



## SUNDAR SRINIVASAN, PH.D.

RESEARCH ASSOCIATE PROFESSOR  
HARBORVIEW MEDICAL CENTER  
RESEARCH  
[WWW.ORTHOP.WASHINGTON.EDU/FACULTY/SRINIVASAN](http://WWW.ORTHOP.WASHINGTON.EDU/FACULTY/SRINIVASAN)

BRANDON J. AUSK, M.S., JITENDRA PRASAD, PH.D.  
THOMAS S. RICHARDSON, PH.D., AND TED S. GROSS, PH.D.

### Every Second Counts - Discovering Mild Physical Activity to Build-Up Bone Mass - Putting Regeneration to Work

- Osteoporosis and resulting non-traumatic fractures are an inevitable consequence of aging and menopause.
- Anabolic options are required to build-up bone mass at adolescence such that non-traumatic fractures can be prevented later in life.
- Physical exercise offers promise as a therapy but requirements for high-impact, strenuous activity have prevented realization of this potential.
- We have therefore sought to design mild exercise based interventions by focusing upon observations that brief exercise (~ 2 – 3 mins) can elicit robust bone adaptation.
- Using this basis, we have developed a novel computational model that simulated activation of the Ca<sup>2+</sup>/NFAT pathway, a signaling mechanism critical in how bone cells and tissue perceive and respond to brief mechanical loading or physical exercise.
- Interestingly, optimization using this model suggested that loading bone once every 10 mins could result in substantially more bone formation than loading bone 1800 times over a 30 min 'exercise' bout.
- Remarkably, our preliminary experiments confirm predictions of this computational model, demonstrate the utility of our approach and suggest that mild activity can indeed be 'engineered' to be potentially anabolic for the skeleton.
- Ultimately, a similar strategy could be used to design mild physical exercise to robustly build-up bone mass at adolescence as a bulwark against the inevitable ravages of age and menopause.

Osteoporosis and related non-traumatic fractures can be thought of as the inevitable consequence of aging superimposed upon a previously insufficient peak bone mass 'bank' balance. As such, the search is on for anabolic therapies that build up this bone 'bank' from adolescence through adulthood, thereby providing a bulwark against the inevitable declines in bone mass accrued over a lifetime. Physical exercise can be substantially osteogenic for bone and holds promise as a non-invasive means to enhance the bone mass at adolescence. However, the vigorous and high impact activities that have proven to be osteogenic are

not trivial to safely implement in the young, growing skeleton. Our group at the OSL seeks to explore bone mechanotransduction function with a view to ultimately discovering physical exercise based strategies that are both mild to perform and substantially osteogenic for young and old alike.

Given this goal, we have focused upon studies that suggest that physical exercise need only be brief, in order to beneficially influence bone mass and structure. Remarkably, loading bone for as little as 5 seconds a day has been found to be sufficient to enhance bone mass and strength. Given this, bone cell activity induced during and by loading (i.e., within

seconds to minutes) must clearly be critical in regulating downstream tissue adaptation. However, the activity and responses induced in bone cells in vivo within this acute time frame are highly inaccessible. Therefore, and using a novel technique suited for the exploration of complex systems, we developed a biophysical agent-based computational model (ABM) for how cell signaling initiated within seconds by mechanical stimuli could influence bone tissue adaptation weeks downstream.

Our ABM for cell signaling induced within seconds simulates activation of the Ca<sup>2+</sup>/NFAT pathway, a critical mechanism known to underlie

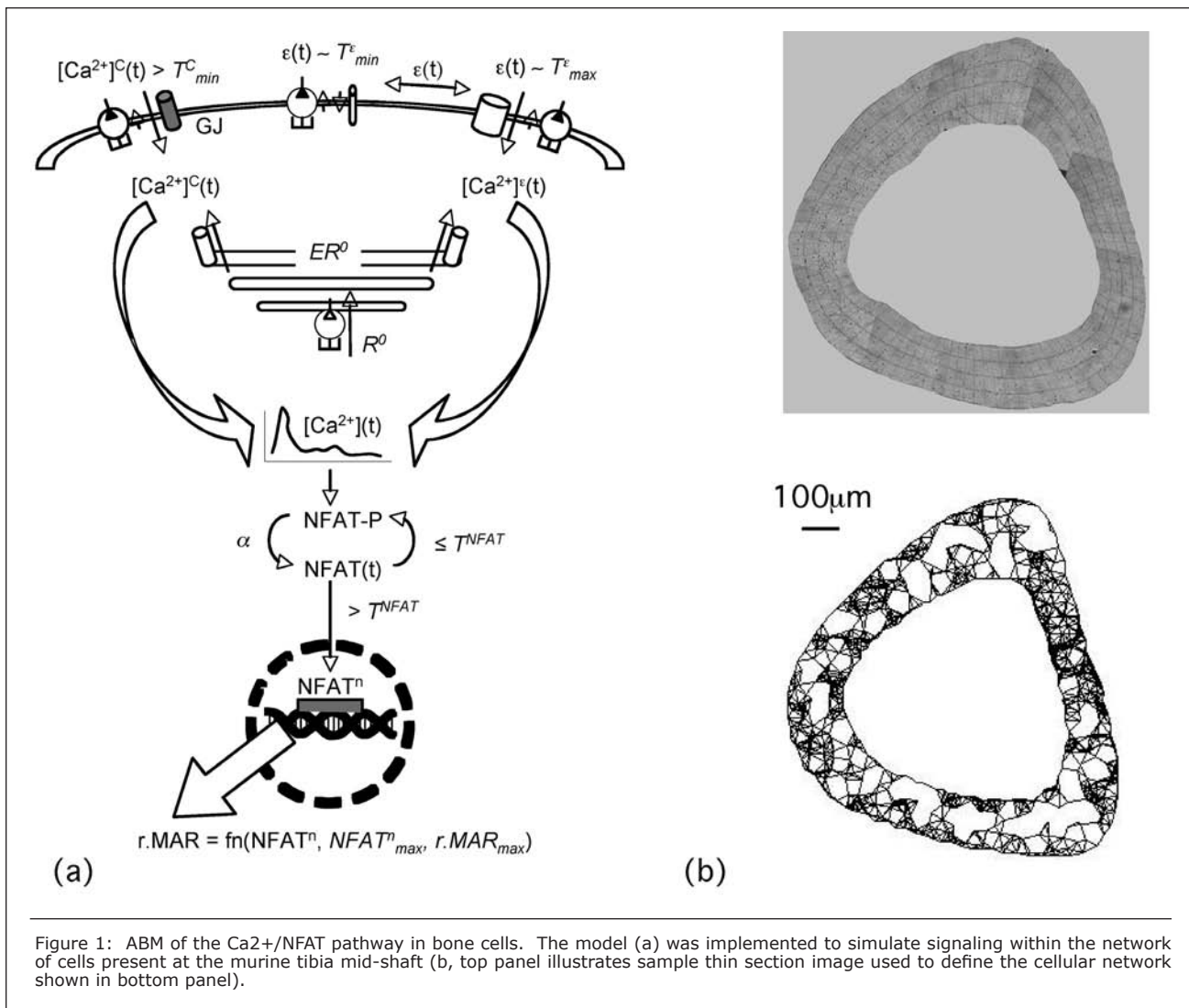


Figure 1: ABM of the Ca<sup>2+</sup>/NFAT pathway in bone cells. The model (a) was implemented to simulate signaling within the network of cells present at the murine tibia mid-shaft (b, top panel illustrates sample thin section image used to define the cellular network shown in bottom panel).

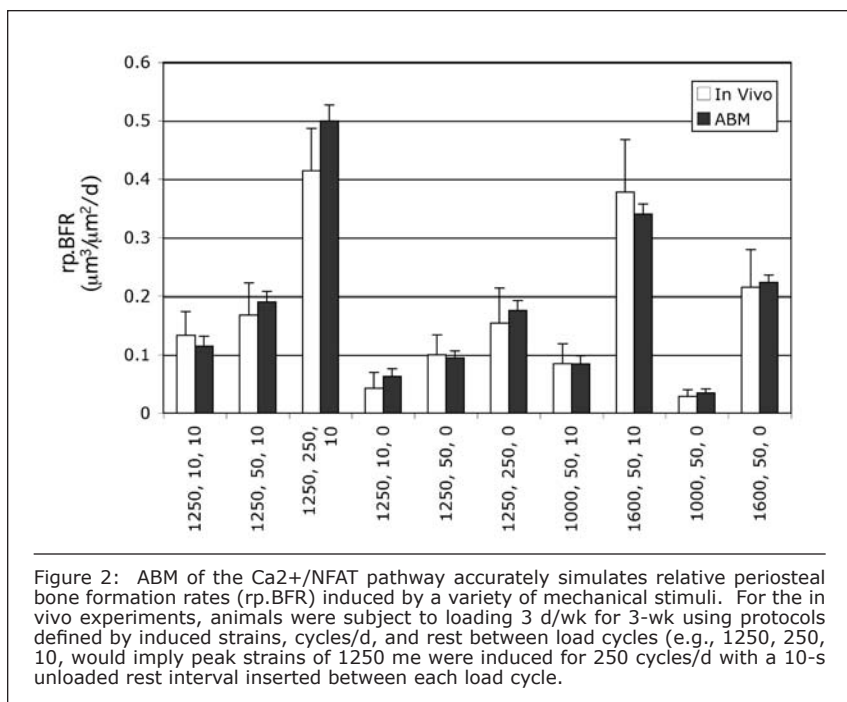
mechanotransduction (Figure 1 a). Briefly, our parametric model is based upon experimental reports and assumes that mechanical strain induced on a cell body causes Ca<sup>2+</sup> oscillations within the cell cytoplasm due to the confluence of 1) Ca<sup>2+</sup> influx into the cell cytoplasm through stretch activated ion channels, 2) Ca<sup>2+</sup> efflux from the endoplasmic reticulum, and 3) influx of Ca<sup>2+</sup> into the cell body from neighboring networked cells via gap junctional exchange of Ca<sup>2+</sup> ions. Downstream of Ca<sup>2+</sup> oscillations, our model simulates the dynamics of de-phosphorylation and nuclear transport of the cytoplasmic protein, NFAT. Finally, given the known biology, our model simulates accumulated NFAT protein binding with DNA and its control over mineral apposition by surface osteoblasts. We have implemented this biophysical model to simulate signaling interactions

within and between cells present at the mid-shaft cross-section of young adult female C57BL/6 mice (4 Mo; Figure 1 b).

As such, the model was designed to simulate loading induced activation of the Ca<sup>2+</sup>/NFAT pathway within and between bone cells at the murine tibia mid-shaft and the bone formation that ensues following repeated bouts of loading over a 3-wk period. To determine model parameters, we ‘trained’ the model using bone formation data derived from young adult animals exposed to 10 different mechanical loading waveforms. The loading waveforms involved subjecting mice to increasing strain magnitudes, loading repetitions and inserting 0 or 10 s unloaded ‘rest’ intervals between each loading event. We found that our model was sufficient to accurately simulate bone formation induced by a variety of loading protocols in young

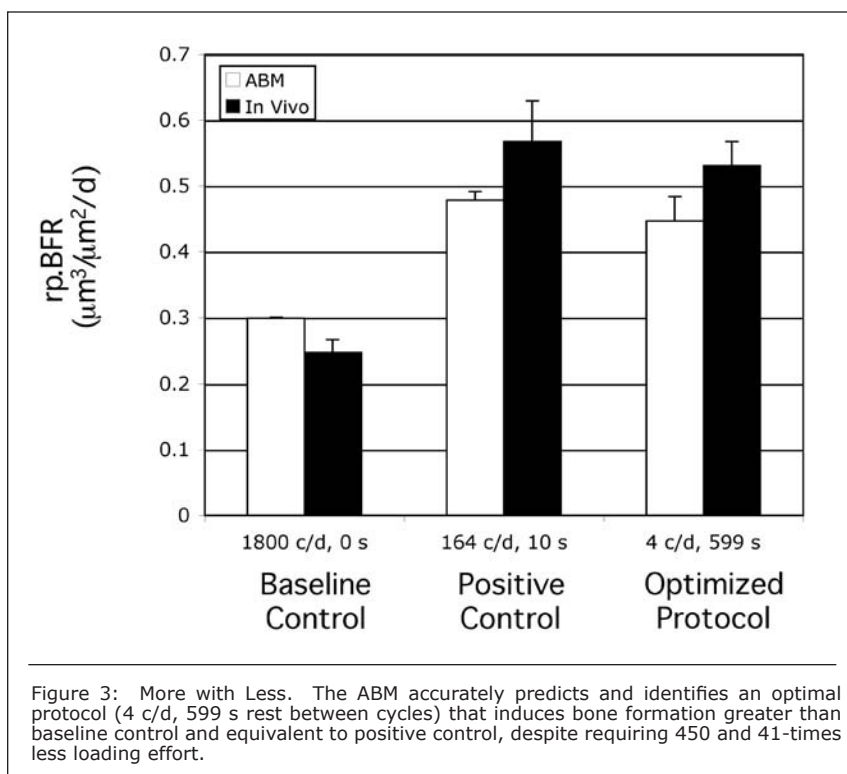
adult animals (error < 15%, p = 0.57; Figure 2). Given this, we sought to use this model in a predictive role and examined our ability to optimize mechanical stimuli in young adult animals.

We utilized our ABM to explore whether a 30 min ‘exercise’ protocol could be optimized for young adults such that bone tissue adaptation would be substantially enhanced while requiring minimal loading ‘effort’. To perform the optimization, we first simulated bone adaptation induced by ‘control’ loading regimens (Figure 3). Bone formation rates induced by a baseline control involving 1800, 1-Hz load cycles provided 3 days/wk for 3-weeks was first simulated. Next, we used the model to simulate bone formation induced by a positive control regimen involving 164 c/d, with a 10-s rest between each cycle, for 3 days/wk for 3-wks. Finally, we used



our ABM to design a protocol that could induce bone formation greater than our baseline control, equivalent to our positive control, while requiring minimal loading effort. Our analysis suggested that loading bone 4 times a day, with a 10 min rest-interval between each loading event, would induce the required bone formation. To test these predicted outcomes, we implemented these regimens in

young adult female C57BL/6J mice (4 Mo, n = 8) and determined bone formation rates in vivo via dynamic histomorphometry. While preliminary, our finding that loading bone every 10 mins during a 30 min 'exercise' period can substantially enhance bone formation was stunning, not just in validating the predictive ability of an in silica model, but in suggesting that a minimal loading effort can indeed



be 'engineered' to be substantially anabolic for bone.

In conclusion, we have focused upon a pathway that is activated during brief mechanical stimuli as a means to both explore mechanotransduction and to design novel approaches to loading bone. Our in silica model has proven to be predictive, and more importantly, has identified that loading bone just 4 times during a 30 min exercise bout, can be substantially osteogenic. While we are seeking to confirm these findings, they indeed offer promise to both our approach of using in silica models to design in vivo experiments and our supposition for the existence of mild loading protocols that would be safe to implement yet substantially anabolic for the young adult skeleton. Ultimately, we expect to be able to use a similar approach in designing mild physical exercise regimens for trials in adolescent and school age populations with the goal of sufficiently building up their bone 'banks'.

#### Acknowledgements

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#### Recommended Reading

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