# *Supplementary Material*

# Simulated interventions to ameliorate age-related bone loss indicate the importance of timing

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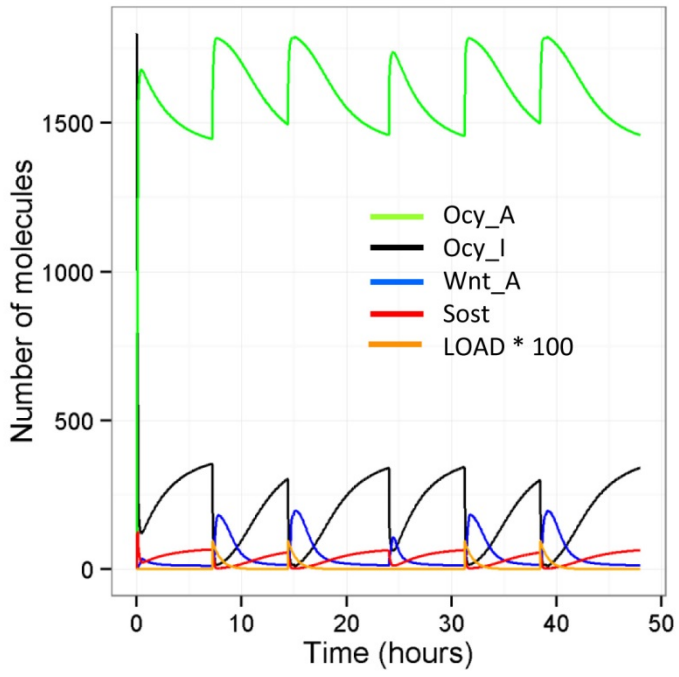
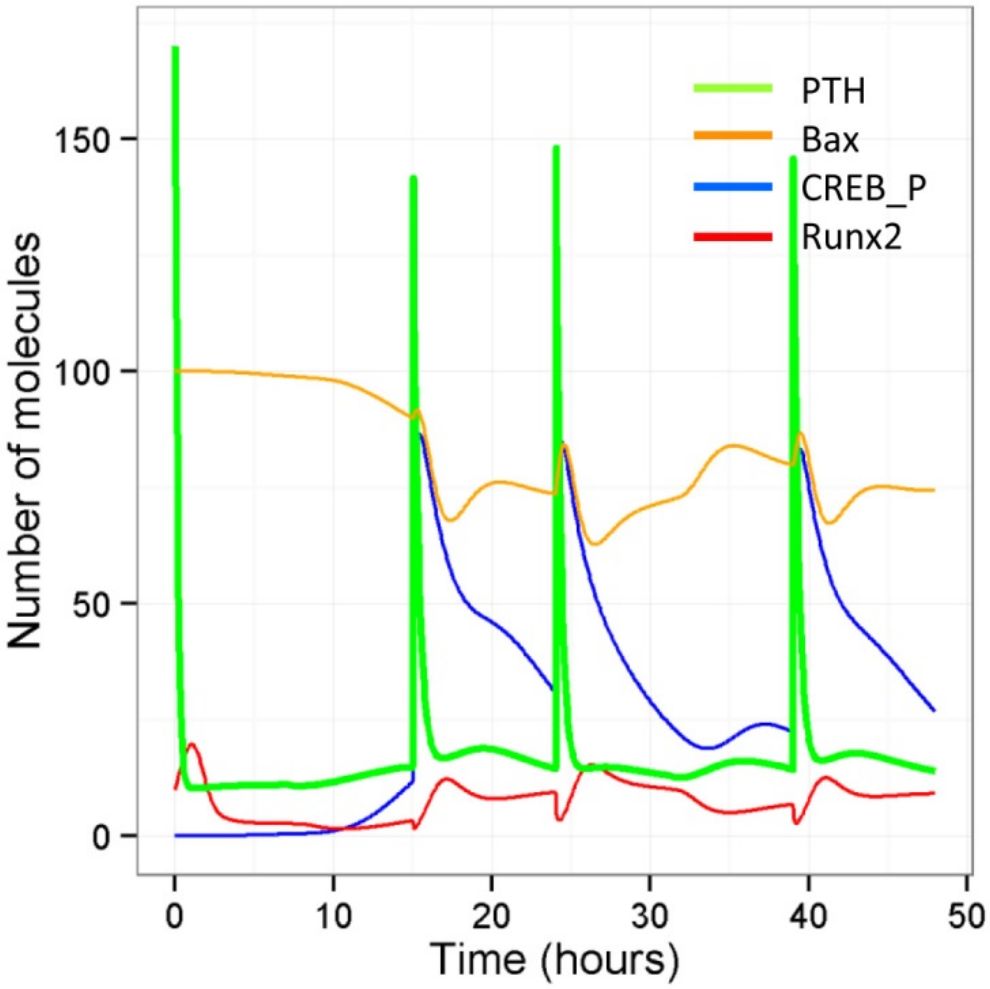
1. **Supplementary Figures**
2. **Supplementary Tables**

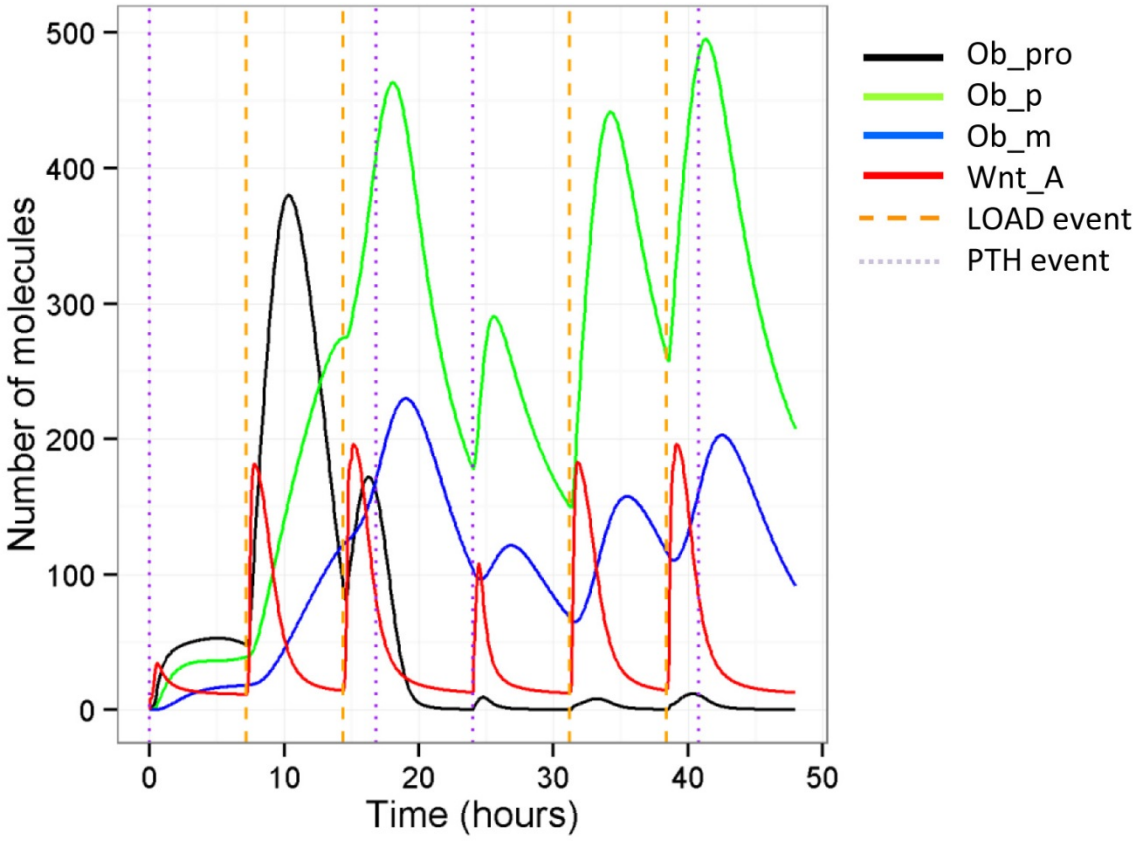
**Supplementary Figure 1 Model output of sub-model "Loading"** This figure shows the output for the model species that are immediately affected by LOAD (see Figure 1 of main manuscript). After each loading event, osteocytes are activated within 1 hour, leading to downregulation of Sost and activation of Wnt. There is then a slower return to basal levels. This agrees with experimental data (Lara-Castillo et al., 2015). LOAD is scaled by 100 for visualisation. Simulations were performed over 2 day period to give a clearer picture of the dynamics.



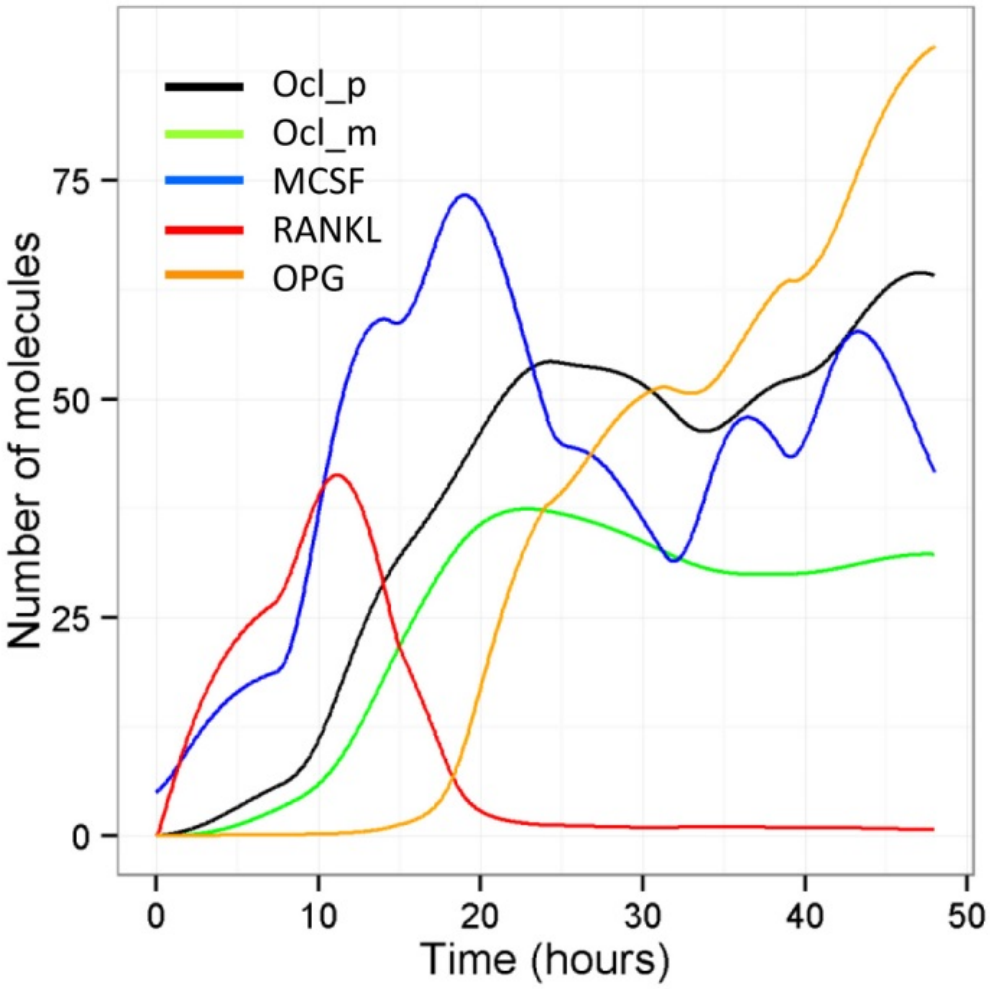
**Supplementary Figure 2 Model output of sub-model "PTH".** After each PTH event, there is a rapid increase in CREB phosphorylation, leading to an increase in Runx2 and inhibition of Bax. There is a slow return to basal levels, so when peaks of PTH are close together, the second peak of Runx2 is slightly higher, leading to lower levels of Bax – this corresponds to the night-time peak of PTH. Simulations were performed over 2 day period to give a clearer picture of the dynamics. Note actual time of simulations starts at 2 am with a peak in PTH.



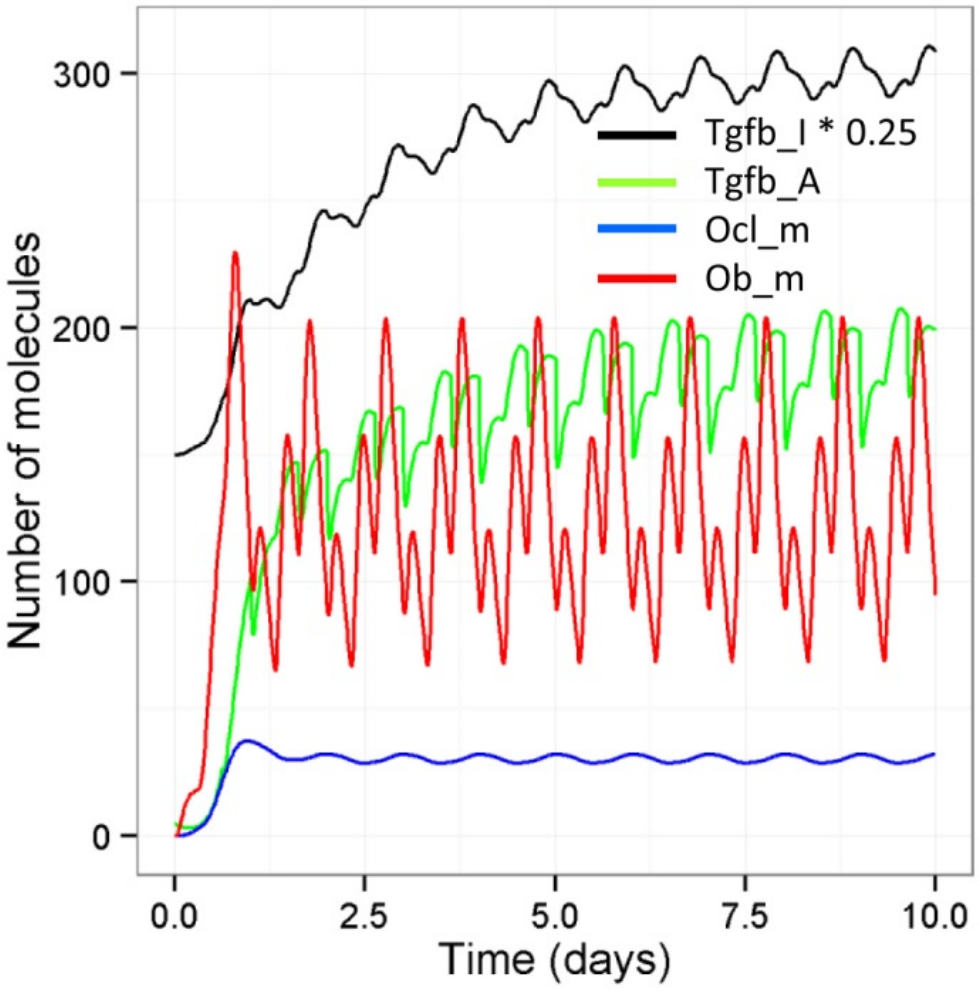
**Supplementary Figure 3 Model output of sub-model "Osteoblast differentiation"** The loading event at about 7 hours (which corresponds to 9am) results in a rapid activation of Wnt leading to MSCs differentiating into osteoblast progenitors (Ob_pro). This is followed by differentiation into osteoblast precursors (Ob_p) and finally mature osteoblasts (Ob_m). The attenuation of the Ob_pro response after 24 hours is due to the increase in Tgfb_A (which increases over the first 24 hour period and then oscillates – see Supp Fig 5) and therefore an increase in the rate at which Ob_pro differentiates into Ob_p. The lack of a regular pattern is due to the irregular spacing between the events for load and PTH. Simulations were performed over 2 day period to give a clearer picture of the dynamics. Note actual time of simulations starts at 2 am with a peak in PTH.



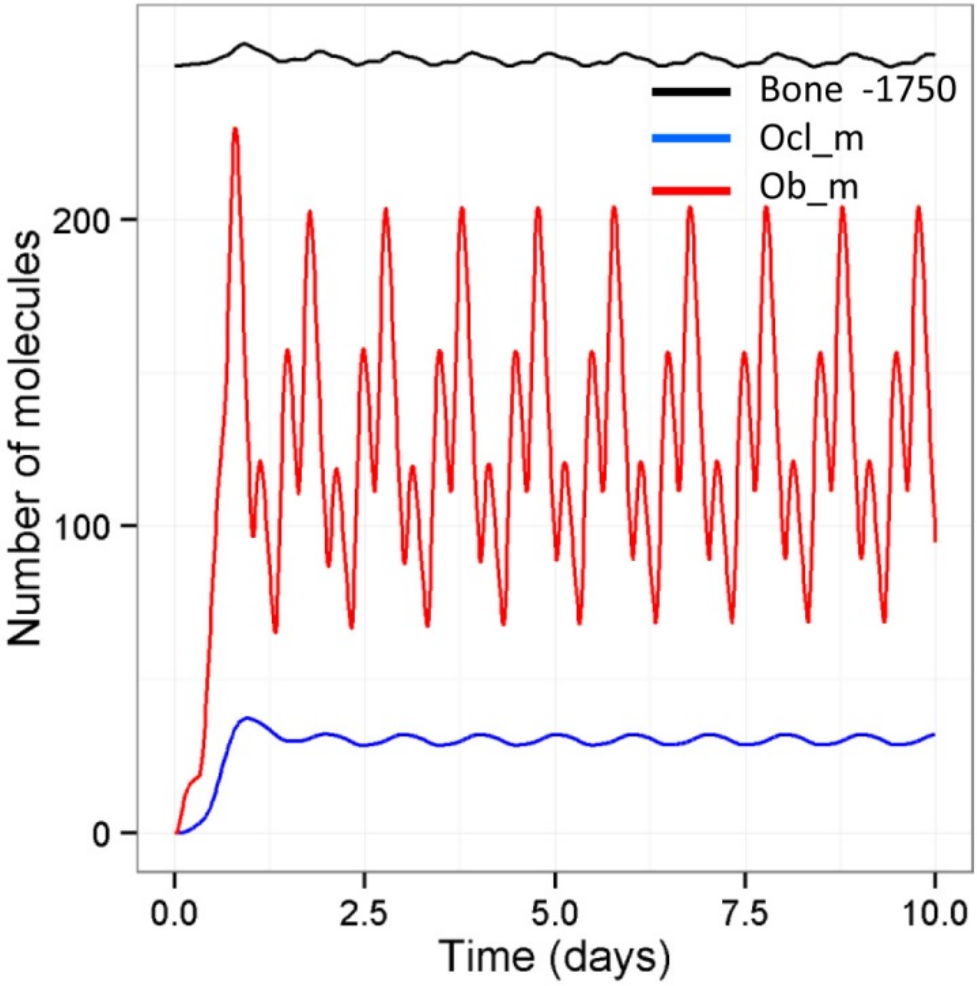
**Supplementary Figure 4 Model output of sub-model "Osteoclast differentiation".** The peak of PTH at the beginning of the simulation leads to an increase in secretion of MSCF and RANKL and both are increased further after the loading event at about 8 hours. This leads differentiation of HSCs into osteoclast precursors (Ocl_p), followed by differentiation into mature osteoclasts (Ocl_m). OPG levels start to increase as mature osteoblasts are formed, leading to less osteoclast differentiation and numbers of Ocl_m decline due to apoptosis. Simulations were performed over 2 day period to give a clearer picture of the dynamics.



**Supplementary Figure 5 Model output of sub-model "Tgfb".** When mature osteoblasts (Ob_m) increase after loading or a peak in PTH levels, levels of secreted (inactive) Tgfb (Tgfb_I) increase, so that peaks of Tgfb_I correspond with peaks of Ob_m. Tgfb is activated (Tgfb_A) by mature osteoclasts (Ocl_m) and so peaks of Tgfb_A correspond to peaks of Ocl_m. Tgbf_I is scaled for easier visualisation. Simulations were performed over 10 day period to show how oscillations of bone cells affect Tgfb levels. Note that Tgfb_A is total pool including that which is bound to Ob_p.



**Supplementary Figure 6 Model output of sub-model "Bone turnover".** During the time course, mature osteoblasts (Ob_m) and mature osteoclasts (Ocl_m) peak at different times so that the Ob_m/Ocl_m ratio changes with time. Bone mass increase when the ratio is high and declines when the ratio is decreased.



## Supplementary Table 1. Details of model reactions and parameter values

| Reaction ID | Reactants and products | Kinetic rate law |
|---|---|---|
| Unloading | LOAD → Sink | $k_{unload}$ * LOAD |
| Osteocyte_activation | Ocy_I + LOAD → Ocy_A + LOAD | $k_{actOcy}$ * Ocy_I * LOAD |
| Osteocyte_inactivation | Ocy_A → Ocy_I | $k_{inactOcy}$ * Ocy_A |
| Ocy_I_binding_by_PTH | Ocy_I+PTH→ Ocy_I_PTH | $k_{binOcyPTH}$ * Ocy_I * (PTH^2/(100^2+PTH^2)) |
| Ocy_I_PTH_release | Ocy_I_PTH → Ocy_I +PTH | $k_{relOcyPTH}$ * Ocy_I_PTH |
| Osteocyte_activation_by_PTH | Ocy_I_PTH → Ocy_A+PTH | $k_{actOcyPth}$ * Ocy_I_PTH |
| Ocy_apoptosis | Ocy_I → Sink | $k_{deathOcy}$ * Ocy_I |
| Sost_secretion | Ocy_I→ Ocy_I+Sost | $k_{secSost}$ * Ocy_I |
| Sost_degradation | Sost → Sink | $k_{degSost}$ * Sost |
| Wnt_activation | Wnt_I → Wnt_A | $k_{actWnt}$ * Wnt_I |
| Wnt_activation_by_PTH | Wnt_I+Ob_m_PTH → Wnt_A+Ob_m_PTH | $k_{actWntPth}$ * Wnt_I * Ob_m_PTH |
| Wnt_inactivation_by_Sost | Wnt_A+Sost → Wnt_I +Sost | $k_{inactWnt}$ * Wnt_A * Sost^2/(50^2+Sost^2) |
| MSC_differentiation_to_Ob_pro | MSC+Wnt_A → MSC+Wnt_A+Ob_pro | $k_{diffMSC}$ * MSC * Wnt_A^2/(50^2+Wnt_A^2) |
| Osteoblast_progenitor_ differentiation_by_Tgfb | Ob_pro+Tgfb_A → Ob_p+Tgfb_A | $k_{diffObproTgfb}$ * Ob_pro * Tgfb_A^2/(50^2+Tgfb_A^2) |
| Ob_precursor_differentiation | Ob_p → Ob_m | $k_{diffObP}$ * Ob_p |
| Ob_p_binding_by_PTH | Ob_p+PTH → Ob_p_PTH | $k_{binObpPTH}$ * Ob_p * (PTH^2/(100^2+PTH^2)) |
| Ob_p_PTH_release | Ob_p_PTH → Ob_p+PTH | $k_{relObpPTH}$ * Ob_p_PTH |
| Ob_p_Tgfb_binding | Ob_p+Tgfb_A → Ob_p_Tgfb_A | $k_{binObpTgfb}$ * Ob_p * Tgfb_A |
| Ob_p_Tgfb_release | Ob_p_Tgfb_A → Ob_p+Tgfb_A | $k_{relObpTgfb}$ * Ob_p_Tgfb_A |
| Ob_maturation_to_Ocy | Ob_m → Ocy_I | $k_{matOb}$ * Ob_m |
| Ob_maturation_to_Ocy_by_Tgfb | Ob_m+Tgfb_A → Ocy_I+Tgfb_A | $k_{matObTgfb}$ * Ob_m * Tgfb_A^2/(50^2+Tgfb_A^2) |
| Ob_m_bound_by_PTH | Ob_m+PTH → Ob_m_PTH | $k_{binObmPTH}$ * Ob_m * (PTH^2/(100^2+PTH^2)) |
| Ob_m_PTH_release | Ob_m_PTH → Ob_m+PTH | $k_{relObmPTH}$ * Ob_m_PTH |
| Ob_m_apoptosis | Ob_m+Bax → Bax | $k_{deathOb}$ * Ob_m * Bax^2/(50^2+Bax^2) |
| Ob_m_PTH_apoptosis | Ob_m_PTH+Bax → Bax+PTH | $k_{deathOb}$ * Ob_m_PTH * Bax^2/(50^2+Bax^2) |
| HSC_differentiation_to_Ocl_p | HSC+MCSF → HSC+MCSF+Ocl_p | $k_{diffHSC}$ * HSC * $MCSF^2/(50^2+MCSF^2)$ |
| Ocl_p_RANKL_binding | RANKL+Ocl_p → Ocl_p_RANKL | $k_{binOclpRANKL}$ * Ocl_p* RANKL |
| Ocl_p_RANKL_release | Ocl_p_RANKL → Ocl_p + RANKL | $k_{relOclpRANKL}$ * Ocl_p_RANKL |
| Osteoclast_precursor_ differentiation | Ocl_p_RANKL → Ocl_m | $k_{diffOclP}$ * Ocl_p_RANKL |
| Ocl_p_apoptosis | Ocl_p → Sink | $k_{deathOclp}$ * Ocl_p |
| RANKL_inhibition | OPG+RANKL → OPG_RANKL | $k_{inhibRANKL}$ * OPG * RANKL |
| OPG_RANKL_dissociation | OPG_RANKL → OPG+RANKL | $k_{relRANKL}$ * OPG_RANKL |
| OPG_RANKL_degradation | OPG_RANKL → Sink | $k_{degOPGRANKL}$ * OPG_RANKL |
| Osteoclast_apoptosis | Ocl_m → Sink | $k_{deathOcl}$ * Ocl_m |
| RANKL_degradation | RANKL → Sink | $k_{degRANKL}$ * RANKL |
| OPG_degradation | OPG → Sink | $k_{degOPG}$ * OPG |
| MCSF_secretion_by_MSC | MSC → MSC+MCSF | $k_{secMCSFbyMSC}$ * MSC |
| MCSF_secretion_by_Ob_pro | Ob_pro → Ob_pro+MCSF | $k_{secMCSFbyObpro}$ * Ob_pro |
| MCSF_secretion_by_Ob_p | Ob_p → Ob_p+MCSF | $k_{secMCSFbyObp}$ * Ob_p |
| MCSF_secretion_by_Ob_p_PTH | Ob_p_PTH → Ob_p_PTH+MCSF | $k_{secMCSFbyObp}$ * Ob_p_PTH |
| MCSF_secretion_by_Ob_m | Ob_m → Ob_m+MCSF | $k_{secMCSFbyObm}$ * Ob_m |
| MCSF_secretion_by_Ob_m_PTH | Ob_m_PTH → Ob_m_PTH+MCSF | $k_{secMCSFbyObm}$ * Ob_m_PTH |
| MCSF_degradation | MCSF → Sink | $k_{degMCSF}$ * MCSF |

| | | |
|---|---|---|
| OPG_secretion_by_Ob_p | Ob_p → Ob_p+OPG | $k_{secOPGbyObp}$ * Ob_p |
| OPG_secretion_by_Ob_p_PTH | Ob_p_PTH → Ob_p_PTH+OPG | $k_{secOPGbyObpPTH}$ * Ob_p_PTH |
| OPG_secretion_by_Ob_m | Ob_m → Ob_m+OPG | $k_{secOPGbyObm}$ * Ob_m |
| RANKL_secretion_by_Ocy_A | Ocy_A → Ocy_A+RANKL | $k_{secRANKLbyOcy}$ * Ocy_A |
| RANKL_secretion_by_Ocy_I | Ocy_I → Ocy_I+RANKL | $k_{secRANKLbyOcyI}$ * Ocy_I |
| RANKL_secretion_by_MSCs | MSC → MSC+RANKL | $k_{secRANKLbyMSC}$ * MSC |
| RANKL_secretion_by_Ob_p | Ob_p → Ob_p+RANKL | $k_{secRANKLbyObp}$ * Ob_p |
| RANKL_secretion_by_Ob_p_Tgfb_A | Ob_p_Tgfb_A → Ob_p_Tgfb_A+RANKL | $k_{secRANKLbyObpTgfb}$ * Ob_p_Tgfb_A |
| RANKL_secretion_by_Ob_p_PTH | Ob_p_PTH → Ob_p_PTH+RANKL | $k_{secRANKLbyObpPTH}$ * Ob_p_PTH |
| RANKL_secretion_by_Ob_pro | Ob_pro → Ob_pro+RANKL | $k_{secRANKLbyObpro}$ * Ob_pro |
| RANKL_secretion_by_Ob_m | Ob_m → Ob_m+RANKL | $k_{secRANKLbyObm}$ * Ob_m |
| RANKL_secretion_by_Ob_m_PTH_enhanced | Ob_m_PTH → Ob_m_PTH+RANKL | $k_{secRANKLbyObmPTH}$ * Ob_m_PTH |
| Tgfb_secretion_by_Obm | Ob_m → Ob_m+Tgfb_I | $k_{secTgfb}$ * Ob_m |
| Tgfb_activation | Tgfb_I+Ocl_m → Tgfb_A+Ocl_m | $k_{actTgfb}$ * Tgfb_I * Ocl_m |
| Tgfb_degradation | Tgfb_A → Sink | $k_{degTgfb}$ * Tgfb_A |
| Tgfb_degradation_by_PTH | Tgfb_A+Ob_m_PTH → Ob_m_PTH | $k_{degTgfbPTH}$ * Tgfb_A * Ob_m_PTH |
| PTH_production | Source → PTH | $k_{synPTH}$ * Source |
| PTH_degradation | PTH → Sink | $k_{degPTH}$ * PTH |
| CREB_activation | Ob_m_PTH+CREB → Ob_m_PTH+CREB_P | $k_{actCreb}$ * CREB*Ob_m_PTH$^2$/ (100$^2$+Ob_m_PTH$^2$) |
| CREB_inactivation | CREB_P → CREB | $k_{inactCreb}$ * CREB_P |
| CREB_Runx2_binding | CREB_P+Runx2 → CREB_Runx2 | $k_{binCrebRunx2}$ * CREB_P * Runx2 |
| CREB_Runx2_release | CREB_Runx2 → CREB_P+Runx2 | $k_{relCrebRunx2}$ * CREB_Runx2 |
| Bcl2_synthesis | CREB_Runx2 → CREB_Runx2+Bcl2 | $k_{synBcl2}$ * CREB_Runx2 |
| Bcl2_degradation | Bcl2 → Sink | $k_{degBcl2}$ * Bcl2 |
| Bax_Bcl2_binding | Bax+Bcl2 → Bax_Bcl2 | $k_{binBaxBcl2}$ * Bax * Bcl2 |
| Bax_Bcl2_release | Bax_Bcl2 → Bax+Bcl2 | $k_{relBaxBcl2}$ * Bax_Bcl2 |
| Runx2_degradation_via_PTH | Ob_m_PTH+Runx2 → Ob_m_PTH | $k_{degRunx2PTH}$ * Runx2 * Ob_m_PTH |
| Runx2_degradation | Runx2 → Sink | $k_{degRunx2}$ * Runx2 |
| Runx2_synthesis | Source → Runx2 | $k_{synRunx2}$ * Source |
| Bone_formation | Ob_m → Ob_m+Bone | $k_{formBone}$ * Ob_m |
| Bone_formation_Obm_PTH | Ob_m_PTH → Ob_m_PTH+Bone | $k_{formBone}$ * Ob_m_PTH |
| Bone_degradation | Ocl_m+Bone → Ocl_m | $k_{degBone}$ * Ocl_m * Bone |

The ODE of each species in the model can be constructed from the reactions in which the species is a reactant or a product. If the species is a reactant, then the result of the reaction is that the species declines by one and so the term is negative and rate is given by the kinetic rate law. If the species is a product, then the result of the reaction is that the species increases by one and so the term is positive and the rate is given by the kinetic rate law. For example, OPG_RANKL is a product in one reaction: RANKL_inhibition, and a reactant in two reactions: OPG_RANKL_dissociation and OPG_RANKL_degradation. Therefore the ODE is:

$$\frac{d[OPG\_RANKL]}{dt} = k_{inhibRANKL}[\text{OPG}][\text{RANKL}] - k_{relRANKL}[\text{OPG\_RANKL}] - k_{degOPGRANKL}[\text{OPG\_RANKL}]$$

All the ODEs can be viewed in the COPASI file (Supplementary file 3).

**Supplementary Table 2. Detail of model parameters**

| Parameter id | Value[a] | Comment |
|---|---|---|
| $k_{actCreb}$ | 9.0E-3 $s^{-1}$ | CREB is activated rapidly (within a few minutes) after PTH binds to mature osteoblasts (Zhang et al., 2011). |
| $k_{actOcy}$ | 4.0E-3 $mol^{-1}s^{-1}$ | Activation occurs within 1 hour of loading (Lara-Castillo et al., 2015) |
| $k_{actOcyPth}$ | 0.08 $s^{-1}$ | Osteocytes are rapidly activated after PTH binding |
| $k_{actTgfb}$ | 2.0E-7 $mol^{-1}s^{-1}$ | Tgfb activation by osteoclasts occurs |
| $k_{actWnt}$ | 0.03 $s^{-1}$ | Activation occurs within 1 hour of loading (Lara-Castillo et al., 2015). This is due to downregulation of Sost |
| $k_{actWntPth}$ | 1.0E-3 $s^{-1}$ | PTH binding to mature osteoblasts activates Wnt but at a lower rate than that induced by loading. |
| $k_{binBaxBcl2}$ | 0.01 $mol^{-1}s^{-1}$ | Assume rapid kinetics of binding/dissociation of Bax/Bcl2 binding |
| $k_{binCrebRunx2}$ | 0.01 $mol^{-1}s^{-1}$ | Assume rapid kinetics of binding/dissociation of CREB/Runx2 binding |
| $k_{binObmPTH}$ | 0.02 $s^{-1}$ | PTH binds strongly to receptors on mature osteoblasts |
| $k_{binObpPTH}$ | 3.0E-4 $s^{-1}$ | PTH binds to receptors on osteoblast precursors with less affinity than mature cells |
| $k_{binObpTgfb}$ | 2.0E-4 $mol^{-1}s^{-1}$ | Tgfb binds to receptors on osteoblast precursors with fast kinetics compared to activation of Tgfb by osteoclasts (Lemaire et al., 2004) |
| $k_{binOclpRANKL}$ | 1.0E-3 $mol^{-1}s^{-1}$ | Assume fast kinetics of binding/dissociation of RANKL/RANK receptors on osteoclast precursors |
| $k_{binOcyPTH}$ | 8.0E-3 $s^{-1}$ | PTH binds to receptors on osteocytes with less affinity than mature osteoblasts |
| $k_{deathOb}$ | 2.4E-4 $s^{-1}$ | Assumed a half-life of about two hours due to simplifying assumptions regarding spatial aspects. |
| $k_{deathOcl}$ | 6.5E-5 $s^{-1}$ | Assumed a half-life of about three hours due to simplifying assumptions regarding spatial aspects. |
| $k_{deathOclp}$ | 1.0E-5 $s^{-1}$ | Assumed a half-life of about one day due to simplifying assumptions regarding spatial aspects. |
| $k_{deathOcy}$ | 1.0E-8 $s^{-1}$ | Half-life is about 25 years (Knothe Tate et al., 2004). This was speeded up about 10 fold for the model due to simplifying assumptions regarding spatial aspects. |
| $k_{degBcl2}$ | 2.5E-3 $s^{-1}$ | Assumed that only free pools of Bcl2 are degraded with a half-life of about 5 minutes. |
| $k_{degBone}$ | 6.5E-9 $mol^{-1}s^{-1}$ | 10% of bone is remodeled in one year. This was speeded up about two-fold to allow shorter simulation times. |
| $k_{degMCSF}$ | 1.0E-4 $s^{-1}$ | Half-life is about 2 hours (Rubin et al., 1998) |
| $k_{degOPG}$ | 4.0E-6 $s^{-1}$ | Half-life is about 2 days (Lemaire et al., 2004) |
| $k_{degOPGRANKL}$ | 1.0E-5 $s^{-1}$ | Assumed OPG and RANKL are both degraded when they are bound (Tat et al., 2006) |
| $k_{degPTH}$ | 2.0E-3 $s^{-1}$ | Half-life is about 5 minutes (Bieglmayer et al., 2002). |
| $k_{degRANKL}$ | 3.0E-5 $s^{-1}$ | Half-life of about 6 hours |
| $k_{degRunx2}$ | 1.0E-4 $s^{-1}$ | Half-life is about 2 hours. |
| $k_{degRunx2PTH}$ | 3.0E-3 $s^{-1}$ | PTH increases Runx2 degradation rate. |
| $k_{degSost}$ | 4.0E-3 $s^{-1}$ | Half-life is about 3 minutes. |

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|---|---|---|
| $k_{degTgfb}$ | 5.0E-5 $s^{-1}$ | Half-life is about 4 hours. |
| $k_{degTgfbPTH}$ | 1.7E-5 $mol^{-1}s^{-1}$ | PTH signaling leads to further Tgfb degradation. |
| $k_{diffHSC}$ | 5.5E-5 $s^{-1}$ | Osteoclast precursors increase rapidly after loading and secretion of MCSF, peaking at about 10-15 hours post-loading. |
| $k_{diffMSC}$ | 6.5E-4 $s^{-1}$ | Osteoblast pro-genitor cells increase rapidly after loading or PTH-induced activation of Wnt and peaks at about 3 hours after Wnt. |
| $k_{diffObP}$ | 1.0E-4 $s^{-1}$ | Mature osteoblasts increase about 10 hours after the precursor cells. |
| $k_{diffObproTgfb}$ | 0.05 $s^{-1}$ | Osteoblast precursors increase rapidly after Tgfb activation, peaking about 10 hours after the peak in progenitor cells |
| $k_{diffOclP}$ | 8.0E-5 $s^{-1}$ | Mature osteoclasts increase after RANKL binds to osteoclast precursors. |
| $k_{formBone}$ | 3.07E-6 $s^{-1}$ | Rate of formation was set to balance rate of degradation under normal loading and an intact PTH circadian rhythm. |
| $k_{inactCreb}$ | 1.0E-4 $s^{-1}$ | Dephosphorylation of CREB has much slower kinetics than phosphorylation (time scale of hours) (Zhang et al., 2011). |
| $k_{inactOcy}$ | 2.0E-5 $s^{-1}$ | Osteocyte activation by loading or PTH is transient so assume a gradual return to an inactive state with half-life of about 10 h in the absence of any further stimuli. . |
| $k_{inactWnt}$ | 0.8 $s^{-1}$ | Wnt is rapidly inactivated in the presence of Sost. |
| $k_{inhibRANKL}$ | 1.0E-3 $mol^{-1}s^{-1}$ | This is the rate at which OPG binds to RANKL to inhibit its activity. Assumed fast kinetics of binding/dissociation of OPG/RANKL binding. |
| $k_{matOb}$ | 2.0E-9 $s^{-1}$ | A low proportion of osteoblast mature into osteocytes. This rate was adjusted so that pool of osteocytes was maintained. |
| $k_{matObTgfb}$ | 1.0E-8 $s^{-1}$ | Tgfb increases the rate of osteoblast maturation |
| $k_{relBaxBcl2}$ | 0.5 $s^{-1}$ | Assume rapid kinetics of binding/dissociation of Bax/Bcl2 binding |
| $k_{relCrebRunx2}$ | 0.01 $s^{-1}$ | Assume rapid kinetics of binding/dissociation of CREB/Runx2 binding |
| $k_{relObmPTH}$ | 5.0E-3 $s^{-1}$ | Assume rapid kinetics of binding/dissociation of PTH with mature osteoblasts. |
| $k_{relObpPTH}$ | 5.0E-3 $s^{-1}$ | Assume rapid kinetics of binding/dissociation of PTH with osteoblast precursors. |
| $k_{relObpTgfb}$ | 0.01 $s^{-1}$ | Assume rapid kinetics of binding/dissociation of Tgfb with osteoblast precursors. |
| $k_{relOclpRANKL}$ | 1.0E-3 $s^{-1}$ | Assume rapid kinetics of binding/dissociation of RANKL with osteoclast precursors. |
| $k_{relOcyPTH}$ | 5.0E-3 $s^{-1}$ | Assume rapid kinetics of binding/dissociation of PTH with osteocytes |
| $k_{relRANKL}$ | 1.0E-3 $s^{-1}$ | Assumed rapid kinetics of binding/dissociation of OPG/RANKL binding. |
| $k_{secMCSFbyMSC}$ | 1.0E-5 $s^{-1}$ | Rate of secretion was set to balance degradation rate. |

| | | |
|---|---|---|
| $k_{secMCSFbyObm}$ | 1.0E-5 $s^{-1}$ | Assumed different types of bone cell secreted MCSF at same rate. |
| $k_{secMCSFbyObm}$ | 1.0E-5 $s^{-1}$ | |
| $k_{secMCSFbyObp}$ | 1.0E-5 $s^{-1}$ | |
| $k_{secMCSFbyObp}$ | 1.0E-5 $s^{-1}$ | |
| $k_{secMCSFbyObpro}$ | 1.0E-5 $s^{-1}$ | |
| $k_{secOPGbyObm}$ | 1.0E-5 $s^{-1}$ | Rate of secretion was set to balance degradation rate. |
| $k_{secOPGbyObp}$ | 2.0E-6 $s^{-1}$ | Assumed less secretion of OPG by osteoblast precursor cells than mature cells. |
| $k_{secOPGbyObpPTH}$ | 1.0E-6 $s^{-1}$ | Assumed PTH inhibited secretion of OPG. |
| $k_{secRANKLbyMSC}$ | 1.0E-6 $s^{-1}$ | Since there is a constant pool of MSC, there is a low basal rate of RANKL secretion which is set to balance degradation rate giving a low basal level of about 2-3 molecules. |
| $k_{secRANKLbyObm}$ | 1.0E-7 $s^{-1}$ | Assume mature osteoblasts secrete low levels of RANKL |
| $k_{secRANKLbyObmPTH}$ | 1.0E-6 $s^{-1}$ | Assume a ten-fold increase in the rate of secretion of RANKL by mature osteoblasts when bound by PTH. |
| $k_{secRANKLbyObp}$ | 3.0E-6 $s^{-1}$ | Assume osteoblast precursors secrete more RANKL than mature cells |
| $k_{secRANKLbyObpPTH}$ | 2.0E-5 $s^{-1}$ | Assume an order of magnitude increase in the rate of secretion of RANKL by osteoblast precursors when bound by PTH. |
| $k_{secRANKLbyObpro}$ | 7.0E-6 $s^{-1}$ | Assume osteoblast progenitor cells secrete more RANKL than unstimulated precursor cells but less than precursor cells bound by PTH. |
| $k_{secRANKLbyObpTgfb}$ | 4.0E-6 $s^{-1}$ | Assume Tgfb increases the rate of RANKL secretion by osteoblast precursors but not to the same extent as PTH. |
| $k_{secRANKLbyOcy}$ | 1.0E-6 $s^{-1}$ | Assume active osteocytes secrete RANKL at 10x the rate for inactive osteocytes. |
| $k_{secRANKLbyOcyI}$ | 1.0E-7 $s^{-1}$ | Assume inactive osteocytes secrete low levels of RANKL. |
| $k_{secSost}$ | 7.5E-4 $s^{-1}$ | Parameter set to maintain basal levels of Sost in the absence of loading or PTH peaks. |
| $k_{secTgfb}$ | 5.0E-5 $s^{-1}$ | Parameter set to maintain basal levels of Tgfb. |
| $k_{synBcl2}$ | 5.0E-3 $s^{-1}$ | Parameter set to maintain basal levels of Bcl2 |
| $k_{synPTH}$ | 0.02 mol $s^{-1}$ | Parameter set to maintain basal levels of PTH |
| $k_{synRunx2}$ | 5.0E-3 mol $s^{-1}$ | Parameter set to maintain basal levels of Runx2 |
| $k_{unload}$ | 3.5E-4 $s^{-1}$ | Period of loading is about 30 minutes |

[a]mol=number of molecules

**Supplementary Table 3. Detail of model events for loading and PTH circadian rhythm**

| **(A) Model with 2 loads and 2 PTH peaks per day** | | |
|---|---|---|
| **Event ID** | **Trigger** | **Assignment** |
| AddLoad1 | X>300 | LOAD=1 |
| AddLoad2 | X>600 | LOAD=1 |
| AddPTH1 | X>700 | PTH=150 |
| AddPTH2 | X>1000 | PTH=170; X=0 |
| **(B) Model with 1 load and 2 PTH peaks per day** | | |
| AddLoad1 | X>300 | LOAD=1 |
| AddPTH1 | X>700 | PTH=150 |
| AddPTH2 | X>1000 | PTH=170; X=0 |
| **(C) Model with 2 loads and 1 PTH peak per day** | | |
| AddLoad1 | X>300 | LOAD=1 |
| AddLoad2 | X>600 | LOAD=1 |
| AddPTH1 | X>1000 | PTH=150; X=0 |
| **(D) Model with 1 load and 1 PTH peak per day** | | |
| AddLoad1 | X>300 | LOAD=1 |
| AddPTH1 | X>1000 | PTH=150; X=0 |

**Supplementary Table 4. Results of sensitivity analysis**

| Parameters with positive effects on bone mass | | Parameters with negative effects on bone ,mass | |
|---|---|---|---|
| kformBone | 0.818 | krelObmPTH | -0.071 |
| kdeathOcl | 0.816 | kdiffOclP | -0.073 |
| kdegMCSF | 0.489 | kdegOPG | -0.074 |
| kdiffObP | 0.425 | krelRANKL | -0.074 |
| kdiffMSC | 0.378 | kbinOclpRANKL | -0.078 |
| kdegSost | 0.281 | kinactCreb | -0.085 |
| ksynPTH | 0.232 | kdegRunx2PTH | -0.108 |
| kactWnt | 0.169 | krelCrebRunx2 | -0.109 |
| ksecOPGbyObm | 0.140 | ksecMCSFbyMSC | -0.111 |
| ksynBcl2 | 0.123 | ksecTgfb | -0.111 |
| kbinBaxBcl2 | 0.123 | kbinObpTgfb | -0.112 |
| ksynRunx2 | 0.114 | ksecRANKLbyObpTgfb | -0.113 |
| krelObpTgfb | 0.112 | ksecRANKLbyObp | -0.122 |
| kbinCrebRunx2 | 0.109 | kdegBcl2 | -0.123 |
| kbinOcyPTH | 0.088 | krelBaxBcl2 | -0.123 |
| kdeathOclp | 0.078 | ksecMCSFbyObm | -0.140 |
| kinhibRANKL | 0.075 | kunload | -0.178 |
| kdegOPGRANKL | 0.074 | ksynX | -0.193 |
| kbinObmPTH | 0.073 | kinactWnt | -0.201 |
| kdegTgfbPTH | 0.073 | kinactOcy | -0.226 |
| krelOclpRANKL | 0.073 | ksecMCSFbyObp | -0.231 |
| kactOcy | 0.072 | ksecSost | -0.278 |
| kactCreb | 0.051 | kdegPTH | -0.290 |
| | | ksecRANKLbyOcy | -0.303 |
| | | kdiffHSC | -0.441 |
| | | kdeathOb | -0.802 |
| | | kdegBone | -0.815 |

Sensitivity analysis of the deterministic model with two loading and two PTH events per day (Table S3) was carried out in Copasi using subtask “Time series”. Parameters which positively or negatively affect bone mass by more than 5%. All sensitivity values are scaled. Green/red indicates parameters which increase/decrease bone mass if their value increases. Full results are shown in Supplementary File 2.

**Supplementary Table 5. Results of parameter scans.** Each parameter which had more than 10% effect on bone mass was varied from half to twice its initial value over ten intervals. The table shows the effect of doubling each parameter value on bone mass, mature osteoblasts and mature osteoclasts compared to default value. Parameters are listed in order of their sensitivities (as shown in Supplementary Table 4), note that in some cases, halving the parameter value had a greater effect on bone mass (e.g. $k_{deathOcl}$).

| Parameter name | Bone mass (% change) | Ob_m (% change) | Ocl_m (% change) |
|---|---|---|---|
| kformBone | 81.46 | 0.00 | 0.00 |
| kdeathOcl | 56.88 | -0.01 | -49.95 |
| kdegMCSF | 62.81 | -0.07 | -53.44 |
| kdiffObP | 26.22 | -0.77 | -27.30 |
| kdiffMSC | 38.00 | 114.77 | 52.07 |
| kdegSost | 23.75 | 74.86 | 38.00 |
| ksynPTH | 47.07 | 113.39 | 39.87 |
| kactWnt | 13.55 | 42.35 | 23.02 |
| ksecOPGbyObm | 12.30 | 0.01 | -13.95 |
| ksynBcl2 | 13.48 | 18.37 | 1.56 |
| kbinBaxBcl2 | 13.48 | 18.40 | 1.55 |
| ksynRunx2 | 13.48 | 18.37 | 1.56 |
| krelObpTgfb | 6.15 | -0.01 | -7.29 |
| kbinCrebRunx2 | 10.71 | 14.66 | 1.23 |
| kdegRunx2PTH | -5.41 | -7.38 | -0.71 |
| krelCrebRunx2 | -5.43 | -7.42 | -0.71 |
| ksecMCSFbyMSC | -8.04 | 0.00 | 10.42 |
| ksecTgfb | -9.45 | -0.02 | 12.66 |
| kbinObpTgfb | -9.23 | -0.03 | 12.36 |
| ksecRANKLbyObpTgfb | -9.33 | -0.01 | 12.56 |
| ksecRANKLbyObp | -9.87 | -0.02 | 13.34 |
| kdegBcl2 | -5.79 | -7.89 | -0.77 |
| krelBaxBcl2 | -5.79 | -7.89 | -0.77 |
| ksecMCSFbyObm | -9.38 | 0.00 | 12.32 |
| kunload | -8.49 | -25.40 | -15.80 |
| ksynX | 29.63 | 84.08 | 36.76 |
| kinactWnt | -11.17 | -34.09 | -21.82 |
| kinactOcy | -11.50 | -41.73 | -29.91 |
| ksecMCSFbyObp | -12.50 | -0.01 | 16.91 |
| ksecSost | -13.59 | -44.85 | -30.78 |
| kdegPTH | -6.30 | -22.64 | -15.29 |
| ksecRANKLbyOcy | -14.94 | -0.03 | 21.20 |
| kdiffHSC | -19.70 | -0.02 | 28.56 |
| kdeathOb | -39.31 | -53.91 | -7.55 |
| kdegBone | -48.24 | 0.00 | 0.00 |

The parameters which were shown to have more than 10% effect on bone mass in the sensitivity analysis (see Supplementary File 2) were varied in the range $0.5k$-$2k$, where $k$ is the parameter value, and deterministic simulations were carried out over 10 intervals in COPASI. The table shows the effect of a 2-fold increase in each of the parameters on bone mass, levels of mature osteoblasts (Ob_m), and levels of mature osteoclasts (Ocl_m) averaged over the final 20 time-points (final 2 days of the simulation) because levels of Ob_m and Ocl_m fluctuated during each 24 h period. The deterministic model with loading (activated every 24 h by an event) and a circadian rhythm of PTH was used for this analysis.

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