Roux-en-Y Gastric Bypass Surgery But Not Vertical Sleeve Gastrectomy Decreases Bone Mass in Male Rats

Kerstin Stemmer, Maximilian Bielohuby, Bernadette E. Grayson, Denovan P. Begg, Adam P. Chambers, Christina Neff, Stephen C. Woods, Reinhold G. Erben, Matthias H. Tschöp, Martin Bidlingmaier, Thomas L. Clemens, and Randy J. Seeley

Institute for Diabetes and Obesity (K.S., C.N., M.H.T.), Helmholtz Centre Munich, 85764 Neuherberg, Germany; Endocrine Research Unit (M.Bie., M.Bid.), Medizinische Klinik und Poliklinik IV, Klinikum der Ludwig-Maximilians-Universität München, 80336 Munich, Germany; Metabolic Diseases Institute (B.E.G., D.P.B., A.P.C., S.C.W., R.J.S.), University of Cincinnati, Cincinnati, Ohio 45237; Department of Biomedical Sciences (R.G.E.), University of Veterinary Medicine Vienna, 1210 Vienna, Austria; and Department of Orthopaedic Surgery (T.L.C.), Johns Hopkins University School of Medicine, Baltimore, Maryland 21287

The most effective treatment for obesity is bariatric surgery. However, there is increasing concern that bariatric surgery can cause nutrient deficiencies that translate into metabolic bone disease. Whether this is true for all surgery types is not yet clear. We therefore investigated the effects of 2 commonly applied bariatric surgeries (Roux-en-Y gastric bypass [RYGB] and vertical sleeve gastrectomy) on energy and bone metabolism in rats 60 days after surgery. Both surgeries resulted in similar reductions of body weight, body fat, and food intake. Glucose tolerance was improved to a similar extent after both surgeries and was accompanied by increased postprandial secretion of glucose-dependent insulinotropic peptide. Using microcomputed tomography, we found that, relative to sham-operated rats, bone volume was significantly reduced after RYGB but not vertical sleeve gastrectomy. RYGB rats also had markedly reduced lipid absorption from the intestine and significantly lower serum 25-hydroxyvitamin D and calcium levels. Importantly, dietary supplementation with calcium and vitamin D could not fully rescue the reduced bone volume after RYGB surgery. Both surgeries resulted in a significant increase in stomach pH, which may have worsened the malabsorption in RYGB rats. Our findings suggest that bone loss in RYGB rats is not exclusively driven by calcium and vitamin D malabsorption but also by additional factors that may not be rescuable by dietary supplementation. These data point toward important similarities and differences between bariatric procedures that should be considered in clinical settings as guidance for which procedure will be best for specific patient populations. **(*Endocrinology* 154: 2015–2024, 2013)**

Obesity and its comorbidities, such as type 2 diabetes, hypertension, dyslipemia, and chronic kidney disease, are health problems of the first order and currently linked to more worldwide deaths than inadequate calorie supply (1). Lifestyle interventions such as hypocaloric diets and increased exercise fail to produce sustained weight loss. Even current pharmacological therapies produce weight loss that is substantially less than what is achieved with bariatric procedures (2–4). In contrast, bariatric surgery results in greater weight loss that is far more durable than other available therapies.

Roux-en-Y gastric bypass (RYGB) surgery is currently the most commonly performed bariatric surgery type in the United States (5). During RYGB, the size of the stom-

ISSN Print 0013-7227 ISSN Online 1945-7170
Printed in U.S.A.

Received November 13, 2012. Accepted March 29, 2013.
First Published Online April 3, 2013

Abbreviations: BMD, bone mineral density; BV/TV, bone volume over total volume; μCT, microcomputed tomography; FGF, fibroblast growth factor; GI, gastrointestinal; GIP, glucose-dependent insulinotropic peptide; HFD, high-fat diet; 25(OH_2)D, 25-hydroxyvitamin D; P1NP, propeptide of type 1 procollagen; RYGB, Roux-en-Y gastric bypass; VSG, vertical sleeve gastrectomy.

ach is reduced to a small pouch, the remainder is connected to the midjejunum, and the pancreaticobiliary flow is delivered to the distal jejunum. RYGB surgery has been hypothesized to combine elements that restrict the amount of food that can be consumed and reduce the intestinal surface area available for nutrient absorption (6).

RYGB is associated with improvements in a wide range of obesity-related comorbidities. This includes a 40% remission rate in patients with type 2 diabetes 1 year after surgery (7). Despite substantial weight loss and metabolic improvements after RYGB surgery, there is growing clinical evidence that it causes abnormalities in bone metabolism. For instance, retrospective studies reveal increased bone resorption and reduced bone mineral density (BMD) after RYGB (8, 9). One suggested mechanism to account for this finding is malabsorption of vitamin D from the manipulated intestine. Subsequent impairments in calcium absorption followed by secondary hyperparathyroidism could then cause increased calcium mobilization from the bones leading to decreased BMDs (10–12). However, quantification of vitamin D in plasma samples from RYGB patients has resulted in conflicting findings of either decreased (10), similar, or even increased (13, 14) vitamin D levels after RYGB. Confounding factors include a high variation in vitamin D plasma levels in obese patients, even without receiving surgery (15–17). Also, the lack of standard approaches for micronutrient supplementation and postoperative evaluation, as well as differences in eating behavior after surgery (18), make it difficult to demonstrate a causal link between bariatric surgery and the development of bone diseases.

A relatively novel, less invasive bariatric surgical technique is vertical sleeve gastrectomy (VSG). In VSG, 80% of the stomach is removed along the greater curvature, whereas the intestinal tract remains unaltered (19). Importantly, RYGB and VSG result in similar changes in gut hormone secretion in humans (20) and rodents (21) and similar metabolic rates of type 2 diabetes remission in humans (7). Notably, 2 recent studies suggested decreased BMD in patients after VSG (22, 23). However, a systematic comparison of effects of both surgery types on bone density has not been done in experimental models.

The use of animal models of bariatric surgery would facilitate comparison of alternative surgical techniques in terms of both metabolic benefits and potential adverse effects under defined experimental conditions. In this study, we compared bone loss in cohorts of rats that underwent VSG, RYGB, or sham surgery. We found a significant decrease in bone volume after RYGB, and this did not occur after VSG. Decreased bone volume in RYGB rats was accompanied by significantly decreased levels of serum calcium and vitamin D. Importantly, dietary supplementation normalized calcium and vitamin D serum levels but did not rescue the decreased bone volume in RYGB compared with sham-operated rats. Overall, our data suggest similar metabolic improvements after RYGB and VSG but a significantly lower risk of bone loss in VSG-operated rats.

Materials and Methods

Animals

Male Long-Evans rats (250–300 g, 8–10 wk of age; Harlan Laboratories, Indianapolis, Indiana) were single housed at the Metabolic Diseases Institute of the University of Cincinnati under standard controlled conditions. All animal experiments were performed in compliance with the University of Cincinnati Institutional Animal Care and Use Committee. To cause overweight and metabolic impairments, rats were ad libitum fed with high-fat diet (HFD) (40% fat; 4.54 kcal/g, D03082706; Research Diets, New Brunswick, New Jersey) for 8 weeks. Three days before surgery, rats were matched for body weight and fat mass and assigned to RYGB, VSG, RYGB sham, and VSG sham surgical groups.

Surgical procedures

VSG, RYGB, and respective sham surgeries were conducted as described previously (21, 24). Briefly, for RYGB surgery, the stomach was divided into an upper small pouch that receives food from the esophagus, and a larger distal portion, physically separated from the pouch that remained connected to the duodenum. The jejunum was then transected 30 cm beyond the suspensory muscle of the duodenum (ligament of Treitz). The distal end of the transected jejunum was then anastomosed to the new pouch, whereas the remaining proximal intestine was anastomosed to the jejunum distal to the initial transection. After surgery, food absorption from the intestine begins at the anastomosis of the jejunum and the pouch, whereas the remainder of the stomach and the duodenum are bypassed. For VSG surgery, the lateral 80% of the stomach was excised using an ETS 35-mm staple gun, leaving a tubular gastric remnant in continuity with the esophagus superiorly and the pyloric sphincter and duodenum inferiorly. For the RYGB sham surgery, the jejunum was transected and reanastomosed. For VSG sham surgery, the stomach was isolated, followed by manually applying pressure with blunt forceps at a vertical line starting at the esophagus superiorly and ending at the duodenum inferiorly. For all experiments, equal numbers of rats that received either RYGB sham or VSG sham surgery were combined to one group, as no differences in any parameters measured were detected between the groups.

Postoperative care

All surgical groups received postoperative care consisting of sc injections of Metacam (0.25 mg/100 g body weight once daily for 4 d), gentamicin (0.8 mg/100 g body weight on the day of surgery), Buprenex (0.3 mL twice a day for 5 d), and warm saline (10 and 5 mL twice daily for d 0–3 and 4–5, respectively). During the 5 days of postoperative care, rats had free access to Osmolite OneCal liquid diet (Abbott, Columbus, Ohio) until they were switched back to a solid diet.

Dietary regimen

The first experimental group included sham (n = 9)-, VSG (n = 8)-, and RYGB (n = 7)-operated rats, which were kept on HFD until the end of experiment, 60 days after surgery. Additional sham (n = 10)- and RYGB (n = 10)-operated rats of the same surgical cohort were switched to an isocaloric HFD, modified by the addition of 2200-IU vitamin D per kg, 2% calcium (calcium phosphate and calcium carbonate), 1.25% phosphorus, and the replacement of 20% of carbohydrates from corn starch, maltodextrin 10, and sucrose with lactose (D03082706; Research Diets) and were kept on this diet until day 60 after surgery. This "rescue diet" was previously documented to normalize calcium homeostasis in vitamin D receptor mutant mice (25).

Body composition and food intake

Body weight was recorded daily from the day of surgery for 2 weeks. From day 14 until the day of killing, rats were weighed every fifth day. Food intake was recorded daily starting at day 5 after surgery, after switching the animals back from liquid diet to solid HFD. Magnetic resonance imaging was performed 3 days before surgery and 5 days before killing to determine body composition using a whole-body composition analyzer (EchoMedical Systems, Houston, Texas).

Mixed meal tolerance test

Rats (n = 7–10) were fasted for 6 hours, and baseline glucose levels (0 min) were measured with a hand-held glucose analyzer (Accu-Chek; Roche Diagnostics, Indianapolis, Indiana) in all groups. All animals were subsequently gavaged with 2.5-mL (3.7 kcal) Ensure Plus liquid diet (21). Blood glucose was again measured at 15, 30, 60, and 120 minutes. At the 0- and 15-minute time points, 180-μL blood was collected into tubes containing 20-μL antiproteolytic cocktail (21). Glucose-dependent insulinotropic peptide (GIP) was measured using an ELISA (Meso Scale Discovery, Gaithersburg, Maryland) according to the manufacturer's instructions.

Intestinal lipid absorption

Dietary lipid absorption from the intestine was assessed as described previously (26). Briefly, rats (n = 7–9) were temporarily placed on a HFD containing 5% sucrose polybehenate (behenic acid). Fecal lipid content was assayed by gas chromatography of fatty acid methyl esters. Fat absorption was calculated from the ratio of behenic acid to other fatty acids in the diet and feces.

Killing and sample collection

Eight weeks after surgery, rats were fasted for 6 hours and then killed by decapitation after CO_2 anesthesia. Trunk blood was collected for serum analysis. Femurs were carefully removed without injuring the acetabulofemoral joint and cooled on ice for later sample processing.

Gastrointestinal (GI) pH measurement

For direct measurement of the stomach pH, immediately after killing, a small cut was made into the center of the intact stomach (sham animals), the center of the sleeve (VSG), or the gastric pouch (RYGB). An electrode was inserted, and pH measures were taken. Similarly, pH was assessed in the upper duodenum (sham and VSG rats) or the bypassed duodenum and the jejunum connected to the gastric pouch (RYGB rats).

Microcomputed tomography (μCT) analysis

Rat femurs were dissected free of soft tissue, fixed in 10% formalin, and scanned using a desktop microtomographic imaging system (SkyScan 1172; SkyScan, Kontich, Belgium) in accordance with the recommendations of the American Society for Bone and Mineral Research (27). The femur was scanned at 50 keV and 200 mA using a 0.5-mm aluminum filter with an isotropic voxel size of 10 μm. The resulting 2-dimensional cross-sectional images are shown in gray scale.

Serum parameters

Handling and storage of serum samples has been performed as previously recommended (28). IGF-I, 25-hydroxyvitamin D (25[OH_2]D), β-CrossLaps, aminoterminal propeptide of type 1 procollagen (P1NP); parathyroid hormone (rat intact PTH, Immunotropics, San Clemente, California); leptin (Alpco Diagnostics, Salem, New Hampshire); and fibroblast growth factor (FGF)21 (Merck Millipore, Darmstadt, Germany) were measured in serum samples with commercially available kits according to the manufacturer's instructions. Total serum calcium, phosphate, high density lipoprotein cholesterol, triglycerides, and albumin were measured using an automated system (Cobas Integra 800; Roche Diagnostics, Mannheim, Germany), as described previously (29).

Statistical analyses

All data are expressed as mean ± SEM. Body weight, fat and lean mass, food intake, and glucose excursions after the mixed meal tolerance test were analyzed via 2-way ANOVA (variables: treatment and time) with a Sidak's multiple comparisons test. Statistical differences between surgical groups of all other measurements were analyzed using 1-way ANOVA followed by a Tukey post hoc test. Analyses were done using GraphPad Prism 6.0 software (GraphPad, San Diego, California). $P < .05$ was considered statistically significant.

Results

RYGB and VSG lead to similar body weight reductions and metabolic improvements in rats

After 8 weeks of HFD, weight- and fat-matched groups of dietary obese rats were subjected to either RYGB, VSG, or sham surgery. Body weights were taken before and continuously after surgery, to compare the efficacy of both surgeries. During the first 10 days of surgical recovery, all surgical groups (sham, VSG, and RYGB) lost significant body weight. During this time, RYGB- and VSG-operated rats displayed a similar but more pronounced reduction in body weight than the sham-operated controls. After 10 days, all groups started to regain body weight while on the HFD until the end of the experiment, 60 days after surgery. The weight regain up to day 60 was significantly lower for

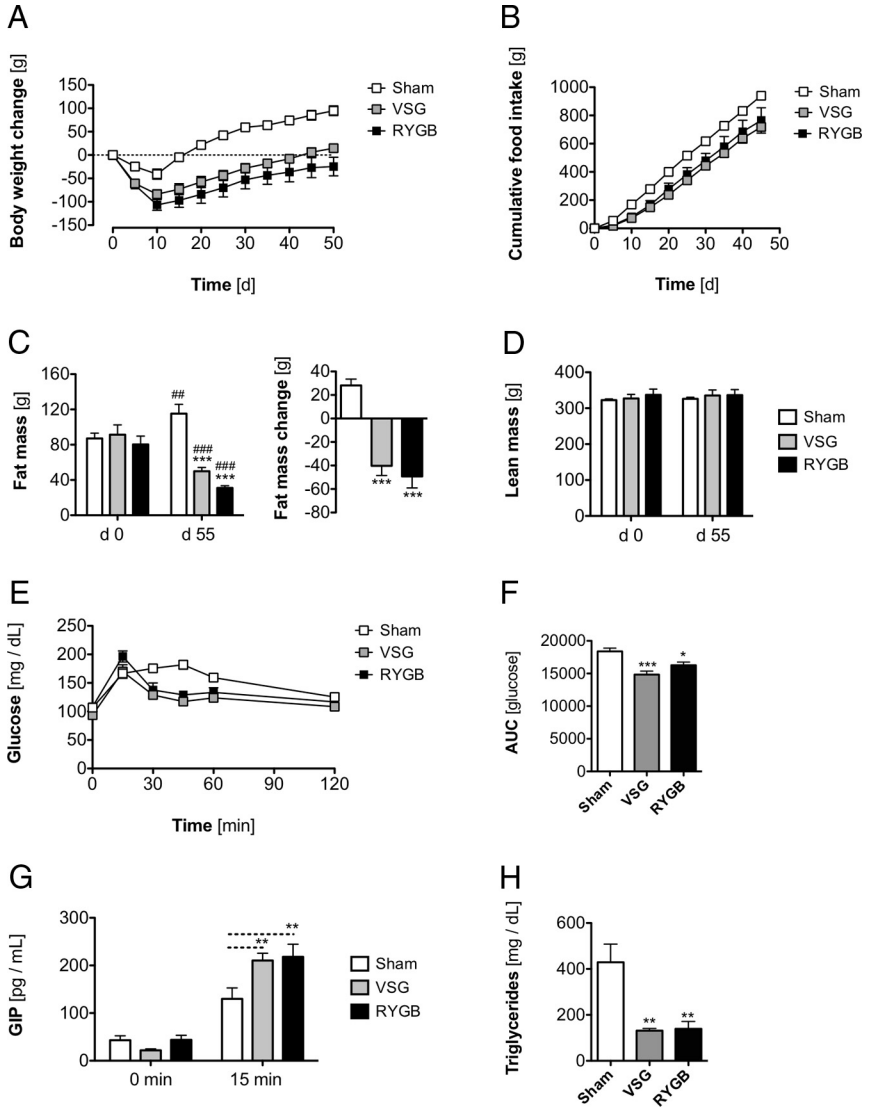

Figure 1. RYGB and VSG lead to similar body weight reductions and metabolic improvements in rats. (A) Similar reductions in bodyweight after VSG (***$P < .001$) and RYGB (***$P < .001$) surgery compared with sham-operated controls. (B) Average cumulative food intake was higher in sham-operated rats vs RYGB vs VSG (***$P < .001$) and RYGB (**$P < .01$). (C) (Left panel) Fat tissue mass was significantly increased in sham-operated rats in response to 55 days of HFD feeding (##$P < .01$) but decreased in RYGB (###$P < .001$) and VSG (###$P < .001$) rats. Accordingly, at day 55, fat tissue mass was substantially lower in RYGB and VSG rats compared with the sham controls (***$P < .001$). (Right panel) Similar and significant fat mass change after RYGB (***$P < .001$) and VSG (***$P < .001$). (D) No significant changes in lean tissue mass were observed in any treatment group. (E) Blood glucose levels before (time 0) and after an oral mixed meal tolerance test indicate that RYGB- and VSG-operated rats had similar but significantly lower glucose excursions compared with the sham-operated controls (*$P < .05$ for RYGB rats; ***$P < .001$ for VSG rats). (F) Area under the curve (AUC) for plasma glucose across the 2-hour glucose tolerance test showed a significant decrease in RYGB-operated (*$P < .05$) and VSG-operated (***$P < .001$) rats vs sham controls. (G) Fifteen-minute glucose-dependent insulinotropic peptide (GIP) excursions after the mixed meal tolerance test: RYGB and VSG produced similar increases in postprandial GIP release that were much greater than changes in sham-operated rats (**$P < .01$). (F) Significantly improved levels of serum triglyceride after VSG and RYGB surgery (**$P < .01$).

both RYGB and VSG rats, compared with the sham controls (Figure 1A). Body weight reduction in RYGB and VSG rats was accompanied by a significant reduction of cumulative food intake. However, there was no difference in food intake between RYGB and VSG rats at any time throughout the experiment (Figure 1B). Body weight changes were reflected by parallel changes in adipose tissue mass. Although sham-operated rats continued to gain body fat on HFD, VSG- and RYGB-operated rats had similarly significant reductions in their adipose tissue mass 55 days after surgery (Figure 1C). Lean mass was not affected by either surgical intervention (Figure 1D). In addition, both types of bariatric surgery improved glucose tolerance after the ingestion of a mixed meal, as indicated by the lower glucose excursions 15, 30, and 60 minutes after the gavage (Figure 1, E and F). Investigation of postprandial plasma glucose-dependent insulinotropic peptide (GIP) levels before and 15 minutes after the intragastric mixed meal revealed a similar and significant increase in VSG and RYGB rats compared with the sham controls (Figure 1G). Furthermore, serum triglycerides (Figure 1H) were significantly decreased in VSG and RYGB rats compared with levels in sham controls. In summary, these findings confirm that RYGB and VSG result in substantial and comparable metabolic improvements in this paradigm.

RYGB but not VSG leads to decreased bone volume

Distal femoral bone volume (expressed as bone volume over total volume [BV/TV]) was similar in sham- and VSG-operated rats. In contrast, RYGB-operated rats had a 50% reduction of their bone volume at day 60 after surgery compared with the sham controls (Figure 2, A and B). Cancellous bone osteopenia was associated with a decrease in trabecular thickness (Figure 2C) and trabecular number (Figure 2D), as well as with an increase in trabecular separation (Figure 2E). To assess the effects of bariatric surgery on bone turnover, we next analyzed gene expression in bone marrow

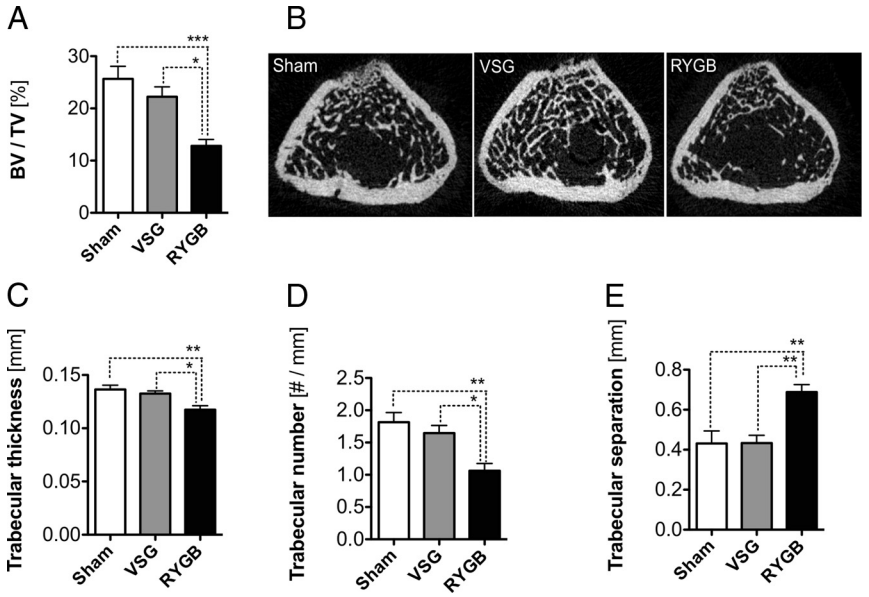

Figure 2. RYGB but not VSG surgery leads to decreased bone densities. (A) Quantitative μCT demonstrated a significant decrease in BV/TV in RYGB rats compared with VSG rats (*$P < .05$) and the sham-operated group (***$P < .001$). (B) Representative μCT images of the trabecular bone structure in sham-, VSG-, and RYGB-operated groups. (C) Trabecular thickness was decreased in RYGB rats compared with sham-operated (**$P < .01$) and VSG-operated (*$P < .05$) rats. (D) Decreased trabecular number in RYGB-operated compared with sham-operated (**$P < .01$) and VSG-operated (*$P < .05$) rats. (E) Increased trabecular separation in RYGB rats compared with sham-operated (**$P < .01$) and VSG-operated (**$P < .01$) rats.

and biochemical bone markers in serum from animals in each surgery group. Gene expression analyses only revealed trends towards a higher bone resorption and lower bone formation in RYGB rats (Supplemental Figure 1, published on The Endocrine Society's Journals Online web site at http://endo.endojournals.org). However, the analysis of serum markers showed significantly increased serum levels of the bone resorption marker β-CrossLaps in RYGB but not VSG rats, whereas the bone formation marker P1NP was not significantly different in animals from each group (Table 1). IGF-I, another important regulator of bone metabolism, was slightly but significantly decreased in VSG rats compared with the sham controls. However, IGF-I levels were much lower in RYGB rats and significantly different from levels in both sham and VSG rats (Table 1). Similarly, RYGB but not sham or VSG rats had significantly decreased serum albumin. In contrast, FGF21, a key mediator of the response to fasting and important regulator of lipolysis (30–33), which was recently discovered to be involved in bone mass loss (34), did not change in response to any surgery, as indicated by similar serum concentrations (Table 1) and similar expression levels in the liver (Supplemental Figure 1A). Serum leptin levels were decreased by a similar extent after RYGB and VSG. Importantly, RYGB but not VSG rats had a small but statistically significant decrease in serum calcium and 25(OH_2D) levels, compared with sham controls and VSG rats, indicating an impairment of their vitamin D and calcium metabolism (Table 1). In contrast, no significant changes were detected for serum phosphate. PTH measures were taken. However, interindividual variation within the surgical groups was too high to make firm conclusions. Together, these findings suggest that RYGB surgery leads to an imbalance in calcium and vitamin D homeostasis, which could be a result of impaired nutrient absorption from their manipulated GI tract.

Table 1. Serum Markers of Bone Turnover

	Sham	VSG	RYGB
β-CrossLaps (ng/mL)	26.16 ± 5.51	30.97 ± 3.45	54.78 ± 8.28[ad]
P1NP (ng/mL)	1.92 ± 0.23	2.38 ± 0.25	1.93 ± 0.15
IGF-I (ng/mL)	1283 ± 55.0	1050 ± 26.6[a]	870.7 ± 66.9[cd]
Albumin (g/dL)	4.13 ± 0.16	3.89 ± 0.08	3.06 ± 0.16[ce]
FGF21 (pg/mL)	564.7 ± 96.4	274.0 ± 62.0	427.1 ± 114.2
Leptin (pg/mL)	908.6 ± 253.8	172.6 ± 40.9[b]	32.2 ± 7.5[b]
Calcium (mmol/L)	2.98 ± 0.03	2.97 ± 0.04	2.81 ± 0.08[ad]
Phosphate (mg/dL)	8.30 ± 0.43	8.86 ± 0.212	8.36 ± 0.30
25(OH_2)D (ng/mL)	9.31 ± 0.63	11.24 ± 0.820	6.52 ± 0.88[ae]
PTH (pg/mL)	1410 ± 279.1	1713 ± 657.9	1183 ± 151.3

Data are presented as mean ± SEM. Differences between groups were calculated by 1-way ANOVA with Bonferroni's post hoc test.

[a] Significantly different vs sham-operated controls ($P < 0.05$).

[b] Significantly different vs sham-operated controls ($P < 0.01$).

[c] Significantly different vs sham-operated controls ($P < 0.001$).

[d] Significantly different vs VSG-operated rats ($P < 0.05$).

[e] Significantly different vs VSG-operated rats ($P < 0.01$).

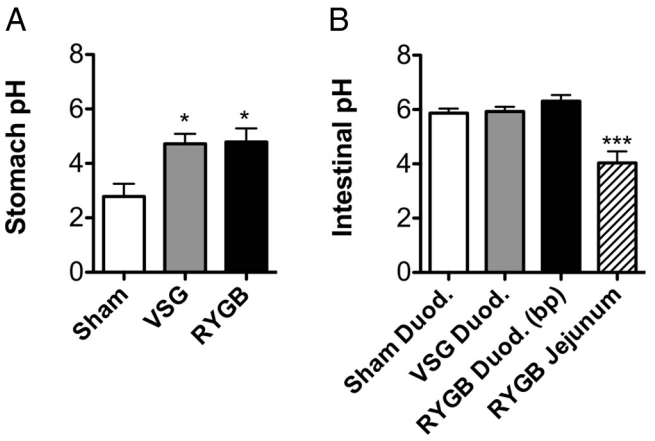

Figure 3. Impaired gastric acidification after RYGB and VSG surgery. (A) Increased stomach pH in VSG- and RYGB-operated rats compared with sham-operated controls (*$P < .05$). (B) pH measures in the bypassed (bp) duodenum (duod.) of RYGB rats after 6 hours of fasting were similar to the duodenal pH of VSG- and sham-operated rats. pH in the jejunal segment reconnected to the gastric pouch was significantly lower than in the duodena of all groups (***$P < .001$).

Impaired gastric acidification after RYGB and VSG surgery

Calcium absorption from the intestine is influenced by the stomach pH, whereby an acidic pH facilitates ion bonding and absorption in the intestine (35). However, no studies have addressed whether surgical manipulation of the GI tract alters gastric intestinal pH. First, we compared stomach pH in the remaining sleeve (for VSG rats), in the gastric pouch (for RYGB rats), or in the center of the intact stomach of the sham-operated group. After 6 hours of fasting, sham rats had a pH at an expected physiological level of 2.79 ± 0.47 (36). Stomach pH was significantly higher in RYGB (4.8 ± 0.50) and VSG rats (4.7 ± 0.37) (Figure 3A). In contrast, pH in the duodenum of sham and VSG rats, as well in the bypassed duodenum of RYGB rats, was in the range of 6.0 and did not differ among groups (Figure 3B). However, pH in the jejunum (which is directly connected to the gastric pouch) of RYGB rats was significantly lower than that in the duodenum distal to the pylorus of all other groups and was similar to the pH in the RYGB gastric pouch (4.03 ± 0.42) (Figure 3B). Together, these findings suggest that RYGB rats lack a physiological pH gradient in the upper GI tract, which may impair passive calcium absorption by the intestine.

RYGB leads to nutrient malabsorption

Besides being absorbed by passive diffusion, calcium is also taken up via active transport in the intestine, a process that is regulated by vitamin D. In addition, vitamin D is also absorbed from the intestine to provide the correct balance of calcium and phosphorus to support bone mineralization (37). Owing to its fat-soluble nature, vitamin D absorption mainly depends on the presence of dietary lipids, and pharmacological blockage of lipid absorption significantly impairs vitamin D absorption (38). We therefore compared intestinal lipid absorption in our cohort of RYGB-, VSG- and sham-operated rats as a surrogate measurement for vitamin D uptake. Although sham and VSG rats had similar percentages of intestinal lipid absorption (82% for sham and 84% for VSG rats), the intestinal absorption of dietary fat was significantly decreased in RYGB rats (Figure 4A). Indeed, circulating 25(OH_2)D, the most commonly used index of vitamin D status, was significantly reduced in the serum of RYGB rats compared with VSG rats or sham controls (Table 1). Together, these findings suggest that vitamin D malabsorption could contribute to bone loss in RYGB rats.

Dietary supplementation increases serum levels of calcium and 25(OH_2)D but does not fully rescue bone density loss in RYGB rats

Our data suggest that an impaired calcium and vitamin D absorption could be involved in bone loss in RYGB rats. If malnutrition is indeed the initiating factor for loss of bone mass after RYGB surgery, it should be possible to rescue the phenotype by supplementing the diet with calcium and vitamin D. Although dietary supplementation did not affect body weight, food intake, glucose tolerance, or triglyceride levels (Supplemental Figure 2), it resulted in an overall increase of serum 25(OH_2)D levels in supplemented sham and RYGB rats compared with the respective nonsupplemented groups (Figure 4B). Similarly, circulating calcium levels were no longer different between the surgical groups (Figure 4C). Although dietary supplementation produced a nonsignificant trend towards higher bone volume in RYGB rats, supplementation could not reverse the detrimental effect of RYGB on bone volume and structure (Figure 4, D–G). Similar serum levels of β-CrossLaps (Figure 4H) in supplemented and nonsupplemented animals were consistent with this finding. Together, these findings suggest that factors other than malabsorption contribute to bone loss in RYGB rats.

Discussion

Bariatric surgical procedures have become the therapy of choice for many severely obese individuals, including those suffering from type 2 diabetes (5, 39). Although RYGB and VSG surgeries are commonly applied, the mechanisms underlying the broad spectrum of metabolic improvements and potential adverse effects are poorly understood. In the present study, we directly compared RYGB and VSG surgery using a rat model of HFD-induced obesity. We demonstrate that both surgeries result

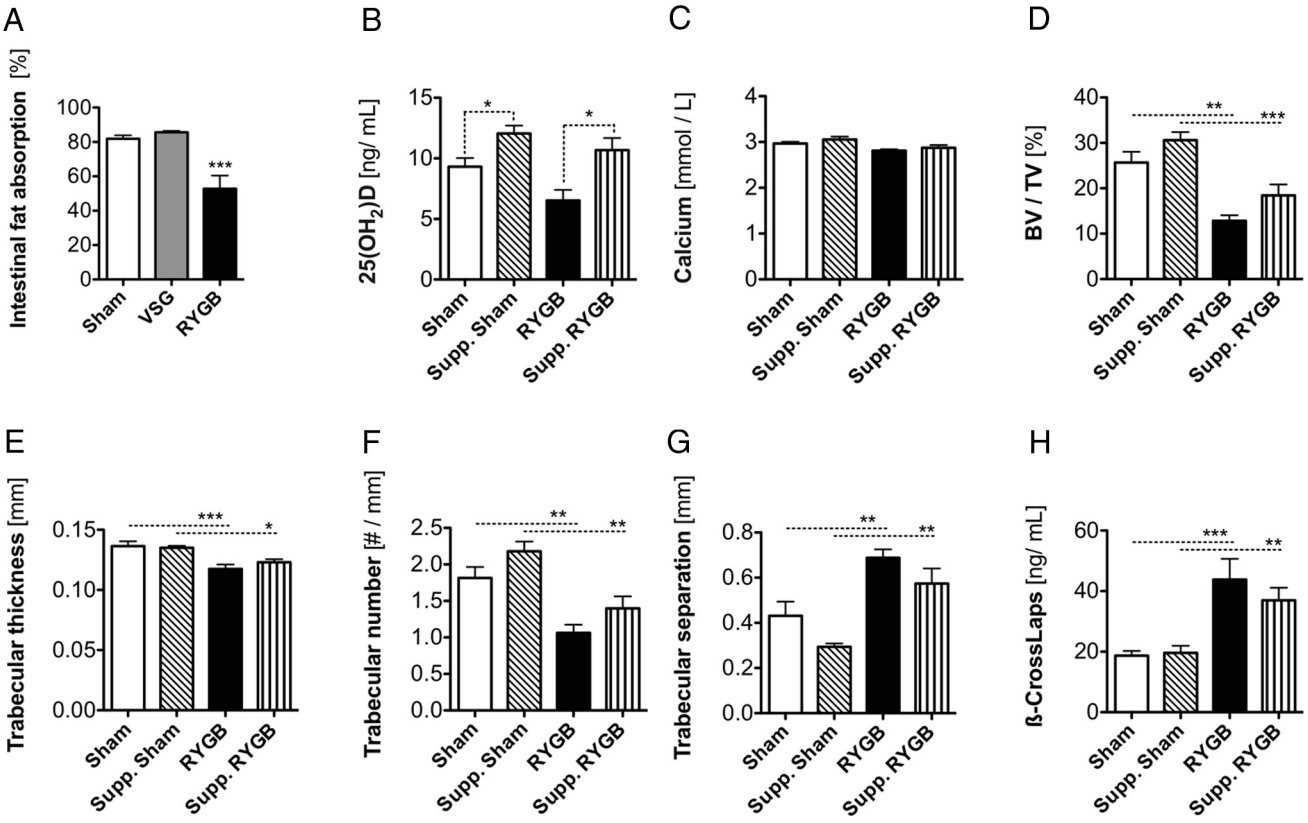

Figure 4. RYGB surgery leads to nutrient malabsorption. (A) RYGB significantly reduced intestinal lipid absorption compared with sham- and VSG-operated rats (***$P < .01$). (B) Dietary supplementation with calcium, vitamin D, phosphate, and lactose resulted in increased serum levels of 25(OH_2)D in RYGB- and sham-operated rats (*$P < .05$) and blunted the decreased serum 25(OH_2)D levels in RYGB rats. (C) Serum calcium levels tended to be increased after dietary supplementation. (D) Dietary supplementation resulted in a slight overall increase of BV/TV but did not rescue the significant decrease (***$P < .001$) in RYGB- vs sham-operated rats. (E) Significantly decreased trabecular thickness in RYGB vs sham operated rats (**$P < .01$) was still abundant after dietary supplementation (**$P < .01$). (F) Dietary supplementation resulted in a slightly increased overall trabecular number per millimeter (#/mm) but did not rescue the significant difference between sham and RYGB surgery (**$P < .01$). Trabecular separation (G) and β-CrossLaps (H) were significantly increased in RYGB- vs sham-operated rats (**$P < .01$), despite dietary supplementation.

in a similar decrease in body weight, fat mass, and cumulative food intake, as well as improved glucose tolerance and serum lipids. In contrast to the comparable beneficial effects of both surgeries, our data demonstrate that RYGB but not VSG results in a significant loss of cancellous bone volume that could not be rescued by dietary supplementation. The increased serum levels of the bone resorption marker β-CrossLaps, together with the unchanged levels of the bone formation marker P1NP, suggest that RYGB-induced bone loss is caused by an imbalance between increased bone resorption and unchanged formation.

BMD and bone metabolism are influenced by age, genetic, mechanical, endocrine, and nutritional factors (40). The current rat model allows us to control for many of these factors by using rats of the same age and genetic background. The finding of similar metabolic improvements in RYGB and VSG rats further excludes several possible factors that may have contributed to the broad difference in bone mass. For instance, body weight, which provides a mechanical force on the bones, has been positively correlated with bone density (41). Similar body weight reductions in RYGB and VSG rats, however, exclude weight loss-induced changes in mechanical factors as major contributors to RYGB-induced loss of bone mass and point towards a critical role for endocrine or nutritional factors.

Consistent with a role for endocrine factors, some of the hormonal changes after bariatric surgery have been directly linked to different bone-controlling mechanisms. One of the best studied examples is leptin (42–44). However, our finding that serum leptin levels declined similarly in RYGB- and VSG-operated rats indicates that leptin does not play a significant role in bone remodeling after bariatric surgery. More recent findings have demonstrated that insulin is also necessary to maintain normal bone mass in mice (45). However, fasting and postprandial insulin levels were changed to a similar extent after VSG and RYGB surgery in rats (46). RYGB and VSG resulted in comparable postprandial plasma levels of the GIP. Although a bone-protective role for GIP has been reported (47, 48), the increase observed here is not sufficient to rescue the bone loss after RYGB surgery. Similarly, the

small change in liver or serum FGF21 in RYGB rats cannot explain their bone phenotype, because previous studies have reported that genetic and pharmacologic FGF21 gain of function causes a significant decrease in bone mass (34).

IGF-I is another well-established bone anabolic hormone (49, 50). We found that serum IGF-I levels were decreased in RYGB rats compared with VSG- and sham-operated rats. Synthesis of IGF-I and its action on different target organs are largely GH dependent (51) but can be also impaired by other factors, such as protein or carbohydrate malnutrition (52, 53). Our finding that serum albumin levels were significantly decreased only in RYGB rats suggests a state of relative protein malnutrition after RYGB that is associated with bypassing protein absorption sites in the duodenum.

Recent studies have raised concerns that some types of bariatric surgery can cause or exacerbate micro- and macronutrient deficiencies (12, 54). One candidate to mediate negative effects of bariatric surgery on bone health is calcium homeostasis. In rats, it was estimated that anywhere from 65% to 88% of calcium absorption occurs in the ileum, 4% to 17% in the jejunum, and 7% to 8% in the duodenum (55, 56). When sufficient amounts of calcium are available, it is primarily absorbed from the ileum and jejunum by passive diffusion. Active and vitamin D-dependent transport in the duodenum is of greater importance under low calcium conditions (57). Accordingly, bypassing the duodenum in RYGB rats mainly affects active calcium transport, which could be specifically detrimental under conditions of low calcium. The increased stomach pH in our RYGB rats could have resulted in lower ionization of dietary calcium, leading to lower levels of absorbable calcium. Although gastric pH was increased to a similar extent in VSG as in RYGB rats, the presence of the active calcium transport in the duodenum could have compensated for the decreased levels of absorbable calcium. This could be reflected by the small decrease in serum calcium levels in RYGB but not VSG rats, 60 days after surgery. Unfortunately, PTH measurements were highly variable, and we were unable to conclude whether low calcium levels were also translated into a secondary hyperparathyroidism.

In addition to calcium and protein absorption, the duodenum is also an important site for the absorption of lipids. Indeed, RYGB but not VSG rats demonstrated a decrease in intestinal lipid absorption. Efficient absorption of lipophilic vitamin D is strongly dependent on the presence of fat in the gut lumen (58). Our finding of decreased lipid absorption in RYGB but not VSG rats is consistent with their decreased serum levels of 25-OH-vitamin D. Furthermore, clinical studies have demonstrated that nutritional deficiencies after bariatric surgery are often proportional to the degree of malabsorption created by the surgical procedure (12, 54). To avoid nutritional deficiencies and loss of bone mass after bariatric surgery, patients are supplemented with multivitamin and mineral tablets in combination with additional calcium (12, 59). Whether these suggestions are sufficient for all patients and for all surgery types remains to be determined. Indeed, our findings indicate that dietary supplementation with an established rescue diet rich in calcium, vitamin D, phosphate, and lactose (25) could normalize serum vitamin D and calcium levels. However, the supplemented diet did not fully prevent the bone loss in RYGB rats 60 days after surgery. Together, these findings suggest that bone loss in RYGB-operated rats could stem from a combination of independent factors, including nutritional and endocrine modulations that do not occur after VSG surgery.

Conclusion

Taken together, our findings demonstrate that RYGB and VSG result in similar metabolic improvements, despite involving very distinct manipulations of the GI tract. This suggests that despite the disparate surgical techniques, there are common metabolic mechanisms that contribute to the potent benefits of both RYGB and VSG. In contrast, bypassing the duodenum causes major impairments in micronutrient and macronutrient balances that negatively affect bone volume. The finding that extensive dietary supplementation with calcium, vitamin D, phosphate, and lactose cannot fully rescue the bone loss points to the contribution of additional factors, such as hormones or changes in stomach pH that could have significantly added to the bone phenotype observed in RYGB rats. In addition to providing important insights into the mechanisms of bone loss after RYGB, the present findings also provide information that may eventually result in clinical guidance as to which patients are most appropriate for which surgical procedure.

Acknowledgments

We thank Jose Berger, Alfor Lewis, Ken Parks, Kathi Smith, and Mouhamadoul Toure (Metabolic Diseases Institute, University of Cincinnati) as well as Sarina Meurer and Amon Horngacher (Medizinische Klinik und Poliklinik IV) for excellent technical support.

Address all correspondence and requests for reprints to: Randy J. Seeley, Metabolic Diseases Institute, University of Cin-

cinnati, 2170 East Galbraith Road, Cincinnati, Ohio 45237. E-mail: randy.seeley@uc.edu.

This work was supported by a grant from Ethicon Endo-Surgery, Inc. K.S. was supported by a fellowship from the German Research Foundation.

Disclosure Summary: R.J.S. consults for Amylin Pharmaceuticals and Johnson & Johnson; is a scientific advisory board member for Eli Lilly, Johnson & Johnson, Zafgen, Inc, Zealand Pharmaceuticals, Boehringer Ingelheim, and Angiochem; is on the speaker's bureau for Novo Nordisk and Merck; has stock options in Zafgen, Inc. R.J.S. was supported by research grants from Johnson & Johnson, Novo Nordisk, Ablaris, and Zealand Pharmaceuticals. All other authors have nothing to disclose.

References

1. **World Health Organization.** Obesity and Overweight. Fact Sheet 311. Geneva, Switzerland: World Health Organization; 2006.
2. **Torgerson JS, Hauptman J, Boldrin MN, Sjöström L.** XENical in the prevention of diabetes in obese subjects (XENDOS) study: a randomized study of orlistat as an adjunct to lifestyle changes for the prevention of type 2 diabetes in obese patients. *Diabetes Care*. 2004; 27:155–161.
3. **Sarwer DB, von Sydow Green A, Vetter ML, Wadden TA.** Behavior therapy for obesity: where are we now? *Curr Opin Endocrinol Diabetes Obes*. 2009;16:347–352.
4. **Proietto J.** Why is treating obesity so difficult? Justification for the role of bariatric surgery. *Med J Aust*. 2011;195:144–146.
5. **Buchwald H, Oien DM.** Metabolic/bariatric surgery Worldwide 2008. *Obes Surg*. 2009;19:1605–1611.
6. **Rubino F, Schauer PR, Kaplan LM, Cummings DE.** Metabolic surgery to treat type 2 diabetes: clinical outcomes and mechanisms of action. *Ann Rev Med*. 2010;61:393–411.
7. **Schauer PR, Kashyap SR, Wolski K, et al.** Bariatric surgery versus intensive medical therapy in obese patients with diabetes. *New Eng J Med*. 2012;366:1567–1576.
8. **Coates PS, Fernstrom JD, Fernstrom MH, Schauer PR, Greenspan SL.** Gastric bypass surgery for morbid obesity leads to an increase in bone turnover and a decrease in bone mass. *J Clin Endocrinol Metab*. 2004;89:1061–1065.
9. **Mahdy T, Atia S, Farid M, Adulatif A.** Effect of Roux-en Y gastric bypass on bone metabolism in patients with morbid obesity: Mansoura experiences. *Obes Surg*. 2008;18:1526–1531.
10. **Johnson JM, Maher JW, DeMaria EJ, Downs RW, Wolfe LG, Kellum JM.** The long-term effects of gastric bypass on vitamin D metabolism. *Ann Surg*. 2006;243:701–704; discussion 704–705.
11. **El-Kadre LJ, Rocha PR, de Almeida Tinoco AC, Tinoco RC.** Calcium metabolism in pre- and postmenopausal morbidly obese women at baseline and after laparoscopic Roux-en-Y gastric bypass. *Obes Surg*. 2004;14:1062–1066.
12. **Bal BS, Finelli FC, Shope TR, Koch TR.** Nutritional deficiencies after bariatric surgery. *Nat Rev Endocrinol*. 2012;8:544–556.
13. **Bruno C, Fulford AD, Potts JR, et al.** Serum markers of bone turnover are increased at six and 18 months after Roux-en-Y bariatric surgery: correlation with the reduction in leptin. *J Clin Endocrinol Metab*. 2010;95:159–166.
14. **Aasheim ET, Björkman S, Søvik TT, et al.** Vitamin status after bariatric surgery: a randomized study of gastric bypass and duodenal switch. *Am J Clin Nutr*. 2009;90:15–22.
15. **Madan AK, Orth WS, Tichansky DS, Ternovits CA.** Vitamin and trace mineral levels after laparoscopic gastric bypass. *Obes Surg*. 2006;16:603–606.
16. **Ducloux R, Nobécourt E, Chevallier JM, Ducloux H, Elian N, Altman JJ.** Vitamin D deficiency before bariatric surgery: should supplement intake be routinely prescribed? *Obes Surg*. 2011;21:556–560.
17. **Ernst B, Thurnheer M, Schmid SM, Schultes B.** Evidence for the necessity to systematically assess micronutrient status prior to bariatric surgery. *Obes Surg*. 2009;19:66–73.
18. **Freire RH, Borges MC, Alvarez-Leite JI, Toulson Davisson Correia MI.** Food quality, physical activity, and nutritional follow-up as determinant of weight regain after Roux-en-Y gastric bypass. *Nutrition*. 2012;28:53–58.
19. **Cottam D, Qureshi FG, Mattar SG, et al.** Laparoscopic sleeve gastrectomy as an initial weight-loss procedure for high-risk patients with morbid obesity. *Surg Endosc*. 2006;20:859–863.
20. **Peterli R, Wölnerhanssen B, Peters T, et al.** Improvement in glucose metabolism after bariatric surgery: comparison of laparoscopic Roux-en-Y gastric bypass and laparoscopic sleeve gastrectomy: a prospective randomized trial. *Ann Surg*. 2009;250:234–241.
21. **Chambers AP, Jessen L, Ryan KK, et al.** Weight-independent changes in blood glucose homeostasis after gastric bypass or vertical sleeve gastrectomy in rats. *Gastroenterology*. 2011;141:950–958.
22. **Pluskiewicz W, Bužga M, Holéczy P, Bortlík L, Šmajstrla V, Adamczyk P.** Bone mineral changes in spine and proximal femur in individual obese women after laparoscopic sleeve gastrectomy: a short-term study. *Obes Surg*. 2012;22:1068–1076.
23. **Nogués X, Goday A, Peña MJ, et al.** [Bone mass loss after sleeve gastrectomy: a prospective comparative study with gastric bypass]. *Cir Esp*. 2010;88:103–109.
24. **Stefater MA, Perez-Tilve D, Chambers AP, et al.** Sleeve gastrectomy induces loss of weight and fat mass in obese rats, but does not affect leptin sensitivity. *Gastroenterology*. 2010;138:2426–2436, 2436 e2421–2423.
25. **Zeitz U, Weber K, Soegiarto DW, Wolf E, Balling R, Erben RG.** Impaired insulin secretory capacity in mice lacking a functional vitamin D receptor. *FASEB J*. 2003;17:509–511.
26. **Jandacek RJ, Heubi JE, Tso P.** A novel, noninvasive method for the measurement of intestinal fat absorption. *Gastroenterology*. 2004; 127:139–144.
27. **Bouxsein ML, Boyd SK, Christiansen BA, Guldberg RE, Jepsen KJ, Müller R.** Guidelines for assessment of bone microstructure in rodents using micro-computed tomography. *J Bone Miner Res*. 2010; 25:1468–1486.
28. **Bielohuby M, Popp S, Bidlingmaier M.** A guide for measurement of circulating metabolic hormones in rodents: pitfalls during the preanalyticalphase. *Mol Metabol*. 2012;1:47–60.
29. **Bielohuby M, Stemmer K, Berger J, et al.** Carbohydrate content of post-operative diet influences the effect of vertical sleeve gastrectomy on body weight reduction in obese rats. *Obes Surg*. 2012;22: 140–151.
30. **Kharitonenkov A, Shiyanova TL, Koester A, et al.** FGF-21 as a novel metabolic regulator. *J Clin Invest*. 2005;115:1627–1635.
31. **Inagaki T, Dutchak P, Zhao G, et al.** Endocrine regulation of the fasting response by PPARα-mediated induction of fibroblast growth factor 21. *Cell Metab*. 2007;5:415–425.
32. **Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E.** Hepatic fibroblast growth factor 21 is regulated by PPARα and is a key mediator of hepatic lipid metabolism in ketotic states. *Cell Metab*. 2007;5:426–437.
33. **Adams AC, Yang C, Coskun T, et al.** 2012 The breadth of FGF21's metabolic actions are governed by FGFR1 in adipose tissue. *Mol Metabol*. 2013:2:31–37.
34. **Wei W, Dutchak PA, Wang X, et al.** Fibroblast growth factor 21 promotes bone loss by potentiating the effects of peroxisome proliferator-activated receptor γ. *Proc Natl Acad Sci USA*. 2012;109: 3143–3148.
35. **Sipponen P, Härkönen M.** Hypochlorhydric stomach: a risk condi-

tion for calcium malabsorption and osteoporosis? *Scand J Gastroenterol*. 2010;45:133–138.
36. **Chu S, Tanaka S, Kaunitz JD, Montrose MH.** Dynamic regulation of gastric surface pH by luminal pH. *J Clin Invest*. 1999;103:605–612.
37. **Peacock M.** Calcium metabolism in health and disease. *Clin J Am Soc Nephrol* 2010;5(suppl 1):S23–S30.
38. **McDuffie JR, Calis KA, Booth SL, Uwaifo GI, Yanovski JA.** Effects of orlistat on fat-soluble vitamins in obese adolescents. *Pharmacotherapy*. 2002;22:814–822.
39. **Santry HP, Gillen DL, Lauderdale DS.** Trends in bariatric surgical procedures. *JAMA*. 2005;294:1909–1917.
40. **Dempster DW, Lindsay R.** Pathogenesis of osteoporosis. *Lancet*. 1993;341:797–801.
41. **Reid IR.** Relationships among body mass, its components, and bone. *Bone*. 2002;31:547–555.
42. **Cornish J, Callon KE, Bava U, et al.** Leptin directly regulates bone cell function in vitro and reduces bone fragility in vivo. *J Endocrinol*. 2002;175:405–415.
43. **Ducy P, Amling M, Takeda S, et al.** Leptin inhibits bone formation through a hypothalamic relay: a central control of bone mass. *Cell*. 2000;100:197–207.
44. **Stunes AK, Westbroek I, Gordeladze JO, Gustafsson BI, Reseland JE, Syversen U.** Systemic leptin administration in supraphysiological doses maintains bone mineral density and mechanical strength despite significant weight loss. *Endocrinology*. 2012;153:2245–2253.
45. **Fulzele K, Riddle RC, DiGirolamo DJ, et al.** Insulin receptor signaling in osteoblasts regulates postnatal bone acquisition and body composition. *Cell*. 2010;142:309–319.
46. **Chambers AP, Stefater MA, Wilson-Perez HE, et al.** Similar effects of roux-en-Y gastric bypass and vertical sleeve gastrectomy on glucose regulation in rats. *Physiol Behav*. 2011;105:120–123.
47. **Tsukiyama K, Yamada Y, Yamada C, et al.** Gastric inhibitory polypeptide as an endogenous factor promoting new bone formation after food ingestion. *Mol Endocrinol*. 2006;20:1644–1651.
48. **Ding KH, Shi XM, Zhong Q, et al.** Impact of glucose-dependent insulinotropic peptide on age-induced bone loss. *J Bone Miner Res*. 2008;23:536–543.
49. **Zhang M, Xuan S, Bouxsein ML, et al.** Osteoblast-specific knockout of the insulin-like growth factor (IGF) receptor gene reveals an essential role of IGF signaling in bone matrix mineralization. *J Biol Chem*. 2002;277:44005–44012.
50. **Zhao G, Monier-Faugere MC, Langub MC, et al.** Targeted overexpression of insulin-like growth factor I to osteoblasts of transgenic mice: increased trabecular bone volume without increased osteoblast proliferation. *Endocrinology*. 2000;141:2674–2682.
51. **Melmed S.** Insulin-like growth factor I–a prototypic peripheral-paracrine hormone? *Endocrinology*. 1999;140:3879–3880.
52. **Oster MH, Fielder PJ, Levin N, Cronin MJ.** Adaptation of the growth hormone and insulin-like growth factor-I axis to chronic and severe calorie or protein malnutrition. *J Clin Invest*. 1995;95:2258–2265.
53. **Bielohuby M, Sawitzky M, Stoehr BJ, et al.** Lack of dietary carbohydrates induces hepatic growth hormone (GH) resistance in rats. *Endocrinology*. 2011;152:1948–1960.
54. **Folli F, Sabowitz BN, Schwesinger W, Fanti P, Guardado-Mendoza R, Muscogiuri G.** Bariatric surgery and bone disease: from clinical perspective to molecular insights. *Int J Obes (Lond)*. 2012;36:1373–1379.
55. **Marcus CS, Lengemann FW.** Absorption of Ca45 and Sr85 from solid and liquid food at various levels of the alimentary tract of the rat. *J Nutr*. 1962;77:155–160.
56. **Cramer CF, Copp DH.** Progress and rate of absorption of radiostrontium through intestinal tracts of rats. *Proc Soc Exp Biol Med*. 1959;102:514–517.
57. **Khanal RC, Nemere I.** Regulation of intestinal calcium transport. *Annu Rev Nutr*. 2008;28:179–196.
58. **Mulligan GB, Licata A.** Taking vitamin D with the largest meal improves absorption and results in higher serum levels of 25-hydroxyvitamin D. *J Bone Miner Res*. 2010;25:928–930.
59. **Heber D, Greenway FL, Kaplan LM, Livingston E, Salvador J, Still C.** Endocrine and nutritional management of the post-bariatric surgery patient: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2010;95:4823–4843.