

Osteocyte-Independent Mechanotransduction of Interstitial Fluid Flow

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Bone contains a porous network of canaliculi that has been shown to facilitate substantial and rapid transcortical interstitial fluid flow (IFF) [1]. This fluid flow originates from leaky venous sinusoids in the intramedullary cavity and is driven radially outward through cortical bone by a transmural pressure gradient between the endosteal vasculature and the lymphatic drainage at the periosteal surface [1-3]. Under mechanical bending or compressive loads, pressure gradients are created that drive fluid from areas of compression to areas of tension which rapidly accelerate fluid at rates of the order of 6 milliseconds [4-5]. High impact exercise such as running and jumping will drive rapid fluid flow, and with associated relaxation phases after loading, results in highly oscillatory flow.

It has been hypothesized that changes in IFF due to intraosseous pressure changes influence bone remodeling [6-9]. Numerous investigations into flow effects on osteoblasts in vitro have shown that osteoblasts exposed to flow exhibit increased prostaglandin E2 and nitric oxide release [10-11]. Fluid shear stress potently stimulates nitric oxide production in pre-osteoclasts as well [12].

It has been speculated that skeletal adaptation to mechanical loading involves IFF stimulation of osteocytes. Recently, Tatsumi and co-workers generated mice possessing a diphtheria toxin (DT) receptor transgene driven by the DMP1 promoter (DMP1-DTR), allowing for inducible osteocyte ablation by administration of DT [13]. While these authors found that osteocyte ablation conferred resistance to bone loss upon hindlimb suspension (HLS), mechanotransduction upon reloading was normal, giving rise to the intriguing possibility that loading-induced IFF may be sensed by cells other than osteocytes.

We recently developed a microfluidic system for modulating femoral intramedullary pressure (ImP) in alert mice, and showed that the generation of dynamic ImP significantly increased IFF within lacunae and protected against bone loss in mice subjected to HLS [14]. Using this system, we investigated the effects of osteocyte ablation on IFF-induced adaptation. 16wk F wildtype (WT) and transgenic (Tg) DMP1-DTR mice were subjected to HLS for 14d. One limb was exposed to dynamic ImP/IFF (3min/d, 5Hz, peak flow rate: 5uL/s); the other limb served as a sham control. Mice were administered DT (10 or 50ug/kg) 1d prior to HLS and a booster 7d later. BMD and structural indices were quantified at the lesser trochanter using pQCT and uCT [2]. Osteocyte ablation was confirmed by observing empty lacunae (~30%) in H&E-stained sections from Tg mice. In both WT and Tg mice, we observed significant gains in BMD, trabecular volume fraction (BV/TV), cortical thickness (Ct.Th), and cortical area (Ct.Ar) in limbs exposed to flow compared to sham controls (Table 1). In addition, a significant increase in trabecular thickness (Tb.Th) was observed in Tg mice. Interestingly, relative gains (i.e., flow-no flow) in all parameters were greater in Tg mice, indicating that osteocyte ablation did not affect, or even enhanced skeletal adaptation to flow. In particular, rBMD and rTb.Th were significantly different between WT and Tg mice administered 10 or 50ug/kg DT (Table 1).

Taken together, osteocyte ablation does not abrogate skeletal adaptation to dynamic ImP/IFF, suggesting that this response occurs independently of flow-induced stimulation

of osteocytes. One cellular target of Imp/IFF may be osteoclastic resorption. Support for this comes from in vitro observations of shear-induced nitric oxide production in pre-osteoclasts, possibly leading to autocrine inhibition of resorption. The role of osteocytes in mechanotransduction in bone remains to be defined.

In addition, osteocyte ablation may enhance the response to dynamic Imp/IFF, perhaps by altering the function and/or number of other types of cells within bone.

References:

- [1] Montgomery, R.J., Sutker, B.D., Bronk, J.T., Smith, S.R. and Kelly, P.J. (1988). Interstitial fluid flow in cortical bone. *Microvasc Res* 35: 295-307.
- [2] Kelly, P.J. (1983). Pathways of transport in bone, Williams and Wilkins.
- [3] Dillaman, R.M., Roer, R.D. and Gay, D.M. (1991). Fluid movement in bone: theoretical and empirical. *J Biomech* 24 Suppl 1: 163-177.
- [4] Piekarski, K. and Munro, M. (1977). Transport mechanism operating between blood supply and osteocytes in long bones. *Nature* 269: 80-82.
- [5] Qin, Y.X., Kaplan, T., Saldanha, A. and Rubin, C. (2003). Fluid pressure gradients, arising from oscillations in intramedullary pressure, is correlated with the formation of bone and inhibition of intracortical porosity. *J Biomech* 36: 1427-1437.
- [6] Reich, K.M., Gay, C.V. and Frangos, J.A. (1990). Fluid shear-stress as a mediator of osteoblast cyclic adenosine-monophosphate production. *J Cell Physiol* 143: 100-104.
- [7] Reich, K.M. and Frangos, J.A. (1991). Effect of Flow on Prostaglandin-E2 and Inositol Trisphosphate Levels in Osteoblasts. *American Journal of Physiology* 261: C428-C432.
- [8] Hillsley, M.V. and Frangos, J.A. (1994). Bone tissue engineering: the role of interstitial fluid flow. *Biotechnol Bioeng* 43: 573-581.
- [9] Turner, C.H., Forwood, M.R. and Otter, M.W. (1994). Mechanotransduction in bone: do bone cells act as sensors of fluid flow? *FASEB J* 8: 875-878.
- [10] Johnson, D.L., McAllister, T.N. and Frangos, J.A. (1996). Fluid flow stimulates rapid and continuous release of nitric oxide in osteoblasts. *Am J Physiol* 271: E205-208.
- [11] McAllister, T.N. and Frangos, J.A. (1999). Steady and transient fluid shear stress stimulate NO release in osteoblasts through distinct biochemical pathways. *J Bone Miner Res* 14: 930-936.
- [12] McAllister, T.N., Du, T. and Frangos, J.A. (2000). Fluid shear stress stimulates prostaglandin and nitric oxide release in bone marrow-derived preosteoclast-like cells. *Biochem Biophys Res Commun* 270: 643-648.

[13] Tatsumi S, Ishii K, Amizuka N, Li M, Kobayashi T, Kohno K, Ito M, Takeshita S, Ikeda K. (2007). Targeted ablation of osteocytes induces osteoporosis with defective mechanotransduction. *Cell Metab.*;5:464-75.

[14] Kwon RY, Meays DR, Tang WJ, Frangos JA. Microfluidic enhancement of intramedullary pressure increases interstitial fluid flow and inhibits bone loss in hindlimb suspended mice. *J Bone Miner Res.* 2010 Feb 23.

		WT (n=15)	Tg+10ug/kg DT (n=11)	Tg+50ug/kg DT (n=5)	One-way ANOVA
Parameter (flow – no flow)	rBMD (mg/ccm)	15.7±4.0**	18.9±6.9*	48.2±17.0*	p<0.05
	rBV/TV (%)	5.8±1.0***	7.0±1.5***	7.0±1.3***	NS
	rTb.Th (um)	0.3±3.1	13.0±3.0**	19.2±1.6***	p<0.01
	rCt.Th (um)	10.9±3.7*	16.2±3.7**	17.3±6.5	NS
	rCt.Ar (mm ²)	0.07±0.02**	0.09±0.02**	0.09±0.03	NS

Table 1. Osteocyte ablation does not affect or enhances skeletal adaptation to dynamic IFF. Values (presented as mean±SE) are relative differences between limbs exposed to dynamic IFF vs. contralateral controls (i.e., flow – no flow) for WT and Tg mice administered 10 or 50ug/kg DT. For WT mice, no differences in relative values were observed for mice administered 10 or 50ug/kg DT, thus for statistical analysis these groups were combined. *, **, or *** indicate p<0.05, p<0.01, or p<0.001 obtained using a one-sample t-test with an assumed zero mean. One-way ANOVA revealed a statistically significant difference between groups (i.e., WT, Tg+10ug/kg DT, and Tg+50ug/kg DT) for rBMD and rTb.Th.