

# Unexpected Effects of Sclerostin Antibody (Sost-Ab) on Glucose Metabolism and Bone Health after Roux-en-Y Gastric Bypass (RYGB) Surgery and Ovariectomy

Huseyin Ozkan<sup>1,2</sup>, Katrien Corbeels<sup>2</sup>, Sai Rama Krishna Meka<sup>1</sup>, Michael Klüppel<sup>1</sup>, Pranav Kumar Mishra<sup>1,2</sup>, Esin Ozkan<sup>1</sup>, Alfonso Torquati<sup>2</sup>, and Anna Spagnoli<sup>1</sup>

<sup>1</sup> Department of Orthopedic Surgery, Rush University Medical Center, Chicago, IL 60612, USA

<sup>2</sup> Department of Surgery, Rush University Medical Center, Chicago, IL 60612, USA

**Introduction:** Roux-en-Y Gastric Bypass (RYGB) surgery has proven to be a valuable treatment option for morbid obesity and to control and even resolve diabetes, but also negatively impact bone health<sup>1</sup>. Fracture risk is increased two-fold after bariatric surgery compared to obese controls or restrictive procedures<sup>2</sup>. Although it has been reported that bariatric surgery is associated with considerable high-cycle bone loss and notable degradation in bone microarchitecture and strength, the underlying mechanisms behind these effects remain incompletely elucidated<sup>2</sup>. We recently reported that in patients that underwent RYGB circulating sclerostin levels were significantly higher in post-menopausal women compared to pre-menopausal<sup>3</sup>. In the present studies, we evaluate the impact of RYGB on the regulation of glucose metabolism and bone health, as well as the effects of sclerostin antibody (Sost-Ab) treatment assessed in a postmenopausal RYGB mouse model.

**Methods:** Female C57BL/6J mice were fed a high-fat diet (HFD) (60 kcal%) for 9 weeks, after which bilateral ovariectomy (OVX) was performed. HFD was then continued, and 5 weeks later mice underwent either RYGB or sham surgery. We designed two set of studies. In the first study (Study 1) we evaluated the effect of RYGB surgery on mice placed on a high-fat diet (HFD) and OVX To correct for food intake and body weight differences between groups, sham mice were either pair-fed (Sham PF) or body weight matched (Sham BWM) to RYGB mice. In the second study (Study 2), we investigated the effects of Sost-Ab treatment. In this group, non-surgery normal chow (NC), sham BWM and RYGB surgery mice were injected subcutaneously with Sost-Ab at the dose of 25 mg/kg 2 times a week, and the control groups were injected with the same amount of saline. Glucose tolerance was assessed by intraperitoneal glucose tolerance test (IPGTT) 7 weeks post-procedures. Bone characteristics and serum were analyzed 8 weeks after surgery. Bone characteristics were assessed by microCT and bone histomorphometry analyses. Data were analyzed using one-way ANOVA and were expressed as mean ± SD (\*p<0.05).

**Results:** After 14 weeks on HFD mice weighed around 35.4 ± 6.1 g. At euthanasia, mice that underwent RYGB and mice in the BWM group weighted around 24.5 ± 3.4 g. While sham PF mice continued to gain weight, and at euthanasia weighted 37.8 ± 5.1 g. IPGTT and HOMA-IR analyses of Study 1 data indicated that RYGB induced a significant improvement in glucose tolerance and insulin resistance when compared to the sham AL, PF and BWM groups. While microCT analyses indicated that RYGB mice had a lower BV/TV and trabecular thickness compared to controls. Furthermore, trabecular porosity was approximately 1,5-fold higher in RYGB mice than controls. Remarkably, analyses of Study 2 data indicated that in RYGB mice. Sost-Ab treatment significantly improved glucose tolerance and insulin resistance compared to RYGB mice that were treated with carrier (Fig. 1). However, when compared to NC treated with Sost-Ab, RYGB mice treated with Sost-Ab had a lower response as indicated by the lower increase in the BV-TV ratio by micro-CT (Fig. 2). Notably, results were confirmed by histomorphometric analyses. Interestingly, when compared to RYGB mice treated with carrier, RYGB mice treated with Sost-Ab mice showed an increase in cortical porosity (Fig.2). Histomorphometric analyses indicated that when compared to RYGB mice treated with carrier, RYGB mice treated with Sost-Ab mice had an increase in the perimeter of osteoclasts per total bone (OcS) indicating that the noted effects of Sost-Ab might be due to an increase in the osteoclastic response.

**Discussion:** Our data demonstrate that RYGB led to improved glucose tolerance and insulin resistance in OVX mice. However, this was combined with an increase in bone loss. Most notably, in RYGB mice, Sost-Ab treatment induced a further improvement in glucose tolerance and insulin resistance, but unexpectedly increased cortical porosity and osteoclast response and overall, a less pronounced response on BV/TV increase was noted.

**Significance/Clinical Relevance:** The mechanisms by which bariatric surgery improves insulin sensitivity while having detrimental effects on bone quality are largely unknown. Here we report an unexpected effect of Sost-Ab in improving glucose metabolism after RYGB that was accompanied with an increase in bone porosity and a blunted response in improving bone loss. The findings are clinically relevant as they indicate that beside its effects on bone, sclerostin has an endocrine role in regulating the inter-organ interface between bone/fat/glucose/insulin metabolisms. Furthermore, the unexpected effects of Sost-Ab on osteoclasts warrant further investigations.

## References:

- Chen Y, Corsino L, Shantavasinkul PC, Grant J, Portenier D, Ding L, Torquati A. Gastric Bypass Surgery Leads to Long-term Remission or Improvement of Type 2 Diabetes and Significant Decrease of Microvascular and Macrovascular Complications. *Ann Surg.* 2016 Jun;263(6):1138-42. doi: 10.1097/SLA.0000000000001509. PMID: 26599565.
- RK, Ghezzi M, Habli D, Alami RS, Chakhtoura M. Fracture risk following bariatric surgery: a systematic review and meta-analysis. *Osteoporos Int.* 2022 Mar;33(3):511-526. doi: 10.1007/s00198-021-06206-9. Epub 2022 Jan 5. PMID: 34988627.
- Tangalakis LL, Tabone L, Spagnoli A, Muehlbauer M, Omotosho P, Torquati A. Effects of Roux-en-Y Gastric Bypass on Osteoclast Activity and Bone Density in Morbidly Obese Patients with Type 2 Diabetes. *Obes Surg* 2020; 30:290-295

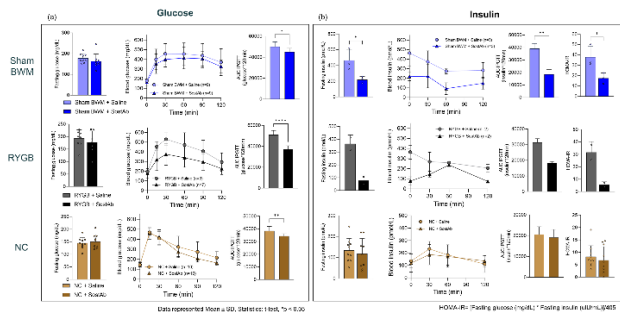


Figure 1: IPGTT, SOST-Ab injections led to improved RYGB mice (a) glucose and (b) insulin

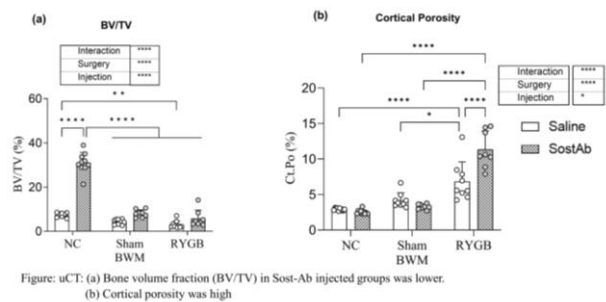


Figure: uCT: (a) Bone volume fraction (BV/TV) in Sost-Ab injected groups was lower. (b) Cortical porosity was high