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# Primary Cilia Mediate $PGE_2$ Release in MC3T3-E1 Osteoblasts

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## 1 Introduction

Bone cells can remodel the mammalian skeleton through changes in gene expression and cellular activity by responding to extracellular mechanical signals such as fluid flow. We hypothesized that bone cells may sense fluid flow via the primary cilium, a microtubule-based organelle that has been shown to mediate flow-induced cellular responses in kidney cells. We asked whether loss of primary cilia affects the response of bone cells to flow *in vitro*.

In studies of kidney cells, mechanical stimulation of primary cilia results in calcium mobilization [2, 3]. Calcium mobilization also occurs following fluid shear stress in bone cells [4]. To determine whether primary cilia act as mechanosensors in bone, we examined two outcome variables, intracellular calcium release and prostogandin $E_2$ release, which have been shown to be independently stimulated by fluid flow [4,5].

## 2 Materials and Methods

*Primary Cilia in Bone Osteocytes:* We confirmed the existence of primary cilia in bone tissue using immunofluorescent detection of acetylated α-tubulin [6]. Dissected mouse tibiae were fixed, cryosectioned, and incubated with 6-11B-1 anti-acetylated α-tubulin (Sigma) *Primary Cilia in MLO-Y4 Osteocytes:* MLO-Y4 cells were grown to 100% confluence, fixed in -20°C methanol and stained for acetylated α-tubulin as described above. *Cilium Removal*: RNA interference was used to reduce protein levels of the ciliary protein polaris. siRNA targeting polaris (Invitrogen) was transfected into MC3T3-E1 cells using Xtreme Transfection Agent (Roche). As an alternative method for cilia removal, 4 mM chloral hydrate [3] was added to cells for 72h, cells were washed with PBS, and fresh media was added for 24h before flow experiments. *Oscillatory Fluid Flow*: Slides were placed in flow chambers 30 min prior to the start of each experiment. Slides were exposed to 1Hz, 10 dynes/cm$^2$ oscillatory fluid flow for 1 hr for $PGE_2$ experiments and for 3 min for calcium experiments [7, 8]. *Quantification of PGE2 release:* $PGE_2$ was quantified using a commercial ELISA kit (Amersham) and normalized to total protein. *Calcium Experiments:* Real-time intracellular levels of calcium were quantified. Cells were incubated with 10 mM Fura-2AM (Molecular Probes) for 30 min at 31°C. The slides were mounted in a parallel plate flow chamber on an epifluorescence microscope. Flow media consisted of MEM-α and 2% FBS. A cell response was defined as a transient increase in fluorescent intensity at least 4 times the maximum oscillation recorded during the pre-flow baseline period.

## 3 Results

*Primary Cilia:* Primary cilia were found in mouse tibia osteocytes and 65% of MLO-Y4 osteocytes (**Fig. 1**). Primary cilia were found in 69% of MC3T3-E1 osteoblasts, and 60% in scrambled siRNA treated MC3T3-E1s. That number dropped to 4.5% in chloral hydrate treated cells and 30% in polaris siRNA treated cells. *$PGE_2$ Release:* After exposure to oscillatory fluid flow we saw a 3.4 fold increase in $PGE_2$ release in untreated cells ($p < 0.001$) and a 1.8 fold increase in $PGE_2$ in scrambled siRNA treated cells ($p < 0.01$). With primary cilia removed using either chloral hydrate or polaris siRNA we saw no significant increase in $PGE_2$ release after exposure to oscillatory fluid flow (**Fig. 2**). *Calcium Response:* Eliminating primary cilia using either chloral hydrate or siRNA against

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polaris did not significantly abrogate cytosolic calcium mobilization in MC3T3-E1 cells (**Fig. 3**).

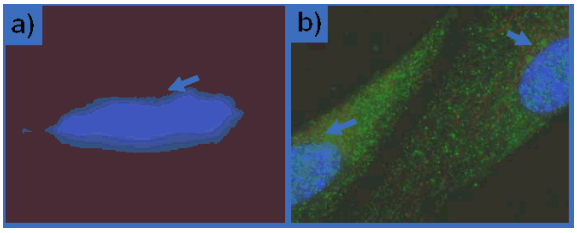


**Figure 1** : a) Mouse tibial osteocyte; and b) MLO-Y4 osteocyte, primary cilia stained in red for acetylated α-tubulin.

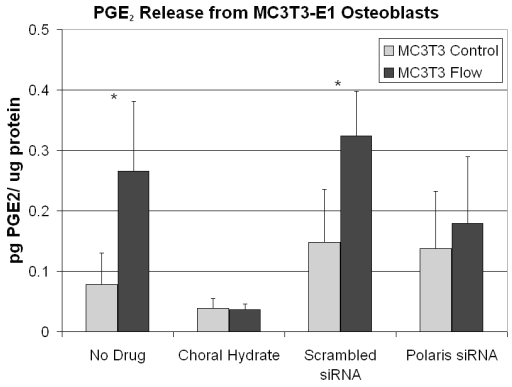


**Figure 2 :** Amount of $PGE_2$ released from MC3T3-E1 osteoblasts.

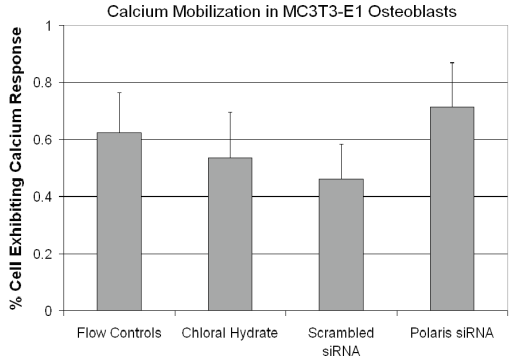


**Figure 3** : Percent of MC3T3-E1 osteoblasts exhibiting a calcium response.

## 4 Discussion

While it has been known for decades that bone is a mechanosensitive organ, the responsible mechanism has been elusive. Primary cilia, once commonly thought to be vestigial organelles, are now being re-examined in development and in kidney tissue as a fluid sensing organelle. We have found primary cilia in both osteoblastic and osteocytic cell lines as well as in osteoblasts and osteocytes *in vivo*.

In kidney cells, primary cilia have been established as a necessary component of the cell's fluid flow induced calcium response through stretch activated channels at the base of the primary cilia. We examined whether primary cilia were necessary for intracellular calcium mobilization and $PGE_2$ release in MC3T3-E1 cells and found that while the primary cilium was necessary for the flow induced $PGE_2$ release, it did not play a role in the cell's mobilization of intracellular calcium. Our findings suggest that primary cilia are a component of the mechanotransduction pathway for $PGE_2$ release, but distinct from the kidney, do not play a role in intracellular calcium mobilization. This independence of calcium and $PGE_2$ has been reported previously [5] and suggests that there are multiple mechanisms by which bone cells sense their mechanical environment.

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