Introduction: It is well accepted that fluid flow is an important mechanical signal in regulating bone structure and function. Primary cilia, which are non-motile, microtubule based organelles that extend from the centrosome and project into extracellular space in many cell types (Fig 1), have recently been shown to mediate fluid flow-induced osteogenic responses in MC3T3-E1 osteoblastic cells. However, primary cilia did not mediate increases in intracellular Ca^{2+} concentration, and the second messenger system(s) involved in primary cilia-mediated mechanosensing has yet to be elucidated. In this study, our goals were to (1) determine whether exposing bone cells to oscillatory fluid flow increases intracellular levels of cyclic adenosine monophosphate (cAMP), another ubiquitous second messenger molecule, and (2) investigate whether this increase is mediated by primary cilia.

Materials and Methods: MC3T3-E1 cells were cultured in growth media containing α-MEM with 10% PBS and 1% penicillin/streptomycin at 37°C and 5% CO_{2}. For fluid flow experiments, cells were seeded on fibronectin coated slides 48hrs prior to experimentation and loaded into parallel plate flow chambers containing flow media consisting of 5mM 3-isobutyl-1-methylxanthine in growth media. The cells were incubated for 30mins and then exposed to 1Pa 1Hz oscillatory fluid flow. Immediately following cessation of flow, cAMP was quantified using an enzyme immunoassay system (Assay Designs) and normalized to total protein as quantified by a Bradford assay. To remove primary cilia, 4mM aqueous chloral hydrate was added to cells seeded on slides 72hrs prior to flow, cells were washed three times with PBS, and fresh medium was added for 24hrs before flow experiments.

Results: Intracellular cAMP levels after exposing cells to 5, 15, and 30mins of oscillatory fluid flow can be seen in Fig 2A. Exposing cells to 30mins of flow increased cAMP levels more than threefold relative to controls (No flow: 5.27pmol/mg, Flow: 18.84pmol/mg, p=0.02, n=6). Flow-induced increases in cAMP relative to controls at other time points were not statistically significant. Treating cells with chloral hydrate eliminated flow-induced increase in cAMP in cells exposed to 30mins of flow (Fig 2B; Chloral hydrate no flow: 2.74pmol/mg, Chloral hydrate flow: 2.72pmol/mg, p=0.98, n=8).

Discussion: In this study, we investigated the role of primary cilia in mediating oscillatory fluid flow-induced increases in intracellular cAMP levels in MC3T3-E1 osteoblastic cells. We found that exposing cells to 30 minutes of oscillatory flow caused the cells to respond with more than a threefold increase in intracellular cAMP. The 30 minutes of oscillatory flow necessary to induce a cAMP response is in contrast to previous investigations that have shown that steady flow induces increases in cAMP as early as 30 seconds in calvarial osteoblasts [1]. The difference in the time of flow exposure necessary to induce increases in cAMP may be due to exposing the cells to an oscillatory versus a steady flow profile. For example, several isoforms of adenylyl cyclase (the enzyme responsible for production of cAMP) are mediated by Ca^{2+} [2], and application of static versus dynamic flow profiles has been shown to substantially alter the Ca^{2+} response in bone cells [3]. We removed primary cilia using a chloral hydrate treatment process that maintains cytoplasmic microtubules and cellular morphology [4], thus minimizing non-specific effects of the drug. We found that removing primary cilia eliminated the flow-induced cAMP response in cells exposed to 30 minutes of oscillatory flow, suggesting that cAMP is a second messenger molecule important for primary cilia-regulated mechanosensing. It has previously been shown that PGE_{2} release is mediated by primary cilia [4], and that extracellular PGE_{2} can increase cAMP formation by binding prostaglandin EP_{2} receptors [5]. Therefore, these data suggest that primary cilia-mediated flow-induced increases in cAMP may be regulated by PGE_{2} release, although further investigations are necessary in this regard.

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