# A SYSTEMS BIOLOGY MODEL TO DESCRIBE LONG-TERM BONE REMODELING EFFECTS OF ESTROGEN IN MENOPAUSAL AND POSTMENOPAUSAL WOMEN

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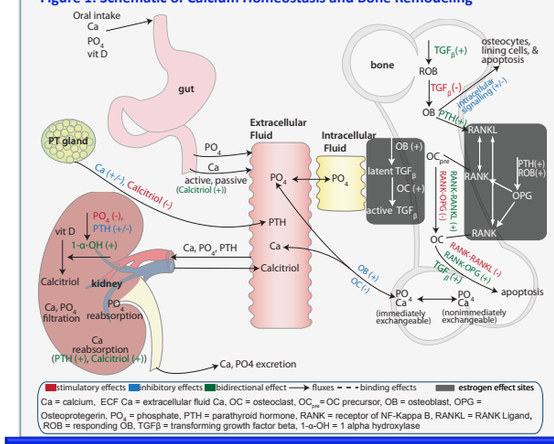
## ABSTRACT

**Background**: Estrogen has been proposed to control bone remodeling through multiple cellular mechanisms, including mediation through receptor activator of NF-κB (RANK)-RANK ligand (RANKL)-osteoprotegerin (OPG) and transforming growth factor beta (TGFβ). Estrogen withdrawal, caused naturally during menopause or through discontinuation of postmenopausal estrogen replacement therapy (ERT), increases bone turnover and decreases bone mineral density (BMD). Conversely, ERT in postmenopausal patients decreases net bone resorption and increases BMD. Quantifying these changes through a systems biology model will allow for appropriate accounting of longitudinal estrogen effects while exploring bone- and/or calcium-related therapeutic interventions and disease progressions. **Methods**: A previously developed systems biology model was the framework for quantifying estrogen effects on bone remodeling. Briefly, this model quantifies the interrelation of osteoclasts and osteoblasts through RANK-RANKL-OPG, TGFβ and parathyroid hormone, in concert with controlling mechanisms for total body calcium homeostasis. Literature data from menopausal and postmenopausal patients with and without ERT followed for up to 3 years were used to estimate alternative model effects of estrogen on RANKL and TGFβ and to discern the plausible roles of each as controlling factors of bone remodeling. **Results**: Models describing estrogen effects through both RANKL and TGFβ were able to estimate bone resorption decreases (40- 60%) related to ERT in postmenopausal patients and increases (100-150%) related to estrogen withdrawal. Visual diagnostics revealed that the time-courses of observed changes were more consistent with mediation through TGFβ. **Conclusion**: Estrogen-related changes in bone remodeling were capable of being estimated through a systems biology model affecting either RANKL or TGFβ, but the time-course of these effects suggested a more predominant role related to TGFβ.

## OBJECTIVE

- Explore proposed pharmacologic mechanisms linking estrogen effects to longitudinal bone remodeling markers.
- Quantify progression of bone remodeling effects following estrogen replacement therapy (ERT) discontinuation in postmenopausal (PM) women, or following initiation of ERT.

**Figure 1: Schematic of Calcium Homeostasis and Bone Remodeling**




## BACKGROUND

- Calcium (Ca) homeostasis and bone remodeling are both physiological requirements
  - Involves intracellular signaling, endocrine feedbacks and multiple organs
  - Maintains tight control of extracellular fluid (ECF) Ca concentration
  - Regulates bone remodeling: maintain bone structure / strength
- Estrogen effects on bone remodeling reported to be mediated through effects on RANK-RANKL-OPG and transforming growth factor beta (TGFβ)[1-5]
- A previously developed Ca homeostasis and bone remodeling systems biology model[6-7] (Figure 1) provided a general platform to evaluate these plausible controlling mechanisms of estrogen on bone remodeling

## METHODS

- Literature reports were reviewed to determine the typical magnitude and time-course of estrogen effects on the following bone remodeling markers:
  - Bone resorption markers (osteoclast function):
    - Urine N-telopeptide (uNTx)
    - Serum C-telopeptide (sCTx)
  - Bone formation marker (osteoblast function):
    - Bone specific alkaline phosphatase (BSAP)
- Literature data digitized: Plot Digitizer 2.4.1
  - http://plotdigitizer.sourceforge.net/
- Graphics and data management: R version 2.7.2
  - http://r-project.org
- Model fitting and simulation: Berkeley Madonna 8.0
  - http://berkeleymadonna.com
- Models components evaluated: ( ) inhibit, ( ) stimulate
  - Direct effects of estrogen on production of TGFβ or RANKL
  - Effect of estrogen-TGFβ interaction on osteoblast survival
  - Goodness-of-fit diagnostics
    - Graphical
    - Akaike Information Criterion (AIC)

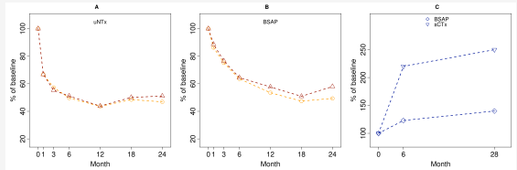
## RESULTS

***OBSERVED CLINICAL DATA***

(--○--) Bone, H.G., et al., J Clin Endocrinol Metab, 2000. 85(2): p. 720-6.
(--△--) Greenspan, S.L., et al., Ann Intern Med, 2002. 137(11): p. 875-883.
(--◇▽--) Sornay-Rendu, E., et al., Bone, 2003. 33(1): p. 159-66.

- Bone remodeling markers continued to decline even after one year of ERT in PM women. After ERT discontinuation markers increased for > 6 months (Figure 2).

**Figure 2: Observed (mean)[8-10] bone remodeling markers following initiation (Panel A and B) and discontinuation (Panel C) of ERT**




***PROPOSED MECHANISM THROUGH RANKL EFFECT***

- ERT initiation: decreased RANKL results in too rapid of a decrease in osteoclast function (Figure 3).

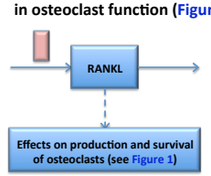
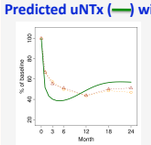



**Figure 3: Estrogen effect on RANKL. Predicted uNTx ( ) with ERT initiation**

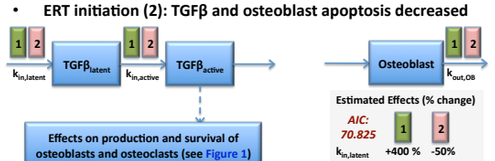


uNTx decrease predicted from ~15% reduction in RANKL production

*AIC: 106.65*

***PROPOSED MECHANISM THROUGH TGFβ EFFECTS***

- ERT discontinuation (1): TGFβ and osteoblast apoptosis increased
- ERT initiation (2): TGFβ and osteoblast apoptosis decreased





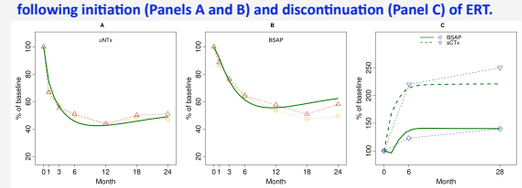


## RESULTS (continued)

***PROPOSED MECHANISM THROUGH TGFβ EFFECTS, continued…***

- Describes time-course and magnitude of bone markers (Figure 4).

**Figure 4: Estrogen effects on TGFβ. Predicted ( ) bone remodeling markers following initiation (Panels A and B) and discontinuation (Panel C) of ERT.**




## SUMMARY

- System model provided tool for evaluating proposed mechanisms.
- Estrogen-related changes in bone remodeling were capable of being estimated through the model, effectively ruling out a mechanism based on RANKL alone. Rather, the time-course of these effects suggested a more predominant role related to TGFβ.
- This quantitative model is useful for simulation following initiation or withdrawal of ER, and can also be translated to simulate natural progression of estrogen loss during menopause.
- Subsequent linkage to bone mineral density will allow quantitative description of osteoporosis disease progression.

## REFERENCES

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