Extension of a Multiscale Model of Calcium Homeostasis and Bone Remodeling to Include the Progressive Effects of Estrogen Loss During Menopause Transition.

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Abstract

Purpose: *Extend an existing multiscale model to describe the effect of naturally declining estrogen on calcium homeostasis and bone remodeling. Inclusion of this natural progression will enhance our understanding of the kinetic-dynamic mechanisms controlling these changes, as well as provide an accounting of these longitudinal changes in typical patients to be studied for related therapies (e.g., SERM, hormonal antagonism), combinations of therapies, or related disease states.*

Methods: *The underlying multiscale model has been published and described previously.[1] For this extension of the model, data relating estrogen changes by age and menopause onset age[2,3] were modeled to describe the natural longitudinal estrogen decline in women during the transition through menopause. Estrogen has been shown to decrease bone turnover, conversely leading to an increase in net bone resorption (loss) when natural estrogen levels decline. Longitudinal changes in bone markers following estrogen replacement therapy in postmenopausal women can be described by directly affecting TGFβ production and through intracellular changes affecting osteoblast apoptosis rates.[4] These same mechanisms were used to quantify the changes in bone makers during progression from peri- to post-menopause in women. In addition to these effects on bone turnover, estrogen inversely affects the renal excretion of calcium.[5,6] To account for these changes within the existing model, an effect relating estrogen levels with the calcium renal tubular reabsorption maximum was investigated. The resulting model was used to simulate longitudinal effects for varying menopausal onset ages with and without subsequent estrogen replacement therapy. These results were compared to reported clinical observations, e.g., (Garnero et al., 1996;[7] Gallagher et al., 2002;[8] Greenspan et al., 2002;[9] Riggs et al., 2002[10]).*

Results: *Longitudinal changes (relative fraction) in endogenous estrogen (E) production were modeled using an ordinary differential equation with first-order elimination (E_{out}, $t_{1/2}$ = 12 hours) and a zero-order endogenous production rate (E_{in}). The rate of production declined after 41 years of age with an additional fractional decline during menopause: $E_{in} = (E_{out})*(Age/41)^{-2.3}*(1 - (0.64*(time_{normo})^{\theta 1}) /((meno_{mid})^{\theta 1} + (time_{meno})^{\theta 1})$, where Age = contiguous patient age (years), $time_{normo}$ = time from the start of menopause, and $meno_{mid}$ = midpoint (0.83 years, total menopause duration = 1.66 years). Therefore, $time_{normo}$ was calculated based on menopause onset age, which itself was an adjustable parameter in the model. Power function models were used to describe the effect of estrogen on latent TGFβ production [$k_{in,latent} = k_{in,latentnorm} \cdot (\frac{1}{E})^{\theta_1}$], the conversion to active TGFβ [$k_{conversion} = k_{conversion,norm} \cdot E^{\theta_2}$], and on responding osteoblast generation [$k_{gen} = k_{in,gen,norm} * (\frac{1}{E})^{\theta_3}$], and osteoblast apoptosis [$k_{apop} = k_{in,apop,norm} \cdot (\frac{1}{E})^{\theta_4}$], where θ_1 = 0.075, θ_2 = 0.045, and θ_3 = 0.16 and θ_4 = 0.000012 (all unitless). The model predicted observed increases from baseline in the bone markers for resorption (telopeptide) and formation (bone alkaline phosphatase) of 96% and 48%, respectively, over the 20-year progression from pre- to post-menopause, consistent with literature reports. Postmenopausal estrogen replacement reversed these increases during therapy with appropriate rebound following discontinuation. The estrogen effect on calcium urinary reabsorption was modeled linearly such that a 90% decline in estrogen decreased maximal reabsorption by 10%.*

Conclusions: *A multi-scale model of calcium homeostasis and bone remodeling has been extended to include the natural effects of aging in woman just prior to, and through, menopause. This model will provide a platform for incorporating these changes within the context of other system changes.*

VIEWPOINT

- Do you use a system of models to understand a drug and its effect on a disease? **OR**
- Do you use drugs and diseases to understand a model system?

Argument: The latter leaves you better informed and with a powerful tool for exploration

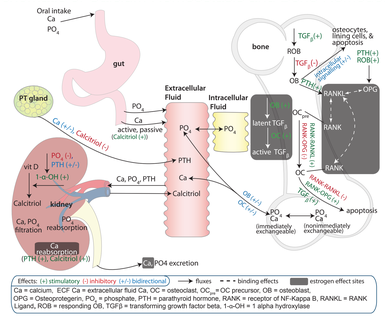
OBJECTIVE

- Link estrogen effects to longitudinal bone remodeling markers
  - Develop longitudinal model of estrogen loss during menopause transition
  - Link longitudinal changes in estrogen with physiologic effects on:
    - Bone remodeling
    - Renal calcium handling

BACKGROUND

- Calcium (Ca) homeostasis and bone remodeling
  - Multiscale involvement: intracellular signaling, endocrine feedbacks, and multiple organs (*Figure 1*)
  - Maintains tight control of extracellular fluid (ECF) Ca concentration
  - Regulates bone remodeling: maintain bone structure/quality
- Estrogen effects:
  - Bone remodeling: mediated through RANK-RANKL-OPG and transforming growth factor beta (TGFβ)[4,11–14]
  - Decrease in calcium renal tubular reabsorption maximum (increased calcium excretion) with estrogen loss[5,6]

Figure 1: Schematic of Calcium Homeostasis and Bone Remodeling.



Modified from Figure 1 of Peterson and Riggs, 2010[1]

METHODS

- Clinical data from literature review:
  - Magnitude and time-course of estrogen loss during the transition through menopause
  - Associated effects on bone remodeling markers:
    - Bone resorption marker (osteoclast function): urine cross-linked N-telopeptide of type I bone collagen (NTx)
    - Bone formation marker (osteoblast function): bone specific alkaline phosphatase (BSAP)
- Extend existing Ca homeostasis and bone remodeling model (*Figure 1*)[1] to evaluate:
  - Longitudinal estrogen (E, relative fraction) described as ordinary differential equation (Equation 1)

$$\frac{d(E)}{dt} = k_{in,E} - k_{out,E} \cdot E$$

$$k_{in,E}(t) = \begin{cases} k_{out,E} & Age < 41 \\ k_{out,E} \cdot (Age/41)^{-2.3} \cdot \left(1 - 0.64 \cdot \frac{(Age_{onset})^2}{(Age_{mid})^2 + (Age_{onset})^2}\right) & Age \geq 41 \end{cases} \quad (1)$$

    - Age_{onset} represents time (years) from the start of menopause onset
    - Age_{mid} represents half of the duration of menopause and is assumed to correspond with the time of final menstrual period (FMP)
  - Direct effects of estrogen on production of TGFβ (Equations 2, 3)

$$\frac{d(TGF\beta_{latent})}{dt} = k_{in,TGF\beta} \cdot \left(\frac{1}{E^{\theta_{TGF\beta_{latent}}}}\right) - k_{conversion,E} \cdot \left(E^{\theta_{TGF\beta_{active}}}\right) \cdot TGBF\beta_{latent} \quad (2)$$

$$\frac{d(TGF\beta_{active})}{dt} = k_{conversion,E} \cdot \left(E^{\theta_{TGF\beta_{active}}}\right) \cdot TGBF\beta_{latent} - k_{out,TGF\beta} \cdot TGF\beta_{active} \quad (3)$$

  - Effect of estrogen-TGFβ interaction on responding osteoblast (ROB) differentiation (Equation 4) and osteoblast (OB) survival (Equation 5)
  - Estrogen-TGFβ interaction included multiplicatively: ROB and OB differentiation and survival rates are affected by $TGF\beta$ (*Figure 1*)[1]

$$\frac{d(Osteoblast_{responding})}{dt} = k_{in,ROB} \cdot \left(\frac{1}{E}\right)^{\theta_{ROB}} - k_{in,OB} \cdot ROB \quad (4)$$

$$\frac{d(Osteoblast_{active})}{dt} = k_{in,OB} \cdot ROB - k_{apop,OB} \cdot \left(\frac{1}{E}\right)^{\theta_{OB}} \cdot OB \quad (5)$$

  - Effect of estrogen on renal calcium reabsorption maximum (Tm) for urinary excretion (Equation 6)

$$Tm_{estrogen} = \theta_{slope,E} \cdot E + \theta_{int,E} \quad (6)$$

Rate constants and all other model parameters for each moiety (TGFβ, ROB, OB, osteoclasts [OC], PTH, extracellular and bone calcium, calcitriol, etc.) were fixed at previously reported values.[1]

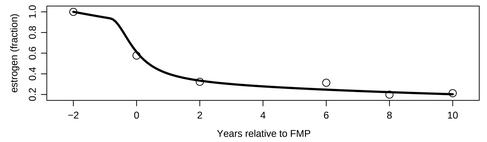
- Software
  - Literature data digitized: Plot Digitizer 2.4.1 (http://plotdigitizer.sourceforge.net/)
  - Graphics and data management: R version 2.7.2 (http://r-project.org)
  - Model fitting and simulation: Berkeley Madonna 8.0 (http://berkeleymadonna.com)

RESULTS

Longitudinal Estrogen Effect

- First-order elimination ($k_{out,E}$, $t_{1/2}$ = 12 hours)
- Age_{mid} = 0.83 years

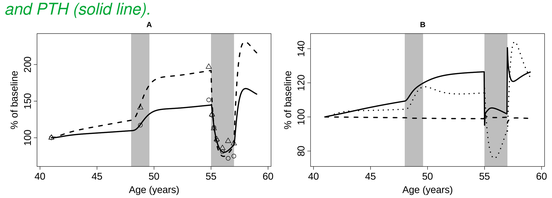
Figure 2: Predicted fractional changes in estrogen during menopause transition (solid line). Observed data (circles) were taken from Sowers et al.[3]



Longitudinal Calcium, PTH and Bone Effects

- Model Estimates (unitless):
  - $\theta_{TGF\beta_{latent}}$ and $\theta_{TGF\beta_{active}}$ = 0.075 and 0.045, respectively
  - θ_{ROB} and θ_{OB} = 0.16 and 0.000012, respectively
  - Urine calcium reabsorption maximum (Tm) decreased linearly:
    - $\theta_{slope,E}$ and $\theta_{int,E}$= 0.0848 and 0.915, respectively
    - ~ 8% decline with a 90% reduction in estrogen

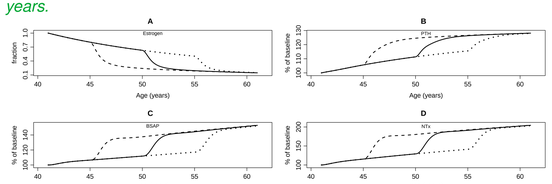
Figure 3: Predicted longitudinal changes in (A) BSAP (solid line) and urine NTx (dashed line), and (B) plasma calcium (dashed line), TGF-β (dotted line) and PTH (solid line).



Shaded area from age 48 to 49.6 years highlights the assumed duration of menopause. Shaded area from age 55 to 57 years represents the period of post-menopausal estrogen replacement therapy. Observed bone marker data (BSAP=circles, NTx = triangles) prior to estrogen replacement therapy were taken from Garnero et al.,[7] and data during estrogen replacement were taken from Bone et al.[15]

Model Simulation: Longitudinal Effects of Varied Menopause Onset Age

Figure 4: Simulated longitudinal estrogen (A), PTH (B), BSAP (C) and NTx (D) for menopause onset ages of 45 (dashed), 50 (solid), and 55 (dotted) years.



Comparison to Observed Clinical Data

- Predicted observed changes in BSAP and NTx over the time-course of menopause transition and during estrogen replacement in post-menopausal women (*Figure 3, Panel A*)
- Nordin et al. reported a 2.1-fold increase in urine Ca excretion related with a 7.4% decrease in the tubular reabsorption maximum.[5] Model predicted an approximate 2.5-fold increase in urine Ca excretion related with a 7.6% decrease in the tubular reabsorption maximum (*Figure 3, Panel B*).
- Riggs et al. reported increased PTH of ~10% and 25% in women of age groups 50–59 and greater than 70 years old, respectively, compared to pre-menopausal women.[10] Model predicted PTH increases of 10–25% in 50-to-59-year-old women who entered menopause a the age of 50, with an asymptotic maximum of ~30% (*Figure 3, Panel B*).

DISCUSSION

- Model can be used to evaluation mechanisms reported to mediate estrogen effects on bone
  - Results support direct effects on TGFβ and through effects controlling OB differentiation.
  - Estrogen-mediated effect on OB apoptosis rate was estimated to very nearly zero, indicating it was either not influential, or not distinguishable from the other estimated estrogen-related effects.
- Model can be used to confirm and quantify known physiology
  - Model described inverse relationship between estrogen level and net bone resorption, and thus net calcium loss from bone to extracellular fluid,
  - Estrogen inversely affects the renal excretion of calcium,[5,6] serving as a compensatory mechanism for extracellular calcium regulation.
  - PTH has widespread involvement in mechanisms regulating oral calcium absorption, renal calcium excretion, and the RANK-RANKL-OPG system in bone. The model consistently adjusts PTH production based on feedbacks from calcium and calcitriol to the parathyroid gland.
  - Estrogen-related model parameters were not fit to clinical data pertaining to Ca, PTH, or off-treatment bone marker rebound,[5,10,14] yet the model generated remarkably consistent predictions of these related factors, serving to further substantiate the appropriateness of the quantified interrelations of the model system compared to observed biologic responses.

SUMMARY

Multiscale Model of Calcium Homeostasis and Bone Remodeling

- Extension describes natural effects of aging in women
- Extension preserved the structure and parameter estimates[1] thereby retaining its ability to describe the previously described therapeutic interventions and disease states.
- Provides simultaneous description of multiple known pharmacological effects of estrogen.
- Serves as platform for incorporating these changes within the context of other (therapeutic, disease, genetic) system changes.

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