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Cortical and trabecular deterioration in mouse models of Roux-en-Y gastric bypass

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ABSTRACT

Roux-en-Y gastric bypass (RYGB) is a profoundly effective treatment for severe obesity, but results in significant bone loss in patients. Developing a murine model that recapitulates this skeletal phenotype will provide a robust tool with which to study the physiologic mechanisms of this bone loss. We studied adult male C57BL/6J mice who underwent either RYGB or sham operation. Twelve weeks after surgery, we characterized biochemical bone markers (parathyroid hormone, PTH; C-telopeptide, CTX; and type 1 procollagen, P1NP) and bone microarchitectural parameters as measured by microcomputed tomography. RYGB-treated mice had significant trabecular and cortical bone deficits compared with sham-operated controls. Although adjustment for final body weight eliminated observed cortical differences, the trabecular bone volume fraction remained significantly lower in RYGB mice even after weight adjustment. PTH levels were similar between groups, but RYGB mice had significantly higher indices of bone turnover than sham controls. These data demonstrate that murine models of RYGB recapitulate patterns of bone loss and turnover that have been observed in human clinical studies. Future studies that exploit this murine model will help delineate the alterations in bone metabolism and mechanisms of bone loss after RYGB.

1. Introduction

More than one-third of U.S. adults have obesity and the subpopulation of individuals with severe obesity (body mass index, BMI ≥ 40 kg/m^2) continues to increase rapidly [1,2]. Bariatric surgery is an increasingly popular and effective treatment for severe obesity, and Roux-en-Y gastric bypass (RYGB) is one of the most commonly performed bariatric procedures in the U.S. and worldwide [3].

Despite major improvements in body weight and obesity comorbidities after this operation, clinical studies have documented striking declines in bone mineral density (BMD) of up to 10% [4–12]. However, clinical studies have been hampered by their observational study designs and by concerns about obesity-related bone imaging artifact [13,14]. Furthermore, these studies have been unable to tease out the physiological mechanisms of RYGB-induced bone loss and have tested associations without the ability to show direct causation.

Rat and mouse models of RYGB have been established that recapitulate the metabolic improvements observed in human clinical studies [15–18]. These surgical models have been utilized in genetically modified mice to identify the physiological and molecular mechanisms through which RYGB imparts its powerful effects on weight loss and improved glucose regulation [19–23]. Declining bone density has been documented in rat models of RYGB [24–28], whose evaluation suggests that neither skeletal unloading nor calcium and vitamin D malabsorption are the primary drivers of the associated bone loss. These studies have raised intriguing questions about the etiology of RYGB-induced bone loss, but they have been limited in their ability to elucidate the underlying molecular mechanism(s). By their ability to harness the power of genetic manipulation, mouse bariatric surgery models provide the opportunity to explore the molecular pathophysiology of bone loss. In particular, targeted manipulation of individual pathways can determine their effects on surgically-induced bone loss. Robust mouse models of RYGB that demonstrably recapitulate human physiology have only recently been established, and the skeletal consequences of bariatric surgery in the mouse have not yet been evaluated. We sought to assess

Abbreviations: RYGB, Roux-en-Y gastric bypass; PTH, parathyroid hormone; CTX, C-telopeptide, CTX; P1NP, type 1 procollagen; BMI, body mass index; BMD, bone mineral density; μCT, micro-computed tomography; Tb. BV/TV, trabecular bone volume fraction; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation; Conn.D, connectivity density; SMI, structural model index; Ct.TMD, cortical tissue mineral density; Ct.Th, cortical thickness; Ct.Ar, cortical bone area; Tt.Ar, total bone area; Ct.Ar/Tt.Ar, cortical bone area fraction; Ct.Po, cortical porosity; J, polar moment of inertia; I_{max}, maximum moment of inertia; I_{min}, minimum moments of inertia.

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the relevance of the mouse model of bariatric surgery for bone disease by determining the effects of RYGB on bone microarchitecture and metabolism in adult mice.

2. Materials and methods

2.1. Animals

We evaluated wild-type mice that were part of a larger study examining the effects of 5HT2C-receptor on metabolism after RYGB [29]. Male C57BL/6J mice (Jackson Labs, Bar Harbor, ME) were placed on a 60% high fat diet (HFD; Research Diets, D12492) at weaning to induce substantial obesity. All surgically treated animals were individually housed and maintained in a 12-h light, 12-h dark cycle under controlled temperature (19–22 °C) and humidity (40–60%). All animal studies were performed under protocols approved by the Massachusetts General Hospital and Harvard Medical School Institutional Animal Care and Use Committees.

2.2. Surgery and postoperative care

Between 23 to 26 weeks of age, diet-induced obese mice were randomized to receive either RYGB surgery ($n = 6$) or laparotomy sham surgery ($n = 5$) under inhalation anesthesia. For these operations, a 2.0–2.5 cm midline abdominal incision was made into the peritoneum and a self-retaining retractor placed at the laparotomy. RYGB anatomy was created as previously described [18]. The total length of the small intestine was measured, and as in the typical human operation, a total of 20–30% of the small intestine was included in the combination of Roux and biliopancreatic limbs (approximately 10–15% in each limb). To construct the Roux limb, the jejunum transected 2–3 cm below the ligament of Treitz. Then, 4–5 cm beyond the transection, a 2 mm incision was made on the anti-mesenteric wall of the jejunum. A running end-to-side jejuno-jejunal anastomosis was created using 8–0 Vicryl suture (Ethicon, Somerville, NJ). The lesser and greater curvature of the stomach were then dissected and minor branch vessels from the main gastric artery were cauterized around the gastro-esophageal junction to facilitate gastric transection. The gastric pouch and distal gastric remnant were created by transecting the stomach, closing the remnant with a running 8–0 Vicryl suture, and constructing an end-to-end gastro-jejunal anastomosis. Finally, the laparotomy was closed using 6–0 Vicryl suture in two layers. Sham-operated mice were treated in a manner similar to the RYGB mice, but the operation was limited to a laparotomy and surgical repair. Mice were provided with liquid diet (40% Vital AF 1.2 Cal, Abbott Laboratories, Abbott Park, IL) during the 2 weeks immediately following surgery, and were gradually weaned back onto the HFD during this time. All mice were continued on the HFD after surgery to avoid an independent or confounding effect of dietary change. Body weight was monitored weekly, and food intake was measured during post-operative weeks 6–8. One mouse in the RYGB group was excluded from the analysis due to apparent poor health (poor body condition, decreased grooming, decreased mobility, and a body weight that declined to less than 25 g), leaving 5 mice in each of the RYGB and sham groups.

2.3. Specimen harvesting and preparation

At 12 weeks after surgery (age 35–38 weeks), mice were fasted for 4 h and euthanized by CO_2 inhalation. Blood was immediately collected via cardiac puncture. Femurs, tibias, and vertebrae were harvested and cleaned of soft tissue, wrapped in saline-soaked gauze, and stored at −20 °C.

2.4. Biochemical assays

Plasma parathyroid hormone (PTH) was assessed by ELISA (Immutopics, San Clemente, CA), an assay with intra-assay precision of 2–6%. Plasma levels of type 1 collagen C-telopeptide (CTX) and amino-terminal propeptide of type I procollagen (P1NP) were measured using mouse ELISA kits (IDS, Fountain Hills, AZ), assays with intra-assay precisions of 5–9%. All assays were batched so as to be performed with a single kit and run according to the manufacturers' protocols.

2.5. Microarchitecture

Micro-computed tomographic (μCT) imaging was performed on the tibia, femur, and the 5th lumbar (L5) vertebra of mice from each group using a high-resolution desktop imaging system (μCT40, Scanco Medical AG, Brüttisellen, Switzerland). The methods used were in accordance with guidelines for the use of μCT in rodents [30]. Scans were acquired using isotropic voxel sizes of 10 μm^3 for the tibia and femur and 12 μm^3 for the L5 vertebra, with 70 kVp peak X-ray tube potential, 200 ms integration time, and were subjected to Gaussian filtration. Trabecular bone microarchitecture was evaluated in semi-automatically contoured regions in the proximal metaphysis of the tibia, distal metaphysis of the femur, and L5 vertebral body. The region of interest for the proximal tibia began 100 μm below the proximal growth plate and extended distally 1 mm. The region of interest in the distal femur started 200 μm above the peak of the growth plate and extended proximally 1.5 mm. The vertebral body region of interest extended from 120 μm below the cranial growth plate to 120 μm above the caudal growth plate. Cortical bone microarchitecture was evaluated in the tibia mid-diaphysis in a region that started 2 mm above the inferior tibiofibular joint and extended distally 500 μm. Cortical bone microarchitecture was also evaluated in the femoral diaphysis in a region that began 55% of the femoral length below the top of the femoral head and extended distally 500 μm. Segmentation thresholds of 330 and 696 mg HA/cm^3 were used for the evaluations of trabecular and cortical bone, respectively, based on adaptive-iterative thresholding (AIT) that was performed on the sham-operated control group. All analyses were carried out using the scanner manufacturer (Scanco Medical) evaluation software. Cancellous bone outcomes included trabecular bone volume fraction (Tb. BV/TV, %), trabecular thickness (Tb.Th, μm), trabecular number (Tb.N, mm^{-1}), trabecular separation (Tb.Sp, μm), connectivity density (Conn.D, mm^{-3}), and structural model index (SMI). Cortical bone outcomes included cortical tissue mineral density (Ct.TMD, mg HA/mm^3), cortical thickness (Ct.Th, μm), cortical bone area (Ct.Ar, mm^2), total bone area (Tt.Ar, mm^2), cortical bone area fraction (Ct.Ar/Tt.Ar, %), cortical porosity (Ct.Po, %), polar moment of inertia (J, mm^4), and the maximum and minimum moments of inertia (I_{max} and I_{min}, mm^4).

2.6. Statistical analysis

Statistical analyses were performed with SAS 9.3 (SAS Institute, Cary, NC). All data were checked for normality, and standard descriptive statistics computed. Wilcoxon rank sum tests were used to compare differences between RYGB and sham-operated groups. Multivariate regression using PROC GLM was employed to adjust results using final body weight as a covariate. The threshold of statistical significance was set at $p \le 0.05$. Data are reported as mean ± SD, unless otherwise noted.

3. Results

3.1. Body weight

Pre-operative body weight was similar in RYGB-treated and sham-operated groups (40.5 ± 4.7 g and 39.2 ± 5.3 g, respectively). Twelve weeks after surgery, RYGB mice weighed an average 38% less than sham controls (30.1 ± 2.5 g vs. 48.2 ± 5.6 g, $p < 0.0001$; Fig. 1). Daily food intake did not differ between groups (RYGB: 3.1 ± 0.6 g/day; Sham: 2.7 ± 0.2 g/day).