Romosozumab Improves Systemic Metabolism Despite Impaired Osteoanabolic Capacity in High Fat Diet Mice

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INTRODUCTION

Skeletal mass and structure are maintained through the process of bone remodeling, wherein old or damaged bone is replaced with new, healthy bone. However, during aging the rates of bone formation and bone resorption become dysregulated. The increase in bone resorption and decrease in new bone formation leads to osteopenia and osteoporosis, resulting in significant health concerns such as skeletal fragility and fracture [1]. Most osteoporosis treatments target osteoclast-mediated bone resorption and prolonged use is often associated with adverse side-effects such as atypical femur fractures [2,3]. Romosozumab, a monoclonal antibody clinically approved for the treatment of osteoporosis, targets the osteocyte-secreted protein sclerostin. Sclerostin is an inhibitor of the Wnt/β-catenin signaling pathway, a key driver for bone formation and osteoblast differentiation [4]. Beyond the anabolic effects on bone, previous studies from our lab demonstrate that sclerostin knockout ($Sost^{-/-}$) mice exhibit resistance to diet-induced diabetes and improved lipid metabolism [5]. Additionally, elevated serum sclerostin levels have been positively correlated with diabetes and obesity in human studies [6,7]. Given the heightened risk of fracture in patients with obesity and diabetes, there is a growing interest in elucidating the mechanisms behind skeletal fragility and metabolic dysfunction. In this study, we examine whether the influence of sclerostin inhibition by Romosozumab reaches beyond bone anabolism by exploring the influence on metabolic parameters during high fat or low fat diet diet feeding in C57BL/6J mice.

BACKGROUND

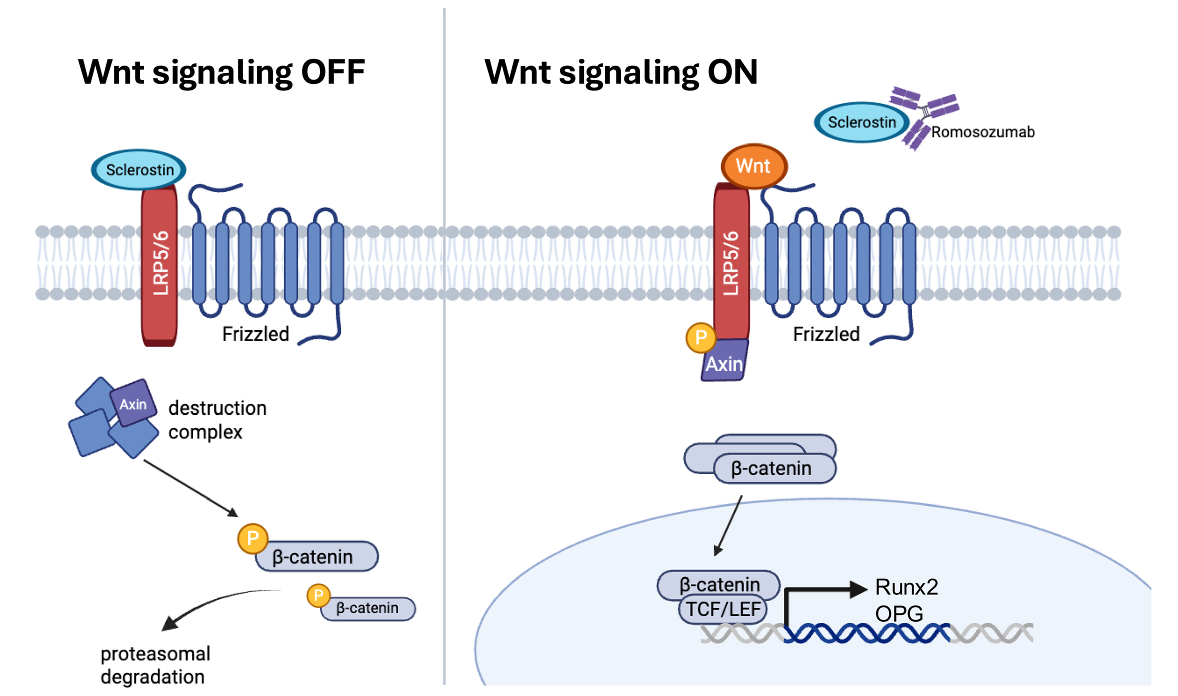
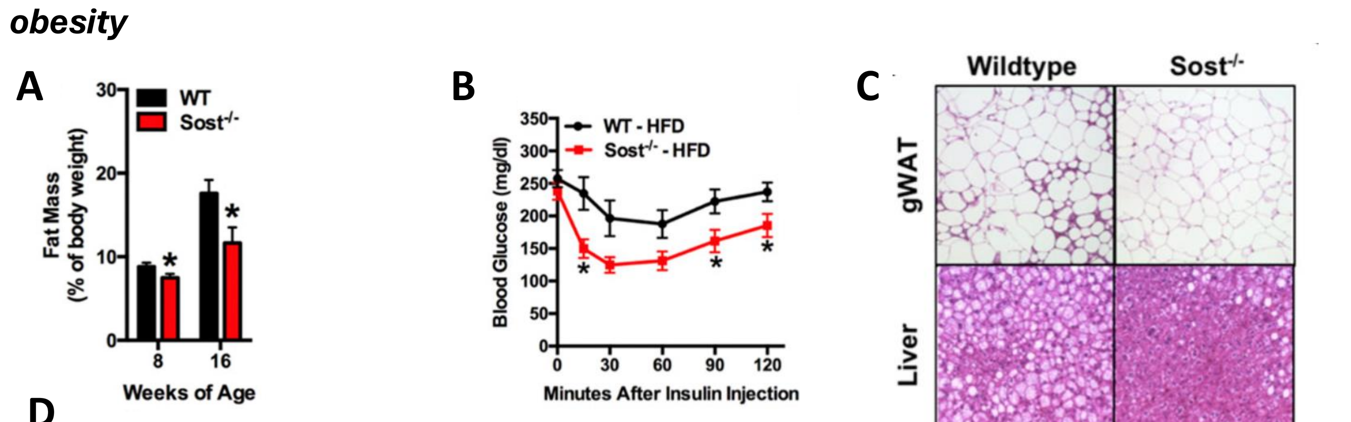


Fig 1. Canonical Wnt Signaling pathway. Wnt signaling off (left): In the absence of a Wnt ligand, or in the presence of a Wnt pathway inhibitor like sclerostin, the destruction complex phosphorylates β-catenin, targeting it for ubiquitination and degradation. Wnt signaling on (right): Binding of Wnt ligand to LRP5/6 and Frizzled receptors allows recruitment and phosphorylation of Axin. Β-catenin escapes proteasomal degradation and can activate the expression of target genes. *Image created in Biorender.com.*

Sclerostin-deficient mice display altered metabolism and resistance to diet-induced obesity



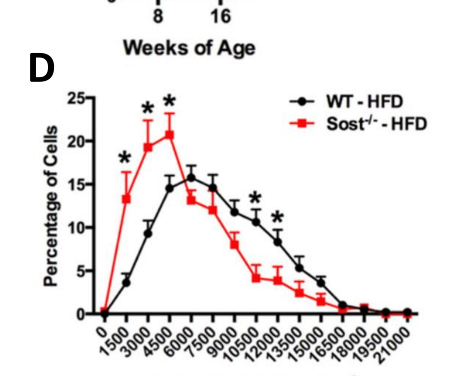


Figure 2. (A) *Sost* KO (-/-) mice show a decreased fat mass as compared to their littermate controls at 8 and 16 weeks old. (B) *Sost* KO mice have increased insulin sensitivity as compared to controls during an insulin tolerance test. (C) *Sost* KO mice have a decrease in gonadal white adipose tissue cell size and reduction in hepatic steatosis. (D) Quantification of adipocyte cell area shown in (C) reveals a higher percentage of smaller average cell area. Data modified from [5]

Experimental Timeline

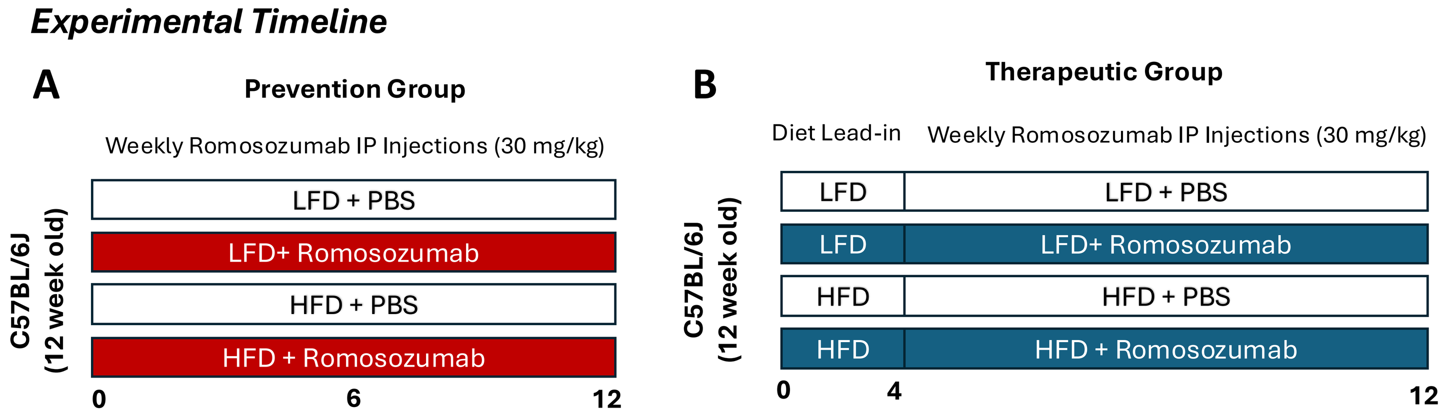


Figure 3. (A) 12-week old C57BL/6J mice in the prevention group receive concurrent diet (high-fat diet or low-fat diet) and weekly IP injections of romosozumab for 12 weeks (30mg/kg body mass). (B) 12-week old C57BL/6J mice underwent a 4 week diet lead-in, followed by 12 weeks of weekly romosozumab injections (30mg/kg).

RESULTS: *Prevention*

DEXA and uCT results indicate a reduced bone anabolic response in HFD + Romo mice

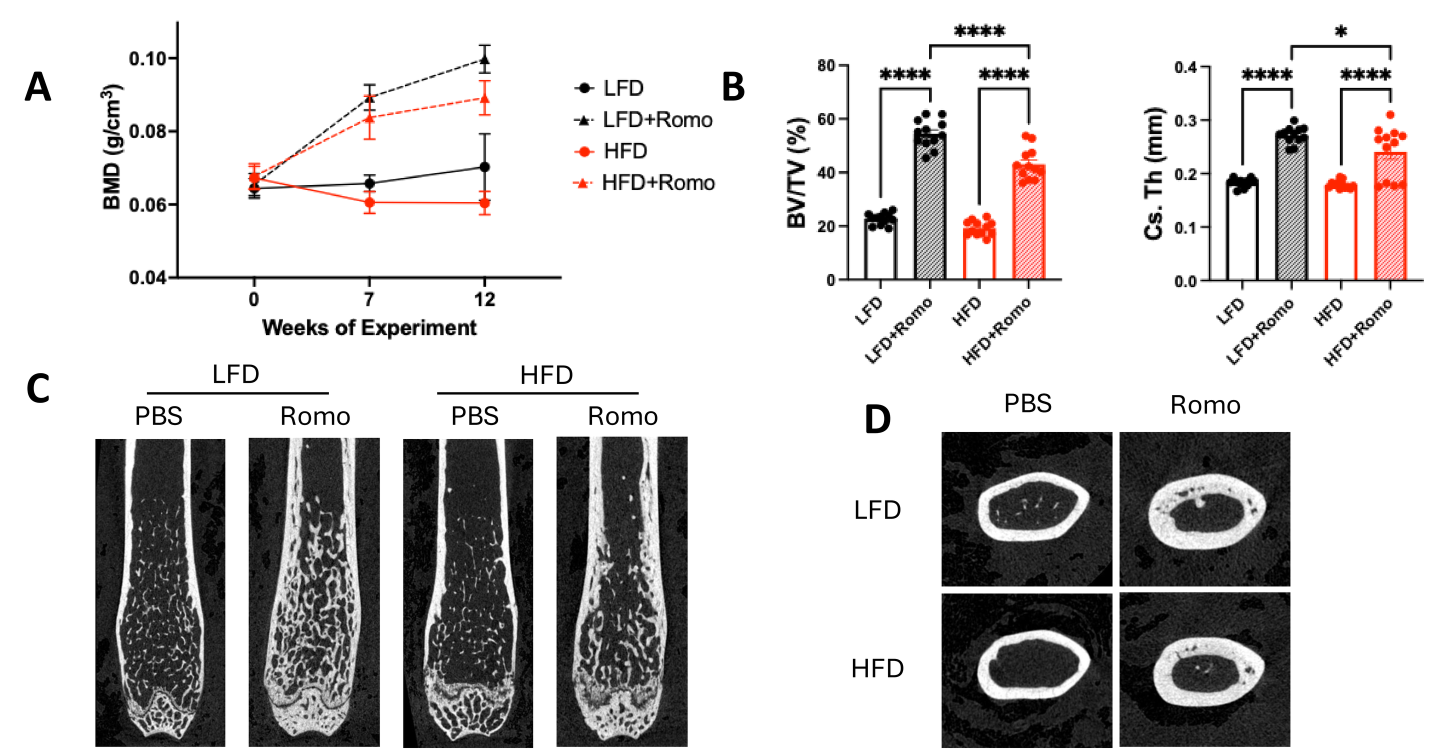


Figure 4. Analyses of bone microarchitecture in prevention treated and untreated mice (A) Whole body bone mineral density was assessed at Week 0, 7 and 12 of the study. (B) MicroCT analyses indicate an increase in trabecular bone volume and cortical thickness in all romosozumab treated groups, with a more robust anabolic response in mice on the low fat diet as compared to high fat diet. (C) Representative microCT trabecular images of the femoral distal metaphysis (D) Representative microCT images of the femoral mid-diaphysis.

Romosozumab improves insulin sensitivity and serum lipid profile in HFD mice

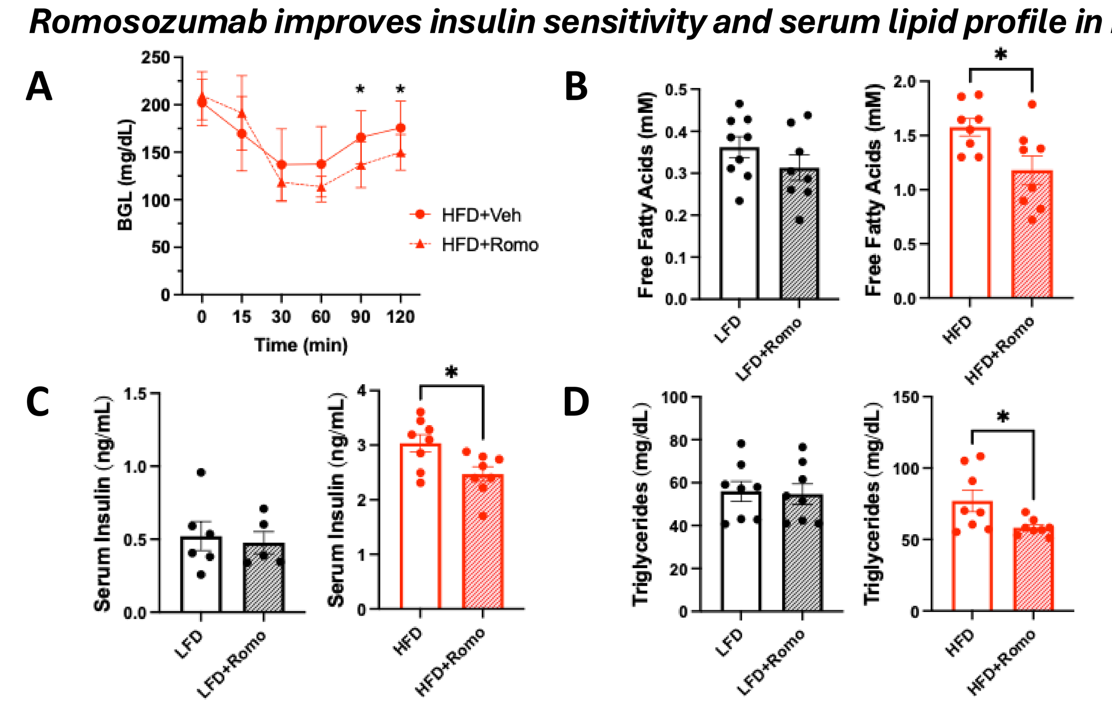


Figure 5. (A) Serum blood glucose levels measurements from an insulin tolerance test indicate an increase in insulin tolerance in mice treated with HFD and romosozumab. Mice treated with high fat diet and romosozumab have reduced serum free fatty acids (B), insulin (C), and triglycerides (D).

Romosozumab treatment reduces adipocyte hypertrophy HFD and LFD fed mice

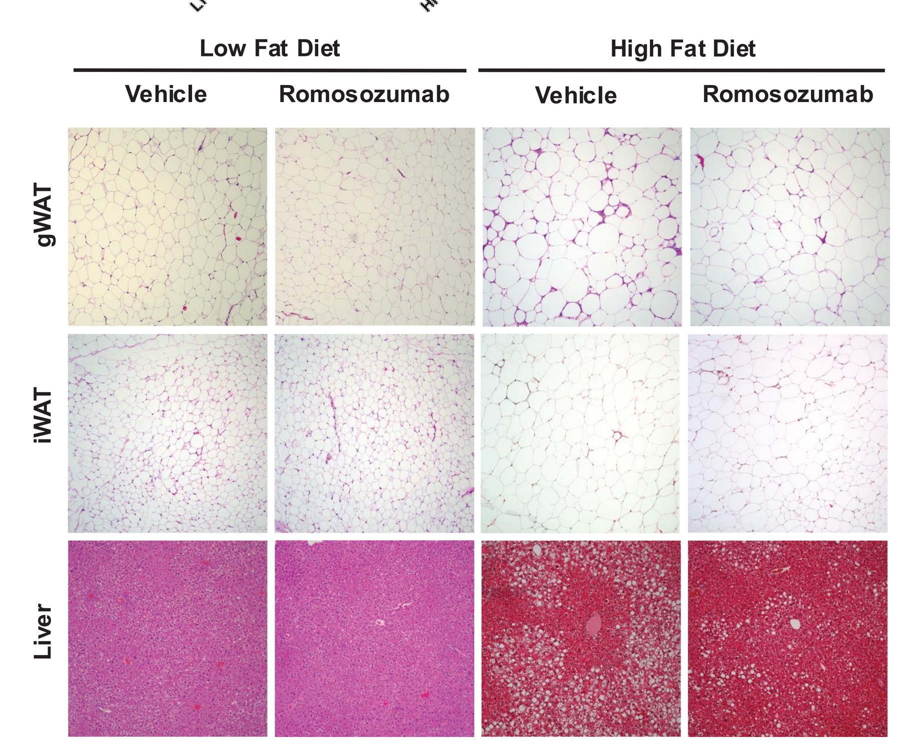


Figure 6. Hematoxylin and eosin staining. Top row: Gonadal white adipose tissue indicates a reduction in adipose tissue inflammation in romosozumab treated mice. Middle row: Inguinal white adipose tissue shows a significant decrease in adipocyte size in both LFD and HFD mice treated with romosozumab. Bottom row: Liver histology indicates a reduction in hepatic steatosis in HFD mice treated with romosozumab.

RESULTS: *Therapeutic*

DEXA and uCT results indicate a reduced bone anabolic response in HFD + Romo mice

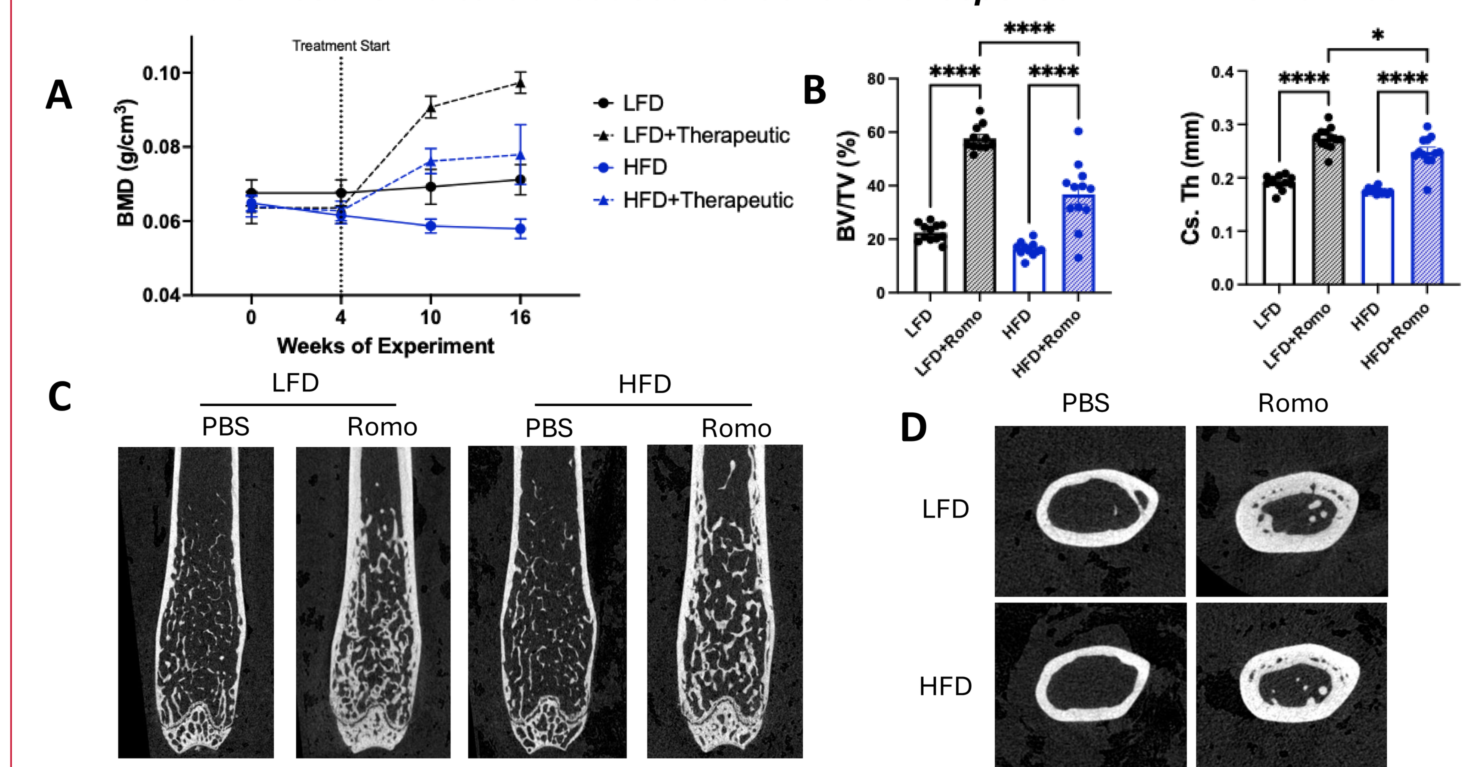


Figure 7. Analyses of bone microarchitecture in therapeutic treated and untreated mice (A) Whole body bone mineral density was assessed at Week 0, 4,10, and 16 of the study. (B) MicroCT analyses indicate an increase in trabecular bone volume and cortical thickness in all romosozumab treated groups, and a more robust anabolic response in the mice on the low fat diet as compared to high fat diet. (C) Representative microCT trabecular images of the femoral distal metaphysis (D) Representative microCT images of the femoral mid-diaphysis.

Romosozumab improves insulin sensitivity and serum lipid profile in HFD mice

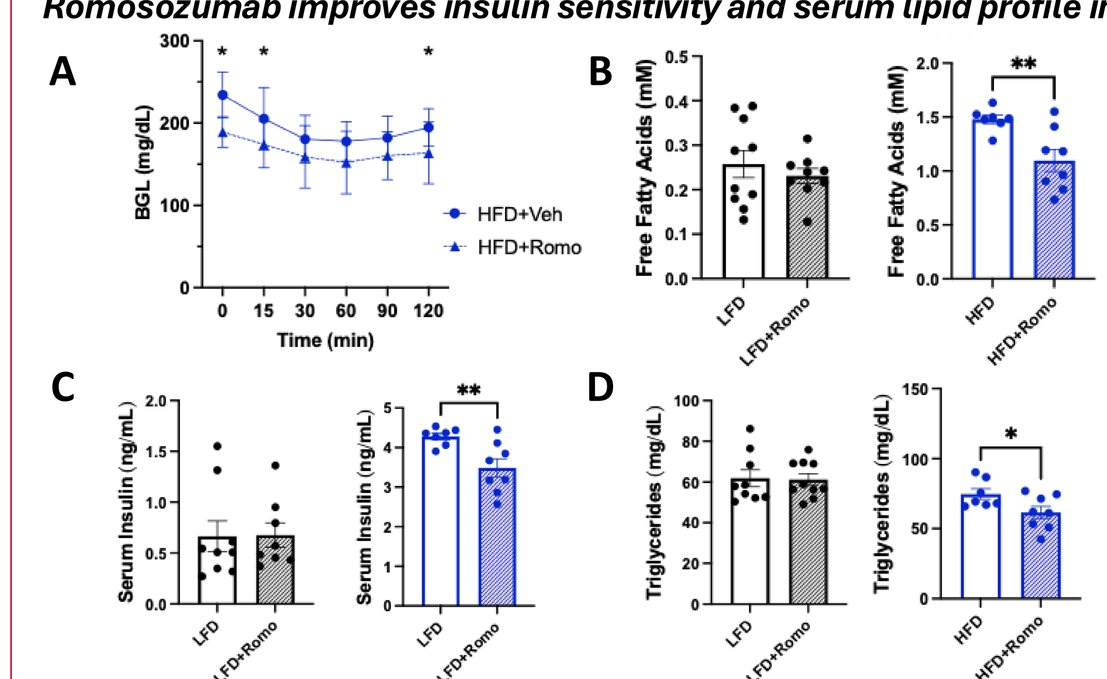


Figure 8. (A) Serum blood glucose levels measurements from an insulin tolerance test indicate an increase in insulin tolerance in mice treated with HFD and romosozumab. Mice treated with high fat diet and romosozumab have reduced serum free fatty acids (B), insulin (C), and triglycerides (D).

Romosozumab treatment reduces adipocyte hypertrophy and inflammation in HFD fed mice

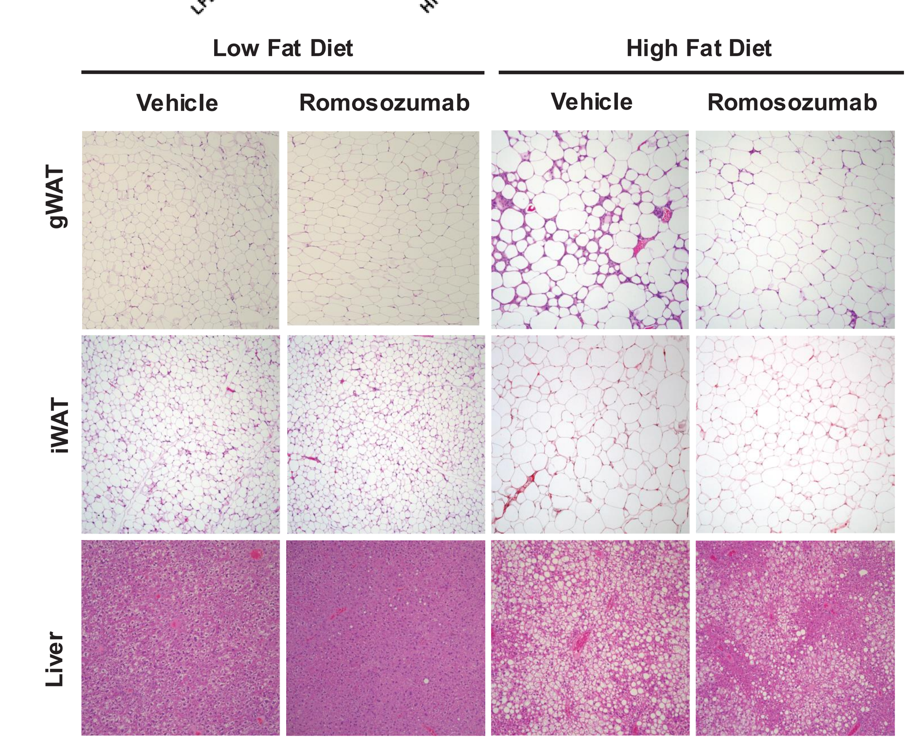


Figure 9. Hematoxylin and eosin staining. Top row: Gonadal white adipose tissue indicates a reduction in adipose tissue inflammation and size in romosozumab treated mice. Middle row: Inguinal white adipose tissue shows a significant decrease in adipocyte size in both LFD and HFD mice treated with romosozumab. Bottom row: Liver histology indicates a reduction in hepatic steatosis in HFD mice treated with romosozumab.

CONCLUSIONS

- Romosozumab produced a prominent bone anabolic response in low fat diet fed mice in both treatment strategies
- In high fat diet obese conditions, mice displayed impairment of bone formation as compared to low fat diet, which was more significant following a high fat diet lead-in period
- Comparable to *Sost* KO mice, romosozumab treatment under high fat conditions improved lipid metabolism and adipocyte inflammation
- Although romosozumab action was suppressed in bone under obese conditions, both diet groups had improvement in lipid mobilization and adipocyte metabolic characteristics
- Future studies will focus on the mechanisms behind the osteoanabolic and metabolic alterations following sclerostin inhibition during obese conditions

References

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