA: acetic acid, propionic acid, isobutyric acid, butyric acid, 2-methylbutyric acid, isovaleric acid, valeric acid, and hexanoic acid by LC-MS/MS (Metabolon Method TAM135) at a commercial laboratory (Metabolon, Inc.). The mass spectrometer was operated in negative mode using electrospray ionization. The peak area of the individual analyte product ions was measured against the peak area of the product ions of the corresponding internal standards. Quantitation was performed using a weighted linear least squares regression analysis generated from fortified calibration standards prepared immediately prior to each run. LC-MS/MS raw data were collected and processed using AB SCIEX software Analyst 1.6.3 and processed using SCIEX OS-MQ software v1.7. Data reduction was performed using Microsoft Excel for Office 365 v.16.

## Statistical methods

Baseline descriptive data were expressed as means ± SD or medians (IQR) depending on normality. Spearman's rank correlation was used to characterize the relationships between preoperative microbial diversity ($\alpha$-diversity) and study parameters. Adjusted baseline associations were determined with Spearman's partial correlation with age, sex, menopause status, and baseline BMI. Permutational multivariate analysis of variance (PERMANOVA) was employed to evaluate factors that significantly explained variation in microbial $\beta$-diversity preoperatively. Adjustment of PERMANOVA with age, sex, menopause status, and baseline BMI were done with adonis2 function in vegan package (version 2.5-7) in R to partition the distance matrix to remove sources of variation.

The paired *t*-test or Wilcoxon signed-rank test was used to examine whether there was a change between preoperative and 6-mo postoperative time points, depending on normality of the variable. Significance level was defined as two-sided *P*-value <.05. Spearman's rank correlation test was used to assess the relationships between the gut microbiome characteristics (changes in $\alpha$-diversity, $\beta$-diversity, and fecal SCFA pre-post LSG, and abundance of specific taxa/SV at the 6-mo postoperative time point) and longitudinal changes in other study parameters. Spearman's partial correlation was performed to evaluate adjusted associations with age, sex, menopause status, and changed in BMI (all in one function). Differentially enriched taxa pre-post LSG were determined with Analysis of Composition of Microbiomes with Bias Correction (ANCOM-BC) method using the ANCOMBC package on GitHub,[46,47] which estimates the unknown sampling fractions and corrects the bias induced by their difference among samples. The predicted functional characteristics of microbial community were performed from the Kyoto Encyclopedia of Genes and Genomes database using Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt2; version 2.5.2), which generates functional predictions of microbial community from amplicon sequences.[48] Visualization of PICRUSt2 analysis was performed with the ggpicrust2 package in R (version 1.7.2).[49] All statistical analyses were performed using Stata 15.1 software (StataCorp) and R (version 4.0.3).

**Table 1.** Baseline characteristics.

| | *N* = 23 |
|---|---|
| Age, yr | 48.0 ± 12.0 |
| Women, *n* (%) | 19 (83) |
| Premenopausal | 10 (53) |
| Postmenopausal | 9 (47) |
| Race, *n* (%) | |
| White | 17 (74) |
| Black/African American | 6 (26) |
| Ethnicity, *n* (%) | |
| Hispanic/Latinx | 3 (13) |
| Weight, kg | 116.4 ± 21.7 |
| BMI, kg $m^{-2}$ | 42.3 ± 5.3 |
| Percent body fat (%) | 43.7 ± 4.1 |
| Waist circumference (cm) | 116.0 ± 11.2 |
| Waist-hip ratio | 0.88 ± 0.11 |
| Diabetes, *n* (%) | 7 (30) |
| Serum calcium, mg $dL^{-1}$ | 9.3 ± 0.4 |
| Albumin, g $dL^{-1}$ | 4.1 ± 0.4 |
| 25OHD upon enrollment, ng $mL^{-1}$ | 26 ± 9 |
| 25OHD at pre-op visit, ng $mL^{-1}$ | 43 ± 12 |
| PTH, pg $mL^{-1}$ | 41 ± 21 |
| $1,25(OH)_2D$, pg $mL^{-1}$ | 67 ± 22 |
| CTx, ng $mL^{-1}$ | 0.19 (0.13-0.28) |
| P1NP, ng $mL^{-1}$ | 40.9 (34.9-60.7) |
| Creatinine, mg $dL^{-1}$ | 0.77 ± 0.19 |
| eGFR, mL $min^{-1}$ 1.73 $m^{-2}$ | 97 ± 25 |
| Urine calcium, mg 24 $hr^{-1}$ | 187 (71-295) |
| Areal BMD, g $cm^{-2}$ | |
| Lumbar spine | 1.118 ± 0.130 |
| Total hip | 1.050 ± 0.125 |
| Femoral neck | 0.875 ± 0.129 |

Values are means ± SD, median (IQR), or count (percentage). 95% reference intervals provided by the test manufacturers: PTH, 11.5-78.4 pg $mL^{-1}$; CTx, 0.142-1.351 ng $mL^{-1}$ (postmenopausal women); P1NP, 27.7-127.6 ng $mL^{-1}$; $1,25(OH)_2D$, 15.2-90.1 pg $mL^{-1}$. Abbreviations: CTx, collagen type 1 C-telopeptide; eGFR, estimated glomerular filtration rate; P1NP, procollagen 1 intact N-terminal propeptide; PTH, intact parathyroid hormone.

# Results

## Baseline participant characteristics

Preoperatively, mean age was 48.0 ± 12.0 yr, weight was 116.4 ± 21.7 kg, and BMI was 42.3 ± 5.3 kg $m^{-2}$ (Table 1). Of the 23 participants, 19 (83%) were women with 9 (47%) of those postmenopausal and 10 (53%) premenopausal. Mean 25OHD level was 43 ± 12 ng $mL^{-1}$ with supplementation at the time of preoperative study measurements. Mean serum calcium, PTH, $1,25(OH)_2D$, serum creatinine, and eGFR levels were within their reference ranges. One of the 9 postmenopausal women and one of the 2 men ≥50 yr old had an areal BMD T-score between −1.0 and −2.4 at any site preoperatively. None had a T-score ≤ −2.5. None of the premenopausal women or men <50 yr old had an areal BMD Z-score ≤ −2.0. Baseline characteristics of participants in this ancillary study did not differ significantly from the participants in the larger cohort study who did not provide stool samples.

Baseline measures of $\alpha$-diversity were positively correlated with bone turnover marker levels (Shannon diversity index: CTx $\rho = +0.45$, $P = .02$; P1NP: $\rho = +0.44$, $P = .02$ and Chao1

**Table 2.** Changes in metabolic parameters, calcium homeostasis, and bone metabolism 6 mo after LSG.

| $N = 23$ | Baseline | 6 mo post-op | % Change | $P$ |
|---|---|---|---|---|
| Body composition changes | | | | |
| Weight (kg) | 116.4 ± 21.7 | 88.4 ± 18.5 | −24.0% ± 6.8% | <.01 |
| BMI (kg $m^{-2}$) | 42.3 ± 5.3 | 32.2 ± 4.9 | −23.9% ± 6.6% | <.01 |
| Percentage body fat (%) | 43.7 ± 4.1 | 36.8 ± 5.8 | −16.1% ± 9.5% | <.01 |
| Waist circumference (cm) | 116.0 ± 11.2 | 95.9 ± 9.3 | −17.1% ± 6.7% | <.01 |
| Waist-hip ratio | 0.88 ± 0.11 | 0.85 ± 0.08 | −2.2% ± 4.4% | .02 |
| *Laboratory changes* | | | | |
| HbA1c (%) | 5.5 (5.1–6.6) | 5.0 (4.9–5.4) | −7.8 (−18.2 to −3.7) % | <.01 |
| IGF-1 (ng $mL^{-1}$) | 127.6 ± 47.9 | 133.7 ± 52.3 | +11.0% ± 32.7% | .29 |
| Calcium homeostasis | | | | |
| Serum calcium, mg $dL^{-1}$ | 9.3 ± 0.4 | 9.3 ± 0.3 | −0.2% ± 4.7% | .40 |
| Albumin, g $dL^{-1}$ | 4.1 ± 0.4 | 4.0 ± 0.3 | −2.0% ± 9.4% | .22 |
| 25OHD, ng $mL^{-1}$ | 43 ± 12 | 44 ± 16 | +8.6% ± 42.4% | .75 |
| PTH, pg $mL^{-1}$ | 41 ± 21 | 45 ± 24 | +3.8% ± 13.0% | .18 |
| 1.25$(OH)_2$D, pg $mL^{-1}$ | 60 (51–75) | 84 (72–105) | +37.3 (+13.6 to +72.4) % | <.01 |
| Creatinine, mg $dL^{-1}$ | 0.75 (0.63–0.92) | 0.70 (0.59–0.83) | −9.5 (−17.3 to 0.0) % | .01 |
| eGFR, mL $min^{-1}$ | 101 (87–116) | 108 (95–118) | +3.2 (−2.1 to +13.2) % | .01 |
| Urine calcium, mg 24 $hr^{-1}$ | 187 (71–295) | 178 (92–302) | +7.78 (−53.2 to +163.0) % | .91 |
| Bone turnover markers | | | | |
| CTx, ng $mL^{-1}$ | 0.19 (0.13–0.28) | 0.50 (0.41–0.83) | +188.4 (+90.8 to +317.6) % | <.01 |
| P1NP, ng $mL^{-1}$ | 40.9 (34.9–60.7) | 72.9 (63.0–92.0) | +61.0 (+32.8 to +109.8) % | <.01 |
| Bone mineral density (DXA) | | | | |
| Lumbar spine | 1.118 ± 0.130 | 1.122 ± 0.120 | +0.7% ± 5.1% | .67 |
| Total hip | 1.050 ± 0.125 | 0.998 ± 0.132 | −5.0% ± 3.5% | <.01 |
| Femoral neck | 0.875 ± 0.129 | 0.845 ± 0.122 | −3.3% ± 5.4% | <.01 |

Values are means ± SDs or median (IQR). $P$-value calculated based on paired $t$-test or Wilcoxon signed-rank based on normality. Abbreviations: CTx, collagen type 1 C-telopeptide; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; P1NP, procollagen 1 intact N-terminal propeptide; PTH, intact parathyroid hormone.

index: CTx $\rho = +0.39$, $P = .04$; P1NP $\rho = +0.54$, $P < .01$), that is, those with lower gut microbial richness and evenness had lower CTx and P1NP levels. After adjustment for age, sex, menopause status, and BMI, the associations remained significant (Shannon's diversity index: CTx $\rho_{partial} = +0.41$, $P = .05$; P1NP $\rho_{partial} = +0.45$, $P = .03$ and Chao1 index: CTx $\rho_{partial} = +0.37$, $P = .07$; P1NP $\rho_{partial} = +0.63$, $P < .01$). The baseline measure of $\beta$-diversity (weighted UniFrac) was significantly associated with P1NP (PERMANOVA, $R^2 = 0.11$, $P < .01$). This relationship also remained significant irrespective of adjustment for age, sex, menopause status, and BMI ($P < .01$).

## Changes in metabolic parameters, calcium homeostasis, and bone metabolism after LSG

Six months after LSG (median 7.5 [6.5–8.2] mo), all participants achieved substantial weight loss, with a mean loss of 28.0 ± 9.2 kg, or a 24.0% ± 6.8% decline from baseline weight ($P < .01$, Table 2). Glycated hemoglobin (HbA1c) decreased ($P < .01$) and eGFR improved slightly ($P = .01$). No statistically significant change in IGF-1 level was observed. All participants maintained a 25OHD level > 25 ng $mL^{-1}$ postoperatively without a statistically significant change in serum calcium, PTH, or 24-hr urinary calcium levels. However, 1,25$(OH)_2$D level increased from a median of 60 (IQR 51–75) pg $mL^{-1}$ to 84 (IQR 72–105) pg $mL^{-1}$ ($P < .01$). Concurrently, bone turnover marker levels increased markedly, by a median of +188.4 (IQR +90.8 to +317.6) % for CTx and +61.0 (IQR +32.8 to +109.8) % for P1NP ($P < .01$ for both). Areal BMD declined significantly at the total hip (by −5.0% ± 3.5%, $P < .01$) and femoral neck (−3.3% ± 5.4%, $P < .01$). There was not a statistically significant change in lumbar spine areal BMD over the 6-mo period.

## Changes in the gut microbiome after LSG

Six months after LSG, there was no significant change in $\alpha$-diversity (richness and/or evenness within a sample) or $\beta$-diversity (overall community microbial composition between samples) compared with paired presurgical samples (Figure 1A and B, Supplementary Figure S2A and B). However, there was a reduction in the relative abundance of bacteria belonging to the phylum *Firmicutes* postoperatively (log2 fold change of −1.08, adjusted $P = .02$) (Figure 1D). At the genus level, there were microbial taxa/sequence variants that changed significantly pre- and post-LSG (Figure 1E). Notably, there was a significant reduction in the relative abundance of *Bifidobacterium bifidum* (log2 fold change of −0.87, adjusted $P < .001$). There was no statistically significant change in dry fecal SCFA concentrations (Figure 1C, Supplementary Figure S2C) or in the functional prediction of microbial SCFA metabolism pathways (Supplementary Figure S3).

Participants with larger shifts in microbial community composition after surgery, as quantified by the Bray-Curtis $\beta$-diversity metric, showed greater percentage increase in P1NP levels ($\rho = +0.48$, $P = .02$) and greater bone loss at the femoral neck ($\rho = -0.43$, $P = .04$; Figure 2A and B). The relationships remained significant with the adjustment for age, sex, menopause status, and BMI loss (ΔP1NP: $\rho_{partial} = +0.53$, $P = .02$; femoral neck bone loss: $\rho_{partial} = -0.45$, $P = .05$). The association between the change in Bray-Curtis and percentage increase in CTx was not statistically significant ($\rho = +0.36$, $P = .09$; Figure 2C). In addition, participants with greater increases in $\alpha$-diversity measures had greater increases in

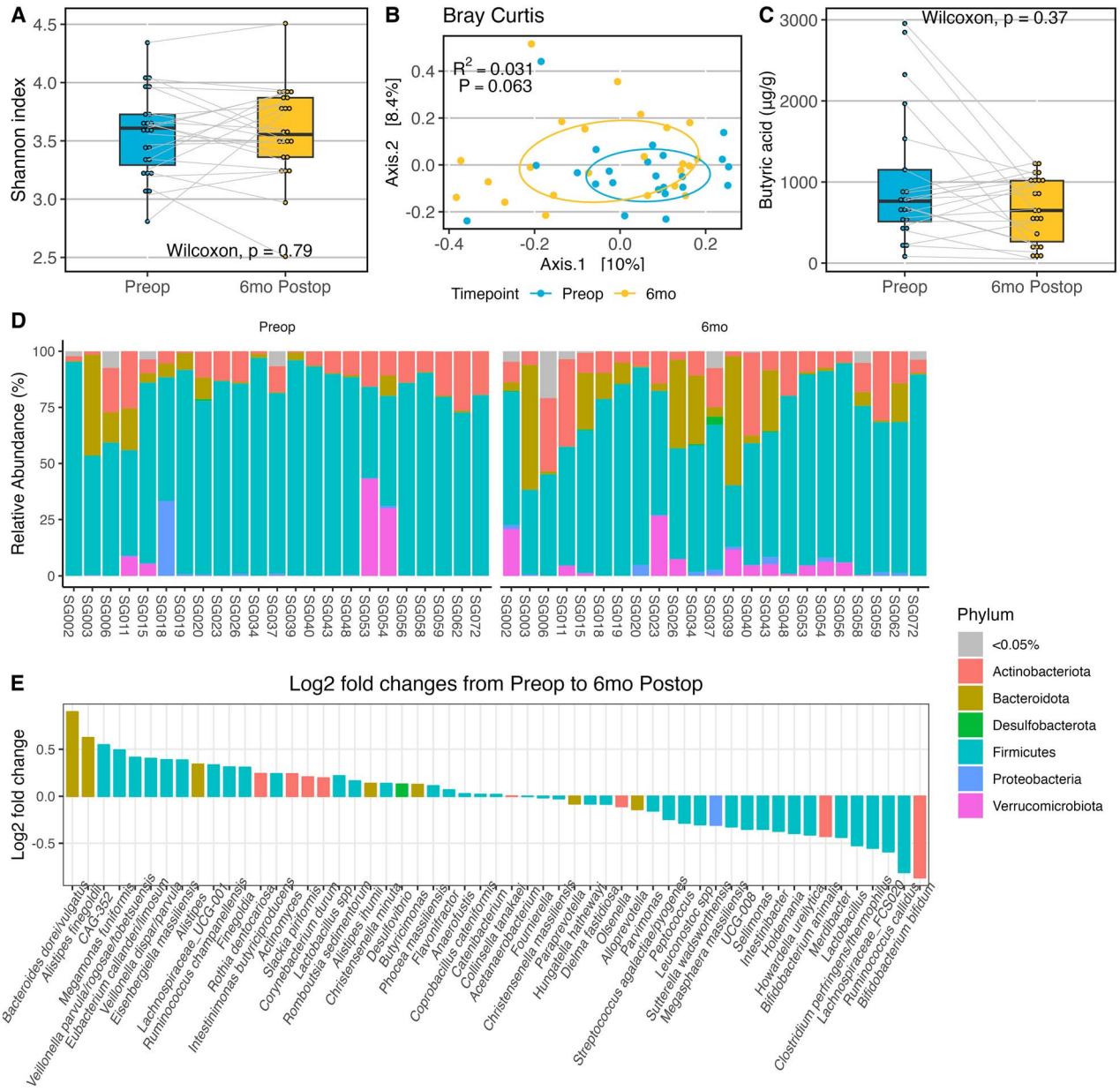



**Figure 1.** Preoperative and 6-mo postoperative changes in (A) $\alpha$-diversity (within-sample diversity) with Shannon's diversity index (other measures shown in Supplementary Figure S2A) using Wilcoxon signed-rank test, (B) $\beta$-diversity (between-sample community compositional dissimilarity) with Bray-Curtis (other measures shown in Supplementary Figure S2B) using PERMANOVA, (C) fecal butyrate concentrations (other SCFA shown in Supplementary Figure S2C) using Wilcoxon signed-rank test, (D) relative abundance of microbial taxa at the phylum level by each participant, and (E) all the differentially enriched microbial taxa at the genus level (using ANCOM-BC, adjusted $P < .05$) arranged in order of log2 fold change, colored by the corresponding phylum level.

IGF-1 levels (Shannon's diversity index: $\rho = +0.56$, $P < .01$; Chao1 index: $\rho = +0.58$, $P < .01$; Figure 3A). The strength of the point estimates for these relationships remained similar with the adjustment for age, sex, menopause status, and BMI loss (Shannon's diversity index: $\rho_{partial} = +0.45$, $P = .04$; Chao1 index: $\rho_{partial} = +0.39$, $P = .09$). Changes in other calciotropic hormones were not associated with postoperative changes in diversity measures.

There were three microbial taxa that were associated with postoperative bone loss. Participants with greater postoperative abundance of *Lachnospiraceae*, which are among the SCFA producers in the human gut, had less femoral neck BMD loss ($\rho = +0.44$, $P = .03$), and participants with greater abundance of *Bacteroides caccae* and *Bacteroides uniformis* postoperatively had greater femoral neck BMD loss ($\rho = -0.46$ and $\rho = -0.58$, $P = .03$ and $.003$). The strength of the point estimates for these relationships remained similar with the adjustment for age, sex, menopause status, and BMI loss (*Lachnospiraceae*: $\rho_{partial} = +0.42$, $P = .10$; *B caccae*: $\rho_{partial} = -0.47$, $P = .04$; *B uniformis*: $\rho_{partial} = -0.63$, $P = .01$).

Regarding fecal SCFA concentration, participants with lower butyrate levels postoperatively had lower IGF-1 levels ($\rho = +0.43$, $P = .04$; Figure 3C). Although not statistically significant, participants with greater decreases in butyrate levels tended to have greater increases in CTx levels ($\rho = -0.39$, $P = .07$; Figure 3B). The decrease in butyrate levels

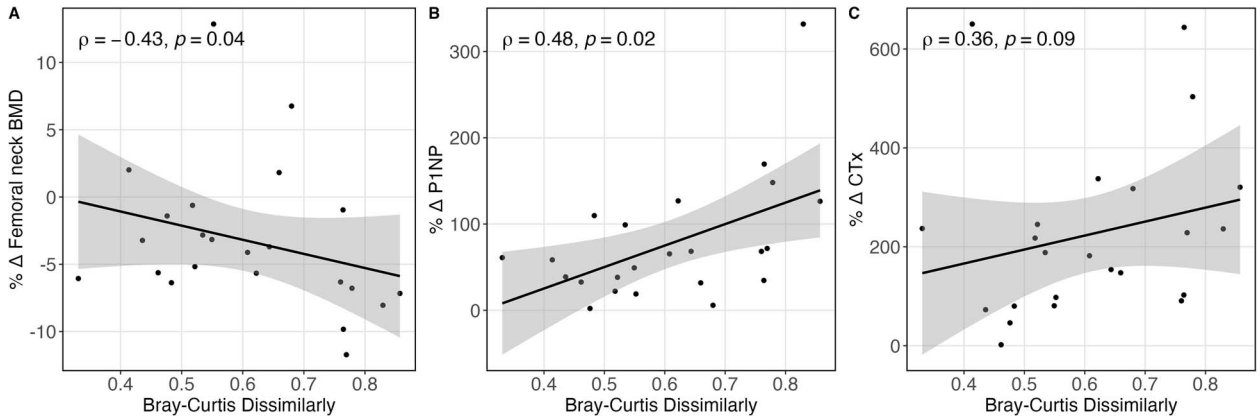



**Figure 2.** Spearman's c rank correlations between 6-mo postoperative changes in microbial community structure and (A) Δ femoral neck BMD, (B) Δ P1NP levels, and (C)Δ CTx levels. The shaded areas represent the 95% confidence intervals for predictions from a linear model.

was negatively associated with the absolute postoperative CTx levels ($\rho = -0.43, P = .04$). This relationship strengthened with adjustment for age, sex, menopause status, and BMI loss ($\rho_{\text{partial}} = -0.50, P = .03$).

## Discussion

We report the first study to explore the relationship between the gut microbiome and skeletal outcomes in patients undergoing LSG for weight loss. In this cohort study of 23 adults with severe obesity undergoing LSG, we found that greater 6-mo changes in overall microbial composition were associated with greater increases in levels of biochemical markers of bone turnover and greater declines in femoral neck areal BMD. Although there was no overall decline in fecal butyrate levels after LSG, the greater the reduction in butyrate levels, the higher the postoperative level of the bone turnover marker CTx. These findings suggest that LSG-induced alterations in the gut microbiome composition and function could contribute to negative skeletal effects.

Prior to LSG, in the setting of severe obesity, we found an association between gut microbial diversities and biochemical markers of bone turnover. Recent studies report an aberrant gut microbiome in individuals with obesity. The gut microbiome in obesity has a greater ability to metabolize and extract energy from food and to produce SCFA from enhanced fermentation of undigested polysaccharide.[50–52] This functional distinction is reflected by a differential gut microbial composition. The gut microbiome in obesity tends to have a lower microbial diversity, reduced abundances of the *Bacteroidetes* phylum, and higher abundances of the *Firmicutes* phylum, which has a dominant role in carbohydrate metabolism.[53–56] These observed obesity-associated gut microbial characteristics and metabolic activities can affect bone metabolism in obesity. We found that those with a lower gut diversity, and thus a gut microbiome that appeared more different than that expected in normal weight, had more suppressed bone turnover marker levels. We postulate that a lower gut microbial diversity in severe obesity may signal a less functionally diverse microbiome with enhanced efficiency in fermentation of substrates and in SCFA production. Preclinical studies have shown that SCFA, in addition to having metabolic effects, decreases bone resorption by inhibiting osteoclast differentiation and thus lowering bone turnover.[34,57] Studies have shown that biochemical markers of bone turnover are lower in adults with obesity compared with normal weight.[58,59] The lower bone turnover may contribute to the seemingly higher BMD in obesity.[58]

The alteration in the gut microbiome we observed 6 mo after LSG is consistent with previous reports.[60–62] We found an overall decrease in *Firmicutes* with a trend toward an increase in *Bacteroidetes* phyla without a change in microbial richness 6 mo after LSG. Reportedly, the gut microbiome profile following LSG approaches that of lean individuals and is associated with favorable metabolic outcomes.[63–65] The specific pattern of microbiota changes is unique to LSG compared to RYGB and dietary weight loss intervention.[61,66,67] Possible mechanisms for LSG-induced changes in the gut microbiome include postoperative dietary changes, altered gastrointestinal function and gastric acidity, and hormonal changes. LSG not only leads to decreased food intake but also changes in food choice and frequency of intake. It is known that diet influences the structure and activity of the human gut microbiome rapidly.[68] In addition, the gut microbiome can be shaped by the altered intestinal lumen environment of LSG, including accelerated gastric emptying, increased intestinal motility, and diminished gastric acid production.[69–71] Studies have also suggested that the changes in hormones such as leptin and ghrelin may influence the postoperative gut microbiome, although this is not fully understood.[72,73]

Six months after LSG, serum bone resorption marker CTx increased by a median of 188.4% and bone formation marker P1NP increased by a median of 61.0%, while DXA-derived areal BMD decreased 5.0% at the total hip and 3.3% at the femoral neck. Many factors likely contribute to BMD loss at the hip after LSG, such as mechanical unloading, hormonal changes, and changes in nutritional factors (vitamin D deficiency, decreased calcium absorption). In the present study, we found that participants with greater postoperative changes in microbial community composition (Bray-Curtis dissimilarly measure) had greater increases in P1NP levels and greater bone loss at the femoral neck. While additional studies demonstrating causality are needed, our correlative findings suggest that gut microbiome alteration may be another contributing factor to LSG's skeletal effects.

The reduction in the gut microbial community's capacity to produce SCFA is a potential mechanism for bone metabolism effects of LSG. After LSG, there was an overall

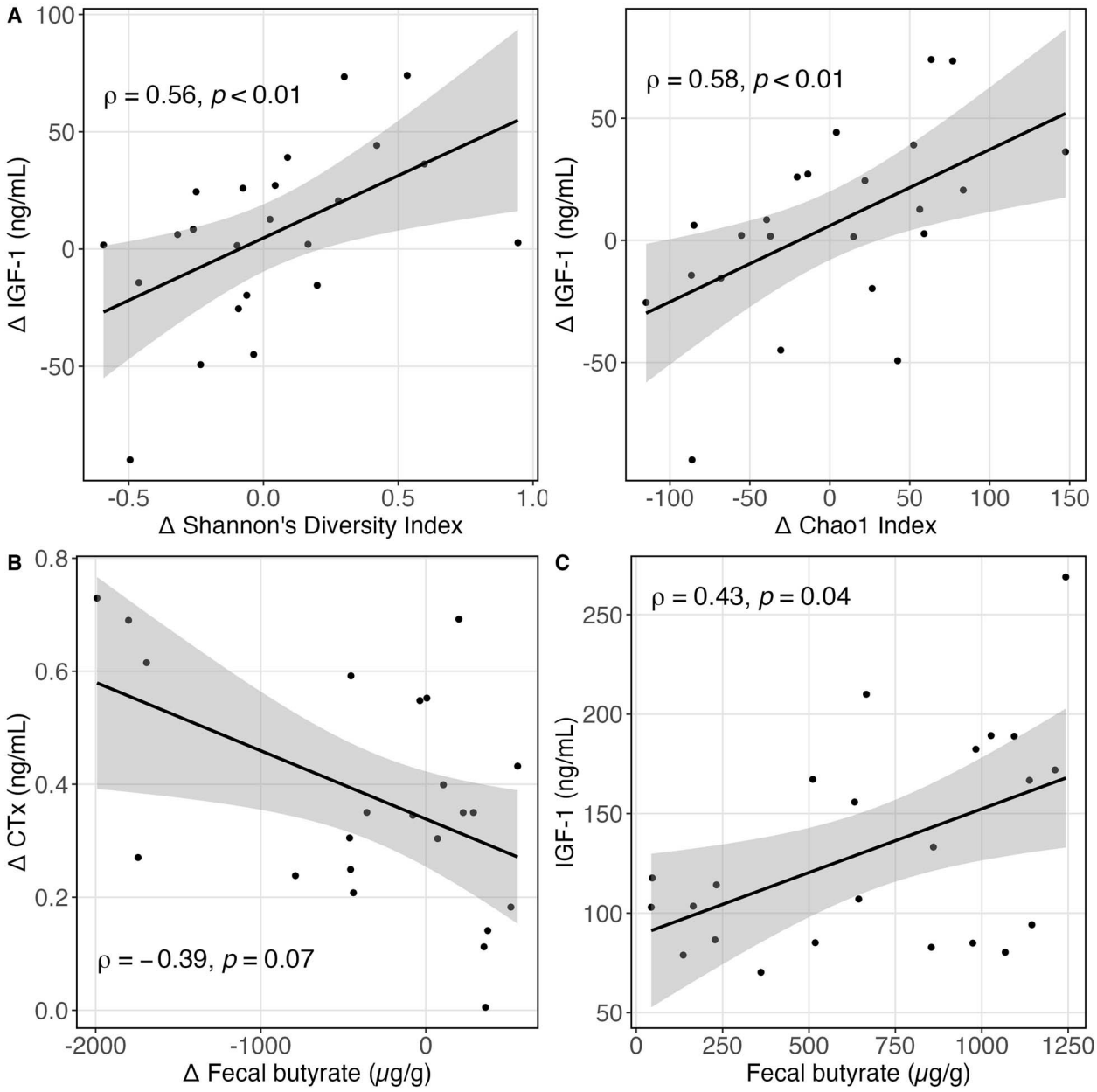



**Figure 3.** Spearman's rank correlations between 6-mo postoperative (A) Δ $\alpha$-diversity measures (Shannon's diversity index on left, Chao1 index on right) and Δ IGF-1 levels, (B) Δ fecal butyrate concentrations and Δ CTx levels, and (C) fecal butyrate levels and IGF-1 levels. The shaded areas represent the 95% confidence intervals for predictions from a linear model.

reduction in the phylum *Firmicutes* and a notable decrease in *Bifidobacterium spp*. The main SCFA-producing bacteria in the human gut belong to the phylum *Firmicutes*, and *Bifidobacterium spp* are known producers of acetate and lactate, which can be converted into butyrate by other colon bacteria through cross-feeding interactions.[74] Although we did not detect a differential predicted enrichment of microbial genes in SCFA metabolism pathways or find a statistically significant decrease in fecal SCFA concentrations, we found that participants with greater postoperative reduction in fecal butyrate concentration had higher serum levels of CTx after LSG. Concordantly, those with the lowest postoperative abundance of *Lachnospiraceae spp*, which are among the main SCFA producers in the human gut, had greater femoral neck BMD loss. A decrease in abundance of *Lachnospiraceae* has been associated with osteoporosis in older adults.[37,75–77] Given SCFAs' inhibitory effects on osteoclastogenesis and anti-inflammatory capacities,[34,57,78] a reduction in levels may lead to higher bone turnover and more bone loss. It should be noted the limitations with the methods of estimating SCFA production: (1) PICRUSt2 and any amplicon-based functional prediction analysis have limited resolution to distinguish strain-specific metabolic pathways and tend to underestimate metabolic diversity and (2) fecal SCFA concentrations reflect a net outcome of the difference between production and absorption, not a direct indicator of intestinal SCFA production.

SCFA have also been shown to modulate levels of hormones including IGF-1, which promotes bone growth and remodeling.[35,79] Yan and colleagues[80] showed that colonization of adult germ-free mice with microbiota and SCFA supplementation of antibiotic-treated mice both lead

to higher IGF-1 levels, suggesting that microbiota-derived SCFA increase IGF-1 levels to promote skeletal growth and development. Consistent with Yan and colleagues, we found a positive association between fecal butyrate and serum IGF-1 levels after LSG. In addition, participants with greater increases in microbial diversity postoperatively had greater increases in IGF-1. Therefore, we postulate that LSG-induced alterations in gut microbial community structure and its ability to ferment complex nondigestible carbohydrate lead to a decline in SCFA production that can impact bone turnover directly and indirectly via IGF-1.

Strengths of our study include its prospective longitudinal design and its comprehensive assessment of bone and calcium homeostasis. Our cohort was representative of the target patient population undergoing bariatric surgery in the United States. A limitation of our study is the small sample size with heterogeneity in terms of sex, age, menopausal status, and race/ethnicity. The study is limited by its 6-mo duration and the absence of a nonsurgical control group. In addition, 16S rRNA amplicon-based microbiota profiles have limited resolution, are reported as relative rather than absolute quantitation, and do not provide insights into microbial functions. Follow-up studies using strategies to assess microbial function would likely provide further insights into the relationships observed in this study.

In conclusion, this hypothesis-generating, exploratory study suggests that alteration in the gut microbiome may be one factor contributing to the negative skeletal consequences of LSG. A potential mechanism is the reduction in the gut microbial capacity to produce SCFA, which can have direct and indirect effects on bone turnover via IGF-1. Our findings thus offer unique insights into the gut-bone axis. Further investigation into the underlying mechanisms and the development of multifaceted prevention strategies are crucial to mitigate the negative skeletal consequences of LSG.

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## Supplementary material

Supplementary material is available at *Journal of Bone and Mineral Research* online.

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## Conflicts of interest

ALS has received investigator-initiated research funding from Amgen. The other authors have nothing to disclose.

## Data availability

The data that support the findings of the current study are not publicly available but are available from the corresponding author on reasonable request.

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