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Juraj Zahatnansky MD
*Western Michigan University Homer Stryker M.D. School of Medicine*

Daniel Dick
*Van Andel Research Institute*

Gabrielle Foxa
*Van Andel Research Institute*

Robert Baker
*Western Michigan University Homer Stryker M.D. School of Medicine*

Bart Williams PhD
*Van Andel Research Institute*

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Western Michigan University

# EXERCISE AS A POTENTIAL TREATMENT FOR WNT INHIBITOR INDUCED BONE LOSS

Juraj Zahatnansky, Daniel Dick*, Gabrielle Foxa*, Cassandra Diegel*, Robert Baker, Bart Williams*

Western Michigan University Homer Stryker M.D. School of Medicine, Kalamazoo, Michigan and *Van Andel Research Institute, Grand Rapids, Michigan

## ABSTRACT

Wnt signaling pathway has been shown to play a role in bone homeostasis (**Figure 1**) and carcinogenesis. On the one hand, a decrease in signaling has been associated with a decrease in bone mass, on the other, an increase in signaling with cancer development.

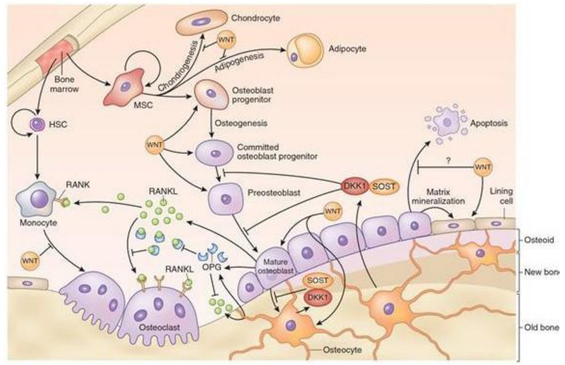



**Figure 1 from Baron, R. and Kneissel, M., *WNT signaling in bone homeostasis and disease: from human mutations to treatments.* Nature Medicine, 2013. 19(2): p. 179-92.**

LGK974 is a Wnt signaling inhibitor currently being investigated as a potential cancer therapeutic agent. This molecule inhibits Porcupine, a transmembrane protein necessary for Wnt ligand secretion (**Figure 2**).
In light of the above and based on our preliminary data (not shown here), treatment with LGK974 leads to significant bone mass loss. Our investigation aims to address whether such bone loss can be prevented by mechanically inducing stress to the bone during the treatment with LGK974.

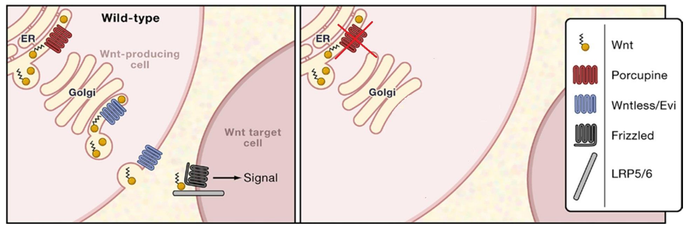
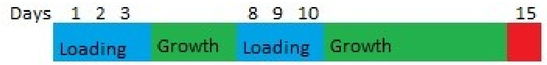


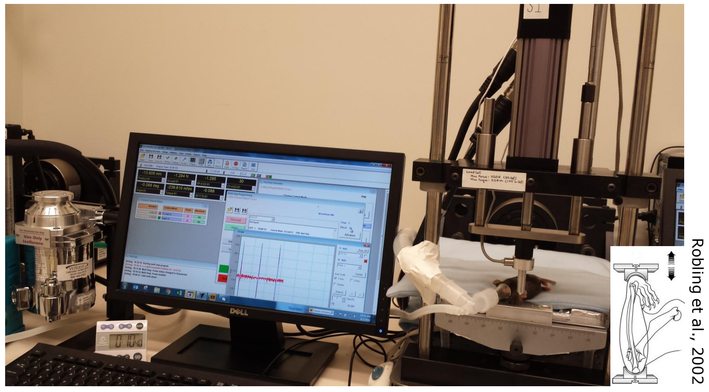

**Figure 2 Adopted from Ching, W. and R. Nusse, *A dedicated Wnt secretion factor.* Cell, 2006. 125(3): p. 432-3.**

## METHODS

We treated twelve 20-week old C57Bl/6J male mice with LGK974 and twelve with a vehicle (0.5% Methyl Cellulose, 0.5% Tween-80 in water) on weekdays for two weeks. During that time, under isoflurane-induced anesthesia, all animals underwent right forearm mechanical loading at 60 cycles per day for 3 consecutive days using a 2-Hz haversine waveform at a peak force of 2.4 N. The non-loaded left forearm served as an internal control. Both loaded and control limbs were harvested 15 days post first loading day and processed for micro-computed tomography (microCT).




**Ulna Loading Schedule Schematic. Loading days are shown as blue boxes and days without loading are shown as green boxes. The mice were sacrificed on the day shown with a red box.**



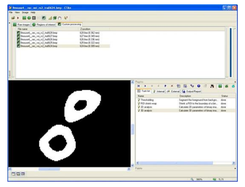
Robling et al., 2002

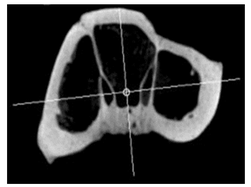
**Loading Apparatus**

Also, for dynamic histomorphometry (quantitative study of the microscopic organization and structure of the bone), we injected all animals with two doses of 1% calcein solution on two different days to allow for measurement of bone formation rate and matrix apposition rate (a measure of the amount of bone matrix deposited per osteoblast cluster). All procedures performed in this experiment were in accordance with the Van Andel Research Institute Institutional Animal Care and Use Committee guidelines.

## RESULTS

Cross sectional microCT analysis was performed on the region of interest which was just distally to the midshaft of the ulna.





**MicroCT analysis (Vandermoren, Andy, Grand Rapids Lecture)**

We were unable to identified statistically significant differences in multiple different bone parameters (Figure 3) between the ulnas that were mechanically loaded and treated with LGK974 versus those treated with LGK974 alone. However, based on the trends we observed, we felt further investigation was warranted.

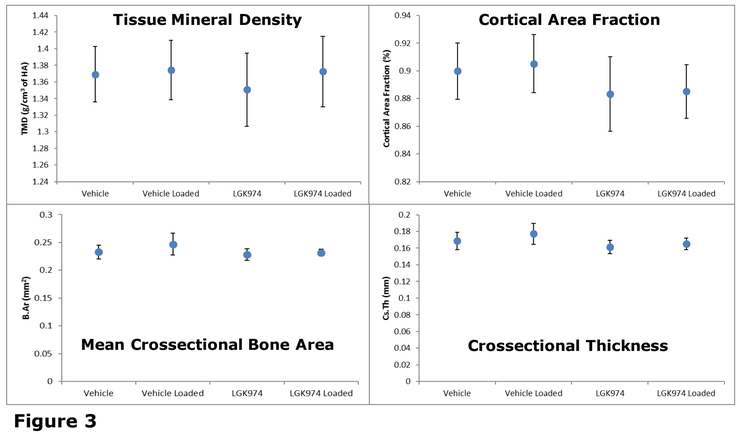



**Figure 3**

We therefore submitted these samples to our outside collaborator for histomorphometry and are awaiting the results. We also went ahead and repeated the loading study without the treatment with the drug. This time we waited till day 42 post the initial loading day before harvesting the bones. We were able to detect statistically significant differences between the loaded and non loaded ulnas by microCT. We are currently evaluating different amounts of maximal force applied to the ulna in order to eliminate woven bone phenotype observed in some of the samples.