SYNCHRONOUS SIGNALING WITHIN THE OSTEOCYTE CELL NETWORK UNDERLIES THE OSTEOGENIC POTENCY OF REST-INSERTED LOADING

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INTRODUCTION: We recently found that when a 10-s rest was inserted between low-magnitude mechanical loading cycles, these otherwise impotent regimens were transformed into stimuli perceived as potently osteogenic (over 8-fold increases in bone formation; Srinivasan et al, 2002). Considering that rest-insertion, per se, is a passive intervention, we hypothesize that this strategy derives its potency by amplifying signal coordination within the mechanosensory osteocytic cell network. Here, we explored this thesis by simulating the response of a population of osteocytic cells to both cyclic and rest-inserted loading utilizing a unique agent based modeling environment (NetLogo1.1; Wilensky, 1999).

METHODS: The response of a network of osteocytes to cyclic and rest-inserted stimuli was simulated within Netlogo 1.1. The Netlogo modeling environment allows exploration of connections between the micro-level behavior of individuals and the macro-level patterns that emerge from the interactions of many individuals. Specifically, within NetLogo, individual osteocytes were assigned localized sets of parametric rules (modeling micro-level behavior), permitted to interact with neighboring osteocytes, and global network (or population) level patterns allowed to emerge over time. For the simulations, each osteocyte was assigned the following parametric rules governing behavior: *1)* osteocytes were assigned internal clocks representing the time required to elapse before individual cells could activate (simulating rest intervals between stimuli; e.g., for a 5-s internal clock or rest interval setting, osteocytes can activate every 5-s); 2) each osteocyte can communicate with cells present within its immediate neighborhood (represented by the dimensionless 'communication length' parameter); 3) lastly, if an osteocyte perceives a given number (two) of simultaneously activated osteocytes within its neighborhood, the cell immediately activates (simulating signaling coordination between a localized grouping of 3 osteocytes). In this context, the activity of populations of 200 osteocytes (generated at random spatial locations and internal clock settings) were modeled and 5 simulations performed per experiment. First, parametric studies were performed for rest intervals of 2 to 30-s. Next, for rest intervals of 2 and 15-s, the influence of neighborhood size upon coordinated cell signaling was examined by varying communication lengths (from 15 to 50). For each simulation, the number of coordinated osteocytes as a percent of the entire population was recorded.

RESULTS: With respect to varying rest periods, synchronization of osteocyte activity displayed a normal distribution (Fig 1). Rest-intervals of 10- and 15-s induced the largest population of osteocytes to synchronize activity (70-80%). In contrast, rest-intervals of 2-, 5-, and beyond 20-s resulted in a markedly reduced and statistically similar number of coordinated osteocytes (50-60%). Additionally, protocols representative of cyclic loading (2-

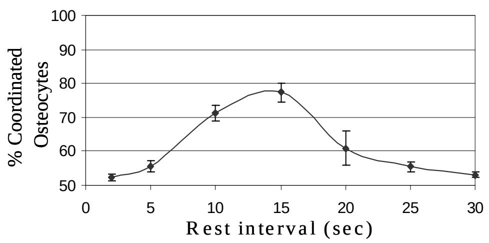

Fig 1: Percent coordinated osteocytes as a function of rest-intervals between stimuli.

s rest) and optimal rest-inserted loading (15-s) display markedly varied sensitivity to extents of osteocyte communication. Osteocytes exposed to cyclic stimuli (2-s rest) displayed reduced and invariant levels of coordination that was independent of communication lengths (Fig 2). In contrast, osteocytes exposed to 15-s rest inserted stimuli displayed markedly enhanced synchronicity (nearly 90%) beyond a threshold length (of 30), with saturation of coordinated activity occurring thereafter.

DISCUSSION: In support of our hypothesis, the presented model appears to indicate that rest-insertion between stimuli enables the osteocytic network to co-ordinate and synchronize its activity. Specifically, optimal synchronization of the network appears to occur beyond a threshold rest interval (~ 10-s), but with the benefits declining beyond 20-s of rest. In this context, *in vivo* observations suggest that while rest-insertion between mechanical load cycles enhances bone formation only beyond 7 – 10s (Srinivasan et al., 2002; Robling et al, 2001), the benefits of rest-insertion saturates between 10- and 20-s (Srinivasan et al, 2003). Further, if intervals of rest were too long (e.g., on the order of minutes), the protocol may cease to be perceived as a continuous train of stimuli that require a bone cell response. As such, the similarity of response trends between our model and *in vivo* data suggests that the ability to synchronize activity within the network of mechanosensory osteocytic cells may partly underlie the dramatic osteogenic potency of rest-inserted mechanical loading. Interestingly, our data indicates that synchronization of the osteocytic network in response to rest-inserted (but not cyclic) stimuli is enhanced and strongly dependent upon the ability of osteocytes to communicate with immediate neighbors. Importantly, this unique capacity of rest-inserted loading to utilize exquisite, interconnected networks of osteocytic cells arrayed *in vivo* may underlie its ability to transform mild mechanical stimuli into potent osteogenic regimens.

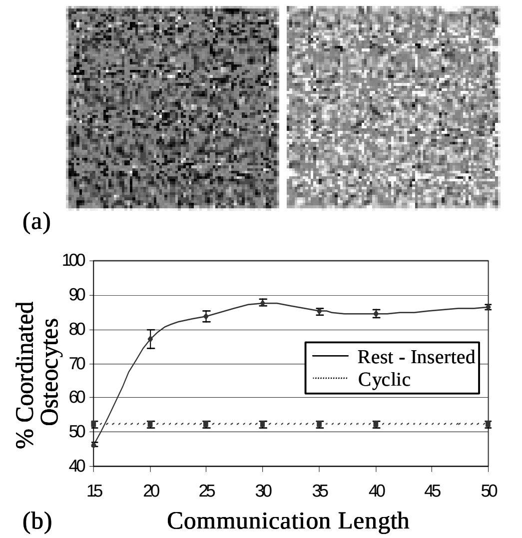

Fig 2: Typical model space screen captures displaying a larger population of activated (white) versus inactive (black) osteocytes when exposed to rest-inserted (15-s; right) versus cyclic stimuli at communication lengths of 30 (a). Similar response is evident in the coordinated activity of osteocytes plotted as a function of communication length (b).

REFERENCES: (1) Srinivasan et al. 2002 JBMR 17:1613; (2) Wilensky, U. 1999 http://ccl.northwestern.edu/netlogo; (3) Robling et al. 2001 J Exp Biol 204:3389; (4) Srinivasan et al. 2003 49th ORS.

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