

Genetic and Pharmacological Ablation of GP130 Tyrosine 814 Prevents Bone Loss Induced by High Fat Diet.

Youngjoo Lee¹, Jade Tassey¹, Joshua Lee¹, Andrew C. Drake¹, Falisha Nguyen¹, Jenny Magallanes¹, Arijita Sarkar¹, Jinxiu Lu¹, Nancy Q. Liu¹, and Denis Evseenko¹

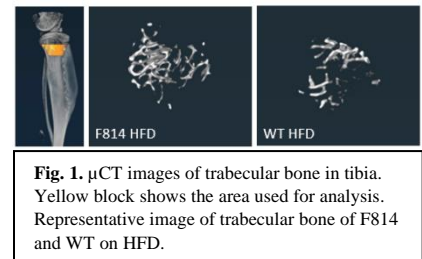
¹University of Southern California (USC), Los Angeles, CA, USA evseenko@usc.edu

Disclosures: DE is a co-founder and a stockholder of Carthronix Inc.

INTRODUCTION. Obesity is a disease endemic in developed countries, and the prevalence of adult obesity in the USA is nearly 42%. In obesity, adipose tissue (AT) becomes hypertrophic and senescent, and a significant number of macrophages are recruited to the AT. These changes make the AT much more immunologically active and increase the secretion of inflammatory signals, mainly interleukin 6 (IL-6). This results in a low-grade systemic and chronic inflammatory condition, which is the main driver for systemic deterioration and diseases. Gp130 is the common co-receptor for IL-6 family cytokines, and prior research in Evseenko lab demonstrated that inhibiting the intracellular tyrosine 814 signaling residue (Y814) of gp130 downregulates proinflammatory pathways and enhances tissue regeneration in acute injury models (1). Building on this work, this project aims to reveal the effects of gp130-Y814 signaling modification on the impact of chronic inflammation and bone loss induced by obesity.

METHODS. Mouse lines and breeding: All procedures involving animals were approved by the IACUC of USC. A mutant gp130-Y814 mouse (F814) was generated using CRISPR-Cas9 (1); C57BL/6J mice were used for wildtype (WT) controls and for pharmacologic experiments. HFD consisted of 60% kCal from fat and the rest of the calories from protein and carbohydrates at approximately 20% each (TD.06414, Envigo Teklad). For pharmacological studies we used a small molecule inhibitor of gp130-Y814 (X-13), a more water-soluble analog of previously reported drug R805 (1), dissolved in 1% Captisol and saline (vehicle). This chemical also inhibits gp130-Y759 but has minimal or no effect on gp130-Y905. X-13 or vehicle alone was injected intraperitoneally twice a week, at a dose of 10 mg/kg body weight for 12 weeks. The BD Aria II flow cytometer was used to acquire flow cytometry data and FlowJo software was used to analyze the data. WBC differential and peripheral blood smear examination was done by IDEXX BioAnalytics. Micro-computed tomography (μ CT) was done in 20 μ m resolution using Nikon XT H 225ST.

RESULTS. As expected, HFD induced weight gain and increased level of systemic inflammation. C-Reactive Protein (CRP), a highly sensitive biomarker for systemic inflammation that is directly induced by IL-6 in the liver, was increased substantially by HFD. However, F814 and X-13 treated mice exhibited significantly lower levels of CRP compared to WT and vehicle-treated mice, indicating the gp130-Y814 mutation and the Y814 inhibitor reduced the level of systemic inflammation induced by HFD. Myeloid immune cell recruitment and polarization in AT are considered the primary source of proinflammatory cytokines in obesity. Assessed by Flow Cytometry, HFD increased the presence of macrophages (CD45⁺, Ly6G⁺, CD11b⁺, F4/80⁺ myeloid) in AT compared to the normal diet (ND). Subpopulations of macrophages can be distinguished further based on the expression of F4/80, where it has been reported that F4/80^{low} macrophages are predominant in adipose tissue of lean mice and produce anti-inflammatory cytokines such as IL-4 (2). On the other hand, F4/80^{high} macrophages are associated with impaired glucose tolerance and produce a greater amount of pro-inflammatory cytokines (2). F814 mice displayed a significantly reduced total number of macrophages in their adipose tissue compared to WT, and they had a higher F4/80^{low} to F4/80^{high} macrophage ratio than the WT control. We confirmed higher pro-inflammatory gene expression, such as TNF- α in F4/80^{high} compared to F4/80^{low}, and only F4/80^{low} cells expressed IL-4. Our findings also showed that F814 AT expressed a substantially lower amount of MCP-1 (CCL2) than WT, corroborating reduced macrophage recruitment to AT and thus decreased systemic inflammation levels in the mutant. 90% of WT animals and only 10% of F814 animals on HFD had mature macrophages present in the peripheral blood, further indicating a significantly decreased activation of the innate immune system in the mutant. Because elevated levels of systemic chronic inflammation markers such as CRP and MCP-1 are also closely associated with bone health, μ CT analysis revealed significantly higher bone density in trabecular bone (bone volume/total volume) in F814 and X-13 treated mice on HFD compared to WT or vehicle-treated mice on HFD (Figure 1). TRAP staining indicated that the mutant and drug-treated mice had reduced osteoclast activity. Gp130-Y814 inactivation also prevented total weight gain in male, but not female mice; this effect was much more pronounced in the drug-treated mice compared to F814 animals.



DISCUSSION. Here we show the impact of the gp130-Y814 residue mutation and drug X-13 on obesity-induced inflammation and its subsequent effect on bone density. Our data reveals that gp130-Y814 signaling plays a key role in macrophage recruitment/polarization in AT of obese mice, and inactivation of this residue favors anti-inflammatory versus pro-inflammatory macrophage polarization in AT. Remarkably, F814 mutants and X-13 treated mice were largely protected from bone density loss induced by HFD; the association between reduced systemic inflammation and preserved bone density is evident in F814 mutant and X-13 treated mice. Furthermore, the implications extend to osteoclast activity, a key player in bone remodeling, with both groups displaying diminished osteoclast activity. This suggests a potential connection between the modulation of macrophage polarization and osteoclast regulation, hinting at a broader mechanism through which the mutation and drug intervention could impact bone health.

SIGNIFICANCE. This study offers a substantial stride toward understanding the intricate interplay between inflammation and bone density regulation in the context of obesity. The demonstrated capacity of the gp130-Y814 mutation and X-13 treatment to curtail inflammation-associated bone density loss holds significant promise for addressing critical challenges in metabolic and age-related health decline. By elucidating the mechanisms through which these interventions reduce osteoclast activity and preserve bone density, this study paves the way for potential therapeutic avenues that target the nexus between chronic inflammation and bone deterioration.

ACKNOWLEDGEMENTS. This work is supported by NIH grants R01AG058624 and R01AG058624 (to DE).

REFERENCES.

1. Shkhyan R, Flynn C, Lamoure E, Sarkar A, Lu J, Liu NQ, Lee Y, Lee S, Li J, Vadivel K, Bajaj P, Harn H, Chuong CM, Van Handel B, Hurtig M and Evseenko M. Inhibition of a signaling modality within gp130 receptor enhances tissue regeneration and mitigates osteoarthritis (2023). *Science Translational Medicine*, Epub: March 22.
2. Bassaganya-Riera J, Misyak S, Guri AJ, and Montecillas R. PPAR γ is highly expressed in F4/80hi adipose tissue macrophages and dampens adipose-tissue inflammation (2009). *Cell Immunol*; 258(2): 138–146.